



# **ACTIVITY REPORT 2009**





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#### **ADMINISTRATION BOARD**

## Members of the Administration Board:

Mr Frank GANSEN President

Dr François SCHNEIDER Vice-President

Mr Serge HOFFMANN Treasurer

Dr André KERSCHEN Secretary

Mr Pierre DECKER Observer

Prof. Jules HOFFMANN Member

Mr Jean-Paul JUCHEM Member

Dr Marc GRAAS Member

Mr Mario GROTZ Member

Dr Michel NATHAN Member

Mr René ZIMMER Member

Dr Jean-Louis ROBERT Expert

## The Chairman's message

Dear reader.

The 2009 annual report of the research activities at the "Centre de Recherche de la Santé", (CRP-Santé), is now available and we are delighted to present here the activities carried out during this year which was a very fruitful one for our research entities

I would like to express my deepest gratitude to all members of CRP-Santé for having contributed actively with success to attempt one of our main objectives which is the promotion and recognition of excellence in European research.

Many initiatives with national and international partners have been set up to increase the visibility and attractiveness of the research of CRP-Santé and thus in Luxembourg. The aim is indeed to highlight the personal achievements of local researchers with a view to supporting their further development and international recognition.

One of the overall targets of CRP-Santé is to create transnational research teams led by researchers who have the potential to reach international excellence in some particular scientific fields clearly defined by its research strategy.

A strong network of collaboration with international research teams who are working on cutting-edge and interdisciplinary research in other countries and especially in the United States is being built. We are looking forward to developing transnational and transatlantic collaborations with all organizations and companies who have already contributed to our research and also to many further fruitful relationships during the coming years. All the projects and activities fit into the broader key research area of biomedical and clinical research of CRP-Santé.

In order to face the main public health issues, the CRP-Santé will also coordinate and organize quality research in health with the clearly identified goal to improve medical treatment and to develop knowledge, to improve health and health equity at a population level. CRP-Santé will develop a high quality public health research in association with the research community in order to improve the health and wellbeing of the users of the health systems. Public health research is fundamental to evidencebased decision making, leading to the development of excellence in policy, practice and public health management according to the main guiding principles of public health research programs which are focusing on innovation and high quality for underpinning effective strategic research and enabling national and international competitiveness.

In the near future we will also have to challenge the impact of the international financial and economic crisis. Even if our Government recently confirmed its commitment to consider public research as being one of the priorities of its policy, the impact of the crisis is likely to diminish or make more difficult access to finance for the public research centres.

As CRP-Santé has been emphasizing during the last years with success the need for reinforcing and developing excellence, efficiency and competitiveness, we continue to have many reasons to stay confident that we are going to reach our ambitious objectives. As a conclusion, the CRP-Santé is ready to respond to international scientific standards required in a more difficult environment.

THE CHAIRMAN
FRANK GANSEN



## THE MANAGEMENT BOARD'S MESSAGE

The year 2009 was the second year of performance contract with government. Again, we were able to meet most of our ambitious targets. Of special note is CRP-Santé's outstanding performance in peer-reviewed scientific publications, the most appropriated indicator of scientific quality. In only two years time. CRP-Santé was able to increase the average impact factor of its publications by 124%, from a modest 2.71 to an excellent 6.07, while at the same time augmenting the number of peer-reviewed publications by 56%. By doing so, CRP-Santé has clearly reached one of the major goals from its 2006 vision statement, i.e. "becoming by 2010 an internationally recognized biomedical research organization". It highlights also again the primary focus of CRP-Santé: excellence in scientific research. Even if we recognize that deriving economical or, more generally, societal value from research is increasingly important and that our researchers should be actively involved in the "value adding" process, we believe that successful valorization is mainly driven by doing high quality scientific research first.

