Discovery consists of seeing what everybody has seen, and thinking what nobody has thought.

Albert Szent-Györgyi, 1937 Nobel Prize for Medicine
The Institute brings together different research groups who work to improve our understanding of disease mechanisms, as well as to discover and/or develop new therapeutic strategies. Basic research joins clinical research in an enriching environment where clinical scientists and basic researchers can exchange their experiences and work on common projects. Our focus is thus clearly on translational research.

Research at our Institute covers a wide range of biomedical problems and is mostly organized in an organ or system specific manner. The Institute is composed of 21 research groups working in close collaboration with the Cliniques universitaires Saint-Luc, in Brussels, and Mont-Godinne, in Yvoir. The Institute brings together more than 500 researchers and PhD students of different horizons and provides logistical support for both basic and clinical research.

Jean-Louis Vanoverschelde
IREC President
ADMINISTRATIVE STRUCTURE

The administrative structure of the Institute is composed of an Institutes Administrative Coordinator, a Clinical Research Unit and the Logistics and Accounts Unit. This structure ensures a transversal support to all Research Groups.

- Institutes Administrative Coordinator (CAI):
  Michel Van Hassel

- Clinical Research Unit: Regulatory Affairs, Quality, Contract-Budget Analysis of Clinical Trials
  Valérie Buchet
  Clémentine Janssens
  Salvatore Livolsi
  Marie Masson
  Dominique Van Ophem

- Logistics and Accounts Unit:
  Maimouna Elmjouzi MBLG/CTMA
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RESEARCH GROUPS

- Cardiovascular Research (CARD) 7
- Computer Assisted Robotic Surgery (CARS) 24
- Experimental Surgery and Transplantation (CHEX) 32
- Endocrinology, Diabetes and Nutrition (EDIN) 39
- Pharmacology (FATH) 50
- Hepato-Gastro-Enterology (GAEN) 62
- Gynecology (GYNE) 71
- Medical Imaging Research (IMAG) 79
- Louvain Centre for Toxicology and Applied Pharmacology (LTAP) 92
- Medical Microbiology (MBLG) 99
- Molecular Imaging, Radiotherapy and Oncology (MIRO) 102
- Morphology (MORF) 115
- Nephrology (NEFR) 123
- Pediatrics (PEDI) 126
- Pneumology, ENT and Dermatology (PNEU) 132
- Rheumatic Pathology (RUMA) 137
- Centre for Applied Molecular Technologies (CTMA) 142
The importance of cardiovascular disease in terms of public health is well established. Cardiovascular diseases, mainly secondary to atherosclerosis, are responsible for about 50% of deaths in industrialized countries. Since it is not possible to study all aspects of the cardiovascular disease, our research unit has determined its main lines of investigation based on its expertise and know-how and also on the clinical applicability of the scientific topics, which are addressed. Our research focus on three main axes:

1. Research on the role of intracellular signalling in cardiovascular diseases like type 2 diabetes, cardiac hypertrophy and heart failure.
2. Research on valvular diseases.
3. Research in cardiac imaging.
4. Research on platelet signaling and metabolism.

These four main research areas are highly interconnected, integrating both clinical and basic research. Our unit has developed a number of skills ranging from molecular and cell biology to biochemistry and population studies. Our researchers bring their own expertise to the entire research program.
**EXPERIMENTAL RESEARCH GROUP**

**Connection between cardiac vascular permeability, myocardial oedema and inflammation during sepsis: role of the α1AMPK isoform**

D. Castanares-Zapatero, N. Marquet, S. Horman, C. Beauloye

Septic cardiomyopathy is a well-recognized cardiovascular complication in patients in severe sepsis, characterized by a reversible decrease in systolic and/or diastolic left ventricular (LV) function. Although its impact on outcome remains to be defined, it at least contributes to haemodynamic impairment in septic patients. Currently, the patho-physiological mechanisms of depressed LV function remain to be identified.

Increased microcirculatory permeability, a major complication in sepsis due to endothelial barrier disruption, contributes to end-organ dysfunction. This is particularly the case in the lungs where enhanced permeability induces lung oedema causing acute respiratory distress syndrome (ADRS). Whether vascular permeability increases in the heart, as it is the case in other tissues, and whether it contributes to LV dysfunction during sepsis remain incompletely explored.

Since AMP-activated protein kinase (AMPK) (more particularly α1AMPK) controls cytoskeleton organization in endothelial cells (ECs), we postulated that it could influence vascular permeability and inflammation during sepsis, and potentially influence heart function. α1AMPK/-/- and α1AMPK+/+ mice were submitted to sepsis (triggered in vivo using a sub-lethal injection of lipopolysaccharide). α1AMPK-deficiency dramatically impaired tolerance to LPS challenge. α1AMPK/-/- exhibited heightened cardiac vascular permeability after LPS challenge compared to α1AMPK+/+. Consequently, an increase in LV mass corresponding to exaggerated wall oedema occurred in α1AMPK/-/-, without any further decrease in systolic function.

Mechanistically, the LPS-induced α1AMPK/-/- cardiac phenotype could not be attributed to major changes in the systemic inflammatory response, but was due to an increased disruption of interendothelial tight junctions.

Our results demonstrate (Figure 1), for the first time the involvement of a signalling pathway in the control of LV wall oedema during sepsis. AMPK exerts a protective action through the preservation of interendothelial tight junctions. Interestingly, exaggerated LV wall oedema was not coupled with aggravated systolic dysfunction. However, it could contribute to diastolic dysfunction in septic patients.

![Figure 1](AMPK controls vascular permeability during sepsis and influences the onset of septic cardiomyopathy. AMPK activators are potential therapeutic targets. Molecular mechanisms involved in the control of vascular permeability by AMPK are under investigation in the lab.)

**Molecular response to substrate overfuel in the heart (glucotoxicity).**

Anne Van Steenbergen, M. Balteau, S. Horman, L. Bertrand, C. Beauloye

Diabetic cardiomyopathy has been defined as ventricular dysfunction (diastolic or systolic) occurring in the absence of coronary artery disease and hypertension. Changes in myocardial structure, calcium signalling and metabolism are early defects that have been described in many animal models and may preceed cardiac dysfunction. Hyperglycemia participates in the pathophysiology of diabetic cardiomyopathy. Hyperglycemia results in toxic effects in several cell types including cardiomyocytes via an oxidative stress-dependent mechanism. NADPH oxidase and more particularly NOX2 iso-
form plays a critical role in high glucose-induced ROS production and glucotoxicity in the heart.

However, the mechanism linking high glucose concentrations to NADPH oxidase activation remains to be elucidated. Therefore, we investigated the connection between glucose transport, metabolism, NADPH oxidase activation and subsequent ROS production under hyperglycemia. Increased glucose metabolism by itself does not trigger NADPH oxidase activation. NOX2 activation results from glucose transport through SGLT, suggesting that an extracellular metabolic signal transduces into an intracellular ionic signal. This signalling pathway is under investigation in the lab. More particularly, we characterize the expression of SGLT isoforms in the heart and evaluate their contribution in this “high glucose sensing” mechanism.

Protection conferred Glucagon-like peptide-1 against cardiac glucotoxicity. Implication of AMPK.

M. Balteau, A. Van Steenbergen, L. Bertrand, C. Beauloye

As stated above, NADPH oxidase and more particularly NOX2 isoform plays a critical role in high glucose-induced ROS production and glucotoxicity. NOX2 activation by hyperglycemia occurred in the caveolar structure.

In this project, we hypothesized that activation of AMPK could influence NOX2 activation and the subsequent ROS production in response to high glucose concentration in cardiomyocytes. The know AMPK activators inhibited hyperglycemia-induced NOX2 activation and ROS production. More, particularly, they blocked the translocation of the NADPH oxidase activating sub-unit, p47phox, closed to caveolin-3. Glucagon-like peptide-1 or GLP-1 also activated AMPK and counteracted p47phox translocation into the caveolar structure. The critical role of AMPK was evidenced using AMPK knockout mice. GLP-1 could therefore protects the heart against diabetic complications independently glycemic control (Figure 2).

AMPK regulates actin polymerization, lamellipodia formation and clot retraction, in thrombin-stimulated platelets.

M.-B. Onselaer, S. Lepropre, C. Beauloye, S. Horman

Platelet activation contributes to thrombotic disorders and can lead to myocardial infarction. Thus, basic research to understand how platelets work and how their function can be blocked is a crucial approach for the prevention and treatment of cardiovascular diseases.

Platelet activation requires sweeping morphological changes, supported by contraction and remodelling of platelet actin cytoskeleton. In epithelial and endothelial cells, AMPK controls actin cytoskeleton organization through the phosphorylation of cytoskeletal targets, namely myosin regulatory light chains (MLC), coflin and the vasodilator-stimulated phosphoprotein (VASP), extending the role of AMPK beyond metabolism.

In this study, we hypothesized that AMPK was activated in thrombin-stimulated platelets and played a role in platelet secretion, aggregation and clot retraction, by regulating polymerization and organization of actin cytoskeleton through the phosphorylation of MLC, coflin and VASP.
We showed that human platelets express exclusively the AMPKα1 isoform. In human purified platelets, thrombin led to a transient activation of AMPKα1 and to phosphorylation of its substrate acetyl coA carboxylase (ACC). Platelets isolated from mice lacking AMPKα1 exhibited reduced aggregation and secretion in response to thrombin, associated with a defect in ACC, MLC, coflin and VASP phosphorylation. These changes were associated with an abrogation of thrombin-dependent F-actin formation. Moreover, the percentage of platelets able to form lamellipodia after immobilization on fibrinogen-coated coverslips and stimulation by thrombin, was significantly reduced in the absence of α1AMPK, indicating an altered cytoskeleton reorganization during spreading. More importantly, clot retraction was slower and less effective in KO platelets (Figure 3).

Our results demonstrate for the first time that AMPKα1 plays a critical role in platelet function through the phosphorylation of cytoskeletal targets and the subsequent regulation of cytoskeleton organization-dependent processes.

Figure 3 Platelet activation requires sweeping morphological changes, supported by contraction and remodelling of platelet actin cytoskeleton. Our study demonstrates for the first time that AMPKα1 plays a critical role in platelet function through the phosphorylation of cytoskeletal targets and the subsequent regulation of cytoskeleton organization-dependent processes, e.g. clot retraction. Clot retraction was assessed at 1 hour after addition of thrombin 0.05 U/ml. The images in lower panel show a representative result. Data are presented as mean ± SEM (n=3, ###P<0.001 represents a statistical difference between platelets from WT and AMPK-α1 KO mice treated with thrombin).

Identification of a new biomarker for in vivo evaluation of blood coagulation and platelet activation.

M.-B. Onselaer, S. Lepropre, P. Buchlin, C. Beauloye, S. Horman

We discovered that thrombin was the only platelet agonist causing AMP-activated protein kinase activation and subsequent ACC phosphorylation in human platelets. Therefore, we postulated that the phosphorylation state of its substrate acetyl-coA carboxylase (ACC) could be considered as a valuable marker of the thrombin response in platelets from patients undergoing major surgery.

To test this hypothesis, twenty-nine consecutive patients scheduled for elective cardiac surgery with cardio-pulmonary bypass (CPB) were recruited in the department of cardiovascular surgery. The human platelets were isolated from citrated blood samples drawn during CPB (under high-dose UFH) and 4 hours after surgery. ACC phosphorylation was assessed by immunoblotting. Activated clotting time (ACT), thrombin time (TT) and activated partial thromboplastin time (aPPT) were measured in a coagulation device. In addition, an estimation of how each patient can generate thrombin during all steps of the protocol has been done, using a fluorogenic assay for the measurement of endogenous thrombin potential (ETP).

Under CPB, mean ACT was 594 ± 100 s, and aPTT and TT were over 180 s and 120 s, respectively. Normal capacity to coagulate was restored 4 hours after surgery (aPTT: 36.3 ± 7.4 s TT: 21.3 ± 5.2 s). ETP values were characterized by a huge heterogeneity in post-operative status, reflecting the random ability of patients to generate thrombin after CBP. ACC phosphorylation was therefore evaluated in 2 extreme groups of patients, recovering more than 80% and less than 20%-of basal ETP, respectively. ACC phosphorylation was significantly higher in the platelets of patients preserving an intact ability to generate thrombin (ETP>80%).

In conclusion, our study reveals that ACC phosphorylation in platelets reflects (i) their activation by thrombin after major surgery and more importantly (ii) an intact ability to generate thrombin in this situation. From a clinical point of view, postoperative bleeding is one the most common
complications of cardiac surgery that requires multiple transfusion or surgical re-exploration. In vivo assessment of ACC phosphorylation could therefore allow discriminating bleeding due to imperfect surgical haemostasis from bleeding attributed to a coagulation disorder. This information could have a direct and crucial impact on therapeutic decisions. ("Marker for blood coagulation", WO/2013/076157).

Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking α1AMPK.

G. Noppe, C. Dufeys, P. Buchlin, N. Marquet, C. Beauloye, S. Horman

Cardiac fibroblasts (CF) are crucial in left ventricular (LV) remodelling after myocardial infarction. They predominantly express the α1 catalytic subunit of AMPK 1, while AMPKα2 is the major catalytic isoform in cardiomyocytes. AMPKα2 is known to protect the heart by preserving the energy charge of cardiac myocytes during injury, but whether AMPKα1 interferes with maladaptive heart responses remains unexplored. In this study, we aimed at further substantiating the role of this AMPK isoform in the pathogenesis of post-MI LV remodelling and more particularly in the regulation of fibrotic properties of CF.

To investigate this question, AMPKα1 knockout (KO) and wild type (WT) mice were subjected to permanent ligation of the left anterior descending coronary artery to mimic MI. Cardiac fibrosis was monitored using QRT-PCR analysis, histology and immunohistochemical staining. LV function and remodelling was assessed by echocardiography.

We showed that, in the absence of AMPKα1, the CF proliferative response was increased in infarcted myocardia. It resulted in elevated levels of fibrotic factors but did not lead to excessive matrix deposition or degradation in KO infarcts. While CF proliferation was increased, expression of the myodifferentiation marker α-smooth muscle actin was decreased. Although infarct size was similar in KO and WT hearts subjected to MI, these changes resulted in defective scar collagen maturation. This was associated with an exacerbated adverse remodelling as indicated by increased LV diastolic dimension 30 days after MI (Figure 4).

In conclusion, these data genetically demonstrate the centrality of AMPKα1 in post-MI scar formation and highlight the specificity of this catalytic isoform in cardiac fibroblast/myofibroblast biology.

Figure 4 AMPKα1 deficiency reduces myodifferentiation of cardiac fibroblasts and alters scar maturation, leading subsequently to exaggerated left ventricular dilation after myocardial infarction.

Control of protein synthesis by AMPK in normoxic and ischemic hearts.

A. Ginion, C. Beauloye, S. Horman, L. Bertrand

Eukaryotic elongation factor 2 (eEF-2) and mammalian target of rapamycin (mTOR)-p70 ribosomal protein S6 kinase (p70S6K) signaling pathways are the main elements controlling protein synthesis in the heart. They are known to be inhibited during myocardial ischemia. Intracellular acidosis and AMP–activated protein kinase (AMPK) activation occur during ischemia and both have been proposed to participate in eEF2 and mTOR/p70S6K inhibition. In this project, we evaluated the contribution of AMPKα2, the main cardiac AMPK catalytic subunit isoform, in eEF-2 and mTOR/p70S6K regulation using AMPKα2 knockout (KO) mice. We have demonstrated that, under ischemic condition, acidosis is the main actor regulating mTOR/p70S6K inhibition whereas eEF2 inhibition is under the control of AMPKα2 (Figure 5).
This challenges the accepted notion that mTOR/p70S6K is inhibited by myocardial ischemia mainly via an AMPK-dependent mechanism. However, we also showed that basal AMPKα2 repress p70S6K under normoxic condition. This p70S6K regulation was associated with the phosphorylation of Raptor, an mTOR partner previously identified as an AMPK target (Figure 5). To summarize, AMPKα2 controls cardiac p70S6K under normoxia and regulates eEF-2 but not the mTOR/p70S6K pathway during ischemia.

![Figure 5 Regulation of heart eEF2 and mTORC1/p70S6K pathways by AMPKα2 under normoxic and ischemic conditions.](image)

**Action of AMPK on stem cell metabolism and proliferation.**

A. Timmermans, C. Beauloye, L. Bertrand

Mesenchymal stem cells (MSCs) are widely used for cell therapy, particularly for the treatment of ischemic heart disease. Mechanisms underlying control of their metabolism and proliferation capacity, critical elements for their survival and differentiation, have not been fully characterized. AMPK is a key regulator known to metabolically protect cardiomyocytes against ischemic injuries and, more generally, to inhibit cell proliferation. We originally hypothesized that AMPK plays a role in control of MSC metabolism and proliferation. We showed that chronic exposure of MSCs to hypoxia failed to induce cell death despite the absence of AMPK activation. This hypoxic tolerance was the consequence of MSC preference towards glycolytic metabolism independently of oxygen availability. On the other hand, we showed that A-769662-induced AMPK activation inhibited MSC proliferation. Proliferation was not arrested in MSCs lacking AMPK expression, providing genetic evidence that AMPK is essential for this process. Among AMPK targets proposed to regulate cell proliferation, we showed that neither the p70S6K/eEF-2-dependent protein synthesis pathway nor p21 was involved, whereas p27 expression was increased by A-769662. Silencing p27 expression prevented the A-769662-dependent inhibition of MSC proliferation. In conclusion, MSCs resist hypoxia independently of AMPK whereas chronic AMPK activation inhibits MSC proliferation via p27 regulation.

**EQUIPMENT**

- Experimental physiologic and biochemistry lab
- Experimental cardiac histology lab (cryosections, paraffin sections, immunohistology)
- Heart perfusion equipment (for mice, rat, rabbit and pig)
- Dedicated research echo machines (including Visualsonics VEVO dedicated to small animals)
- Three cath-labs
- Computerized system for rest and stress ECG-VCG analysis
- Clinical database
Drug-resistant hypertension and renal sympathetic denervation

A. Persu, J. Renkin

Resistant hypertension is a blood pressure that remains above goal on three or more antihypertensive medications, preferably including a diuretic. The Symplicity HTN-1 and HTN-2 studies show that catheter-based endovascular sympathetic renal denervation (RDN) by means of low-frequency energy is feasible in this indication. It entails a 25–30 mm Hg mean decrease in office systolic blood pressure, with a rate of procedural adverse events lower than 5% assessed 6 months after RDN. However, the proportion of patients eligible for RDN, the size and durability of the antihypertensive, renal and sympatholytic effects of RDN and the long-term safety of the technique still remain to be firmly established.

The Symplicity trials have important limitations and most studies are small, industry-driven, and purely observational. Since 2012, we discussed in depth the weaknesses and potential biases of the current evidence supporting the use of RDN in ten reviews, position papers and letters published in peer review journals.

In order to assess the safety and efficacy of RDN, we initiated the European Network Coordinating research on REnal Denervation (ENCOREd) consortium.

Our first aim was to investigate the proportion of patients eligible for RDN and the reasons of non-eligibility. The analysis included 731 patients from 11 European centres (Figure 6). Age averaged 61.6 years, office blood pressure at screening was 177/96 mm Hg, and the number of blood-pressure lowering drugs taken was 4.1. Seventy-six % of patients were referred by specialists.

The proportion of patients eligible for RDN according to the Symplicity HTN-2 criteria and each centre’s criteria was 42.5% and 39.7%, respectively. At the first visit to the referral centre, drug treatment was clearly suboptimal: 14.9% of patients had no drug inhibiting the renin-angiotensin system, 27.2% had no calcium antagonist and 18.0% had no diuretic.

Furthermore, 21.5% were prescribed two or even three drugs inhibiting the renin angiotensin system, a non-synergic and potentially dangerous drug combination. By contrast, spironolactone, a drug recommended for the treatment of resistant hypertension was prescribed in only 26.0% of patients. Eventually, the main reasons of non-eligibility for RDN were normalization of blood pressure after treatment adjustment in the expert centre (46.9%), unsuitable renal arterial anatomy (17.0%), and previously undetected secondary causes of hypertension (11.1%) (Figure 7).
Our findings highlight that hypertension centres with a record in clinical experience and research should remain the gatekeepers before RDN is considered and that a substantial proportion of patients with resistant hypertension are amenable to blood pressure control by skilful treatment adjustment.

In a second time, we performed a subject-level meta-analysis of the changes in blood pressure observed 6 months after renal denervation (RDN) at 10 European centres. Recruited patients (n=109 46.8% women mean age 58 years) had essential hypertension confirmed by 24-h ambulatory blood pressure. From baseline to 6 months, treatment score declined slightly from 4.7 to 4.4 drugs per day. Systolic/diastolic blood pressure fell by 17.6/7.1 mm Hg for office blood pressure, but only by 5.9/3.5 for 24-h ambulatory blood pressure (P≤0.03, Figure 8).

Figure 8  Mean changes in blood pressure.

Blood pressure changes after RDN were highly variable (Figure 4). For office blood pressure, at 6 months, normalization, improvement or no decrease occurred in 22.9%, 59.6% and 22.9% of patients for 24-h ambulatory blood pressure, these proportions were 14.7%, 31.2% and 34.9%, respectively. Higher baseline blood pressure predicted greater blood pressure fall at follow-up higher baseline serum creatinine was associated with lower probability of improvement of 24-h blood pressure (odds ratio for 20 µmol/L increase, 0.60  P=0.05) and higher probability of experiencing no blood pressure decrease (OR, 1.66  P=0.01).

Figure 9  Individual changes in blood pressure.

These results suggest that blood pressure responses to RDN include regression-to-the-mean and remain to be consolidated in randomized trials based on ambulatory blood pressure monitoring.

For now, RDN should remain the last resort in patients in whom all other ways to control blood pressure failed and must be cautiously used in patients with renal impairment.

Long term risk of valve related event, pericardial patch repair and ventriculo-aortic junction annuloplasty

L. De Kerchove, P. Noirhomme, G. El Khoury

From 1995 to 2013, 650 patients underwent aortic valve repair (AVR) in our institution. This series represents one of the largest in the world. All those patients are followed closely and constitute the substrate for continuous research in that field. The indication and techniques of aortic valve repair have evolved during these two decades. The development and refinement of the repair technique have led to extend indication of AVR like in reoperation after Ross procedure or after failed AVR. Research has allowed identifying the prerequisite for durable repair and to understand the mechanisms of failure.
A recent retrospective analysis of our entire cohort showed that AVR is safe with a hospital mortality rate of 0.8% only. At 10 years, freedom from cardiac death and valve-related death was 81% and 90% respectively. Reoperation was needed in 28 patients; all of them survived reoperation and 8 had repeat repair. Ten-year freedom from AV reoperation and AV replacement was 86% and 90% respectively. Freedom from AV reoperation was similar in tricuspid and bicuspid valve. During the follow-up period, linearized rate of thromboembolic event, bleeding, and AV endocarditis was 0.7%, 0.23%, and 0.19% per year, respectively. The study concludes that AV repair is a solid alternative to replacement in young patients as it is associated with low mortality, low risk of valve-related events and acceptable durability.

Pericardial patch repair allows extending the indication of AVR to patients with partial valve destruction or restriction by fibrosis or calcification. However, the use of patch has been associated with a higher rate of repair failure. We retrospectively analyzed our experience with pericardial patch in AVR for non-rheumatic AV disease. Patients had unicusp (12%), bicuspid (53%) or tricuspid (35%) AV. At 8 years, freedom from reoperation was 75%. Freedom from reoperation was slightly better in tricuspid compared to non-tricuspid valves (92% vs 68%, P = 0.18) and slightly better for bovine compared to autologous pericardium (95% vs 73%, P = 0.38). In tricuspid valves, freedom from reoperation was significantly better in perforation repair compared to other techniques (100% vs 50%, P = 0.02). In bicuspid valves, freedom from reoperation was similar between different repair techniques (P = 0.38). The study concludes that AVR with patch is feasible for various etiologies. The techniques are safe and long-term durability is acceptable or even excellent in certain indication like perforation in tricuspid AV. Treated autologous pericardium is the first choice for patch repair, but bovine pericardium is an acceptable second choice.

In a retrospective propensity match study, we demonstrate that circumferential annuloplasty (the valve sparing reimplantation technique, VSR) improve durability of bicuspid AVR compared to non-circumferential annuloplasty (the subcommissural annuloplasty technique, SCA). In a subsequent study, we analyzed the role of aortic annulus dimension and type of annuloplasty on repair durability. Mean preoperative aortic annulus was 28 ± 3 mm which is larger than in normal tricuspid AV. Annulus was larger in younger patients (<40 years) and in patients with severe aortic regurgitation (AR) (P < 0.01). The VSR reduced more the annulus size compared to the SCA (21 mm vs 24 mm, P < 0.01). By univariate analyses, SCA, preoperative annulus size ≥30 mm and cusp repair with patch were predictive of recurrent AR ≥1+. In the SCA, annulus ≥30 mm was associated with decreased 6 years freedom from recurrent AR≥1+ (<30 mm: 74% vs ≥ 30 mm: 39%, P = 0.01). Whereas in the VSR, VAJ dimension had no effect on recurrent AR >1+ (P = 0.93). The study concludes that in bicuspid AVR, large aortic annulus (≥30mm) was predictive of AR recurrence after repair with the SCA technique, yet it had no impact on repair durability after the VSR technique. VSR can be a recommended treatment for BAV regurgitation in patients with large annulus and no-to-moderate aortic root dilatation.

Pathophysiology of aortic stenosis evaluated by cardiac MRI.

B. Gerber, A. Pasquet, J-L Vanoverschelde

Severe degenerative aortic stenosis is the most frequent valvular heart disease in industrialized countries and its prevalence steadily increases with age. The increased pressure afterload and ventricular wall stress of this condition stimulates left ventricular hypertrophic remodelling. Such remodelling is frequently associated with development of adverse intramyocardial fibrosis, which may lead to alterations of both systolic and diastolic left ventricular function and functional status of patients with aortic stenosis. When patients develop symptoms due to severe aortic stenosis, the valve needs to be replaced to prevent death or heart failure.

Clinical guidelines define severe aortic stenosis as aortic valve area < 1.0 cm² or <0.6 cm²/m² with mean transvalvular gradient > 40 mm Hg and transvalvular velocity > 4 m/s. According to the Gorlin formula, the pressure gradient across a stenotic valve is directly related to magnitude of flow rate and inversely related to the square of valve area. Thus for a given transvalvular flow, valve area and pressure gradient are directly related by an inverse polynomial relationship. However in clinical practice, the relation between transvalvular pressure gradient and aortic valve area may often be discordant. Such discordance between pressure gradients and estimated surface area makes it often difficult to grade the
severity of aortic stenosis, hampering decision on whether the valve should be replaced or not.

The problem most often occurs in patients with poor ejection fraction (EF) and low transvalvular flow. Yet lower than expected gradients have also been observed in some patients with severe aortic stenosis and preserved left ventricular function. Pibarot and Dumesnil named this condition “paradoxical low flow low gradient” aortic stenosis, and reported that such patients would have more pronounced left ventricular concentric remodelling, smaller left ventricular cavity size, lower left ventricular function although remaining within the normal range, and more interstitial fibrosis. They hypothesized that paradoxical low flow low gradient aortic stenosis would represent a more advanced state of valvular and ventricular disease, in which the ventricle would be failing because of increased afterload and hypertrophy. However discordance between transvalvular gradients and estimated aortic valve area could also result from confounding situations, such as small body size, measurement errors of stroke volume, trans-valvular flow and effective aortic area by trans-thoracic echography. Because these measurements are evaluated from left ventricular outflow tract diameter and velocity time interval, underestimation of these parameters by trans-thoracic echography could explain such discordance between gradients and valve area.

Cardiac MRI allows direct planimetry of the anatomical valve area and of the left ventricular outflow tract area, and assessment of aortic stroke volume by phase contrast imaging. Therefore it may verify the accuracy of these measurements by transthoracic echocardiography in paradoxical low flow aortic stenosis. Hence we performed a study in 128 consecutive patients with severe aortic stenosis defined as aortic valve area<0.6 cm/m2 and preserved ejection fraction (>50%) using cine, phase contrast and, contrast enhanced CMR to evaluate the pathophysiology of paradoxical low-flow low gradient aortic stenosis. In particular we wanted to 1) to evaluate the accuracy of aortic stenosis classification by echocardiography by comparing measurement of effective orifice area and of left ventricular outflow area and aortic stroke volumes by echocardiography and against MRI. 2) to explore the pathophysiology of different types of aortic stenosis by cardiac MRI aiming at better understanding the differences between high and low gradient and normal and low flow aortic stenosis with preserved left ventricular function. We compared the severity of left ventricular hypertrophy and of patterns of remodeling and the degree of focal myocardial fibrosis among these different types of aortic stenosis.

Our study showed that MR confirmed that classification of aortic stenosis patterns by echocardiography was overall accurate. Indeed we observed a good correlation between measurements of aortic stroke volume and aortic valve area between echocardiography and MRI, suggesting that measurement errors are not the major explanation of paradoxical low flow aortic stenosis, when exams are carefully performed.
Early surgical intervention vs watchful waiting and outcomes for mitral regurgitation

J-L Vanoverschelde, A. Pasquet

The optimal management of severe mitral valve regurgitation in patients without class I triggers (heart failure symptoms or left ventricular dysfunction) remains controversial in part due to the poorly defined long-term consequences of current management strategies. In the absence of clinical trial data, analysis of large multicenter registries is critical.

To ascertain the comparative effectiveness of initial medical management (nonsurgical observation) vs early mitral valve surgery following the diagnosis of mitral regurgitation due to flail leaflets, we conducted a retrospective multicentric study, using the data from the Mitral Regurgitation International Database (MIDA) registry. This registry includes 2097 consecutive patients with flail mitral valve regurgitation (1980-2004) receiving routine cardiac care from 6 tertiary centers (France, Italy, Belgium, and the United States). Mean follow-up was 10.3 years and was 98% complete. Of 1021 patients with mitral regurgitation without the American College of Cardiology (ACC) and the American Heart Association (AHA) guideline class I triggers, 575 patients were initially medically managed and 446 underwent mitral valve surgery within 3 months following detection. Outcomes measures were survival, new onset heart failure, and new-onset atrial fibrillation.

We found no significant difference in early mortality (1.1% for early surgery vs 0.5% for medical management, P=.28) and new-onset heart failure rates (0.9% for early surgery vs 0.9% for medical management, P=.96) between treatment strategies at 3 months. In contrast, long-term survival rates were higher for patients with early surgery (86% vs 69% at 10 years, P <.001), which was confirmed in adjusted models (hazard ratio [HR], 0.55 [95% CI, 0.41-0.72], P <.001), a propensity-matched cohort (32 variables HR, 0.52 [95% CI, 0.35-0.79], P = .002),

More importantly our study demonstrated that patients with paradoxical low flow low gradient aortic stenosis had less rather than more severe aortic stenosis severity.

We also found they had less hypertrophy and remodeling and similar amount of focal fibrosis than high gradient aortic stenosis.

The findings of our study thus contradict the view that paradoxical low flow low gradient aortic stenosis is a more severe rather form of aortic stenosis than high gradient aortic stenosis. On the contrary, and in agreement with the long known hemodynamic relationship between aortic stenosis severity and pressure gradients our data suggest that paradoxical low flow low gradient aortic stenosis is less rather than a more advanced form of aortic stenosis. Our works also demonstrated that cardiac MRI could be a valuable approach for assessing patients in which assessment of aortic stenosis severity by transthoracic echocardiography is difficult, in particular those where gradients and surface severity of the valve and clinical symptoms are discordant.

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and an inverse probability-weighted analysis (HR, 0.66 [95% CI, 0.52-0.83], P < .001), associated with a 5-year reduction in mortality of 52.6% (P < .001).

Similar results were observed in relative reduction in mortality following early surgery in the subset with class II triggers (59.3 after 5 years, P = .002).

Long-term heart failure risk was also lower with early surgery (7% vs 23% at 10 years, P < .001), which was confirmed in risk-adjusted models (HR, 0.29 [95% CI, 0.19-0.43], P < .001), a propensity-matched cohort (HR, 0.44 [95% CI, 0.26-0.76], P = .003), and in the inverse probability-weighted analysis (HR, 0.51 [95% CI, 0.36-0.72], P < .001).

Reduction in late-onset atrial fibrillation was not observed (HR, 0.85 [95% CI, 0.64-1.13], P = .26).

We concluded that among registry patients with mitral valve regurgitation due to flail mitral leaflets, performance of early mitral surgery compared with initial medical management was associated with greater long-term survival and a lower risk of heart failure, with no difference in new-onset atrial fibrillation.

Central and peripheral pulse wave velocities are associated with ankle-brachial pressure index.

A. V. Lacroix, E Marchandise

Central Pulse Wave Velocity (PWV) is considered to be the gold standard measurement of arterial stiffness. In healthy subjects, cardiovascular risk factors such as age, hypertension, diabetes and end-stage renal disease are associated with increased central (Carotid-Femoral) and peripheral (Femoral-Ankle) PWV. However, little is known about PWV in patients with peripheral arterial disease and pathological Ankle-Brachial Index (ABI).

The aim of this study was to study central and peripheral PWV in a population with various degree of peripheral arterial disease. Central and peripheral PWV were measured in sixty-two hospitalized patients. Half were admitted for symptomatic peripheral vascular disease and the remainder for cardiac or carotid disease. The population was classified on basis of the Framingham-derived risk score for claudicants and on the ABI. For all patients, PWV was assessed on electrocardiogram ultrasonographic images acquired at the four following sites: carotid, radial, femoral and tibial arteries.

Carotid-Femoral PWV increased significantly with the Framingham-derived global risk score (p < 0.0001) but Femoral-Ankle PWV did not (Fig.1).

With respect to the Ankle-Brachial Index, Carotid-Femoral and Femoral-Ankle PWV significantly increased (p = 0.05 and p = 0.02 respectively) with the severity of peripheral arterial scoring (Fig.2).
These results confirm that central PWV is the best indicator of general atherosclerosis, even in the presence of peripheral arterial disease. Both central and peripheral PWV can be considered as indicators of the severity of peripheral vascular disease.

**SELECTED PUBLICATIONS**


15. Astarc P, Glineur D, De Kerchove L, El Khoury G. Transcatheter valve used in a bailout technique


63 | Persu A, Scavée C, Staessen JA, Blankestijn PJ. Electric nerve stimulation to monitor the efficacy of renal


EQUIPMENT

- Experimental physiologic and biochemistry lab
- Experimental cardiac histology lab (cryosections, paraffin sections, immunohistology)
- Heart perfusion equipment (for mice, rat, rabbit and pig)
- Dedicated research echo machines (including Visualsonics VEVO dedicated to small animals)
- Three cath-labs
- 9 high-end echocardiographic systems
- 3T cardiac magnetic resonance system
- 258-slice MDCT
- Computarized system for rest and stress ECG-VCG analysis
- Valve disease registry

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In the research group CARS, we are engineers from UCL and surgeons from the Cliniques universitaires Saint-Luc. We have close collaborations with other institutes from UCL, such as IONS, IMMC, ICTM, ISBA, Louvain Bionics, Louvain School of Engineering, etc. We have international collaborators in Europe, North and South America, and Asia.

In terms of research activities, we aim to promote a collaborative research framework enabling engineers and surgeons to work together in the field of CAOS. We supervise research of Master and PhD students in engineering and surgery, from the Louvain School of Engineering and the School of Medicine. We have two axes of research: we are developing new assistance technologies for surgery, and we are developing new standard protocols to evaluate quality in CAOS. We have seven fields of expertise: surgical planning and simulation, medical imaging and data processing, patient-specific instrumentation, surgical navigation and robotics, mechanical design in biomedical engineering, metrology and standards, and technology assessment. Currently, we have 11 ongoing research projects in various surgical applications, such as bone tumors, scoliosis, vascularized bone grafting, bone assembly, fracture reduction, spine trauma, TKA, pedicle screw insertion and early-onset scoliosis.

As part of the UCL, we are also following three missions, each at a different level. At the institutional level, we are developing new teaching of computer-assisted and robotic surgery at the Louvain School of Engineering, particularly through the course LMECA2355 “Mechanical design in biomedical engineering”, using the latest technologies available in our lab such as intraoperative fluoroscopic imaging, optical navigation, 3D printing and mechanical measuring machine. At the national level, we are creating “CAOS-Belgium”, the first Belgian Scientific Society for Computer Assisted Orthopaedic Surgery, in collaboration with other Belgian universities. And finally, at the international level, we are aiming to setting up a new technical committee within the International Organization for Standardization ISO, with the mission to develop new ISO standards for quality evaluation in CAOS.
ISOCAOS - New ISO Standards for Quality Evaluation in Computer Assisted Orthopaedic Surgery

O Cartiaux

In orthopaedic surgery, there exist several assistance technologies such as imaging and navigation systems, positioning robots and additive-manufacturing patient-specific instrumentation (PSI). These generic technologies can be used for different applications, i.e. knee arthroplasty, spine instrumentation and bone tumor resection. Practically, these technologies enable surgeons to identify patient anatomy, define preoperative surgical planning and intraoperatively replicate this planning on the patient.

Since 2010, there exists a standard protocol from ASTM society to assess technical specifications of navigation systems and positioning robots in CAOS. The standard presents both parameters to be used and tracking tests to be performed to define accuracy and precision (bias and variability) of navigation and robotic systems. The logical continuation of this first ASTM standard is to develop new standard protocols to evaluate quality of bone-preparation tasks, i.e. bone cutting, milling, drilling and assembly of bone fragments, performed with the aid of such CAOS systems. This project introduces a practical guide for orthopaedic surgeons to plan and evaluate bone-preparation tasks in CAOS. The practical guide consists in a systematic six-steps approach to plan and evaluate bone-preparation tasks in CAOS. The practical guide provides guidelines to implement the systematic approach for specific task and surgical application, i.e. femoral and tibial cutting in knee arthroplasty, vertebral drilling in spine instrumentation, and bone cutting and assembly in tumor surgery.

Practically, for surgeons, such a practical guide can serve as a standard application-specific protocol to evaluate how accurately a preoperative planning can be intraoperately replicated on the patient. For researchers, this can be a standard protocol to investigate performances of new CAOS technologies that are still in prototyping in laboratories. For industrials, this can be useful for the certification of new CAOS technologies that are ready for marketplace.

In 2014, joint application to ISO and CAOS societies will aim the setting-up of a new international technical committee composed of voluntary academicians and industrials, surgeons and engineers, to develop new application-specific ISO standards dedicated to the planning and quality evaluation in CAOS.

