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

Albert SZENT-GYORGYI, 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**
  Clémentine Janssens
  Valérie Buchet
  Salvatore Livolsi
  Marie Masson
  Dominique Van Ophem

- **Logistics and Accounts Unit:**
  Maimouna Elmjouzi MBLG/CTMA
  Myriam Goosse-Roblain MIRO
  Eric Legrand CHEX
  Cyril Mougin FATH/RUMA
  Michel Notteghem CARD/EDIN
  Dora Ourives Sereno GYNE/PEDI/GAEN
  Benjamin Verpeut LTAP

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  1200 Woluwe-Saint-Lambert

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  http://www.uclouvain.be/irec.html
RESEARCH GROUPS

- Cardiovascular Research (CARD) 09
- Computer Assisted Robotic Surgery (CARS) 26
- Experimental Surgery and Transplantation (CHEX) 38
- Endocrinology, Diabetes and Nutrition (EDIN) 46
- Pharmacology (FATH) 56
- Hepato-Gastro-Enterology (GAEN) 67
- Gynecology (GYNE) 74
- Medical Imaging Research (IMAG) 83
- Louvain Centre for Toxicology and Applied Pharmacology (LTAP) 97
- Medical Microbiology (MBLG) 105
- Molecular Imaging, Radiotherapy and Oncology (MIRO) 109
- Morphology (MORF) 123
- Nephrology (NEFR) 135
- Pediatrics (PEDI) 137
- Pneumology, ENT and Dermatology (PNEU) 145
- Rheumatic Pathology (RUMA) 150
- Centre for Applied Molecular Technologies (CTMA) 156
**RESEARCH EXPENDITURES AT INSTITUTIONAL LEVEL 2010-2013 (K€):**

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**NEW RESEARCH CONVENTIONS CONCLUDED 2010-2013:**

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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 4 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 signalling and metabolism.

These 4 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.
AMP-activated protein kinase (AMPK) signalling in platelets: From bench to bedside

M.-B. Onselaer, S. Lepropre, S. Kautbally, D. Castanares, P. Buchlin, C. Beauloye and S. Horman

Platelet activation requires sweeping morphological changes, supported by contraction and remodelling of platelet actin cytoskeleton. Since the CAMKKβ-AMPK-α1 pathway is a key factor in cytoskeleton organization, we hypothesized that it regulates platelet activation and aggregation by controlling the phosphorylation and activity of cytoskeletal proteins, namely MLC, coflin and VASP. In this study, we established that thrombin activated the CAMKKβ-AMPKα1 axis in human platelets. Pharmacological or genetic inhibition of this pathway decreased platelet aggregation modified MLC, VASP and coflin phosphorylation states and altered actin polymerization upon thrombin stimulation. Interestingly, the AMPK pathway was activated in vivo, in platelets from patients undergoing major surgery.

Since thrombin was the only platelet agonist causing AMPK activation in human platelets and subsequent phosphorylation of acetyl coA carboxylase (ACC), its “bona fide” substrate, we postulated that the phosphorylation state of ACC could be considered as a valuable in vivo marker of the thrombin response in platelets from patients undergoing major surgery (Patent application WO/2013/076157 - UCL-036). In the medical context, management of bleeding complications after cardio-pulmonary bypass (CPB) surgery still remains a challenging issue. Differentiating between inadequate surgical haemostasis (breeches of the vasculature) and coagulopathies as a cause of post-CPB bleeding, is often difficult. New suitable tests to identify haemostatic disturbances and predict excessive bleeding are therefore desirable in order to help physicians to take the right decision in front of bleeding complications. Our future work will evaluate whether P-ACC assessment in platelets could have a potential clinical utility to predict bleeding tendency within the first hours following surgery.

Figure 1  Control of platelet function by AMPK α1 through regulation of cytoskeleton-dependent processes.

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.

Control of fibroblastic properties by AMPKα1: Impact on LV remodelling post-MI

G. Noppe, C. Dufey, N. Marquet, C. Beauloye, S. Horman

Cardiac fibroblasts (CF) are crucial in left ventricular (LV) healing and remodelling after myocardial infarction (MI). They are typically activated into myofibroblasts that express alpha-smooth muscle actin (α-SMA) microfilaments and contribute to the formation of contractile and mature collagen scars that minimize the adverse dilatation of infarcted areas. CF predominantly express the α1 catalytic subunit of AMP-activated protein kinase (AMPKα1), 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 investigated the role of AMPKα1 in modulating LV dilatation and CF fibrosis during post-MI remodelling. Our data indicate that in AMPKα1-deficient hearts (knockout, KO), AMPKα1 paucity is associated with increased number of proliferative CF, fewer myofibroblasts and altered collagen maturation in infarcted areas. All these changes lead to increased end-diastolic volume (EDV) of KO hearts after MI. Our results highlight the role of AMPKα1 in CF/myofibroblast biology, providing new perspectives and potential therapeutic approaches that could counter the adverse LV remodelling of infarcted hearts.

The A-769662 compound potentiates the effect of other AMPK activators and increases cardiomyocyte survival during ischemia/hypoxia

A. Timmermans, C. Beauloye, L. Bertrand

AMPK is activated during myocardial ischemia. This activation is been shown to be protective for the heart. We were interested to see if pharmacological (over)activation of AMPK by the specific and direct AMPK activator A-769662 could increase the protective action of AMPK during ischemia/hypoxia. We mainly showed that, when combined with classical AMPK activators, such as metformin or hypoxia, A-769662 induced more profound AMPK phosphorylation and subsequent glucose uptake and glycolysis stimulation, known to be implicated in the protective effect of AMPK.

Interestingly, the synergistic action of A-769662 under such ischemia-mimetic conditions protected cardiomyocytes against ROS production and cell death. The ability of A-769662 to potentiate the action of other AMPK activators makes it a potentially useful therapeutic agent for the protection of the ischemic heart (Figure 3).
Figure 3 Pharmacological AMPK over-activation promotes cardiomyocyte survival during myocardial ischemia. AMPK overactivation increases ATP production via the stimulation of the anaerobic glucose metabolism. AMPK also inhibits the ischemia-mediated production of reactive oxygen species (ROS). These actions contribute to the protective action of AMPK activators.

AMPK activation by glucagon-like peptide-1 (GLP-1) prevents NADPH oxidase activation induced by hyperglycemia in adult cardiomyocytes

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

Exposure of cardiomyocytes to high glucose concentrations stimulates ROS production by NADPH oxidase (NOX2). NOX2 activation is triggered by enhanced glucose transport through a sodium-glucose co-transporter (SGLT) but not by a stimulation of glucose metabolism. AMPK-activated protein kinase (AMPK) activation by A769662 or phenformin nearly suppressed ROS production induced by hyperglycemia in adult cardiomyocytes. Interestingly, GLP-1, a new anti-diabetic drug, concomitantly induced AMPK activation and prevented the hyperglycemia-mediated ROS production. Anti-ROS properties of AMPK activators were not related to changes in glucose uptake or glycolysis. Using in situ proximity ligation assay, we demonstrated that AMPK activation prevented the hyperglycemia-induced p47phox translocation to caveolae, whatever the AMPK activators used. NOX2 activation by either α-methyl-D-glucopyranoside, a glucose analog transported through SGLT, or angiotensin II was also counteracted by GLP-1.

Figure 4 Pharmacological AMPK activation by GLP-1 counteracts hyperglycemia-induced NADPH oxidase (NOX2) activation and ROS production in cardiomyocytes. AMPK activation by GLP-1 blocks p47phox translocation to the caveolar structure (plasma membrane) in response to hyperglycemia (HG). p47phox translocation to the plasma membrane is required for NOX2 activation. Red dots in the pictures correspond to p47phox co-localization with caveolin-3 in adult cardiomyocytes in culture.

The crucial role of AMPK in limiting HG-mediated NOX2 activation was demonstrated by overexpressing a constitutively active form of α2AMPK using adenoviral infection. This overexpression prevented NOX2 activation in response to hyperglycemia, whereas GLP-1 lost its protective action in AMPK deficient mouse cardiomyocytes. Under hyperglycemia, the GLP-1/AMPK pathway inhibited PKCβ2 phosphorylation, a key element mediating p47phox translocation.

In conclusion, AMPK is key signalling element downstream of GLP-1, blocking hyperglycemia-induced p47phox translocation to the plasma membrane, NOX2 activation and, thereby preventing glucotoxicity.
PATENT

- WO/2013/076157 - UCL-036

KEY WORDS

- AMPK, heart remodelling, platelet, thrombosis, glucose metabolism, stem cell proliferation.

EQUIPMENT

- Experimental physiologic and biochemistry lab
- Microplate reader including injectors (Victor X and Enspire)
- Heart perfusion equipment (for mice, rat, rabbit and pig)
- Dedicated research echo machines (including Visualsonics VEVO dedicated to small animals)
- Two channel whole blood/optical lumi-aggregometer (Chrono-Log model 700, Stago)
- CELL-DYN Emerald (Abbott Diagnostics Division) for automated hematology analysis (human and murine)
- Three cath-labs
- Computarized system for rest and stress ECG-VCG analysis
- Clinical database

CLINICAL RESEARCH GROUP


Fabian Demeure, Jean-Louis Vanoverschelde, David Vancraeynest

18F-Fluorodeoxyglucose-Positron emission tomography/Computed tomography (18F-FDG-PET/CT) can be used to detect arterial atherosclerotic plaque inflammation. However, avid myocardial glucose uptake may preclude its use for visualizing coronary plaques. Fatty acid loading or calcium channel blockers could decrease myocardial FDG uptake, thus assisting coronary plaque inflammation identification.

We conducted a prospective randomized trial to compare the efficacies of different interventions for suppressing myocardial FDG uptake. We also investigated whether circulating free fatty acid (cFFA) levels predicted the magnitude of myocardial FDG uptake.

![Figure 4](image-url) Study protocol.
Thirty-six volunteers ate a high-fat low-carbohydrate meal, followed by a 12-hour fasting period. They were then randomized to one of four intervention groups. Group 1 received no additional preparation and served as reference. Groups 2 and 3, respectively, received a solution containing 43.8 g lipids (KetoCal®) or 50 mL olive oil one hour before FDG injection to evaluate the impact of fatty acid loading on myocardial FDG uptake. Group 4 received verapamil to evaluate the effect of calcium channel blockers. Cardiac PET/CT was performed after administration of 370 MBq 18F-FDG. Myocardial uptake suppression was assessed using a qualitative visual scale and by measuring the myocardial Standardized uptake value. Insulin, glucose, and cFFA were serially measured (Figure 4).

0.65 mmol/L cFFA as the best cut-off value to predict adequate FDG uptake suppression (PPV 89%).

We were able to conclude that a high-fat low-carbohydrate meal followed by a 12-hour fasting period effectively suppressed myocardial FDG uptake in a majority of subjects. Neither complementary fatty acid loading nor verapamil administered 1 hour before FDG injection conferred any additional benefit. Myocardial FDG uptake was inversely correlated with cFFA level, representing an interesting way to predict myocardial FDG uptake suppression. We believe that these data can refine FDG-PET/CT technique in the search of the vulnerable coronary plaque.

Drug-resistant hypertension and renal sympathetic denervation

A. Persu, J. Renkin, M. Azizi and J.A. Staessen

In 2009–2010, the Symplicity HTN-1 and HTN-2 studies reported a mean 25–30 mm Hg decrease in office systolic blood pressure (BP) after renal sympathetic denervation (RDN) in patients with drug-resistant hypertension, with a rate of procedural adverse events < 5% at 6 months. Despite limited evidence and serious methodological concerns, RDN was rapidly diffused in Europe and Australia. Nevertheless, in 2014, Symplicity HTN-3, a large US randomized sham-controlled trial which was meant to confirm on a larger scale the benefits of RDN failed to meet its primary efficacy endpoint.

Other subsequent, well-designed RCTs comparing RDN with drug treatment alone or drug treatment intensification showed either no benefit or a modest overall benefit of RDN (~6 mmHg in the French trial DENERHTN). The latter seems to be driven by a low number of extreme responders. Accordingly, most expert centres have witnessed spectacular BP decreases after RDN in a minority of patients. Identification of the characteristics of those few patients likely to draw a substantial benefit from the technique is thus a priority.

Hyper-responders vs. non-responder patients after Renal Denervation: do they differ?
In order to further explore the predictors of blood response to RDN, we compared baseline characteristics and analysed BP responses in extreme BP responders vs. non-responders to RDN within the European Network Coordinating research on Renal Denervation (ENCoReD) consortium. Extreme responders and non-responders according to office BP decrease were defined as patients belonging to the first and fifth quintile of office systolic BP decrease, respectively. Similarly, extreme and non-responders according to ambulatory BP decrease were defined as patients belonging to the first and fifth quintile of 24-h ambulatory systolic BP decrease.

Twenty-one extreme-responders and 22 non-responders according to office BP were identified. Extreme responders defined according to office BP were characterized by a huge white-coat effect at baseline, with dramatic shrinkage at 6 months, suggesting a major overestimation of office BP decrease after RDN. The latter likely reflects overestimation of office BP at baseline, or underestimation of office BP at 6 months, due to regression to the mean, the Hawthorne effect, physician-related biases, and possibly other unidentified confounders.

In contrast, extreme responders defined according to ambulatory BP had similar baseline office and ambulatory BP and showed an almost identical BP decrease (~ 30/17 mmHg) six months after RDN (Figure). Our findings suggest that extreme responders defined according to ambulatory, not office BP are the “true” responders to RDN. They support the use of ambulatory BP in upcoming RDN trials, both for patients’ selection and evaluation of efficacy.

Furthermore, compared to non-responders, extreme responders defined according to office BP were more frequently women, had higher baseline office - but not ambulatory - BP and higher estimated glomerular filtration rate (eGFR). The association of lower eGFR with poor response to RDN is consistent with our previous analysis, as well as pre-specified analysis performed in the Symplicity HTN-3 trial.

Even though it was initially hypothesized that patients with chronic kidney disease may be prime candidates for RDN due to increased sympathetic activity, in the setting of resistant hypertension, altered renal function may prove a surrogate marker of irreversible renal and vascular damage, or increased sodium retention, predicting a poor BP outcome after RDN. If confirmed, the increased proportion of women in extreme responders may reflect sex differences in drug adherence.

Renal artery stenosis after RDN - a novel differential diagnosis of Fibromuscular Dysplasia?

Before considering RDN in a patient with resistant hypertension, physicians should not only take into account potential benefits but also safety issues. The latter include the risk of renal artery stenosis after RDN. At least 22 cases of renal artery stenosis occurring after RDN were reported using 4 different renal
ablation systems. Most of them were discovered following a rise in BP after an initial decrease. However, in the absence of state-of-the-art imaging of renal arteries in most studies, the prevalence of such stenosis remains unknown.

We reported a case of de novo multiple bilateral stenosis associated with severe hypertension and fluid retention six months after RDN using the Vessix-Boston® radiofrequency balloon catheter, as well as unexpected progression of mild baseline stenosis using the same RDN system in 3 patients from a series of 13 patients treated in Brussels and Paris, who underwent systematic CT-angiography, both at baseline and after RDN. This gives a prevalence of renal artery stenosis of 30.7%. Furthermore, the multiple stenoses documented in the first case had a unique aspect, similar to that observed in multifocal fibromuscular dysplasia (Figure), and were associated with significant morbidity. While it is difficult to draw definitive conclusions from this small cohort, our study pinpoints the importance of independent registries such as ENCOReD to assess the true prevalence and incidence of RDN-related complications using different existing RDN systems.

In conclusion, overall, our studies made a significant contribution to the current debate on RDN, and contributed to influence the design of new RDN trials aiming to identify the small niche of high-risk patients likely to benefit from this approach, with limited risks of complications.

Prognostic value of Detection of Myocardial Fibrosis by cardiac MRI in patients with Severe Aortic Stenosis undergoing Aortic Valve Replacement

Bernhard Gerber, Agnès Pasquet, Jean-Louis Vanoverschelde

Degenerative stenosis of the aortic valve is the most frequent valvular heart disease in industrialized countries and its prevalence steadily increases with age. In response to the chronic pressure overload of this condition, the left ventricle reacts by compensatory concentric hypertrophic remodeling. This phenomenon not only involves increase in myocyte volume, but also coordinated remodeling and increases of the extracellular matrix.

By histopathology, hearts with severe AS present both interstitial diffuse reactive fibrosis as well as focal replacement fibrosis (Figure 1). Late Gadolinium enhanced MRI is the technique of choice to non-invasively detect myocardial fibrosis and necrosis in the myocardium. Indeed, the Gadolinium based contrast agents have extravascular distribution volume, which increases when extracellular matrix proliferates or when myocytes are replaced by focal fibrosis, and thus cardiac MR has the ability to detect focal fibrosis in the myocardium of patients with aortic stenosis (Figure 8).

We therefore evaluated whether the quantitative assessment of fibrosis by CMR, can predict survival in patients with severe AS undergoing aortic valve replacement. We prospectively studied 2 cohorts of patients: 1) 154 consecutive patients with aortic stenosis but without prior myocardial infarct using late gadolinium cardiac MRI before surgical aortic valve replacement over a median follow-up of 2.9 years and 2) a second cohort of 40 patients undergoing trans-catheter aortic valve replacement (TAVR), followed over a median duration of 3.9 years. We then used uni- and multivariate survival analysis to evaluate the role of fibrosis by MRI and other predictors of overall and cardiovascular survival in these patients.
Figure 8  Pathophysiology of myocardial fibrosis in aortic stenosis. In response to the chronic pressure overload of severe aortic stenosis, the left ventricle reacts by compensatory concentric hypertrophic remodeling. This phenomenon involves not only increased myocyte volume (second panel from the left) but also coordinated remodeling and increased extracellular matrix, with development of both diffuse interstitial and focal replacement fibrosis. Diffuse interstitial fibrosis consists of increased deposition of collagen in interstitial spaces (third panel from the left). Focal replacement fibrosis consists of replacement of myocytes by fibrotic tissue (right panel). This latter form of fibrosis can be detected by late gadolinium enhancement on cardiac magnetic resonance.

Our study showed that in patients undergoing surgical aortic valve replacement, presence of preoperative fibrosis by MRI predicted significantly worse overall and cardiovascular survival than patients without fibrosis (Figure 3).

By univariate Cox analysis, presence of fibrosis by MRI, New York Heart Association (NYHA) class III/IV and presence of left bundle branch block were the sole predictors of overall survival.

Multivariate Cox analysis demonstrated that presence of fibrosis by MRI and NYHA functional class were the only independent determinants of overall survival. Also for cardiovascular survival presence of fibrosis by MRI was the sole predictor in multivariate Cox analysis. Indeed presence of preoperative fibrosis by MRI predicted a 10.9 times (95% CI [1.18-100], p=.02) higher risk of perioperative risk, while risk of late overall (p=.25 by log rank test) and cardiovascular death (p=.60 rank test) was not significantly increased.

Figure 9  Examples of different patterns of fibrosis by MRI. (A) Absence of LGE. (B-D) non infarct patterns: (B) Focal nodular non-infarct LGE (C) Linear midwall LGE predominantly affecting the LV septum (D) Diffuse LGE pattern. (E) Typical transmural infarct-like pattern.

Figure 10  Kaplan-Meier graphs of overall a) and cardiovascular survival b) of patients undergoing surgical AVR according to presence or absence of fibrosis by MRI.
Also, in the subgroup of 25/40 patients undergoing transfemoral aortic valve replacement, presence of fibrosis by MRI was associated with significantly worse (p=0.045) cardiovascular survival and a non-significant (p=0.09) trend for worse overall survival (Figure 4). By contrast, survival in the 15 patients undergoing transapical TAVR, was not predicted by MR-fibrosis. In fact this occurred probably because these patients had significantly higher perioperative mortality (25% vs 0% in transfemoral TAVR, p=0.04), likely reflecting higher comorbidity and our early learning curve with the transapical technique.

Our study thus showed that, myocardial fibrosis detected by MRI is a significant marker of risk in patients with aortic stenosis undergoing both surgical and transcatheter valve replacement. This has not yet been reported for a population of patients with degenerative AS only. Our data thus supports that fibrosis by MRI can detect high-risk aortic stenosis patients prior to valve replacement.

Currently the clinical management of patients with aortic stenosis is based mainly on the assessment of valvular parameters, ejection fraction, and symptom. Indeed the appearance of symptoms identifies a critical point in the natural history of aortic stenosis, predicting a high risk of dying unless the valve is replaced. Therefore the guidelines consider severe symptomatic aortic stenosis and decrease of left ventricular ejection fraction as class I indication for surgery. However when surgery is performed at this point, ie in symptomatic patients and in the setting of low ejection fraction, surgical risk is increased and long term survival may be compromised.

Therefore it could be better to offer surgery at earlier time points, when patients are still asymptomatic. Our present study suggests that fibrosis by MRI identifies higher surgical risk and worse long term survival. This supports that identification of primary structural abnormalities of the myocardium by MRI could be useful for timing of surgery and for selecting the operative approach. Indeed absence of fibrosis by MRI in patients with severe aortic stenosis appears to identify patients at low risk for surgery with excellent long-term survival, which might thus benefit from early surgery. On the other hand, patients with high degrees of fibrosis, indicating higher postoperative risk and lower survival, could be candidates for other techniques such as transcatheter valve replacement. Yet these findings need to be confirmed by larger studies.

In conclusion, these findings suggest that evaluation by fibrosis by MRI could be a new method of risk stratification in patients with aortic stenosis undergoing valve replacement, and that this technique might allow better choosing the time of surgery.

**Figure 11** Kaplan-Meier graphs of overall a) and cardiovascular survival b) in the subgroup of 25 patients undergoing transfemoral TAVR to presence or absence of fibrosis by MRI

**Can LV filling pressures be non-invasively assessed during exercise?**

Agnès Pasquet, Sébastien Marchandise, Jean-Louis Vanoverschelde

In patients with exertional dyspnea who have normal hemodynamics at rest, assessment of left ventricular (LV) filling pressures during dynamic exercise can be useful to establish the diagnosis of heart failure and to evaluate its severity. Pulmonary capillary wedge pressure (PCWP) measurement by right-sided cardiac catheterization is usually considered as the gold standard for assessing exercise hemodynamics in these patients. Unfortunately, this is an invasive procedure that cannot be applied of screening of therapy guidance.
in patient with congestive heart failure. Some studies have shown that LV filling pressure at rest could be estimated by the ratio \((E/e')\) of early diastolic transmitral \((E\) wave measured by pulsed Doppler in the mitral inflow) to mitral annular velocities \((e'\) measured by tissue Doppler at the level the septal mitral annulus). Data regarding exercise are limited. Therefore we tested the ability of the ratio \((E/e')\) to provide reliable estimates of PCWP during symptom-limited maximal exercise. Forty patients with severe LV dysfunction and heart failure symptoms underwent simultaneous Doppler assessment of \(E/e'\) and right-sided cardiac catheterization at rest and during a symptom-limited exercise test, at steady state levels of 30%, 60%, and 90% of their maximal exercise capacity.

The ratio \(E/e'\) at rest correlated well with PCWP at rest \((r = 0.75, p < 0.01)\) (figure 1). During exercise, the correlation between \(E/e'\) and PCWP was weaker \((r = 0.57, p < 0.01)\) and was shifted to the right (figure 2). In conclusion, in patients with severe LV dysfunction, although \(E/e'\) allows accurate estimation of PCWP at rest, it appears less reliable for estimating LV filling pressure during exercise. This may impair its use during exercise to estimate filling pressure in patient with heart failure and exercise dyspnea.

### Impact of preoperative symptoms on post-operative survival in severe aortic stenosis: implications for the timing of surgery

**Sophie Piérard, Jean-Louis vanoverschelde**

The impact of symptoms on the natural history of patients with severe aortic stenosis (SAS) has been well documented. By contrast, the implications of preoperative symptoms on postoperative outcomes remain poorly defined. We therefore conducted a study to evaluate the long-term survival of 812 patients greater than 65 years old with SAS undergoing bioprosthetic aortic valve replacement (AVR) and to test the influence of preoperative symptoms.

Operative mortality was larger in New York Heart Association (NYHA) III-IV than in NYHA I-II patients (10% vs 6%, \(p = 0.036\)). Abrupt symptomatic deterioration from NYHA I to NYHA III-IV within the month preceding surgery was observed in 18% of NYHA III-IV patients and resulted in an increased operative mortality (17% vs 5% in NYHA I, \(p = 0.035\)). Long-term survival was also significantly worse in NYHA III-IV than in NYHA I-II patients (56% vs 72%, \(p = 0.002\)). Reduced long-term survival of NYHA III/IV patients was observed in subgroups with a left ventricular ejection fraction (LVEF) 0.50 or greater (58 vs. 74%, \(p = 0.008\)) and in those with a systolic pulmonary artery pressure (SPAP) less than 40 mm Hg (60% vs 74%, \(p = 0.014\)). By contrast, the presence of class III-IV symptoms did not influence outcome in patients with a LVEF less than 0.50 (51 vs. 55%, \(p = 0.34\)) or with a SPAP 40 mm Hg or greater (43% vs 48%, \(p = 0.78\)).