In 2009 CRP-Santé made major efforts to initiate an economical valorization process of its research results. Besides the constant involvement of its senior leadership in public efforts towards valorization (e.g. Luxembourg BioHealth Cluster, the government-driven Luxembourg Biotechnology Initiative and multiple transnational projects (Interreg) in the Greater Region), CRP-Santé has signed a collaboration agreement with Luxinnovation and significantly extended its relationship with the private venture capitalist Vesalius Biocapital. The recruitment of a Technology Transfer Officer, who will further build on this promising publicprivate partnership, was a logical next step we have taken recently. We are confident that these combined efforts will result in tangible economic outcomes in the short term. In addition, CRP-Santé has created value for the Luxembourg society over the last years by building IT-based disease registries, by evaluating the Luxembourg health offer and by contributing to the launch of the "Portail Santé" in 2009.

2009 was also the year of internal communication. Our Communication Manager started with an extensive audit involving about half of our personnel in order to understand needs and perceptions. Based on this survey, we defined our amended internal communication strategy. A major tangible outcome is a complete overhaul of our intranet which will be accomplished during spring 2010. CRP-Santé made also meaningful progress in the implementation of the "European Charter for Researchers" in 2009. We completed our internal analysis process as recommended by the European Union. The resulting strategy document has been reviewed by our board and published on our website. Our efforts were recently rewarded by the attribution of the recognition "HR Excellence in Research" by European authorities.

As a learning organization which emphasizes flexibility as a way to adapt to changing conditions, CRP-Santé has improved is internal structure in 2009. It has created two new competence centers to be implemented in 2010, one on "Methodology & Statistics", and a second on advanced, high value adding technologies, called "Luxembourg Biomedical Research Resources (LBR2)". Methodology & Statistics is a key capability already partially implemented at CRP-Santé. Developing it now as a separate competence center to be headed by an expert senior scientist, will allow an improved service offer for internal and external clients. The new LBR2 technology center, focusing on human expertise and cuttingedge competences while relying on state-of-the-art equipment, will bring together key technological capabilities currently distributed across several research departments, allowing for the deployment of a coordinated strategy. The technology center will also include the new Luxembourg Clinical Proteomics (LCP) initiative headed by Bruno Domon, who joined us recently from the ETH in Zurich, attracted by excellent research opportunities in Luxembourg and encouraged by a FNR PEARL grant. Clinical Proteomics, aiming at eventually introducing a promising technology into clinics and thus being a key enabler of

personalized medicine, will leverage already existing efforts in proteomics in Luxembourg. In addition, LCP will support the Luxembourg Center for Systems BioMedicine in its endeavor to implement a metabolomics program. Bruno Domon has been appointed as a quest professor at the University of Luxembourg in the framework of the master of systems biology training. The two new competence centers at CRP-Santé complement the initial service offer by our first competence center, the Center for Clinical and Epidemiological Investigations (CIEC), already created in 2008.

Another important development is the implementation of the Laboratory of Research in Sports Medicine, headed by Daniel Theisen in collaboration with clinicians from a major hospital. The inception of this research unit was based on a perceived need to evaluate, and in a second step to control, the risk factors for injuries in people practicing sports and to address the challenge of preserving physical mobility in an aging population.

CRP-Santé has extended its national and international collaborations in 2009. This includes an intense collaboration with the Integrated Biobank of Luxembourg (IBBL) on biological sample collection, an enhanced coordination with the University of Luxembourg on scientific projects and training and an improved networking with hospitals for clinical research. Internationally, CRP-Santé aimed at leveraging collaborations, mainly in the fields of oncology and health economics, with the TGen Foundation in Phoenix, Arizona and the Fred Hutchinson Cancer Research Center and the Institute for Systems Biology, both in Seattle, Washington.

Finally, it is worth to highlight that CRP-Santé's management has proactively initiated a major strategic reflection process in preparation of the next performance contract covering the period 2011 to 2013. We are looking forward to the upcoming negotiations with government representatives to define the limits of an even more challenging second performance contract.