OP&RA – 3D Planning and Patient Specific Instrumentation for Bone Tumor Surgery

L Paul

Pelvic bone tumor resection is challenging due to complex geometry, limited visibility and restricted working space of the pelvis. Accurate resection in safe margin is required to reduce the risk of local recurrence. Preoperative planning and intraoperative navigation technologies have been developed for pelvic bone tumor surgeries, and clinical studies have demonstrated the feasibility of achieving clinically adequate (tumor-free) resection margins. Patient-specific instrumentation (PSI) technology has been developed and adapted to bone tumor surgery as a cheaper and time-saving alternative to intraoperative navigation. A recent experimental study has assessed an equivalent value-added of both PSI and navigation technologies in terms of achieved surgical margins during simulated bone tumor resections of the pelvis. This study reports 11 clinical cases surgically treated for a bone tumor resection using PSI within the pelvis and assesses how accurately a preoperative resection strategy can be replicated intraoperatively on actual patient with the PSI. PSI were quick and easy to use with a positioning onto the bone surface in less than 5 minutes for all cases. The positioning of the PSI was considered unambiguous for all patients. Histopathological analysis classified all achieved resection margins as R0 (tumor-free), except for two patients. One patient had an urgent morcelized tumor because of severe bleeding, inevitably inducing R2 bone margins. A second patient had R1 resection because of soft tissues margins between 0 and 1 mm, although bone margins were classified R0. The errors in safe margin averaged -0.8 mm (95% CI: -1.8 mm to 0.1 mm). The maximum positive error was 0.3 mm (patient #7), while the maximum negative error was -3.4 mm (patient #5). The location accuracy of the achieved cut planes with respect to the desired cut planes averaged 2.5 mm (95% CI: 1.8 to 3.2 mm). The maximum inaccuracy was found for patient #5 with a difference of 4.4 mm between desired and achieved cut planes. Results in terms of the errors in safe margin or the location accuracy demonstrated how PSI enabled the surgeon to intraoperatively replicate the resection strategies with a good cutting accuracy.
second, the use of formalin to increase the MRI-contrast at the edge of the resected specimen: In the previous experiments, the specimens are acquired with MRI immediately after surgical resection without any tissue fixation. In case of some delay between MRI and tissue resection, some tissue necrosis or desiccation could occur. The contrast at the edge of the specimen is not always good enough to measure precisely the margin of safe tissue around the tumor. We hypothesized that putting the specimen in a formalin bath will increase the contrast and facilitate the margin evaluation with MRI. Preliminary tests have been performed and show the benefit of formalin. The experience with rats will be realized again but with the use of formalin.

3. Finding a MRI-marker to enhance the edge of the resection specimen: In clinical practice, the pathologist inks the border of the specimen that will be apparent on the histological slices. So he can easily determine the distance between the tumor and the edge of the specimen. Tests have been made with ink in MRI but ink is not visible. We have to find another MRI-visible substance that could be used in clinics. This marker would be applied at the surface of the specimen by a paintbrush. Researches are made to find a MRI-marker visible on T1 and T2-weighted sequences.

Figure
Postoperative evaluation for patient #2: the achieved cut plane was identified and compared to the desired cut plane.

SAFEMARGIN - Extemporaneous specimen MRI: application on bone and soft tissue sarcoma surgery

PL Docquier, SY Traore

1. MRI compared to histology on rodent tumor model: A rodent model is used (WAG male rats, Harlan, Horst, The Netherlands). One fragment of syngenic rhabdomyosarcoma is grafted in the glutei muscles of the thigh muscles. The tumor grows to a volume of 6 to 7 cm3 after 2 weeks. The rodent is sacrificed after 2 weeks and the tumor resected with margin. In some cases no margins are respected, in other cases good margins are obtained. The specimens are acquired with MRI. After MRI acquisition, the specimens are fixed within formalin. Different bath (formalin, ethanol) are used to dehydrate the specimens. They are then included in methylmetacrylate. After polymerization of the methylmetacrylate, slicing is performed. Coloration is processed with methylene blue to be able to analyse histologically the slices. The slices are digitized with a flatbed scanner. Comparison between MRI and histology has been performed, with comparison of the margins measured by MRI, macroscopy (flatbed scanner pictures) and microscopy (pathology). These experiments are still in process.

Figure
Example of a histological slice (below) associated with its corresponding MRI slice (above) of the same excised tumor specimen for the comparison of the two measurement methods.
SCOLIOSIS - Sensitivity analysis of geometric and dynamic variables of the scoliotic spine on the computation of intervertebral efforts

G Abedrabbo, O Cartiaux, M Mousny

In scoliotic spine surgery, the quantification of intervertebral efforts acting between vertebrae can be useful to improve the preoperative surgical planning and define the instrumentation and correction maneuvers to be performed perioperatively. In idiopathic scoliotic patients, the intervertebral efforts seem to be correlated with the severity of spinal deformities. Considering spine as an articulated multibody mechanical system, the computation of the intervertebral efforts can be implemented in a 3-step process: (1) construction of a 3D multibody model of the spine using standing biplanar radiographs, (2) analysis and prediction of the spine kinematics (i.e. motion) during gait, and (3) computation of dynamic intervertebral efforts in terms of forces (N) and torques (Nm) acting between vertebrae. Step #1 has already been implemented for scoliotic patients using the standing position with possible bending and an optimization process based on geometrical data reconstructed from preoperative biplanar radiographs.

The present study aims to investigate the sensitivity of the proposed 3-step process for the computation of intervertebral efforts within the spine. Practically, the work investigated the effect on the intervertebral efforts, of two main factors: (1) the inaccuracies in the identification of the geometrical data in the preoperative biplanar radiographs, and (2) the level of stiffness given to the intervertebral discs in the multibody model of the spine.

The sensitivity analysis demonstrated that the intervertebral efforts acting between vertebrae during simulated motion are significantly influenced by the level of longitudinal stiffness given to the intervertebral discs in the multibody model of the spine. This suggests for further investigations that modeling intervertebral discs with rigid elements could be sufficiently reliable to compute the intervertebral efforts acting between vertebrae during normal gait. Although all these results clearly have to be validated in a larger number of scoliotic patients during gait, they may be straightaway useful to assess the level of accuracy that the multibody modeling of the spine needs to be provided with in terms of geometric and dynamic properties.
ZEEGO SPINE - Quantitative accuracy assessment of pedicle screw insertion in spine surgery using Artis Zeego intraoperative imaging robotic system

X Banse, JC Boulanger, A Boutchichi, O Cartiaux

In spine surgery, intraoperative computed tomography (CT) and fluoroscopy-based navigation systems have demonstrated significant improvements in accuracy and safety of pedicle screw placement when compared to freehand technique. Evaluation of pedicle screw placement is assessed in terms of pedicle breaches typically detected through visual inspection of the CT and fluoroscopic images. However, it is not yet possible to use intraoperative images to quantitatively assess the accuracy of pedicle screw insertion by comparing with a predefined insertion planning. This study aims to demonstrate the feasibility to quantitatively assess the accuracy of pedicle screw insertion using intraoperative fluoroscopic images and compare the achieved screw placement with a predefined insertion planning.

The study was conducted using a synthetic model of a lumbar spine. The testbed consisted of a clamping device with five template supports, produced by additive manufacturing, to rigidly fix the lumbar spine. The test bed was scanned using a CT-scanner and a virtual 3D CT model of the test bed was reconstructed for the planning of the pedicle screw insertion. One operator freehandly performed the insertion of the pedicle screws. Fluoroscopic images of the test bed with the inserted screws have been acquired using the new Siemens Artis Zeego II intraoperative imaging robotic system. The errors in the desired pedicle screw insertion computed numerically with the intraoperative fluoroscopic images were compared to reference mechanical measurements using a Microscribe coordinate measuring machine.

Visual inspection of the synthetic lumbar spine and visual inspection of the intraoperative fluoroscopic images did not reveal any pedicle breach. The difference between the errors computed numerically with the intraoperative fluoroscopic images and mechanically with the coordinate measuring machine, averaged -0.8 mm for the entry points, -0.1° for the orientation axes and -0.3 mm for the target points of the inserted screws. The maximum differences were found in the right pedicle of L5 vertebra (-3.3 mm, 1.8° and 0.9 mm for entry point, orientation axis and target point respectively).

This study showed the feasibility to compute the achieved errors on a predefined pedicle screw insertion planning using intraoperative fluoroscopic images with a very good accuracy when compared to reference mechanical measurements. The results observed here are currently undergoing complementary in vivo studies. Once completed, the quantitative accuracy measurement methodology using intraoperative fluoroscopic images may be useful to investigate further pedicle screw insertion performed with the aid of several assistance technologies such as navigation and robotic systems.

Figure (a) 3D virtual model of the test bed (lumbar spine and reference block) and planning of the pedicle screw insertion (blue and yellow) in Paraview visualization software. (b) Test bed and the inserted pedicle screws (freehandly performed). (c) Accuracy measurements using intraoperative fluoroscopic images from Zeego interventional imaging robotic system. (d) Reference mechanical measurements with Microscribe coordinate measuring machine. (e) Errors in the desired insertion planning (entry and target points and orientation axis) computed in Matlab computation software for comparison between image-based and reference mechanical measurements.
HIP FRACTURE - Feasibility study of a new semi-automatic detection method of joint penetration during triple-screw internal fixation for femoral neck fracture

O Cartiaux, O Cornu, A Englebert

During a triple-screw internal fixation of femoral neck fracture, joint penetration is difficult to detect without imaging technologies and manual measurements. The screws may appear on the standard antero-posterior and lateral radiographs to be within the femoral head while they are actually penetrating the articular joint. The objective of this paper is to study the feasibility of a new semi-automatic detection method of joint penetration during triple-screw internal fixation of femoral neck fractures.

The method requires the computation of the tip-to-surface distance (TSD). A two-step process for the TSD computation was implemented. First, the tip position of the inserted screw can be manually identified on intraoperative antero-posterior and lateral 2D radiographs. Second, the Euclidean coordinates of the screw tip can be reconstructed in 3D and expressed in the reference frame of the intraoperative imaging system. These coordinates enable the automatic TSD computation. Five cases were simulated in a CAD software using virtual femur and screw. Three cases (cases #2, 4 and 5) were designed with penetrating screws, while two cases (cases #1 and 3) had no penetration. TSD corresponding to simulated cases were computed. Two perpendicular 2D snapshots of the 3D scenario with no visually detectable joint penetration, especially for the simulated cases #2, 4 and 5, were defined to simulate intraoperative antero-posterior and lateral radiographs. TSD were computed from manual identification of screw tips and average value (semi-automatic measurement) was compared to corresponding theoretical TSD (reference). Correlation coefficient between the two operators who identified the screw tip for the simulated cases was 0.96. All penetrating screws were detected (cases #2, 4 and 5). Maximum difference between reference and semi-automatic measurements was 1.2 mm for case #5. There was no negative difference between reference and automatic measurements. Moreover, no unpenetrating screw was considered penetrating.

This study showed the feasibility to detect joint penetration during simulated internal screw fixation of femoral neck fracture by using 2D radiographs to identify the position of the inserted screw tip and compute the 3D distance relative to the articular surface of the hip joint.

The results suggest that the proposed method to detect joint penetration is conservative, by ensuring the recognition of actual joint penetration and reducing the risk to recognize false joint penetration. Although these results clearly have to be validated clinically, they may be straightforward useful to assess the level of accuracy that the semi-automatic detection method needs to be provided with.
Select References


Equipment

- Serial 6-dof robot.
- 6-axis force sensor.
- 3D rapid-prototyping printer.
- 3D visualisation, simulation and planning platform.
- 3D measurement tool.
- Dedicated softwares for image analysis and CAD/CAM.
- 3D haptic system.
- Intraoperative surgical navigation system (sawing, milling).

Fundings

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- CenTIS (Louvain-la-Neuve, Belgium)
The main goal of the Experimental Surgery and Transplantation Unit is to study pathophysiological problems related to visceral surgery in order to develop new therapies implying technical progress and/or improved fundamental knowledge. In the laboratory, the techniques used combine sophisticated surgical procedures (cardiac surgery, liver transplantation ...) and biochemical, physiological, immunological and basic molecular biological methods.

The first domain of particular interest is "organ transplantation (allografts and xenografts)". The mechanisms of tolerance induction and maintenance to primarily (liver, kidney) vascularized allografts are under investigation in a unique miniature swine model (MGH-pig) which has been selected on MHC antigens.

The second domain of particular interest is the implantation of encapsulated Pig Pancreatic cells graft into diabetic primates in order to correct diabetes without the need of chronic immunosuppression. Although unmodified pigs are and have been used successfully to transplant pancreatic pig islets into primates, we recently had access to several genetically engineered pigs such as Galactosyl Knock Out pigs which should provide organs or xenogeneic cells less sensitive to hyperacute rejection. We have also access to multiple transgenic pigs for several genes involved in xeno- or allo-rejection (CD55, Thr, CTLA4Ig, ...) and in the near future, transgenic pigs which overexpress GLP-1 to amplify the pig insulin response to hyperglycemic challenges will be available.

In addition, several pre-clinical and clinical studies are undertaken by members of the University Hospital such as the study of systemic and hepatic hemodynamics in cirrhotic children, Fibrosis of allograft in pediatric liver transplant recipients, bioartificial face transplantation, oral or maxillofacial surgery, implantation of devices for correcting sleep apnea or valves repair in pigs.

Additional activity of the Laboratory is to develop and characterize rat or mouse monoclonal antibodies against multiple targeted antigens: Rat anti-mouse, rat anti-human and anti-baboon Igs, mouse anti-rat Igs, Rat anti-bacteria.
N. Mourad, P. Gianello

Pig islets represent a promising alternative to human islet transplantation in diabetic patients since they can be obtained in large quantities without raising ethical questions. Insulin produced by porcine beta-cells differs from human insulin by only one amino acid and has long been used to treat diabetic humans. Furthermore, genetic modifications of pig cells are technically possible and should solve several problems related to discordant islet xenotransplantation. Several preclinical pig-to-non-human primate studies including our own have been published during the last decade, with promising results regarding the production of insulin in the recipient. However, pig islets show a relatively weak response to glucose stimulation. When isolated pig islets are stimulated by increasing glucose concentration from resting (1-2 mM) to stimulatory (8-15 mM) levels, the increase in insulin secretion is between 1.5 and 3-fold. In comparison, insulin secretion is increased by 12 to 16-fold when human, primate or rodent islets are challenged with a similar increase in glucose concentration. This property of pig islets has sometimes drawn doubts regarding their usefulness as a treatment for diabetes when transplanted into more insulin-demanding organisms such as non-human primates and possibly humans. In particular, the lower response to blood glucose of porcine islets compared to human islets leads to the need of transplanting a high number of pig islets to adequately correct the human glucose level, which is also a drawback of the treatment method as several pigs are currently used to transplant one patient. Our ongoing work then focuses on two aspects:

Isolation and maturation of neonatal pig islets which have been reported to survive for longer periods than adult islets when transplanted in the liver or under the renal capsule. Another advantage of using neonatal pig islets is that it is easier and more cost-effective to obtain SPF-certified animals of 1-4 weeks old than to maintain a herd of adult pigs under the same conditions. After isolation, piglet islet-like cell clusters are cultured for 8 days in a modified medium promoting beta-cell proliferation and differentiation of precursor endocrine cells into insulin-secreting cells.

Production of genetically modified pig islets with targeted modification of islet beta-cell function rendering the islets more responsive to glucose stimulation. The rationale behind this strategy is that pathways other than those activated by glucose can modulate insulin secretion from beta-cells. Our in vitro experiments show that pig islet secretory response to glucose is enhanced when the islets are treated with forskolin to increase intracellular cAMP and thus activate protein kinase A (PKA) and Epac2 or in the presence of PMA which directly activates protein kinase C (PKC). It thus seems beneficial to activate these pathways in pig beta-cell in an attempt to render them more responsive to glucose stimulation. Therefore, we produced vectors carrying a sequence encoding glucagon-like peptide 1 (GLP-1), natural ligand to the GPCR which activates adenylly cyclase, as well as a sequence encoding a constitutively activated type 3 muscarinic receptor which leads to activation of phospholipase C (PLC) which cleaves membrane phospholipids and produces diacylglycerol, natural ligand and activator of PKC. Both sequences were placed under the control of an insulin promoter to target their expression.
expression to pancreatic beta-cells. These vectors will be used to produce transgenic pig islets which will be tested in-vitro then used in our pig to primate xenotransplantation model to assess their ability to control glycemia in diabetic non-human primates.

The current work is funded by the European research grant UE P7 Xenoislet 601827.

A bioartificial pancreas to treat type 1 diabetes: optimization of cell survival and function in preclinical and clinical phases

P. Gianello

MAILPAN (MAcroencapsulation of PANcreatic Islets) is a prototype of bioartificial pancreas usable in the human designed to treat type 1 diabetic patients. Next step is now to bring the prototype to the pre-clinical and clinical phases necessary to the ensuing commercialization of MAILPAN whose ultimate goal is to improve the life of million persons in the world. In order to reach this goal, CeeD and Defymed gathered a consortium made of seven partners from academia, clinical/public health research sector and industry/SMEs from three different European countries – Belgium, France and UK. The expertise gathered includes encapsulation techniques, islet isolation, cell engineering, islet transplantation, islet preconditioning, surgical implantation, and medium formulation. The project proposal of a 36-month duration intends to bring the most modern and up to date improvements that the bioartificial pancreas still needs and can receive such as to enhance cells survival inside the device by formulating a new adapted cell culture medium, to further lower the rejection risk by studying the biocompatibility and anti-inflammatory mechanisms, to test the prototype in primates, and to validate its further use in humans. Safety, bio-compatibility and interoperability of MAILPAN device combined to the islets/pseudo-islets, will be assessed, in respect to the applied regulatory directives. At UCL, MAILPAN is implanted in large animal and will be filled with either pig islet cells, human endocells and human islets in our diabetic animal models at CHEX.

MAILPAN implanted during 3 months without any immunosuppression does not show any fibrotic reaction.

This work is funded by the European research grant UE P7 BioSid 305746.

Project website: http://defymed.com/biosid/

Bio-artificial face transplantation

J. Duisit, P. Gianello, B. Lengelé

Although providing a revolutionary reconstructive option for severely disfigured patients, wide-spreading of facial transplantation still has to face the need of an immunosuppressive treatment. This is a limitation for any organ transplantation, is particularly critical in Composite Tissues Allotransplantation (CTA), relying on both skin component and non-vital aspect of such procedures. We have no doubt that, even though not being a life-threatening condition, a patient’s life is dramatically impaired by loss of facial integrity.

In order to counteract immunosuppression, we are developing new strategies to provide full immuno-compatibility between the graft from a deceased donor and the recipient. For this mean, we are using the surgical knowledge from facial transplantation legacy, treated with new technologies arising from Tissue Engineering: the principle is to remove ex vivo the entire cellular compartment, leaving the Extra-Cellular Matrix (ECM) intact. In vitro, the obtained ECM will be reseeded with stem cells harvested on the recipient, prior to in vivo transplantation. The animal models used are rat and pig, with different CTA models.
This project is guided by a partnership between MORF pole (Pr B. Lengelé - expertise in Facial Transplantation and Anatomy), CHEX pole (Pr P. Gianello - expertise in Organ Transplantation and Immunology) and Wake Forest Institute for Regenerative Medicine, USA (Pr G. Orlando – expertise in Organs Tissue Engineering). Dr J. Duisit, PhD student, is funded by Fondation Saint-Luc research grant.

Pre-clinical development in pigs of a medical device for sleep apnea

P. Gianello, A. Mashiach (Nyxoah S.A.)

The system consists of an Implantable Stimulator (IS), which is implanted and placed on one of the tongue muscles and an External Patch (EP). The system applies electrical stimulation to the tongue muscle in an open-loop mode, with parameters set by the physician. The stimulation of the tongue prevents it from obstructing the airway.

The Implantable Stimulator is powered by the External Patch. The Implantable Stimulator is implanted and fixed on genio-glossus muscles. The External Patch is configured and adjusted via an external configuration console. This system is now validated to implantation and has been safely implanted in four humans.

PEDIATRIC SURGERY AND TRANSPLANT UNIT

Systemic and hepatic hemodynamics in cirrhotic children: Clinical contributions to the physiopathology, and to the surgical algorithm in pediatric liver transplantation

PhD Research project – C. de Magnée, promoter R. Reding

This work is going to bring a better knowledge of the diverse systemic and hepatic hemodynamic modifications associated with liver cirrhosis in children, and more particularly in infants.

The hemodynamic observations are correlated to the biological and histological parameters of cirrhosis, considering specifically biliary atresia (the most frequent cause of cirrhosis, and the most frequent indication of liver transplantation in children).

The classical non-invasive methods used to investigate hemodynamic parameters (liver Doppler ultrasound and cardiac Doppler ultrasound) are completed by, and correlated to intra-operative hemodynamic invasive measures (collected by ultrasound transit time flowmeter on the liver vessels, and by transpulmonary thermodilution), done on the occasion of paediatric liver transplantation.

The badly understandable phenomenon of portal vein hypoplasia (defined as a portal vein of less than 4mm of diameter), anatomical modification only observed in cirrhotic infants and children, is particularly studied at a physiologic, anatomopathologic, diagnostic, and therapeutic point of view.
Study of The Factors Influencing The Progression Of Allograft Fibrosis In The Long-Term Follow-Up Of Pediatric Liver Transplant Recipients. Impact of the immunosuppressive therapy and role of hepatic stellate cells

PhD Research project – C. Venturi, promotor R. Reding

Progressive liver allograft fibrosis (LAF) has shown high prevalence in late post-transplant liver biopsies (LB). The clinical significance of long-term fibrosis and its influence in the graft survival remain to be fully understood. A rigorous evaluation and quantification of LAF was performed analyzing clinical biochemical and serologic screening as well as, LB histology at 6 months, 1, 2, 3, 5, 7 and 10 years post-LT in 139 primary pediatric liver transplant (LT) recipients, who underwent a LT at Saint-Luc University Clinics, between April 1999 and June 2005. Protocol LBs were reviewed assessing LAF using the METAVIR and Ishak systems. A novel LAF scoring system, Liver Allograft Fibrosis Score, (LAFSc) which score LAF separately in portal (0-3), sinusoidal (0-3) and centrolobular areas (0-3) was designed and validated (Am J Transplant 2012 Nov;12(11):2986-96). The influence of clinical variables and the immunosuppression on sinusoidal, centrolobular and portal fibrosis was studied in the long-term. A total of 595 LB in 139 patients were reviewed. Progressive LAF was found in 74% of patients in the long term, 70% of whom had unaltered liver enzymes. Peri and post-LT-associated factors were found to promote fibrosis development in a specific area of the liver parenchyma. Steroid therapy was not associated with reduced fibrosis (p=0.178). Proper-tolerance status, defined as normal liver enzymes at least 12 months before LB, and TAC monotherapy with levels below 4 ng/ml did not contribute to increase fibrosis in this population (p=0.566). This study would allow the author to infer about pediatric graft evolution dividing it in three periods as follows; the first two years post-LT, period of injuries with fibrosis accumulation; a second period, (3 to 7 years), which there is no great risk of complications, the IS remains stable and LAF would continue stable or even decrease; however, as soon as a new injury is established or IS levels fluctuate, LAF would progress; and the third period, more than 7 years when the graft is going to be accepted, when low tough levels of IS are aimed at, and when LAF may progress slowly in case of new injuries or remain stable with normal liver function (Am J Transplant 2014: in press). Future steps: to analyze the role of hepatic stellate cells as LAF triggering factor and to develop preventive strategies to avoid fibrosis progression.

ABDOMINAL SURGERY AND TRANSPLANTATION

Liver transplantation

J. Lerut

The clinical research in the field of liver transplantation is focused on two important themes:

The place of liver transplantation (LT) in hepatobiliary oncology.

LT takes a more and more important place in the treatment of primary hepatobiliary tumors. Our research is concentrated at the extension of inclusion criteria based on a combination of clinical, imaging and biological markers as well as implementation of an aggressive neo-adjuvant locoregional tumor treatment. Recently there is also a renewed interest in liver transplantation as a possible curative treatment of neuro-endocrine and colorectal secondaries.

The project of minimal immunosuppression.

The most frequent cause of liver allograft loss relates today to lethal cardiovascular, infectious and oncologic events in the presence of a well-functioning graft. As all these events are related to the chronic immunosuppressive therapy, minimizing immunosuppressive treatment is therefore of utmost importance especially in view of the more and more frequent long-term survival rates. This policy is also an important step in the project to obtain allograft tolerance.
ORAL AND MAXILLOFACIAL SURGERY RESEARCH LAB

R. Olszewski, H. Reychler

The main areas of interest for OMFS Lab in 2013 were 1) computer-assisted maxillofacial surgery (2 PhD theses ongoing with Pomeranian University-Szczecin, Poland with Pr R. Olszewski as a copromotor), 2) three-dimensional (3D) dento-maxillofacial imaging (1 PhD thesis ongoing with ULB-Brussels with Pr R. Olszewski as a copromotor, and 1 PhD thesis ongoing with Iasi University, Romania, with Pr R. Olszewski as a copromotor), 3) three-dimensional cephalometric analysis (1 PhD thesis ongoing with KUL-Leuven with Pr R. Olszewski as a copromotor, and 1 PhD theses ongoing with Pomeranian University-Szczecin, Poland with Pr R. Olszewski as a copromotor), and 4) development of new research unit inside OMFS Lab on 3D paper-based medical models with a low cost ecological 3D printer (Matrix 300, Mcor technologies, Eire) with 1 PhD thesis ongoing with University of Lodz, Poland, with Pr R. Olszewski as a copromotor. The OMFS Lab team in 2013 was represented by 2 professors: Pr R. Olszewski, chairman and co-founder of OMFS Lab, and Pr H. Reychler, co-founder of OMFS Lab, and reached 7 PhD students which are financed on external founds. The OMFS Lab obtained a grant (10.000 euros) from Fondation Saint-Luc - Fonds Hervé Reychler to develop international research collaboration in maxillofacial surgery on 3D models from low-cost paper-based 3D printer. OMFS Lab reached in 2013 four international publications with IF, 1 proceedings published, 2 invited speeches, 3 national meetings, 1 poster, 9 oral presentations, and 1 national television interview by RTBF on paper-based 3D printing. Pr R. Olszewski was invited to join the Collège des Alumni of the Royal Academy of Belgium.

TECHNOLOGIES LINKED TO MOUSE, RAT AND HUMAN MONOCLONAL ANTIBODIES DEVELOPMENT

Y. Nizet, C. Lecuivre, F. Nisol, P. Gianello

We are specialized in the development, production and marketing of monoclonal antibody from mouse, rat, or possibly human. We currently offer a catalogue of more than 100 monoclonal antibodies which are commercialized by our-self or through various companies. We also offer a service of custom antibodies, available for the laboratories of our university, or for public and private institutions in Belgium or abroad.

In addition to the development of monoclonal antibodies, we are also specialized in the development of new methods of immunization including DNA vaccination for which we have developed a new plasmid vector patented and licensed to a Belgian biotechnology firm (Delphi Genetics) to develop a veterinary vaccine. We have also recently developed and patented magnetic beads able to bind bacteria with a high affinity. These beads are currently investigated for the rapid analysis of bacteria in biological fluids by mass spectrometry.

Clinical Research

in the field of Digestive Surgery, Pediatric and Adult Liver/Kidney Transplantation, Urology and Maxillofacial Surgery is ongoing and achieved by several members of the Unit:

**TRAININGS AND SERVICES**

- Surgical Training for new surgical technologies: microsurgery, implantation of artificial heart, cardiac valves repair, laparoscopic surgery.
- Test of new surgical devices (OPUS MEDICAL)
- Use of the technique ICE* (St-Jude Medical)
- Partnership Workshop of techniques of sutures (Ethicon)
- Training VENTRACOR
- Organ Recovery System (ORS)
- New techniques in laparoscopy ((Ethicon + Coviden: Johnson et Johnson), Single Access Laparoscopy (Olympus))
- Repair of porcine cardiac valves (Medronic)

**EQUIPMENT**

- 2 surgery rooms and post-operative care
- Possibility of hosting for large mammals (pig, calf)
- 6 operating microscopes for microsurgery
- Beta and gamma counter
- Centrifuges
- 2 rooms of cellular culture with laminar flow and incubators
- Equipment for molecular basic biology (PCR, Northern, Western blots ...)
- Irradiator for cells and animals
- Flow cytometer (FACS, BECTON DICKINSON)

**SELECTED REFERENCES**


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Ongoing research projects in the pole are dealing with the control of production and action of various hormones and paracrine factors and their alteration in endocrine diseases, with a particular focus on diabetes, obesity, muscle atrophy, pituitary adenomas and thyroid diseases. These projects encompass basic research carried in vitro and in vivo, as well as clinical research.

**Experimental Research Themes:**
1. Regulation of insulin and glucagon secretion and their alteration in type 2 diabetes
2. Regulation of adipokine secretion and their role in obesity and type 2 diabetes
3. Regulation of muscle mass and metabolism by IGF-1 and Myostatin

**Clinical Research Themes:**
1. New approaches in prognosis and treatment of pituitary adenomas
2. Regulation and role of vasoactive peptides in cardiovascular diseases and metabolic syndrome
3. New approaches in the treatment of diabetes mellitus (continuous glucose monitoring, functional insulin therapy, pancreatic islet transplantation, new drugs)
4. New approaches in the treatment of thyroid cancer and thyroid ophthalmopathy
5. Metabolic and endocrine perturbations in malignant hemopathies of the young
6. Characterization of endocrine factors involved in human cancer cachexia

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- Ana GOMEZ RUIZ
- Chae HEEYOUNG
- Hilton TAKASHI
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How do chronic changes in nutrient supply alter survival and function of insulin-secreting pancreatic β-cells?

J Duprez, LRB Santos, LP Roma, HK Takahashi, JC Jonas

In type 2 diabetes (T2D), hyperglycaemia results from the combination of insulin resistance, defective insulin secretion, and increased glucagon secretion. Besides genetic risk factors, the reason why insulin-secreting β-cells fail to compensate for insulin resistance in some individuals may include lipotoxicity, low grade systemic inflammation and β-cell exhaustion. It is also clear that, once present, hyperglycaemia further reduces β-cell mass and function through a process called glucotoxicity (Fig. 1).

Figure 1 Pathophysiology of insulin-secreting pancreatic β-cells in type 2 diabetes.

Among the mechanisms that may contribute to β-cell glucotoxicity, oxidative stress has been studied for many years but the results are highly controversial, largely due to the lack of sensitive and specific tools to measure reactive oxygen species and their impact on cellular constituents in various subcellular compartments. Using new genetically-encoded ROS-sensitive fluorescent probes derived from Green Fluorescent Protein, we have recently characterized how changes in nutrient availability affect glutathione oxidation in the cytosol and mitochondrial matrix of pancreatic β-cells. Building on these data, we tested the ability of various antioxidant drugs/enzymes targeted or not to the mitochondrial matrix to improve β-cell function and survival under in vitro conditions that mimic the metabolic stress of T2D. The main results and conclusions are briefly depicted in figure 2. For a full description of our recent studies, the reader is referred to publications 1 to 3 from the Pôle.

The control of glucagon secretion by glucose and KATP channel modulators

A Gómez-Ruiz, HY Chae, P Gilon

Glucagon secreted by pancreatic α-cells is a major hyperglycemic hormone. Its secretion is stimulated by a decrease of the blood glucose concentration. The regulation of glucagon secretion is markedly impaired in diabetic patients in which hyperglucagonemia coexist with chronic hyperglycemia. Furthermore, glucagon secretion in these patients does not increase during hypoglycemic episodes triggered by excessive insulin administration, thereby contributing to diabetes morbidity.

The mechanisms by which glucose inhibits glucagon secretion are poorly understood. In particular, it is still unknown whether the glucagonostatic effect of glucose results from a direct action of the sugar on α-cells or an indirect action involving the release of an inhibitory paracrine factor from neighbouring islet cells, such as insulin released by β-cells or somatostatin released by δ-cells. The specific inhibitory effect of glucose on glucagon secretion is all the more intriguing since KATP channels are present in α-, β- and δ-cells, and since these channels play a
key role in the stimulatory effect of glucose in these two latter cell types. Indeed, their closure transduce the glucose-induced increase in the cytosolic ATP/ADP ratio into a depolarization of the plasma membrane, which opens voltage-dependent Ca\(^{2+}\) channels, increases [Ca\(^{2+}\)]\(_{c}\), and triggers insulin and somatostatin release.

Using various transgenic mouse models and pharmacological agents, we showed that glucose is poorly effective in isolated α-cells, whereas it strongly inhibits glucagon release from whole islets. Its glucagonostatic effect does not seem to result from a modulation of α-cell K\(_{ATP}\) channels. We also observed that direct closure of K\(_{ATP}\) channels by tolbutamide (a sulfonylurea) controls glucagon secretion by two mechanisms: a direct stimulation of α-cells and an indirect inhibition via somatostatin released from δ-cells. The net effect on glucagon release results from a balance between both effects (see diagram in Fig. 3). This direct glucagonotropic effect of tolbutamide should be considered during treatment of type 2 diabetic patients by sulfonylureas because stimulation of α-cells by the drugs could, in some situations, aggravate the unwanted hyperglucagonemia found in diabetes.

**Figure 3** Mechanisms by which glucose and tolbutamide (a blocker of K\(_{ATP}\) channels) control the secretion of insulin, somatostatin and glucagon. For a full description of the results, the reader is referred to publication 4 and 5 from the Pôle.

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**Adipokines in health and diseases**

M Abou-Samra, R Boursereau, S Lecompte, SM Brichard

Adipose tissue (AT) secretes adipokines, which play central roles in energy and vascular homeostasis as well as in immunity. Deregulation of these adipokines triggers the development of a low-grade pro-inflammatory state, which is considered to build the common soil for the development of obesity-linked disorders such as insulin resistance, type 2 diabetes and atherosclerosis, which are components of the metabolic syndrome (Fig 4). Resetting the immunological balance in AT may be a crucial approach for the future management of this syndrome.

Adiponectin (ApN), which is decreased in the metabolic syndrome, is a potent enhancer of insulin action and fatty acid oxidation. Recently, this adipokine has also emerged as a master regulator of inflammation/immunity in various tissues, including AT, its own production site. We have recently shown, thanks to our transgenic mice overexpressing ApN specifically in white AT, that ApN regulates in vivo the secretory profile of downstream adipokines, decreasing those with pro-inflammatory properties while up-regulating those with anti-inflammatory action. Yet, the mechanisms by which ApN shifts the immune balance of adipocytes toward a less inflammatory phenotype are not fully elucidated. We are currently working on this topic. Besides studying adipokines in AT, we are also exploring their effects on skeletal muscle where ApN may also be a promising anti-inflammatory tool.

**Figure 4** Potential mechanisms leading to adipose tissue inflammation in obesity - Lipid accumulation leads to adipocyte hypertrophy, initiating a state of cellular stress and activation of pro-inflammatory pathways, especially Nuclear Factor-κB (NF-κB). This upregulates the production of pro-inflammatory adipokines. Some of these are chemokines that bind to specific receptors (CCR and CXCR) of monocytes-macrophages to recruit them into AT. The majority of macrophages in obese AT aggregate in “crown-like structures” completely surrounding dead adipocytes and scavenging adipocyte debris. Increased lipolysis by hypertrophied adipocytes and metabolic endotoxemia (increased circulating lipopolysaccharides, LPS) also contribute to inflammation through activation of Toll-like receptor 4 (TLR4). Moreover, hypoxia may also be a very early contributor. Local inflammation worsens and propagates systemically via adipokines. CCR and CXCR designate chemokine receptors; Mo, monocytes, FFA, free fatty acids. (reprinted from publication 6).
Role of Myostatin/Activin and their inhibition in the regulation of the skeletal muscle mass: a new pathway to mitigate muscle atrophy

S Kalista, A Loumaye, C Barbé, M de Barsy, JP Thissen

MMuscle atrophy, observed in catabolic states such as cachexia, immobilization or aging is associated with muscle functional loss contributing to morbidity and mortality. Molecular and cellular mechanisms responsible for muscle atrophy are still unraveled, explaining why present therapies are relatively inefficient. Due to lack of effective treatments, new approaches have been actively investigated. Attention has been oriented towards the potential benefits of the Myostatin (Mstn) inhibition. This growth factor, a member of the TGFβ superfamily, strongly inhibits the muscle mass development. Given its marked anabolic effect, Mstn inhibition appears as a promising way to treat muscle atrophy. The deciphering of the mechanisms by which Mstn inhibition stimulates muscle mass should provide critical informations not only for understanding the control of muscle size in general but also for developing new therapeutic strategies. In an early work, we showed that Mstn inhibition prevents the muscle mass loss caused by glucocorticoids, a classical model of muscle atrophy. More recent work of our lab demonstrated the crucial role of the IGF-I pathway in the muscle hypertrophy induced by Mstn inhibition. Evidence suggests that the interaction between the two pathways, IGF-I and Mstn, should take place just downstream of the IGF-I receptor. Using gene expression profile analysis by microarray, our current research focuses on the identification of the molecules regulated by Mstn inhibition and contributing to its hypertrophy effect. In a parallel study, we showed that Activin A (ActA), a molecule closed to Mstn, could also cause muscle atrophy by binding to the same receptor. The interest of this observation is further enhanced by the recent finding that ActA might contribute to cancer cachexia in several animal models. Indeed, recent observations indicate that the production of ActA by some tumors cause skeletal muscle atrophy and contribute to cancer cachexia and hence to the cancer-related mortality. Therefore, our current goal is to characterize the role of ActA in human cancer cachexia. This hypothesis seems particularly attractive to be tested, given the large number of agents capable of blocking the ActA/Mstn signaling pathway, some of which being currently tested by our lab in animal models. This translational work might lead to the identification of a new biomarker predictive of cachexia, allowing the selection of patients susceptible to benefit from ActA antagonists presently in clinical investigation. These results will help to guide therapeutic strategies targeting this pathway in conditions where muscle atrophy impairs survival or quality of life.

Figure 5 Molecular signaling of Activin A and Myostatin on skeletal muscle cells - Activin A (ActA) produced by the tumor and Myostatin (Mstn) produced by skeletal muscle fibers bind the type 2B Activin receptor (ActRIIB) and stimulates Smad-2/3 phosphorylation. This leads to the activation of an atrophy gene program responsible of muscle atrophy. Inhibition of Mstn/ActA by Follistatin (FS) or the soluble ActRIIB (sActRIIB) stimulates muscle hypertrophy and mitigates muscle atrophy.
Laboratory medicine is an important contributor for the evaluation of endocrine disorders and cardiovascular diseases. New biomarkers and innovative assays for measurement of hormones and neurohormones are emerging and must be evaluated for their analytical and clinical performances. Our ongoing evaluations are related to heart failure (HF), diabetes, pregnancy related disorders, update of reference values and the potential impact of assays based on mass spectrometry.

HF is characterized by a neurohormonal activation which plays a significant role in myocardial and multi-organ adaptations to the disease. Circulating levels of aldosterone, arginine-vasopressine, endothelin and natriuretic peptides are also increased in HF. B-type natriuretic peptide (BNP) is synthesized in the ventricles as a 108 prohormone, undergoing a cleavage generating the C-terminal 32 amino-acids active peptide (BNP) and the inactive N-terminal fragment (Nt-proBNP). Rapid and sensitive tests for BNP and Nt-proBNP are now routinely used for the evaluation of cardiovascular disorders. BNP and Nt-proBNP are useful for ruling out the presence of heart failure. We have demonstrated that circulating levels of proBNP 1-108, myostatin and C-terminal fibroblast growth factor 23 are triggered in HF patients, are related to well established biomarkers of the worsening course of HF and might contribute to the prognosis of patients with systolic HF. New challenges in HF are related to the risk stratification of the patients and treatment selection through the integration of patient characteristics and biomarkers in multimarker strategies (MMS). MMS represent the integration of quantitative results of laboratory tests, alone or in combination with patient characteristics and medical/family history, to support a medical decision and to facilitate the physicians’ work of interpretation of multiple sources of information. Our objective is to integrate the information and communication technologies for the selection of the biomarkers for MMS and to define a profile of biomarkers from several pathways (natriuretic peptides, PTH, FGF23, myostatin, Galectin-3, chromogranin, etc.), which might help to identify high risk patients and thereby might aid the selection of appropriate therapy.