In patients with SAS, preoperative NYHA III-IV symptoms, particularly of recent onset, are independently associated with excess short- and long-term postoperative mortality. This was particularly evident in patients with normal LV function or pulmonary artery pressures. These findings plead in favor of an earlier surgical correction of SAS, before the onset of severe symptoms, especially in low-risk patients.

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CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; SPAP = systolic pulmonary artery pressure.
Figure 12  Kaplan-Meier survival curve showing overall postoperative survival in patients with no or minimal symptoms, normal preoperative left ventricular ejection fraction (LVEF > 0.50) and no evidence of pulmonary hypertension (systolic pulmonary artery pressure <40 mm Hg) undergoing isolated aortic valve replacement. Dotted line shows the survival of the age- and gender-matched Belgian population. Numbers at bottom indicate patients at risk.

Natural History of Paradoxical Low Gradient « Severe » Aortic Stenosis

Frédéric Maes, Jamila Boulif, Jean-Louis Vanoverschelde

In up to 30% of patients referred for echocardiographic evaluation of the severity of aortic stenosis (AS), the clinician is confronted with lower than expected peak transvalvular velocities and mean pressure gradients despite apparently severe AS (SAS) based on aortic valve area (AVA). Because transvalvular pressure gradients are notably flow-dependent, it has long been recognized that patients with left ventricular (LV) dysfunction and low cardiac output may present with relatively low transvalvular pressure gradients, despite SAS. Recently, several retrospective studies have shown that such low transvalvular gradients can also be observed in patients with preserved LV ejection fraction (LVEF), presumably as a result of small cavity sizes and low stroke volumes, typically < 35 mL/m². Because it is observed in patients with normal LVEF, this new subset of SAS has been termed “paradoxical low gradient” (PLG)-SAS, in contrast with the “low flow (LF) - low gradient (LG)” subset seen in patients with LV dysfunction.

There is considerable debate as to the clinical significance of this new AS constellation. Some authors indeed consider this new entity as a more advanced form of AS, with increased interstitial fibrosis, reduced LV longitudinal function 8-9 and poor prognosis; whereas others believe it represents a relatively benign form of AS, with an outcome similar to that of moderate AS.

In view of this controversy, the objective of the present study was to compare the natural history of patients with PLG-SAS, with that of patients with a high gradient SAS (HG-SAS). By censoring patients at the time of aortic valve replacement (AVR), we sought to minimize the effects of treatment and of its potential differential use in the 2 groups on outcome. Because in AS patients, natural history is dramatically influenced by symptoms, we also paid a particular attention to the outcome of initially asymptomatic patients. Finally, we investigated how transvalvular gradients evolved overtime in the 2 groups, to test which of the 2 conditions could be considered as a more advanced stage of the disease.

We prospectively studied 349 patients with SAS and preserved LVEF. Patients were categorized into HG-SAS (n=144) and PLG-SAS (n=205) according to mean transvalvular gradient (mean gradient > or ≤40 mmHg). Primary end-points were all causes mortality and echocardiographic disease progression. To evaluate natural history, patients undergoing aortic valve replacement were censored at the time of surgery (n=92). During a median follow-up of 28 months, 148 patients died.

Kaplan Meier survival curves showed better survival in PLG-SAS than in HG-SAS, both in the overall population (48 vs 31%, p<0.01) and in the asymptomatic subgroup (59 vs 35%, p<0.02). In asymptomatic patients, Cox’s analysis identified age, diabetes, left atrial volume and mean gradient as independent predictors of death. Finally, at last echocardiographic follow-up, PLG-SAS demonstrated significant increases in mean gradient (from 29±6 to 38±11 mmHg, p<0.001).
Our study indicates that PLG-SAS is a less malignant form of aortic stenosis than HG-SAS, as their spontaneous outcome is better. We further demonstrated that patients with PLG-SAS are "en route" toward the more severe HG-SAS form, as the majority of them evolve into HG-SAS overtime.

**Importance of catheter contact force during radiofrequency ablation of atrial fibrillation**

Jean-Benoît le Polain de Waroux, Christophe Scavée

Atrial fibrillation (AF) is the most common arrhythmia and is associated with increased risks of death, heart failure, stroke and cognitive impairment. During the last decade, pulmonary vein isolation (PVI) has emerged as a promising therapy for patients with paroxysmal AF. Using radiofrequency energy, PVI is usually easily achieved. However, acute pulmonary vein reconnection frequently occurs and is associated with unfavourable long-term clinical results. This phenomenon of acute electrical disconnection and later recovery of the pulmonary veins is related to a state of functional block called "dormant conduction" (DC). Typically, DC progresses to permanent conduction recovery during the healing process occurring in the weeks and months following ablation.

In the present study, we hypothesize that low catheter contact force (CF) during radiofrequency ablation potentiates oedema formation rather than deeper, permanent lesions and that areas of low CF will demonstrate either a recovery or dormant conduction early after PVI. Using a specific force sensing catheter, we explored the relationship between CF and DC during catheter ablation for paroxysmal AF.

Our results demonstrate a clear relationship between the absence of CF during PVI and the occurrence of early recovery or dormant conduction. Using a fixed target power of 27 watts, a mean CF < 5g was highly predictive of ER or DC (Se: 71%, Sp: 81%). In contrast, the incidence of ER or DC was very low in the group of lesions created with a mean CF > 10g (negative predictive value: 98.7%). For the group of lesion created with > 5g but less than 10g, the application duration and the subsequent force time integral were also predictive of dormant conduction (cut-off value of 800g.s). These target values were therefore proposed to optimize the efficacy of the pulmonary vein isolation.
Predictive value of the heart rate reserve in patients with permanent atrial fibrillation treated according to a strict rate-control strategy

Jean-Benoît le Polain de Waroux, Sébastien Marchandise, Christophe Scavée

Atrial fibrillation patients treated according to a rate-control strategy seem to have excellent outcomes as long as their ventricular response is kept low. However, the stringency of the rate control to adopt with pharmacologic agents is not clearly defined. In particular, the clinical importance of preserving a heart rate (HR) reserve (HRR) during exercise remained unexplored. In the present study, we analysed the HR response profiles during exercise in a large group of patients with permanent AF for whom a strict rate-control strategy was the preferred treatment option. Patients were asked to perform an exercise test on a cycle ergometer until exhaustion. Doing so, we demonstrated that both a low exercise capacity and a low HRR were significantly associated with the risk of heart failure or death. Moreover, No correlation was found between the treatment category (i.e. beta-blockers, calcium channel antagonist, and digoxin) and the HRR.

This prospective, randomized, single center trial enrolled 64 consecutive patients with paroxysmal AF undergoing a cryo-isolation of the PVs. Patients were randomized to receive (Cryo+) or not (Cryo) an extra cryo-application after PV disconnection.

Pulmonary vein isolation using the second-generation cryoballoon: do we still need a second set of lesion to warrant favourable early and long term results

Jean-Benoît le Polain de Waroux, Cynthia Barbraud

Pulmonary vein isolation to treat paroxysmal atrial fibrillation can be performed by using RF or Cryo-energy. Cryo-isolation of the PV present several advantages over RF procedures. However, with the first generation device, consecutive freeze-taw-freeze cycles were required to achieve favourable long term results. Recently, Medtronic launched on the market a second-generation cryoballoon that present technical solutions designed to optimize the clinical outcome after cryo-isolation of the pulmonary veins. The principal modifications consisted in doubling and better positioning the ports used to deliver the refrigerant to the inner lumen of the balloon. As a result, in comparison with the first generation CB, the CB-A demonstrates important improvement in temperature uniformity and a larger freezing zone at the balloon apex. The aim of the present study was to estimate the rate of early recovery, dormant conduction and Mid-term outcome after cryo-isolation of the pulmonary vein (PV) with the second-generation cryoballoon (CB) and to determine the impact of a second set of cryo-application for the patient outcome.

2014 PUBLICATIONS


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.
New ISO Standards for Quality Evaluation in Computer Assisted Orthopaedic Surgery

O Cartiaux, L Joskowicz, G De Jongh

In orthopaedic surgery, the complexity and frequency of interventions involving bone-preparation tasks such as bone-cutting, bone-drilling and bone-assembly have spawned an important research area. Since the 1990s, there has been extensive development of computer and robot assistance technologies to improve clinical and functional outcomes through increased accuracy and reproducibility. Numbers of surgical navigation systems, active or passive robots, and rapid-prototyping devices are undergoing clinical trials, or are already in use, for procedures such as knee and hip arthroplasties, osteotomies, tumour resections...

The parameters for evaluating performances of computer-assisted orthopaedic surgery systems have typically been defined according to the desired functional outcomes of the surgical procedure. However, by evaluating the functional outcomes of the procedure, it is not possible to assess the specific accuracy of the bone-preparation tasks with respect to the desired outcome (for instance, errors between achieved and desired bone cuts). Moreover, despite the fact that this functional evaluation methodology is still largely in use, there is a significant controversy surrounding the added value of surgical assistance technologies, hindering their integration and use in clinical routine. In consequence, there is a significant need for undertaking standardization activities in this particular field. The goal of the standardization project is to produce a new consensus-based international ISO standard on accuracy measurements in computer-assisted orthopaedic surgery (CAOS). The standard will include the terms and definitions regarding accuracy and accuracy measurements in CAOS, and the methods for measuring accuracy of bone-preparation tasks in CAOS, e.g. bone.

Intraoperative quantitative measurement method of pedicle screw insertion accuracy in spine surgery

V Cordemans, P Paladin, X Banse, O Cartiaux, M von Roden, L Kaminski

The aim is to develop a new intraoperative quantitative measurement method of pedicle screw insertion accuracy using 3D fluoroscopic images.

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 1**

### Assistance device for surgical reduction of fracture/dislocation of the ankle-foot complex

**P Etienne, T de Walque, T Paquet, D Putineanu, O Cartiaux**

Surgical treatment of a fracture/dislocation of the ankle-foot complex is carried out in two stages. Initially, the fracture or dislocation is reduced by means of an external fixator for repositioning the bone fragments in their natural positions. The patient then remains bedridden for several days to several weeks to allow time for the soft tissue to heal. Secondly, if necessary, the surgeon places an internal fixation with nails or osteosynthesis plates to promote bone fusion of the fracture.

The project has already covered the design, manufacture and validation of a demonstrator allowing the surgeon to reposition the foot and ankle bones in their natural positions. Based on this first demonstrator manufactured by 3D printing, the project aims now to:

1. design and build a more successful version of the device by implementing the entirety of the technical specifications that are not yet covered by the demonstrator (material selection, sterilization, assembly / disassembly ...)

2. assess the added value of the device for Trauma Surgery using synthetic or cadaver bone models to quantify the performance of the surgical procedure performed.

**Figure 2**
Assistance device for surgical reduction of distal femur fractures

H Allard, Q Wala, D Putineanu, O Cartiaux

Surgical treatment of a fracture of the distal femur is carried out in two stages. Initially, the fracture is reduced by means of nails and pins for repositioning the two condyles in their natural positions. In a second step, the surgeon fixes the complex formed by the two condyles to the diaphysis by means of a bone plate or an intramedullary nail to promote bone fusion of the fracture.

Presently, the surgeon has difficulty in the second phase of the operation. Indeed, if he manages to correctly replace both condyles in the axis of the diaphysis, assembly of the condyles to the diaphysis by means of a plate or a nail is problematic in that both condyles tend to move when fixing. A displacement of the condyles has to be avoided since this causes problems during revalidation of the patient.

The project has already covered the design, manufacture and validation of a first demonstrator allowing the surgeon to reposition the femoral fragments in their natural positions. Based on this first demonstrator manufactured by 3D printing, the project aims now to:

1. design and manufacture a more developed version of the device for repositioning the femoral fragments in their anatomical position during the installation of an internal fixator by the surgeon.

2. evaluate the added value of the device using synthetic or cadaver bone models to quantify the performance of the surgery.

New semi-automatic detection method of joint penetration during triple-screw internal fixation for femoral neck fractures

A Englebert, O Cornu, O Cartiaux, K Tribak, D Putineanu

During triple-screw internal fixation of femoral neck fractures, hip joint penetrations are difficult to detect without medical imaging or manual measurements. The screws may appear on conventional two-dimensional radiographs as properly inserted in the femoral head, when in reality they penetrate the articular surface of the hip joint. A new method of joint penetration detection was developed and implemented in the form of computer software using the two-dimensional X-rays to identify the positions of the screws and measure the distances to the articular surface of the hip.

The software has been validated on femoral neck fractures on simulated synthetic bone models. The correlation coefficient between the two operators who identified the positions of the screws was 0.99. The mean difference between software measurements and mechanical reference measurements was 0.72 ± 0.51 mm. All penetrating screws were detected. No non-penetrating screw was considered a penetrating screw. The project now aims to validate and evaluate the clinical relevance of the new detection method by studying its accuracy and inter- and intra-observer reproducibility on real clinical cases radiographs.

Figure 3

Figure 4
Assistance device for vascularized bone grafting in hand surgery

F Jacquet, D Willame, O Barbier, X Libouton, O Cartiaux

Vascularized bone grafting is now the best conservative way to treat diseases of the wrist bones such as scaphoid and lunate. The surgical technique involves harvesting and fixing a vascularized bone graft in the carpal bones. However, surgeons do not have tools (computer, mechanical or electromechanical) sufficient to perform the procedure with adequate accuracy, the expulsion of the graft out of the recipient site being the main complication.

The project aims to design and manufacture a functional prototype of a new surgical tool for the harvesting of vascularized bone grafts and stabilization in the recipient site. The project also includes a validation phase of harvested grafts using synthetic bone models to quantify the performance of the surgical procedure and investigate the added value of the new device for hand surgery.

Computation of spine intervertebral motions and efforts in scoliotic patients: a multibody approach

G Abedrabbo, C Vanoorenberghe, P Fisette, O Cartiaux, M Mousny, M Raison, P Mahaudens, C Detrembleur

The intervertebral efforts quantification in scoliotic spines, before and after spine arthrodesis, appears to be useful for surgical planning. An increase of 30% of the energetic cost for adolescent idiopathic scoliosis patients, as well as its consequences in ordinary living, suggest that gait is a relevant motion to be considered in our study. The accurate computation of those efforts strongly depends on 4 pillars: geometrical identification, spine and pelvis kinematics, patient physiology and muscular forces. The geometrical identification of the spine, using bi-planar X-rays, as well as the computation of its kinematics from a limited amount of data, has been addressed in previous studies. The present work focuses on the validation of the spine kinematics identification during gait, for a scoliotic patient.

The geometrical identification of the spine is based on bi-planar X-rays, whereby the center of the intervertebral discs and the position of metallic markers stuck on the skin at the level of the 18 (from L5 to C7) spinous processes are identified. Reflective markers are stuck at the same place (for only 8 spinous processes) for the gait analysis.

The followings steps are carried out: 1. A multibody model (MBS) is developed based on the bi-planar X-rays. 2. An optimization algorithm is then used to fit the center of the discs in the model, to those measured on the standing-up patient. 3. The position of the spinous processes is then used to compute the intervertebral torsion along the spine. This torsion is particularly important in scoliotic patients.

Due to experimental limitations during gait, it is impossible to obtain the same amount of information as from radiograph, leading to an underdetermined problem. In the current protocol, we are only able to stick up 1 reflective marker on the spinous process per two vertebrae. For this reason some additional information is required to predict the lacking kinematic data of the spine during gait.

Since it is experimentally complicated to place a marker on each vertebra, in order to estimate the impact of the lack of kinematic information, we suggest to work in the opposite direction. In other words, we have fixed one reflective marker each 2 vertebrae from L4 to C7, then 2 different optimizations have been carried out: the first one includes all the reflective markers and the second one uses 1 reflective marker for each 4 vertebrae only i.e. neglecting the information of one every two markers. Finally, both models are compared to analyze the sensitivity of the kinematics to this lack of information.

In this study, two different kinematical reconstructions have been achieved. One using the information from markers stuck on: the pelvis, L4, L2, T12, T10, T6, T4 and T2 and another one using the information from markers stuck on: the pelvis, L4, T12, T8, T6 and T2. To analyze the difference between both reconstructions, the relative rotation between vertebrae has been compared. The vertebra rotation results from the above optimization process that fits the multibody models with the filtered experimental data. Angular velocity and accelerations, also required for the future spine analysis, are obtained via curve fitting and differentiation.
Although some discrepancies are observed for velocity and acceleration, it can be said that the spine kinematics is rather well-captured to predict the overall motion of the spine during gait. The future work will focus on the spine dynamics, for which the present kinematic computation represents a fundamental input. It will be important to keep in mind the sensitivity of the kinematic accuracy, tackled in this study, on the final results, i.e. the intervertebral efforts in the dynamic context of gait.

The patient series consisted in 11 patients eligible for curative surgical resection of primary bone tumor of the pelvis. Eight patients had a bone sarcoma of iliac bone involving the acetabulum, two patients had a sacral tumor, and one patient had a chondrosarcoma of proximal femur with intra-articular hip extension. For all cases, magnetic resonance imaging (MRI) and computerized tomography (CT) were acquired preoperatively for diagnosis. The tumor volume was first delineated on the MRI. The set of MRI and CT images were fused to produce 3D models of bone and tumor volume. Resection planning consisted in desired cut planes positioned close to the boundary of the tumor (from 1 up to 6 planes) defining the desired bone cutting with a safe margin defined by the surgeon from 3 up to 15 mm. PSI were designed in computer-aided design software according to the desired resection strategy and produced by additive manufacturing technology.

PSI were designed to have bone-specific surfaces to fit in unique position on the bony structure of the patient. PSI were equipped with cylindric guides for 2-mm diameter Kirschner wires to be pinned on the bony structure and flat surfaces to materialize the desired cut planes. Intraoperatively, PSI were positioned freehand by the surgeon and fixed on the bone surface using the K-wires. Once the resection was achieved, both K-wires and PSI were taken off. The standard surgical approach has been used for each patient. Dissection of soft tissue for bone exposure was in accordance with the routine technique. There was no additional bone exposure to position the PSI.

Histopathological analysis of the resected tumor specimens was performed to evaluate the safety of the achieved resection margins. Postoperative CT were acquired to assess the local control of the tumor. 3D bone models were reconstructed from the postoperative CT of the patient and registered with the corresponding preoperative bone model. Two parameters were measured: achieved resection margin (RM) and location accuracy (L). RM was defined as the minimum distance (mm) between the achieved cut plane...
and the boundary of the tumor. Consequently, the error in the desired safe margin (ESM) was defined as the difference (mm) between RM and the desired safe margin. L was used in accordance with the ISO1101 standard to evaluate accuracy between achieved and desired cut planes. L was defined as the maximum distance (mm) between the achieved cut plane and the desired cut plane.

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 unambiguous for all patients. Histopathological analysis classified all achieved resection margins as R0 (tumor-free), except for two patients. Patient #8 had an urgent morcelized tumor because of severe bleeding, inevitably inducing R2 bone margins. Patient #5 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 ESM or the location accuracy L demonstrated how PSI enabled the surgeon to intraoperatively replicate the resection strategies with a very good cutting accuracy. These findings are consistent with the levels of bone-cutting accuracy already published in the literature on the clinical use of PSI and navigation technologies for bone tumor surgery. Ritacco et al. reported a series of 28 navigation-assisted bone tumor resections with an average cutting error of 2.5 mm between planned and achieved resection planes. Khan et al. also investigated bone-cutting accuracy in accordance to the ISO1101 standard and reported a 2-mm location accuracy during a PSI-assisted multiplanar resection on a cadaveric femur.

PSI technology described in this study achieved clear bone margins for all patients. Longer follow-up period is required but it appears that PSI has the potential to provide clinically acceptable margins.

**Figure 6**

Intra-operative MRI-based measurements of safe margins during resection of bone tumors

SY Traore, JC Boulanger, S Vandergugten, L Bellanova, PL Docquier, O Cartiaux, F Lecouvet, C Galant, T Schubert, X Banse

Complete surgical resection is the standard treatment for sarcomas of soft and hard tissue. During the procedure, the surgeon removes the tumor en-bloc with a continuous layer of healthy tissue, the healthy margin. As most of these tumors are very close to important vessels and nerves, careful pre-operative planning is required to save these structures. Currently surgeons delineate the tumor boundaries on MRI images to plan the surgery.

After surgery, the resected tumor specimen undergoes pathological examination under the microscope. The goal of surgery is to leave a margin of at least 1 mm of healthy tissue around the tumor (R0 resection). When there is some residual tumor tissue (microscopically R1 and macroscopically R2), revision surgery or postoperative radiotherapy is usually recommended. Pathological examination of bone sarcomas is technically difficult and time consuming in case of large sarcomas. The main objective of the project is therefore to design a tool to control the healthy margin for resection of bone tumors using magnetic resonance imaging. The sub-objectives are to optimize the quality of MRI images (environmental air, water ...) for the delimitation of the volume of the resected tumor specimen, and to design and implement a methodology for measuring surgical margin of a resected tumor specimen from MRI images.

The first studies (conducted on small animals and patients) compared the MRI-based measurements with conventional measurement method on histological sections. These studies
have shown a very good correlation between the methods of measurement (kappa 0.84, P <0.05). MRI can be considered a relevant tool to provide rapid information on the surgical margins and help the pathologist to focus on suspicious areas and thus save time in the specimen analysis. This study shows that MRI analysis can be effective in the treatment of bone tumors in cancer surgery if done by an experienced radiologist in conjunction with the pathologist.

Figure 7

Anthropometric measurements of the knee: time to make it fit

E Thienpont, R Becker

Anthropometry is the study of the measurement of the human body in terms of the dimensions of bone, muscle and adipose tissue. The word derives from a composition of the Greek words anthropos or “man” and metron or “measure”. It is a science which measures the range of body sizes within populations. Therefore, anthropometry plays a vital role in industrial design, clothing design, ergonomics and architecture whereby the statistical data gathered with respect to the distribution of body dimensions in a population are used to optimize product development. The most commonly studied parameters are human height, weight, organs and finally human aesthetics. For most of the parameters, it is understandable that there will be morphologic differences between different ethnic groups.

Obviously, there is a difference in size between the Asian and European populations. Asians require smaller component sizes in total knee arthroplasty (TKA). The correct fit of the Duracon®, Scorpio®, NexGen®, PFCSigma® and the UKnee® was studied in the Asian population. Mediolateral undersizing was found for the smaller implants, but overhang in the larger sizes. Anthropometric dimensions for a specific population can be ranked by size and described in terms of percentiles. In furniture design, general dimensions are chosen as such so that they fit the fifth percentile of the female population and the 95th percentile for the male population. This rationale accommodates approximately 90% of the population.

The question in knee surgery is of course, should an implant be designed to accommodate 90% of the population or should it cover the entire population. Early knee designs were developed “down the middle” using the mean sizes for males and females and as a result could not fit everyone perfectly.

The mismatch of femoral components, especially in the mediolateral dimension of female patients, was one of the main reasons for the need to design specific implants in order to reduce component overhang in female patients. Gender differences were also observed in the anatomy of the trochlear groove. A strong correlation between the morphology of the proximal femur and the trochlea was reported.

A meta-analysis on the outcome of TKA using gender implants was performed recently and showed that gender specific TKA indeed reduces component overhang, but without any positive effect on clinical outcome.

However, the functional evaluation of patients after TKA revealed an overall significant number of patients (regardless of gender) who presented with mediolateral overhang of either the femoral or tibial component. There was also a significant negative correlation between the over-
An overhang of the femoral component of more than 3 mm appears to be associated with an almost twofold increased risk of knee pain.

One may presume that the mismatch between component design and bone morphology could have a significant impact on patient satisfaction. However, it remains unclear how much of a mismatch between the implant and the morphology of the knee might be tolerable as many other contributing factors might also have a significant impact on the clinical outcome as well.

With the introduction of patient-specific instrumentation, more attention was paid to bone morphology. Patient specific instrumentation is based on 3D MRI or CT images, and thousands of these 3D images are available for research today which gives us a unique opportunity for detailed analysis of human bone morphology. It also allows us to identify differences between ethnic groups, which could have a significant impact on component design for globally active orthopaedic companies. Osteoarthritic changes of the knee also cause changes to the bony geometry. Significant differences were found with regards to a few parameters between the arthritic and the non-arthritic knee.

Tibial designs have moved more recently from the symmetric non-anatomical tibial plateau to the more anatomical plateau, showing smaller AP dimensions on the lateral side. The Natural knee®, which was developed in 1985, used already an asymmetric plateau successfully. With this design, studies showed less impingement at the area of the popliteus tendon and a better fit to the tibial plateau.

This finding has been confirmed recently. The coverage of the tibial bone by a non-anatomical component is about 85–87 %. This bony coverage increases up to 92 % when anatomical designs are used. The non-anatomical design requires internal rotation of more than 5° for compensation in 39–60 % of patients in order to improve the bony coverage.