#### THE MANAGEMENT BOARD

DANIEL CARDAO

JEAN-CLAUDE SCHMIT

MARIE-LISE LAIR



#### SCIENTIFIC ADVISORY BOARD

2009 Composition

Professor Jules Hoffmann CNRS, Former President of the French Academy of Sciences Strasbourg, France

Professor Anders Soennerborg Karolinska Institute Stockholm, Sweden

Professor Hans Maurer Saarland University Homburg, Germany

Professor Jean-François Bach Académie des Sciences de France Paris, France

Professor Vladimir Benes European Molecular Biology Laboratory (EMBL) Heidelberg, Germany

Professor Dirk Inzé University of Ghent and VIB Ghent, Belgium

Professor Bernard Swynghedauw Hôpital Lariboisière Paris, France

Professor Alain-Jacques Valleron Hôpital Saint Antoine Paris, France

Professor Nikolaus Müller-Lantzsch Saarland University Homburg, Germany

Professor Eric Van den Neste Cliniques Universitaires Saint Luc, Université de Louvain Brussels, Belgium

Professor Kurt Blaser Swiss Institute of Allergy and Asthma Research, Davos, Switzerland

Professor Gaetano Finocchiaro National Neurological Institute "Carlo Besta", Milano, Italy



#### **PROJECT REVIEWERS**

CRP-Santé is grateful to all external project reviewers who helped to evaluate the scientific quality of our work.

Name	Organisation	Country
AKDIS CZEMI A.	SWISS INSTITUTE OF ALLERGY AND ASTHMA RESEARCH - DAVOS	Switzerland
ALBERT Jan	KI DEPT. OF MICROBIOLOGY, TUMOR AND CELL BIOLOGY - STOCKHOLM	Sweden
ARENZANA-SEISDEDOS Fernando	INSERM INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE - PARIS	France
BACH Jean-François	ACADEMIE DES SCIENCES DE FRANCE - PARIS	France
BALLING Rudi	UNIVERSITE DU LUXEMBOURG	Luxembourg
BENFENATI Emilio	ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" - MILAN	Italy
BIEBER Pascal	INSTITUT NATIONAL DE CHIRURGIE CARDIAQUE ET DE CARDIOLOGIE INTERVENTIONNELLE - LUXEMBOURG	Luxembourg
BISDORFF Alexandre	CENTRE HOSPITALIER EMILE MAYRISCH - ESCH/ALZETTE	Luxembourg
BLASER Kurt	SWISS INSTITUTE OF ALLERGY AND ASTHMA RESEARCH - DAVOS	Switzerland
BOHLE Barbara	MEDICAL UNIVERSITY OF VIENNA - VIENNA GENERAL HOSPITALI, AKH	Austria
BÖHM Michael	KLINIK FUR INNER MEDIZIN III - HOMBURG	Germany
BOUCHARD Stéphane	UNIVERSITE DU QUEBEC EN OUTAOUAIS - GATINEAU	Canada
BRON Dominique	INSTITUT JULES BORDET - BRUXELLES	Belgium
CAPRON Monique	INSTITUT PASTEUR - LILLE	France
CARLBERG Carsten	UNIVERSITE DU LUXEMBOURG	Luxembourg
CHARPIOT Anne	HOPITAUX UNIVERSITAIRES DE STRASBOURG	France
DADOUN Frédéric	CENTRE HOSPITALIER UNIVERSITAIRE NORD MARSEILLE	France
DECKMYN Hans	LABORATORY FOR THROMBOSIS RESEARCH - KORTRIJK	Belgium
DEMEULEMEESTER René	INPES - PARIS	France
DUNLOP Thomas	UNIVERSITY OF KUOPIO	Finland
EHRLICH Joachim	KLINIKUM DER GOETHE UNIVERSITAET FRANKFURT AM MAIN	Germany
FERREIRA Fatima	UNIVERSITY OF SALZBURG	Austria
FISZMAN Marc	HOPITAL PITIÉ-SALPETRIERE - PARIS	France
GÄRTNER Barbara	UNIV SAAR - INSTITUT FUR VIROLOGIE - HOMBURG	Germany
GLUKHOVA Marina	INSTITUT CURIE SECTION DE RECHERCHE - PARIS	France
GOUBAU Patrick	CLINIQUES UNIVERSITAIRES SAINT LUC - BRUXELLES	Belgium
GRESTY Michael	IMPERIAL COLLEGE OF SCIENCE AND MEDICINE - LONDON	United Kingdom
GRIFFIOEN Arjan W.	UNIVERSITY HOSPITAL MAASTRICHT	Netherlands
INZE Dirk	VIB - DEPARTMENT OF PLANT SYSTEMS BIOLOGY - GENT	Belgium
JANDA Kim D.	THE SCRIPPS RESEARCH INSTITUTE - LA JOLLA	United States of America
JOSEPHSON Filip	KI DEPT. OF CLINICAL PHARMACOLOGY - STOCKHOLM	Sweden
JOUVENT Roland	HOPITAL PITIÉ-SALPETRIERE - PARIS	France
KAUFMANN Gunnar	THE SCRIPPS RESEARCH INSTITUTE - LA JOLLA	United States of America
KIRSCH Monique	MUSEE NATIONAL D'HISTOIRE NATURELLE - LUXEMBOURG	Luxembourg
KLEINE-TEBBE Jörg	ALLERGIE-UND ASTHMA-ZENTRUM WESTEND - BERLIN	Germany
KLYS Malgorzata	INSTITUTE OF FORENSIC MEDICINE JAGIELLONIA UNIVERSITY - KRAKOW	Poland
LOPATA Andreas	RMIT UNIVERSITY - MELBOURNE	Australia
LOSCH Serge	ADMINISTRATION DES SERVICES VETERINAIRES - LUXEMBOURG	Luxembourg
MACGOWAN Guy A.	FREEMAN HOSPITAL - NEWCASTLE UPON TYNE	United Kingdom
MALMBERG Kalle	KAROLINSKA INSTITUTET - STOCKHOLM	Sweden
MAURER Hans	UNIV SAAR - INSTITUT FUR EXPERIMENTELLE UND KLINISCHE PHARMAKOLOGIE UND TOXIKOLOGIE - HOMBURG	Germany
MEYER Jobst	UNIVERSITÄT TRIER	Germany