In patients with type 2 diabetes (T2D), circulating biomarkers are increasingly used for patient risk stratification, cardiometabolic risk estimation and to support primary and secondary prevention initiatives. Our objective is to determine the circulating levels of cardiac biomarkers and bone turnover markers in T2D patients and assess their potential for risk stratification of such patients. Our results have recently shown that serum osteocalcin levels are associated with glucose metabolism parameters and cardiovascular biomarkers in T2D.

The determination of the reference values is therefore important for the appropriate use of emerging biomarkers and might contribute to a more personalised laboratory medicine. The emphasis should also be placed on the evaluation of new methods such as mass spectrometry to introduce more reliable and sensitive measurement of hormones in clinical practices.

The development of a multidisciplinary and integrated platform for laboratory testing within the IREC should also contribute to the evaluation of the potential added value of these emerging biomarkers and technologies.

Further details about this research program can be found in references 13 to 15 of the Pôle.

### Equipment

- Cell culture and molecular biology
- Construction and generation of defective adenovirus (biosecurity level 2)
- Evaluation of islet cell biology (dynamic hormone secretion in perifusion)
- Hormone RIA and ELISA assays (automatic pipetting, gamma and beta counters)
- Electrophysiology (patch-clamp)
- Live-cell imaging systems (excitation and emission fluorescence ratio, highly sensitive EMCDD cameras, photon counting mode)
- Confocal microscopy (spinning disc), TIRF
SELECTED PUBLICATIONS


FUNDING SOURCES

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  - Action de Recherche Concertée ARC 13/18-051 to C Beauloye, D Dufrane and P Gilon. Coordinator P Gilon «Glucose homeostasis: from its physiological control to the consequences of its dysregulation in diabetes»
  - Crédit aux Chercheurs F.R.S.-FNRS 1.5012.11 to JC Jonas «Molecular mechanisms of the phenotypic plasticity of pancreatic beta cells under pathophysiological conditions»
  - Crédit aux Chercheurs F.R.S.-FNRS 1.5097.12 to S Brichard «Adiponectin and skeletal muscle: potential role in muscular diseses»
  - Convention FRSM 3.4521.12 to JC Jonas «Molecular mechanisms of the phenotypic plasticity of pancreatic beta cells under pathophysiological conditions»
  - Convention FRSM 3.4554.10 to P Gilon «Le couplage stimulation-sécrétion des cellules à insuline et à glucagon dans des conditions normales et physiopathologiques liées au diabète»
  - Convention FRSM 3.4539.12 to JP Thissen «Caractérisation des mécanismes d’action et du rôle de l’Activine A dans l’atrophie musculaire de la cachexie cancéreuse»
  - Grands-Fonds_FRSM_T.0212.13 to S Brichard «Novel targets for controlling adipose tissue inflammation and the metabolic syndrome: miRNAs regulated by Adiponectin»
  - Fonds Spéciaux de Recherche – UCL (Ph.D. fellowship to M Sadoine, Promotor P Gilon) «Mécanismes de contrôle de la sécrétion de glucagon par le glucose et les modulateurs des canaux KATP»
  - F.R.I.A. – Ph.D. fellowship to J Duprez, Promotor JC Jonas «Effects of zinc and metallothioneins MT1a and MT2a in the alterations of pancreatic β-cell survival and function by extreme glucose concentrations»
  - Société Francophone du Diabète to JC Jonas «Mélatoninhémine, zinc et stress oxydatif dans les cellules β pancréatiques en conditions extrêmes de glucose»
  - Fondation contre le Cancer to JP Thissen «Rôle de l’Activine A dans la cachexie cancéreuse humaine»
  - Fondation St-Luc to JP Thissen «Rôle de l’Activine A dans l’atrophie musculaire de la cachexie cancéreuse humaine»
  - Téléthon Belge et Association Française contre les Myopathies (AFM-Téléthon) to S Brichard

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The clinical division of endocrinology and nutrition of the UCL Saint-Luc university hospital is tightly connected to several research areas developed in the pole of endocrinology, diabetes and nutrition (EDIN) and all the academic staff members working in our division are members of the Institute of Clinical Research (IREC). These close relationships provide large opportunities to develop common translational research projects, to collaborate with the other members of the Institute and to further expand our knowledge and understanding of the pathophysiological processes operating in diabetes, obesity and many endocrine diseases.

The division also provides a wide range of services for patients with endocrine diseases. Areas of special expertise include the management of patients with type 1 diabetes, severe metabolic syndrome and obesity, other specific causes of diabetes (i.e. mucoviscidosis and haemochromatosis), thyroid cancer, thyroid ophthalmopathy, as well as adrenal and pituitary tumors. For many of these diseases, an optimal decision-taking process is made in the frame of regular multidisciplinary team meetings.

Our clinical research activities are also conducted in collaboration with colleagues from our own and other departments (i.e. cardiology, biology, pediatrics, endocrine surgery and neurosurgery, ophthalmology,...) and have led to several important publications in the field (see below). Among others, recent studies have focused on new therapies in type 2 diabetes, reducing cardio-vascular risk in patients with the metabolic syndrome, evaluation of hypogonadism in obese men, optimizing treatment of advanced thyroid cancer and Grave’s ophthalmopathy, studies of disease activity markers in acromegaly, outcomes of trans-sphenoidal surgery in pituitary tumors and characterization of endocrine and metabolic complications in childhood cancer survivors.
SELECTED PUBLICATIONS


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Our activities are firstly those of clinicians who, owing to a staff of two, must deal with all fields of endocrinology including diabetes, thyroid, pituitary and adrenal diseases as well as obesity. Therefore, this allows us to look after a large number of patients in whom sometimes, rare diagnoses are made providing a questioning and a thorough search for underlying pathophysiological mechanisms. We thus favour this daily thought process that may lead to report interesting cases (for instance about multiple endocrine neoplasia, Cushing syndrome, phaeochromocytoma etc). We also help the trainee doctors to write them, which is a part of our teaching mission. Beyond these reports, the review and analysis of clinical series enables us to elucidate the characteristics of some diseases and draw important messages. As an example, we studied the incidence and the risk factors of new onset diabetes after lung transplantation, which was presented at the annual meeting of the European Association for the Study of Diabetes. We also carried out studies in close co-operation with teams of other specialities such as endocrine surgery or neurosurgery. The former allowed us to disclose in a series of patients with hyperparathyroidism the importance of magnetic resonance imaging to locate parathyroid adenoma, the latter to write a review paper including guidelines about the management of pituitary incidentalomas. Other works were done in collaboration with other institutions either in Belgium or abroad. In this respect, studies in the field of prolactinomas are worth mentioning. So, we analysed the characteristics and the prognosis of giant prolactinomas in women. We also linked our activities with those of basic science thanks to specialised techniques (molecular biology or cell cultures) performed in research laboratories. For instance, a large amount of our research focused on the role of the endothelin system in the pathogenesis of thyroid cancer and on the search for prognosis factors in pituitary tumours. Finally, we created a thyroid clinic providing a multidisciplinary evaluation of thyroid disease including notably fine-needle aspiration cytology and discussion of recently published research in that field.

The Service of Endocrinology and Diabetes
CHU Dinant-Godinne UCL Namur
SELECTED PUBLICATIONS


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Traditionally oriented towards cell signaling, the 4 groups of the Pole of Pharmacology and Therapeutics have diversified their fields of interest with 2 groups mainly involved in cardiac and vascular biology and 2 groups in cancer biology and metabolism. While doing so, the realization that many biological paradigms are common to the 2 fields and that their study requires similar experimental approaches was an impetus to develop trans-disciplinary projects that would allow scientific cross-fertilization and easier access to a larger panel of shared equipment and techniques. Typical examples include collaborative projects on angiogenesis, vascular-parenchyma/stroma interactions, tissue/cellular responses to oxidant stress or hypoxia and reciprocal regulation of metabolism and signaling.
Biology of nitric oxide synthases (NOS)

J.-L. Balligand, C. Dessy, O. Feron, P. Sonveaux

The synthases producing nitric oxide (NO), an ubiquitous messenger radical, belong to a family of three isoforms, each encoded by a specific gene. Our laboratory has a long-standing interest in the molecular regulation of the activity of these NOS, particularly eNOS (the isoform originally identified in endothelial cells), through post-translational mechanisms. These include allosteric modulation through protein-protein interactions with the chaperone, hsp90 and the caveolar-coat protein, caveolin. Recently, we focused our analysis on the impact of co-localization in rafts/caveolae of eNOS with other effectors, such as isoforms of NADPH oxidase on NOS coupled activity (i.e. its ability to produce NO vs. superoxide anions), NO bioavailability and N0-dependent endothelial function. We found that vasoactive peptides such as Angiotensin II promote the assembly and activation of NADPH oxidase together with eNOS in rafts/caveolae, resulting in NOS uncoupling, ROS production and endothelial dysfunction. Similar mechanisms are being dissected in response to other receptor-mediated signaling, e.g. beta3-adrenoreceptors, in other cellular contexts. In the field of cancer, we have recently identified the lack of caveolin and acidic conditions both observed in the tumor vasculature as a favorable ground for nitrite-driven angiogenesis and vasodilation; these pathways are currently explored for therapeutic purposes.

Mechanistic studies of the determinants of the adaptive versus maladaptive remodeling of the stressed heart

E. Dubois, H. Ding, H. Esfahani, C. Dessy, J.-L. Balligand

In response to neurohormonal, inflammatory or mechanical stress, the heart undergoes cellular and tissular remodeling that initially participates to the adaptation of cardiac pump function to increased load but ultimately may lead to cardiac failure. Although probably part of a continuum, each stage is associated with cumulative, albeit distinctive molecular events and identification of critical signaling driving towards a more adaptive or deleterious phenotype would improve both early diagnosis and therapy of heart failure. We used a number of genetically-modified mouse models and compared their phenotype in response to standardized stresses that promote remodeling (i.e. minipump infusion of neurohormones, infarction from LAD ligation, trans-aortic constriction, calibrated chronic exercise). We have identified several regulators of hypertrophic remodeling, e.g. involving cardiac beta3-adrenergic receptors and nitric oxide synthases, associated with activation of distinctive intracellular signaling. In collaboration with colleagues at ULB, the role of purinergic receptors on cardiac endothelial cells was identified. In a larger collaborative effort at the European level, we will use the output from large genomic studies in cohorts of heart failure patients to select few putative target genes, and combine these with transcriptomic datasets to identify co-regulated genes that will help define signaling networks putatively involved in remodeling. These will be reconstituted for validation in vitro in cellular models (primary cardiac myocytes and iPS cells) and in vivo with gain- and loss-of-function in zebrafish and mice.

Figure 1 Integrated influence of paracrine and autocrine NO on cardiac function. Endothelial NO production paracrinally increases myocyte distensibility. eNOS in caveolae activated by muscarinic and beta-adrenergic receptors (i.e. beta3-AR) attenuates the beta1/2-adrenergic inotropic effect and potentiates the cholinergic effect, resulting in attenuated chronotropic effects, cooperatively with pre-synaptic nNOS. The overall effect is increased lusitropy with increased diastolic interval, which promote ventricular perfusion and filling. The increased distensibility also promotes the recruitment of contractile reserve by stretch, which activates eNOS, a mediator of the slow increase in calcium transient and contraction force (Anrep effect). SR re-filling, in turn, is promoted by nNOS-derived NO, so that both isoforms contribute to potentiation of EC coupling and diastoly and attenuate remodeling. ec: endothelial cell; cm: cardiac myocyte; ps: parasympathic; os: orthosympathic; sr: sarcoplasmic reticulum; ach: acetylcholine; nad: noradrenaline. Adapted from J.-L. Balligand et al (2009) Physiol Rev. 89(2):481-534
Pathogenic mechanisms of endothelial dysfunction in atherosclerosis and metabolic syndrome

M. Romero Perez, G. Rath, I. Lobysheva, J.-L. Balligand, C. Dessy

Numerous studies have emphasized the pivotal role of endothelial dysfunction in the development, progression or clinical complications of atherosclerosis. Although it plays multiple functions, a reduced vasodilatory response to pharmacological stimulation constitutes a recognized indicator of a dysfunctional endothelium. It results from a rupture of the controlled balance between production and release of endothelial relaxing (NO, EDHF, PGI2) and contracting factors (ET-1, TxA2, and PGs). The formation of prostacyclin (PGI2), thromboxane (TxA2), and isoprostanes is markedly enhanced in patients with atherosclerosis. Activation of TxA2 receptors (TP receptors) causes potent vasoconstriction and induces increased formation of superoxide anions (O2-) and peroxynitrite (ONOO-), a product of rapid reaction of O2- with NO that accelerates NO degradation and reduces its availability. In the last decades, numerous reports have suggested that TP-receptors antagonism (with sulotroban or terutroban) or direct inhibition of thromboxane synthase (with furegrelate) can not only have antiplatelet effects but also impact endothelial dysfunction as well as the inflammatory component of atherosclerosis. Failure to inhibit deleterious isoprostanes synthesis explains why the latter components did not live up to the expectations in clinical trials. Therapeutic interest has thus switched to compounds that combine thromboxane synthase inhibition and TP receptor antagonism, such as BM-573. In previous in vitro and ex vivo studies, BM-573 has been demonstrated as a potent dual compound able to reduce TxA2 production by TxAS inhibition and to prevent the action of TxA2 by blocking the TP receptors. In addition to its antiplatelet and antithrombotic effects, BM-573 has proven to be effective in different animal models of cardiovascular diseases where levels of TxA2 are increased. We have recently evaluated the effects of acute and chronic treatments with BM-573 on endothelial function, NO bioavailability, oxidative stress and systolic blood pressure in apolipoprotein E-deficient (ApoE-KO) mice. Both treatments were able to reduce endothelium-derived contractile factor(s) and restore endothelial function in the microcirculation of ApoE-KO mice. Acute effects of BM-573 were mediated by an increased phosphorylation of both eNOS and Akt whereas BM-573 chronic treatment reduced oxidative stress and restored NO bioavailability. Together with a previous report showing a prevention of plaque progression by BM-573 in conductance vessels in the same mouse model, our data provide additional rationale to combine antagonism of TP receptors and TxAS inhibition as a therapeutic modality to prevent the vascular deleterious consequences of atherogenesis.

The metabolic syndrome combines several risk factors for endothelial dysfunction and atherosclerosis, including dyslipidemia. Statins (HMG-CoA inhibitors) are classically used to treat lipid disorders, but are endowed with ancillary (“pleiotropic”) effects on cardiac and vascular biology. Using genetically modified mouse models that are resistant to lipid lowering effects of statins (e.g. LDLr or ApoE KO mice) we identified several lipid-independent effects of statins on blood pressure regulation, vascular NO production and myocardial fibrosis. We assigned specific mechanisms for these beneficial effects, e.g. statin-induced PPAR-gamma activation of superoxide dismutase (SOD) expression or activation of AMPK in cardiac fibroblasts.

We have developed a EPR-based subtraction technique to quantitate nitrosyl-heme complexes (Hb-NO) from hemoglobin in intact venous erythrocytes, that we correlated with NOS activity and NO production in vessels ex vivo and with endothelium-dependent systolic blood pressure variability. Recently, similar Hb-NO signals were obtained in humans and correlated with endothelial function measured with peripheral arterial tonometry (PAT). Biochemical determinants of Hb-NO formation in erythrocytes in pathophysiologic conditions are further studied before the development of this technique as a biomarker of NO-dependent endothelial function.
Molecular pharmacology of adrenergic receptors in heart and vessels

L. Vanhoutte, S. Moniotte, O. Feron, C. Dessy, J.-L. Balligand

Catecholamines released from sympathetic nerve activation are key regulators of cardiovascular function. We focus on the functional role of the third isotype of beta-adrenoceptors (beta3-adrenoceptors), that we identified in cardiac myocytes and coronary microvascular endothelium (including in humans). Its activation induces both endothelium-dependent vasodilation, angiogenesis and opposes the positive inotropic and remodeling effects of catecholamines on beta1/beta2 adrenoceptors. Several downstream effectors account for these effects, e.g. NOS activation (in cardiac and endothelial cells) and EDH (in endothelium). Additional signaling pathways are being identified, including through phospho- and nitroso-proteomic approaches. Metabolic effects on both cell types, as well as on distant tissues, are being examined. As beta3-adrenoceptor proteins are upregulated in failing hearts (contrary to beta1/beta2), the interplay between adrenoceptors on the expression of the respective isotypes, as well as the effect of specific beta-blockers is examined.

We already mentioned the implication of cardiac beta3-adrenoceptors in cardiac remodeling. In addition, cardiac autoantibodies, e.g. resulting from autoimmunity against the cardiac beta1-adrenergic receptor were documented to play an active role in the pathogenesis of dilated cardiomyopathy. We have generated a mouse model of hypertrophic remodeling associated with immunization against a peptide sequence of the second extracellular loop of beta1-adrenoceptors. The pharmacological properties of these auto-antibodies against the beta-adrenoceptor isotypes is being characterized, as well as comparative effects of similar immunization in several genetic mouse models. Effects on several aspects of remodeling (hypertrophy, fibrosis, function/flow) are being characterized in these mice with a 11.7T MRI equipped with a cardiac antenna, together with specific algorithms for optimal imaging.

Alteration of the endothelial phenotype in response to ischemia/reperfusion, intermittent hypoxia or radiotherapy

G. Rath, V. Montiel, P. Sonveaux, O. Feron, J.-L. Balligand, C. Dessy

Ischemia/reperfusion.

The treatment of ischemic heart diseases relies on an early return of blood flow to ischemic zones of the myocardium. However, reperfusion on its own has the potential to cause further irreversible myocardial cell injury and endothelial dysfunction as the consequence of a burst of reactive free radicals such as reactive oxygen species (ROS), and pro-inflammatory cytokines. In 1986, the pioneer work of Murry demonstrated that exposing the heart to transient sublethal ischemia and reperfusion, protects the myocardium against functional damage and cell death caused by a subsequent sustained ischemia. This phenomenon, called ischemic preconditioning, has since proven to be true in both animals and humans, and in many organs including the vascular endothelium. Nitric oxide (NO) being the best characterized and, likely, the most important endothelial factor, many studies hypothesized its involvement in the molecular cascade that leads to preconditioning in both myocardial and vascular tissues. Indeed, in the vasculature, a protective effect of ischemic preconditioning on the endothelium relaxation has been documented to be NO-mediated. In resistance arteries, however, the control of vascular tone not only depends on NO bioavailability but also on the generation of endothelium-derived hyperpolarization (EDH(F)). The key mediators of EDH(F) signaling are nowadays clearly identified. Accordingly, EDH(F) is triggered by an elevation of the cytoplasmic Ca2+ concentration in endothelial cells ([Ca2+]i) and the final opening of Ca2+-activated potassium channels (KCa) expressed either on endothelium or on smooth muscle cells. In this context, we have demonstrated the obligatory role of the TRPV4 channels in the endothelium-dependent vascular relaxation, as their genetic deletion affected both the NO and EDHF components of the relaxation to muscarinic cholinergic stimulation. Also, in many resistance vessels, vascular gap junction integrity is a requisite for vascular smooth muscle cell hyperpolarization to occur.
We have demonstrated that the EDH(F)-mediated relaxation was totally absent in caveolin-1 (cav-1) deficient mice, where the expression of connexins (Cx43, 40, and 37) was reduced and myo-endothelial gap junctions were altered. As the consequences of ischemia/reperfusion and ischemic preconditioning in resistance arteries remain virtually unaddressed, we have investigated the impact of hypoxia and reoxygenation on endothelial relaxation to specifically clarify the role of TRPV4 channels and gap junctions. By mimicking ischemia-reperfusion we have documented impairment in NO-mediated relaxation and an up-regulation of EDHF-mediated relaxation. Hypoxic preconditioning however restored the NO mediated relaxation and further improved the EDHF-mediated response. An increase in expression and activity of the TRPV4 channels associated with a higher concentration of caveolae at the membrane probably potentiate the EDHF response under hypoxia and promotion of inter-cellular coupling through gap junctions most probably trigger the vascular protective effect of preconditioning. Thus our work provides further evidence on how TRPV4 and connexins may participate to preserve vasorelaxation under ischemic conditions and restore the NO-mediated pathway in ischemic preconditioning conditions. Unexpectedly, pointing out caveolae as a common signaling platform, our results further suggest an intimate relationship between NO and EDH(F) signaling that remains to be investigated.

Another aspect of ischemia/reperfusion is the formation of tissue edema, with adverse consequences on perfusion and cardiac function. Trans-membrane water fluxes are acutely regulated by aquaporins, some isoforms of which are expressed in heart and vessels. In collaboration with NEFR/IREC, we have focused on the characterization of expression, cellular localization and functional role of aquaporin-1, as deduced from the cardiovascular phenotype of AQP1 KO mice. We found unexpected influences of AQP1 on cardiac and vascular function, probably subserved by its co-localization with other effectors in rafts/caveolae.

**Radiotherapy.**

Currently, the radiation protection system is based on the assumption that for noncancer effects there is a threshold of low dose radiation below which no significant effects are observed. Recent years however witnessed growing epidemiological evidence of excess risk of late occurring cardiovascular disease at much lower doses without a clear cut threshold. Until now, these epidemiological data are suggestive rather than persuasive due to a lack of knowledge about the underlying mechanisms. For the benefit of public health, it is therefore now of utmost importance to investigate these biological and molecular mechanisms in order to obtain a more accurate risk assessment in the low dose region and thus to improve radiation protection. We are currently investigating the effects of low dose radiation on endothelial cell biology.

**Metabolic regulation of stem cells plasticity**

A. De Pauw, E. Andre, P.E. Porporato, O. Feron, J.-L. Balligand, P. Sonveaux

Metabolism is a basic characteristic of all cell types controlling not only energy homeostasis but also redox homeostasis, biosynthesis, and protein expression/modifications to name only few aspects. Intracellular metabolic fluxes are wired with intercellular nutrient exchanges, oxygen delivery and waste clearance, rendering cells metabolically dependent of their (micro) environment. Although these external influences are often well characterized in many differen-
tiated tissues, there is still much to learn about metabolic rearrangements occurring during cell differentiation and pathology. On one hand, there is increasing evidence that stemness in normal and cancer tissues is associated with metabolic rewiring, but most of the phenotype remains to be characterized and little is known about the external metabolic influences on stemness and differentiation. On the other hand, several conditions such as obesity, cachexia, cancer and inflammation – e.g. during wound healing, are critically influenced by metabolism. If the metabolic response to existing treatments often remains poorly characterized and may influence outcome, metabolic characterization will also allow the identification of new therapeutic strategies. Stem cells are subject to intense research in regenerative heart medicine and in cancer. While in the heart stem cell engraftment could limit the functional consequences of injury and in tumors stem cell impairment could offer new therapeutic perspectives, the molecular mechanisms governing stem cell maintenance and differentiation are still elusive, making it difficult to anticipate cell responses to selected treatments. We are undertaking a thorough metabolic characterization of adult cardiac stem cells and selected cancer stem cell lines with state-of-the-art technologies aiming to identify common metabolic characteristics of both cell types and defined metabolic and/or molecular interventions modulating stemness.

Metabolism and signaling pathways driven by glucose, lactate and glutamine in tumors


Cancer is a metabolic disease striving to match ATP production and demand and to meet the biosynthetic needs of unbridled cell proliferation. Glycolysis and the use of glutamine when they are both uncoupled from oxidative phosphorylation largely account for the aggressiveness of tumor cells providing them with an oxygen-independent source of ATP production and biosynthetic inter-mediates. These metabolic peculiarities also correlate with tumor progression, metastatic burden, resistance to treatments and tumor recurrence. We have shown that the glycolytic end-product lactate shuttles between hypoxic and oxygenated tumor areas where it fuels oxidative tumor cells and further initiates several signaling pathways in tumor and endothelial cells. Lactate thereby strongly supports tumor growth, an activity that requires the expression of the inward lactate transporter monocarboxylate transporter 1 (MCT1) at the outer membrane of tumor cells and tumor-associated endothelial cells. MCTs are also involved in the release of lactate from glycolytic tumor cells. Silencing MCT expression shows strong antitumor effects through combined anti-metabolic and anti-angiogenic activities. This work led us to launch a drug discovery program together with CD3 (KULeuven) to identify new MCT inhibitors. Three distinct families of MCT inhibitors were recently obtained, with one of them offering inhibitors of lactate influx but not efflux. This latter family is made of 7-amino carboxycoumarins and was shown to sensitize mice bearing human tumors to the effects of chemo- and radiotherapy.

We are also pursuing the evaluation of the involvement of lactate signaling in aerobic glycolysis and the study of the mechanisms that regulate MCT1 expression in tumor cells. An area of investigation is the coupling between the metabolic pathway of lactate and autophagy, a process of cellular self-eating actively recycling damaged proteins and organelles, thereby promoting tumor cell survival.

Figure 3 Model depicting the pro-angiogenic activity of lactate and the anti-angiogenic activity of MCT1 inhibitors in cancer. Lactate activates HIF-1 in normoxic tumor and endothelial cells, thereby stimulating angiogenesis through the paracrine VEGF pathway and the endothelial autocrine bFGF and IL-8 pathways. These activities of lactate can be blocked with MCT1 inhibitors.
In parallel, we are working on glutamine, the second major fuel for tumor and endothelial cells. The focus of this research is a better understanding of the fate of glutamine and glutamate (the first metabolite resulting from glutaminolysis) in their capacity to feed a variety of metabolic pathways but also to promote the transport of essential amino acids and cysteine for production of glutathione, respectively. This work recently led us to identify tumor acidosis as a trigger of the metabolic shift from glucose to glutamine metabolism. Histone deacetylase SIRT1 was further identified as the master regulator of this plasticity (i) providing acetate as a counteranion to transport protons out of the cells and (ii) regulating the expression and activity of HIF-1α and HIF2α that in turn (oppositely) influence glucose and glutamine metabolism.

Cancers also evolve a subpopulation of tumor cells that metabolically rely on glycolysis uncoupled from oxidative phosphorylation (OXPHOS) irrespectively of oxygen availability (aerobic glycolysis). Given that most metastases are avid for glucose and because clinical data show a positive correlation between lactate production and tumor metastasis, cells performing aerobic glycolysis could constitute a population of metastatic progenitor cells that would remain glycolytic in the blood stream. We found a different metabolic phenotype, though. Indeed, we identified a mitochondrial switch corresponding to an overload of the electron transport chain with preserved mitochondrial functions (including ATP production) but increased mitochondrial superoxide production. The switch provided a metastatic advantage that was phenocopied by moderate OXPHOS inhibition associated with mild mitochondrial superoxide increase. Thus, two different events, OXPHOS overload or moderate OXPHOS inhibition, promote superoxide-dependent tumor cell migration, invasion, clonogenicity, and metastasis; demonstrating the central role of mitochondrial superoxide generation in the pathogenesis of metastasis. Based on these data, we are now working on the development and validation of metastasis-prevention therapies.

To exploit or to destroy the tumor vasculature: provascular, vascular targeting and anti-angiogenic strategies

P.E. Porporato, P. Sonveaux, O. Feron

Tumors are highly heterogeneous in all phenotypic features including the tumor vasculature that encompasses both mature vasoactive and immature angiogenic blood vessels. In line with its physiological roles, the tumor vasculature actively participates in metabolite and oxygen delivery and in waste removal but these functions in tumors are under malignant influence. Derailed angiogenesis is a typical example of an attempted (although largely failed) enslaving of the host vasculature by tumor cells. More successful from the metabolic point of view is the cooption by tumors of preexisting blood vessels, even if – and we were instrumental to show it – tumor cell activities profoundly alter the reactivity of otherwise perfectly mature blood vessels. The distribution of tumor blood vessels into mature and immature populations influenced by unique tumor microenvironmental features offers a rationale for specific therapeutic interventions aimed at modulating metabolite and oxygen delivery. Histone deacetylase SIRT1 was further identified as the master regulator of this plasticity (i) providing acetate as a counterion to transport protons out of the cells and (ii) regulating the expression and activity of HIF-1α and HIF2α that in turn (oppositely) influence glucose and glutamine metabolism.

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either theranostic radioisotopes or siRNA targeting major determinants of angiogenesis.

### Tumor hypoxia: a major hallmark of cancer progression and a possible Achille’s heel


Hypoxia is nowadays described as a hallmark of tumors. Tumor angiogenesis and glycolytic metabolism are two extensively studied responses of cancer cells to a deficit in oxygen. The building of new blood vessels to bring $O_2$ and the uncoupling of glycolysis from mitochondrial oxidative phosphorylation to survive under low $O_2$ are actually two complementary tumor responses to hypoxia. These somehow opposite modes of adaptation account for local and temporal heterogeneities in tumor $O_2$ distribution. As a corollary, the extent of hypoxia and in particular its cycling nature reflect tumor plasticity and thus measure the capacity of tumor cells to survive and to proliferate in a hostile environment.

We are conducting both basic and translational research programs aiming to explore the determinants and to exploit the consequences of hypoxia in tumors. We are studying how autophagy and ER stress, both induced by a deficit in oxygen, influence the response to anticancer strategies including mTOR inhibition.

Together with chemists from UCL, we have also developed a program of phenotypic screening for the identification of hypoxia-selective anticancer compounds, either acting as hypoxia-activated prodrugs or specifically targeting hypoxia-driven prosurvival pathways in endothelial and tumor cells. In another project, we integrated the hypoxia parameter in our search for diagnostic and prognostic biomarkers of a variety of human cancers in close collaboration with clinical oncologists (Cliniques St Luc, UZ Brussel, CHU Liège). We are for instance studying how low tumor $pO_2$ influences the immunoproteome and thereby leads to the production of autoantibodies directed against hypoxia-induced antigens occurring at early stages of colorectal cancer progression. In another project, we are working with engineers of the Ecole Polytechnique de Louvain on the prognostic potential of a transcriptomic signature associated with cycling hypoxia in order to optimize the stratification of breast and colon cancer patients.

**Figure 2** Overview of the main alterations in cell phenotype induced by hypoxia. While a variety of adaptive mechanisms can in fine account for more aggressive tumors (blue), the bioreductive environment associated to low $pO_2$ leads to cellular stress (green) compromising tumor cell viability and thus offering new opportunities for treatment.
SELECTED PUBLICATIONS


EQUIPMENT

- Molecular biology equipment including adeno-, retro- and lentivirus technology
- Real-time PCR (Biorad IQ5 & AB ViiA7) and Gradient PCR (Biorad C1000)
- Microplate Readers incl. injectors (Victor 5 and Spectramax i3 with Minimax imaging cytometer module)
- Telemetry equipment (DataScience Intl.)
- Hypoxia workstations (Ruskinn In Vivo 400 and 500)
- EPR spectrometer (Magnetech S400) for free radicals (e.g. NO) measurements
- Enzymatic analyzes of glucose, lactate, pyruvate, glutamate and urea (CMA600)
- Microdialysis (CMA400, CMA402 and refrigerated fraction collector)
- 96-well luminometer (GloMax)
- Bioenergetic analyzer (Seahorse XF96, 4 injection sqports)
- Bioplex (Biorad)
- MACS for cell immuno-isolation
- Biochemistry: equipment for ELISA development and MiniProtean III and transfer units for immunoblotting Ultracentrifuges
- Microscopy (Zeiss): Axio-Imager, Pseudo-confocal Apotome
- 2D-gel platform incl. IpgPhor III, ETTAN DALT6, TE77 transfer units, SE600 electro-phoresis unit, SG100 gradient maker (GE)
- Laser Scanner Typhoon FLA9500 incl. Decyder analysis software
- Spot picker (Ettan)
- Akta Microscale liquid chromatography
- Intravital microscopy (Zeiss Axioskop + Hamamatsu EBCD camera)
- In vivo bioluminescence (IVISSO, Xenogen)
- Laser Doppler imaging (Moor)
- Pressure and wire myographs and cardiac myocyte contractility (incl. fluorimetry) setup (Ionoptix)


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The pole of Research in Hepato-Gastroenterology brings together clinicians and scientists for the study of diseases of the liver, pancreas and digestive tract. Our goals are, by a better understanding of the diseases’ mechanisms, to improve diagnosis and identify new therapeutic targets, and by evaluating new drugs and therapeutic strategies, to shape better care and treatments for patients suffering from these diseases. To meet our goals our pole of research has established fruitful collaboration with other teams within IREC and UCL, with scientific organizations, with international teams of clinicians and scientific leaders.

Clinical, translational and fundamental research projects co-exist and interconnect. Our research unit is pioneer in endoscopic procedures for the treatment of diseases of the digestive tract, biliary tree and pancreatic diseases. It largely invests in the evaluation of new therapeutics and pharmacological treatments through clinical studies, drug trials and strong interaction with the unit of clinical pharmacology. Our experimental research unit has developed, constantly invests in and masters numerous animal models for the study of liver diseases, ranging from metabolic disorders, fibrogenesis, liver regeneration and carcinogenesis. Indeed, those represent valuable tools for the understanding of pathogenic mechanisms at play in the appropriate environment of the (diseased) organ or organism.
Clinical research focuses on treatment of neoplastic and preneoplastic tumours in the gastrointestinal tract, in particular with evaluation and optimization of pioneer therapeutic endoscopy procedures. This includes animal experiments evaluating NOTES (Natural Orifice Transluminal Endoscopic Surgery) and the acute phase response associated with endoscopic and laparoscopic accesses. Prospective clinical studies have been initiated in stenting biliary benign and malignant strictures and esophageal stenoses with newly designed devices including biodegradable self expandable stents, in evaluating new advanced imaging techniques in endoscopy and endoscopic ultrasonography (elastography, contrast enhanced echoendoscopy) and confocal endomicroscopy in pancreatic cysts and tumours.

The expertise in advanced therapeutic endoscopy is further applied in benign conditions such as the development of innovating treatment modalities of achalasia (per oral endoscopic myotomy). The pathogenesis of achalasia is also studied as part of an international consortium for GWA studies.

Clinical research is performed on evaluation of tumor response in metastatic colorectal treated by different chemotherapeutic regimens in collaboration with experts of quantitative radiology and radiomics.

Clinical research is also ongoing for a better understanding of the pathogenesis of chronic pancreatitis and pancreatic cancers arising as a complication of genetically determined pancreatitis. Special focus is given to the study of Intraductal mucinous and papillary neoplasms (IPMN), through a collaborative with the team of P Jacqmain (LPAD, de Duve Institute). Basic (i.e. the building of a transgenic mouse model) and translational work (analysis of the different phenotypes of IPMN resected in Clin St-Luc) are part of the research goals. Endocrine pancreatic function is also explored in this context, as diabetes is a dreadful complication of chronic pancreatic disease. In particular, the plasticity of endocrine Langerhans islets is studied and compared, in a same organ, between healthy area and area of chronic pancreatitis caused by obstructive adenocarcinoma. In addition, Dr Sempoux has a long standing interest in the study of islet function, regulation and plasticity in congenital hyperinsulinism of infancy.

**SELECTED PUBLICATIONS**


Numerous clinical studies are performed in the field of viral hepatitis (HBV and HCV) including since the beginning of 2013 studies using antivirals without interferon administration (interferon free regimens). Studies on clinical pharmacokinetics of new drugs in cirrhotic patients and healthy volunteers are also regularly performed (Investigator, Y Horsmans).

Our unit (Investigator, I Borbath) participates to several phase 2 and phase 3 therapeutic trials in the field of treatment of hepatocellular carcinoma, cholangiocarcinoma, pancreatic cancers and endocrine tumors of the digestive tract for which results have been (or will soon be) communicated at international conferences and published; and is a driving force for establishing registries for endocrine tumors both at the Belgian (www.bgdo.org/dnet) and European (www.enets.org) levels.

The unit is also very active in the field of inflammatory bowel diseases with participation to clinical trials evaluating new treatments and new therapeutic strategies for Crohn’s disease and ulcerative colitis (Investigator, O Dewit), as well as participation to multicentric studies aiming at a better delineation of the epidemiology of those diseases and of genetic determinants. Similarly, the unit is at the forefront of pharmacologic clinical research in functional gastro-intestinal disorders as principal investigator in several phase II and phase III trials.

**SELECTED PUBLICATIONS**


**TRANSLATIONAL RESEARCH**

**Biological and behavioral control of alcohol-dependent subjects – pathophysiology of alcoholic and non-alcoholic fatty liver diseases**

*P Starkel, N Lanthier, C De Saeger*

Dr Starkel is studying, together with Dr Ph de Timary, co-founders of the clinical alcohology unit, the mechanisms implicated in biological and behavioral control of alcohol-dependency. They highlighted the role of increased intestinal permeability and inflammation and documented the role of circulating inflammatory cells in the systemic inflammation that characterized chronic alcohol abusers. Studies based on animal models aim at a better understanding of the role of the gut, gut permeability and changes to the gut microbiota in alcohol dependancy and alcohol-induced liver diseases and their metabolic consequences.

Dr N Lanthier joined the clinical team contributing to studies of physiopathological mechanisms in alcoholic steatohepatitis in collaboration with the University of North Carolina. He also actively works at developing translational research in non-alcoholic fatty liver diseases and hepatic complication of obesity.
SELECTED PUBLICATIONS


EXPERIMENTAL RESEARCH

Non-alcoholic fatty liver disease and related fibrosis.

*N Lanthier, V Legry, L Poekes, V Lebrun, N Fezabding, Y Horsmans, I Leclercq*

The laboratory has a long standing interest and expertise in the study of the pathogenesis of fatty liver diseases, non-alcoholic steatohepatitis (NASH) and associated fibrosis, which represent the hepatic manifestations of the metabolic syndrome. We contributed to major milestones in the field such as the discovery of CYP enzymes as source of noxious lipoperoxides in NASH, the demonstration of the lipotoxicity, the identification of leptin as a key component of the signaling network controlling liver fibrogenesis, hepatocytes proliferation and NASH. Now we have identified the pivotal role of activated Kupffer cells in the initiation of hepatic insulin resistance and adiposity in response to a high fat diet and provided experimental support to the concept of liver-derived factors able to influence metabolism and inflammation in peripheral tissues. We are now working at the identification of such factor(s).

**Figure 1** Upon transition from a normal to a high fat diet, mice develop liver steatosis, activation of Kupffer cells and hepatic insulin resistance. Kupffer cell depletion by injection of liposome-encapsulated clodronate prevents the initiation of hepatic insulin resistance. The graph displays the results of the hyperinsulinemic-euglycemic clamp study (GIR: Glucose infusion rate; HGP: hepatic glucose production) in mice subjected to a standard chow (ND), a high fat diet (HFD and HFD+PBS) or a high fat diet with Kupffer cells deplketion (CLO+HFD).
Research in the field of NASH has suffered from the lack of appropriate animal model as most of the rodent models recapitulating the metabolic disturbances do not develop a hepatic phenotype, while the dysmetabolic context is missing when liver injury mimicking steatohepatitis is induced. Through international collaboration, we now house the foz/foz mice, a colony of mice harboring a spontaneous mutation in the Alström gene and developing spontaneously upon high fat feeding, obesity, diabetes and progressive fibrosing steatohepatitis. Those mice are used (1) to understand the mechanistic relationship between altered function of the Alström gene product and the metabolic and hepatic phenotype, as model to evaluate (2) the role of ER stress in liver disease associated with obesity and (3) the effects of drugs targeting insulin sensitivity, lipid / glucose metabolism, ... on liver disease progression and reversal in the dysmetabolic context.