There seems to be a renaissance of the more asymmetric anatomical tibial design in TKA.

However, it remains questionable whether the return from the non-anatomical to the anatomical design will really improve knee function and clinical outcome. Anthropometric data will help to improve the understanding of the bony morphology in relation to the knee.

A systematic review and meta-analysis of patient-specific instrumentation for improving alignment of the components in total knee replacement

E Thienpont, PE. Schwab, P Fennema

We conducted a meta-analysis, including randomised controlled trials (RCTs) and cohort studies, to examine the effect of patient-specific instruments (PSI) on radiological outcomes after total knee replacement (TKR) including: mechanical axis alignment and malalignment of the femoral and tibial components in the coronal, sagittal and axial planes, at a threshold of > 3° from neutral. Relative risks (RR) for malalignment were determined for all studies and for RCTs and cohort studies separately.

Of 325 studies initially identified, 16 met the eligibility criteria, including eight RCTs and eight cohort studies. There was no significant difference in the likelihood of mechanical axis malalignment with PSI versus conventional TKR across all studies (RR = 0.84, p = 0.304), in the RCTs (RR = 1.14, p = 0.445) or in the cohort studies (RR = 0.70, p = 0.289). The results for the alignment of the tibial component were significantly worse using PSI TKR than conventional TKR in the coronal and sagittal planes (RR = 1.75, p = 0.028; and RR = 1.34, p = 0.019, respectively, on pooled analysis). PSI TKR showed a significant advantage over conventional TKR for alignment of the femoral component in the coronal plane (RR = 0.65, p = 0.028 on pooled analysis), but not in the sagittal plane (RR = 1.12, p = 0.437). Axial alignment of the tibial (p = 0.460) and femoral components (p = 0.127) was not significantly different.

We conclude that PSI does not improve the accuracy of alignment of the components in TKR compared with conventional instrumentation.
Rotational alignment of the distal femur: anthropometric measurements with CT-based patient-specific instruments planning show high variability of the posterior condylar angle

E Thienpont, PE Schwab, F Paternostre, P Koch

Finding the anatomical landmarks used for correct femoral axial alignment can be difficult. The posterior condylar line (PCL) is probably the easiest to find during surgery. The aim of this study was to analyse whether a predetermined fixed angle referencing of the PCL could help find the surgical epicondylar axis (SEA) and this based on a large CT database with enough Caucasian diversity to be representable.

A total of 2,637 CT scans and 3D reconstructions from patients on four continents, executed for preoperative planning and creation of patient-specific instrumentation, were used to perform anthropometric measurements and to measure the posterior condylar angle (PCA) between the surgical epicondylar angle and the PCL.

The mean (SD) PCA was 4° (1.4°) of external rotation. A significant correlation was found between more external rotation of the SEA and more proximal varus of the tibia or more distal valgus of the femur. For 59% of the study population, 4° external rotation from the PCL would be the right amount of axial rotation to align the femoral component in line with the SEA. Nine per cent needs less, and 32% needs more than 4° of axial rotation. On 105 (4%) CT-based 3D models, external rotation between 7° and 11° was measured and 77 (73%) of those cases were in varus or neutral alignment. In 132 patients, bilateral measurements were available and 94 (71%) had rotation within 1° of the opposite side. This last finding underlines that there is even an intra-individual difference in distal femoral anatomy that can range from 1° to 5°.

This study was performed on a very large anthropometric CT and 3D models database and showed that there is a 41% risk of malalignment if a fixed PCA referenced of the PCL is used in total knee arthroplasty. The clinical importance of this study is the observation that femoral axial anatomy is individual and also that it is determined by the tibial anatomy. A group of patients needs more than the average external rotation because they have more distal femoral valgus with dysplastic condyles or more proximal tibial varus with a bigger medial condyle.

Total knee arthroplasty in patients with substantial deformities using primary knee components

J De Muylde, J Victor, O Cornu, L Kaminski, E Thienpont

Although advocated for severe varus and valgus deformities, constrained implant designs are associated with a number of disadvantages in total knee arthroplasty (TKA). Combining a minimally invasive surgical approach with an interchangeable posterior stabilized (PS) implant design may allow adequate soft tissue balancing with a minimal amount of constraint and without residual instability. Retrospectively 51 patients operated with the minimally invasive far medial subvastus approach for severe varus or valgus deformity, who underwent primary TKA with a fully interchangeable PS implant (Vanguard, Biomet Inc., Warsaw IN, USA) between 2009 and 2013 were examined. Soft tissue releases was performed using a piecrust needling technique. Preoperative alignment and surgical parameters were collected for all patients. All patients underwent preoperative and follow-up radiographic assessment and completed a battery of clinical assessments.

All procedures were performed successfully, with alignment improving from a preoperative mean (SD) varus deformity of 165° (3°) and a mean (SD) valgus deformity of 196° (4.5°) to an overall mean (SD) postoperative mechanical alignment of 179.5° (3.0°). Nine patients had postoperative varus, while three patients had a postoperative valgus deviation from neutral alignment >3°. The mean change in joint line position in extension was −0.0 ± 0.6 mm. Clinical scores at final follow-up were excellent for both groups.

Good TKA outcomes can be achieved in patients with substantial varus or valgus deformities using a combination of a minimally invasive far medial subvastus approach, interchangeable PS implants and soft tissue releases with a piecrust needling technique.
The combined Whiteside’s and posterior condylar line as a reliable reference to describe axial distal femoral anatomy in patient-specific instrument planning

F Paternostre, PE Schwab, E Thienpont

Aligning the femoral component in the axial plane parallel to the surgical epicondylar axis (SEA) has been generally recommended. In this retrospective study on the axial anatomy of the distal femur, as determined by the patient-specific instruments (PSI) planning tool based on MRI and 3D reconstructions, the different rotational axes were compared. The purpose of this study was to compare the impact of posterior axial anatomy on anterior anatomy and to compare the different angles of rotation obtained by a PSI-planning engineer.

The preoperative planning of 77 PSI patients with a mean (SD) age of 65.6 (9.6) years undergoing primary total knee replacement for osteoarthritis was analyzed for rotational anatomy of the distal femur. The angles between the posterior condylar line (PCL) and the SEA called posterior condylar angle (PCA), between Whiteside’s line and the SEA and finally between Whiteside’s line and the PCL, were retrieved from the PSI axial rotation planning screen.

The mean (SD) PCA was 3.2° (1.4°). The mean (SD) angle between Whiteside’s line and the SEA was 91.4° (2.2°), and the mean (SD) angle between Whiteside’s line and the PCL was 94.5° (2.3°). No significant difference for this last rotational parameter was found in between varus and valgus knees.

Patient-specific instrument’s preoperative planning found consistent angles to describe the distal femoral anatomy as previously published in the literature. The angle between Whiteside’s line and the PCL as measured on PSI planning is a mean angle of 94.5° (2.3°) for both varus and valgus knees. Setting a fixed PCA of 5° of external rotation referenced of the PCL makes this planning repeatable during conventional surgery.

SELECTED REFERENCES


COLLABORATORS

- Bureau de Normalisation NBN (Brussels, Belgium)
- International Society for Computer Assisted Orthopaedic Surgery (CAOS International)
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PATENTS


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).
- Intraoperative robotic imaging system

FUNDINGS

- Fondation Saint-Luc (Brussels, Belgium)
- Fondation belge contre le cancer, grant SCIE2010-184 (Brussels, Belgium)
- First Spin-Off, Walloon Region, Belgium
- Brains Back To Brussels Grant, Brussels-Capital Region, Belgium
- Meditis Training Program in Biomedical Sciences and Technology (NSERC/ FONCER, Canada)
- Siemens S.A./N.V. (Beersel, Belgique)

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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 successfully transplanted 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 more recently, transgenic pigs which overexpress GLP-1 to amplify the pig insulin response to hyperglycemic challenges.

In addition, several pre-clinical and clinical studies are undertaken by members of the University Hospital such as the impairment of hepatic regeneration by chemotherapy in rats, research in urology areas, the 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.
Functionally-enhanced pancreatic islets from adult and neonatal pigs for xenotransplantation

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 raised 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 porcine pancreatic cell clusters (NPPCs)** 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. Pancreatic cell clusters obtained immediately after isolation were heterogeneous in shape, aspect and size. The smaller ones (50-200 µm diameter) presented a rather rounded shape, a translucent appearance and a few were positive for dithizone (DTZ) staining (DTZ binds to the zinc core of insulin crystals thus staining insulin-containing cells in orange) but most of them were bigger in size (>400 µm), presented irregular shapes and a compact, darker appearance. Such clusters were not stained by DTZ and consisted mainly of exocrine pancreatic tissue. We regularly examined the aspect and purity of our neonatal pig pancreas preparations during their culture period. The amount of exocrine tissue within each preparation started decreasing on the first day following isolation. This loss of exocrine tissue continued through the culture period so that by the fifth day after isolation, most of it had already disappeared. In parallel to this disappearance of exocrine tissue, the number of insulin-positive clusters and the amount of positive cells within the clusters increased over the culture period as shown by DTZ staining (Fig. 1).

**Figure 1** Dithizone staining of live NPPCs on the first day after isolation (left panel) and after 8 days of culture (right panel). Orange coloration indicates insulin-containing cells.

The function, i.e. insulin secretion, of isolated NPPCs was also evaluated. Following the 8-day culture period, NPPCs were washed with Krebs buffer then counted to determine the number of IEQ per mL. NPPCs were then divided into groups of 100 IEQ and preincubated for 1 hour in low (1 mM) glucose Krebs buffer. The islets were then incubated in quintuplicates for 2 hours at 37°C in a Krebs buffer containing different insulin secretagogues such as glucose, forskolin or high potassium. At the end of the 2 hours incubation period, supernatants were kept to measure secreted insulin and the
islets were lysed in acid ethanol to determine intracellular insulin content. As shown in figure 2, insulin secretion from NPPCs increased by 2.9-fold in the presence of stimulatory (15 mM) glucose concentration compared to resting (1 mM) conditions. The magnitude of NPPCs response to glucose is in line with previous data on pig islet insulin secretion and indicates that these pancreatic cell clusters contain mature, responsive beta-cells. Interestingly and as previously described for adult pig islets, glucose-induced insulin secretion from NPPCs was greatly increased (stimulation index = 12.2) in the presence of 1 µM forskolin which activates beta-cell adenyl cyclase thus increasing intracellular cAMP. NPPCs were also found to be responsive to high potassium stimulation which depolarizes cell membranes, increases intracellular calcium and induces insulin granule exocytosis. 30 mM KCl thus increased insulin secretion by 6.7-fold in the presence of 1 mM glucose. Altogether, these data show that NPPCs isolated and cultured for 8 days in a specific maturation medium are responsive to glucose stimulation and secrete insulin when challenged with an increase of ambient glucose concentration or KCl-induced membrane depolarization and that their secretory response is greatly amplified by cAMP-increasing agents such as forskolin.

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. As shown in figure 3, forskolin increased glucose-induced insulin secretion by 1.5-fold in adult pig islets and by 4.5-fold in neonatal pig islets. Direct PKC activation by PMA increased glucose-induced insulin secretion by 2-fold in islets isolated from adult pigs and up to 8-fold in islets isolated from neonatal pigs, thus improving porcine islet responsiveness to glucose. Interestingly, when we exposed pig islets to both PMA and forskolin in the presence of 15 mM glucose, we observed a synergy in the secretory response which was then augmented by almost 6-fold in adult islets and 25-fold in neonate islets. These results thus demonstrate an unexpected synergistic effect of PKC activation and PKA activation on insulin secretion.
GLP-1, natural ligand and activator of the GPCR (G-protein-coupled receptor) which activates adenylyl cyclase has low endo- 
genous expression levels in islet cells. Moreover, GLP-1 is rapidly degraded (half-life = 2 min) 
by dipeptidyl peptidases (DPP) which further shortens its effects on target cells. It would 
thus be beneficial for beta-cell function to increase GLP-1 production within islets and 
to preserve it from enzymatic degradation. A plasmid harbouring a sequence encoding the 
active GLP-1 fragment (amino acid 7 to 37) was produced. In this plasmid, the alanine 
residue at position 8 of the GLP-1 sequence was replaced by a serine to make GLP-1 Ser8 
resistant to degradation by DPP.

PKC pathway can be activated upstream by engineering constitutively active muscarinic 
receptors (M3R) on beta-cells membrane. By activating the pathway at the receptor level, 
we avoid the negative feedback loop as well as possible tumorigenesis related to prolonged 
direct PKC activation and benefit from the calcium-increasing effect of phospholipase C 
(PLC) activation. To study the effects of GLP-1 and M3R expression in vitro in isolated pig 
islets, GLP-1 Ser8 and M3R sequences were inserted in a pENTCMV adenoviral vector to 
permit expression of transgenic GLP-1 (GLP-1 Ser8) and activated muscarinic receptor 
(M3R) in primary islet cells. For co-expression of GLP-1 and M3R, the two sequences were 
inserted in the same bicistronic vector to study the effect of concomitant activation of PKA 
and PKC on pig islet insulin secretion.

Adult pig islets cultivated in RPMI were ex-
posed to GLP-1, M3R or GLP-1 + M3R viral 
expression vectors at a multiplicity of infec-
tion of 200 (MOI = 200) during 48 hours before 
glucose challenge. Islets were placed in pe-
rifusion chambers sealed with 0.2 µm filters. 
They were first perfused with 1 mM glucose 
(G1) krebs medium during 30 minutes for equi-
librium then during 10 minutes in G1 with 
media collection every 2 minutes followed by 
30 minutes stimulation with G15. As shown 
in figure 4, GLP-1 expression had virtually 
no effect on acute insulin secretion. M3R 
expression increased both phases of glucose-
induced insulin secretion but this increase 
was greater when pig islets co-expressed 
GLP-1 and M3R.

Figure 4  Insulin secretion from isolated adult pig islets. 
Isolated islets were exposed to 200 MOI viral expression 
vectors carrying sequences coding for GLP-1 (GLP-1 Ser8), 
activated muscarinic receptor (M3R) or both (GLP-1 + M3R) 
during 48 hours. Batches of 600 islets were perfused in 
krebs medium containing 1 mM glucose (G1) then 15 mM 
glucose (G15) as indicated on top of the figure. Insulin se-
cretion was then measured in the effluent fractions. Values 
are means ± SEM for n=3-4 from 4 different preparations.

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

**BIOactive implantable Capsule for PANcreatic islets 
immunosuppression free therapy (BIOCAPAN)**

M. Henry, P. Gianello

BIOCAPAN is a European project proposal of a 
48-month duration, which aims at deve-
loping an innovative treatment for diabetes. 
The therapy is based on the implantation of 
smartly microencapsulated allogeneic islet 
cells, which will allow an effective long-lasting 
blood glucose normalisation and stabilisation, 
without the need for immunosuppression. 
The first objective is the design a GMP-grade 
bioactive microcapsule that will maximize 
the long-term functionality and survival of 
pancreatic islets by prevention of pericapsu-
lar fibrotic overgrowth, in situ oxygenation, 
innovative extracellular matrix microenviron-
ment reconstruction and immune-system 
modulation. A GMP-grade microfluidic mi-
croencapsulation platform will be developed 
to protect freshly harvested islets quickly in 
a standardized and reproducible way. We aim 
for full preclinical validation and we will esta-
blish a complete protocol in accordance with
the provisions of the Advanced Therapy Medicinal Products Regulation, in order to start clinical trials within one year after the end of the project. The consortium is made of nine partners from academia, public research sector and industry from Europe and USA. This project will start in June 2015. Two UCL labs will be involved in BIOCAPAN. BSMA (Bio and Soft Matter) Unit will participate to the development of the smart bioactive treatment of the microcapsule surface. In CHEX, empty and filled microcapsules with human, pig or rat islets will be evaluated in several in vivo models including rodents, pigs and eventually primates.

This work will be funded by the European research grant UE H2020 Biocapan 646272.

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-months 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 is filled with either pig islet cells, human endocells and human islets in our diabetic animal models at CHEX.

This work will be 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-spread 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.

Pig auricular flaps designed for the study.

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 Germany in five humans with a significant effect on apnea index.

Hepatic regeneration is impaired in a rat model by chemotherapy agents used in metastatic colorectal cancer

C. Hubert, C. Dahrenmüller, L; Marique, N. Jabbour, P. Gianello, I. Leclercq

Oxaliplatin before resection of colorectal liver metastases (CLRM) in human can induce Sinusoidal Obstruction Syndrome (SOS) that can affect postoperative outcome. Bevacizumab can improve SOS lesions and post-operative liver regeneration. Here we investigate in a rat model whether Oxaliplatin has a direct impact on liver regeneration and whether this is modified by the administration of Bevacizumab.

Rats underwent a 70% partial hepatectomy (PH) 3 days after intraperitoneal administration of chemotherapy or saline. Chemotherapy agents included Oxaliplatin, 5 fluorouracyl (5FU) and Bevacizumab. Liver regeneration was evaluated by the weight of the remaining lobe and by the liver mass recovery. DNA synthesis was determined by immunodetection of BrDU incorporation. Hepatic mRNA expression levels of VEGF-a and of its 2 receptors (fIT-1 and KDR) were quantitated by PCR technique.

Liver regeneration was altered in groups of rats having received high doses of Oxaliplatin alone (20mg/kg) and in groups having received combined 5FU 100 mg/kg and Oxaliplatin (10 or 20mg/kg) but without associated alteration of liver sinusoids. Bevacizumab administration did not improved liver regeneration caused by chemotherapy. Chemotherapy administration has no effect on VEGF-a and receptors expression, compared to control. VEGF-a expression and receptor 2 (KDR) expressions are decreased 24h after PH in controls. Similarly, chemotherapy regiments including Oxaliplatin 20 and 5FU 100 + Oxaliplatin 10 were also associated, 24 h after PH, with a decreased expression of VEGF-a and KDR or KDR alone expression.
Oxaliplatin, alone in a dose-dependent manner, or combined to 5 FU, causes impairment in hepatocytes proliferation in early phase post- PH in a rat model that is not associated to sinusoidal alteration. Bevacizumab do not worsen nor improve liver regeneration altered by chemotherapy. The mechanism of this hepatocytes proliferation impairment cannot be explained by a change of VEGFa signaling.

This work is funded by Roche® and Dr C. Hubert is funded by “Patrimoine” – UCL - Faculty of Medicine research grant (2010).

B. Tombal

The activity of the Department of Urology is deployed in four areas:

1. Detection of genetic signatures on circulating blood by analyzing SNPs for the diagnosis of prostate cancer and the prediction of occurrence and intensity of side effects to hormone deprivation by androgen1 (Mrs. Valentina Butoescu: PhD thesis). The Maisin Foundation and multicenter study supported by a Grant from the educational Ferring firm.


3. The development of a urinary quality of life questionnaire for patients with multiple sclerosis. IEP Collaboration, C. Detrembleur (PhD Student: Mr. Laurent Gaspard). Internally funded. 6,7

4. The development of new generation anti-androgen treatment strategies (coordinated multicenter phase II study and initiated by UCL, PI of an international phase III). Studies sponsored by Estella’s and Bayer.8–10

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:


EQUIPMENT

- 2 surgery rooms and post-operative care
- Possibility of hosting for big 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)
TRAININGS AND SERVICES

- Surgical Training for new surgical technologies: microsurgery, implantation of artificial heart, cardiac valves repair, laparoscopic surgery.
- Training on surgical robotic (Intuitive Surgical, American Motion, Lapman ...)
- 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 + Covidien: Johnson et Johnson), Single Access Laparoscopy (Olympus))
- Repair of porcine cardiac valves (Medtronic)

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
7. Effects of prebiotics on the metabolic syndrome in obese patients
**Nutrients acutely reduce mitochondrial glutathione redox state in rodent and human insulin-secreting pancreatic β-cells?**

HK Takahashi, LRB Santos, LP Roma, JC Jonas

The glucose stimulation of insulin secretion by pancreatic β-cells depends on increased production of metabolic coupling factors, among which changes in NADPH and reactive oxygen species (ROS) may alter the glutathione redox state (EGSH) and signal through changes in thiol oxidation. However, whether nutrients affect EGSH in β-cell subcellular compartments is unknown. Using redox-sensitive GFP2 fused to glutaredoxin 1 and its mitochondria-targeted form, we studied the acute nutrient regulation of EGSH in the cytosol/nucleus or the mitochondrial matrix of rat islet cells. These probes were mainly expressed in β-cells and reacted to low concentrations of exogenous H₂O₂ and menadione. Under control conditions, cytosolic/nuclear EGSH was close to -300 mV and unaffected by glucose (from 0 to 30 mM). In comparison, mitochondrial EGSH was less negative and rapidly regulated by glucose and other nutrients, ranging from -280 mV in the absence of glucose to -299 mV in 30 mM glucose. These changes were largely independent from changes in intracellular Ca²⁺ concentration and in mitochondrial pH. They were unaffected by overexpression of SOD2 and mitochondria-targeted catalase, but were inversely correlated with changes in NAD(P)H autofluorescence, suggesting that they indirectly resulted from increased NADPH availability rather than from changes in ROS concentration. Interestingly, the opposite regulation of mitochondrial EGSH and NAD(P)H autofluorescence by glucose was also observed in human islets isolated from two donors (Fig. 1). In conclusion, this study demonstrates that glucose and other nutrients acutely reduce mitochondrial but not cytosolic/nuclear EGSH in pancreatic β-cells under control conditions. For further details, see publication 1 of the Pole.

**Figure 1** Effects of glucose on NAD(P)H autofluorescence, intracellular Ca²⁺ concentration and (mt-)GRX1-roGFP2 fluorescence ratio in perifused human islets
- A, effects of increasing glucose concentrations on NAD(P)H autofluorescence expressed as a percentage of the average fluorescence level during initial perifusion with G0.5. B, effects of glucose on fura-2 LR fluorescence ratio as an indicator of [Ca²⁺]. C, effects of glucose on normalized mt-GRX1-roGFP2 fluorescence ratio. Results are means ± SE for 7-15 islets from two donors. A, B * P<0.001 vs. G0.5 and # P<0.001 for the effect of azide or high potassium. C, * P<0.001 vs. G0.
The control of glucagon secretion by glucose and $K_{ATP}$ 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 $K_{ATP}$ 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$^{2+}$], 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 release from δ-cells. The net effect on glucagon release results from a balance between both effects (see diagram in Fig. 2). 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.

Adipokines in health and diseases

M Abou-Samra, R Boursereu, 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 3). 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.
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

Muscle 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. 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 and Mstn might contribute to cancer cachexia in several animal models. To investigate the role ActA and Mstn in human cancer cachexia, we assessed changes in body composition and circulating concentrations of ActA and Mstn in cancer patients. Our results indicate that ActA is increased in cachectic patients and positively correlated with weight loss and anorexia. Given the known muscle atrophic effects of ActA, our study suggests that increased circulating concentrations of ActA may contribute to the development of cachexia in cancer patients. These results are particularly interesting, given the large number of agents capable of blocking the ActA/Mstn signalling pathway, some of which being currently tested by our lab in animal models. In addition, this translational work supports the possible role of ActA as a new biomarker predictive of cachexia, allowing the selection of patients susceptible to benefit from ActA antagonists. These results will help to guide therapeutic strategies targeting this pathway in conditions where muscle atrophy impairs survival or quality of life.
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.

**Evaluation of Laboratory Innovative Technologies for testing in Endocrinology (ELITE)**

D Gruson

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. We have demonstrated that circulating levels of proBNP 1-108, myostatin, GDF-11, GDF-15 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 (Fig. 5).

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, which might help to identify high risk patients and thereby might aid the selection of appropriate therapy. We recently demonstrated the synergism between natriuretic peptides and PTH(1-84) for the prediction of cardiovascular death in HF patients (Fig. 6).

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 publications 12 to 14 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 EMCD cameras, photon counting mode)
- Confocal microscopy (spinning disc), TIRF

FUNDING SOURCES

Research carried out in the Pôle EDIN was supported by the following grants:

- 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 diseases».
- 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 «Metallothioneine, zinc and 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.
SELECTED PUBLICATIONS


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The Division of Endocrinology and Nutrition
UCL Saint-Luc University Hospital

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


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

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 hyper-parathyroidism 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.

### 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 transdisciplinary 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.
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 NO-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-adrenoceptors, in cardiac myocytes. 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, J. Hammond, N. Hermida, 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 particular, we found that cardiac beta3AR attenuate hypertrophy through activation of eNOS and nNOS and downstream activation of cGMP-dependent pathways that inactivate pro-hypertrophic signalling, such as NFAT: Strikingly, cardiac myocyte expression of beta3AR modulates paracrine signalling that attenuates fibrosis. We modelled inter-cellular cross-talk by building a superfusion model in vitro, and through proteomic analysis of the “secretome” of myocytes, we identified critical pro-fibrotic factors that are attenuated by cardiac beta3AR. In collaboration with colleagues at ULB, the role of purinergic receptors on cardiac endothelial cells was also identified. We participated in a larger collaborative effort at the European level, using 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.