**SELECTED PUBLICATIONS**


Hepatocellular carcinoma represents a major public health problem and currently available therapeutic options are largely deceitful. In most instances, it arises in a setting of liver cirrhosis. Therefore, prevention of HCC in cirrhotic patients is of major importance. We have previously demonstrated that somatostatin analogues as well as the Ras oncogene inhibitor FTS inhibit hepatic fibrosis and prevent cancer in animal models. This anti-proliferative effect has also been demonstrated on human cancer cells. Interestingly, we have shown that FTS sensitize transformed malignant cells to apoptosis and that combination therapy with a death-receptor (Trail) agonist induced apoptosis in malignant but not in healthy hepatocytes. Those results prompted us to further investigate combination therapy in HCC in animal pre-clinical models and to analyse the mechanisms of action of potential anti-HCC drugs. In parallel, we also aim at a better understanding of the synergistic relationship between HCC and the fibrotic environment as targeting fibrosis may have a synergistic effect on the control of cancer cell growth. In collaboration with international team, prof C Sempoux participate to work aiming at a better classification of liver tumors to provide better diagnostic, predict outcome and tailor treatment to disease phenotype.

SELECTED PUBLICATIONS


Liver regeneration, contribution of liver progenitor cells and liver cell therapy

N Van Hul, R Espanol Suner, AC Dusabineza, V Legry, N Feza-Bingi, I Leclercq

Rapidly after liver damage, unharmed hepatocytes divide to compensate for the endured cell loss and regain normal function. However, in case of massive and/or chronic injury this process is insufficient due to either paucity of hepatocytes able to engage into the regenerative process or replicative inability of the remaining hepatocytes. In those conditions, a dormant compartment of liver progenitor cells (LPC) is activated, giving rise to transit amplifying cells (termed ductular reaction in human pathology) and considered as a possible rescue mechanism for liver mass regeneration.

Using specific transgenic model enabling lineage tracing studies (in collaboration with prof F Lemaigre DDVE/UCL), we recently demonstrated that liver progenitor cells LPC derive from the embryonic ductal plate. We provided the first experimental evidence that LPC /biliary cells are able to differentiate into functional hepatocytes in specific conditions of hepatocellular damage, but do not participate to physiological liver mass homeostasis. We also provided experimental evidence that the microenvironment (extracellular matrix, laminin, Kupffer cells) controls de lineage cell commitment of LPC. Current work aims at investigating whether and how modulation of the microenvironment may increase hepatocellular differentiation of LPC and improve functional regeneration of the diseased liver.

LPC have been proposed as been the cell of origin of (some) hepatocellular carcinoma. This burning question is currently being addressed using specific cell lineage tracing tools.

Liver cell transplantation represents an alternative to orthotopic liver transplantation for the treatment of inborn error of metabolism. Mature hepatocytes are commonly used for liver cell therapy but the results of such a procedure remain poor, in part because they engraft poorly into the host liver. We demonstrated that hepatic stellate cells and the matrix they produce protect hepatocytes and improve their homing and engraftment into a host liver.

**SELECTED PUBLICATIONS**


PhD awarded in 2013-2014


- Nicolas Charrette, 12/2013 “The effect of S-farnesyl-thiosalicylic acid (FTS, Salirasib) on proliferation, apoptosis and signalling pathways in humans hepatocarcinoma cell lines”.

- Nicolas Lanthier, 05/09/2013 “Insulinorésistance intrahépatique : Rôle des macrophages et cellules de Kupffer”.

- Regina Español Suñer. 20/06/2013. “Origine and fate of liver progenitor cells”.

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IL is a FRS-FNRS research associate, PS, IB, NL are clinician-post-doctoral researcher financed by IREC and FNRS. VL is a post-doctoral researcher financed by IREC.
The GYNE unit has focused its research activities on four main topics related to male and female infertility:

1. Ovarian tissue and ovarian follicle cryopreservation and transplantation in order to preserve fertility in female cancer patients.
2. Pathogenesis of endometriosis and endometriotic nodules, which are among the most frequent benign gynecological diseases affecting women of reproductive age.
4. Andrology: Testicular tissue cryopreservation and transplantation, and differentiation of primordial germ cells derived from pluripotent cells in order to restore fertility in male cancer patients.

In the GYNE unit, a pluridisciplinary team (gynecologists, molecular biologists, clinical biologist and veterinary surgeon) investigate reproductive tissue physiology at the molecular and cellular level, both on patient biopsies and in experimental animal models. The team involved in these projects works in close cooperation with the gynecology department of the Cliniques universitaires Saint-Luc.
Ovarian tissue and ovarian follicle cryopreservation and transplantation

Cryobanking
MM. Dolmans, P. Jadoul

Ovarian tissue cryopreservation is offered to young women at risk of premature menopause and sterility after gonadotoxic therapies such as chemo- and radiotherapy. Cryopreservation and transplantation of ovarian tissue is a promising approach to preserve fertility in young cancer patients undergoing gonadotoxic treatment. Transplantation of cryopreserved ovarian tissue allowed restoration of ovarian function, and fertility in more than 30 patients so far worldwide, with 6 babies for Saint-Luc. The ovarian tissue bank at Cliniques Universitaires St Luc (one of the first and largest in the world) contains tissue from more than 600 patients, with around 100 patients having donated their tissue for research purposes and 500 for fertility preservation and long-term cryopreservation. Pathologies are various and include both malignant and benign diseases requiring chemotherapy. The most frequent indications are hematological malignancies and breast cancer.

A) Assembling a transplantable artificial ovary
C. Chiti, MM. Dolmans, C.A. Amorim

AIM
The aim of this project is to develop a bioinspired artificial ovary that offers an environment in which follicles can survive and grow. It is essential to bear in mind that just like the natural ovary, the artificial ovary should maintain the original structure of follicles, preserving contact between granulosa cells and oocytes and preserving follicular interaction with the extracellular matrix (ECM). In other words, the artificial ovary should spatially and temporally mimic the ECM. In order to do so, it needs to include certain design specifications, such as interaction with cells, physical support of follicles, porosity and biodegradability, which are all interconnected and influence each other. It must also be biocompatible and, from a practical point of view, capable of being sterilized and handled.

BACKGROUND
Although safe xenotransplantation of ovarian tissue from lymphoma patients has been reported in SCID mice, the possibility of reintroducing tumor cells into cancer patients by autografting of ovarian tissue cannot be excluded for other indications, such as leukemia. To avoid transferring malignant cells, grafting of isolated follicles may be considered.

Matrices tested for the artificial ovary
We recently encapsulated isolated mouse follicles and ovarian cells (OCs) in alginate or fibrin matrix and autotransplanted to immunocompetent mice. After one week of transplantation, we observed follicle survival and development up to antral stage, survival and proliferation of grafted cells, vessel formation and matrix degradation. Such encouraging results demonstrated that the artificial ovary can be a feasible option to restore fertility in cancer patients.

Stromal cells for the artificial ovary
Follicles need OCs to support their growth. OCs are recruited to differentiate into thecal cells and a previous study demonstrated the essential role of isolated OCs, including stromal and endothelial cells, in the formation of a well vascularized and structured ovary-like stroma after one week of grafting. The aim of this study is to determine the best origin of stromal cells for the artificial ovary. In order to do so the viability and in-vivo growth and vascularization of OCs isolated from fresh or frozen ovarian tissue (cortex or medulla) will be compared after 7 days of xenotransplantation to nude mice in a fibrin matrix.

B) Ovarian tissue vitrification
C.A. Amorim, M. Soares, MM. Dolmans

AIM
The aim of this project is to investigate vitrification approaches to cryopreserve ovarian tissue fragments, with a view to increasing survival of the follicle population and ovarian tissue quality after thawing/warming.

BACKGROUND
The lifesaving treatment endured by cancer patients leads, in many women, to early menopause and subsequent infertility. Loss of fertility potential is a difficult issue to understand for young children, but potentially traumatic for them as adults. In clinical situations where patients are prepubertal girls or where chemotherapy cannot be delayed, ovarian tissue cryopreservation has emerged as a promising option to restore fertility. In our research unit, we perform cryopreservation and transplantation of ovarian tissue fragments, and were the first in the world to obtain a live birth using this technique. Nevertheless, we are continually developing new
studies in order to improve the protocol of cryopreservation and grafting. For this reason, we have now decided to focus on a novel approach: vitrification of ovarian tissue fragments. Vitrification is defined as the conversion of a system from a fluid to a solid solely by an increase in viscosity, without a phase change, without any crystallization of water, and therefore in the complete absence of ice.

We have conducted a number of experiments to test the toxicity of various vitrification solutions and to compare different vitrification protocols. Based on our encouraging results, we are now vitrifying and autografting non-human primate ovarian tissue over the longer term. For this, we are using baboons, a large primate commonly utilized as an animal model for studies of human reproduction.

C) Search neoplastic cells in the ovarian tissue
M. Soares, Y. Iwahara, C.A. Amorim, MM. Dolmans

Our aim is to identify the risk of transmission of malignant cells through the grafted ovarian tissue, and develop protocols to purge selectively malignant cells from the cryopreserved-thawed ovarian tissue before transplantation.

In most centers, hematological disease represents the most frequent indication for ovarian tissue cryopreservation. For leukemia, our experimental studies showed that ovarian tissue reimplantation cannot be safely performed in young women with the acute lymphoblastic form because of the risk of reintroducing the disease. For ovarian tissue from hematologic cancer patients, it is therefore of primordial importance to identify minimal residual disease (MRD) before ovarian transplantation. Markers used to detect MRD in ovarian tissue are disease-specific, requiring a patient-oriented case-by-case approach.

Our current research investigates how to purge the follicle suspension:

-1st step. Improving our follicle isolation procedure to obtain completely purified (disease-free) follicles. Isolation of preantral follicles from an ovarian cortical fragment is a well-established protocol in our laboratory. This procedure however was found to be unsafe in a model of ovarian tissue contaminated with leukemic cells. An improved technique with 3 additional washes proved to be effective in eliminating the leukemic cells taken along with the follicles.

-2nd step. Application of the improved follicle pick-up technique in human ovarian tissue from deceased leukemic patients and evaluation of the efficacy by immunofluorescence and by xenografting to immunodeficient mice. (collaboration with the Hematology Laboratory, Cliniques universitaires Saint Luc, Dr P. Saussoy, for the flow cytometry and for determining the most suitable markers to be used for each type of leukemia, depending on the primary disease)


Dolmans MM., Marotta ML, Pirard C, Donnez J, Donnez O. Ovarian tissue cryopreservation followed by COS and pick-up of mature oocytes does not impair the number or the quality of the retrieved oocytes. 2014, submitted.


Patents

European Patent Application n° 07117661.4-1219: “Scaffolds for follicle transplantation”

Partnership

- Inter-university: ULg, ULB & UCL
- Entreprises: Baxter and SMI

Fundings

- FNRS – Télévie
- FRSM-FNRS
- Région Wallonnie, Biowin
- Fondation Saint-Luc
- Fondation belge contre le cancer

Main Equipment

- Programmable freezers
- Facilities for cell and follicle culture
- Facilities for cryopreservation of isolated cells and tissue

Product and Services

- Scaffold for human ovarian follicle grafting

Key words for R&D

- Cryopreservation
- Vitrification
- Transplantation
- Fertility preservation
- Post-chemotherapy
- Follicle isolation
- Artificial ovary
- Scaffold
- Ovarian tissue

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Endometriosis is one of the most frequently encountered benign gynecological diseases. It is now well established that three different types of endometriosis must be considered in the pelvis: peritoneal endometriosis, ovarian endometriosis and deep endometriotic nodules of the rectovaginal septum. Rectovaginal endometriotic nodules are strongly associated with pelvic pain and dysmenorrhea in 95% of cases, rectal dyschezia in 25% of cases, and infertility. They are characterized by nodular aggregates of smooth muscle cells, with islets or strands of endometrial-type stroma and glandular epithelium. Most deep endometriotic nodules originate from the posterior part of the cervix (types II & III) and secondarily infiltrate the anterior wall of the rectum (type III). Surgery is the gold standard for the treatment of rectovaginal endometriotic nodules, but treatment has so far proved relatively ineffective, highlighting the importance of developing new treatment strategies.

In order to gain further insights into the etiology of nodules and identify potential therapeutic targets, we investigated vascularization, expression of developmentally regulated HOXA genes, and more recently, steroidogenesis and neurogenesis in nodules collected from patients.

Our study demonstrated relatively low nerve fiber density in peritoneal and ovarian lesions, while it was significantly higher in deep nodular lesions, as well as their direct environment. To characterize nerve fibers detected, we investigated the presence of NF protein and found that in lesions, around 30% of PGP9.5-positive nerve fibers were NF-positive, and hence myelinated. Nerve fibers in lesions were therefore mostly unmyelinated, and possibly implicated in pain. While glandular nerve growth factor expression levels were in the same range as in other lesion types, stromal expression levels were found to be higher in case of deep-infiltrating lesions.

However, as the time from the onset of endometriosis to its diagnosis has been evaluated to be 8-11 years, a model was clearly needed to study its origin and early development. Our group recently developed the first experimental model for induction of nodular endometriosis, obtaining a 100% induction rate. Microscopic results reveal that nodular lesions induced after grafting specimens containing the junctional zone (JZ) are statistically significantly larger, have greater glandular density, and can invade surrounding organs in more than 40% of cases. These data clearly underline the importance of the endometrium-myometrium interface (JZ). While the baboon model has mainly been used for the study of peritoneal endometriosis, our new model allows investigation of deeper nodular lesions, as well as invasion phenomena associated with nodular lesions. Further studies will focus on the involvement of the JZ and its ability to induce deep lesions in this animal model by invasion of surrounding organs by nodular endometriosis.

SELECTED PUBLICATIONS


Evaluation of the Mechanism of Action of Selective Progesterone Receptor Modulators in Leiomyoma Treatment

G. Courtoy, O. Donnez, M. Luyckx, J. Squifflet, MM. Dolmans

Uterine myomas are benign monoclonal hormone-sensitive smooth muscle tumors of the uterus. This is the most commonly found tumor of the female reproductive tract in premenopausal women and is mostly asymptomatic. When symptomatic, its main symptoms are heavy uterine bleeding, anemia, abdominal pain, urinary frequency and infertility. Alleviating patient symptoms prior to surgery is a key concern. Stopping or reducing bleeding, moderating pain and decreasing myoma and uterine size are considered to be beneficial to patient health and quality of life, and favorable in terms of surgical risk, offering the potential for less invasive surgery (e.g. vaginal rather than abdominal route for hysterectomy).

The main pharmacodynamic property of PGL4001 (ulipristal acetate) is to reversibly block progesterone receptors (PRs) in its target tissues (uterus, cervix, ovaries, hypothalamus) and act as a potent orally active and selective PR modulator (SPRM, Figure 2). PGL4001 has the potential to provide therapeutic effects similar to GnRH agonists (the most common drugs used for fibroid treatment) without reducing circulating estrogen levels to castration levels, hence significantly increasing safety and tolerance of the treatment. PGL4001 immediately stops uterine bleeding, while GnRH agonists produce an initial flare-up leading to an additional episode of bleeding that can sometimes be heavy. PGL4001 also has the advantage of being an orally active compound, whereas GnRH agonists have to be injected intramuscularly or subcutaneously.

Given the promising pharmacological and pharmacodynamic profile of PGL4001, the compound was tested as a treatment for the management of symptoms caused by uterine myomas prior to surgery. In a phase III study, it was recently demonstrated that PGL4001 treatment for 13 weeks effectively controlled excessive bleeding due to uterine fibroids and reduced the size of fibroids [1]. Moreover, the 5mg and 10mg daily doses of PGL4001 were not inferior to once-monthly leuprolide acetate in controlling uterine bleeding, and were significantly less likely to cause hot flashes [2].

The mechanisms leading to these observations are, however, unknown and our current research is focused on their identification. We are particularly investigating the ability of PGL4001 to inhibit proliferation and induce apoptosis of leiomyoma cells, as well as their impact on PR expression (and known PR co-regulators), on fibrosis and on vascularization of myomas.

Figure 1 Activation of the progesterone receptor (PR) by progesterone receptor ligands. (A) Binding of progesterone to the inactive receptor complex induces a conformational change, which leads to receptor dimerization, DNA binding, and recruitment of co-activators. (B) PR can also interact with transcription co-repressors. This usually occurs in the presence of an antagonist ligand, resulting in the absence of transcription activation. The interaction and recruitment of co-regulators, as well as post-translation modifications of PR and its co-regulators, appear to be the main factors determining agonist or antagonist activity.

Selected Publications


**ANDROLOGY**

Due to remarkable advances in cancer therapies, we have seen great improvements in survival rates of pediatric and reproductive-age male patients. Unfortunately, fertility in adult life might be severely impaired by these treatments. Gonadotoxic therapy is also used to cure a variety of non-malignant disorders such as hemoglobinopathies, aplastic anemia, autoimmune diseases, resulting in a growing population affected by fertility-threatening therapies. Knowledge and understanding of fertility preservation and restoration approaches therefore merits broader diffusion in clinical practice.

**Our research focuses on three main axes:**

> **1** Optimization of fertility preservation methods for prepubertal boys by cryopreservation of immature testicular tissue.

> **2** Development of fertility restoration techniques from cryopreserved immature testicular tissue by autotransplantation and in vitro maturation

> **3** Development of fertility restoration techniques from alternative stem cell sources, i.e induced pluripotent stem cells

**A) Fertility preservation and restoration from cryopreserved immature testicular tissue (ITT)**

*J. Poels, C. Wyns*

We developed a slow-freezing protocol for prepubertal human testicular tissue that has yielded good structural integrity of cells and tissue after evaluation in an in vivo xenotransplantation model.

Consequently, indications for spermatogonial stem cells (SSC) banking were established and banking of ITT from prepubertal boys undergoing gonadotoxic treatments was started.

Further evaluation of the functional capacity of cryopreserved human ITT after long-term xenografting was subsequently performed. Although seminiferous tubule integrity and ability of spermatogonial cells to proliferate were well preserved, complete normal spermatogenic differentiation could not be achieved, as spermatids were slightly smaller than in situ controls and spermatozoon-like cells with small heads and short tails were observed. In addition, a high proportion of spermatogonial cells were lost. Studies aimed at optimization of cryopreservation protocols were therefore conducted. The potential of vitrification (a technique preventing ice crystal formation by use of high concentrations of cryoprotectants and ultrafast cooling velocity, which could minimize cellular damage) was evaluated. Vitrification of non-human primate ITT allowed survival of spermatogonia able to proliferate and functional Leydig cells. Moreover, in humans, integrity of seminiferous tubules and survival and proliferation of spermatogonia in long-term organotypic culture were observed, showing vitrification to be a promising alternative strategy to slow-freezing in the emerging field of ITT cryopreservation. Unexpectedly, our comparative studies of cryopreservation methods in our in vivo xenotransplantation model led to the conclusion that the grafting method and the transplantation environment were at least partially responsible for the spermatogonial cell loss and their incomplete differentiation, stressing on the urgent need to develop a robust controlled environment for transplanted tissue before considering autotransplantation of cryopreserved ITT to our patients.

Successful fertility restoration with frozen-thawed ITT in humans has not yet been reported. Our current research focuses on two different fertility restoration strategies from cryopreserved ITT:

> **1** autotransplantation of the stored tissue for patients in whom there is no risk of contamination of the tissue by cancer cells

> **2** in vitro maturation of the SSC contained in the stored tissue yielding in vitro-derived male haploid gametes available for ICSI. This procedure circumvents the risk of reintroducing malignant cells, making this approach potentially highly beneficial in cancer patients.

**B) Characterization and differentiation of primordial germ cells from induced pluripotent stem cells to restore fertility in patients with cancer rendered sterile by radio- and/or chemotherapy**

*O Botman, C Wyns*

For those patients who could not benefit from prior cryopreservation of their spermatozoa or immature germ cells, use of alternative stem cell sources, i.e. induced pluripotent stem cells (iPSCs) capable of differentiating into male gametes, might be a potential strategy to allow these patients to become parents.
Indeed, iPSCs have the capacity to differentiate into cells of the three embryonic layers. Since 2007, several laboratories have proved that somatic cells can be reprogrammed into iPSCs, after integration of a small set of transcription factors (Takahashi et al., 2007). More recently, postmeiotic cells were successfully obtained after in vitro differentiation of iPSCs (Eguizabal et al., 2011). Further assays will nevertheless be needed to assess the developmental and reproductive ability of these post-meiotic cells.

As a first step, we sought to create an iPSC strain from adult fibroblasts (fig 2). The integration of the vector into the specific cells, the expression of undifferentiation and pluripotency markers, AP, REX1, Oct3/4, SOX2 and SSEA4 and the capacity to form cells from the three germ layers confirmed the reprogramming of our fibroblasts into a pluripotent state. Moreover, we showed that our iPSCs express TEKT1, a spermatid specific marker. These encouraging results confirm the potential of our iPSCs to differentiate into putative germ cells. Further investigations will be necessary to isolate and enrich the germ cell population since embryoid bodies obtained after spontaneous differentiation of reprogrammed skin fibroblasts consist of a heterogeneous population of cells including both PSCs and differentiated cells from all lineages.

The objectives of this research project are to:

> 1 develop a method of propagation and in vitro maturation of germ stem cells derived from iPSCs to restore fertility in cancer survivors.

> 2 apply this reprogramming and culture technique to infertile patients in order to better understand the mechanisms of spermatogenesis.


SELECTED PUBLICATIONS


10 | Wyns C, Botman O. Induced pluripotent stem cell potential in medicine, specifically focused on reproductive medicine. Frontiers in Surgery. 2014;1(5)1-10.
IMAG is the medical imaging research group of the Université Catholique de Louvain originating from and embedded within the Radiology Department of the Cliniques Universitaires Saint-Luc. IMAG supports active research programs in Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and Ultrasound Imaging (US) in relying on state-of-the-art facilities and by getting involved together physicists, radiologists, MD residents, PhD students and staff technologists. By the diversity of expertise of its investigators, IMAG can rely on knowledge in several fields such as neuroimaging, abdominal and thoracic imaging, musculoskeletal imaging, pediatric imaging, women's imaging, vascular and interventional imaging, animal experimentation, physics, signal and image processing, and data mining. Research axes within IMAG are therefore numerous. Among these axes, a privileged area of research is the development of MRI as a non-invasive morphologic and functional imaging tool for the diagnosis, staging, treatment monitoring and follow-up of oncological patients. The mainlines adopted by IMAG can be summarized as follows:

1. To develop, optimize and translate advanced imaging technologies into clinical practice and patient care, and contribute to the future shape of radiological imaging.
2. To constitute an open technical platform, offering the opportunity to work with research groups within the UCL and beyond, and favor innovation in biomedical research.

Additional activities of IMAG include the participation in multicenter trials (with other universities, EORTC, pharmaceutical industry) and the collaboration on technological tests and optimization with major imaging companies (GE, Siemens, Philips). IMAG investigators also provide expert advice in the various fields of medical imaging techniques.
One step MRI for the staging of prostate cancer and assessment of metastatic response


This project has financial support from Télécie (PhD thesis).

In cancer patients, a precise and exhaustive tumoral staging is crucial in order to plan the adequate treatment. Magnetic Resonance Imaging (MRI) is a highly sensitive method for the early detection of bone metastasis as it is sensitive to the infiltration of the bone marrow by malignant cells before bone remodelling occurs. Whole-body (wb) MRI has been proven an efficient and cost-effective tool for the screening of bone metastasis in patients with breast or prostate cancer (PCa).

This technique represents an alternative to current diagnostic modalities, i.e. bone scintigraphy and targeted x-rays, which lack specificity and sensitivity for the detection of bone metastasis. More recently, the performance of wbMRI has been highlighted for the concurrent evaluation of bone and visceral – in particular nodal – metastases, in great part thanks to the development of Diffusion-weighted Imaging (DWI) that complement morphologic MRI sequences. In patients with high-risk PCa, wbMRI outperforms the current multimodality work-up used for the evaluation of bones and lymph nodes. wbMRI with DWI challenges PET-CT as the future imaging method of choice for the metastatic screening in many cancers, and for the assessment of response to treatment. Despite these technical advances, much remains to be done to optimize wbMRI protocols, to validate wbMRI as a single step modality for the staging of PCa (in a multicentric context especially) or to formally assess the value of wbMRI in treatment monitoring and follow-up of oncological patients.

Therefore, a first research project was initiated with the aims to demonstrate the fundamental feasibility of “One-step” TNM staging of high-risk PCa, then to optimize the wbMRI protocol in order to improve the detection and quantitative evaluation of lymph nodes and bone metastases from PCa [1, 2]. The validation of wbMRI as a single step modality for the staging of PCa, for the diagnosis of metastases and for their monitoring under therapy would be a major improvement of patient management in terms of adequation of treatment to the real stage of the disease, and in terms of time saving, convenience and minimization of irradiation. This would also represent a model of “One-step” staging transposable to other cancers.

Diagnostic performance of CT-arthrography and 1.5T MR-arthrography for the assessment of glenohumeral joint cartilage

P. Omoumi, J. Malghem, B. Vande Berg, F. Lecouvet

This project has financial support from the Fond de la Recherche Clinique (PhD thesis). This project is developed in collaboration with JE. Dubuc (UCL/SSS/MEDE/Orthopedy).

Magnetic Resonance Imaging (MRI) of the glenohumeral cartilage is challenging, because of the relative thinness of this cartilage, but also because of the configuration of the shoulder, away from the isocenter of most magnets, and from the coils. As a result, the diagnostic performance of MRI is only moderate. Magnetic Resonance Arthrography (MRA) and Computed Tomography Arthrography (CTA) may have the potential to improve the visualization of cartilage lesions. Both benefit from the intra-articular injection of contrast material, while CTA has the advantage of a high spatial resolution as well as a high contrast between the hypodense cartilage and hyperdense surrounding bone.
and contrast material. Both MRA and CTA have proven to be valuable diagnostic tools for the evaluation of internal derangement of the shoulder, particularly in the preoperative setting. Therefore, the aim of this study is to compare the diagnostic performance of CTA and MRA in assessing the entire glenohumeral cartilage, by prospectively acquiring examinations with the two techniques in the same series of patients, with arthroscopic findings taken as a reference.

The cartilage surface was divided in 18 anatomical areas and two musculoskeletal radiologists reviewed the data. It was observed that 46% < SeCTA < 82% and 89% < SpCTA < 96%, while 32% < SeMRA < 66% and 91% < SpMRA < 98%. Diagnostic performance of CTA was significantly better than MRA for both readers. In both techniques, inter-observer agreement for the evaluation of cartilage lesions was moderate while intra-observer agreement was almost perfect. This prospective study showed that the diagnostic performance in detecting glenohumeral cartilage lesions was moderate with both technique, but statistically better with CTA. Future investigations should evaluate the performance of 3.0T-MRA (with the use of newly developed gradient echo or spin echo based isotropic 3D sequences), balanced steady-state free precession (SSFP) imaging and of traction imaging in the detection of cartilage lesions of the shoulder.

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Femoroacetabular impingement morphotype and early osteoarthritis

P. Omoumi, F. Lecouvet, N. Michoux, J. Malghem, B. Vande Berg

This project has financial support from the Fond de la Recherche Clinique (PhD thesis).

Femoroacetabular impingement (FAI) has recently been described as a cause of labrochondral damage, which in turn could predispose to early hip osteoarthritis (OA). Surgery is considered by many as the treatment of choice to relieve symptoms, and in some cases, prevent the progression towards hip OA. The diagnosis and surgical indications are partly based on the evaluation of imaging findings, including many radiological, CT and MRI signs of FAI. However, recent studies have shown that the prevalence of these imaging signs can be quite high in young asymptomatic populations, suggesting that these findings can represent anatomic variants. The pathogenic role of FAI in hip OA has also been controverted by some authors.

Therefore, the following study aims at indirectly assessing whether FAI morphotype leads to early OA in asymptomatic patients in a cross-sectional study by testing both of the following hypotheses: i) the presence of FAI morphotype leads to early OA and, ii) the presence of FAI morphotype by itself does not lead to early OA. Two groups of asymptomatic individuals, adults below 40 (group 1) and adults above 60 years old (group 2), were compared. Patients with hip OA were excluded. It is generally accepted that FAI morphotype is acquired before adulthood. The prevalence of FAI should then remain constant over age and be similar in the two age groups. However, if FAI morphotype leads to early OA, patients with FAI in group 2 would have developed OA and would therefore be excluded. Thus, in their first hypothesis, the prevalence of FAI should be lower in group 2 compared to group 1. Conversely, in the second hypothesis, if FAI morphotype alone in asymptomatic patients does not lead to early OA, the prevalence of FAI morphotype should remain similar in the two age groups. Hypotheses were tested for different cutoff values of FAI signs as well as for the measurement values of these signs.
The statistical analysis of the data brings an indirect argument against a pathogenic role of FAI morphotype alone in the development of early hip OA in asymptomatic patients. However, other associated factors such as activity level, genetics and environmental ones may contribute to the development of OA in a subset of patients with FAI morphotype. Future longitudinal studies focusing on the pathogenic role of FAI in both symptomatic and asymptomatic subjects should include the evaluation of these factors.

More recently Off-Resonance Suppression (ORS), using different gradient amplitudes for excitation and refocusing, has been proposed to provide well-defined spatial-spectral selectivity in SEMAC and MAVRIC to allow scan-time reduction and flexibility of scan orientation.

The aim of this study is to compare high-bandwidth multi-slice TSE, ORS-MAVRIC and ORS-SEMAC sequences in prosthetic hip joint, for coronal T2 and STIR acquisitions. A qualitative analysis is provided, based on the radiologist’s ability to detect relevant anatomical and pathological structures, taking CT arthrography, surgery and panel review of images as reference standard to define hip status. Also, the extent of residual metallic artifact is used as a quantitative measure of image quality. The model of prosthetic hips undergoing CT arthrography and further surgery (gold standard) is chosen.

Metal artifact reduction in MR Imaging of prosthetic hips: comparison of high-bandwidth TSE, SEMAC and MAVRIC with off-resonance suppression

P. Omoumi, F. Lecouvet

This project is developed in collaboration with O. Comu and M. Vancauter (UCL/SSS/MEDE/Orthopedy), and V. Denolin (Philips Medical systems).

Though Magnetic Resonance Imaging (MRI) offers very good visualization of soft tissues (also in native hips) and uses non-ionizing radiation, it is limited by metal, which reduces the potential role of MRI in important preoperative evaluation (fluid, synovitis, osteolysis, tendon abnormalities). Several techniques have been developed to reduce artefacts due to metallic implants in MRI. View Angle Tilting has been introduced as a way to correct for in-plane distortions, followed by Multi-Spectral Imaging (MSI) techniques aimed at resolving through plane signal displacements: slice encoding for metal artifact reduction (SEMAC), multi-acquisition with variable resonances image combination (MAVRIC) and hybrid techniques such as volume selective 3D multispectral imaging (VS-3D-MSI), or SVATSPACE.

Improving the tracer kinetic modelling in dynamic contrast-enhanced MRI

N. Michoux

This project is developed in collaboration with E. Lefrançois (UMR 6253, Université de Technologie de Compiègne, France).

Mass transport in tumors depends on the physicochemical and geometrical characteristics of the vascular and extravascular spaces as well as on the hydrodynamic characteristics of the flow, this latter exhibiting coupling with tissue structure. Transport itself is regulated by advection and diffusion. To reduce the computational complexity of the modelling problem, advection is omitted in perfusion MRI, a quantitative imaging technique recommended to evaluate tumor angiogenesis and monitor the therapeutic response under treatment. Simple compartmental models with diffusion as the prevailing mechanism of mass transport are thus used. A consequence is that mass transport based on pressure gradients and the role of interstitial fluid pressure is not assessable formally. A more exhaustive model which includes advection is thus implemented. Mathematically, the solution of the model results from a finite element approach that consists to rewrite equation (1) in a set of algebraic relations, applied on a mesh
made of elementary segments. The originality of the approach is to assimilate each segment to a dedicated finite element in order to benefit from a well-known numerical implementation. The set of segments is a mesh composed of \( n \) nodes and \( e \) elements (connections). The global process may then be decomposed on three phases.

Phase 1, the pressure on each node of the mesh is calculated based on mass flow conservation. Phase 2, the velocity distribution is deduced from the nodal pressure by solving Darcy’s law. Phase 3, the nodal concentration \( C \) is calculated from an implicit temporal resolution. The MRI data set used to test the model was obtained from a patient with a squamous cell carcinoma (SCC) of the head and neck region.

A transversal T1-weighted spoiled 3D gradient echo sequence with GD-DTPA injection was performed at 3T. During the curve fitting, the following parameters were optimized: the length of each element, the hydraulic conductivity surface area product of each element, the molecular diffusion and the source/sink term. It is observed that the model fits the perfusion curve reasonably well, suggesting that the transport of the contrast media within neoplastic tissues can be described by advection and diffusion mechanisms [3]. Theoretical work continues to dimension the model more accurately and adapt the model to various types of tumors. It is hoped that the additional parameters included in the model will constitute pertinent imaging biomarkers for the diagnosis, staging, treatment monitoring and follow-up of oncological patients.

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**Predicting the response to neoadjuvant chemotherapy in patients with breast cancer**

*N. Michoux, L. Fellah, I. Leconte*

Neoadjuvant chemotherapy (NAC) has a major role in the treatment of breast cancer. However, the rate of response to NAC is limited and dependent of the subtypes of cancer. The identification of non-responding patients is important, especially as it may allow considering alternative therapeutic options. The predictive value of Magnetic Resonance Imaging (MRI) and in particular of Dynamic Contrast-Enhanced MRI (perfusion MRI) has been reported. However, most of these studies were performed after the first courses of NAC. Few studies reported that certain pre-NAC semi-quantitative perfusion parameters were significantly different in chemo-sensitive and chemo-resistant breast lesions and may contribute to the prediction of disease free survival and overall survival. Besides functional imaging, quantitative post-processing of MR images such as visual texture analysis has also been reported to be useful for the characterization of cancerous breast lesions.

Therefore, a predictive model of non-response to NAC combining visual texture, perfusion and BI-RADS parameters measured from dynamic MRI is implemented and assessed. Sixty-nine patients with invasive breast ductal carcinoma who underwent pre-treatment MRI were studied. Pathological response was defined as the absence of invasive and in situ cancer in breast and nodes. Pathological non-responders, partial and complete responders were identified. Dynamic imaging was performed at 1.5T with a 3D axial T1W GRE fat-suppressed sequence. Visual texture, kinetic and BI-RADS parameters were measured in each lesion. ROC analysis and leave-one-out cross-validation were used to assess the performance of individual parameters, then the performance of multi-parametric models in predicting non-response to NAC.

A model based on four pre-NAC parameters (inverse difference moment, GLN, LRHGE, wash-in) and k-means clustering as statistical classifier identified non-responders with 84% sensitivity. BI-RADS mass/non-mass pattern, biological markers and histologic grade did not contribute significantly to the prediction. This pilot
study shows that pre-NAC texture and kinetic parameters help predicting non-benefit to NAC [4]. Further testing including larger groups of patients with different tumor subtypes are ongoing to improve the generalization properties and validate the performance of the predictive model.

Comparison between Contrast Enhanced Spectral Mammography (CESM) and Magnetic Resonance Imaging (MRI)

I. Leconte, M. Teodorescu, L. Fellah, N. Michoux

This project aims at comparing a new imaging technique - Contrast Enhanced Spectral Mammography (CESM) - to Contrast-Enhanced Magnetic Resonance Imaging (CE-MRI) in the assessment of tumor size in patients with breast cancer. This comparison is motivated by the fact that breast CE-MRI (the most sensitive imaging modality available today) is often limited by availability, expensive, long to perform and uncomfortable for the patient.

Though CESM implies an increase in breast radiation dose, this technique, in addition to its practicality, may provide an accurate preoperative staging and treatment planning in breast cancer patients if its performance in terms of lesion detection and tumor size measurement is demonstrated. Therefore, a first assessment was undertaken. During twelve months, 22 patients with 30 breast tumors (29 malignant, 1 benign) underwent CESM and CE-MRI before surgery. Images were read twice by two senior radiologists. Inter-rater agreement and inter-technique agreement (mean measurement from both raters) with regard to the size of the lesions were assessed based on the concordance coefficient $pc$ (product of the Pearson correlation coefficient $\rho$ measuring the precision with a correction factor $Cb$ measuring the accuracy of the measurements) and its 95% confidence interval [ref]. Inter-rater agreement was excellent for both modality (MRI: $pc = 0.995$ [0.989 – 0.997], $\rho = 0.996$, $Cb = 0.999$. AM: $pc = 0.997$ [0.994 – 0.999], $\rho = 0.998$, $Cb = 0.999$). Inter-technique agreement was also found to be excellent between AM and histopathology ($pc = 0.941$ [0.898 – 0.966], $\rho = 0.968$, $Cb = 0.972$) as well as slightly superior to the one observed between MRI and histopathology ($pc = 0.846$ [0.785 – 0.890], $\rho = 0.966$, $Cb = 0.875$). Both CESM and MRI offer reliable means of evaluation of tumor size with an excellent reproducibility of measurements. CESM is at least as efficient as MRI for evaluating tumor size [5].

Improving MR imaging criteria for assessing the inflammatory activity in multiple sclerosis

N. Michoux, T. Duprez

This project is developed in collaboration with A. Guillet (UCL/SSH/IMAQ/SMCS).

Multiple Sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system implying several complex mechanisms: disruption of axonal structures, with and without the presence of concomitant myelin pathology, inflammation resulting in vasogenic edema and accumulation of scar tissue. Despite the sensitivity of Magnetic Resonance Imaging (MRI) in the detection of white matter lesions, inflammatory activity in MS patients cannot be identified without gadolinium injection. Inflammatory activity becomes detectable due to the leakage of the gadolinium from the vascular space resulting in a T1 shortening effect in the area of the inflammatory activity that appears hyperintense on contrast-enhanced T1-weighted (CE-T1) images.

Besides imaging, quantitative post-processing of MR images such as visual texture analysis has been reported to be useful in issues such as the detection of areas (core, rim) with different behaviours within lesion undergoing demyelination or the search for surrogate markers of lesion load and tissue integrity in MS. Therefore, a model assessing the inflammatory activity of MS lesions based on visual
texture analysis of T2-weighted MR images is thus implemented and assessed. Twenty one patients with MS were imaged at 3.0T using T2-weighted, FLAIR, diffusion-weighted imaging from which a mapping of the apparent diffusion coefficient (ADC) is derived, and CE-T1 MR sequences. Lesions and corresponding contra-lateral regions in the normal appearing white matter were segmented.

Each region of interest was characterized using textons computed from the gray level co-occurrence matrix and the run length matrix, and the ADC. ROC analysis and leave-one-out cross-validation were used to assess the performance of individual parameters, then the performance of multi-parametric models in identifying Gd-enhancing lesions. Using linear discriminant analysis as statistical classifier, a model identifying Gd-enhancing lesions using 8 textons and performing with Se = 70% and Sp = 76% was built. Using logistic regression, a model using 10 textons performed with both higher Se = 86% and Sp = 84%.

Using partial least squares, a more parsimonious model using six textons achieved the classification of MS lesions with the highest sensitivity (Se = 88%, Sp = 81%). Textons can be computed to detect changes in the T2-weighted MR appearance of the white matter. Such post-processing dedicated to MR images may contribute to a practical (without gadolinium injection) clinical diagnosis of MS patients and help monitoring the subtle changes of texture properties within the white matter as those occurring during disease progression/regression [6].

MRI of acute ischemic stroke patients: comparison of diffusion related parameters

G. Duchêne, F. Peeters, T. Duprez

MR diffusion-weighted imaging (DWI) has proven major relevance in acute ischemic stroke patients. In routine practice, only the Apparent Diffusion Coefficient (ADC) is measured. The mechanism explaining the decrease in ADC observed in the acute phase is still controversial, and the most commonly proposed model is that of a transfer of water molecules between the extra- and intra-cellular spaces. Therefore, analysis of the MR signal in terms of a bi-exponential decay, separating a fast from a slow diffusion compartment could be relevant.

Moreover, significant changes of fractional anisotropy over time have been described in stroke patients. Unfortunately, when restrictions or hindrance of the water molecules are present, these models cannot lead to an accurate description of diffusion changes because they assume a Gaussian probability density function (PDF) of the diffusion process. The PDF of the diffusion process can be directly measured using q-space imaging (QSI) which yields parameters reflecting the shape of the distribution (height, width, kurtosis, etc...) obviating inexact assumption of a Gaussian distribution of the diffusion. Therefore, different analyses (2-point ADC, bi-exponential fits, Diffusion Kurtosis Imaging (DKI) and QSI) were implemented and compared in a preliminary cohort of hyperacute (<6 hours) stroke patients [7,8].

Institut de Recherche Expérimentale et Clinique B5
**Estimation of cell sizes and distributions with double wave vector encoded diffusion magnetic resonance imaging and application to acute stroke and oncology patients**

G. Duchêne, F. Peeters, T. Duprez

Diffusion weighted magnetic resonance imaging (DW-MRI) has the potential of probing the microstructure of biological tissues. Standard DW-MRI methods are based on the pulsed gradient spin echo (PGSE) sequence and interpret data in terms of simple models such as a Gaussian (free) diffusion propagator. More adequate methods for restricted/hindered diffusion such as q-space imaging (QSI) are difficult to apply on clinical MR scanners. Moreover, QSI is relatively insensitive to the microstructure. A promising method is the double PGSE (or double wave vector encoded) diffusion sequence that has the potential of measuring cell sizes and distributions. Therefore, following project was undertaken with the financial support of the FSR. The double wave vector diffusion echo planar imaging sequence will be implemented on a clinical Philips Achieva 3T scanner (with powerful gradients of 80 mT/m). The sequence will be optimised for maximum image quality within clinically acceptable scan times. Simulations will be performed for simple cell geometries, ideal and realistic gradient durations in order to facilitate interpretation of the data.

Software will be developed that estimates parameter maps for cell sizes and distributions from the DW images. Then, the method will be applied on two patients’ cohort. On acute ischemic stroke patients, the aim will be to compare the sensitivity of the estimated parameters with those obtained from standard DW-MRI methods and investigate whether the method performs better in predicting ‘ischemic penumbra’. On oncology patients, the aim will be to assess the value of the method for quantifying the cells size/distribution (relevance for differential diagnosis) and early treatment response (relevance for early triage between responders and non-responders).

**Translational implementation of non-invasive Magnetic Resonance brain oxygen mapping in acute (72 hours) ischemic stroke patients**

M. Safronova, T. Duprez

This project has the financial support from the PLAN CANCER 027 SPF-SP (PhD thesis).
This project is developed in collaboration with B. Gallez (UCL/LDRI/REMA).

Mapping brain oxygenation is a key-challenge in the clinical work-up of many cerebral disorders. Measurements of proton T1 relaxation induced by paramagnetic molecular oxygen have previously demonstrated capability to monitor changes in tissue oxygenation. The MOBILE (Mapping of Oxygen by Imaging Lipid relaxation Enhancement) sequence increases the sensitivity of this measurement by selectively calculating the spin–lattice (T1) relaxation of lipids after suppressing water signal. We hereby investigated in a translational “proof of concept” way whether MOBILE enabled detection of brain oxygen deprivation in the clinical setting of ischemic acute ischemic stroke patients. Therefore, 12 healthy volunteers, and nine acute stroke patients (48-72 hours) underwent a standardized protocol at 3T including sequences aimed at measuring global T1, lipids T1, and T2* in brain together with morphological and diffusion-weighted imaging.

ROIs contouring infarcted areas were drawn on DW images and overlaid on parametric global R1 (R1 = 1/T1), lipids R1, and R2* (R2* = 1/T2*) maps. Contralateral mirror-ROIs within unaffected brain tissue were generated. Comparison of the medians of the diseased areas versus those of the mirror ROIs was performed using paired t test. Histogram analysis of the ROIs was also performed and comparison of values within patients’ unaffected mirror-ROIs and within brain tissue of healthy volunteers was made. Selective measurements of lipids R1 demonstrated strongly higher statistical significance (median difference: 0.408 s⁻¹) than global R1 (median difference: 0.154 s⁻¹). Global and lipids R1 values within unaffected brain tissue of stroke patients were not different from those of healthy volunteers. Better sensitivity in oxygenation level mapping by selectively measuring the relaxation of the lipids protons was demonstrated in normal and acutely infarcted human brain [9].

Institut de Recherche Expérimentale et Clinique
MOBILE allows a follow-up of brain oxygen variations during a 100% O2 breathing

M. Safronova, T. Duprez

This project has the financial support from the PLAN CANCER 027 SPF-SP (PhD thesis).
This project is developed in collaboration with B. Gallez (UCL/LDRI/REMA).

The availability of an uninvasive andrepeatable quantitative technique for monitoring tumor oxygenation throughout therapeutic course should be of major impact on the therapeutic strategy. The aim of this study was to assess the applicability of MR-based MOBILE (Mapping of Oxygen By Imaging Lipids relaxation Enhancement) sequence for mapping oxygenation of primary central nervous system malignancies in a routine clinical setting.

Therefore, 27 patients with brain neuroepithelial tumors were examined on a clinical 3T MR system with morphological acquisitions together with sequences aimed at calculating global R1 (water + lipids), lipids R1 (MOBILE) and T2*. Regions of interest (ROIs)contouring contrast-enhanced tumor (C+), unenhanced tumor (C-), peritumoral edema, and normal appearing white matter (NAWM) areas were drawn on post-contrast FLAIR and T1-weighted images. Means of the global R1, lipids R1 and R2* values were compared between the sub-areas and to normal brain parenchyma of 17 healthy volunteers using paired t test. Both global R1 and lipids R1 values were significantly lower within tumoral areas thereby reflecting lower oxygenation levels. Lipids R1 allowed additional differentiation between tumor and peritumoral edema. None measurement showed significant difference between C+ and C- tumor areas. Global R1 and lipids R1 measurements allowed estimation of tumor hypoxia within primary brain tumors, with the added value of the latter to enable differentiation between tumor and peritumoral edema [10].

Feasibility of a volumetric measurement of the left liver lobe with 3D ultrasound compared with MRI

E. Danse, N. Michoux

This project is developed in collaboration with R. Reding (UCL/SSS/IREC/CHEX).

The measurement of the functional liver volumes of donor and recipient is a critical part of preoperative evaluation in adult living donor liver transplantation. Volumetric measurements need to be accurate to reduce surgical complications. The aim of this project is to assess the feasibility of the volumetric estimation of the left liver lobe with 3D ultrasound (3D-US) compared to Magnetic Resonance Imaging (MRI). This comparison is motivated by the fact that MRI (the reference method) is often limited by availability and expensive compared to liver volumetric measurements performed with 3D-US device.

Moreover, errors associated to volumetry made with MRI and dedicated image processing software, as well as the repeatability and reproducibility of the measurements are not well evaluated. Discrepancies between intraoperative graft volume and MRI preoperative assessment have been reported, justifying further evaluation of MRI as well as of alternative imaging technique such as 3D-US. Thus, the data of 19 consecutive patients referred for giving their left liver lobe to a child were reviewed (living donor transplantation program). The measurements of the 3D volumes of the segments II and III were performed retrospectively with comparison of the same evaluation performed with MRI (the reference and routine technique for the preoperative assessment of the liver size). All the examinations were done
twice by 2 experts radiologists and radiologists in training. Then, 3D-US and MRI measurements were compared and related to surgically proved volume quantification. Gage R&R analysis shows that repeatability and reproducibility are both above 25ml (up to 54ml) whatever the technique/observer considered. Compared to MRI, the repeatability of 3D-US is found to be higher and the reproducibility is found to be lower in 3D US. Bland-Altman analysis reveals a mean underestimation (bias) of volume measurement of 18ml using 3D-US compared to MRI (interval of agreement ranging from -110ml to +74ml). From this preliminary assessment, it appears that correct estimation of the left liver lobe volume performed with 3D-US or MRI is related to a low repeatability and a low reproducibility for both techniques, suggesting that the post-processing of image data as well as the training of the operators need to be improved [11].

Ultrasound in the diagnosis of pneumonia in children

D. Dumitriu, A-S. Claes, R. Menten, P. Clapuyt

The aim of this project is to determine the performance of ultrasound in the detection of alveolar condensation in children with acute respiratory infection, in comparison to the gold standard technique, chest x-ray. Between October 2013 and February 2014, 127 patients aged between 3 months and 14 years sent by the pediatrician for a chest x-ray with suspicion of pneumonia were examined. A frontal chest x-ray was followed by a thoracic ultrasound, performed by a different radiologist, blinded to the chest x-ray. Among the 127 pairs of exams, 118 agreements in detection and 9 disagreements were recorded, with 1 false negative and 8 false positives of ultrasound, as compared to the chest x-ray. The agreement between the two methods was calculated by Scott’s pi at 0.837.

The sensitivity of ultrasound was calculated at 97.3%, with a specificity of 91.1%, PPV = 81.8%, NPV = 98.8%. Ultrasound is a very accurate technique in the detection of pneumonia in children, without the use of ionizing radiation. Given its great negative predictive value, ultrasound is especially useful to exclude pneumonia and could reduce the number of x-rays in children in the future.

Toward an optimized abdominal organ enhancement in multi-detector rows Computed Tomography scanner

E. Coche, N. Michoux

This project is developed in collaboration with A. Vlassenbroek (Philips Medical System) and C. Kemper (Global Medical and Clinical Affairs Radiology, Bayer HealthCare).

Administration of intravenous contrast agents in multi-detector rows CT (MDCT) requires integration of many different parameters including the volume and the concentration of contrast agent, the rate of injection, the patient’s weight, the time of CT acquisition, the kVp settings, the cardiac output, and the site of injection. All these factors influence the quality of both the vascular and parenchymal enhancement and are sometimes difficult to control in daily routine practice because of their complexity and their possible mutual interactions.

The introduction of commercial software solutions (SyncRight) that enable the individual calculation of patient- and procedure-specific injection protocol parameters directly at the point of care within the existing CT scanning workflow represents an important step forward for the management of patients in CT. Therefore the comparison of abdominal organ enhancement at MDCT using a routine standard injection protocol and using a CT Scanner integrated injector system that automates the use of weight-based injection software (P3T) was undertaken. 82 oncologic patients were enrolled in this study and divided into two equal cohorts.

The first cohort was injected with the standard injection protocol: 120mL of Iobitridol 350 injected at a rate of 2.5mLs-1 with a delay of
70s. The second cohort underwent an automatic customized weight-based injection protocol (SyncRight) using Iobitridol 350 using the following injection protocol: 1.51mL Iodine/kg injected at a rate of 0.031mL Iodine.kg-1.s-1 with a delay of 70s. CT acquisition parameters were 64x0.625mm (Brilliance 64), 3 mm slice thickness reconstruction, 120kVp, mAs (depending on body habitus). Regions of interest were placed in the liver, spleen and renal cortex and Hounsfield Unit (HU) density was recorded. It was observed that enhancement in the liver and in the spleen, but not in the renal cortex, was significantly higher with SyncRight compared to the routine non weight-based injection protocol. Further investigation is ongoing to optimize injection protocols for abdominal CT examinations.

Low contrast detectability improvements in Computed Tomography images

D. Millon, N. Michoux, E. Coche

This project is developed in collaboration with A. Vlassenbroek (Philips Medical System).

Improvements in Computed Tomography (CT) scanner technology has led the radiology community to pay attention to radiation exposure from CT examinations according to ALARA (As Low As Reasonably Achievable). In order to deal with this major public health challenge, CT companies have introduced CT iterative reconstruction (IR) techniques. This technology maintains image quality at reduced dose using reconstruction time consistent with daily-life CT workflow. The aim of this study is to evaluate and compare the low contrast detectability of Multi Detector Computed Tomography (MDCT) images reconstructed with an iterative reconstruction algorithm and with a standard filtered back projection algorithm both in standard and high resolution. The phantom study was performed on a 256-slice MDCT (Philips).

A Catphan phantom (The Phantom Laboratory) containing a low contrast module was imaged with decreasing dose (48.8mGy down to 0.7mGy) and parameters typical of a chest examination: 3-mm-thick «smooth» images optimal for the mediastinum visualization and 1-mm-thick «sharp» images optimal for the lung parenchyma, were reconstructed both with standard filtered back projection and iterative reconstruction algorithm (IMR, Philips Healthcare). Scan parameters were 120kVp and mAs ranging from 10 up to 720mAs. Three independent raters counted the number of low contrast objects visible on each reconstructed dataset. It was observed that the proportion of detected objects was significantly higher (from 11% up to 23%) whatever the slice thickness or rater considered, supporting the iterative reconstruction technique. Though further testing is needed, these preliminary results show that iterative reconstructions improve the image quality of MDCT images and enable to limit the number of reconstructed series to a single sharp-thin slice serie with improved low contrast detectability.
EQUIPMENT

- 3 clinical MRI systems (two 3.0T and one 1.5T)
- 1 clinical MRI system (3.0T) with surgical suite for intraoperative cerebral and spinal studies
- Several clinical multislice CT scanners (40, 64 and 256-slice configurations, single-source)
- Several digital mammography systems (one based on Contrast Enhanced Spectral Mammography)
- Several Ultrasound systems (UI22 Philips, Toshiba Aplio, Supersonic Aixplorer)
- Transient Elastography (FibroScan), Elastography Point Quantification (ElastPQ), Shearwave elastography (Supersonic)
- Equipment for animal study (rodent anesthesia module, biosafety cabinet, stereo microscope, surgical instruments)
- Software solutions for numerical MRI simulations, scientific programming, image processing and visualization, statistics

SELECTED PUBLICATIONS


The Louvain Centre for Toxicology and Applied Pharmacology (LTAP) was established in 2009 to conduct advanced research on the effects of xenobiotics on human health. It addresses both intended (applied pharmacology) and adverse health effects of chemicals (toxicology).

The centre integrates interdisciplinary expertise (from clinical, to analytical, experimental, and epidemiological sciences) and is actively engaged in commissioned projects and research for international, European, national and regional government bodies, major companies, trade associations, etc.
Therapeutic drug monitoring: A multidisciplinary approach to reach personalized medicine

V. Guy-Viterbo, MT Thi Nguyen, F. Musuamba, A. Capron, P. Wallemacq

Therapeutic drug monitoring is a fast growing field, which involves not only analytical expertise but also pharmacokinetics (population pharmacokinetics, modelisation, pharmacogenetics), pharmacodynamics (PD biomarkers), and the evaluation of disease evolution, aiming at optimizing and personalizing drug dosage regimens. “Pharmacometrics”, is the term referring to this emerging area.

Our institution played a pioneering role in this discipline more than 30 years ago, and our group is maintaining this leading position by contributing to research efforts to optimize immunosuppressants and antibiotics drug dosages and regimens in specific subpopulations (e.g. pediatric liver transplantation, septic patients). Also, our group has demonstrated the interest of measuring intracellular drug concentrations as better marker of drug exposure/efficacy, than blood or serum concentrations.

About 15 peer reviewed contributions were published in 2012, together with a couple of textbook chapters. Various members of this group participated actively in international congresses, workshops, scientific committees and roundtables. A collaboration has been initiated with the two largest French paediatric transplantation centres (Bicêtre and Necker) to identify the evolution of the main drug-disposition covariates during the first years of age. Another long-term collaboration has been reinforced with the University Hospital Erasme in Brussels in the field anti-infectious therapeutics.

We also develop a new approach based on dry blood spot testing which allows reduced blood volume sampling, and stable samples shipment. These advantages are explored in our group, in collaboration with a Brasilian center (supported by a FNRS/CNPq sponsoring).

Human pharmacogenomics and personalized medicine: Experimental and clinical studies on the influence of genetic polymorphisms on drug metabolism and transport.

G. Dessilly, L. Belkhir, L. ELens, V. Haufroid

The development of new active substances is a continuous source of progress in pharmacotherapy. However, the search for an optimal use of existing molecules constitutes another avenue of improvement. In conventional medicine, a common practice is to recommend a single dosage regimen determined as an average in a given population, assuming that patients respond in a similar way on drug therapy. However, inter-individual variations exist in drug response, making adverse drug reactions and treatment failure major issues in daily clinical practice. Pharmacogenomics uses genomics information to predict response to therapy and might certainly contribute to improve the concept of personalized medicine. The promise of pharmacogenomics is that both the choice of a drug and its dose will be determined based on the genetic make-up of a given patient.

Our group has been actively involved in the pharmacogenomics of immunosuppressive drugs in solid organ transplantation for more than 10 years proposing, for instance, internationally recognised dosing guidelines for tacrolimus therapy based on CYP3A5 genotype. (Haufroid et al. Am J Transplant. 2006). From January 2011 until October 2013, a clinical study (CYRANO study) on 150 new patients undergoing kidney transplantation and treated by tacrolimus using our CYP3A5-based dosing recommendation was initiated. Preliminary results were presented in 2013 at two international congresses (American Society of Transplant Surgeons and American Society of Transplantation, Seattle, USA, May 18-22, 2013 and 16th Congress of the European Society for Organ Transplantation (ESOT), Vienna, Austria, September 8-11, 2013). The publication of the first follow-up of the CYRANO study is in progress.

International collaborations have also been reinforced, more particularly with the Erasmus Medical Centre Rotterdam (Dr R.H.N. van Schaik) where Laure Elens completed a post-doctoral
fellowship and investigated pharmacogenetic determinants of drug response in several cohorts of patients. Pharmacogenomics research has been continued on two others classes of drugs with a low therapeutic index: antiretrovirals (funded by Saint-Luc Foundation, doctoral thesis of Leïla Belkhir) and anticancer agents, more particularly tyrosine kinase inhibitors (funded by Télévie, FNRS and FSR, doctoral thesis of Géraldine Dessilly).

In parallel with those clinical studies, experimental approaches on modified cell lines (including selected SNPs of interest) are used to more deeply explore clinically relevant associations observed in vivo (ABCB1, ABCC1, ABCC2,...). In particular, we showed that ABCB1 1199G>A genetic polymorphism (rs2229109) influences the intracellular accumulation of tacrolimus in HEK293 and K562 recombinant cell lines (G. Dessilly et al. 2014, PLOS One) confirming previous associations observed in vivo by our group and opening the way to new clinical applications.

HEK293 cells transfected with ABCB11199G and stained with anti-ABCB1 and analyzed by microscopy (left) or FACS (right). Dessilly et al., 2014.

Cystic Fibrosis therapy: “From bench to bed”

S. Noel, B. Dhooghe, T. Leal

We have used for the last 10 years genetically modified mice under the scope of translational research exploring cystic fibrosis (CF) lung pathology and tracheal dysmorphogenesis, testing fundamental therapeutic strategies and characterizing imbalanced fatty acid metabolism in CF. Our F508del breeding colony has the major advantage of harbouring a specific clinically relevant mutation found in at least 85% of CF patients. Additionally, the F508del mouse model displays several advantages: survival to maturity is almost completely preserved (~95%) and mRNA levels are preserved in all relevant target organs and tissues. The model has proven to be extremely valuable as a human-equivalent disease model.

Thanks to this mouse model, we have shed some additional light on the pathophysiology of lung inflammation in CF by showing that a pro/anti-inflammatory imbalance is evident in CF under naive, non-stimulated conditions, even in the absence of any detectable infection. We also have shown that inflammatory responses are exaggerated in CF. Our work identified that CF lung inflammation is a complex process involving the contribution of distinct cell types. Macrophages play a key role in the inflammatory process in CF: infiltrated alveolar and peritoneal macrophages are more numerous in CF as compared to wild-type mice and the macrophage-related chemokine, CCL-2/MCP-1, is about 14-fold more abundant in the alveolar space of mutant mice. We are interested in demonstrating that the biology of CF macrophages is dysregulated and that CFTR mutations may alter in particular the pro/anti-inflammatory balance. This project is financially supported by the French cystic fibrosis foundation Vaincre la mucoviscidose.

Another current topic of research is the potentiality of phosphodiesterase type 5 inhibitors (PDE5i), a class of drugs initially approved for improving erectile dysfunction, as a fundamental therapy for CF. When applied to F508del-CF mice at clinical doses, by intraperitoneal injection or by local deposition at the nasal mucosa, vardenafil corrects CFTR-dependent chloride transport, which is abolished or blunted in CF (Lubamba et al., 2011). The effect was identified by means of the nasal potential difference, an extremely delicate diagnostic test that has been also used to evaluate therapeutic efficacy of potential drugs during clinical trials. We have acquired great expertise for this test both in mice and in patients. With the same topic line, we have shown that vardenafil is able to attenuate inflammatory responses in CF mice (Lubamba et al., 2012). Thanks to collaboration with the Cell Imaging platform of the IREC, we have shown that vardenafil increases the expression of F508del-CFTR protein to the apical membrane of epithelial cells from mouse colon (Dhooghe et al., 2013). Because vardenafil and analogs are in clinical use for other clinical applications, research on this class of drugs might speed up the development of new therapies for CF. We are currently receiving funding from FRIA (doctoral fellowship for B. Dhooghe) to investigate the mode of action of vardenafil on F508del-CFTR to particularly determine if vardenafil promotes proper folding of the mutant protein.
Evaluation of health hazards and risks of chemical substances

P. Hoet, G. Van Maele-Fabry

Our focus is the human health hazards and risks of chemicals, and we develop exposure and health monitoring programmes for human health risks associated with industrial and environmental chemicals. Two complementary approaches are applied: (1) conducting epidemiological studies in occupational or environmental settings and (2) assessing existing data through critical reviews, systematic reviews and meta-analyses. We recently conducted studies on the biomonitoring of trace elements (TEs), indium (In) and manganese (Mn) exposure, cadmium (Cd) nephrotoxicity in acute liver disease patients and conducted systematic reviews on the epidemiological associations between pesticide exposure and cancer.

TEs are ubiquitous and concerns about their potential impacts on human health have been constantly growing. Biological monitoring is generally considered a useful tool to assess human exposure to chemicals for risk assessment both at occupational and environmental levels. However, the determination of accurate reference values, which may vary across countries or regions, is a prerequisite for a correct interpretation of biomonitoring data and these were not available in Belgium. We have determined the reference distribution and the upper reference limits for 26 TEs (Al, As, Sb, Ba, Be, Bi, Cd, Cr, Co, Cu, In, Li, Mn, Hg, Mo, Ni, Pd, Pt, Pb, Se, Te, Ti, Sn, U, V, Zn) in the urine of 1022 adults from the general population residing in Belgium (Hoet et al., 2013).

Urinary levels are often adjusted to creatinine concentration to integrate the effects of fluid balance on spot samples. Hence, reference limits were established for both measured (µg/L) and creatinine-adjusted (µg/g creat) levels. Carrying out this adjustment systematically for all biomarkers is however questionable. The validity of creatinine adjustment is, among others, dependent on whether the TE is excreted in urine via the same pathway as creatinine, which for many of them is not established. This issue, along with correlations between unadjusted and creatinine-adjusted levels of TEs is currently investigated in our group.

Excessive occupational, iatrogenic or environmental exposure to cobalt (Co) has been associated with several adverse effects on a.o. the respiratory tract, hematopoietic system, thyroid gland, myocardium, central and peripheral nervous system. It has been shown that Co implants, in particular cobalt–chromium metal–metal (MoM) implants, could produce wear debris and increase blood and urinary Co concentrations. There is currently no data allowing recommending a threshold to prevent systemic toxicity. We have launched a study aiming at assessing, in a cohort of patients with hip implants, the levels of Co exposure and the possible relationship between exposure to Co and incipient or established signs of adverse effects on the thyroid, the red blood cells and the myocardium (in particular signs of dilated cardiomyopathy). This research aims at providing additional data to help determining the acceptable levels of endogenous exposure and possibly to warn patients of the risks associated with their implants.

A growing number of epidemiological and molecular studies have focused on the role of environmental factors in the onset of cancers and other diseases. Increasing attention has been given to pesticides used in agricultural, commercial, home and garden applications. Given the increasing body of publications on this issue, it was hard to keep track of the scientifically established knowledge about these important issues, and to consider measures based on sufficient evidence. It was our aim to enhance our understanding of the potential involvement of pesticide exposure on the induction and development of cancer. To this end, comprehensive systematic reviews and meta-analyses focusing on the association between occupational and/or residential pesticide exposures and several cancers (prostate, leukaemia and brain [in adults and/or in children]) were performed. These analyses provided refined estimates of the effects and thus more reliable results upon which to draw conclusion (Van Maele et al., 2013). Our group participated to an "expertise collective INSERM (Institut National de la Santé et de la Recherche Médicale)" on the relationship between pesticide exposure and health, recently published. The group also contributed to the presentation of an advisory report on "childhood leukaemia and environmental factors" within the framework of EuSANH (European Science Advisory Network for Health) (2012). Similar investigations were conducted for the alleged association between pesticides and Parkinson disease.
Development of non-invasive tests to detect effects of environmental pollutants: validation and application in human subjects

A. Bernard

Biomarkers of effect correspond to changes that are qualitatively or quantitatively predictive of health impairment or potential impairment resulting from exposure. They must be sensitive, specific, robust and measurable in a minimally invasive way. We are currently using such biomarkers to evaluate the health risks of exposure to various environmental stressors including cadmium, tobacco or wood smoke, fine particles and chlorination products. Our recent observations support the hypothesis that early age exposure to stressors linked to hygiene, especially chlorine-based oxidants, can cause airways epithelium defects promoting the development of allergic sensitization (Bernard et al., 2011). We also found evidence of biological interactions between tap water hardness and atopy in the development of childhood eczema (Chaumont et al., 2012). Observations among adults and adolescents also suggest that lifestyle stressors linked to some sports, including swimming in chlorinated pools, can cause detrimental effects on the testicular function.

The study of urinary cadmium levels in adolescents has revealed the existence of physiologically driven associations that call into question the paradigm currently used for assessing the health risks of environmental cadmium.

Experimental models of particle-induced lung inflammation and fibrosis: health benefits from basic discovery.

S. Lo Re, V Rabolli and F. Huaux

Our group has developed a specific expertise in the respiratory toxicity of micrometric- and nanometric-materials such as silica, asbestos and carbon nanotubes (CNT). Several experimental projects are currently running to further investigate the mode of action of toxic particles, with a particular interest in the fibrotic and carcinogenic activity of these materials. The main issue investigated is the role of immune processes in these reactions in the respiratory system. We have accumulated over recent years experimental evidence that immune suppressive mediators contribute to the development of particle-induced chronic diseases. We have newly discovered that IL-10-producing macrophages are key immunosuppressive cells implicated in the fibrotic responses to silica. We have also demonstrated that CD4+ Foxp3+ regulatory T cells (Treg) persistently recruited during long-term responses to particles highly express the growth factors PDGF-B and TGF-β1, directly stimulate fibroblast proliferation and increase lung collagen deposition. In addition, we have recently observed that besides Treg, Myeloid-Derived Suppressor Cells (MDSC), another immunosuppressive cell population, also accumulate in the lung of mice treated with particles. The main function of these cells is their ability to inhibit both the innate and adaptive immune responses, subverting immune surveillance. To examine the immunosuppressive responses over the whole course of the pathological process induced by fibers and particles, we have validated experimental models in mice and rats. These projects are funded by the following research grants: FNRS PDR T.0119.13 – Role of Myeloid Derived Suppressor Cells (MDSC) in the lung fibrosis induced by silica particles in mice. Fondation contre le Cancer 2012-219 - Implication of anti-inflammatory and immunosuppressive responses in the pathogenesis of asbestos-related mesothelioma. Umicore 2013 – Indium Tin oxide particles and Pulmonary Alveolar Proteinosis.
Nanotechnology is impacting on virtually all types of industrial and domestic products, and developments are expected to increase sharply in the coming years. The same unique physico-chemical properties that make nanomaterials (NM) so technologically attractive may also represent potential challenges to human health and the environment. There is, therefore, an immense demand for nanotoxicology evaluations, and our group has been involved in this effort for more than 10 years.

In 2013, we further investigated in vitro and in vivo the capacity of carbon nanotubes to stimulate the proliferation of fibroblasts (Vietti et al., 2013). The mechanisms of this effect are currently investigated at the cellular level, and the predictivity of the in vitro observations are evaluated in vivo in a mouse model.

Through a collaborative project funded by ANSES (France) involving scientists at CEREGE (Aix-en-Provence, Dr J. Rose) and CEA (Paris, Dr A. Thill) we have investigated the toxicological effects of a new class of Aluminogermanate nanotubes, named imogolites. These nanotubes have multiple potential industrial applications, including e.g. gas storage, insulation material sheathing, and the possible health risks of human exposure under occupational scenarios or indirectly via the release in the environment should be investigated. We have characterized the effect of these materials on the respiratory system using in vitro and in vivo (rat) models. Single walled (40x3.7 nm) and double walled (15x4.2 nm) imogolite nanotubes were found to induce inflammatory and fibrotic lung responses as well as genotoxic damage (micronuclei) in epithelial lung cells in vivo.

In view of some structural similarities with asbestos fibres, a serious concern exists about the potential of nanotubes to induce cancer (lung cancer and mesothelioma). The capacity of nanotubes (carbon or imogolites) to induce mesothelioma, is investigated by our group in rat models in a project funded by the Fonds de la Recherche scientifique médicale (FRSM) and by the Fondation contre le Cancer.

LTAP coordinated the NANO-IRIS project (in collaboration with profs Leyns L. and Kirsch-Volders M. at VUB) funded by the Brussels Capital Region (INNOVIRIS). This initiative aimed at establishing in Brussels a centre of expertise in nanotoxicology research to bridge the gaps between academic research and the practical needs of regulators and industry in terms of hazard and risk assessment. Several Standard Operating Procedures (SOP) for in vitro tests specifically adapted and internally validated for NM have been produced in this context, these include SOPs for assessing fibroblast proliferation, inflammasome activation in macrophages and NM dissolution.

### EQUIPMENT

- Forensic toxicology
- ICP-MS for the analysis of metallic elements in biological matrices (urine, blood, ...)
- Chromatography (GC,FPLC, GC-MS, HPLC, UPLC, LC-MS/MS)
- Genotyping and phenotyping of drug- metabolizing enzymes and transport proteins
- Modelisation and population pharmacokinetics (NONMEM, WinNonLin)
- Sensitive latex immunoassays for specific proteins in various matrices (rodent and human Clara cell protein and albumin, retinol-binding protein, beta2-microglobulin,...)
- Equipment for measuring exhaled nitric oxide, collecting EBC and NALF
- Automated samplers for immunoassays
- Equipment for protein purification
Transportable analyser for monitoring ozone in ambient air
Fully equipped and accredited facilities for in vivo toxicology studies
Cell culture, isolation and purification (MACS, FACS), in vitro toxicology
Molecular biology: nucleic acid extractions, realtime PCR, immunoassays, ...


10 | van den Brule S., Huaux F., Uwambayinema F., Ibouraadaten S, Yakoub Y., Palmai M., Trottein F., Renaud J.-C., Lison D. (2013) Lung inflammation and damage to the thymus after bleomycin are controlled by the PGD2 receptor DP1. Am J Respir Cell Mol Biol 50:212-22

* : for a complete list of publications at LTAP in 2013 see http://www.toxi.ucl.ac.be/publication/publication.html
The pole of microbiology includes the virology and the bacteriology groups and is devoted to clinical microbiology research. It acts as a Belgian National AIDS Reference Laboratory (ARL), and houses the National Reference Centers for \textit{Clostridium difficile}, \textit{Yersinia} and \textit{Borrelia}.

\textbf{MEDICAL MICROBIOLOGY (MBLG)}

The pole of microbiology includes the virology and the bacteriology groups and is devoted to clinical microbiology research. It acts as a Belgian National AIDS Reference Laboratory (ARL), and houses the National Reference Centers for \textit{Clostridium difficile}, \textit{Yersinia} and \textit{Borrelia}.

\textbf{RESEARCH GROUP OF BACTERIOLOGY}

\textbf{Diagnosis and epidemiology of \textit{C. difficile} infections (CDI).}

\textit{C. difficile} is the main cause of hospital acquired diarrhea and has become one of the most frequent bacterial pathogen isolated in healthcare settings.

During the last ten years the epidemiology of \textit{C. difficile} infections dramatically changed with the emergence of a hyper-virulent clone called “\textit{ribotype 027}” that is characterized by an increased production of toxins and a resistance to fluoroquinolones. A mortality rate over 10\% in elderly has been reported for CDI caused by this clone.

A rapid diagnosis of CDI is mandatory to allow an adequate treatment of the patient but also...
to implement prevention measures to avoid outbreaks in hospitals. Our laboratory has developed and validated algorithms of diagnosis tools allowing to reduce the turn around time below 2 hours in more than 90% of the cases. Classical approaches like culture are combined with toxin detection on colonies (toxigenic culture) and direct toxin detection in faecal specimens are performed with immuno-assays and molecular biology techniques. In 2013, PCR detection of toxin genes in stools has been included in the routine diagnosis and evaluated.

As the Belgian national reference center (NRC) for *C. difficile*, the laboratory pursued in 2013 its collaboration with the national public health institute (IPH). The national surveillance program allows the epidemiologic survey of CDI. A clear trend to a decrease of ribotype 027 has been observed.

A new typing approach has been developed: multilocus variable-number tandem repeats (MLVA) which allows to discriminate strains among ribotypes. In collaboration with the veterinary faculty of the ULG, this technique has been used to compare strains of ribotype 027 from two different outbreaks.

### Identification of rare micro-organisms

The laboratory is internationally recognized for the identification and taxonomic classification of rare bacteria. Phenotypic and immunologic characterization are combined with genome sequencing approaches and mass spectrometry.

In 2013, the reputation of our laboratory has been internationally recognized by the denomination of two new bacteria with the name of Pr. Georges Wauters who is the past director of our unit: *Acidovorax wautersii* and *Yersinia wautersii*.

### New projects

Two new programs have been initiated in 2013. Dr Alexia Verroken started a PhD thesis project devoted to the study of the clinical impact of the application of Maldi-tof mass spectrometry in routine microbiology.

Dr Emmanuel André started as well a PhD thesis studying the impact of technology and end-user innovations on tuberculosis control in South-Ki-vu, DRC.

Both fellows received a grant from the St Luc Foundation.

### Borrelia burgdorferi

In 2013 the diagnostic algorithm for *Borrelia* serology was evaluated and general epidemiology in Belgium was established together with the laboratory of the KULeuven and the IPH. An evaluation of the presence of tick-borne encephalitis was done in exposed people with the IPH.

### Antiretroviral drug resistance

The AIDS reference laboratory (ARL) is active in the surveillance of drug resistance transmission. In collaboration with the other Belgian ARLs and the Scientific Institute of Public Health, we track the generation of transmission clusters in the population using phylogenetic analysis and epidemiological data. We described a large transmission cluster of a NNRTI-resistant strain in the region of Namur. The ARL of UCL is also the Belgian board for the ESAR (European Society for Antiviral Resistance), a collaborative group studying drug resistance spread.

The laboratory also takes part to a project in South-Africa initiated by pediatricians of the Cliniques St-Luc. The prevalence of antiretroviral drug resistance in infants contaminated with HIV at birth is investigated in order to establish better guidance for their treatment.

### Towards an HIV cure

Although antiretroviral drugs considerably changed the disease prognosis, the HIV infection cannot be currently cured. Diverse projects aiming at cure need specific laboratory tools that are not available for clinical routine today. In this field, we particularly focus on the detection of residual viremia on therapy and its clinical significance by the validation of ultra-sensitive methods for genome quantification.
**HIV-2**

Over recent years the ARL has become the reference for HIV-2 in Belgium and Luxemburg, for both research and laboratory tests linked to the clinical follow-up. The laboratory coordinates clinical data for the international collaboration AcHleV2e and takes part to the elaboration of genotypic interpretation rules for antiretroviral drug resistance (HIV-grade). HIV-2 may also cause AIDS, but it often leads to a controlled infection without progression to AIDS on the long term. Host factors are responsible for that control; the laboratory studies some antiviral restriction factor that interferes with the viral replication. Particularly, we focus on the interaction between a viral envelope glycoprotein and the cell protein BST-2/tetherin, interfering with virion release. Site-directed mutagenesis, viral culture and protein interactions studies are performed at the lab, while modeling studies are conducted in collaboration with the CRP-Santé in Luxembourg.

**EQUIPMENT**

In 2013, the pole acquired the digital PCR technology and has started its evaluation

- Safety laboratory (P3 level)
- Nucleic acid sequencing facilities

**SELECTED PUBLICATIONS**


2 | Vaneechoutte M, Janssens M, Avesani V, Delmée M, Deschaght P. Description of *Acidovorax wautersii* sp. nov. to accommodate clinical isolates and an environmental isolate, most closely related to *Acidovorax avenae*. Int J Syst Evol Microbiol. 63: 2203-6, 2013


The pole of Molecular Imaging, Radiotherapy and Oncology includes two independent laboratories, the laboratory of Molecular Imaging and Radiation Oncology led by Prof. V. Grégoire, and the laboratory of Medical Oncology led by Prof. JP Machiels. The driving force of these two laboratories including both clinical and basic scientists is to build bridges between the clinical applications and the bench within their specific research areas, which are introduced hereafter.
Cancer is among the leading causes of death in western countries, and its incidence is progressively increasing in part due to aging of the population. Radiation Oncology -delivered as single modality or in combination with surgery and/or medical treatments- represents one of the most effective options to cure cancer at a local or loco-regional stage. It also has a prominent palliative role for the management of patients with metastatic disease.

Although indisputable progresses have been made over the last few decades in the treatment of cancer, patients still die from uncontrolled loco-regional disease. Inaccurate definition of the target volumes, insufficient or sub-optimal radiation dose distribution, and intrinsic radiation resistance are, among others, factors that explain these treatment failures.

In this framework, the Laboratory of Molecular Imaging and Radiation Oncology developed several lines of research aiming at 1) improving the radiation delivery, 2) at a better understanding of the role of tumour microenvironment in radiation response, 3) in comparing the biological efficacy of Hadron and photon beams, and 4) at integrating molecular imaging with various PET tracers in the radiation treatment process.