- E 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; rad: 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


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.

Many “aggressors” to the endothelium, such as high glucose, oxidized lipoproteins or elements of the renin-angiotensin-aldosterone system leave enduring activation marks on the endothelium that persist despite interruption of the endothelial stress. This results in permanent endothelial dysfunction that may even be inheritable through epigenetic regulation of specific gene expression in endothelial cells. We have established in vitro and in vivo models of “metabolic memory” in endothelial cells that result in such enduring dysfunction, e.g. with increased production of reactive oxygen species. Current work aims at unveiling the epigenetic marks in endothelial cells that may be common or specific to these stressors, so as to identify potential nodal points possibly amenable to therapeutic modulation.
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.

Ischemia/reperfusion.

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 hy-
perpolarization 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, V.L. Payen, 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 a 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 differentiated 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.

In search of epigenetic regulation of cardiac progenitor cells (CPC) plasticity, in collaboration with colleagues at the de Duve Institute, we found that Sca1+ CPC isolated from adult hearts exhibit constitutively active canonical Wnt/beta-catenin pathway that needs to be inactivated to allow their differentiation to cardiac myocytes, thereby recapitulating the extinction of Wnt signaling during the specification of the primary cardiac mesoderm in embryogenesis. In search of regulators of Wnt in CPC, we found that Wif-1 is upregulated during differentiation and that this is driven by de-methylation of the Wif-1 gene. This is driven by downregulation of a methyltransferase, Dnmt through upregulation of a specific microRNA. siRNA targeting of the methyltransferase promotes CPC differentiation in vitro and in vivo, after CPC injection in infarcted hearts. This is also accompanied with beneficial alterations of cardiac remodeling. We are now examining putative paracrine factors differentially released by the CPC upon this epigenetic modulation.

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 intermediates. 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 antimetabolic 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.
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 mitochondrial superoxide production. The switch provided a metastatic advantage that was pheno-
copied 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.

A growing body of evidence also indicates that mitochondria within cancer cells actively participate in treatment resistance. It is well exemplified with chemotherapy where the plastic modulation of mitochondrial activities, including ATP generation, the production of reactive oxygen species (mtROS) and the control set by mitochondria on apoptosis, profoundly affects the therapeutic outcome. However, how mitochondria influence the response of tumors to radiotherapy is still largely unknown. In addition to the oxygen-sparing effect associated to high tumor radiosensitivity, we investigate the existence of additional metabolic determinants of tumor radiosensitivity that could be targeted concomitantly with radiotherapy.

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 aminoacids and cystine 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 counter-anion 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
mature and immature populations influenced by unique tumor microenvironmental features offers a rationale for specific therapeutic interventions aimed at modulating metabolite, drug and oxygen delivery, the latter impacting the clinical response to radiotherapy.

We contributed to the field by (i) proposing and validating a new therapeutic approach aimed at exploiting the differential reactivity of mature tumor blood vessels in combination with conventional anticancer therapies (now termed the “provascular approach”), and (ii) identifying and validating new targets for anti-angiogenic therapy. Our research further led to the demonstration that a FDA approved polymer can be used as a systemic agent to trigger reparative angiogenesis and to accelerate the healing of ischemic and superficial wounds in mice. Together with a consortium of partners, we are currently translating several of these findings into new therapeutic applications. The knowledge derived from this dissection of the tumor vascular phenotype allowed us to embark in several research programs aiming to use nanoparticles to deliver therapeutic entities directly in the tumor. In particular, we are working together with colleagues from UCL (IMCN, LDRI) and UNamur on the optimization of nanocarriers coupled to RGD peptidomimetics or antibodies against tumor-associated endothelial antigens. As payload of the nanoparticles, we are using 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 O2 and the uncoupling of glycolysis from mitochondrial oxidative phosphorylation to survive under low O2 are actually two complementary tumor responses to hypoxia. These somehow opposite modes of adaptation account for local and temporal heterogeneities in tumor O2 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 pO2 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 4 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 pO2 leads to cellular stress (green) compromising tumor cell viability and thus offering new opportunities for treatment.
EQUIPMENT

- Molecular biology equipment including adeno-, retro- and lentivirus technology
- Real-time PCR (Biorad IQ5 & AB ViiA7) and Gradient PCR (Biorad C1000)
- Microplate Reader incl. injectors (Victor 5)
- 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 ports)
- 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 (IVISS0, Xenogen)
- Laser Doppler imaging (Moor)
- Pressure and wire myographs and cardiac myocyte contractility (incl. fluorimetry) setup (Ionoptix)

SELECTED PUBLICATIONS


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 those diseases. To meet those 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 identification of neo-
plastic and preneoplastic tumours in the upper
and lower gastrointestinal tract, in particular with
evaluation and optimization of pioneer therapeu-
tic 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 ad-
vanced therapeutic endoscopy is further applied in
benign conditions such as the development of in-
novating 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 ex-
erts of quantitative radiology and radiomics.

Clinical research is also ongoing for a better under-
tstanding 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 papil-
ary 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 phe-
notypes 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 par-

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observer agreement of the Ki-67 labelling index. Cytopathology. 2013 Nov 15. |
Clinical trials and pharmacokinetics

Y Horsmans, I Borbath, O Dewit, H Piessevaux, N Lanthier

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.

Our unit also participates to a phase 2 trial in non-alcoholic steatohepatitis (investigator N. Lanthier).

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 dependency and alcohol-induced liver diseases and their metabolic consequences.

Dr N Lanthier collaborates with the University Hospitals of Geneva in the field of alcoholic hepatitis. He recently published the results of one study performed both in Geneva and in Brussels. He also 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 Feza-Bingi, 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 depletion (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 steatophatitis 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

P Starkel, I Borbath, C Sempoux, B Delire, O Ciccarelli, C De Saeger, MP Berghmans, I Leclercq

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.

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 Le-
maigre 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.

Figure 2. Upon-recovery from CDE-induced liver injury, identification of YFP+/HNF4a+ hepatocytes (Hep) derived from liver progenitor cells.

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.

PhD awarded in 2013-2014


SELECTED PUBLICATIONS

1 | Dusabineza AC, Najimi M, van Hul N, Legry V, Ngoc Khuu D, van Grunsven L, Sokal E, Leclercq IA


Financial support

- The D.G. Higher Education and Scientific Research of the French Community of Belgium (grant #12/17-047),
- The Fund for Scientific Medical Research (FRS-FNRS-Belgium), grants # 3.4520.10; 3.4570.11; 3.4544.11; PDR T.1067.14-P, Televie 7.4584.12.
- La foundation contre le cancer (C/2014/207)
- Grant télévie - FNR (22602291)
- The Université catholique de Louvain,
- The “centre du cancer”, Cliniques universitaires St-Luc,
- ESGE-EURO NOTES grant
- Unrestricted research grants from Janssens Pharmaceutica Belgium, MSD and Roche Belgium.
- IL is a FRS-FNRS research associate, PS, IB, NL are clinician-post-doctoral researcher financed by IREC and FNRS.

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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, hematology and oncology departments 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 chemotherapy. Cryopreservation and transplantation of ovarian tissue is a promising approach to preserve fertility in young cancer patients undergoing gonadotoxic treatment and the only option for prepubertal patients and patients who have no time to undergo stimulation for embryoocyte cryopreservation. Transplantation of cryopreserved ovarian tissue allowed restoration of ovarian function and fertility in more than 40 patients so far worldwide, with 8 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
Transplantation of cryopreserved ovarian tissue allows restoration of ovarian function and fertility. 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 and immunodeficient 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. Moreover, we observed that secondary follicles seem to survive better than small primordial and primary follicles. 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 ovarian stromal cells (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.

A study was recently conducted in order to determine the best origin of stromal cells for the artificial ovary. The viability and in-vivo growth and vascularization of OCs isolated from fresh or frozen ovarian tissue (cortex as well as medulla) was compared after 7 days of xenotransplantation to nude mice in a fibrin matrix. We found that fresh medulla was the best source of ovarian cells as they could be isolated in higher numbers, showed higher cell viability and improved graft vascularization. The ability of these cells to be recruited into thecal cells, necessary for follicle development is currently being studied using a co-culture system.
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. Based on our previous encouraging results on vitrification and autografting of baboon ovarian tissue, we decided to repeat our experiments and increase the period of transplantation (up to 18 months) to evaluate the long-term survival and development of the follicles.

C) Minimal residual disease in the ovary
M. Soares, C.A. Amorim, MM. Dolmans

AIM
- Evaluate the risk of disease retransmission through the graft
- Obtain disease-free ovarian follicle suspensions from ovarian tissue of leukemia patients for grafting.

BACKGROUND
In most centers, including ours, 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. Given the presence of leukemic cells and the possibility of disease transmission, reimplantation of ovarian tissue in young women with the acute form of leukemia is not recommended. One option to restore fertility in these patients could be the grafting of isolated preantral follicles.

RISK OF DISEASE RETRANSMISSION THROUGH THE GRAFT
Recently, a study was conducted in order to determine if a few leukemia cells grafted in an artificial ovary are able to reintroduce disease. We found that ten and 100 BV-173 cells encapsulated in a fibrin matrix along with other ovarian cells were not able to induce leukemia in SCID mice after 20 weeks of grafting. In a previous xenografting experiment, risk of ovarian tissue was assessed for patients with LLA and LMC. We are currently completing this study by evaluating this risk with ovarian tissue from deceased LMA patients.

SAFE FOLLICLE ISOLATION IN LEUKEMIA PATIENTS
We have recently investigated the safety of our follicle pick-up procedure in a model of ovarian tissue suspension artificially contaminated with leukemic cells. We showed that this procedure was not safe in case of a relatively important contamination. However, 3 additional washes proved effective in eliminating the leukemic cells taken along with the follicles. This improved follicle isolation technique has been applied to follicle suspensions obtained from the ovarian tissue of 10 leukemia patients. Suitable markers to be used for detecting leukemia cells in each patient has been determined with the help of Pr. Pascale Saussoy, hematology laboratory, Cliniques Universitaires St; Luc) and the disease free nature of these follicles are currently being tested by PCR.
### SELECTED PUBLICATIONS


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**Patents**

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

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**Partnership**

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

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**Fundings**

- Mécénats
- FNRS
- Fondation contre le Cancer
- Fondation Saint Luc
- Wallonie-Bruxelles International (WBI)
- Région Wallonne (Pôle BioWin)

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**Main Equipment**

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

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**Product and Services**

- Scaffold for human ovarian follicle grafting

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**Key words for R&D**

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

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ETIOLOGY OF THE ADENOMYOTIC NODULE OF THE RECTOVAGINAL SEPTUM

O. Donnez, R. Orellana, J. Squifflet, MM. Dolmans

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 sometimes disappointing, 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. Another advantage is that this model allows the identification and analysis of glands that invade surrounding organs, and compare with those that remain in the site of implant. Both differs in the expression of cell adhesion proteins, mitotic activity and morphology, suggesting the participation of a collective cell migration process.

Figure: Laparoscopic view of induced lesions and type III lesions in humans

Macroscopic view of nodular lesions from baboon (A) and human (B) subjects, showing striking similarities between lesions induced in baboons and type III nodules in patients (nodular lesion between the white arrows).

REPRESENTATIVE REFERENCES


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EVALUATION OF THE MECHANISM OF ACTION OF SELECTIVE PROGESTERONE RECEPTOR MODULATORS IN MYOMA TREATMENT

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

Uterine myomas (fibroids) 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. Depending on their number, size and location in the uterus, symptomatic myomas are responsible for 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, UPA) is to reversibly modulate progesterone receptors (PRs) activity in a selective manner depending on the tissue. In its target tissues (uterus, cervix, ovaries, hypothalamus) UPA acts as a potent orally active and selective PR antagonist. UPA 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. UPA immediately stops uterine bleeding, while GnRH agonists produce an initial flare-up leading to an additional episode of bleeding that can sometimes be heavy. UPA 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 UPA, 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 demonstrated that UPA treatment for 13 weeks effectively controlled excessive bleeding due to uterine fibroids and reduced the size of fibroids. Moreover, the 5mg and 10mg daily doses of UPA were not inferior to once-monthly leuprolide acetate in controlling uterine bleeding, and were significantly less likely to cause hot flashes. More recently, a long-term study demonstrated that the volume of myoma was dramatically reduced after UPA-treatment. Pregnancies were achieved after this therapy without tumor regrowth, attesting the sustained effect. The mechanisms leading to the myoma volume reduction are, however, unknown and our current research is focused on their identification. Using the tissue microarray method to compare untreated myomas with short-term or long-term UPA-treated myomas, we have provided evidences that UPA treatment in vivo reduces the proliferation of myoma cells. We also reported an increase of apoptotic index of myoma cells in short-term treated myomas but not in the long-term treated myoma cells. Most of all, we highlighted a dramatic reduction of the ECM in long-term treated myomas and proposed that matrix metalloproteinase 2 (MMP-2) is involved in this phenomenon. Together, these findings suggest a dynamic and multifactorial combination of factors involved in myoma size reduction.

We are particularly investigating the ability of PGL4001 to regulate the expression of ECM component and an in-depth analysis of the tissue remodeling processes is currently ongoing (Figure 1). This work aims at (1) better understanding which molecular mechanisms are involved in the volume reduction of these tumors after UPA treatment, and (2) at elucidating the fundamental mechanisms of growth of theses tumors.

Figure 1 Working model of the myoma ECM remodelling under UPA (PGL4001) treatment. Based on our results and recent literature, we build this working model of UPA mechanism of action in myoma cells. PGL4001 (a) directly inhibit the synthesis of collagen, (b) stimulates the expression of matrix metalloproteinases (MMPs) that degrade collagen fibers, (c) stimulates the expression of the MMP inducer EMMPRIN and (d) inhibits the MMPs inhibitor (TIMPs).
A) Fertility preservation and restoration from cryopreserved immature testicular tissue (ITT)
J. Poels, C. Wyns, F. De Michele

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 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 started.

**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 Optimalization 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.

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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:

The objectives of this research project are to:

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 spermatogonial stem cell 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 (Eqvizabal 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 1). 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
2007-2014


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**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 support 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.**
Diagnostic accuracy of wbMRI to detect bone metastases

V. Pasoglou, N. Michoux, F. Lecouvet

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

This project is developed in collaboration with JF. Jamar (UCL/SSS/IREC/MIRO) and B. Tombal (UCL/SSS/IREC/CHEX).

In adults, bone metastases (BM) are more common than primary bone cancers. Skeletal metastatic disease occurs in 30-70% of all cancers. Breast cancer is the first cause of BM in women and prostate cancer in men, followed by lung cancer [1]. In many patients, bone involvement is diagnosed after a complication such as pain, pathological fracture or compression of adjacent structures. Early and reliable detection of bone involvement is essential to plan the most appropriate therapy given the disease stage and to prevent complications. Numerous imaging modalities are used to detect bone involvement. Computed Tomography (CT), Bone Scintigraphy (BS), Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET)-CT, Magnetic Resonance Imaging (MRI) and more recently whole-body (wb) MRI with diffusion-weighted imaging. It seems that the imaging choice for BM screening is progressively shifting to wbMRI and/or PET-CT.

A review of the literature focusing on wbMRI and comparing it to BS, PET or PET-CT for BM screening, and updates the information on their respective diagnostic accuracy for BM detection is undertaken to confirm this observation. From this review, it appears that there is still a critical need for multicentric and mono-cancer studies respecting more accurately the guidelines for implementing performance studies to enhance knowledge on the field. Studies assessing DWI and comparing wbMRI to PET with new promising radiotracers such as 18NaF, 11C and 18F Choline and labeled PSMA are still missing, preventing to reach a definitive conclusion on which of these emerging methods will become the standard of choice in each primary cancer for BM screening.

Nevertheless, recently published performance studies bring additional information on the diagnostic accuracy of wbMRI. The evidence supporting the superiority of wbMRI over BS for BM detection, independently of the primary cancer, is today very robust. DWI alone does not demonstrate a sufficiently high diagnostic accuracy. DWI must be embedded in wbMRI as it improves the sensitivity of the examination. Regarding the practical issue of the imaging pathway, i.e. when considering wbMRI instead of PET-CT for BM screening, this review provides some additional answers.

Although their diagnostic accuracy appears roughly equivalent, it appears that wbMRI is superior in lesion-based analysis, while PET-CT seems to keep some advantage in patient-based analysis, especially in breast and neuroendocrine tumors. Notwithstanding this advantage and apart from all other considerations (radiation exposure, local availability of the techniques, availability of dedicated / tumor specific PET tracers, local experience, cost), wbMRI might be a more “universal” solution as it is sensitive to bone marrow infiltration by any metastasis regardless of the primary cancer, while PET-CT definitively relies on the affinity of the cancer and its metastases for a given tracer.

From a more general standpoint, the comprehensive review of the diagnostic performances suggests that the work-up of metastases will not remain “mono-technique” as it has been the historical case for BS; it will most likely be shared between wbMRI and PET according to the primary cancer. This scenario is further supported by the perspective offered by both techniques of an all-organ (non-bone limited) screening of metastasis [1].
Performance of imaging modalities in bone metastases
One step MRI for staging of prostate cancer


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

The first aim of this thesis was to provide a critical review of the literature on the subject of bone metastases/imaging detection, and input on most recent imaging modalities, WB-MRI and PET. The second aim was to improve Prostate Cancer (PCa) N and M staging by developing a new 3D whole-body T1-weighted (wb3DT1) MRI sequence. The results suggest that whole-body MR imaging is feasible with a 3D T1-weighted sequence and provides better quality of image when compared with 2D sequences, with a diagnostic performance that is as good or better for the detection of bone metastases and better for the detection of lymph node metastases [2] (ESUI Vision award 2015, European Association of Urology).

The third aim of the thesis was to combined the wb3DT1 protocol with an optimized multi-parametric MRI (mpMRI) of the prostate for the local (T) staging and we created a new “All in one” protocol for the global TNM screening of patients with PCa. Thirty patients with “high-risk” PCa prospectively underwent mpMRI of the prostate and wbMRI, which was compared to the routine protocol for the staging of PCa including a 99mTc bone scan (BS), completed with standard X-rays (TXR) and contrast enhanced CT for distant staging. The results show that AJCC M and N staging using wbMRI is feasible during the same imaging session as mpMRI performed for T staging, in less than one hour. wbMRI outperforms BS and TXR and abdomino-pelvic CT work up for discriminating subsets of patients with or without distant spread of the cancer [3].

Whole body MRI (WB-MRI) assessment of metastatic spread in prostate cancer patients: implications for the treatment, with special focus on oligometastatic disease

A. Larbi, V. Pasoglou, N. Michoux, B. Vande Berg, F. Lecouvet

This project is developed in collaboration with B. Tombal (UCL/SSS/IREC/CHEX), O. Rahier (Visceral, vascular, abdominal surgery and urology, Cliniques Saint Pierre, Ottignies) and B. Dallaudière (Department of diagnostic and interventional imaging in adults CHU Bordeaux).

This work has been supported by grants from the Fonds de Recherche Clinique (PhD thesis) and Société Française de Radiologie (SFR, bourse de recherche 2013).

Whole body magnetic resonance imaging with diffusion-weighted imaging (WB-MRI/DWI) allows the early detection of metastatic spread in high-risk prostate cancer (PCa) patients. This opens perspectives for new metastatic targeted therapeutic strategies, also in patients with “oligometastatic” disease. The first aim of this thesis is to determine the proportion of oligometastatic status (≤ 4 metastases) and impact on treatment of the distribution of metastatic deposit in PCa patients. Two musculoskeletal radiologists reviewed WB-MRI/DWI studies in 96 newly diagnosed metastatic PCa patients; 46 patients with newly diagnosed PCa (mHNPC); 50 patients with first appearance of metastasis during monitoring for non-metastatic castration resistant PCa (mCRPC).

The anatomical distribution of metastatic deposit was assessed and the proportions of patients with ≤ 3 or ≤ 4 metastases were measured. 28% and 30% of mHNPC and 50% and 52% of mCRPC entered metastatic disease with ≤ 3 and ≤ 4 sites, respectively. Bone metastases (BM) were identified in 68.8% patients; 71.7% of mHNPC and 66% of mCRPC patients. Most commonly involved areas were left ilium (43.8%) and lumbar spine (38.5%). Enlarged lymph nodes (LN) were detected in 68.7% of patients; 69.6% of mHNCP and 68.0% of mCRPC. Most commonly involved areas were
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.

Low-dose multidetector computed tomography of the cervical spine: optimization of iterative reconstruction strength levels

P. Omoumi, Y. Ben Salah, B. Vande Berg, F. Lecouvet, J. Malghem

This project is developed in collaboration with F.R. Verdun and J.G. Ott (Institute of Radiation Physics, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland), R. Meuli and F. Becce (Department of Diagnostic and Interventional Radiology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland).

Iterative reconstruction (IR) techniques reduce image noise in multidetector computed tomography (MDCT) imaging. They can therefore be used to reduce radiation dose while maintaining diagnostic image quality nearly constant. However, CT manufacturers offer several strength levels of IR to choose from. The aim of this work is to determine the optimal strength level of IR in low-dose MDCT of the cervical spine. Thirty consecutive patients investigated by low-dose cervical spine MDCT were prospectively studied. Raw data were reconstructed using filtered back-projection and sinogram-affirmed IR (SAFIRE, strength levels 1
to 5) techniques. Image noise, signal-to-noise ratio (SNR), and contrast-to-noise ratio (CNR) were measured at C3-C4 and C6-C7 levels. Two radiologists independently and blindly evaluated various anatomical structures (both dense and soft tissues) using a 4-point scale. They also rated the overall diagnostic image quality using a 10-point scale. As IR strength levels increased, image noise decreased linearly, while SNR and CNR both increased linearly at C3-C4 and C6-C7 levels (P < 0.001).

For the intervertebral discs, the content of neural foramina and dural sac, and for the ligaments, subjective image quality scores increased linearly with increasing IR strength level (P ≤ 0.03). Conversely, for the soft tissues and trabecular bone, the scores decreased linearly with increasing IR strength level (P < 0.001). Finally, the overall diagnostic image quality scores increased linearly with increasing IR strength level (P < 0.001). The optimal strength level of IR in low-dose cervical spine MDCT depends on the anatomical structure to be analyzed. For the intervertebral discs and the content of neural foramina, high strength levels of IR are recommended.

Computed tomography of the cervical spine: comparison of image quality between a standard-dose and a low-dose protocol using filtered back-projection and iterative reconstruction

B. Vande Berg, Y. Ben Salah, F. Lecouvet, P. Omoumi.

This project is developed in collaboration with F. Becce and R. Meuli (Department of Diagnostic and Interventional Radiology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland), F.R. Verdun (Institute of Radiation Physics, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland).

The aim of this work is to compare image quality of a standard-dose (SD) and a low-dose (LD) cervical spine CT protocol using filtered back-projection (FBP) and iterative reconstruction (IR). Forty patients investigated by cervical spine CT were prospectively randomised into two groups: SD (120 kVp, 275 mAs) and LD (120 kVp, 150 mAs), both applying automatic tube current modulation. Data were reconstructed using both FBP and sinogram-affirmed IR. Image noise, signal-to-noise (SNR) and contrast-to-noise (CNR) ratios were measured. Two radiologists independently and blindly assessed the following anatomical structures at C3-C4 and C6-C7 levels, using a four-point scale: intervertebral disc, content of neural foramina and dural sac, ligaments, soft tissues and vertebrae.

They subsequently rated overall image quality using a ten-point scale. For both protocols and at each disc level, IR significantly decreased image noise and increased SNR and CNR, compared with FBP. SNR and CNR were statistically equivalent in LD-IR and SD-FBP protocols. Regardless of the dose and disc level, the qualitative scores with IR compared with FBP, and with LD-IR compared with SD-FBP, were significantly higher or not statistically different for intervertebral discs, neural foramina and ligaments, while significantly lower or not statistically different for soft tissues and vertebrae.

The overall image quality scores were significantly higher with IR compared with FBP, and with LD-IR compared with SD-FBP. LD-IR cervical spine CT provides better image quality for intervertebral discs, neural foramina and ligaments, and worse image quality for soft tissues and vertebrae, compared with SD-FBP, while reducing radiation dose by approximately 40%.
Femoroacetabular impingement: normal values of the quantitative morphometric parameters in asymptomatic hips

A. Larbi, F. Lecouvet, B. Vande Berg, P. Omoumi

This project is developed in collaboration with M. Lepage-Saucier (CuSL/ Hôpital Notre-Dame - Centre hospitalier de l’Université de Montréal, Québec, Canada) and C. Thiery (Radiology CHWAPI/ CuSL).