This laboratory includes various scientists with as different background as physicians, biologists, physicists, radio-chemists and engineers. A short description of the various ongoing projects in the Laboratory of Molecular Imaging and Radiation Oncology is presented hereafter.

Influence of tumor microenvironment on radiosensitivity

Tumor radioresponse and metabolic profile

(Vanesa Bol in collaboration with Anne Bol, Daniel Labar, John Lee, Caroline Bouzin, Paolo Porporato, Pierre Sonveaux and Olivier Feron)

The Warburg phenotype identified decades ago describes tumor cells with increased glycolysis and decreased mitochondrial respiration even in the presence of oxygen. This particular metabolism also termed ‘aerobic glycolysis’ reflects an adaptation of tumor cells to proliferation in a heterogeneous tumor microenvironment. Although metabolic alterations in cancer cells are common features, their impact on the response to radiotherapy is not yet fully elucidated. We produced Warburg-phenotype tumor cells with impaired mitochondrial respiration (MD) and after characterization of their metabolism we compared the response of MD cells to irradiation in vivo and in vitro to the genetically matched parental cells (WT). In vivo, tumor growth delay was increased in MD group, indicating an increased radiosensitivity compared to WT, while in vitro clonogenic survival showed no difference between the two cell lines. We then hypothesized that the increased radiosensitivity of MD tumors could be partially explained by the impaired oxygen consumption of these cells. Therefore, we tested this hypothesis by using different cell lines irradiated in a closed system and then correlated their survival fraction to their respiratory rate. These results demonstrate that in addition to intrinsic parameters, tumor response to radiation depends also on metabolic profile.

Identification of the mechanisms of radiosensitization by human papilloma-virus (HPV) in cancers cell lines

(Vanesa Bol and Florence Masquelier)

It has been shown in several clinical studies that HPV-positive head and neck squamous cell carcinoma (HNSCC) have a more favorable outcome and greater response to therapy. The reason for improved prognosis of HPV-related H&N cancers remains speculative. One hypothesis is that HPV-positive cells are intrinsically
more sensitive to standard therapies and thus, respond better to treatment. Clinical studies suggest indeed that HPV-related cancers actually display enhanced sensitivity to concurrent chemoradiation therapy. The goal of this project is to verify this hypothesis.

For this purpose, we determined radiosensitivity by clonogenic survival of two HPV positive HNSCC cell lines (UPCI-SCC-154 & UPCI-SCC90) compared to two HPV negative ones (SCC-61 & SQD9). Cell cycle distribution and G2/M checkpoint were assessed by flow cytometry. DNA damage repair was evaluated by gamma-H2Ax assay. In addition, apoptosis was investigated in the four cell lines together with mitotic catastrophe and senescence. Our results indicate an increased radiosensitivity of HPV+ cells and an impaired DNA damage repair although at this point further investigation is needed to elucidate the exact mechanism.

Characterization of the biological efficacy of HadronTherapy beams

(John Gueulette)

Our laboratory is committed in the radiobiological study of clinical hadron beams (beam of particles heavier than the electron) since the seventies, when we developed fast-neutron-therapy at the cyclotron Cyclone of Louvain-La-Neuve.

Keeping the momentum and our expertise in non-conventional radiation beams, the laboratory is now participating in different international research programs aiming at defining whether or not proton and/or carbon-ion beams would be beneficial for the treatment of selected tumors. Due to the variation of the ionization density in comparison with photons, the physical dose of radiation (in Gy) is no longer sufficient to monitor adequately the biological effect of these beams. The application of a weighting biological factor is compulsory, which depends on numerous physical and biological parameters specific to each type of machine and each clinical indication. Hence the difficulty to transfer clinical and radiobiological information from one hadron therapy center to another, and to build reliable clinical experience through multicentric trials.

In line with our early intercomparisons of clinical neutron beams – the procedure of which was internationally recognized –, our laboratory is now intercomparing the relative biological effectiveness (RBE) of the different hadron beams presently operational worldwide. This study will contribute to normalize the biological equivalent doses and to harmonize the treatments.

Radical reduction of particle range uncertainties in proton therapy

(Stefaan Vynckier, Edmond Sterpin, Jefferson Sorriaux, Sèverine Rossomme)

Proton therapy is a promising external radiotherapy treatment modality for improvement of tumor local control with minimal side effects. Protons stop in matter at well-defined positions depending on the initial energy of the particles and the materials along their tracks. Compared to photon-based modalities, proton therapy shows a reduced dose before the tumor volume and almost no dose beyond the tumor.

However, the finite range of the protons makes the quality of proton therapy treatments very sensitive to the uncertainties related to modification of patient anatomy, and to the dose calculation process. The medical physics team aims at reducing radically the uncertainties on the range of the protons by 1) studying new types of detectors for accurate dosimetry of proton beams 2) implementing an accurate and super-fast Monte Carlo simulations of proton therapy treatment; 3) incorporating actual patient data acquired while the patient is on the treatment couch for dose calculation; and 4) performing in vivo range verification by comparing measured and simulated prompt gammas emitted by the protons along their tracks. Monte Carlo simulations are the most accurate dose calculation engines because they are based on the direct sampling of physical laws of transport.

The medical physics team of MIRO is expert in dosimetry and Monte Carlo simulations of advanced radiotherapy treatments. The expertise was acquired during collaboration between MIRO, the University of Wisconsin and Accuray aiming at developing an efficient Monte Carlo model for helical TomoTherapy treatments.
Schematic representation of the prompt gamma imaging process using a prototype slit camera. Prompt gammas are emitted along the proton track and can be imaged during treatment (upper figure, from Smeets et al. Phys. Med. Biol. 2012). The detection profiles can be computed by Monte Carlo simulations (down-left and down-right figures) and used as the reference for further comparisons with measurements in clinical conditions.

**PET image analysis**

**Improvement of PET image reconstruction**

(Anne Bol, Kevin Souris)

Correction of preclinical images acquired on the Mosaic animal PET system with CT-based attenuation correction rather than using an external positron-source. This method results in less noisy images and will be validated on phantoms and animal measurements using the micro-CT from the recently installed animal SPECT/CT. A Monte Carlo model of the MOSAIC camera has been set up (using GEANT4/Gate) in order to determine some physical characteristics of the camera, such as the mean interaction depth in the scintillation crystals.

**Processing of PET images to better delineate target volumes in radiotherapy treatment plan**

(John A. Lee, Anne Bol, Sarah Differding, François-Xavier Hanin, Stéphanie Servagi, Marie Wanet)

PET images convey very useful functional information about tumour. Depending on the tracer, glucose metabolism, proliferation, and hypoxia can be revealed. However, these images suffer from a rather low resolution that prevents a spatially accurate delineation of the tumor extension. Specific image processing tools have been developed (denoising, deblurring) in order to increase this accuracy. These tools have been integrated in a segmentation method that has been recently validated for lung tumors. Ongoing work aims at applying these tools on PET images acquired with an hypoxia tracer (FAZA). Other tools are also in development in order to implement dose painting approaches. In these treatment strategies, the dose prescription includes a heterogeneous increment, which follows the PET tracer uptake, and then concentrate the dose on sub-regions suspected of radio-resistance.

Various imaging modalities along the course of a radiotherapy treatment. A patient with a Head & Neck tumor is imaged with computed tomography (CT), Magnetic resonance imaging (MRI), and positron emission tomography (PET). The images show the progressive tumor shrinkage. An accurate delineation of the tumor on the PET images has been demonstrated to be closer to the actual volume than with other modalities. This allows for both a better targeting in the treatment plan and reduced side effects.

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Automatic segmentation of CT images using a registration-free atlas

(John A. Lee, Guillaume Bernard)

This project aims at addressing the weaknesses of automatic segmentation methods based on the non-rigid registration of previously delineated images (atlases). Contours obtained with these methods are often inaccurate, due to simplistic regularization schemes in registration algorithms. The approach developed in the project relies on machine-learning techniques.

Improvement of atherosclerotic plaque imaging with clinical FDG PET/CT

(Anne Bol, François-Xavier Hanin)

FDG-PET is a potential useful tool to detect the atherosclerotic plaque in the myocardium. In order to allow a better plaque detection, the intense uptake of FDG in the myocardium must be reduced. Therefore, the impact of different dietary preparations on myocardial FDG uptake is compared in a randomized controlled trial (collaboration with CARD).

Starting with $^{18}$F-fluoride, the radiopharmaceutical tracers commonly prepared are: $^{18}$F-FAZA (hypoxia), $^{18}$F-FLT (proliferation), $^{18}$F-fluorocholine (phospholipids in the cell membranes), $^{18}$F-NaF (bones), $^{18}$F-FPA (lactate) and $^{18}$F-FHBG (expression of the herpes simplex virus type-1 thymidine kinase (HSV1-tk) gene.

All these tracers are used in pre-clinical studies for in vivo characterisation of different pathologic models. $^{18}$F-FAZA, $^{11}$C-CO$_2$, $^{11}$C-methionine and $^{11}$C-acetate are prepared in GMP conditions and are thus also used for clinical applications. For pre-clinical studies, all tracers are prepared by using homemade remote control systems developed by NI LabVIEW program.

GMP syntheses are performed in clean rooms by fully automated systems also developed and piloted by NI LabVIEW program. Beside this logistic activity, we are also involved in fundamental research.

High specific activity $^{89}$Zr (T 1/2: 78.4 h) is produced for monoclonal antibodies labelling. In a first study, the biodistribution of $^{89}$Zr-labelled cetuximab before and after coupling reaction to gold nanoparticles was compared and the quantitative imaging performance of $^{89}$Zr immuno-PET was evaluated.

The second research area is the synthesis of silicon analogues of $^{[18F]}$-fluoro-misonidazole in order to develop new radiolabelled compounds for the detection of tumour hypoxia.

Radiochemistry

(Daniel Labar, Thomas Doumont, Jacques Gillart)

The radiochemistry team carries out the synthesis of radiotracers labelled by positron emitter isotopes for applications in positron emission tomography. The isotopes routinely produced are: fluorine-18, carbon-11, nitrogen-13, produced using the IBA 18/9 cyclotron, and zirconium-89 and germanium-68, produced using the IBA Cyclone 30. Our role is initially logistic with the synthesis of radiopharmaceuticals known for their potential in PET imaging.
Aseptic dispensing cleanroom for human use tracers
Antibody-functionalized nanoparticles for imaging cancer: influence of conjugation to gold nanoparticles on the biodistribution of 89Zr-labeled cetuximab in mice. Karmani, Linda ; Labar, Daniel ; Valenbois, Vanessa ; Bouchet, Virginie ; Nagaswaran, Praveen Ganesh ; Bol, Anne ; Gillart, Jacques ; Levêque, Philippe ; Bouzin, Caroline ; Bonifazi, Davide ; Michiels, Carine ; Feron, Olivier ; Grégoire, Vincent ; Lucas, Stéphane ; Vander Borght, Thierry ; Gallez, Bernard. In: Contrast Media & Molecular Imaging, Vol. 8, no. 5, p. 402-408 (September/October 2013).


Adaptive PET-guided intensity modulated radiation therapy in head and neck, and non-small-cell lung cancer: towards an individualized treatment
(Sarah Differding, Xavier Geets, Vincent Grégoire, Samuel Goossens, Guillaume Janssens, John Lee, Stéphanie Servage, Edmond Sterpin, Marie Wanet)

Radiation therapy (RT) combined with chemotherapy or targeted agents has been recognized as one of the main treatment modalities for locally advanced head and neck (H&N) and non-small-cell lung cancer (NSCLC). However, local tumor failure remains high in these patients, with loco-regional failure rates in the order of 30-50%. This justifies pursuing strategies to increase local tumor control that can be integrated with systemic treatment. The clinical implementation of dose-intensification protocols however remains challenging. More specifically, a uniform dose escalation delivered to the whole tumor would certainly end up with unacceptable radiation-induced toxicities. In that regard, the so-called “dose painting” approach appears particularly promising. It consists in identifying tumor areas with a potential radioreistance phenotype that might benefit from selective and targeted additional doses, with the ultimate goal of safely improving the local tumor control. However, many methodological issues and clinical validations have still to be addressed before such a strategy becomes a clinical reality.

In this framework, in locally advanced H&N tumors, a randomized phase II studies has been designed to tackle the issue of dose-painting escalation to voxels expressing high FDG uptake. The main objective of the study is to demonstrate the benefit of a molecular imaging-based adaptive dose escalation in patients with HPV-negative locally advanced squamous cell carcinoma of the oropharynx. Considering the expected changes both in the patient’s anatomy and in the tumour biology with time during treatment, the dose-painting will be adapted throughout the treatment in both arms.

The primary endpoint of the study will be the primary tumor control probability at 1 years after completion of treatment. Patients will be randomized between a standard arm treatment with IMRT to a total dose of 70 Gy in 7 weeks. In the experimental arm, a graded boost dose (i.e. dose painting by number) will be delivered on the tumor GTV based on the signal intensity of FDG-PET images acquired before and early on during treatment (i.e. after 10, 20 and 30 Gy). The boost dose intensity will vary from 0 to 16 Gy based on the relative signal intensity of the PET images. In both arm, Erbitux®, an EGFR inhibitor will be injected weekly during radiotherapy treatment starting with a loading dose given one week prior to the start of treatment. Two hundreds patients are required to show a 20% improvement in local control at 1 year.

In NSCLC, a prospective phase II study has thus been designed to assess the impact of FDG-PET subvolume dose boosting on the local tumor control (local progression free survival), as well as the safety of this approach (acute and late toxicities). In this protocol, a total dose of 62.5 Gy is delivered in 5 weeks to the primary tumor and positive nodes delineated on a gated 4D planning CT with iv contrast. The dose per fraction is then selectively escalated on the pre-treatment FDG-PET-based target volumes using a simultaneous integrated boost (SIB) IMRT technique, until a set of predefined normalized dose-limiting normal tissue constraints is reached for the considered organs (spinal cord, lungs, heart, esophagus...).

A step further will involve the integration of a hypoxia PET tracer (FAZA), and the morphological/biological changes throughout the RT course, in the treatment planning process. First, a planning study will address the methodological aspects and the technical feasibility of this approach. Schematically, FDG and FAZA PET-CT will be performed prior to treatment and at weeks 2 and 3. Based on pre-treatment images, the dose will be gradually increased from the GTV defined on FDG-PET, which has been shown to best estimate the true macroscopic tumor, to the intra-tumor voxels with the highest FAZA uptakes, which are believed to correspond to the most hypoxic regions.
The dose will be prescribed according to an optimized tomotherapy voxel based prescription, and will be further adapted to the morphological and biological changes identified on the per-treatment images (figure). As soon as the optimal methodology is found out, a prospective phase II trial will be initiated at a European level to assess the impact of such an approach on the patient outcome.

In parallel, various strategies will be deployed and validated for the integration of motion-related geometric uncertainties in tomotherapy treatment planning and delivery, including the probabilistic “MidPosition” approach and a new robust dose prescription specifically developed for heterogeneous dose prescription in dose-painting by numbers.

Schematic view of dosimetric aspects related to the FDG & FAZA PET-based adaptive dose painting by numbers for NSCLC. The black grid and the red contour represent the GTVCT and GTVFDG-PET, respectively. The red square corresponds to the highest FAZA uptake within the primary tumor. PET-CT images are acquired prior to treatment, and at the beginning of weeks 2 and 3. The dose distribution at each time point is computed according to a voxel-based approach in which the dose is linearly increased from the boundaries of GTVFDG-PET (green voxels) to the voxel with the highest FAZA uptake (red voxels).


Nuclear medicine projects

(François Jamar, François-Xavier Hanin, Renauld Lhomme, Stanislas Pauwels, Stéphane Walrand in collaboration with CARD and the University of Tours)

The Nuclear medicine Unit is involved in preclinical or analytical work dedicated to two main topics: dosimetry and image acquisition. Dosimetry in radionuclide therapy has been a hallmark of the unit’s scientific production over the last decade.

The development of dosimetry models that enable estimates of the biological effective doses led in particular to the first ever real assessment of the oxygen effect in internal therapy: tumor response assessed using $^{18}$F-FDG PET, following intrahepatic administration of $^{90}$Y-labelled microspheres showed to be better predicted if the hemoglobin concentration was taken into account.

In addition, this dosimetric modelization was made possible by the use of PET for imaging $^{90}$Y, a method developed in our laboratory. Further work is ongoing for allowing accurate imaging of $^{90}$Y radio-pharmaceuticals using modified Bremsstrahlung acquisition and processing; Monte-Carlo simulations are underway to find the optimal collimator design and acquisition settings. Imaging of high energy photons (>1 MeV) has been made possible using a modified gamma camera.

This is important in view of the potential future use of $^{125}$Sn, in combination (cocktail) with the radionuclide of choice nowadays, $^{177}$Lu. The laboratory also continues work on imaging of small animals, more recently, using a $^{99m}$Tc-RGD-derivative binding to integrin (in collaboration with CARD), for the assessment of atherosclerosis and especially the vulnerable plaque. Results are promising and ex vivo work on human artery specimens encourage a move in the near future to human in vivo imaging.

Finally, the laboratory is involved in the development and preclinical evaluation of nebulizers for more efficient local vs peripheral lung deposition of small particles, such as antibiotics [ref 3].

Cancer Immunotherapy

(JF Baurain, AM Feyens, J Degueldre, F. Hammouch, K Amman, A Devalckeneer)

Our research program is based on several small clinical trials with translational research performed in collaboration with other teams. In the early nineties, the identification of tumour antigen recognised by autologous cytolytic T lymphocytes (CTL) has allowed the development of new immunotherapeutic strategies.

Melanoma vaccines comprising tumour antigens have been investigated in numerous clinical trials. Even though, these vaccines have been generally very well tolerated, their clinical effectiveness has remained low, with a minority of patients showing objective tumour responses and clinical benefit. These vaccines were poorly immunogenic and only a minority of patients presented with anti-vaccine lymphocyte responses. But it is also likely that many melanomas acquire the ability to resist immune destruction during their evolution, which might also explain the low efficacy of cancer vaccines.

Current strategies explore the effectiveness of more immunogenic vaccines (project 1), the capacity of melanoma vaccines to prevent relapse in high-risk patients who are disease-free after complete surgery, associations of vaccines with drugs aimed at blocking tumour resistance mechanisms (project 2 & 3).

Meanwhile, we are also exploring new agents able to revert anergy of CTL (project 4), drugs able to change the homing of CTL (project 5), and the role of antibodies binding CTLA-4 (Ipi-limumab) on the functionality of anti-tumour CTL (project 6). Finally, the progress of genetics and molecular biology has allow the identification of activating mutations, BRAF, NRAS, MEK and c-KIT, in melanomas. We are studying the impact of these mutations on the survival of melanoma patients but also on the response to immunotherapy (project 7).
Project 1  New cancer vaccines - study LUC09-003 : Theravac In collaboration with Pr. C. Leclercq (Institut Pasteur, Paris) and Pr. B. van den Eynde (LICR, Bruxelles)
The Pasteur Institute of Paris in collaboration with the LICR in Bruxelles has developed a new vaccine called CyaA-Tyr (Theravac) which is a recombinant protein derived from the adenylate cyclase of Bordetella Petussis. This protein targets dendritic cells (DC) by binding to CD11b. Once internalized, the toxin kills DC thus preventing the immune system to mount a response against the pathogen. Preliminary in vitro data and mice models have shown that CyaA-Tyr is the most immunogenic vaccine never obtained. This new vaccine is tested in melanoma patients as a monocentric phase I study, first in human.

Eleven patients have been already included. Inclusion at the maximum dose level is ongoing to further assess clinical and immunological benefit of this new vaccine.

Project 2  Local Immunomodulation associated with cancer vaccines - study LUC10-002. In collaboration with Pr. P. Coulie (GECE, DDUV, UCL) and Dr. N. van Baren (LICR, Bruxelles)
Under normal conditions, female CBA mice don’t reject a male skin graft. This situation is puzzling since female mice have an immune response against the antigen H-Y, a minor histocompatibility antigen present only on male tissue. The local injection of a combination of Interleukin-2, alpha Interferon and GM-CSF associated with the application of Imiquimod on the graft was able to induce skin rejection due to activation of anti-H-Y CTL. Therefore, the idea was to use the same combination associated with a tumour vaccine on cutaneous melanoma metastases.

Two patients have been already included and received the complete treatment.

Project 3  Local Immunomodulation associated with cancer vaccines - study LUC10-001. In collaboration with Pr. P. Traber (Galectine therapeutics, USA) and Pr. P. van der Bruggen (LICR, Bruxelles)
Recently, the team of P. van der Bruggen has identified a new mechanism causing CTL anergy and found chemical agents able to revert their status. Co-localisation of the T-cell receptor (TCR) and the CD8 coreceptor is key for T-cell activation. Ovarian infiltrating CTL have their TCR locked in a network of glycoproteins mediated by Galectins. Once the TCR is far from the CD8, the CTL is anergic. But by co-incubating these anergic CTL with sugars that bind to galectin, we are able to revert this anergy. GM-CT-01 is a soluble polysaccharide that binds to galectine-3 which is produced also by many melanomas. This sugar has already been used in human in combination with a chemotherapy and no side-effect has been reported. We have proposed to combine a peptide cancer vaccine with GM-CT-01 that will be injected intravenously or locally. This is a Belgian multicentric study where 12 patients will be included.

Six patients have already been included and three of them have received all the injections of GM-CT-01.

Project 4  Local Immunomodulation with new ligands of Galectins. In collaboration with Pr. P. Traber (Galectine therapeutics, USA), Pr. P. van der Bruggen (LICR, Bruxelles), Pr. J. Squifflet (CUSL, Bruxelles), Pr. H. Schambye (Galecto, Sweden) and Pr. T. Sehti (King’s college, UK)
Based on the discovery made by Pr. P. van der Bruggen and described above, one may speculate that one could enhance immune mediated tumour eradication in cancer patients by blockading galectin-3. Therefore, one will test different concentrations of GM-CT-01 in ovarian cancer patients and analyse in parallel the functionality of tumour infiltrating...
lymphocytes. This multicentric phase I study will include up to 30 ovarian cancer patients. One also want to test other ligands of galectin-3 that are under development at Galecto (European FP-7 project - Galectonco).

**Project 5**  Using anti-EGFR antibodies to modify homing of anti-cancer T cells. In collaboration with Dr. K. Segers (Amgen, USA) Squamous Cell Carcinoma (SCC) is one the most common malignancies in Caucasian population. The effect of the immune system on the development of skin tumors has been demonstrated in transplant patients taking immunosuppressive agents (65 fold risk increase). It has been reported that activation of EGFR and RAS signaling pathways play an important role in disease progression maybe through downregulation of the immune system. We want to treat unresectable SCC patients with an antibody against EGFR ( Vectibix, panitumumab). This antibody induce tumor regression in metastatic colorectal and has been approved for that indication. We want to measure the response rate but also analyze the modification of expression profile of some key proteins involved or supposed to be involved in the signaling pathways of EGFR and in the regulation of the immune system. Some chemokines such as CCL27 has been shown to play a critical role in the skin-associated immune response by regulating T cell homing. The downregulation of CCL27 is mediated by activation of EGFR/RAS/MAPK signaling pathways. This open multicentric study in squamous cell carcinoma will evaluate the clinical efficacy (overall response rate) of Panitumumab in these patients but will also study the modification of expression profile of several key tumour genes during treatment. The presence of CTL in the tumour and their functionality will also be recorded. Five patients have already been included.

**Project 6**  Anti-tumour CTL in melanoma patients treated with Ipilimumab. In collaboration with Pr. P. Coulie (GECE, DDUV, UCL, Bruxelles)

Recently, Ipilimumab has emerged as a new treatment for metastatic melanoma. It is a humanised antibody that binds to CTLA-4, a protein that becomes expressed on T lymphocytes upon activation and acts as a negative feedback by inhibiting the immunostimulatory CD28-B7 pathways. Ipilimumab is though to restore T cell function in tumours. Two recent pivotal phase III trials with repeated infusions of Ipilimumab in melanoma patients have demonstrated a benefit in terms of response and survival. Prolonged remission was observed in some patients. The treatment can cause severe toxicities consistent with auto-immune reactions. We want to analyse the anti-tumour T cell repertoire of melanoma patients before and after 4 infusions of Ipilimumab. The first step in this project is to establish a tumour cell line from a metastasis of a patient. This step is crucial and is also the limiting factor since we can derive a permanent cell line in less than half of the patients. In order to compare the frequency of anti-tumour CTL before and after Ipilimumab, we have to stimulate CTL derived from peripheral blood in limited dilution condition. This will allow us to assess the functionality of these T cells will be tested in terms of cytokines production and expression of activating markers.

**Project 7**  Correlation between survival and presence of BRAF mutation in melanoma. In collaboration with Dr. I. Theate (IPG, Loverval) and M. Vikkula (GEHU, DDUV, UCL, Bruxelles)

Recent progress in genetithics and molecular biology has allowed to identify oncogenes and tumour suppressor genes that are frequently mutated in melanoma. For example, activating mutations in BRAF, NRAS, MEK and c-KIT are present approximately in 50%, 20%, 8% and 1% of melanomas, whereas inactivating mutations are found in 60%, 30% and 5% of the PTEN, CDKN2A and TP53 genes, respectively. These genes identify the MAPK and PI3K signal transduction pathways, as well as cycle regulation and DNA repair, as key factors in melanocyte oncogenesis. More importantly, they have paved the way for the clinical development of specific pathway inhibitors. Several of these drugs, Vemurafenib, Dabrafenib, Trametinib, have recently shown a high therapeutic potential in metastatic melanoma. Presently, there is no evidence that the presence of these mutations are correlated with a poorer or better prognosis, nor if melanoma patients harbouring these mutations respond differently to immunotherapy. We will retrospectively go for a «fishing expedition» in order to make correlation between the mutanome of our melanoma patients and their survival or response to therapy.

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**Molecular targeted therapies for cancer treatment**

*(JP Machiels, F. Mardjuadi, S. Schmitz, A. Gillain, ML Verhaegen, R Albu, R-M Goebbels)*

**Squamous cell carcinoma of the head and neck**

We have conducted (are conducting) several academic clinical trials investigating targeted agents in Head and Neck SCC (SCCHN). We are studying novel agents that target either the vascular endothelial growth factor receptor (VEGFR) or the Insulin Growth Factor-1 receptor (IGF-1 R) or the Epidermal Growth Factor Receptor (EGFR). In these studies, paired tumor biopsies (before and after treatment) are obtained for translational research with the aims of analyzing the molecular pathways involved and the potential resistance mechanisms.

**Our results indicate that:**

1. A long-term benefit of anti-EGFR monoclonal antibodies is observed in a minority (10-15%) of the patients with recurrent SCCN.
Sunitinib is an orally bioavailable molecule that inhibits multiple tyrosine kinase receptors, including, among others, VEGFR-1, VEGFR-2, VEGFR-3 and platelet derived growth factor (PDGF) receptor-, PDGFR receptor -. We showed that this therapy could lead to significant tumor regression in recurrent SCCHN but with unacceptable toxicities (i.e. NCI-CTC grade 4 and 5 bleeding). We found a significant decrease in tumor perfusion by DCE-MRI in some patients after treatment. The molecular analyses of paired tumor biopsies collected before and after sunitinib administration demonstrated that sunitinib has a significant impact on the tumor vascularisation with tumor necrosis.

Figitumumab is a fully human monoclonal antibody targeting the IGF-1R. We tested this compound in recurrent SCCHN. We found no clinically significant activity. Translational research showed that fitigumumab down regulated IGF-1R at the surface of tumor cells. However, we observed activation of the EGFR pathway, as shown by the upregulation of p-EGFR in tumor cells (p=0.016) and an increase in the plasma level of Tumor Growth Factor-alpha (p=0.006). cDNA microarray and qRT-PCR also performed on paired biopsies confirmed the activation (instead of an inhibition) of the PI3K/AKT pathway (3).

Toward better study designs....

Targeted agents are often investigated in unselected end-stage cancer patients. This trial design has some major limitations in regard of translational research.

First, most patients have received radiation and/or chemotherapy and/or surgery, and so have developed multifactorial resistance and are less likely to respond to new agents effectively. Second, feasibility of conducting translational research is hampered by ethical considerations in obtaining iterative tumor biopsies in palliative patients. One way to resolve some of these issues and to explore these pathways is to perform “window studies” where a targeted agent is given during the incompressible period of time between the diagnosis and surgery in patients selected for a primary surgical treatment.

Evaluation of compounds in the pre-operative window setting could maximize the chance of observing tumor response. In addition, the collection of biopsies before treatment and after treatment may provide insights into the pharmaco-dynamic effects of novel agents as well as their mechanisms of action and also help to identify some hypotheses regarding treatment resistance. We have performed a pilot « proof of concept » study with cetuximab given pre-operatively to 33 untreated SCCHN. Cetuximab, a monoclonal antibody that targets the EGFR, was administered during two weeks between the diagnosis and the curative surgery. We have shown that this approach is feasible.

Today, we are continuing to improve the design of clinical trials that investigate new molecular targeted agents in SCCHN. Within the EORTC framework, we have created a platform to perform “window opportunity study” with new agents in a multicenter setting. We are leading several clinical trials in this context and the tumor biopsies are analyzed in our laboratory. In addition, to better understand the resistance mechanisms to anti-EGFR therapies, we are establishing a biobank of human xenografts representing the different subsets of SCCHN. The aims of this project are: (i) to study primary and secondary (acquired) resistance to anti-EGFR monoclonal antibody; (ii) to preclinically evaluate the efficacy of new specific targeted agents; and (iii) to identify relevant biomarkers that could lead to stratified patient trials in clinical trials.

**Anti-EGFR treatment to radiosensitize rectal cancer**

Epidermal Growth Factor Receptor (EGFR)-targeting monoclonal antibodies (moAbs) have demonstrated radiosentingizing properties. Several trials, including one conducted by our group, have investigated EGFR-targeting moAbs in combination with preoperative chemoradiation regimen in rectal cancer. However, the addition of these moAbs to preoperative chemoradiation in rectal cancer has failed to improve outcome.

We hypothesized that the radiosensitizing activity of anti-EGFR moAbs could be optimised by prospectively selecting KRas wild-type tumors and by clinically applying the treatment sequence that yield the best anti-tumor response in vivo. First, we conducted in vivo experiments to explore several treatment sequences of combined anti-EGFR moAbs and radiotherapy in murine xenografts of KRas wild-type human colorectal cancer. Next, based on our pre-clinical findings, we designed the study treatment for a phase 2 clinical trial in which panitumumab, a IgG2 EGFR-targeting moAbs, was given in association with fractionated radiotherapy to treat patients with cT3-4/N+ KRas wild-type rectal cancer,
followed by surgery. Unfortunately, despite the optimization of the study design, the trial was prematurely closed for lack of activity as it failed to achieve its primary endpoint. Currently, we are working towards unlocking the potential resistance mechanisms to the study regimen. For instance, although it is still constantly debated, the presence of BRAf and PI3KCA mutations as well as the loss of PTEN expression in metastatic colorectal cancer are frequently associated with poor response to EGFR inhibitors. There is also the possible activation of other alternative growth factor receptors, such as the insulin-like growth factor-1 (IGF-1) and the hepatocyte growth factor (HGF) receptor, to compensate the EGFR blockade, therefore inducing tumor resistance to EGFR inhibitors.

**Targeting the mTOR pathway to treat bladder cancer**

We conducted a phase II trial to investigate a mTOR inhibitor (everolimus) in advanced transitional cell carcinoma. Twenty-seven percent of the patients experienced a clinical benefit from this new approach. Interestingly, PTEN loss was found exclusively in patients with noncontrolled disease at week 8. This observation could highlight the importance of the negative feedback mediated by S6 Kinase 1 towards the PI3K/Akt pathway. Indeed, it could be postulated that everolimus induces Akt activation via the removal of this negative feedback. Akt activation could be sustained in case of PTEN loss and therefore might act as a cancer cell survival mechanism inducing everolimus resistance. Based on this background, a trial with an inhibitor that blocks mTOR and PI3KCA has just been initiated. The addition of PI3KCA inhibition could interfere with this postulated resistance mechanism and therefore improve patient outcome.

**Identification of new constitutional genetic alterations in patients with a family history of breast cancer**

*(F. Duhoux and L. Delle Vigne in collaboration with M. Vikkula (GEHU, DDUV, UCL, Bruxelles)*

This study aims at the identification of new constitutional genetic alterations predisposing to breast cancer in patients at increased familial breast cancer risk in whom no germline BRCA1 nor BRCA2 alterations were identified. Whole-exome sequencing is currently performed on up to 200 patients who are being enrolled in a dedicated breast cancer consultation at the Cliniques universitaires Saint-Luc and Grand Hôpital de Charleroi.

The analysis will first be conducted on a prespecified set of genes already described in the literature in families with genetic cancer predisposition, with a special focus on genes involved in the different pathways of DNA repair, or genes functionally associated with BRCA1 and BRCA2. Candidate genes will be validated in other affected family members, and subsequently in a cohort of non-related breast cancer patients. An independent validation will be performed at Institut Curie in Paris, in collaboration with Professor Dominique Stoppa-Lyonnet. Identifying novel genetic alterations predisposing to breast cancer will enable physicians to offer patients personalized screening and prevention, potentially leading to a reduction in the incidence of breast cancer cases and to an improvement in the management of some high-risk breast cancer patients.

**Characterization of immune infiltration in the treatment of advanced colorectal cancer**

*(M. Van den Eynde, D. Debetancourt, JP Machiels in collaboration with Jerome Galon and Franck Pages (Centre de Recherche des Cordeliers, INSERM-Université Paris-Descartes)*

Increasing literature supports the hypothesis that colorectal cancer (CRC) development is influenced by the host immune system. A common idea has emerged, emphasizing the critical need to evaluate systemic and local immunological biomarkers. It is in agreement that this
may offer powerful prognostic information and facilitate clinical decision-making regarding the need for systemic therapy. For early and localized colorectal cancer, numerous data collected from large cohorts of human cancers, demonstrated that the number, type and location of tumor immune infiltrates in primary tumors, are prognostic for Disease-Free Survival (DFS) and Overall Survival (OS). A potential clinical translation of these observations is the establishment of a simple immune score, quantifying the density and location of immune-cells within the operated colorectal tumor. This immune score, based on the density of Th1/cytotoxic and memory T cells (CD3/CD8/CD45RO), both in the center and the invasive margin of the tumors, has important prognosis value that may be superior to the AJCC/UICC TNM –classification. Tumor invasion was shown to be, in fact, statistically dependent on the host-immune reaction. The prognostic role of the tumor immune infiltration in the metastases and the possible relation with systemic therapy (chemo and targeted therapies) is little studied in this setting.

A few publications seem to indicate that immune infiltration is able to predict a response to postoperative chemotherapy and better its outcome. In this project, we currently analyse the tumor infiltrating lymphocytes (type, location and density of immune cells) in the curatively resected tumor and liver metastases after preoperative treatment. We will to compare this immune infiltration to the pathological response of the tumor and the patient disease outcome after preoperative treatment (see Figure).

However, the prognostic impact of immune infiltration remains a post-surgical analysis on the whole resected tumor. As demonstrated in other tumor types (such as breast cancer), the tumor immune infiltration evaluated by tumor biopsies could potentially help the treatment decision making it a prognostic and possibly predictive marker. Therefore, this project proposes also to assess and correlate the colorectal tumor infiltrating lymphocytes (CD3/CD8/CD45RO) on the tumor biopsies before any preoperative treatment and to compare it with the resected tumor (surgical specimen). As demonstrated for other tumor, the aim is to validate the immune quantification on biopsies as a reliable predictive tool for clinical patient outcome and response to therapy.
The research themes of the morphology pole focus on the tissular, cellular and molecular interactions in several experimental models and are grouped into 5 main axes:

1. Angiogenesis, oxidative stress and tumorigenesis in thyroid gland.

2. Causes, consequences and improvements of oxidative stress in eye muscles and orbitary fat in cases of Graves’ orbitopathy.

3. Implantation of miniaturized neuromuscular stimulators.

4. Adaptive mechanisms of the skeletal tissues from development and growth to senescence, in pathological conditions and at the bone-implant interface.

5. Morphological (anatomic, histologic and X-ray imaging) description of particular regions of the body in order to develop new clinical and surgical approaches.

Most of our studies are currently conducted in collaboration with other research poles in order to privilege multidisciplinary approaches.
Angiogenesis, oxidative stress and tumorigenesis: the thyroid model

A.C. Gérard, J. Craps, J. Vanderstraeten, B. Dejongh, M.C. Many, I. Colin

1. Role of caveolin-1 and PPARγ in the maintenance of thyroid cell homeostasis

J. Craps, M.C. Many

With the models of caveolin-1 knockout mice (Senou et al., 2009) and of human Pendred disease (Senou et al., 2010), we have demonstrated that caveolin-1 has a key role to maintain a coherent organization of the proteins involved in thyroid hormone synthesis. Caveolin-1 is a component of the thyroxisome assembling thyroperoxidase (TPO) and Dual oxidases (Duox) at the apical pole of the thyrocytes where iodination takes place in normal conditions. The disruption of the thyroxisome leads to intracellular iodination and aberrant intracytoplasmic localization of TPO and Duox which generates the cytotoxic peroxide H2O2, responsible for oxidative stress. If not compensated by the cell antioxidant defenses, this leads to cell apoptosis aggravating the hypothyroidism due to the loss of hormone synthesis.

We are currently comparing caveolin-1 expression in two opposite autoimmune diseases, TH2 Graves’ disease with hyperthyroidism and TH1 Hashimoto’s thyroiditis with hypothyroidism. This in vivo study is completed by an in vitro analysis of the effects of TH1 and TH2 cytokines on human thyrocytes.

The regulation of caveolin-1 expression must still be elucidated and one important factor that we are studying is PPARγ. We analyze its expression in vivo in TH1 and TH2 autoimmune diseases and in vitro on human thyroid cells.

2. Iodine deficiency and angiogenesis

A.C. Gérard, J. Craps, J. Vanderstraeten, B. Dejongh, I. Colin

Despite the efforts to introduce salt iodization in iodine insufficient countries, iodine deficiency (ID) remains a global problem. Beside its well-known effects on fetal development, ID is also involved in several thyroid disorders. In our laboratory, its impact on microvascular changes in thyroid has been extensively studied. It has been observed that thyroid cells can react to ID by secreting VEGF through HIF-1α stabilization, resulting in an increased thyroid blood flow. However, other organs express the sodium/iodide symporter and are able to take up iodide. As different disorders in those organs have been linked to ID, we have decided to study the effects of ID on the vascularization of three of these organs (stomach, salivary and mammary glands) and compare it with the effects observed in thyroid.
Eight-week-old NMRI mice were fed with iodide deficient diet and perchlorate containing water (NaClO₄, a sodium/iodide symporter (NIS) inhibitor) during one to ten days. VEGF expression was studied by immunochemistry in the three organs and blood flow was measured with a laser Doppler in mammary and salivary glands before sacrifice at day 0, 1, 2, 4 and 10. NIS and HIF protein levels were measured by western blot in salivary glands.

In the stromal and epithelial cells of the salivary glands an increase in NIS and HIF-1α protein expression was observed after 1 day of treatment and was followed by an increase in VEGF expression at days 2 and day 4. Moreover, a significant rise in blood flow was observed at days 2 and 4. The blood flow then returned to control level at day 10. Differences were noticed between males and females. In mammary glands, an increase in blood flow was observed from day 1 to day 2. However, there were no differences in VEGF expression between control and ID mice. In gastric mucosa, VEGF expression was strongly enhanced at day 1 and day 2 and then dropped at days 4 and 6.

These data indicate that cells other than thyroid cells can react to ID by inducing microvascular changes, probably trying to adapt iodide inflow. The increase in VEGF and blood flow seems to be transient in the three organs, which is similar to what was observed in thyroid upon ID exposure. However, the rise in mammary blood flow does not seem to involve VEGF. Further investigation needs to be done to determine the actors in this pathway.

**Causes, consequences and improvements of oxidative stress in eye muscles and orbital fat in cases of Graves’ orbitopathy**

M.C. Many, C. Behets, B. Lengelé

Graves’ orbitopathy (GO) causes disturbance of visual function and facial disfigurement in about 25% of people with Graves’ hyperthyroidism. Its an inflammatory condition of the orbital soft tissues, due to an autoimmune reaction against TSH receptor; this autoantigen being also responsible for Graves’ hyperthyroidism. Indeed, we have demonstrated the abnormal expression of TSH receptor on fibroblasts in eye muscles from GO patients (Boschi et al., 2004).

Many studies investigate the role of oxidative stress as a pathogenetic mechanism for GO, by analyzing *in vitro* eye fibroblasts and preadipocytes, but little is known about the *in vivo* modifications of the eye muscular cells and adipocytes.

**Our work follows 3 main axes:**

1. To demonstrate oxidative stress in GO muscular cells and adipocytes, to characterize the types of ROS (Reactive Oxygen Species) which are involved and to analyze the cellular antioxidant defenses.
2. To analyse the impact of the reduction of glucose supply, as a cause of oxidative stress, due to downregulation of caveolin-1 expression and Glut-4 expression.
3. To analyse the impact of the reduction of T3 supply, as a cause of oxidative stress, due to disruption of the balance between deiodinases (D) 2 and 3, D2 generating active T3 from T4, and D3 inactivating T4 into rT3.

Our approach mainly consists in a morphological analysis (optic and electronic microscopy, immunohistochemistry, immunofluorescence) of human eye muscles and fat from GO and control patients. The expression of the proteins involved in the pathogenetic mechanisms and the quantification of their mRNA are also analyzed by Western Blot and RT-PCR. The final goal of this study would be to limit the production of ROS and/or to increase the cellular antioxidant defenses for example by selenium supplementation, selenium being a cofactor of deiodinases. This work is made in collaboration with EUGOGO (European Group of Graves Orbitopathy).
Adaptive mechanisms of skeletal tissues

The skeletal tissues organization depends on several genetic, biochemical and mechanical factors. The adaptive capacity towards specific functions or environment is studied through different physiological, pathological or therapeutic models.

Hoxa2 and embryonic development: patterning and control of chondrogenic differentiation, in collaboration with R. Rezsöhazy – ISV

C. Behets, P. Deprez, B. Lengelé

This work addresses the relationship between the activity of Hoxa2 and chondrogenesis. Hoxa2 is a homeotic gene known to pattern embryonic territories, but its expression ceases once chondrogenic differentiation gets started. Using transgenic mice ectopically expressing Hoxa2 all along chondrogenesis, we associated the animal phenotype to human idiopathic proportionate short stature (Deprez et al 2012). Our analysis showed that this overall size reduction was correlated with a negative influence of Hoxa2 at the first step of endochondral ossification. However, the molecular pathways leading to such phenotype are still unknown. Using protein immunodetection and histological techniques comparing transgenic mice to controls, we showed that the persistent expression of Hoxa2 in chondrogenic territories provokes a general down-regulation of the main factors controlling the differentiation cascade, such as Bapx1, Bmp7, Bmpr1a, Ihh, Msx1, Pax9, Sox6, Sox9 and Wnt5a (Deprez et al. 2013).

These data confirm the impairment of chondrogenic differentiation by Hoxa2 overexpression. They also show a selective effect of Hoxa2 on endochondral ossification processes since Gdf5 and Gdf10, and Bmp4 or PthrP were up-regulated and unmodified, respectively. Since Hoxa2 deregulation in mice induces a proportionate short stature phenotype mimicking human idiopathic conditions, our results give insight for understanding proportionate short stature pathogenesis by highlighting molecular factors whose combined deregulation may be involved in such a disease.

Immunohistochemistry (A) and western blotting (B) on E13.5 βS-Hoxa2-lacZ (left) and Col2a1/Hoxa2-lacZ (right) vertebral bodies. (A) Runx2 was unmodified at E13.5 but reduced in pre- and hypertrophic chondrocytes as shown in E15.5 and E16.5 limbs, respectively. (B) Immunoblots are featured as follows (from top to bottom): the targeted molecule, β-actin as a control and the semi-quantification results using mean gray values. (a) P<0.05, (b) P<0.005 and (c) P<0.0005. Magnification 40x; scale bar = 0.1cm; n=5. (Deprez et al. 2013).
Stimulation of osteogenesis via the Wnt canonical pathway in oim/oim mouse (osteogenesis imperfecta), in collaboration with D. Manicourt – RUMA

C. Behets, D. Manicourt, M. Cardinal, T. Roels, S. Lafont

Sclerostin is an antagonist of the canonical Wnt pathway, a powerful bone formation pathway. Its inhibition by a monoclonal antibody has been shown to increase dramatically bone formation, bone mass and bone strength throughout the skeleton of ovariectomized osteoporotic rats (Li et al. 2005, 2008, 2009). Accordingly, this project wants to evaluate the anabolic potential of sclerostin inhibition in oim/oim mice whose phenotype is most similar to the clinical features of the human nonlethal form of type III osteogenesis imperfecta (OI) and characterized by small body size, low bone mass, skeletal fragility and spontaneous fractures. To address this issue, 4- to 7-week-old oim/oim and normal control mice are either treated or not with a sclerostin neutralizing monoclonal antibody for 4 to 8 weeks.

Preliminary morphological analyses show that the long bones of the oim/oim mice are less radiopaque and have thinner cortical bone than those of the normal animals. The treatment improves these features and the bones of the treated oim/oim mice have a similar radiological aspect than those of the normal untreated mice. The therapeutic effects are further assessed by clinimetry, photoabsorptiometry, plain X-rays (fractures count), peripheral quantitative computerized tomography and histology of calcified tissues.

New approaches for peri-implantitis treatment, in collaboration with M. Brecx - EMDS-SLUC

S. Toma, C. Behets, M. Brecx

Peri-implantitis is characterized by inflammatory lesions in peri-implant tissues associated with loss of supporting bone (Zitzmann and Berglundh 2008). Peri-implantitis is diagnosed when there is bleeding on probing (BoP) in addition to radiographic evidence of loss of supporting bone.

The primary etiological factor of peri-implantitis is the colonization of the implant surface by pathogenic bacteria organized in biofilm, similar to the etiology of periodontitis (Heitz-Mayfield and Lang 2010). Therefore the removal of the dental biofilm has become a major goal for the treatment of peri-implantitis.

A surgical approach combined with an effective elimination of the oral biofilm from rough implant surfaces is recommended to manage peri-implant disease. Nevertheless, at the moment, no consensus is reported about a more reliable decontamination procedure.

Four modalities of implant surface treatment will be investigated (plastic curettes, air-powder abrasive system, Ti-brush® and implantoplasty bur). The purpose of this project is to assess the influence of these different treatment modalities on (1) the removal of dental biofilm grown on titanium surfaces and (2) the biocompatibility of the instrumented titanium surfaces.
Anatomical research related to the development of new clinical tools

Actualization of anatomical knowledge is susceptible to improve diagnostic and/or therapeutic techniques.

**Segmental anatomy of the face and facial allografts**

A. Gerdom, B. Lengelé, C. Behets

The ultimate aim of face transplantation is to restore the functional relationships between the neuro-embryonic facial compartments and the underlying brain. Therefore, we revisited the segmental neurovascular anatomy of the face in order to provide a rationalized classification of facial allografts (FAGs). According to their neurovascular architecture, FAGs were classified as lower- (type I), mid- (type II), upper- (type III), hemi- (type IV) and full face (type V) allografts. Subtype A grafts included only soft tissues; subtype B involved their bone support. After selective injection of the facial artery, microradiographic and histological analyses of skeletal tissues showed that their arteriolar system was filled, in both cortical bone and bone marrow, through musculo-periosteal and perineural-endosteal anastomosing vessels.

In these composite tissue transfers, bone fragments are thus fully supplied by superficial vessels only. In contrast with previous assertions, they are prone to survive independently of the deep maxillary artery system. Acting as vascularized bone marrow allografts, they may play a role in long-term tolerance induction. As clinical application of the above described results, we were involved in the surgical planification and clinical inset of the world first face transplantation procedure carried out in Amiens (type I allograft, 2005) and in the Harvard face transplantation programme for midfacial (type II, 2009) and full face (type V, 2011) allotransplantations.

Similar studies are carried out for the advancement of ovarian, laryngeal and tracheal allotransplantation.

**Morphology**

This project includes three parts:

1. De novo biofilm formation on various titanium surfaces previously treated, as well as biofilm elimination on various titanium surfaces using different treatment modalities.
2. In vitro (SaOs-2 cells) evaluation of the biocompatibility of titanium implant surface previously treated: adhesion, proliferation, cell morphology and phenotypic expression.
3. Clinical and radiological evaluation of various surface modalities during surgical treatment of peri-implantitis. The clinical results obtained with the air-powder abrasive system showed some improvement of the peri-implantitis features, but did not succeed in reducing the biofilm activity (Toma et al 2014).

The other procedures are currently under study.

**Microscopic study of the vascular supply of the mandible after selective injection of contrasting agent in facial artery.** Contrast dots emphasize the filling of the endosteal (EO) and periosteal (PO) vessels. Higher magnification shows arteriolar vessels entering the Haversian bone canals. Left: Micro-CT, Top right: Microradiography, Bottom right: Methylene Blue staining of the corresponding area. Arrows: Periosteal vessels containing contrasting agent.
Bio-artificial face transplantation

J. Duisit, P. Gianello, B. Lengelé

Although providing a revolutionary reconstructive option for severely disfigured patients, wide-spread use of facial transplantation still has to face the need of an immunosuppressive treatment. This is a limitation for any organ transplantation, is particularly critical in Composite Tissues Allotransplantation (CTA), relying on both skin component and non-vital aspect of such procedures. We have no doubt that, even though not being a life-threatening condition, a patient’s life is dramatically impaired by loss of facial integrity.

In order to counteract immunosuppression, we are developing new strategies to provide full immunocompatibility between the graft from a deceased donor and the recipient. For this mean, we are using the surgical knowledge from facial transplantation legacy, treated with new technologies arising from Tissue Engineering: the principle is to remove ex vivo the entire cellular compartment, leaving the Extra-Cellular Matrix (ECM) intact. In vitro, the obtained ECM will be reseeded with stem cells harvested on the recipient, prior to in vivo transplantation. The animal models used are rat and pig, with different CTA models.

This project is guided by a strong partnership between MORF pole (Pr B. Lengelé - expertise in Facial Transplantation and Anatomy), CHEX pole (Pr P. Gianello - expertise in Organ Transplantation and Immunology) and Wake Forest Institute for Regenerative Medicine, USA (Pr G. Orlando – expertise in Organs Tissue Engineering). Dr J. Duisit, PhD student, is funded by Fondation Saint-Luc research grant.

SELECTED PUBLICATIONS


EQUIPMENT

- Anatomy laboratory
- Animal anaesthesia and dissection equipment (mice)
- Cell cultures
- Histology lab (cryosections, paraffin sections, immunohistology)
- Hard (calcified) tissues histology (resin embedding, sectioning, staining, microradiography)
- Electron microscopy techniques
- Peripheral Quantitative Computed Tomography (pQCT)
- Molecular biology (PCR, western blotting, electrophoresis)

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MORPHOLOGY

9. TOMA S., LASSERRE J.F., TAIEB J., BRECX M.C.
   - Evaluation of an air-abrasive device with amino acid
glycine-powder during surgical treatment of peri-
GREGOIRE V., ANG K., BUDACH W., GRAU C., HAMOIR
M., LANGENDIJK J.A., LEE A, LE Q.T, MAINGON P,
NUTTING C., O’SULLIVAN B., PORCEDDU S.V., LENGELE
B. - Delineation of the neck node levels for head and
neck tumors: A 2013 update. DAHANCA, EORTC,
HKNPCSG, NCIC CTG, NCRI, RTOG, TROG consensus

10. GREGOIRE V., ANG K., BUDACH W., GRAU C.,
    HAMOIR M., LANGENDIJK J.A., LEE A, LE Q.T,
    MAINGON P, NUTTING C., O’SULLIVAN B., PORCEDDU
    S.V., LENGELE B. - Delineation of the neck node levels
    for head and neck tumors: A 2013 update. DAHANCA,
    EORTC, HKNPCSG, NCIC, NCRI, RTOG, consensus
Regulation of body fluid homeostasis is of vital importance for all terrestrial organisms. In most mammals, the maintenance of the hydration status and normal plasma electrolyte levels critically depends on the appropriate handling of water and ions by the kidneys. This essential function involves specific transport systems operating in the epithelial cells lining kidney tubules. In the past two decades, our understanding of the transport mechanisms across biological membranes has substantially improved with the molecular identification and structural characterization of key proteins (channels, transporters, or their regulators) that are expressed in the nephron. The discovery of these molecules, initiated by classical biochemical approaches, has benefited from the molecular genetics analysis of rare genetic diseases. The analysis of such diseases has provided essential informations about the mechanisms of water and solute handling by the nephron. In turn, these insights improved the diagnosis, follow-up and treatment of renal diseases and associated conditions such as dehydration, electrolyte disorders, hypertension, growth retardation, nephrolithiasis, and progressive renal failure.

Understanding the nature and clinical relevance of fluid and ion transport across biological membranes has driven our research since the early 1990’s. Based on a multi-disciplinary approach including studies on patients, human and mouse genetics, and analysis of mouse and cellular models, we have investigated the transport mechanisms operating in various segments of the kidney, their regulation and ontogeny, and the pathophysiology of inherited renal disorders including tubulopathies and polycystic kidney disease. Insights obtained through these investigations are relevant for common conditions such as blood pressure regulation, kidney stones, progression of renal failure, and cardiovascular complications of renal diseases. The knowledge of transport mechanisms also led us to work on the molecular basis of water and solute transport across the peritoneal membrane, with the aim of improving peritoneal dialysis, a therapeutic modality for patients with end-stage renal disease.

Over the years, our studies benefited from fruitful international collaborations, leading us to initiate and participate in several European networks. These collaborations allow us to develop our projects using genome, transcriptome and proteome analyses; genome-wide association studies; conditional KO and randomly mutagenised mice; in translation with studies of human tubular disorders collected at the European level.
Research projects

1. Physiology of transport mechanisms:
   - Mechanisms and regulation of endocytosis in the proximal tubule
   - Salt and water handling by distal nephron segments
   - Mechanisms of differentiation of epithelial cells
   - Genome-wide association studies for renal function parameters

2. Pathophysiology of inherited kidney disorders:
   - Mechanisms and consequences of proteinuria
   - Pathophysiology of inherited forms of chronic interstitial nephritis, including uromodulin-associated kidney disorders
   - Gitelman syndrome and salt-losing tubulopathies
   - Clinical and genetic aspects of polycystic kidney and liver diseases

3. Mechanisms of water and solute transport across the peritoneal membrane:
   - Improving the efficiency of water and solute removal during peritoneal dialysis
   - Role and regulation of water channels in endothelial cells
   - Development of mouse models of peritoneal dialysis
   - Mechanisms of osmosis, characterization of alternate osmotic agents
   - Genetic influence on transport parameters.

Methodology and resources

- Transgenic mouse models, conditional knock-out, segment-specific invalidation
- Immortalized cell lines and primary cell culture systems
- Manipulation of gene expression in cell lines and primary cultures
- Promoter analysis (in silico, in vitro)
- In situ hybridization, advanced quantitative RT-PCR
- Immunoblotting, immunoprecipitation, and immunohistocytochemistry
- Intracellular distribution studies: subcellular fractionation, immunogold, biotinylation
- Transport studies in cells and native tissues (Ussing chamber)
- Mouse phenotyping: metabolic cages, special diets, pharmacology interventions
- Multisystemic phenotyping: cardiovascular, osmoregulation & thirst
- Biochemical profiling on dedicated platform optimized for rodent samples
- Development and automation of ELISA
- Water and solute transport in mouse model of peritoneal dialysis
- Biobanking: end-stage kidney samples (200+); kidney biopsies (500+); urine samples from isolated populations (N=6000); peritoneal biopsies (100+)
- DNA cohorts: ADPKD (N=300); rare inherited kidney disorders (N=200); renal transplant (N=300); peritoneal dialysis (N=800)
- EU-funded EUNEFRON, Genecure, EUROSPAN and EURenOmics consortia

SELECTED PUBLICATIONS 2010-2014


EU-funded programs:
EUNEFRON: www.eunefron.org
Genecure: www.genecure.eu
EUReNomics: www.eurenomics.eu

Other multi-centric programs:
NCCR Kidney.CH: www.nccr-kidney.ch
ERA-EDTA Working Group on Inherited Kidney Disorders: WGiKD

Scientific societies:
International Society of Nephrology: www.isn-online.org
American Society of Nephrology: www.asn-online.org
European Renal Association-EDTA: www.era-edta.org
American Physiological Society: www.the-aps.org
International Society for Peritoneal Dialysis: www.soc-nephrologie.org
Société de Néphrologie: www.fondation-du-rein.org
Académie Royale de Médecine de Belgique: www.armb.be

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- Pôles d’Attractions Interuniversitaires (PAI)
- Région wallonne
- Baxter Extramural Grant Program
The Pole of Pediatrics works on the development of liver regenerative medicine by means of cell therapy. Our research activity focuses on understanding and improving how hepatocytes and hepatic stem/progenitor cells participate in liver parenchymal regeneration. The developed research projects aim at i) characterizing the best candidate stem cells regarding their expansion stability, differentiation into mature hepatocytes and their therapeutic potentialities, ii) reconstituting their niche and cell interactions in 3D culture models, iii) investigating their biodistribution as well as engraftment in vivo using imaging and chimerism detection approaches and iv) evaluating their immunogenicity before and after transplantation. The laboratory of Pediatric Hepatology & Cell Therapy is isolating and studying stem cells from several adult tissues like bone marrow, umbilical cord, skin, pancreas and liver. The advanced equipped cell culture platform has large experience in optimizing their expansion and differentiation. Hepatic stem cells are also used to study drug metabolism and reproduce in vivo the replication of hepato-tropic viruses.

A second research program aims at understanding the immunological environment at birth and in the first years of life that triggers or protect infants against the development of allergy.

The Pole of Pediatrics has established multiple collaborations within IREC and other UCL Institutes, universities and biotech partners. The lab works in close collaboration with the Paediatric department, the Paediatric Clinical Investigation Center and the Tissue Bank of hepatocytes and hepatic stem cells of Cliniques Universitaires Saint-Luc.

In 2009, PEDI launched its spinoff “Promethera Biosciences” which develops the regenerative medicine of the liver from candidate stem cells identified in PEDI into clinics.
Liver cell and liver stem transplantation

Liver cell based therapy has been initially developed to correct hepatic functional defects in patients with inborn errors of metabolism. The approach is based on the transfer, via the portal route, of a cell suspension into the diseased liver. The success of the technique closely depends on the ability of transplanted cells to integrate and repopulate the target liver. Initial clinical trials have been performed using isolated adult hepatocytes, the smallest and pivotal functional entity of the liver, and demonstrated that the technique is feasible, safe, and able to bring the missing metabolic functions. The wide implementation and routine clinical use of liver cell transplantation was however hampered by increasing donor shortage and altered quality of hepatocytes (after primary culture & cryopreservation). The need to preferentially use fresh cells and the limited durability of functional improvement with mature hepatocytes led to consider alternative sources of cells, i.e. stem cells. The laboratory is also investigating the origin of the stem/progenitor cells isolated from the adult healthy liver. An extensive comparison with other fibroblastic liver cell populations such as stellate cells confirmed the singularity of our isolated mesenchymal cell population.

Improvement of the quality of cell suspension

The laboratory is coordinating the BruStem collaborative project funded by “Région Bruxelloise” and involving teams from UCL (1), VUB (2) and ULB (1). The project is aiming to develop the best cell suspension mixture that can be used as alternative to mature hepatocytes for the development of liver cell-based therapies. The cell mixture, which may contain mature hepatocytes, stem/progenitor cells and/or stellate cells, is currently investigated both in vitro and in vivo. The data obtained revealed significantly improved hepatocyte engraftment upon co-transplantation with hepatic stellate cells compared to transplantation of hepatocytes alone. The data from this research project may represent thus a promising strategy for the improvement of liver cell therapy efficacy and durability.

Improvement of the differentiation potential of the selected stem/progenitor cells

Liver cell-based assays are nowadays available, yet they have serious limitations. The lab is a partner of the Biowin Valostem project consortium (including Promethera Biosciences, UCL, FUNDP and UCB) which principal aim is the development of cell-based assays for early drug metabolism and toxicity screening using differentiated stem/progenitor cells (Figure 1).

Figure 1. Development of HepaScreen technology. 2D and 3D cultures of a human hepatic progenitor cell types suitable for in vitro pharmaco-toxicological studies.

Liver based metabolic diseases are caused by one enzyme/protein defect while the other metabolic functions and liver architecture, are normal. In those cases, it is postulated that a small number of normal cells, less than 5% of the total liver cell mass, could be sufficient to restore the metabolic function. We are currently exploring the ability of ADHLSC to exhibit such metabolic activities both in cells from expansion conditions as well as after in vitro differentiation.

For example, we have demonstrated ADHLSCs’ ability to synthesize glucose, metabolize ammonium into urea, metabolize phytanic acid (Figure 2), and conjugate bilirubin.

Figure 2. ADHLSC are able to metabolize VLCFA as evidenced by a decrease in phytanic acid concentration in culture supernatants after 24h incubation with 1µM phytanic acid. Healthy donors n=3, Refsum disease donor n=1.
Biodistribution, Engraftment and in situ differentiation of transplanted stem/progenitor cells

In order to evaluate new strategies to improve human adult liver progenitor cell engraftment, we are working on developing an organotypic model of the liver microenvironment in vitro by generating spheroids of human hepatocytes and human umbilical vein endothelial cells (HUVECs) in the presence or absence of extracellular matrix components such as Matrigel™ (Figure 3).

One of our lab main interests is to understand the mechanisms triggering hepatogenic differentiation of stem/progenitor cells. This issue would be useful for both studying post-natal liver regeneration as well as the development of liver cell-based therapies. We recently demonstrated that the transcription factor Sox9 may play a pivotal role in hepatocyte lineage development including for adult liver mesenchymal stem/progenitor cells. Further studies on the identification of pathways regulated by or regulating Sox9 are ongoing to gain insight into the molecular networks deeply controlling the hepatogenic differentiation.

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Biodistribution of ADHLSC was also assessed in patients. ADHLSC from large scale GMP compliant cultures (from hepatic stem cells bank) were efficiently labelled with 111-Indium DTPA radiotracer and infused through the portal vein. After acquisition of whole body imaging using SPECT imaging, we demonstrated that ADHLSC diffused homogeneously throughout the recipient liver and remained strictly within the targeted organ up to 5 days post-injection. We also noticed that no signal was observed in any other organs. The data of this study supports the previous preliminary clinical as well as the preclinical safety data towards the clinical use of ADHLSC in future cell-based therapy.
Human hepatocytes are difficult to obtain and maintain in culture, while other models such as HepAD38 (HBV) or HUH7 (HCV) present specific limitations. We developed two progenitor cell models, the first derived from the liver (ADHLSC), the second from the umbilical cord (UCMSC) that are easy to harvest and maintain in culture. Both showed interesting hepatocyte like differentiation potential. Recently we demonstrated that differentiated UCMSC were permissive to HBV and able to sustain the entire viral cycle. The aim of our current studies is to better understand the molecular steps underlying UCMSC’s susceptibility to HBV. We are currently investigating the involvement of NTCP transporter in the early events related to HBV entry in differentiated UCMSC.

**Immunogenicity of human hepatic cells**

The success of cell transplantation relies on several factors, including the ability of the transplanted cells to integrate the host tissue without being rejected by the immune system. In this study, we first demonstrated that human hepatic cells (hepatocytes and progenitors cells) are poorly immunogenic. In fact, we have shown that human hepatocytes can induce tolerance via the generation of IL-10 producing DC, leading to CD4 T cell hyporesponsiveness in vitro. In addition, we found that human liver progenitor cells can reduce the proliferative response of peripheral blood mononuclear cells (PBMCs) in a mixed lymphocyte reaction in vitro suggesting that these cells have immunomodulatory properties. However, human liver progenitor cells could still induce an immune response in vivo. We are therefore currently monitoring the immune response of patients treated with human liver progenitor cell based cell transplantation, in order to find potential early signs of immune tolerance or rejection. In addition, we are currently developing and validating a digital PCR assay allowing for the detection of donor/recipient chimerism in liver biopsies of the transplanted patients to determine whether we can correlate presence or absence of metabolic effect with presence or absence of donor cells and signs of tolerance or rejection.

**Evaluation of the anti-fibrotic properties of adult-derived human liver stem/progenitor cells**

Chronic liver diseases represent the 7th cause of mortality worldwide and may have different etiologies including viral, autoimmune, drug induced, cholestatic and metabolic diseases. Liver fibrosis, which can evolve towards cirrhosis, is characterized by an accumulation of extracellular matrix in the liver parenchyma, a consequence of an activation of hepatic stellate cells (HSC) into myofibroblasts.

Orthotopic liver transplantation remains the only treatment option nowadays. However, this procedure is invasive and organ shortage is a daily reality. A promising perspective is currently emerging for the treatment of liver fibrosis: mesenchymal stem cells (MSC) injections. A regression of liver fibrosis was observed in animal models but the exact mechanisms are not yet fully understood. Clinical trials are ongoing with MSC derived from the human bone marrow.

The purpose of this research project is to evaluate the potential of Adult-Derived Human Liver Stem/progenitor Cells (ADHLSC) to inhibit the activation of HSC into myofibroblasts. The influence of ADHLSC is currently studied in vitro (co-culture systems) as well as in vivo by transplanting a mouse model of liver fibrosis. The in vitro data demonstrated the ability of ADHLSC to inhibit the proliferation of activated stellate cells by blocking their cycle and modulating their secretion of anti-fibrotic molecules. We are currently assessing in vivo, if this modulation of HSC activation may be critically related to an efficient targeting of liver fibrosis.

**Use of stem/progenitor cell cultures as a model for the study of HBV and HCV infection events**

HBV and HCV infection are global health problems. Both viruses have a hepatocyte tropism. In vitro models of HBV and HCV infection are crucial to better study the physiopathology of viral infection, which is needed for the further development of specific novel drugs against the disease. However, adequate models are currently lacking. Hepatocyte tropism of HBV and HCV seems to be related to the existence of liver-specific viral receptors limiting viral entry.

**Figure 5** Whole body images after infusion of labelled ADHLSC. (A) Whole body anterior image acquired at time of infusion. (B) Whole body anterior image acquired 120 h after infusion.

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Pediatric immunology

Early biomarkers of future allergic development

The CRISTALL cohort comprises 200 children that we have been monitoring from birth to age five to determine whether we can unravel early signs that a child will become allergic. A classification of the children into “allergic” and “non-allergic” groups was performed at 18 and 36 months. Allergic patients with positive plasma levels of allergen specific IgE and/or skin prick tests were considered “atopic”. The study so far has shown an influence of acetaminophen intake on the development of allergy. In addition, we have been able to show differences in the cytokine profiles of cord blood mononuclear cells from allergic and non-allergic patients as well as from atopic and non atopic patients based on the classifications established at 18 and 36 months. Finally, gene expression analysis of PBMCs collected at 6 months using microarray technology has allowed us to establish a signature of 52 genes that can predict whether a child will be allergic by age 3 with 82% accuracy, 67.7% balanced classification rate (BCR) and a stability of 0.753.

Biomarkers of tolerance or rejection in liver transplanted children

Although the liver is a well-tolerated organ, some children can present chronic rejection or progressive liver fibrosis on long-term follow-up. Blood biomarkers that can efficiently predict whether a patient is at risk for these complications are still lacking and liver biopsies, which are invasive, remain the only way to diagnose them. In this project, established as collaboration between the PEDI unit and ImmuneHealth, we are monitoring pediatric liver transplanted patients in order to try to correlate blood biomarker(s) of tolerance or rejection with the results of the hepatic biopsy. More than 200 samples are already available.

Liver Immune Mediated Fibrosis post liver transplant

Progressive liver fibrosis and non-specific inflammatory anatomo-pathological changes are seen in most children within 10 years following liver transplantation. The basis for these non-specific inflammatory changes and consequent fibrosis is presumed to be immunological, the reasoning being that these changes are partially reversible with increased immune suppression and associated with the presence of HLA antibodies. In addition, the immunosuppressive regimen currently used is thought to cause a change in the ratio of the pro-inflammatory Th17 cells and anti-inflammatory T reg cells. The presence of HLA antibodies, imbalance of Th17:Tregs in the setting of the inflammatory milieu of grafted liver may cause a stimulation of the hepatic stellate cells that secrete collagen type I and cause fibrosis. The aims of this study are: 1) to elucidate the immunological basis of liver graft fibrosis and 2) to understand the mechanisms of stellate cell activation and its implication in the development of fibrosis.

Accredited Bank of Hepatocytes and Hepatic Stem Cells

We routinely isolate hepatocytes coming from human livers. These hepatocytes are thereafter either transplanted or cultured to generate hepatic stem cells. Both types of cells are used to cure children suffering from liver diseases. During the years 2011 and 2012, our services conducted over nineteen isolation processes. Since the Bank’s inception, 11 patients have been transplanted with hepatocytes and 3 others with hepatic stem cells expanded in the tissue bank. Beside hepatocytes, human stellate cells are also isolated for research.

Pediatric Clinical Investigation Center

The Pediatric Clinical Investigation Center brings together the strengths of all paediatric subspecialties aiming to provide an efficient platform for high quality clinical investigations in accordance to ICH-GCP rules. The Center currently manages more than 20 paediatric clinical protocols in every field of the Paediatrics, from phase I to IV.
**SELECTED PUBLICATIONS**


3 | Sana et al. Adult human hepatocytes promote CD4+ T cell hyporesponsiveness via interleukin-10 producing allogeneic dendritic cells. *Cell Transplant.* 2013 Apr 12


5 | Khuu et al. Adult human liver mesenchymal stem/progenitor cells participate to mouse liver regeneration after hepatectomy. *Cell Transplant.* 2012 Dec 4


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**FUNDING**

- REGENESTEM : PPP Région Wallonne-PEDI-PROMETHERA Biosciences : “Cellules progenitrices pour le traitement des maladies acquises du foie”
- CRISTALL : PPP-Région Wallonne-PEDI-GSK : “Détermination des critères de risque d’allergie chez le nourrisson et stratégie de prévention”
- VALOSTEM : projet Biowin Région Wallonne
- BRUSTEM : Région Bruxelloise ; UCL-ULB-VUB : “Regenerative medicine of the liver using a mixture of mature and immature hepatocytes with MSC or with hepatic stellate cells”
- IMMUNE FU : CWALity Région Wallonne : PEDI-PROMETHERA Biosciences : “Evaluation de la réponse individuelle d’un produit de thérapie cellulaire hépatique dans le but d’optimiser le traitement immunosupresseur concomitant”
- IMTOX : Programme de recherche collective Région Wallonne : “Evaluation de l’immunotoxicité de nouveaux traitements”
- FNRS CC : “Suivi immunologique post-infusion”
- FNRS CDR : Improving progenitor cell engraftment
- FNRS FRIA : “Defining the role of asialglycoprotein receptor in determining hepatitis B virus hepatotropism”
- FNRS Télévie : “VHC et précurseurs hépatiques”
The importance of respiratory diseases for public health is increasingly recognized. This ranges from lethal disorders such as lung cancer or severe COPD which continue to increase despite current treatments, to chronic diseases that affect a large part of the population such as asthma, sleep apnea, rhinitis or atopic dermatitis (WHO predicts allergy will affect 50% of the population by 2020).

Our research pole has been focusing on the study of:

1. Physiology and pathology of breathing and sleep.
2. Mucosal immunobiology and inflammation of the airways and skin.
Physiology and pathology of breathing and sleep:

(a) pitfalls of CPAP treatment in sleep apnea

B. Mwenge, G. Desuter & Ph. Rombaux

Support : Financement extérieur (firme Imthera).

Obstructive sleep apnea (OSA) represents the paradigm of the complex interactions between breathing and sleep. Some people develops asphyxia when asleep, resulting in sleep destruction and reduced survival. Treatment with continuous positive airway pressure applied all and every night normalizes sleep and breathing as well as survival. However, a third of patients is unable to accept/tolerate the treatment.

Firstly, the effect of a specific training of oropharyngeal muscles on OSA syndrome is currently evaluated, as well as assessment of compliance to specific measures in postural OSA.

Secondly, new treatments are needed for patients with obstructive sleep apnea intolerant to CPAP. A new modality of treatment consists of electro-stimulation of the hypoglossal nerve either on its proximal main trunk or its terminal branches, via an implanted stimulator and electrode with internal battery or via an implanted stimulator using transdermic electrical transmission. Our studies are the first world assessments of this type of treatment. The initial results show a significant improvement in a large majority of patients, with a benefit persisting several days after stimulation has been stopped (1).

Third, interactions between non-invasive ventilation and sleep are studied, in patients with respiratory failure due to restrictive or obstructive disorders and in obese patients with hypoventilation syndrome. Both the effects of sleep on respiratory failure and the effects of non-invasive ventilation on breathing and sleep are assessed (2).

(b) Drug delivery to the lung

G. Reychler, Ph. Rombaux, F. Jamar & G. Liistro.

Support : Diffusion Techniques Française, SSS-IREC (mandate G. Reychler).

Drug delivery to the lung has been mainly studied in spontaneously breathing patients and in patients who are mechanically ventilated through endotracheal tubes, while nebulization through tracheostomy in spontaneously breathing tracheostomized patients has been poorly studied. We first showed that unvented jet nebulizer with a corrugated piece of tubing delivered a higher amount of drug compared to unvented jet nebulizer alone or open vent jet nebulizer (figure 1). We also showed that the inner cannula should be removed before the nebulization in order to increase the amount of drug delivered.

Figure 1 Improving drug delivery to the lung. Unvented jet nebulizer with a corrugated piece of tubing was shown to deliver a higher amount of drugs compared to the other settings.

The impact of different nebulization devices and modalities are studied (3), both on the emitted dose and on lung deposition by using nuclear imaging and pharmacokinetics. A unique research track concerns the study of deposition of nebulized drugs into sinonasal cavities, for the topical treatment of chronic rhino-sinusitis.

Finally, exercise capacity is assessed in patients with chronic airflow limitation and undergoing pulmonary rehabilitation.
Mucosal immunology and inflammation in the airways and the skin: altered function of the respiratory epithelium and dendritic cells


Asthma and chronic inflammatory diseases of the airways (chronic rhino-sinusitis, COPD) or skin (dermatitis) are very common conditions that affect many people usually throughout lifetime, although with a highly variable clinical expression.

Our first focus assessed in the bronchial epithelium expression of the pIgR (polymeric immunoglobulin receptor), the receptor transcytosing into secretions IgA, the main immunoprotein protecting mucosal surfaces against inhaled materials. We showed that the impaired bronchial expression of the pIgR correlates with disease severity. In addition, this epithelial defect was recapitulated in the in vitro reconstituted bronchial epithelium from such patients, as a consequence of TGF-β upregulation. In the upper airway, pIgR downregulation is also observed in a subset of patients with chronic rhinitis, namely allergic rhinitis and eosinophilic rhino-sinusitis (4), suggesting an unexpected link between pIgR downregulation and eosinophilic/Th2 inflammation.

In patients with allergic rhinitis and asthma, we previously showed that myeloid dendritic cells (DCs), which are rapidly recruited upon allergen exposure, display impaired expression of IL-10 and type I signals (IL-12) and induce in allogeneic CD4+ T cells a preferential Th2 and Th17 polarisation. However, plasticity of these cells is suggested by showing that IgA may inhibit the pro-inflammatory programming of DCs upon endotoxin and IFN-γ activation, by interfering with STAT1 phosphorylation (5). Understanding how the airway epithelium and DCs are aberrantly programmed in asthma (Fig. 2) and chronic lung disease should provide new therapeutic strategies to these disorders.

Occupational asthma represents another phenotype of the disease (10%) and a unique model to study the epidemiology, role of diagnostic procedures (6) and critical biomolecular pathways for asthmagenesis, as most patients have persisting disease despite complete avoidance of the offending allergen.

Patients with allergic contact dermatitis to corticosteroids have been reclassified, with important consequences for clinical management. In addition, detailed tissue immunophenotyping has been carried out in collaboration with L. Dumou-tier (DDUV), who showed that skin infiltration is dominated by Th2-biased T cells and includes IL-4 producing γδ T cells (7). This unique observation is the ground of further investigation with other contact allergens.

Figure 2 Hypothesis of conditioning of dendritic cells by the airway epithelium in asthma. The epithelium, activated by environmental exposure to proteolytic allergens such as dust mite, releases cytokines that activate DCs. This crosstalk could recapitulate the aberrant function observed in these cells from patients with atopic asthma (5), which are primed to induce the polarisation of CD4+ T cells into Th2 (producing IL-13) and Th17 cells.
Novel biological targets in lung cancer: the FAK pathway in SCLC.

F. Aboubakar, M. Lecocq & S. Ocak


Small cell lung cancer (SCLC) is the most aggressive subtype of lung cancer, with a five-year overall survival as low as 5%. Molecular determinants of SCLC behaviour are still poorly understood and this deficiency has translated into the absence of targeted therapies.

We identified 70 regions of copy number gain and 55 regions of copy number loss (8). Using molecular pathway analysis, we found a strong enrichment in these regions of copy number alterations for 11 genes associated with the focal adhesion pathway. We verified these findings at the genomic, gene expression, and protein level. Focal Adhesion Kinase (FAK) was commonly expressed in SCLC tumors (8) and constitutively phosphorylated in SCLC cell lines. Those were poorly adherent to most substrates but to laminin-322. Inhibition of phosphorylation of FAK at Tyr397 by a small-molecule inhibitor, PF-228, induced a dose-dependent decrease of adhesion and an increase of spreading in SCLC cell lines on laminin-322 (Fig. 3). Cells that tended to spread also showed a decrease in focal adhesions, as demonstrated by a decreased vinculin expression.

Based on these results, we hypothesized that FAK activation plays a key role in SCLC cells to develop their highly invasive behaviour and that FAK may represent a good target for therapeutic interventions. Therefore, our current research focuses on the three following specific aims. We propose to determine (a) a role of FAK in SCLC progression by testing cell motility, invasion, proliferation, cell cycle, and apoptosis in cells where FAK has been downregulated or blocked. The hypothesis is that inhibition of FAK will decrease cancer cell motility, invasion, and proliferation, as well as increase apoptosis; (b) signalling events downstream of FAK responsible for the main phenotypic changes observed; and (c) the mechanisms responsible for increased FAK activity in SCLC cells. The hypothesis is that aberrant expression or mutation of TP53 and/or NFkB, and/or stimulation of G-protein-coupled receptors by neuro-mediators play a role in increased FAK activity in SCLC. Understanding the role of FAK in SCLC may provide greater insight into the molecular steps leading to SCLC progression and, ultimately, may justify the development of FAK-targeted therapeutic strategies to reduce mortality from SCLC.