The aim of this work is to determine the means and the reference intervals of the quantitative morphometric parameters of femoroacetabular impingement (FAI) in normal hips with high-resolution computed tomography (CT). We prospectively included 94 adult individuals who underwent CT for thoracic, abdominal or urologic pathologies. Patients with a clinical history of hip pathology and/or with osteoarthritis on CT were excluded. We calculated means and 95% reference intervals for imaging signs of cam-type (alpha angle at 90° and 45° and femoral head-neck offset) and pincer-type impingement (acetabular version angle, lateral centre-edge angle and acetabular index). The 95% reference interval limits were all far beyond the abnormal thresholds found in the literature for cam-type and to a lesser extent for pincer-type FAI.

The upper limits of the reference intervals for the alpha angles (at 90°/45°) were 68°/83° (men) and 69°/84° (women), compared to thresholds from the literature (50°, 55° or 60°). Reference intervals were similar between genders for cam-type parameters, and slightly differed for pincer-type. The 95% reference intervals of morphometric measurements of FAI in asymptomatic hips were beyond the abnormal thresholds, which was especially true for cam-type FAI. Our results suggest the need for redefining the current morphometric parameters used in the diagnosis of FAI.

Anatomic features associated with femoroacetabular impingement are equally common in hips of old and young asymptomatic individuals without CT signs of osteoarthritis

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

This project is developed in collaboration with C. Thiery (Radiology CHWAPI/ CuSL).

The purpose of this work is to evaluate and compare the prevalence and measurement values of CT signs of femoroacetabular impingement (FAI) in asymptomatic hips without CT signs of osteoarthritis between two age groups: younger than 40 years and older than 60 years. We prospectively included patients undergoing thoracoabdominopelvic MDCT for nonorthopedic indications with asymptomatic hips and excluded hips with signs of osteoarthritis seen on CT. Two age groups including 75 hips each were enrolled (< 40 years old: mean age, 31 years; 15 women; > 60 years old: mean age, 66 years; 21 women). Two observers independently performed the image analysis. Prevalences and quantitative values of the cam (alpha angle and femoral head-neck offset) and pincer (acetabular version angle, acetabular index, lateral center-edge angle, crossover sign, and posterior wall sign) FAI morphotypes were compared using both difference and equivalence tests. Intraobserver agreement was assessed. The prevalence of CT signs of FAI were high and showed great variation depending on the signs and cutoff values, in both groups (9-63% for cam; 3-50% for pincer). The prevalence and measurement values of CT signs of the cam morphotype were equivalent between the two age groups. The prevalence and measurement values of CT signs of the pincer morphotype were statistically equivalent between the age groups except for the acetabular version angle, lateral center-edge angle, and crossover sign for which no statistical difference was found, but statistical equivalence was not reached. Interobserver and intraobserver agreement were moderate to almost perfect (κ = 0.72-0.89; intraclass correlation coefficient, 0.42-0.94). The prevalence and measurement values of most CT signs of FAI morphotypes were high and equivalent between the two age groups of patients with asymptomatic nonosteoarthritic hips.
Predicting non-response to NAC in patients with breast cancer using 3D texture analysis

N. Michoux, L. Fellah, I. Leconte

This project is developed in collaboration with H. Mueller (HES-SO, Sierre, Suisse) and A. Geissbuhler (HUG, Genève, Suisse).

Neoadjuvant chemotherapy (NAC) has a major role in the treatment of breast cancer. However, the rate of response to NAC is limited and dependent on the subtypes of cancer. Therefore, the identification of non-responding patients is important, especially as it may allow considering alternative therapeutic options. Pre-NAC semi-quantitative DCE-MRI parameters have been reported to be significantly different in chemosensitive and chemoresistant breast lesions. First studies on breast MR images showed that alternative post-processing approaches such as texture analysis may help evaluate tumor response to NAC.

The aim of the study is to investigate the value of MRI texture analysis in predicting non-responders to NAC, especially in comparing the predictive performance of...
pre-NAC 3D texture parameters with that of pre-NAC 2D texture parameters. MRI examinations were performed using a 1.5T whole-body imaging system and a breast coil. Patients were imaged with a 3D gradient echo axial T1-weighted sequence with fat suppression (SPAIR). Analyses were performed on subtracted images. The intra-lesional texture was assessed as follows. From the grey level co-occurrence matrix (GLCM), 11 texture parameters (i.e. textons) describing the grey levels interdependence in the lesion were estimated. From the run length matrix (RLM), 11 textons describing the distribution of runs of grey levels were estimated with the same computation parameters. From the Riesz wavelet transform, 30 textons characterizing the important orientations and scale properties of grey levels were estimated. Two multi-parametric classifiers were used to predict the non-responders to NAC: a logistic regression model and a support vector machine (SVM) model.

As one cannot know a priori how many and which parameters are important to the classification, all possible combinations of 2 to 5 parameters among 52 parameters) were submitted to the classifiers successively. To estimate how accurately the predictive models would perform in practice, a leave-one-out cross-validation was applied. Using SVM as classifier, a predictive model relying on 3 Riesz parameters was found to perform with a predictive accuracy of 81%. Se = 47% (9/19 NR) and Sp = 94% (48/51 PR+CR). Using the logistic regression as classifier, a better model for identifying NR patients based on 5 textons (1 RLM + 4 Riesz) was found to perform with a predictive accuracy of 76%. Se = 89% (17/19 NR) and Sp = 71% (36/51 PR+CR). The usefulness of pre-NAC texture parameters in predicting response to NAC has been proven already but based on 2D analysis of breast MR images. In a pilot work, we combined kinetic and texture parameters extracted from a single subtracted MR image showing the largest area of the breast lesion with a high enhancement. Using k-means clustering as statistical classifier, a predictive model relying on 4 parameters (1 GLCM, 2 RLM, 1 kinetic) was found to perform with Se = 84% and Sp = 62%. The predictive accuracy of the present 3D analysis is superior to that of 2D analysis (76% vs 68%). However, the gain in performance remains modest. While a predictive model based on textons only improves the practicality of the analysis, the 3D segmentation of breast lesions lengthened the processing time of MR images substantially. The rationale behind these investigations is the development of a computer-assisted solution based on the texture analysis of MR images that may contribute to an appropriate treatment outcome for patients with breast cancer initially eligible for NAC [5].

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. A promising method is the double wave vector encoded diffusion sequence that has the potential of measuring cell sizes and distributions. 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 diffusion weighted images. The method will be validated on phantoms and rat tumours. The method will be applied on two patients’ cohort. On acute ischemic stroke patients 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’, and on oncology patients 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) [6].

**Postendoscopic duodenal hematoma in children: ultrasound diagnosis and follow-up**

*D. Dumitriu, R. Menten, P. Clapuyt*

This project is developed in collaboration with F. Smets (UCL/SSS/IREC/PEDI).

Intramural duodenal hematomas have most frequently been reported in children in a traumatic setting. We present two cases of duodenal hematoma that occurred after upper gastrointestinal tract endoscopy with biopsy in children without significant prior medical history. The diagnosis was made by ultrasound, in correlation with the clinical presentation. Because the patients were hemodynamically stable, they were treated conservatively and the regression of the hematoma was followed up with ultrasound until its complete resolution. These cases demonstrate the risks of endoscopy, which are not to be neglected even in children without impaired coagulation, and the manner in which ultrasound can provide the correct diagnosis and follow-up.

**Chondrosternal arthritis in infant: an unusual entity**

*D. Dumitriu*

This project is developed in collaboration with P-L. Docquier (UCL/SSS/IREC/CARS) and A. Nikolarakou (CuSL, Pédiatrie).

Primary arthritis of chondrosternal joint is very rare and occurs in infants less than 18 months of age. Presentation is most often subacute but may be acute. Child presents with a parasternal mass with history of fever and/or local signs of infection. Clinical symptoms vary from a painless noninflammatory to a painful mass with local tenderness and swelling, while fever may be absent. Laboratory data show low or marginally raised levels of white blood cells and C-reactive protein, reflecting, respectively, the subacute or acute character of the infection. It is a self-limiting affection due to the adequate immune response of the patient. Evolution is generally good without antibiotic therapy with a progressive spontaneous healing. A wait-and-see approach with close follow-up in the first weeks is the best therapeutic option.

**Pitfalls in the diagnosis of common benign bone tumours in children**

*D. Dumitriu, R. Menten, P. Clapuyt*

Benign bone tumours in children are frequent lesions, often with a typical and very identifiable radiological presentation. However, their natural evolution and complications may be the source of variations and errors in interpretation. It is therefore important to understand the possible sources of change in the radiological aspect and to be familiar with common pseudotumoral lesions. The main aim of this work is to review typical aspects of the most common benign bone tumours in children, as well as less frequent variants of these tumours. Teaching points

**Intra-articular osteoid osteoma mimicking juvenile arthritis**

*D. Dumitriu, R. Menten, P. Clapuyt*

This project is developed in collaboration with P-L. Docquier and S.Y. Traore (UCL/SSS/IREC/CARS).

In case of intra-articular osteoid osteoma, misdiagnosis as juvenile arthritis may occur, delaying adequate treatment. We report cases of intra-articular osteoid osteomas in children that were misdiagnosed and initially inappropriately treated with intra-articular corticoid injection. Diagnosis of osteoid osteoma was finally given by CT-scan and appropriate treatment by radiofrequency ablation or surgical ablation was performed. Clinicians and radiologists should be aware of the potentially confusing clinical and imaging findings associated with intra-articular osteoid osteoma.
**Imaging findings of ischemic cholecystitis following transarterial chemoembolisation prior to liver transplantation for hepatocarcinoma**

E. Danse, X. Pavard, P. Goffette, P. Trefois, L. Annet, C. Dragean, N. Michoux

This project is developed in collaboration with J. Lerut (UCL/SSS/IREC/CHEX).

Ischemic cholecystitis (IC) is a complication of transarterial chemoembolisation (TACE). Its radiological diagnosis is different from acute cholecystitis related to stone and is frequently a matter of debate mainly due to the lack of pathological confirmation. The aim of this work is to identify imaging features helping diagnose IC post TACE. A retrospective review of TACE procedures prior to treat hepatocarcinoma was performed including 46 patients (35 transplanted, 2 died, 9 on the waiting list for liver transplantation). Post TACE US/MRI- derived features were correlated to the final diagnosis based on pathologic analysis (35) or biological and clinical follow up (11). Were considered at US/MRI: wall thickening (≥3mm), gallbladder distension (short axis>4cm), pericholecystic fluid and infiltration, striated wall, wall irregularities, at MRI: T1 hyper-signal of the gallbladder wall on unenhanced sequence, T1 hyper-attenuation of the gallbladder wall and/or of the adjacent liver parenchyma on Gd-enhanced sequence, at Sonography: Murphy sign, increased color Doppler signal of the gallbladder wall. Scott’s pi was used to assess inter-modality agreement. A logistic regression was performed to identify imaging features which contribute significantly to the prediction of IC. IC was diagnosed in 12 patients. The most common features (frequency/Se/Sp) were wall thickening (91%/83%/91%), striated wall (82%/74%/96%), wall irregularities (73%/75%/95%). These features were significantly more present in positive patients (p<0.005). Within a logistic model, they predict IC with a good performance level (Youden index=0.91). Imaging-Pathology agreement was good (pi=0.8) as well as US-MRI agreement (0.6<pi<0.8). A model based on three imaging features may allow predicting acute ischemic cholecystitis with US or MRI [7].

**Mesenteric inflammatory myofibroblastic tumor: MRI and CT imaging correlated to anatomical pathology**

T. Kirchgesner, E. Danse, L. Annet, C. Dragean, P. Trefois

This project is developed in collaboration with C. Sempoux (UCL/SSH/FIAL) and A. Kartheuser (UCL/SSS/IREC/CHEX) and N. Abbes Orabi (CuSL, Surgery and Abdominal Transplant).

Inflammatory myofibroblastic tumor (IMT) is a rare tumor, classified by WHO of intermediate biological potential with tendency for local recurrence and small risk for distant metastasis. Histologically IMT is a mixture of inflammatory cells and myofibroblastic spindle cells proliferation. To our knowledge there is no MRI description of mesenteric IMT in the literature. We would like to emphasize the correlation between medical imaging and anatomical pathology based on our experience of a mesenteric IMT in a 28-year-old patient.

**Does the site of platelet sequestration predict the response to splenectomy in adult patients with immune thrombocytopenic purpura?**

E. Danse

This project is lead by N. Jabbour and developed in collaboration with JF. Jamar (CuSL, Nuclear medicine and UCL/SSS/IREC/MIRO), J.F. Gigot (UCL/SSS/IREC/CHEX), N. Navez, J. Navez and C. Hubert (CuSL, Surgery and Abdominal Transplant), C. Lambert (CuSL, hematology), V. Lannoy (CuSL, Cancer center).

Splenectomy is the only potentially curative treatment for chronic immune thrombocytopenic purpura (ITP) in adults. However, one-third of the patients relapse without predictive factors identified. We evaluate the predictive value of the site of platelet sequestration on the response to splenectomy in patients with ITP. Eighty-two consecutive patients with ITP treated by splenectomy between 1992 and 2013 were retrospectively reviewed. Platelet sequestration site was studied by 111Indium-oxinate-labeled platelets in 93% of patients. Response to splenectomy was defined at last follow-up as: complete response (CR) for platelet count (PC) ≥100×10^9/L, response (R) for PC<30×10^9/L and <100×10^9/L with absence of bleeding, no response (NR) for PC<30×10^9/L.
Castrate-resistant prostate cancer with peritoneal metastases treated with docetaxel-based chemotherapy

E. Danse

This project is developed in collaboration with B. Tombal (UCL/SSS/IREC/CHEX), J-P. Machiels (UCL/SSS/IREC/MIRO), R. Rizk (CuSL, Oncology) and S. Aydin (CuSL, Anatomical pathology).

The aim of this work is to identify the risk factors, characteristics and prognosis of patients treated with docetaxel-based chemotherapy for peritoneal carcinomatosis due to metastatic castrate-resistant prostate cancer (mCRPC). We retrospectively reviewed our series of mCRPC patients with peritoneal metastases treated with docetaxel-based chemotherapy between 2004 and 2010. Six patients were identified from our institutions’ internal cancer registry. Three out of these patients had been treated with laparoscopic radical prostatectomy (LRP). In addition to peritoneal metastases, other metastatic sites were mainly visceral. Only 1 patient developed bone metastases. Peritoneal carcinomatosis occurred mainly in patients with a high Gleason (= or ≥6) score since 5 out of our 6 patients had a Gleason score ≥7. All 6 patients were treated with docetaxel-based chemotherapy when they developed castration resistance. Five patients benefitted from chemotherapy according to their PSA or RECIST responses. Median survival from the start of docetaxel therapy was 24.5 months. Our retrospective analysis suggests that peritoneal carcinomatosis occurs mainly in patients with a high Gleason score. It is also possible that tumor seeding occurs during LRP. Patients with peritoneal carcinomatosis resistant to castration seem to benefit from docetaxel-based chemotherapy.

Low contrast detectability improvements with iterative reconstructions of multi detector computed tomography images: a phantom study

D. Millon, N. Michoux, E. Coche

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

The aim of this work is to compare the low contrast detectability of MDCT images reconstructed with an iterative reconstruction algorithm and with a filtered back projection algorithm in standard and high resolution. The experimental study was performed on a 256-slice MDCT (Philips Healthcare, Cleveland, OH). A Catphan phantom (The Phantom Laboratory, Salem, NY) containing a low contrast (LC) module was imaged with decreasing dose (48.8 mGy down to 0.7 mGy) 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 with standard filtered back projection (FBP) and iterative reconstruction algorithm (IMR, Philips HealthCare). Three independent readers counted the number of LC objects visible on each reconstructed dataset. Tests on paired proportions for comparing the number of objects detected given a slice thickness using either standard or iterative reconstruction technique, were performed. Intra-class correlation (ICC) assessing the inter-readers reliability was estimated from all settings. Proportion of detected objects was statistically significantly different whatever the slice thickness or reader considered (p< 0.0001), supporting the iterative reconstruction technique. On 1-mm-thick slices, proportion differences ranged from 13.8% (95% Cl: 9.50% - 18.1%) to 23.0% (95% Cl: 17.9% - 28.0%) between readers. On 3-mm-thick slices, proportion differences ranged from 11.3% (95% Cl: 6.49% - 16.1%) to 20.0% (95% Cl: 14.8%-25.0%) between readers. Inter-reader reliability were ICCstandard =0.78 and ICCiterative = 0.80 respectively. Iterative reconstructions improve LC detectability in standard and high resolution MDCT images.
L’effet du constructeur dans la mesure des flux vasculaires et de LCR en IRM en Contraste de Phase

L. Bogdan, N. Michoux, C. Gandin, P. Ngenzi, T. Duprez

This project is lead by S. Elsankari (UCL/SSS/IONS/NEUR).


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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 for optimizing immunosuppressants and antibiotics drug dosages and regimens in specific subpopulations (e.g. pediatric liver transplantation, septic patients, elderly).

Also, our group has demonstrated the interest of measuring intracellular drug concentrations as better reflection of drug exposure/efficacy, than blood or serum concentrations. About 17 peer reviewed contributions were published in 2014, 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 sampling reduced blood volumes, and stable samples shipment. These advantages were explored in our group, in collaboration with a Brasilian center (supported by a FNRS/CNPq sponsoring). Finally our group, is involved in a FP7 multicenter project, MON4STRAT, aiming at optimizing antibiotic therapy in severe pneumonia by ultra-fast TDM with PK-PD modelling.

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 (ADRs) 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 recognized 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 the CYP3A5-based dosing recommendation established at LTAP 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 expected for 2015.

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. In this respect, our group (V. Haufroid and L. Elens) is actively involved in a European project dealing with the clinical implementation of pharmacogenetics (Eu-Pic; http://www.eu-pic.net/). This project has been selected for the phase II call (H2020-PHC-2015: category: piloting personalized medicine in health and care systems). 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. PLOS One 2014) confirming previous associations observed in vivo by our group and opening the way to new clinical applications.

The effect of the PDE5i was identified by measuring the nasal potential difference, an extremely delicate diagnostic test that has also been used to evaluate therapeutic efficacy of potential drugs during clinical trials. We have acquired great expertise in this test, both in mice and in patients. In the same line, we have shown that vardenafil is able to attenuate inflammatory responses in CF mice (Lubamba et al., 2012). Thanks to collaboration with the IREC Cell Imaging platform, we have shown that vardenafil increases the expression of F508del-CFTR protein at 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 and from the Belgian CF Association (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.

Thanks to our F508del breeding colony, we have contributed to a better understanding of the complex cascade of events intricately involved in the pathophysiology of lung inflammation in CF. We have shown that inflammation is present in CF under naive, non-stimulated conditions, even in the absence of any detectable infection (Legssyer et al, 2006). We have identified that inflammatory responses occur earlier and are exaggerated in CF, and that CF lung inflammation is a complex process involving multiple and cellular players and distinct pathways. Macrophages play a key role in the inflammatory responses in CF: infiltrated bronchoalveolar, lung resident and peritoneal macrophages are more numerous in CF than in wild-type mice, and the macrophage-related chemokine, CCL-2/MCP-1, is about 20-fold more pathophysiology and testing therapeutic strategies able to address the basic loss-of-function of the CFTR protein or to circumvent exaggerated inflammatory responses that progressively disrupt pulmonary architecture finally leading to respiratory failure. A current topic of our 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. CF mice homozygous for the F508del mutation, the most common CFTR mutation in humans, treated with vardenafil, at clinical doses, by intraperitoneal injection (Lubamba et al, 2009) or by local deposition at the nasal mucosa (Lubamba et al, 2011), showed correction of CFTR-dependent chloride transport, which is abolished or blunted in CF.

Cystic Fibrosis therapy:
“From bench to bed”

S. Noel, B. Dhooghe, T. Leal

Under the scope of translational research, genetically modified mice are used as models of cystic fibrosis (CF) disease with the aim at studying lung pathophysiology and testing therapeutic strategies able to address the basic loss-of-function of the CFTR protein or to circumvent exaggerated inflammatory responses that progressively disrupt pulmonary architecture finally leading to respiratory failure. A current topic of our 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. CF mice homozygous for the F508del mutation, the most common CFTR mutation in humans, treated with vardenafil, at clinical doses, by intraperitoneal injection (Lubamba et al, 2009) or by local deposition at the nasal mucosa (Lubamba et al, 2011), showed correction of CFTR-dependent chloride transport, which is abolished or blunted in CF.

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abundant in the alveolar space of F508del-CF mice (Meyer et al, 2009). Cellular differentiation processes of CF macrophages are deregulated and are polarized through a pro-inflammatory profile favoring perpetuation of inflammation and triggering the development of fibrosis. Excess fibrogenesis in CF is not fully understood and may represent a therapy target. We have demonstrated that the phenotype of lung and skin fibroblasts from mice homozygous for F508del is altered. Using an experimental mouse model of bleomycin-induced fibrosis, we showed that markers of pro-inflammatory and pro-fibrotic responses are overexpressed in CF. Cell proliferation and differentiation into myofibroblasts, a specialized type of fibroblasts activated during wound healing, were deregulated in CF fibroblasts (Huaxu et al, 2013). Interestingly, vardenafil reverted these over-responses. These findings provide compelling new support for targeting cGMP signaling pathway in CF pharmacotherapy.

We have originally provided evidence that CFTR protein, deficient in CF disease, plays a role during organogenesis (Bonvin et al, 2008). Structural tracheal abnormalities characterized by disrupted or incomplete cartilage rings, detected in adult and newborn CF mouse models, may represent congenital malformations related to CFTR dysfunction. These findings indicate that CFTR protein, in addition to mainly functioning as a chloride transporter, governs lung and extrathoracic airway development also contributing to the clinical expression of CF respiratory disease. We have shed some additional light on the major impact of CF disease on the nutritional status. As an essential fatty acid deficiency has been increasingly reported in CF, we have studied fatty acid status in CF and extrathoracic airway development also contributing to the clinical expression of CF respiratory disease. We have demonstrated practical benefits of omega3 supplementation in CF (Minouin et al, 2009).

More recently, a new topic of research has been integrated in our group: the deregulation of the expression of microRNA (miRNA) in CF lung disease. miRNAs are short, noncoding RNAs with pleiotropic effects dependent on posttranscriptional regulation of gene expression. By interfering with multiple transcripts, miRNAs have the potential to regulate virtually all cellular mechanisms, and have been identified as key players in producing rapid adaptation to changing environmental conditions. Our preliminary findings support the view that miRNAs act as phenotype modifiers of CF lung disease.

Evaluation of health hazards and risks of chemical

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, we 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 in Belgium and conducted systematic reviews on the epidemiological associations between pesticide exposure and cancer.

Urinary concentrations of analytes are often adjusted to creatinine concentration to integrate the effects of fluid balance on spot samples. Carrying out this adjustment systematically for all biomarkers is questionable and concerns about the appropriateness of such procedure have been emerging over the last years. This issue of the relevance of systematic creatinine adjustment was raised again when determining the reference distribution for trace elements (TEs) in the urine of the general adult population in Belgium. Considering the distribution of these TEs, it was evident that creatinine adjustment of their urinary concentrations entailed very different patterns of distribution according to the TE considered.

Based on the assumption that 24h is the gold standard to assess exposure to TEs, we investigated a) whether the unadjusted concentrations of TEs in random spotU reflect their 24-hour excretion rate, b) whether the relation is improved by creat- and SG-adjustment. The aim of our study was not to perform sophisticated statistical analyses but to answer questions routinely raised by a large number of health professionals from the clinical biologist to the epidemiologist as well as stakeholders establishing guidance values or the physician who has to interpret a result for his patient. The results add to the doubts on the reliability of a systematic creatinine adjustment of...
urinary tests performed on spot samples. Such an approach may introduce a bias and lead to a misestimation of the exposure level and misinterpretation of associations in epidemiological or clinical studies. These results have been recently accepted for publication (Hoet et al., 2015).

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.

Immunoregulation during particle-induced lung fibrosis and cancer: a new pathological pathway

F. Huaux

We have accumulated over recent years experimental evidence that immune regulatory responses contribute to the pulmonary fibrotic process induced by inhaled particles. IL-10-producing macrophages and CD4+ Foxp3+ regulatory T cells (Treg) persistently recruited during long-term responses to silica highly express growth factors such as PDGF-B and TGF-β1, directly stimulate fibroblast proliferation and increase lung collagen deposition. The main function of these immunosuppressive cell subpopulations during long-term responses to particles is their ability to inhibit both the innate and adaptive immune responses, subverting immune surveillance.

In 2014, we have observed that besides regulatory macrophages and Treg, Myeloid-Derived Suppressor Cells (MDSC), another immunosuppressive cell population, also accumulate and participate in the formation of granuloma development by producing osteopontin and TGF-β1 in the lung of mice treated with particles (project funded by Fédération Wallonie Bruxelles (ARC) and by the FNRS).