Figure 3  Effect of FAK inhibition on cell phenotype in vitro. NCI-H69, NCI-H146, NCI-H209 cells were plated for 12h on laminin-332-coated dishes and then treated with 10μM PF-228 or DMSO for 24h. Pictures of unstained cells were taken after 24h treatment and show that SCLC cells treated with PF-228 spread on laminin-332, losing their rounded shape and presenting extended processes. Pictures magnification: x20.

**EQUIPMENT**

- Experimental cellular bench (primary cultures of lung or blood cells);
- Chromatography for IgA purification
- Model of in vitro reconstituted human bronchial epithelium upon air liquid interface
- Experimental histology (paraffin sections, immunohistology)
- Lung tissue biobanking
- Lung function for humans (including inhalation challenge rooms, CHU Mont-Godinne) and for small animals
- In-patient studies of sleep
- Sputum induction & analysis
- Bronchial endoscopy with bronchoalveolar lavage and endo/transbronchial biopsies
- Skin allergy testing
- Clinical databases
SELECTED PUBLICATIONS


Very much in contrast to a common belief, rheumatologists take care not only of elderly patients suffering from the consequences of aging of their musculoskeletal system but also – and nowadays mainly – of (very) young adults with inflammatory and auto-immune diseases, such as rheumatoid arthritis, systemic lupus erythematosus or systemic sclerosis, which may considerably impact not only their quality of life but also their life span.

Over the last decade, our interest has moved towards translational and clinical research in these diseases. Clues to success have been our databases, the systematic use of an appropriate clinimetrics, the possibility to harvest synovial tissue by mini-arthroscopy or PBMC from active patients through leucopheresis and the set-up of an European network of clinical researchers willing to collaborate in investigator-initiated randomized trials.
Clinical and molecular effects of new rheumatoid arthritis therapies

P. Durez, I. Faille, B.R. Lauwerys, C. Galant, A. Nzeusseu Toukap, J. Ducreux

Rheumatoid arthritis (RA) is a disease of unknown etiology, affecting 1% of the population in Western countries. The disease leads to joint destruction, and functional disability when insufficiently treated. Although the progression of the disease displays significant variability in terms of severity, strong evidence indicates that early aggressive therapy is necessary in order to avoid structural damage and loss of function.

Biologic therapies that target inflammatory cytokines, such as TNF-\(\alpha\) or IL-6, have greatly improved the care of RA patients; however, there is no clear consensus on when biologic therapy should be initiated. When a biologic agent is added early on to first-line treatment with methotrexate (MTX), significantly greater improvements in disease activity status, rates of remission and radiographic outcomes are observed compared to MTX alone. Unfortunately, not all RA patients respond to current biologic therapies, and responses are not always maintained, suggesting that there are alternative drivers of RA pathogenesis that might serve as promising therapeutic targets.

Taking advantage of our large recruitment of patients in sponsored phase I to III clinical trials (1, 2), we have developed several academic protocols in early RA and in refractory cases. Among them, TOMERA compares head to head therapy with tocilizumab (anti-IL6R antibody) or MTX in early naïve RA patients, with induction of clinical remission as primary outcome. From 2014, we will be supported by CAP48 (RTBf) for the recruitment of a large, nation-wide cohort of young patients with early arthritis, in order to evaluate the implementation and success rates of remission-inducing therapeutic strategies in Brussels and Wallonia.

In the context of TOMERA, we also collected synovial biopsies in RA patients, prior to and 12 weeks after initiation of therapy, using a needle-arthroscopic procedure (Figure 1). These samples enabled us to study the molecular effects of MTX and tocilizumab therapies, using high-density microarrays, and compare them to the effects of other biologics that we had analyzed in previous studies (adalimumab, a TNF-blocking agent, and rituximab, a anti-CD20 depleting antibody). Strikingly, we found that methotrexate, tocilizumab and rituximab display highly similar molecular effects in the RA synovium, i.e. a decrease in the expression of genes involved in T cell activation. By contrast, the molecular effects of TNF blockade are characterized by a strong decrease in fibroblast cell proliferation (3). These results lead to the identification of T cells and fibroblasts as the main therapeutic targets in RA, and open new therapeutic and theranostic perspectives for the care of the disease.

Figure 1 Synovial biopsies are harvested in ambulatory patients by needle-arthroscopy. The biopsy samples are used in histological, immunohistochemistry, proteomic and transcriptomic experiments. Synovial fibroblasts are isolated and cultured.

Imaging and molecular biomarkers in systemic sclerosis: a tissue-based approach

M. Stoenoiu, C. Galant, P. Durez, B.R. Lauwerys, A. Nzeusseu Toukap, F.A. Houssiau, in collaboration with F. Lecouvet

Systemic sclerosis (SSc) is by far the most devastating systemic rheumatic disease. The cause of SSc is elusive, and the mechanisms underlying inflammation and fibrosis are largely ignored. No specific treatment is available. The severe form of the disease is characterized by intense inflammation, followed by a scarring (so-called fibrotic) process, involving not only the skin and the subcutaneous tissue but also other major organs, such as the lungs, the oesophagus, the gut and the kidneys, thereby
leading to multiple organ failure in the worse case scenario. The presence of palpable tendon friction rubs (TFRs) is associated with disease activity, and is a significant predictor of involvement of internal organs and increased mortality. The genesis of TFRs is not clearly understood. Together with TFRs, tendon and joint involvement is common in patients with SSc and is frequently disabling.

We hypothesised that TFRs, tenosynovitis and synovitis are an important part of the pathophysiology of the disease process, and are underestimated by clinical evaluation. Since TFRs are predictive of diseases activity, we aimed to understand the genesis and the anatomical substrate of TFRs.

Firstly, the frequency of tenosynovitis and synovitis involvement was assessed in consecutive patients suffering from diffuse SSc. Both ultrasound and magnetic resonance imaging detected tenosynovitis in half of the patients and synovitis in a quarter of patients (Figure 2). Flexion contractures and increased skin thickness explain the high rate of false negative clinical diagnosis of arthritis. Moreover, the presence of deep connective tissue infiltrates, detected by imaging is associated with the presence of TFRs, which might be a surrogate marker for more extensive and deeper involvement of connective tissue (4).

Second, imaging findings were compared with histology. Biopsies of synovial tissue from joint and tendon sheath were analysed. Both inflammatory and non-inflammatory changes are present in the synovium from SSc patients including increased vascularity, inflammatory infiltrates, collagen deposition and fibrosis. This pattern is distinct from that described in lupus, rheumatoid and psoriatic arthritis. In long-standing arthritis, onion-like skin appearance of vessels, collagen deposits and fibrosis predominate. In both recent and long-standing arthritis, the synovium is invaded by T cell lymphocytes (CD3), macrophages (CD68) and immature vessels (WT1).

Finally, our current project is aimed at finding new molecular pathways playing a pathogenic role, which could then be specifically targeted. Total body imaging performed in patients with extensive disease will allow us to score skin and musculoskeletal involvement and to validate new imaging biomarkers. Deep subcutaneous and synovial biopsies will be performed and analysed using high-density techniques in order to unmask molecular signatures, i.e. genes and proteins that are either over-expressed or down-regulated. The relevance of these inflammatory and fibrotic pathways will be further tested in animal models and cell cultures in collaboration with KUL colleagues.

Clinical developments in the care of patients with systemic lupus erythematosus

F.A. Houssiau, G. Depresseux, B. R. Lauwerys

Systemic lupus erythematosus (SLE) is a prototypical autoimmune systemic disease with potentially severe and life-threatening manifestations such as glomerulonephritis (Figure 3), cytopenias and central nervous system involvement. Our department has gained an internationally recognized expertise in the management of SLE thanks to optimal clinical care and to basic (see next paragraph), translational and clinical research, mainly in the field of lupus nephritis (LN). This success story stems from a well organized multidisciplinary Lupus Clinic (unique in our country), from access to prospectively collected and electronically computed
clinical data, from availability of serum, lymphocytes, and kidney biopsies obtained from newly diagnosed untreated patients and from numerous laboratory and clinical collaborations, in particular through national and international networks which we created, such as the Euro-Lupus Group and the Lupus Nephritis Trials Network (www.lupusnephritis.org). After the Euro-Lupus Nephritis Trial (comparing two doses of intravenous cyclophosphamide as induction treatment) (5) and the MAIN-TAIN Nephritis Trial (comparing azathioprine and mycophenolate mofetil as maintenance therapy) (6), we are now launching a third European multicenter randomized clinical study aimed at testing the potential value of rituximab in refractory LN.

In 2013, special attention was paid to the long-term prognostic value of subsetting proliferative LN into different pathological subclasses, taking advantage of the Louvain Lupus Nephritis Inception Cohort. We came to the unexpected conclusion that the current classification of LN has no real prognostic value (7), thereby indicating that biomarkers would be most welcome. In this respect, we recently found that elevated soluble IL-7 receptor (sIL7R) concentrations are strongly associated with nephritis in SLE patients (8). In a longitudinal study, we could recently confirm that serum sIL7R titers not only are commensurate with the extent of renal disease activity but even predict the occurrence of renal flares (Lauwerys BR et al., manuscript in preparation).

New insights in the pathogenesis of systemic lupus erythematosus

B.R. Lauwerys, F.A. Houssiau, J. Ducreux, A. De Groof

A majority of SLE patients display a spontaneous expression of type I interferon (IFN)-induced genes in circulating mononuclear cells and peripheral tissues, and type I IFNs play a role in SLE, via the sustained activation of autoreactive T and B cells necessary for the production of pathogenic autoantibodies. There are several types of type I interferons: IFN-α (13 subtypes), -β, -ε, -κ and -ω, and it is not clear which of them are involved in the pathogenesis of the disease. We recently demonstrated that administration of IFN-α kinoid, a therapeutic vaccine that induces the production of a polyclonal antibody response neutralizing all IFN-α subtypes, results in a significant decrease in the expression of type I IFN-induced genes in whole blood RNA samples, harvested after administration of the drug (Figure 4). Interestingly, patients with an elevated IFN signature at baseline produce higher amounts of anti-IFN antibodies after vaccination, and display a stronger neutralization of their IFN signature compared to patients with a low IFN signature (9, 10).
Serum samples, harvested in SLE patients included in this study, contain significant levels of IFN-α, IFN-β or IFN-ω. Intriguingly, only serum IFN-α displays a significant correlation with the expression of type I IFN-induced transcripts in whole blood cells. By contrast, serum IFN-β is associated with mucocutaneous flares of the disease. Taken together, these results show that different mechanisms are involved in the pathogenesis of SLE, according to the site of inflammation, thereby opening very new avenues for future research (tissue-based approaches).

Several mechanisms contribute to the production of type I IFNs in SLE, in particular toll-like receptors (TLR) and other danger-recognition molecules, such as PKR (interferon-inducible RNA dependent protein kinase). We recently identified a SNP in the coding region of TLR3 that is involved in the production of anti-Ro/SSA antibodies in SLE, through an increased antigen-presentation activity of dendritic cells, in response to UV irradiation. The association of this new TLR3 SNP with susceptibility to SLE is currently tested in a large cohort of SLE patients and controls in the context of an European collaboration. We also generated PKR -/- SLE-prone mice, in which we are evaluating the role of PKR in the development of the disease, with a strong focus on the effects of the molecule on dendritic cell functions.

SELECTED PUBLICATIONS


1. STRUCTURE

“The Centre de Technologies Moléculaires Appliquées (CTMA - Centre for Applied Molecular Technologies)” mixed academic-clinical-military biotechnological platform mutualizes the resources of three partners: IREC/UCL (Université catholique de Louvain), CUSL (Cliniques universitaires St Luc) and Belgian Defence (BE-MOD).

CTMA is indeed the reference biotechnological platform (genetics and molecular genetics) for IREC/UCL. Accordingly, CTMA develops proprietary research while supporting a large spectrum of IREC-related research activities. CTMA has also a strong clinical activity in the field of genetics and molecular genetics. This activity is carried out to support the medical activity of the academic hospital CUSL.

Finally, CTMA hosts several research projects and activities for the BE-MoD. As Such, CTMA is the “Biothreat control unit of Defence of Laboratory Department (DLD)” and is therefore specifically named DLD-Bio, from there its full acronym CTMA/DLD-Bio.

Figure 1 Shows the working architecture of this integrated CTMA/DLD-Bio platform with NATO-, EU-, ESA-agencies or organizations, BE governmental authorities, academic-, industrial- and military-partners.

According to its integrated working structure, CTMA/DLD-Bio hosts at the same location researchers from the Belgian Defense (MOD) and UCL/IREC/CTMA as well as the clinical staff working for the academic hospital (Cliniques universitaires St Luc - CUSL). Accordingly, this biological platform benefits from a genetics-dedicated infrastructure, sophisticated emerging technologies developed through successive projects, expensive equipment acquired to fulfill its academic, military and clinical missions. Taking advantage of this mixed academic-clinical-military platform and associated multidisciplinary activities, CTMA/DLD-Bio has progressively developed a strong and extensive clinical, academic and military national and international networking leading to several fruitful multinational partnerships and projects and also elective bilateral partnerships. Table 1 shows the funding of CTMA.

2. MISSIONS

CTMA/DLD-Bio main missions are (See Figure 2):

- Dual military-civilian R&D activities: New emerging technologies, including nanotechnologies and operational tools enabling better detection and protection against known and unknown threatening infectious agents; Low/high density gene expression profiling (biomarkers in malignant and inflammatory diseases); Genome characterization by re-sequencing. Related signal processing, machine learning and biostatistical analysis. Related expertise in Security: study of Belgian and European preparedness and responses to B-threats; scientific, technical and operational support to Belgian Defense Laboratories (CBRN);

- Clinical activities: Diagnostic applications and operational deliverables (infectious and genetic diseases, pharmacogenomics & new biomarkers, array, pyro-sequencing, quantitative real-time PCR...). More than 2000 tests per year are performed for the benefit of patients at the CUSL;
• Service activities: CTMA/DLD-Bio offers expertise and technological support to UCL-researchers and more particularly to IRE-researchers; CTMA has also actively developed service activity for industry such as the fungal biomass production for the preparation of vaccines (the latter activity is located at UCL/CT-MA-MYCO premises –Louvain-La-Neuve);

• Academic courses in Molecular Biology, Genetics, in Statistical Genetics & Multivariate Data Analysis and CBRN topics and Training to Defense units.

3. RESEARCH ACTIVITIES

The global research activities of CTMA is integrated into a global R&D matrix which interconnects each project to all the other in terms of technologies and/or expertise and/or know how. CTMA benefits directly from Belgian Defense grants but is also reinforced and supported by several R&D grants obtained at regional (Walloon Region, BioWin and Marshall Plan), federal (BELSPO) or international (EC, EDA and ESA) level.

Figure 3 shows the strong interrelationship of the whole research activity of CTMA and the link with national and international organizations (for funding and cooperation) in order to spread costs, to mutualize the benefits and to decrease the failure rate. Table 2 presents doctoral thesis works presented in 2012 and on-going thesis works.

Table 2 : Thesis works carried out in CTMA

A) CTMA/DLD-Bio research studies within the frame of the Belgian Defense Research Program

MED 20 - Profile of genetic bacterial resistance to beta-lactamases and aminoglycosides.

(2010-2013) (478 k€)
Yann DECACCHE
Cooperation: Department of Epidemiology and Hygiene (Belgium Ministry of Health), Military Medical Academy (Sofia, Bulgaria), Spitalul Clinic de Urgenta (Bucharest, Romenia).

The detection of bacteria resistant to aminoglycosides relies on the evidence of the presence of specific enzymes destroying antibiotic. This project exploits the theoretical, practical and technical knowledge acquired in prior research works in order to implement rapid DNA-based tests for detecting β-lactams and aminoglycoside-resistant microorganisms.
Figure 4: Decision three of ESBL - Molecular detection of the determinants of the ESBL bacterial resistance through identification of resistance enzymes that undergo specific mutations leading to the expression of ESBL phenotype

CTMA involvement: (1) Phenotypic and genetic characterization of clinical strains documented with resistance patterns (2) development of rapid testing by pyrosequencing.

DLD 04 - Development of a mobile platform for simultaneous identification of main pathogenic biological agents under operational conditions (bacterial agents of Class A CDC and WHO list of 12 bastards).

(2012-2015) (688 k€)
Cathy DELCORPS, Anne-Sophie PIETTE, Stéphane VAN CAUWENBERGHE

This study develops a portable microarray detection platform of all biological agents during a single test, using patented sequence (CTMA/DLD-Bio WO/2005/090596). Previous studies have developed an operational identification of hazardous biological agents capacity, but often detecting only one agent at a time. In the absence of clinical or epidemiological guidance, the identification of biological agents is done sequentially, which may require the completion of dozens of tests. This leads to very high expenses and waste of time, limitation in sample analysis rate according to the expending number of analyses required and the risk of contamination. This study combines the Rolling Circle Amplification (RCA) with the tridimensional microarray Pamgene ® for the development of tests enabling simultaneous identification of main biological agents on a single platform and a single multiplex assay.

DLD05 - Rapid detection and characterization of micro-organisms responsible for infections orthopedic

(2013-2016) (566 k€)
Catherine DUMONT, Elodie CARLIER

The aim of this project is to validate the diagnostic value of transcriptomic and/or proteomic profiles of synovial material in early inflammatory or infectious disease (arthritis). It is based on preliminary data showing that gene expression profiles in synovial biopsies from patients with arthritis are able to discriminate the samples according to the underlying disorder. The large-scale confirmation of these data after will lead to the development of a prototype of a diagnostic tool to be used in routine rheumatology practice.

B) Research within the frame of the European Space Agency (ESA)

B-LiFE- Biological Light Fieldable Laboratory for Emergencies – Phase 1 Feasibility Study

(2012-2013) (Total consortium : 399 k€ / CTMA : 220 k€)
Jean-Luc GALA, Jean-Paul MARCEL, Catherine DUMONT, Jean-François DURANT, Pierre-Alain FONTEYNE
Consortium: CTMA (Coordinator), VUB – ETRO (Brussels), SES TechCom (Bezdorf – Grand Duchy of Luxembourg)
The project is based on the development of a light fieldable laboratory integrating several space based technologies that are critical to provide rapid in-field operational capabilities (satellite communication, Global Navigation Satellite System or Global Positioning System (GNSS/GPS) and Earth Observation (Figures 4a-b and 5). The goal is to optimize communications between the field teams and the command and control centers to provide geological information and maps of the crisis area in order to monitor the outbreak evolution, and to georeference the data samples while keeping real-time contact between the medical/biological and sampling team. CTMA/DLD-Bio technologies developed for the B-LiFE project enable us to develop operational services that could be used in a wide range of endemic or epidemic biohazards.

CTMA develops the light fieldable biological laboratory and manages the project. The Vrije Universiteit Brussel provides expertise in remote sensing and image processing. The consortium is completed with SES TechCom, one of the largest satellite operators, based in Luxembourg.

Phase II / Demonstration Phase aiming at delivering a demonstrator at the highest Technology Readiness Level (TRL 9) is under preparation to start in 2014.

Figure 6: B-LiFE - Transmission flow between the Sampling Team, the CTMA Crisis Center and the Mobile Laboratory

CTMA is leader of WP1-Innovation Management Practices, contributes to WP2-Operational Needs and Innovation Uptake and organizes and/or animates roundtables.

PRACTICE - Preparedness and Resilience against CBRN Terrorism using Integrated Concepts and Equipment

(2011-2014) (Total consortium: 8.424 k€ / CTMA: 403 k€)
Pierre-Alain FONTEYNE, Olga VYBORNOVA
Consortium: EC European CBRNE Center (Coordinator), UmU (Sweden), FFI (Norway), EADS Astrium S.A.S. (France), TNO (The Netherlands); Kings College (United Kingdom), HCFD (France), CBRNE ltd (United Kingdom), Demokritos (Greece), FOI (Sweden), NFI (The Netherlands), SUJCHBO (Checozvajia), Elsag Damat (Italy), Selex Galileo (Italy), PolSpace (Poland), CEN (Belgium), Main School of Fire Services (Poland), Mid-Sweden University (Sweden),
The PRACTICE project will improve the preparedness and resilience of EU member states and associated countries from an attack by a terrorist group using non-conventional weapons, specifically an attack with CBRN (Chemical, Biological, Radiological and/or Nuclear) materials. This Coordination and support action aims at promoting the development of mobile laboratories, structures and functions to support rapid assessment of CBRN events with a cross-border or international impact within and outside Europe. The overall objective of this feasibility study is to provide a global deliverable “CBRN mobile laboratory architecture(s)” that relies (a) on a better understanding and definition of the need and optimal solutions for mobile lab, and (b) on a clear and straightforward interface with existing EU capabilities / structures.

CTMA is the partner in charge of development of a Light Fieldable Laboratory tool for detection and identification of B threats, and development of the CBRN domain ontology as a knowledge base for the PRACTICE toolbox.

The overall objective of this feasibility study is to provide a global deliverable “CBRN mobile laboratory architecture(s)” that relies (a) on a better understanding and definition of the need and optimal solutions for mobile lab, and (b) on a clear and straightforward interface with existing EU capabilities / structures.

CTMA is the coordinator of the project aiming at the harmonization of the definition of a CBRN mobile laboratory and identification of the needs and solutions for deployment in and outside the EU.

Development of mobile laboratories, structures and functions to support rapid assessment of CBRN events with a cross-border or international impact.

The overall objective of this feasibility study is to provide a global deliverable “CBRN mobile laboratory architecture(s)” that relies (a) on a better understanding and definition of the need and optimal solutions for mobile lab, and (b) on a clear and straightforward interface with existing EU capabilities / structures.

EDEN – End-user driven DEmo for CBRNE

FONTEYNE Pierre-Alain, VYBORNOVA Olga
Consortium: BAE Systems (United Kingdom), Astrium-SAS-AST (France), FFI (Norway), Technoalimenti (Italy), Selex (Italy), University Paris XII - SAMU (France), Skola Glowna Sluzby Pozarniczej SGSP (Poland), Centre for Science, society and citizenship (CSSC) (Italy), Astri Polska Spolka Z Ograniczona Odpowiedzialnoscia APL (Poland), Instituto Affari Internazionali IAI (Italy), CBRNE Ltd (United Kingdom), CTMA, LDI Innovation OU LDI2 (Estonia), Fraunhofer-Gesellschaft zur Foerderung der Angewandten Forschung E.V (Germany), Teknologian Tutkimuskeskus VTT (Finland), Fondation sur la recherche straégique (FRS) (France), Indra Sistemas (Spain), Institut national de l'environnement et des risques (INERIS) (France), SICPA Product Security
EDEN aims at demonstrating the added value of a Light Fieldable Biology Laboratory (LBFL) for the response to specific B threat scenarios. The LBFL integrates a set of bricks either operational or at least characterized by high TRL. Short cycle R&D in collaboration with EDEN partners is required to allow full integration of innovative system (e.g. rapid low cost bio inactivation assessment).

CTMA is in charge of testing and validating the LFL in the integrated demonstration of CBRN resilience along the whole food chain, from suppliers to potential casualties and integrates the LFL tool in the EDEN toolbox.

CAERUS aims to identify humanitarian relief actions that pave the way for human development and stability in post-conflict societies. Why have some countries successfully escaped the cycle of violence and conflict where others seem to be trapped? What has been the specific role of national, international and particularly European post-conflict relief action and development cooperation in these cases? This project will undertake humanitarian policy analysis on a global and regional scale, examining ways in which these policies support or undermine development and international security. It will also implement population-based studies in key crises-affected areas to obtain field evidence.

CTMA is in charge of case studies of LFL solution in different missions, such as response to outbreak, support to care management, surveillance of drug resistance, training and education, etc. in post conflict area.
D) European Defense Agency (EDA) Research

BFREE (Biological Free mixed CBRN samples for safe handling and analysis)
– European Defense Agency (EDA) 1st Joint Investment Programme on CBRN Issues (JIP-CBRN1).

Mostafa BENTAHIR
International cooperation: FFI (Norway) (Coordination), Swedish Defence Research Agency (FOI) (Sweden), CTMA, Bundeswehr Research Institute for Protective Technologie NBC Protection – WIS (Germany), TNO (The Netherlands), Ministère de la Défense - DGA - CBRN Defence – CEB (France), Austrian Federal Ministry of Defence and Sport – BMLVS (Austria)

The project aims at obtaining an efficient sample processing and risk mitigation method for both ensuring safe handling and the following analysis of CBRN mixed samples. It will focus on developing a set of validated procedures agreed among a network of European nations to separate a potential mixture of CBRN agents into distinct C, B, RN aliquots that are further prepared and analysed simultaneously, in parallel and/or successively, independent of sample matrix and reducing the turn-around-time for analysis.

The scientific and technological innovation is highlighted and edged on the development of methods/protocols for removal of B agents, and which do not have a negative impact on the CRN agents, to ensure safety of personnel when performing the analysis of C and R agents. Various methodologies will be tested among several European nations to recommend the most optimal methods for rapid, reliable, sensitive, specific, efficient and cost effective analysis of CBRN mixed samples. BFREE will consider previous studies and results from NATO, EDA and EU projects while focusing on improving one of the first crucial steps in preparing the mixed CBRN samples for analysis.

Figure 10: Filtration model developed by CTMA for separation and safe analysis of CBRN mixed samples.

The outcome of BFREE will provide European harmonized approaches for civilian and military laboratories and standardized operating procedures for handling such samples.

Risk Assessment for CB Exposure after Decontamination (RACED) – European Defense Agency (EDA) 2d Joint Investment Programme on CBRN Issues (JIP-CBRN2)
– European Defense Agency (EDA) 1st Joint Investment Programme on CBRN Issues (JIP-CBRN1).

CBRN Issues (JIP-CBRN2).
Mostafa BENTAHIR
International cooperation: TNO (The Netherlands) (Coordination), FFI (Norway), CTMA, Instituto de Tecnologia Química e Biológica (ITQB - UNL) research centre of Universidade Nova de Lisboa (Portugal), Centro de Investigação da Academia Militar (CINAMIL) Laboratório de Bromatologia e Defesa Biológica (Portugal), Integrated Microsystems Austria GmbH (IMA) (Austria)

Integrated Microsystems Austria GmbH (IMA) (Austria). In military protection against chemical and biological (CB) warfare agents, decontamination is a crucial step. In case of exposed surfaces, this process aims at removing chemical and biological hazards from equipment, vehicles, buildings and outdoor areas. Essential for successful response to an attack involving CB agents is to recover contaminated surfaces into assets sufficiently clean to return for use. Ideally, decontamination is quick, extremely thorough and environmentally inert.

However, removal of the last molecule or last viable cell is utopic. This does not need to be
a danger, as long as the remaining number of agent molecules or viable cells is below a critical level and does not pose a health hazard. The challenge is to obtain insight into the status decontaminated objects with regard to the remaining hazard. This exactly formulates the problem the RACED project intents to tackle. In an operational military setting it is not possible to assess the remaining hazard. Moreover, even in state-of-art laboratories it is very difficult to measure the residual contamination after a standard decontamination procedure. And even if residual contamination is known, it is not possible to relate that to the remaining health hazard, let alone how to handle the forthcoming risk. The overall challenge can subsequently be formulated as: the need to find out how much of what is left, how that can reach and affect humans and how can that risk be managed.

To counteract this cascade of challenges, RACED takes the following staged approach: 1. Decontaminate a representative number of CB agents / surfaces by standard means and procedures. 2. To apply state-of-the art analytical and micro/molecular biological assays to identify and quantify residual agent. 3. Simulate and understand transport from decontaminated surface to exposure of human airways and skin. 4. Relate exposure to toxicity and infectiousness, respectively. 5. Design a risk profile and identify measures to mitigate or at least manage those risks.

The end-result is a risk management tool that allows the operational decision maker to rationally and confidently declare an asset clean, or to re-launch a decontamination step or to abandon an asset as too dangerously contaminated to maintain. In achieving this, RACED will deliver a crucial contribution towards answering the how-clean-is-clean paradigm.

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**EBLN – European Biodefense Laboratory Network**

(On going activity since 2008)

Leonid IRENGE, Anne-Sophie PIETTE, Mostafa BENTAHI, Elodie CARLIER.

International cooperation: Armament and Defence Technology Agency - NBC & Environmental Protection Technology Division (Austria), CTMA, Centre for Military Medicine - CB Defence and Environmental Health Centre (Finland), DGA Maîtrise NRBC Le Bouchet (France); Institut für Mikrobiologie der Bundeswehr (Germany); Army Medical and Veterinary Research Center (Italy); FFI (Norway); Ministry of National Defence, Science and Military Education Department (Poland)

The objective of this project is to contribute to the establishment of a laboratory network and common genetic database. The project will improve the EU capability to verify the use of biological agents (B – agents) in the military and civil context such as international regulations, e.g. BTWC (Biological and Toxin Weapon Convention). In the case of a suspected use of B-agents, unambiguous identification of the agent has to be performed. The forensic proof of use of these agents must be such that it cannot be refuted. Microbial forensics has been implemented in the US to ascertain whether an event was natural or intentional and to verify the intentional use of B-agents. Currently, Europe has capability gaps caused by a lack of coordination, standardization, and evaluation of methods to detect, identify type B-agents. Coordinated efforts will contribute to discourage B-terrorism and improve European bio defense capabilities.

Identifying agents and sources in a forensic context relies on a spectrum of features, including epidemiological data and high-resolution analysis. A secure database on B-agents will be established (e.g. sample handling and processing, detection and diagnostic methods, genome sequence and other typing data) to further strengthen the European bio defense capability. In addition, implementation of technical developments in terms of more rapid analysis and higher resolution will be pursued. Sharing experiences on standardization and quality controls are also essential elements of the project. Creation of a strategic European bio defense network around the database based on agent specific expertise will be the end results of the project.
E) Walloon Region (RW), Regional R&D Programme (WALLEO3, Biowin, Other)

University's development cooperation (UDC) - Targeted Interuniversity Pole

CRISTALL
- Evaluation of risk factors for development of allergy in young children.

(Phase I: 2006 – 2011) (Total consortium: 2633 k€) (Phase II: 2011-2013) (Total consortium: Funding 301 k€ by RW / 264 k€ by GSK Biologicals)

Bertrand BEARZATTO
National Consortium: CTMA, Institute for Pediatric Research (Woluwe-Saint-Lambert)

A significant number of children have allergic disorders that tend to evolve and persist into adulthood. This disease affects significantly patient’s quality of life. Cristall aims to study and characterize the immunological and genetic factors associated with the development of allergy in order to identify early those who are at risk. CTMA has assessed gene expression profile in young children using high density microarray (GeneChip HGU 133 Plus 2.0). Successive analyses were carried out to identify a set of predictive markers of allergic development in young children aged 0 to 6 months. The signature will be used as a low-density array diagnostic assay (using customized arrays) for early detection of this allergic predisposition in order to improve prevention strategies in children at risk.

- BIOSE
  - Development of an optic biosensor with high sensitivity for rapid detection of ambulatory pathogens in biological liquids.

(2009 - 2013) (Total consortium: 1.400 k€/CTMA: 280k€)

Catherine DUMONT, Anne-Sophie PIETTE
National cooperation: CTMA (coordination), Zentech S.A (Liège), Wow Company S.A (Namur), Faculté Universitaire Notre-Dame de la Paix (FUNDP) (Namur), LISE (Namur), UCL DICE and POLY (Louvain-la-Neuve), MULTITEL (Mons), SIRRIS (Seraing)

Rapid detection of chemical and biological threats and substances appears increasingly as a key concern in improving and anticipating problems related to health, both in the field of medicine and environment quality. The current analysis techniques are very expensive and require staff and specialized infrastructure. The main need is to perform tests that are sensitive, rapid (<1 hour), low cost (<50 € per test), bedside or outside specialized infrastructure (e.g. in a care center, school or at home in case of emergency) before unambiguous identification in a specialized laboratory.

- ORTHOGEN
  - Integrated Traceability and Management Information System.

(2010 - 2013) (Total consortium: 980 k€ / CTMA: 300 k€)

Leonid IRENGE, Jérôme AMBROISE, Florence MARIEN, Jean-François DURNANT, Jean-Paul MARCEL
International Cooperation: Hôpital Ambroise Paré -Paris (France), CTMA (Coordination), UCL TELE (Louvain-la-Neuve), UCL / Cliniques universitaires de Mont Godinne - Service de radiologie, Faculté Universitaire Notre-Dame de la Paix - PRECSE (FUNDP) (Namur)

Development and validation of an integrated system of traceability and multidisciplinary inference information analysis approach to optimize the diagnosis and treatment of infections of osteoarticular prostheses. This work includes the development of DNA-based algorithms for identifying causative agents for chronic or acute orthopedic infections.
Figure 11: Detection of a 22mm granuloma infection close to the screw in cortile boon.

**RHEUMAGEN – Development of a new Method for Diagnosis of Arthritis.**

(Total consortium: 1.923 k€ / CTMA: 194 k€)  
Bertrand BEARZATTO, Jérôme AMBROISE, Jamal BADIR  
National Consortium: CTMA, UCL Service de Rhumatologie (Coordination) (Woluwe-Saint-Lambert), Eppendorf array technologie (Namur), Eurogentec SA (Liège), ULG Service de Rhumatologie (Sart-Tilman), CHU-Brugmann Service de Rhumatologie (Bruxelles), UZ Gent, Service de Rhumatologie (Gent).

The aim of this project is to validate the diagnostic value of transcriptomic and/or proteomic profiles of synovial material in early inflammatory or infectious disease (arthritis). It is based on preliminary data showing that gene expression profiles in synovial biopsies from patients with arthritis are able to discriminate patient samples according to the underlying disorder. The large-scale confirmation of these data after this two-year project will lead to the development of a prototype of a diagnostic tool to be used in routine rheumatology practice.

**PIC – Support to improve the capacity for detecting and identifying infectious agents in the province of South Kivu in the Democratic Republic of Congo**

(2012 - 2016) (Total consortium: 330 k€ / CTMA: 200 k€)  
Leonid IRENGE  
International Cooperation: CTMA, ULB Ecole de santé Publicque (Bruxelles), Université catholique de Bukavu Laboratoire biologie Clinique (Bukavu, RDC), Institut National de Recherche Biomédicale (Kinsasha, RDC)

Africa is the cradle of some of the most deadly infections. Management of infectious diseases in the province of South Kivu (DR Congo) is a challenge according to the serious impact of infectious disease on related morbidity and mortality and the risk of extension of outbreaks from remote areas to crowded cities and from RDC to European countries. The goal of the project is to improve the capabilities of identifying infectious agents in each health district hospital in the province of South Kivu.

**ALLERT – Handheld Allergens Detector**

Accepted in 2013 to start in 2014 (2014 – 2016)  
(Total consortium: 1.538 k€ / CTMA: 350 k€)  
Jamal BADIR, Bertrand BEARZATTO, Jérôme AMBROISE  
National Consortium: ZENTECH SA, LAMBDA-X, CER GROUPE, CTMA

The scope of ALLERT project is to provide a practical, portable, rapid and effective diagnostic system to detect allergens in foods. This project does not focus on the IgE detection against specific allergens. The first level is our answer to the need of testing quickly several allergens in the same time. The second level includes innovation in photonic allowing a better collection of image data to enhance quality of detection adapted to a mobile testing. The third innovative level will be the preparation of samples. By using a standard preparation device and a standard sample collection and filtration technique we will avoid the extreme variation in sample preparation quality. The fourth innovative level will be in the data analysis using specific algorithms to clean images, analyze multiplexed spots and delivering a result with traceability, communication features.
**INFRASTRUCTURE & EQUIPMENT**

**Infrastructure:**
- Distant pre- and post-PCR rooms;
- Specific rooms for DNA extraction;
- PCR amplification and post-amplification activities;
- Several Biosafety level 2 (BSL2) rooms and one biosafety glove box;
- Access to academic and the federal Laboratoire fédéral d'orientation (FOL) BSL3 facility.

**Major equipment:**
- Standard molecular genetic laboratory equipment: conventional DNA sequencer, pyrosequencer, several PCR and quantitative real-time PCR cyclers, spectrophotometer and synchronous fluorimeter, nucleic acid and protein extraction robots and quantification apparatus, etc…;
- Automated Luminex bead plate multiplex reader;
- Automated Enzyme-linked immunospot (ELISpot) reader;
- HPLC Prominence Liquid chromatograph (Shimadzu)
- FreeZone 2.5 Liter Benchtop Freeze Dry System (LabCongo)
- Emerging technologies:
  - two dimensional low and high density microarray scanner, colorimetric array scanner, tridimensional-microarray scanner, automatic spotters for large scale and micro-piezoelectric spotting, hybridization station, probe station.

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**BIOBACTIL WB – Health Optofluidic biosensor immunoassay for detecting and identifying bacteria in human samples matrixes.**

Accepted in 2013 to start in 2014 (2014 – 2016) 
(Total consortium : 1.000 k€ / CTMA : 180 k€)

Catherine DUMONT
Consortium: UCL TELE, CTMA, MULTITEL, SIRRIS, L. FUNDP, ULG Microsys Lab

The aim of the project is to develop a lab-on-chip demonstrator for detecting and identifying the presence of Neisseria meningitidis in cerebrospinal fluid samples. The untreated sample is deposited on the chip, than a “all or nothing” diagnostic answer is provided within 15 minutes. During the development, the effectiveness of the system will be compared to a standard enzyme-linked immunosorbent assay.

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**E) Industry**

**Stallergènes**

(2013 – 2016)

Marc DILLENBOURG, Amandine DUPRAZ-FRAIZIER, Karine MAJOR, Audrey SINON

The project aims at producing freeze dried, gamma inactivated, fungal raw material for use in allergy research & treatment, starting from pure cultures & inert substrates. A service type contract has recently been signed with a biopharmaceutical industry leader specialized in the treatment of severe respiratory allergies. Consequently, selected strains have been deposited at Mycothèque de l'Université catholique de Louvain (BCCM/MUCL). The production of biomasses can be adjusted to the specificities of any customer (scientific community or industrial sector) in order to guarantee the quality of allergen extracts made using our products. UCL-CTMA/MYCO meets strict quality & safety standards, in compliance with European regulatory requirements (origin, processing, identification & purity). It has the equipment & expertise allowing detection, identification & monitoring of microbial contaminants of indoor & outdoor air. Detection & monitoring is based on surface & air sampling methods. Identification of airborne particles is achieved by standard light microscopy, culture, SDS-PAGE profiling & DNA signature sequences.

Another goal of the project is to perform research on the quantification and analysis of proteins for test and control purposes and in the context of allergy test.
### PUBLICATIONS

**Publications 2013:**


### Selection over the last 3 years

- CTMA first and/or last authorship:


### Patents

*Method for normalization of quantitative PCR and microarrays.*
Filed under No. 61/556.655 (U.S. provisional filed 07/11/2011).

*Method for analysing a pyro-sequencing signal*
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