Through a collaborative project funded by Foundation belge contre le cancer, we have also found that immunosuppressive macrophages and mononuclear cells are associated to the very early immune reaction of the mesothelial tissue exposed to asbestos fibers or carbon nanotubes (see figure below). Our hypothesis is that these regulatory macrophages and monocytes also participate to the initial pathogenic events and in turn contribute to the carcinogenic process and the development of mesothelioma. We are now determining whether these suppressive cells also infiltrate human mesothelioma and may represent new opportunities for early detection and therapy of fiber-induced cancer.
Inorganic particles used in many industrial applications and technological developments present a potential risk for human health, especially via exposure of the respiratory tract. Nowadays, nanotechnology is also impacting on many 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 hazard assessments. Our group has been dedicated in the evaluation of particle and nanomaterial toxicity for more than 30 years.

In 2014, we investigated the direct and indirect effects of carbon nanotubes on fibroblasts. We identified membrane receptors and an intracellular signaling pathway responsible for the proliferative activity of CNT on fibroblasts. We showed that this mechanism was predictive of the lung fibrogenic activity of CNT (Vietti et al, submitted).

Activity of MWCNT on fibroblast proliferation in vitro correlates with the induction of lung fibrosis. On the left, fibroblasts were exposed to different MWCNT samples (NM400, long or short). Cell viability was assessed by the WST-1 assay on fibroblasts primed with a low concentration of PDGF (3 ng/ml) and then exposed to MWCNT (15 µg/cm²) for 24 h. PDGF (30 ng/ml) was used as a positive control. On the right, Sirius red (type I collagen staining) lung sections 2 m after pharyngeal aspiration of 100 µg MWCNT/mouse.

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 carbon nanotubes with different lengths 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.

We have also initiated a project to determine the effect of nanoparticles on the gut microbiota. Silica and silver nanoparticles are commonly added directly to food or packaging for their anti-caking and antibiotic properties, respectively. However, nothing is known on the potential effects of NM on the microbiota and associated disorders, such as obesity and diabetes.
As part of the NANO-IRIS project (in collaboration with profs Leyns L. and Kirsch-Volders M. at VUB) funded by the Brussels Capital Region (INNOVIRIS, ended December 2013), several Standard Operating Procedures (SOP) for in vitro tests specifically adapted and internally validated for NM have been produced for assessing NM cytotoxicity, fibroblast proliferation, inflammasome activation and NM dissolution. In line with this project, our group is still aiming at developing NM-adapted assays to improve the quality of nanotoxicology data (Vietti et al, submitted).

LTAP is also involved in a research project financed by the Walloon region to valorize industrial steel slag dusts through a new process of carbonation (CARMAT project). Mineral carbonation can stabilize industrial residues and, in the steel industry, may contribute to simultaneously valorize CO₂ emissions and slag. We hypothesized that, by restricting the leaching of metals of toxicological concern such as Cr and V, carbonation can suppress the toxicity of these materials. The cytotoxic activity (WST1 assay) of slag dusts collected from a stainless and a Linz-Donawitz (LD) steel plant, before and after carbonation, was examined in J774 macrophages.

The release of Cr, V, Fe, Mn and Ni was measured after incubation in artificial lung fluids mimicking the extracellular and phagolysosomal milieu to which particles are confronted after inhalation. LD slag had the higher Fe, Mn and V content, and was more cytotoxic than stainless steel slag. The cytotoxic activity of LD but not of stainless dusts was reduced after carbonation. The cytotoxic activity of the dusts towards J774 macrophages necessitated a direct contact with the cells and was reduced in the presence of inhibitors of phagocytosis (cytochalasin D) or phagolysosome acidification (bafilomycin), pointing to a key role of metallic constituents released in phagolysosomes. This in vitro study supports a limited reduction of the cytotoxic activity of LD, but not of stainless, steel dusts upon carbonation. This research was recently accepted for publication (Ibouraadaten et al., 2015).

Finally, LTAP has joined an Excellence project funded by the Walloon region on the development of lithium-ion batteries paintings for the local storage of energy. Our task is to determine the potential respiratory toxicity of Li particles and to identify an in vitro endpoint to compare the toxic potential of different particles developed during the project.

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**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 extraction, realtime PCR, immunoassays, ...

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**SELECTED PUBLICATIONS**


*: for a complete list of publications at LTAP in 2014 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 *Clostridium difficile*, *Yersinia* and *Borrelia*. Since 2014, the group has developed important activities in the field of Mycobacteriology and rapid diagnosis of sepsis.

**MEDICAL MICROBIOLOGY (MBLG)**

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**RESEARCH GROUP OF BACTERIOLOGY**

**Diagnosis and epidemiology of *C. difficile* infections (CDI)**

*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 *C. difficile* infections dramatically changed with the emergence of a hyper-virulent clone called “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 turnaround 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 which is performed with immuno-assays and molecular biology techniques. PCR detection of toxin genes in stools has been included in the routine diagnosis.

As the Belgian national reference center (NRC) for *C. difficile*, the laboratory pursued in 2014 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. The center also collaborated in several European projects studying the global epidemiology and in the redaction of European guidelines for diagnosis, treatment and prevention.
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 2014, a new software for genome sequence comparison has been implemented.

Sepsis and MALDI-TOF MS

Sepsis is a severe infection associated with high morbidity and mortality rates. The rapid instauration of an appropriate and targeted antimicrobial treatment is of major importance. In 2014, our laboratory developed and validated rapid identification and susceptibility testing tools aiming at rendering results within the day of blood positivity detection. Identification of positive blood cultures is performed with MALDI-TOF MS directly from blood or on a 5-hour subculture. According to the identification result, chromogenic tests are similarly performed allowing the detection of respectively third generation cephalosporin resistant Enterobacteriaceae/Pseudomonas aeruginosa and methicillin resistant Staphylococcus aureus. Antimicrobial prescription changes and time gain are compared to the classical blood culture workflow. Preliminary results conclude that improving TAT of positive blood cultures speeds up the prescription of a targeted antimicrobial treatment with more than 1 day in 31.7% of the septic episodes hereby potentially improving patients clinical outcome.

MALDI-TOF MS is furthermore challenged as a rapid and easy typing tool in acute hospital-outbreaks.

Mycobacteriology

In 2014, a series of innovative developments have been launched in the field of mycobacterial infections. The aim of our group is to improve the rapidity and the quality of diagnosis, in order to diminish the risk of transmission of the disease and improve the clinical outcome of patients.

At the international level, our group has developed well-recognized e-health solutions for distant monitoring of laboratories in developing countries. We collaborate with major international partners, including the World Health Organization, the US Center for Disease Control and the International Union against tuberculosis and Lung Diseases. The GenXchange software is currently used for the remote supervision of over 30 GeneXpert PCR automates in the Democratic Republic of Congo, and is being used for several international research initiatives in the field of multi-drug resistance tuberculosis diagnostic and treatment.

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.

RESEARCH GROUP OF VIROLOGY

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 ultrasensitive 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 in the lab, while modeling studies are conducted in collaboration with the LIH in Luxembourg.

**SELECTED PUBLICATIONS**


LARGE EQUIPMENT

- Nucleic acid sequencing facilities
- Safety laboratory (P3 level)
- Digital PCR technology

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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 micro-environment 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
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. Last, the influence HPV viruses may have on stimulating the immune host response after ionizing radiation is also investigated in various cell lines engineered for expression of the viral proteins E6 and/or E7.

Characterization of the biological efficacy of HadronTherapy beams

(John Gueulette)

One knows that protons were introduced in radiation therapy because of their ability to enhance the ballistic selectivity of the irradiations. This is due to the fact that their path length is limited and that they deposit the main part of their energy at the end of their path. To be an incontestable advantage, high ballistic precision is nonetheless a double-edged sword. Two reasons for that. The first is related to the uncertainty of the path length itself (which is a pure physical problem) ; the second with the uncertainty about the Relative Biological Effectiveness (RBE) of protons, especially at the end of their path (which is a radiobiological problem). These two uncertainties oppose the precise contouring of the target volume and could result in a deficit of dose in the tumour (origin of a possible recurrence) and/or in an excess of dose outside the tumour (possible cause of unexpected side effects).

Our laboratory has a longstanding commitment in RBE studies in clinical hadron beams. Many radiobiological experiments have been performed in the proton beam of our South African collaborators, at iThemba LABS (Laboratory for Accelerator Based Sciences) in Cape Town. These studies aimed at determining the variation of the proton RBE in the Spread-Out-Bragg-Peak (SOBP), that is the place where the tumour is positioned. This place is also the area of the highest variation of Lineal Energy Transfer (LET) and, therefore, of the highest possible RBE variation.

Intestinal crypt regeneration in mice was used as biological criterium to determine the variation of the proton RBE throughout the SOBP. In the early experiments the animals were irradiated to the whole-body, which investigations showed a 6 - 7% increase in RBE from the beginning to the end of the SOBP. However, due to the thickness of the mouse, the intestine could not be positioned at the very end of the SOBP, so that the preceding values are likely not representative of the full RBE variation over the SOBP.

For these reasons a novel irradiation technique was developed for irradiating small volumes of intestine. In this, a section of jejunum is externalized and positioned in a plane perpendicularly to the axis of the beam. This makes it possible to irradiate small segments of intestine that are positioned as close as 2 - 3 mm from the very distal edge of the SOBP. With this method, the increase in RBE for the 200 MeV clinical proton beam at iThemba LABS was investigated for crypt regeneration in mice at the very end of a 3-cm and a 7-cm SOBP. The method showed itself accurate and easily practicable. The data of the investigations are currently under analysis.

To be noted that the ex-vivo method will be used in another ongoing experimental program aiming at studying the influence of the size of the irradiation field on the response to irradiation. This study, which is particularly relevant to IMRT (Intensity Modulated Radiation Therapy), forms part of the general problem of the “volume effect”.

Radical reduction of particle range uncertainties in proton therapy

(Stefaan Vynckier, Edmond Sterpin, Jefferson Sorriaux, Kevin Souris, Anna Barragan, Séverine 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) in collaboration with IBA, 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.
Since a few years, the treatment of cancers using hadron beams takes an important place in the world. At present, the majority of particle therapy centres worldwide focused on proton therapy, but a few centres in Japan, Germany and Italy treat the patients using carbon ion therapy. This interest for hadrontherapy requires important efforts to improve the accuracy of absorbed dose received by patients.

Thanks to various collaborations, MIRO performed experimental research in different hadron therapy centres in Japan, in Sicily and in UK, with two independent calorimeters: a graphite calorimeter developed at National Physical Laboratory in UK and its water calorimeter. During these studies, the first direct comparison between a graphite calorimeter, a water calorimeter and ionization chambers has been performed. The goals of these investigations was (i) to determine physical properties of beams, such as the mean energy expended in air per ion pair formed, to reduce their uncertainty and improve the accuracy of the calculation of physical absorbed dose and (ii) to calibrate the ionization chambers. Our studies have been performed in various beams: electron beams, photon beams, clinical and non-clinical carbon ion beams and clinical proton beams.


Microdosimetry of hadron therapy

(Sabina Chiriotti, Edmond Sterpin, Stefaan Vynckier)

Ion-beam therapy is increasing worldwide for treating solid tumours that are resistant to low-LET radiations because of their favourable dose depth distribution, enhanced biological effectiveness and reduced oxygen enhancement ratio. Due to the strong dependence of the linear energy transfer (LET) with energy for charged particles, the radiation quality of these particle beams varies significantly within the irradiated tumour. Moreover, nuclear reactions occurring along the particle track give rise to a mixed and complex radiation field. Therefore, a complete characterization of the clinical beam radiation quality in terms of measurable physical properties of charged particles at the cellular/subcellular scale could be useful for improving treatment plans.

In particular, microdosimetry can be useful for specifying the radiation quality, because it studies the spatial and temporal distributions of the energy imparted at the microscopic (i.e. cellular) level. Variations of the radiation quality of complex radiation fields can be measured with tissue-equivalent gas proportional counters (TEPCs). TEPCs are the reference devices in experimental microdosimetry for characterizing the radiation quality in radiation protection and radiotherapy environments. Usually, they are filled with tissue equivalent gases to simulate tissue sites comparable to the cell size (1 or 2 µm). Since clinical beams are usually characterized by high particle fluence rates (~ 10⁶ particles cm⁻² s⁻¹), only miniaturized TEPCs (mini TEPC) of about 1 mm³ can be used in radiotherapy to minimize signal pile-up effects when exposed to these high intensity beams. Different mini TEPC designs have been developed at Legnaro Laboratories (LNL, Italy) in order to minimize the cavity and its external sizes [1]. Experimental measurements in the past proved that the mini TEPC can properly measure in both therapeutic photon and proton beams [2].

The aim of this current research, in collaboration with the group of LNL, is to contribute to the development of miniaturized microdosimeters, in particular mini-TEPCs, for measuring the radiation quality of charged particle beams namely carbon ions. To this end, the response of the mini TEPC was characterized mainly with experimental measurements in known radiation fields but also with simulation techniques [3]. All the physical parameters affecting the measured microdosimetric spectrum such as gas multiplication characteristics, the calibration procedure [4], the detector’s geometry, the simulated site size and the gas filling type have been carefully studied during this project. Then, the first microdosimetric measurements with the mini TEPC at the Italian therapeutic carbon-ion beam (Centro Nazionale di Adroterapia Oncologica, CNAO) were performed with monoenergetic carbon ions [5] proving its feasibility to measure the radiation quality at various depths in a water phantom. Figure 1 illustrates a typical example.
Left panel: penetration in water of a 195.1 MeV/um
$^{12}\text{C}$ beam illustrating the positions where the microdosimetric measurements were performed; right panel: resulting microdosimetric spectra.


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.

PET images convey very useful functional information about tumours. Depending on the tracer, glucose metabolism, proliferation hypoxia, or other cellular mechanisms can be revealed. However, these images suffer from a rather low resolution that prevents a spatially accurate delineation of the tumour 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 validated for head&neck and lung tumors. Ongoing work aims at improving these tools, in particular deblurring, in collaboration with EPL/SST/ICTEAM/ELEN. New constraints and regularization schemes are investigated in order to avoid deconvolution artifacts in the deblurred image. Ultimately, the goal is to recover not only the external boundary of the tumour but also tracer uptake heterogeneities within the tumour. Improved heterogeneity rendering is useful for hypoxia tracers and also 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 concentrates the dose on sub-regions suspected of radio-resistance. Ongoing work has investigated the methodological issues of dose painting in H&N tumours.

S. Chiriotti, D. Moro, E. Motisi, M. Ciocca, P. Colautti, V. Conte. First microdosimetric measurements at CNAO. Annual Report INFN 2014.

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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 deformable registration of previously delineated images (called atlases). Contours obtained with these methods are often inaccurate, due to simplistic regularisation schemes of the deformation map. The approach developed in the project relies alternatively on machine-learning techniques (classifiers). The first results have shown that these techniques can compete with registration-based atlases. They are more generic but require more exhaustive data.

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).

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 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. 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$_2$, $^{18}$F-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) has been 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.

$^{18}$F labelling methodology is a permanent research area. It has been applied to the synthesis of silicon analogues of $^{18}$F-fluoro-misonidazole in order to develop new radiolabelled compounds for the detection of tumour hypoxia and more recently to the development of a $^{18}$F-trifluoromethylating agent for aryl boronic acids and aryl iodides. Trifluoromethylarenes are key moieties for the development of bioactive compounds, especially in medicinal chemistry.

Our last research concerns the synthesis of $^{18}$F-fluorolactate, a new potential radiolabelled compounds for the monocarboxylate transporter 1 (MCT1) imaging.
Adaptive PET-guided intensity modulated radiation therapy in head and neck, and non-small-cell lung cancer: towards an individualized treatment

(Sarah Differding, Dario Di Perri Xavier Geets, Vincent Grégoire, Samuel Goossens, Guillaume Janssens, John Lee, Stéphanie Servagi, Edmont Sterpin)

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 radioresistance 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 valuations 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 recently assessed the impact of FDG-PET sub-volume 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 was 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 was 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 was reached for the considered organs (spinal cord, lungs, heart, esophagus...). Mean doses to the PTV-PET reached 75 Gy and 90 Gy for central and peripheral tumors. With a median follow-up of 30 months, only 23% of patients recurred locally. Acute and late toxicities rates were similar to conventional regimens, except one grade 5 fatal hemoptysis in a central tumor. These results encourage larger
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 18F-FDG PET, following intrahepatic administration of 90Y-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 90Y, a method developed in our laboratory. Further work is ongoing for allowing accurate imaging of 90Y 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 125Sn, in combination («cocktail») with the radionuclide of choice nowadays, 177Lu. The laboratory also continues work on imaging of small animals, more recently, using a 99mTc-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 prospective trials on individualized dose escalations.

A step further involves the integration of a hypoxia PET tracer (FAZA), and the morphological/biological changes throughout the RT course. To investigate to which extent the uncertainties related to imaging and dose delivery impact on the DP process, we have designed the following planning study in non-small cell lung cancer (NSCLC): FAZA-PET and FDG-PET are acquired prior to treatment and during weeks 2 and 3 of conventional chemoradiotherapy. Based on these images, the tracer uptake distributions are compared on a voxel-by-voxel basis between both FDG and FAZA tracers, and the spatio-temporal stability of each tracer is assessed as well. Then, DPBN planning studies are performed to determine whether FAZA and FDG-PET imaging lead to significant differences in dose distribution under realistic treatment conditions (including geometrical uncertainties). Last, these DPBN strategies are compared to simple geometrical dose escalation based on the planning CT, in which the dose is linearly increased from the border to the center of the tumor. Indeed, in spite of all efforts, dose distributions might demonstrate to small differences to justify hypoxia-guided DPBN in NSCLC.

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

Schematic view of FDG & FAZA PET-based adaptive dose painting by numbers for NSCLC. Images are acquired prior to treatment and at weeks 2 and 3. Images are aligned, and uptake distributions are compared on a voxel-by-voxel basis between both tracers and over time.


Geets X. 4D PET-CT guided radiation therapy
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, E. Carrié, A Devallckeneer)

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 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 (Ipilimumab) on the functionality of anti-tumour CTL (project 6). Finally, the progress of genetics and molecular biology has allowed 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.

All the patients were included. This vaccine induces strong antibody response but weak CTL response against the vaccine. The clinical benefit is weak but since there is no toxicity, it can be associated safely with checkpoint inhibitors.

**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.

Three patients have been included and received the complete treatment. No bystander effect was observed. Further development will be done by combining these cytokines with checkpoints inhibitors.

**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 galectin-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 CM-CT-01 that will be injected intravenously or locally. This is a Belgian multicentric study where 12 patients will be included.

Amongst the 6 patients included no clinical benefit was observed. This study is closed and the research will continue in ovarian cancer in order to find the correct dose to revert T cell anergy in vivo.

**Project 4**  
Using anti-EGFR antibodies to modify homing of anti-cancer T cells  
In collaboration with Dr. K. Segers (Amgen, USA)  

B-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 EGF 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. Twelve patients have already been included with a high response rate.

**Project 5**

Correlation between survival and presence of BRAF mutation in melanoma. In collaboration with Dr. I. Theate (IPG, Lo-veral) and M. Vikkula (GEHU, DDUV, UCL, Bruxelles)

Recent progress in genetics 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.

**Molecular targeted therapies for cancer treatment**

*(JP Machiels, S. Schmitz, A. Gillain, G van Caloen, X Caignet, R-M Goebbels, M El Baroudi)*

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

We are conducting several academic clinical trials investigating targeted agents in squamous cell carcinoma of the head and Neck (SCCHN). We are studying novel agents that target either the cell cycle 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.

In addition, to better understand the resistance mechanisms to anti-EGFR therapies and to test novel 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) re-
sistance 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.


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

(F. Duhoux and C. Schoonjans 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 pre-specified 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 4 main axes:

- Oxidative stress, angiogenesis and tumorigenesis in thyroid gland and mammary gland.
- Causes, consequences and improvements of oxidative stress in eye muscles and orbital fat in cases of Graves’ orbitopathy.
- Adaptive mechanisms of the skeletal tissues from development and growth to senescence, in pathological conditions and at the bone-implant interface.
- Morphological (anatomic, histologic and X-ray imaging) description of particular body regions or organs in order to develop new therapeutic approaches.

Most of our studies are currently conducted in collaboration with other research poles in order to privilege multidisciplinary approaches.
Oxidative stress, angiogenesis and tumorigenesis in thyroid gland and mammary gland

1. Role of caveolin-1 in autoimmune thyroid disorders: Hashimoto’s thyroiditis and Graves’ disease

J. Craps, V. Joris, 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 oxidase2 (Duox2) 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 provokes cell apoptosis aggravating the hypothyroidism due to the loss of hormone synthesis.

We also analyzed the caveolin-1 expression in two opposite human autoimmune diseases, Th1 Hashimoto’s thyroiditis (HT) with hypothyroidism and Th2 Graves’ disease (GD) with hyperthyroidism. This in vivo study was completed by an in vitro analysis of the effects of Th1 and Th2 cytokines on human thyrocytes in primary cultures. We have demonstrated that, in HT, the histophysiologival pattern was similar to that described in caveolin-1 knockout mice: loss of caveolin-1 expression, intracellular iodination, T4 accumulation in the cytoplasm, and oxidative stress as shown by the increased expression of 4-Hydroxynonenal, a marker of lipid peroxidation. The oxidative stress was not adequately compensated by antioxidant defenses (peroxiredoxin and catalase, known to detoxify H2O2), and this led to cell apoptosis and endly follicular destruction (Marique et al, 2014).

Th1 cytokines (IL1α, IFNγ) dramatically decreased the caveolin-1 expression in human thyrocytes in primary cultures whereas Th2 cytokine (IL4) had no effect (Marique et al, 2014). In GD thyroids, caveolin-1 was properly located at the apical pole of the thyrocytes, as well as TPO and Duox, and T4 was detected in the follicular lumina of the hyperactive follicles. However, the thyrocytes were the targets of a huge oxidative stress. We are currently studying the pathways involved in this oxidative stress in GD thyroid cells.

2. PPARγ agonists and caveolin-1

PPARγ is a nuclear transcription factor regulating numerous genes and it is known to have anti-inflammatory properties. By immunohistochemistry, we showed its high expression in GD thyroids, correlating with the apical expression of caveolin-1. At the opposite, PPARγ was not detected in HT thyrocytes. Moreover, its mRNA and protein expression in human thyrocytes in primary cultures was decreased by Th1 cytokines.

We are studying the effects of PPARγ agonists, pioglitazone and rosvastatin, on the histophysiologival of the thyroid cells. Pioglitazone has been shown to increase both PPARγ and caveolin-1 expressions in human thyrocytes in primary cultures. It also increased the expression of catalase. PPARγ agonists could thus be considered as potent therapeutic agents to treat Hashimoto’s thyroiditis.
3. Iodine deficiency and microvascular activation in the thyroid gland

J. Craps, I. Colin, M.C. Many

Iodine deficiency (ID) induces micro-vascular changes in the thyroid gland via a TSH-independent reactive oxygen species - hypoxia inducible factor (HIF-1α)-vascular endothelial growth factor (VEGF) pathway. The involvement of nitric oxide (NO) in this pathway and the role of calcium (Ca\textsuperscript{2+}) and of ryanodine receptors (RYRs) in NO synthase 3 (NOS3) activation were investigated in a murine model of goitrogenesis and in three in vitro models of ID including primary cultures of human thyrocytes.

ID activated NOS3 and the production of NO in thyrocytes in vitro and increased the thyroid blood flow in vivo. Using bevacizumab (a blocking antibody against VEGF-A) in mice, it appeared that NOS3 is activated upstream of VEGF-A. L-nitro arginine methyl ester (L-NAME, a NOS inhibitor) blocked the ID-induced increase in thyroid blood flow in vivo and NO production in vitro, as well as ID-induced VEGF-A mRNA and HIF-1α expression in vitro, while S-nitroso-acetyl-penicillamine (SNAP, a NO donor) did the opposite.

Ca\textsuperscript{2+} is involved in this pathway as intracellular Ca\textsuperscript{2+} flux increased after ID, and thapsigargin activated NOS3 and increased VEGF-A mRNA expression.

Two of the three known mammalian RYR isoforms (RYR1 and RYR2) were shown to be expressed in thyrocytes. RYR inhibition using ryanodine at 10 µM decreased ID-induced NOS3 activation, HIF-1α and VEGF-A expression, while RYR activation with ryanodine at 1nM increased NOS3 activation and VEGF-A mRNA expression.

In conclusion, during the early phase of TSH-independent ID-induced microvascular activation, ID sequentially activates RYRs and NOS3, thereby supporting ID-induced activation of the NO/HIF-1α/VEGF-A pathway in thyrocytes.

4. Iodine deficiency and angiogenesis in the mammary gland, stomach and salivary gland

J. Vanderstraeten, M.C. Many

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 independently of TSH 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 two of these organs (stomach and salivary glands) and compare it with the effects observed in thyroid.

Eight week NMRI mice were fed with an iodide deficient diet and perchlorate containing water (a NIS inhibitor), with/without bevacizumab (a VEGF inhibitor). In salivary glands, ID induced a transient increase in HIF-1α protein expression associated with a transient, VEGF-dependent increase in blood flow. In gastric mucosa, VEGF expression was temporarily enhanced in the mucin secreting epithelium and in gastrin secreting endocrine cells during ID.

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. As different disorders such as stomach tumor have been related to ID and thyroid enlargement, our results could provide enlightenment to understand this correlation.

A second part of this project addresses the hypothetic synergy between iodine deficiency (ID) and ionizing radiations (IR) on tumor development. ID and IR are two risk factors responsible for the increased incidence of thyroid cancers that were shown to act synergistically by recent epidemiological and experimental data. The aim of our project is to study the consequences of ID and combined ID/IR on signaling and regulating pathways of the VEGF gene, as well as on microvascular and cell cycle changes, and on DNA damage, in thyroid and in iodine accumulating organs: salivary glands and stomach, based on the assumption of a mutual reinforcement between these two risk factors.
Causes, consequences and improvements of oxidative stress in eye muscles and orbitary 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. It is 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 analyze 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 analyze 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.

1. Sclerostin antibody improves bone parameters of vertebrae in Oim/Oim mouse model of osteogenesis imperfecta

M. Cardinal, C. Behets, D. Manicourt (RUMA)

Osteogenesis imperfecta (OI), a genetic bone disorder causing weak bone quality, engenders a high rate of fractures. Antibody-mediated sclerostin inhibition (Scl-Ab) has been shown to improve bone parameters of long bone in mouse models of mild and severe OI. We studied the effects of weekly intraperitoneal Scl-Ab injections on 4-week-old mice with a spontaneous severe OI, oim/oim mice. Mice growth (weight, length and bone mineral content) was measured at study entrance and at necropsy, 9 weeks later.

Sagittal pQCT slices of lumbar vertebrae and sacrum of WildType and oim/oim mice treated with either vehicle (PBS) or Scl-Ab. Radiopacity shows effects of 9 weeks Scl-Ab treatment on BMD.

Vertebral bone parameters were assessed with ex vivo pQCT analysis. Overall, OI mice were shorter and weighed less than WildType (Wt) mice. A difference of vertebrae length was found only in lumbar vertebrae. These parameters did not significantly improve with the Scl-Ab treatment. Bone mass, however, showed a significant positive response to the Scl-Ab therapy in OI and healthy mice. Scl-Ab therapy significantly improved whole-body bone mineral content (BMC) and bone mineral density (BMD) in vertebrae. The similar results were found for mean cross sectional bone area (CSA) from third lumbar vertebrae to the end of sacrum. At the same level, OI Scl-Ab had the same BMC and CSA as the control group (Wt vehicle). More preferably, their BMD was significantly higher.

Scl-Ab increases whole-body bone mineral content and bone mass parameters in vertebrae of OI mice. This could improve the mechanical properties of the vertebral bodies, preventing spine deformities and subsequent physical disability and respiratory distress.

2. Inhibition of osteoclastic resorption by cathepsin K gene knockout in long bones of osteogenesis imperfecta mice

T. Roels, C. Behets, D. Manicourt (RUMA)

Osteogenesis imperfecta is characterized by low bone mass and high bone fragility leading to multiple fractures. In osteoporotic women, inhibition of cathepsin K (CatK) by an antibody is known to increase bone mineral density (BMD) and to reduce the number of fractures. In mice, CatK knockout (KO) results in high bone mass due to impaired bone resorption.

In the present experiment, we used Oim (osteogenesis imperfecta mice), which constitute a valuable experimental model of osteogenesis imperfecta, and crossed them with CatK KO mice. We hypothesize that CatK KO in the osteogenesis imperfecta mice would reduce the number of fractures and increase the BMD of long bones.

Preliminary morphological analyses show that CatK KO (cat(-/-) ) in Oim reduces the fracture number of long bones and increases BMD in the femur diaphysis (cortical bone) and epiphysis (trabecular bone), but does not modify the total bone area. These data suggest that CatK KO in the Oim improves bone quality rather than to increase bone quantity. This better bone quality could be attribu-
ted to the reduced resorption activity, allowing bone to improve biological and mechanical maturation. These modifications will be investigated in further analyses.

3. The role of acid hyaluronidase (PH20) in osteoarthritis development after meniscectomy and section of anterior cruciate ligament in mice

S. Lafont, D.H. Manicourt (RUMA), C. Behets

Osteoarthritis (OA) is a joint disease characterized by degenerative wearing of articular cartilage as well as modification in subchondral bone structure which leads to whole joint deformation. Articular cartilage is a connective tissue composed of chondrocytes and an extracellular matrix (ECM) which provides a smooth surface with a very low coefficient of friction. Hyaluronan (HA), one of the main constituents of this ECM, is regulated by the hyaluronan synthases (HAS) and the hyaluronidases (HYAL). Hyaluronidases have been suggested to play a role in the HA destruction associated with OA development. In mice deficient in hyaluronidase 3 (HYAL3 KO) and in acid hyaluronidase (PH20 KO), we induce OA by resection of the medial meniscus and section of the anterior cruciate ligament at 10 weeks of age in order to highlight a possible delay in OA occurrence. OA development is analyzed at several times after experiment through periperal Quantitative Computed Tomography (pQCT) in order to investigate the subchondral bone density, and through histology and immuno- histochemistry in order to define the modifications in all soft tissues of the joint (cartilage, menisci, joint capsule,...).

Coronal section through the operated knee of a PH20 KO mouse sacrificed 4 weeks after surgery. In the medial (M) compartment of the joint, articular cartilage is absent (arrows), the subchondral bone density is increased and osteophytes (Op) are visible.

Preliminary data show that isolated knockout of one hyaluronidase does not prevent OA development and suggest a possible enzymatic compensation by the remaining hyaluronidases.

4. In vitro evaluation of peri-implantitis treatment modalities on titanium surface properties and biocompatibility to Saos2 osteoblasts

S. Toma, M. Brecx (SLUC), C. Behets

Peri-implantitis is characterized by inflammatory lesions in peri-implant tissues associated with loss of supporting bone. 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. 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 are currently investigated: plastic curette, air-powder abrasive system, Ti-brush® and implantoplasty bur, in order to assess their influence on (1) the removal of dental biofilm grown on titanium surfaces and (2) the biocompatibility of the instrumented titanium surfaces.
First, the air-powder abrasive system led to some clinical improvement of the peri-implantitis features, but did not succeed in reducing the biofilm activity (Toma et al. 2014).

Surgical treatment of peri-implantitis using the air-powder abrasive system.

The other procedures are currently under study in patients.

Secondly, titanium disks treated in vitro with the same procedures, plastic curette, air-abrasive device (Perio-flow®), titanium brush (Ti-brush®), and implantoplasty, were analyzed in order to define their physical properties and biocompatibility to Saos2 osteoblasts.

Titanium disks treated with plastic curette, Perio-flow® and Ti-brush® and analyzed using SEM showed the same complex microstructure, including craters and micropits, as the control, untreated disks, whereas disks treated by implantoplasty appeared smoother.

Contact angle measurement, using a drop shape analysis system and indicating titanium surface wettability, showed that implantoplasty treated disks were significantly more hydrophilic than the control ones, while those treated with plastic curette appeared more hydrophobic.

Contact angle measured in the 5 groups. *p<0.001 (ANOVA, Tukey’s).

SEM aspect of titanium surface after treatment.

SEM aspect of the SaoS2 osteoblasts (arrows) after 24h of seeding on the treated disks.
Saos2-osteoblasts seeded on disks of the different groups showed no morphological difference under SEM at 1h; at 24h, they were stretched in the implantoplasty group, whereas they remained round shaped in the control, plastic curette, Perio-flow® and Ti-brush® groups.

At 6 days, the Saos2 viability in contact with the titanium surfaces was almost similar as their viability in vitro without titanium.

All treatment modalities promoted cell differentiation towards osteoblastic phenotype by supporting ALP, OPG and OCN production by SaoS2 in the same range as cells seeded without titanium.

In conclusion, increased hydrophily after implantoplasty seems to improve the osteoblasts spreading and do not affect their blastic differentiation.

5. Microradiographic and histological evaluation of the bone-screw and bone-plate interface of orthodontic miniplates in patients

S. Vanderguchten, M.A. Cornélis (Aarhus University), P. Mahy (SLUC), C. Behets

Skeletal anchorage is now part of contemporary orthodontics because of its advantages over traditional anchorage systems. Conventional orthodontics relies on the use of several teeth as an anchorage unit to move other teeth. Additional compliance-dependent devices such as intermaxillary elastics or headgear are often necessary to obtain therapeutic success.

Furthermore, traditional anchorage tools reach their limitation when dental anchorage is lacking, for example in periodontally compromised patients. In these cases, the use of skeletal anchorage can be considered. Prosthetic, retromolar and palatal implants, still useful in limited indications, have been progressively substituted by miniscrews and miniplates which are smaller, less expensive, less traumatic, and can provide a direct anchorage. We use the term “miniplate system” to refer to the miniplate or the fixation screws.

Although these miniplates were shown to be efficient and innocuous in patients, no microscopic data are available about their tissue interface in humans. Moreover, the duration of use of these miniplates in clinical conditions is usually longer than what has been reported in animal models.

Therefore, the purpose of the present study was to evaluate the tissue reactions at the bone-screw and bone-plate interface of miniplates in humans, in order to compare the observations obtained in animals to longterm evaluation of the bone-titanium interface of an orthodontic miniplate system, in humans.

We analyzed 42 samples, consisting of tissue fragments attached or not to miniplates or their fixation screws, which were collected from 24 orthodontic patients treated with miniplate anchorage, at the time of removal of their miniplates. The samples were embedded in methylmethacrylate and cut into undecalcified sections which were submitted to microradiographic analysis. The sections were also stained and examined under ordinary light.

Three types of reactions were observed.

1/ The majority of the stable miniplates were easy to remove (34/42). The tissue samples collected consisted mainly in lamellar bone with some medullary spaces containing blood vessels.

2/ Two screws were highly osseointegrated and required the surgeon to remove them by trephining (2/42). They were surrounded by bone tissue which extended to the miniplate. The histological features were similar to the previous group, though the bone-screw contact was higher.

3/ In 6 samples obtained after unstable miniplate removal during the treatment, we observed either some woven bone trabeculae or loose connective tissue, without any histological sign of inflammation.

In conclusion, the healing reactions observed in human samples in the present study are similar to the patterns previously described in animals. The majority of the miniplates were easy to remove, even if some bone apposition was found on the miniplates. Some miniplates were integrated to the point that their removal was problematic.
Anatomical research related to the development of new clinical tools

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

Bio-artificial face transplantation

J. Duisit, P. Gianello (CHEX), 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 immuno-suppression, 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.

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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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 electrolytes 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 immunohisto-/cyto-chemistry
• Intracellular distribution studies: subcellular fractionation, immunogold, biotinylation
• Transport studies in cells and native tissues (Ussing chamber)

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: WGKD

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 Société de Néphrologie: www.soc-nephrologie.org
Fondation du Rein: www.fondation-du-rein.org
Académie Royale de Médecine de Belgique: www.armb.be

Editorial Boards:
Kidney Int: www.nature.com/ki
Pflügers Arch: http://www.springerlink.com/content/100448/
Perit Dial Int: www.pdiconnect.com

Patient organizations and resources:
Orphanet: www.orpha.net
AIRG Europe: www.airg-france.org/airg_europe.htm
PKD Foundation: www.pkdcure.org

Financial support
- Actions de Recherche Concertées (ARC), Communauté Française de Belgique
- Commission européenne, FP7
- Fondation Roi Baudouin, Fonds Alphonse et Jean Forton
- Fondation St-Luc
- Fonds de la recherche scientifique - FNRS et FRSM
- Pôles d’Attractions Interuniversitaires (PAI)
- Région wallonne
- Baxter Extramural Grant Program

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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 hepatotropic 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.
Biodistribution, Engraftment and in situ differentiation of transplanted stem/progenitor cells

Biodistribution

Ensuring that injected cells engraft in the target organ without spreading to surrounding organs is a major safety aspect in cell therapy. In order to study the biodistribution of liver progenitor cells, one million ADHLSCs were marked with a reporter gene and injected directly into the left lateral lobe of the liver of scid-beige mice. Positron emission tomography (PET) combined with computed tomography (CT) imaging were applied to non-invasively monitor injected ADHLSCs following addition of a radiotracer. Pre-radiolabelled ADHLSCs clearly showed migration from the injection site to the right side of the liver. (Figure 1)

In addition, intravenous administration of the radiotracer one day after cell transplantation also allowed the tracking of cells, with acceptable radioactivity in the gallbladder and other surrounding organs, which was consistent with the control mice without surgery, or with vehicle injection. Together, these results suggest that liver progenitor cells are successfully retained into the liver following intrahepatic injection. However, experiments remain to be carried on to further understand the biodistribution over a longer period of time.

Engraftment

Engraftment is one of the keys to achieving an efficient cell therapy. Unfortunately, MSC (Mesenchymal Stem Cells) infusions have been shown to lead to poor engraftment levels. Our laboratory is studying liver progenitor cell engraftment and evaluating strategies to improve it. To this end, we have focused our work on the first part of the engraftment process, the adhesion of the cells to the endothelium. First, we have confirmed that ADHLSCs, like most MSCs, do not express any Selectin ligand that would allow them to roll on the endothelium. Our preliminary results suggest that using the cell surface’s biochemical properties to add a Selectin Ligand (Sialyl Lewis X) leads to improved liver progenitor cell adhesion to E-selectin, the main selectin of the endothelium.

Second, we hypothesize that the enzymatic dissociation used to detach cells damages the integrins involved in adhesion to endothelial proteins (mainly VCAM-1). We are trying to circumvent this negative effect by growing ADHLSCs on a thermosensitive polymer, which changes conformation at room temperature and gently releases the cells in the culture medium. This method seems to slightly increase the expression of some adhesion proteins and improve cell adhesion to the corresponding ligands.

Finally, our experiments suggest that CXCR4, which is an important receptor involved in stem cell migration and adhesion through the activation of integrins, gets internalized during the culture process. We plan to use a cocktail of cytokines to try to externalize CXCR4, activate it and consequently improve adhesion.

Study of the mechanism of thrombosis induced by transplantation of ADHLSC using intravital microscopy

Our laboratory studies the development of hepatocytes and hepatic stem cell transplantation as a treatment for human metabolic diseases. Nowadays when the usual treatment that consists of strict diets and scavenger treatment fails, orthotopic liver transplantation is the only treatment option left. However, this procedure is invasive and organ shortage is a real problem. Cell transplantation is a rapidly expanding al-
ternative treatment, but the thrombogenic risk induced by these cells is a major concern. The principal goal of our research is to reduce the thrombogenic risk in patients who received hepatic stem cells, without reducing the implantation of the cells. First, we want to study the mechanism of thrombus formation in vivo during these cell infusions with anatomopathological examinations and "live" microscopy, called intravital microscopy. In this model, we would also like to confirm the prevention of thrombosis induced by the cellular infusion by using a combination of anticoagulant drugs (antithrombin activator and thrombin inhibitor).

Currently we are marking endothelial cells and hepatic stem cells with fluorescent trackers, to visualise hepatic stem cells in the vessels of the mouse after an intrasplenic injection (fig2). Then, we would like to study the expression of tissue factor (TF), a protein that triggers the coagulation cascade and seems to play an important role in the procoagulant activity of the hepatic stem cells. We will study the expression of TF and its natural inhibitor tissue factor pathway inhibitor (TFPI), with flow cytometry, quantitative and qualitative PCR at different stages of cell culture (from P2 to P7). Finally, the beneficial effect of the activation of coagulation and the influence of the anticoagulant drugs on cell implantation will be studied in animal hepatic cell transplantation models.

Potential use of bioscaffolds to increase human liver progenitor cell retention, activity and differentiation

Research from our laboratory has shown that ADHLSCs have the capacity to differentiate into hepatocyte like cells when subjected to a specific protocol in vitro. They are also able to engraft into the host liver following cell transplantation and to differentiate in vivo. However, the level of engraftment is fairly low and cell differentiation remains partial. We are therefore studying the possibility of increasing cell retention and improving differentiation using bioscaffolds. We are currently comparing scaffolds generated from a lyophilized, demineralized bone matrix and scaffolds made of alginate.

Our preliminary studies have shown that both types of scaffolds are favorable to sustained cell viability and lead to improved expression of hepatic markers. However, cell retention was low in the bone-derived scaffolds, while alginate scaffolds showed a limited seeding capacity and only allowed for small numbers of cells. We are currently working on confirming these results and trying to combine the use of alginate and bone-derived scaffolds to optimize cell seeding and cell retention.

Role of exosomes and microparticles in ADHLSC-derived therapeutic effects

Despite using cutting edge techniques to try to accurately evaluate the amount of donor progenitor cells engrafted in the host liver, the numbers obtained in pre-clinical and clinical settings seem to remain low in comparison to what could be expected from the improvement seen in the recipient’s hepatic parameters, suggesting that more intricate mechanisms maybe at play than initially thought. Recent evidence suggests an important role for the molecules secreted by the injected cells in cell therapy. In addition to cytokines and growth factors, increasing interest is currently being paid to extracellular vesicles such as microparticles (MPS) and exosomes. We are currently optimizing protocols for the isolation of extracellular vesicles from human liver progenitor cell culture supernatants. Preliminary data have shown that
these particles contains mRNAs of interest for various hepatic disorders, such as UGT1A1 (which is mutated in Crigler Najjar syndrome, a bilirubin conjugation disorder), PEX12 (which is deficient in some types of infantile Refsum disease, a congenital peroxisomal biogenesis disorder) and coagulation factor VIII (which is deficient in hemophilia A), suggesting a potential role of human liver progenitor cell-derived extracellular vesicles in the correction of these diseases. Studies are currently underway to demonstrate horizontal mRNA transfer between donor and target cells.

**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, 36 and 60 months. Analyses performed using the classifications established at 18 and 36 months have shown an influence of acetaminophen intake on the development of allergy. Further analyses are underway to determine whether this effect is still present when using the classification established at 60 months.

In addition, allergic patients with positive plasma levels of allergen specific IgE and/or skin prick tests were considered “IgE-mediated allergic”. We found significant differences in the cytokine profiles of IgE-mediated allergic when compared to the rest of the cohort both when analysing the results using the classifications established at 18 and 36 months. We are currently analysing the data using the classification established at 60 months. Finally, we have also found that a deficit in plasma IgA at 2 months of age was correlated with the development of allergy at age 5.

**Use of stem cells as an in vitro tool to decipher HBV infection events**

Hepatitis B virus (HBV) is a human pathogen with a restricted host range and a high selectivity for the major liver cell type: the hepatocyte. Therefore, the gold standard for in vitro HBV infection studies is freshly isolated primary human hepatocyte (PHH) cultures. However, limited access due to significant organ scarcity, rapid dedifferentiation in culture as well as a poor resistance to cryopreservation has pushed researchers to develop new cell models for HBV in vitro infection studies.

The aim of the laboratory is to study the molecular pathways related to HBV early infection events as we recently demonstrated that human umbilical cord mesenchymal stem cells (UCMSCs) are permissive to HBV and supportive of the whole viral cycle upon hepatogenic differentiation. Worth of note, recently, the Sodium Taurocholate cotransporter polypeptide (NTCP) was shown to be a functional receptor for HBV. Therefore, it was of major interest to confirm if differentiated UCMSCs express this NTCP receptor. Our recent data showed that NTCP expression is up-regulated post-differentiation both at the mRNA and protein levels which may explain why our cell model was found to be susceptible to HBV. We are currently studying the interaction of this receptor with HBV by investigating the key players required for an efficient HBV uptake.

**Evaluation of the anti-fibrotic properties of ADHLSC**

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. Although orthotopic liver transplantation remains the only treatment option nowadays, its wide clinical access is significantly limited by several parameters. Supportive and consistent current preclinical data allow positioning stem cell transplantation as an emerging perspective to inhibit fibrosis while the exact involved mechanisms are not yet fully understood. The laboratory is evaluating the potential of Adult-Derived Human Liver Stem/progenitor
Cells (ADHLSC) to inhibit the activation of HSC into myofibroblasts both in vitro and in vivo. In vitro, we are assessing both the proliferation and the secretion potential of activated hepatic stellate cells (HSC) as well as the signaling pathways involved. In vivo, we are selecting the optimal animal model and cell transplantation protocol to appraise the potential usefulness of ADHLSC in inhibiting liver fibrosis.

## Type 1 diabetes

### Beta-cell engineering

Type 1 diabetes (T1D) results from an inadequate mass of functional β cells therefore the replacement of pancreatic β cells is an attractive potential therapy. Because of current limitations of human islet transplantation, there is a need for new sources of transplantable cells with β cell-like functionality. The pancreas itself contains cells with β-cell differentiation potential, the most promising being epithelial duct cells and acinar cells. Although easily isolated, these cell types have not yet been successfully expanded in vitro. In our laboratory, we showed the possibility to derive cells from human pancreatic ducts that have sustained proliferation capacities and produce immature β cells.

Purified human CA19-9+ duct cells were subjected to growth factor-based differentiation and produced insulin-positive cells with secretion capacities while remaining glucose-insensitive. In order to increase the output of pancreatic endocrine cells from our original duct cell populations, we developed transcription factor-based differentiation strategies and observed that fast and reliable endocrine reprogramming can be obtained after over-expression of NGN3 or MAFA factors. In a collaborative effort with the Broad Institute of Harvard and MIT in Boston, we are evaluating the potential of new small molecules to drive our duct cells towards β cells in a timely fashion. Preliminary results show that the BRD-7552 compound is able and sufficient to obtain β-like cells in vitro. Our future objective is to evaluate whether our transcription factor and small molecule-based differentiation strategies allow robust differentiation and persistence of functional insulin secretion activity after transplantation into murine models of diabetes.

### Immunogenicity of Hepatic cells

#### Biomarkers of tolerance or rejection in Liver Cell Therapy patients

The success of cell therapy relies on several key points, including the capacity of the injected cells to engraft into the target organ and differentiate without triggering a response from the host immune system. The liver is traditionally regarded as an immunoprivileged organ. Indeed, transplanted livers are less frequently rejected than other organs. In fact, the liver is sometimes co-transplanted with a second organ such as the kidney to induce tolerance of the latter. In addition, our research so far supports the idea that human liver progenitor cells are poorly immunogenic in vitro.

However, immunosuppressors are still administered in the context of liver cell transplantation as a precautionary measure. Our project, funded by the Region Wallonne, aims to determine whether it is possible to identify potential markers of tolerance or rejection in patients undergoing liver cell therapy. We have recruited 12 liver cell therapy patients, who were seen before cell transplantation, as well as 14 days, 1, 3, 6, 8 and 12 months post infusion. For each patient, we harvested serum samples to detect potential anti-donor HLA antibodies as well as HLA-G (a marker of tolerance), sCD30 and CD40L (markers of rejection), and harvested blood samples in order to isolate peripheral blood mononuclear cells (PBMCs).

The patients’ PBMCs were frozen until all the timepoints from the same patient were available, and then placed in co-culture with the human hepatic progenitor cells that were used in the infusion. The immune response of the patient was evaluated by ELIspot for IFN-γ, proliferation assay, and measure of cytokine production in the supernatants. In addition, the presence of donor DNA in liver biopsies of the recipient was evaluated using droplet digital PCR. Analyses are underway to determine whether we can correlate a specific immune response to the absence or presence of donor cells in the liver of the recipient and the absence or presence of clinical effects.
**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. We monitor pediatric liver transplant patients in order to correlate blood biomarker(s) of tolerance or rejection with the results of the hepatic biopsy. More than 200 samples are already available.

**Accredited Bank of Hepatocytes and Hepatic Stem Cells**

Human adult hepatocytes are routinely isolated whole or resected human livers. The recovered hepatocyte suspensions are transplanted, banked or cultured to generate hepatic stem cells. Both types of cells are used to cure children suffering from liver diseases. More than 115 liver cell isolations were conducted so far. Since the Bank’s inception, 15 patients have been transplanted with hepatocytes and 3 others with hepatic stem cells.

**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**


FUNDING

- REGENESTEM : PPP Région Wallonne-PEDI-PROMETHERA Biosciences : « Cellules progénitrices 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 »
- 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 immunosuppresseur concomitant »
- IMTOX : Programme de recherche collective Région Wallonne : « Evaluation de l’immunotoxicité de nouveaux traitements »
- SCAFFOSTEM : PPP Région Wallonne-PEDI-PROMETHERA Biosciences : « Potentiel de colonisation d’un scaffold bio-compatible humain par les ADHLSC pour utilisation dans la thérapie cellulaire en site ectopique, en particulier pour le traitement des hémophiles »
- EXOHEP : BEWARE Academia Région Wallonne; PEDI-PROMETHERA : “Exosomes or microvesicles derived from hepatic progenitor cells for improving stem-cell based treatments of metabolic disorders”
- FNRS CC : “Suivi immunologique post-infusion”
- FNRS : mandat de spécialiste postdoctorant
- FNRS CDR: “Improving progenitor cell engraftment”
- FNRS FRSM : “Biodistribution et efficacité de la transplantation intra-portale de cellules progénitrices hépatiques dans deux modèles murins mimant la maladie de Crigler Najjar et la phenylcétonurie chez l’homme
- FNRS CDR: “New technologies for beta-cell engineering from human pancreatic duct cells”
- FNRS: “Defining the role of asialoglycoprotein receptor in determining hepatitis B virus hepatotropism”
- FNRS Télévie : « VHC et précurseurs hépatiques »
- Mandat de Recherche Doctorale de la fondation St Luc: « Study of the mechanism of thrombosis induced by transplantation of ADHLSC by intravital microscopy »
- Mandat IREC de Logisticien de Recherche à 50 % : « projet de soutien logistique pour la plateforme de cytométrie en flux et immunologie de l’IREC »
- Mandat IREC de chercheur post-doctorant en mobilité internationale
- Mandat IREC de Clinicien Chercheur : « Etude en microscopie intravital du mécanisme de thrombose induite par la transplantation de cellules souches/progénitrices hépatiques »
- Financement de la Fondation Salus Sanguinis : « Développement d’un support biologique humain pour la thérapie cellulaire dans le traitement des exérèses osseuses et le traitement de l’hémophilie »
- Belgian Society for Pediatric Endocrinology Research Grant

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The importance of respiratory and skin 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) – and to orphan diseases such as lung fibrosis.

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 develop asphyxia when asleep, resulting in sleep destructuration 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). New hypoglossal trial (IMThERA III), peripheral nerve stimulation (NYXOAH) and mechanical advancement of the base of the tongue (REV-ENT) are also under investigation.

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. New technology (drug targeting) and specific conditions as sinus nebulization or combination of a nebulizer to non-invasive ventilation were investigated.

We showed for the first time that choosing 2 different specific drug targeting nebulization modes does not influence the amount of drug delivered into the lung in healthy male subjects. Moreover, the modes do not modify the site of deposition under the conditions of our study.

The effectiveness of sonic nebulized and oral administration of corticosteroids in chronic sinusitis was demonstrated on orthonasal olfaction. The clinical benefit is better than with nasal spray.

In another study, we showed that with vibrating mesh nebulizers, their position between the exhalation port in the NIV circuit and lung model are more efficient for drug delivery compared with jet or ultrasonic nebulizers. In this position, the improved efficiency of vibrating mesh nebulizers was due to an increase in the inhaled dose and a reduction in the exhaled wasted dose compared with placement between the ventilator and the expiratory port. Because of the high total lost dose, the ultrasonic device should not be recommended. Nebulizer placement before the exhalation port increased the inhaled dose and decreased the expiratory wasted dose, except for the jet nebulizer.

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


Supports: FNRS (FRSM 3.4540.11, 3.4512.12 & 3.4522.12), Région wallonne (WELBIO 2012-037, FIRST postdoc “ITARA”), UCL-FSR (mandate S. Gohy), SSS-IREC (C. Shen), Fondation St-Luc (A. Froidure).

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 (4). In the upper airway, pIgR downregulation is also observed in a subset of patients with chronic rhinitis, namely allergic rhinitis and eosinophilic rhino-sinusitis, suggesting an unexpected link between pIgR downregulation, epithelial dedifferentiation, and eosinophilic/Th2 inflammation (4).

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 1 signals (IL-12) and induce in allogeneic CD4+ T cells a preferential Th2 and Th17 polarisation. We observed that this proTh2 programming relates to the aberrant expression of TSLP-R (5), and DCs from patients with occupational asthma (6) and persisting disease despite strict allergen avoidance also display these features. As TSLP is released by epithelial cells, understanding how the airway epithelium and DCs are aberrantly programmed in asthma (Fig. 1) and chronic lung disease should provide new therapeutic strategies to these disorders.

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. Dumoutier (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 1 Hypothesis of conditioning by the airway epithelium of dendritic cells 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, 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 NFκB, 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.

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.
Diagnosis and treatment of lupus nephritis

F.A. Houssiau, F. Tamirou, S. Nieuwland-Husson

This topic remains one of the most successful area of research in our Pole, due to the important recruitment of patients, far beyond our local boundaries, the efficient organization of our specialized outpatient clinics, our internationally recognized expertise for more than 15 years, and excellent collaborations with nephrologists, nephropathologists and our translational research laboratory.

In 2014, we re-evaluated the validity of the ISN/RPS (International Society of Nephrology/Renal Pathology Society) classification of lupus nephritis (NL). Between 1995 and 2012, we identified 98 incident cases of « proliferative » LN, diagnosed in our hospital, and entered them in the LOULUNIC (LOUvain Lupus Nephritis Inception Cohort) cohort. We demonstrated that the histological grade (III, IV-S or IV-G) had no influence on the speed of response to immunosuppressive therapy (the same treatment is administered in all three classes), and – more importantly –, long-term prognosis. Pejorative outcomes (death, terminal kidney failure, irreversible loss of kidney function) were not more prevalent in the supposedly more severe histological groups (IV > III ; IV-G > IV-S). By contrast, the presence of – even minimal – fibrotic changes in the baseline kidney biopsies was clearly predictive of a worse renal outcome. Overall, this study demonstrates that LN classification must be revisited, since it does not deliver relevant prognostic information (1).

In addition, we obtained and published the 10-years results of the MAINTAIN European study, a randomized clinical trial that we coordinated, in which two immunosuppressive maintenance therapies of LN were compared, azathioprine (AZA) versus mycophenolate mofetil (MMF), after induction of remission using IV cyclophosphamide pulses, according to the now standard Euro-Lupus protocol that we validated in a previous clinical trial. After 10 years, only 10% of the patients were lost of follow-up. Intention to treat analysis at 10 years confirmed the results observed after 5 years : MMF is not superior to AZA in preventing renal relapses in an European population (Fig. 1). More importantly, we confirmed the excellent positive predictive value of a proteinuria drop in the first year of immunosuppressive therapy (Fig. 2). Patients with a proteinuria at 12 months < 0.5 g/day have a 92% probability of displaying favorable outcomes at 10 years, i.e. of having a perfectly normal kidney function (2).

Figure 1  Kaplan–Meier analysis of the probability of an absence of all type of renal flare. All patients received Euro-Lupus intravenous cyclophosphamide, followed by azathioprine or mycophenolate mofetil as maintenance therapy. Survival curves were statistically tested with the log rank test. HR: hazard ratio (95% CI). Numbers shown in abscissa are the number of patients at risk in each group at each timepoint. Analysis was by intention-to-treat.

Figure 2  Differential kinetics of 24-h proteinuria decrease in patients with a good and poor long term renal outcome. Data are shown at baseline and after 3, 6 and 12 months of treatment for patients with good long term renal outcome (serum creatinine ≤120% of baseline value; n=83) or poor long term renal outcome (serum creatinine >120% of baseline value; n=21). P values indicated above the columns were calculated by Mann-Whitney tests.
Clinical effects of new rheumatoid arthritis therapies

P. Durez, M.S. Stoenoiu, L. Meric de Bellefon, A. Avramovska, B.R. Lauwerys, F.A. Houssiau

Biologic therapies that target inflammatory cytokines such as TNF or IL6 have greatly improved the treatment of rheumatoid arthritis (RA). These agents are essential in severe and refractory RA but their initiation could be an option in selected patients with early RA. However, there is no clear consensus on when biologic therapy should be introduced in the course of disease. When a biologic DMARD is added early to methotrexate (MTX) therapy, significantly greater improvements in disease activity status, rate of remission and radiographic outcomes are observed as compared to MTX monotherapy. 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. In 2014, we have participated in 6 sponsored phase II to III clinical trials aiming to test the efficacy of biologic agents such as anti-IL6R antibodies, TNF Kinoïd and JAK inhibitors (3, 14-21).

Thanks to our large recruitment of RA patients, two academic protocols in early and refractory RA are ongoing and two others will start to include patients in 2015. Among them, we have implemented a comparative head-to-head treatment with Tocilizumab (anti-IL6R antibodies) or Rituximab (anti-CD20 antibodies) in severe RA patients refractory to TNF-blocking agents (collaboration with C. Pitzalis, UK)
The main objective of this study is to identify gene expression biomarkers from the synovial tissue that can predict the response to the treatment. We had also the opportunity to develop a medical research program in which young patients with early RA are included in a prospective cohort and followed for five years (collaborative project with ULg and ULB, supported by CAP48 and the RTBF). The clinical information collected on the activity of the disease, joint destruction, and functional capacity will serve as a basis for the development of new molecular biomarkers.

Translational research in rheumatology


Our access to biological samples from a large number of patients recruited at the Lupus and the Rheumatoid arthritis clinics, gives us opportunities to perform biomarker and mechanistic studies in these complex fields, in order to optimize medical decisions and personalized therapeutic approaches. In the field of LN, we confirmed the diagnostic value of serum soluble Interleukin-7 Receptor (sIL7R) concentrations as a marker of a LN flare. In LN, sIL7R is exclusively produced in the kidney, upon stimulation by pro-inflammatory cytokines, and is therefore a sensitive and specific marker of renal disease activity, potentially useful in doubtful diagnostic situations (6).

Kidney biopsies (together with other biological samples) from patients with SLE are also used in a large Innovative Medical Initiatives-funded European project (PRECISESADS) dedicated to the development of a new taxonomy of systemic autoimmune disorders. The aim of this ambitious project is to define diseases based on molecular mechanisms (which can be targeted by specific therapies, in particular biologics), rather than clinical or biological categories that overlap many of these disorders. Finally, evidence indicates that the lupus kidney is not just a target of autoimmunity in SLE, but is involved in the systemic autoimmune response through the activation of specific T and B cells in tertiary lymphoid structures. In particular, we performed high-throughput transcriptomic studies in the blood of LN patients before and after initiation of immunosuppressive therapy, and found that the presence of a CD8 T cell signature is strongly associated with the development of kidney failure. This observation is at the basis of a new PhD project in the laboratory, aiming at the isolation and characterization of renal CD8 T cells from SLE nephritis patients. This project is supported by Cap48.

In the field of RA, we use synovial biopsies in order to detect molecular patterns associated with disease activity, and the effects of therapies.
We usually obtain synovial biopsies through a conventional needle-arthroscopy procedure, but in 2014, we also mastered a new minimally invasive ultrasound-guided biopsy procedure, making possible to obtain material from small metacarpophalangeal joints as well. Using synovial biopsy material, we demonstrated that many therapies used in RA (tocilizumab, an anti-IL6R antibody, but also Rituximab, a depleting anti-CD20 antibody, or even methotrexate, a first line disease-modifying anti-rheumatic drug) target IL6-dependent T cell activation pathways in the synovium (Fig. 3), at the exception of TNF blockers, which rather inhibit cell proliferation and innate inflammation. These observations are an important step forward in the development of individualized therapeutic decisions in RA patients (7). In this context, we are participating in a large international OMERACT (outcome measures in rheumatoid arthritis clinical trials) initiative, aiming at the validation of synovial biopsy markers as surrogates of disease activity and response to therapy. We also recently joined R4-RA, an academic multicentric protocol which intends to validate the expression of specific synovial molecules as predictive markers of response to Rituximab versus Tocilizumab therapy in RA (see above).

We found that the expression of specific molecular pathways in the synovium is diseasespecific (8), and we used this observation to support the development of the Rheumakit, a microfluidics multiplex qPCR now marketed by DNAlytics (Louvain-La-Neuve) for the diagnosis of undifferentiated arthritis (www.rheumakit.com). We recently found that disease activity is driven by the expression of TNFα- and IL6-dependent pathways in the synovium. By contrast, disease severity, and overall response to therapy, is strongly influenced by TNFα-driven inflammation and activation of synovial cells. We confirmed by immunohistochemistry in two independent cohorts of patients that over-expression of TNFα-dependent molecules in the synovium is associated with higher disease severity and poor therapeutic outcomes.

Taken together, these observations illustrate how the development of a new molecular approach of diseases influences medical decisions in rheumatology.

Figure 3 Genes differentially expressed before (T0) and 12 weeks after initiation of tocilizumab therapy (T12) in synovial biopsies from 12 previously untreated RA patients. Pathway analyses indicate that tocilizumab preferentially down-regulates chemokines (A), transcripts involved in T cell activation (B), and up-regulates transcripts involved in healing processes.
**SELECTED PUBLICATIONS**

**FIRST AND LAST AUTHOR PUBLICATIONS**


**CO-AUTHOR PUBLICATIONS**


CENTRE FOR APPLIED MOLECULAR TECHNOLOGIES (CTMA)

CUSL : Cliniques universitaires Saint-Luc,
MoD : Belgian Ministry of Defense
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 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.

2. MISSIONS

- 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/CTMA-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.

Figure 1 CTMA/DLD-Bio Organization Architecture

Figure 2 Missions - multidisciplinary support to the biological spectrum of activities
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 1 presents on-going doctoral thesis works where Prof Jean-Luc GALA is promotor or co-promotor.

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<th>Promotor</th>
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Table 1 : On-going doctoral thesis at 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 DECACCHIE
Cooperation: Department of Epidemiology and Hygiene (Belgium Ministry of Health), Military Medical Academy (Sofia, Bulgaria), Spitalul Clinic de Urgenta (Bucharest, Romania).

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.

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 expanding 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.

Figure 5 : Result from the multiplex PCR multiplex

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.

HFM14/8 - Novel multiplex method for identification of genetically modified or acquired bacterial resistance mechanisms

(2014-2018) (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 purpose of this new study is to integrate the different tests created and validated during the previous studies (MED-04 and MED-20) in a multiplex test single, simple, rapid and sensitive. This test will be adapted to the clinical samples (hospital use or in an operational setting) and environmental (intentional dispersion or accidental biological agents in infrastructure). It will allow to clarify the priori antibiotics ineffective or inefficient panel in a therapeutic setting.

This project targets 2 goals:

The first one is the identification of bioterrorism bacteria and the bacteria responsible for nosocomial infections (clinical samples). The result of this research will be applicable to the medical sector (e.g. bacteria EBLN) and the operating environment. For clinical samples, the objective will be to establish the respective detection limits of tests on real biological samples and to adapt the test conditions accordingly.
For the fight against bio-terrorism, the aim is to develop a protocol for identifying fast, reliable and operational resistance markers of the bioterrorism-related infectious agents of class III (B. anthracis, Y. Pestis, F. tularensis, B. melitensis et B. Mallei). The objective is to transfer the tests validated clinical strains from class II to class III strains: gene sequences used in valid tests will be compared to the new target strains sequences and tests will be adapted and validated on basis of DNA extracted or inactivated cultures. The second one aims to develop a new methodology called “multiplex pyrosequencing”. Several successive parameters will be tested, compared and validated in order to optimize the quality of the signals of pyrosequencing obtained: the ratio of various products of differential gene amplification, order of dispensation of the nucleotide and the quantity of each pyrosequencing primer, the amount of DNA necessary for amplification... These signals will be then handled by a bio-informatics software which has been developed within the CTMA and which allows to break a global signal of pyrosequencing in each of its components, each component corresponding to a particular target sequence.

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

**B-LiFE- Biological Light Fieldable Laboratory for Emergencies – Phase II / Demonstration Phase/ESA IAP/ARTES2**

Nicolas DUBOIS, Jean-Luc GALA, Jean-Paul MARCEL, Leonid IRENGE, Olga VYBORNOVA
Consortium: CTMA (Coordinator), Aurea Imaging (Belgium), nazka mapps (Belgium), SES TechCom (Bezdorf – Grand Duchy of Luxemburg)
Phase II / Demonstration Phase aims at delivering a demonstrator at the highest Technology Readiness Level (TRL 9).

The successful management of sanitary crises such as CBRN threats, life threatening emerging diseases, outbreaks in remote areas, relies on the ability to perform rapid detection and identification of pathogens. National and international agencies dealing with the response to bio-security crises will need mobile laboratory capacities rapidly deployed close to the crisis area, autonomous and transmission and geo-location capabilities.

The B-LiFE project motivation is to bring the diagnostic capacity as close as possible to the crisis area, thus providing an essential element of the fast response. The B-LiFE project is adding to the bio-laboratory a set of space technologies and functions improving considerably the quality of the offered services (See Figure 6): satellite telecommunications to communicate with the distant reach back home base laboratory, stakeholders and end users, GNSS (Global Navigation Satellite System) for geo-location and Earth Observation for site selection and monitoring.

**Figure 6 : B-LiFE – Integration of Space Assets to a Biological Lab**

The proposed B-LiFE system will deliver its services to the end-user based on geographical distant units (see Figure 7): the light mobile field laboratory B-LiFE on one side and various local and distant command and control centers on the other, representing the backend of the applications and services connecting on the one hand to additional medical / biological expertise and on the other hand to local/regional/national emergency response authorities.

The demonstration phase methodology aims to develop and/or integrate stepwise each sub-system of the B-LiFE system in order to reach at the end of the process a full validated demonstrator at a maturity level TRL (technology readiness Level) 9. Step-wise validation against the specified B-LiFE performance requirements will be applied during the Pre-operational Pilots on the field in Democratic Republic of Congo. The Pre-operational Pilots will allow to demonstrate that integration of three categories of space assets (satellite communication, satellite navigation and EO/GIS) to a laboratory platform will result in a highly performant field capacity for rapid assessment of bio-threats anywhere in the world.
The main tasks of Phase II will focus on development/integration of satellite communication and navigation tools, integration of laboratory and mission management software into communication systems for interoperability purposes, operational site selection and monitoring, optional UAVs, development of inactivation system for biological samples, possible transfer and integration of technologies developed for space applications for power supply, portable glovebox and reduction of cold chain dependency.

This Ebola mission is a new task (first addendum to the B-LiFE Phase II contract (CCN#1)), performed in parallel with the planned activities in the B-LiFE Demonstration Project. The CCN#1 provides the opportunity of a precursor deployment in the frame of which user needs and requirements will be gathered and consolidated in a realistic scenarios.

B-LiFE- Biological Light Fieldable Laboratory for Emergencies
– Ebola Mission at N’Zerekore (Guinea Conakry)
Phase II – CCN#1 /Demonstration Phase/ESA IAP/ARTEST20
Jean-Luc GALA, Mostafa BENTAHIR, Elodie CARLIER, Yann DECCACHE, Catherine DUMONT, Jean-François DURANT, Leonid IRENGE, Jean-Paul MARCEL, Anne-Sophie PIETTE, Nora TOUFIK, Stéphane VAN CAUWENBERGHE

The B-LiFE consortium [CTMA (Coordinator), Aurea Imaging (Belgium), nazka mapps (Belgium), SES TechCom (Bezdorf – Grand Duchy of Luxemburg)] is operating in cooperation with B-FAST and emergency. In order to support the French NGO ALIMA

Following international request assistance and approval of the Belgian government authorities, the B-LiFE laboratory is deployed since 20 December 2014 in Guinea (NZERE KORE) as part of the huma-
In addition to its rapid diagnostic capacity of Ebola virus, it also features premium satellite communication capabilities provided by the Grand Duchy of Luxembourg Government emergency.lu that enable secure communications at very high speed to Belgian and international operational centers. It also has an epidemiological mapping capability of the disease through its collaboration with the European Space Agency, the European Commission (DG ECHO and ERCC) and COPERNICUS.

ARCHIMEDES is an integrating project, bringing together 6 European cities to address problems and opportunities for creating environmentally sustainable, safe and energy efficient transport systems in medium sized urban areas. The objective of ARCHIMEDES is to introduce innovative, integrated and ambitious strategies for clean, energy-efficient, sustainable urban transport to achieve significant impacts in the policy fields of energy, transport, and environmental sustainability. CTMA is leader of WP1-Innovation Management Practices, contributes to WP2-Operational Needs and Innovation Uptake and organizes and/or animates roundtables.

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.

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

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.

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 Glosnja Slubzy Pozarniczzej 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 EV (Germany), Hotzone Solutions BENELUX (HZS) (The Netherlands), Agenzia Nazionale per le Nuove Tecnologie, L’ENERGIA - ENEA (Italy), Société Nuclétudes (NUC) (France), Omnidata (OMNI) (Romania), Universidaio del Pais Vasco UPV/EHU (Spain), University of Reading (URED) (United Kingdom), Bruker Daltonics (BRU) (United Kingdom), Ldiaron (Estonia), Microfluidic Chipshop (Germany), Robert Koch Institut (RKI) (Germany), European Virtual Institute for Integrated Risk Management (EU-VRi) (Germany), Centrum Badan Kosmicznych Polskiej Akademi Nauk (Poland), Asociacion de Investigacion de la Industria Agroalimentaria (AINIA) (Spain), Universita Cattolica del Sacro Cuore (UCSC) (Italy), Umea University (UMU) (Sweden)

Figure 11 PRACTICE Toolbox

MIRACLE – Mobile Laboratory for the Rapid Assessment of CBRN Threats Located within and outside the EU

Pierre-Alain FONTEYNE, Olga VYBORNOVA
Consortium: Astrium-SAS-AST (France), Bundes-ministerium der Verteidigung–IMB (Germany), Forsvarets forskningsinstitutt (FFI) (Norway), Totalförsvarets forskningsinstitut (FOI) (Sweden), Netherlands Forensic Institute (NFI) (The Netherlands), Public Health Agency Canada (PHAC) (Canada), Police Service of Northern Ireland (PSNI) (Ireland), Institute for Public Health and the Environment (RIVM) (The Netherlands)
EDEN aims at demonstrating the added value of a Light Fieldable Biology Laboratory (LBFL) for the response to specific B threat scenarios. The LFBL 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.

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**Figure 12**: The Toolbox provides a common gate-way for CBRNE service providers and users to easily access data and added value products used and developed during the project (Communication, Track & Trace, Situation Awareness, Modeling, Procedures, Protocols, Guidelines...)

**Figure 13**: Architecture overview of Data Service Platform
PANDEM - Pandemic Risk and Emergency Management

Accepted in December 2014 to start in 2015 (2015-2016) (Total consortium: 1.410 k€ / CTMA and CRED: 188 k€)
Anne-Sophie PIETTE
Consortium: Coordinator - National University of Ireland Galway (NUIG), Intelligence & Science Applications (ISA), IGS Consulting, London School of Hygiene and Tropical Medicine (LSHTM), Public Health Agency of Sweden (FOHM), Swedish Defence Research Agency (FOI), UCL/CTMA, World Health Organization/EURO (WHO/EURO)

The European Union (EU) faces a growing health security threat posed by pandemics due to the convergence of risk factors driving disease emergence, amplification and dissemination of pathogens with pandemic potential. Protecting the health and security of citizens in the EU in the face of these pandemic threats requires a coherent response by all stakeholders driven by effective pandemic risk management. PANDEM aim is to contribute to the reduction in the health, socio-economic and security consequences of future pandemics so that society will be better prepared at regional, national, EU and global level. PANDEM will assess current pandemic preparedness and response tools, systems and practice at national, EU and global level in priority areas including risk assessment and surveillance, communication and public information, governance and legal frameworks. PANDEM aims to identify gaps and improvement needs leading to the development of viable innovative concepts and analysis of the feasibility of a future demonstration project to strengthen capacity-building for pandemic risk management in the EU.

PANDEM specifically addresses the needs and priorities detailed in the Horizon 2020 Work Programme crisis management topic DRS-4. PANDEM will focus on the needs and requirements of users and first responders across the spectrum of pandemic risk management. PANDEM will bring together highly skilled and multi-disciplinary senior experts from the health, security, defence, microbiology, communications, information technology and emergency management fields. Given the cross-border and multi-sectoral context of the health and security challenge for building pandemic risk management capacity, a systems-based methodology will be applied and the final outcome will be developed for use in a pan-European setting.
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).

(2012-2014) (Total consortium: 1.200 k€ / CTMA: 200 k€)
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 15: 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 Quimica 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)

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.

EBLN – European Biodefense Laboratory Network

(On going activity since 2008) Leonid IRENGE, Anne-Sophie PIETTE, Mostafa BENTAHIR, 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

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.

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

(2014 – 2016) (Total consortium : 1,000 k€ / CTMA : 180 k€)
Mostafa BENTAHIR, Olga MINEEEVA
Consortium: UCL TELE, CTMA, MULTITEL, SIR-RIS, 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.
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.

**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.

*Figure 17: Conidia of Alternaria alternata (standard light microscopy, x 400)*
PUBLICATIONS

Publications 2015 :


2 | Vybornova O, PA Fonteyne, Gala JL. Ontology-Based Knowledge Representation and Information Management in a Biological Light Fieldable Laboratory. 12th International Conference on Information Systems for Crisis Response and Management. May 24-27, 2015, Kristiansand, Norway.


Publications 2014 :


Selection over the last 4 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

Figure 17: CTMA mobile laboratory B-LiFE