Name	Organisation	Country
MOELLER Manfred	UNIV SAAR - INSTITUT FUR EXPERIMENTELLE UND KLINISCHE PHARMAKOLOGIE UND TOXIKOLOGIE - HOMBURG	Germany
MOUTSCHEN Michel	UNIVERSITÉ DE LIÈGE	Belgium
MUENZ Christian	INSTITUTE OF EXPERIMENTAL IMMUNOLOGY - ZÜRICH	Switzerland
MÜLLER Ulrich R.	SPITAL BERN ZIEGLER - BERN	Switzerland
MÜLLER-LANTZSCH Nikolaus	UNIV SAAR - INSTITUT FUR VIROLOGIE - HOMBURG	Germany
NIELSEN Lars P.	STATENS SERUM INSTITUT - COPENHAGEN	Denmark
PANAGIOTAKOS Demosthenes B.	HAROKOPIO UNIVERSITY OF ATHENS	Greece
PANCINO Gianfranco	INSTITUT PASTEUR - PARIS	France
PAULI Gabrielle	HOPITAUX UNIVERSITAIRES DE STRASBOURG	France
PECHNICK Robert	CEDARS-SINAI MEDICAL CENTER - LOS ANGELES	United States of America
PEYTAVIN Gilles	HOPITAL BICHAT - PARIS	France
RINGELSTEIN E.B.	UNIVERSITAETSKLINIKUM MUENSTER	Germany
ROSSIGNOL Patrick	CENTRE D'INVESTIGATION CLINIQUE NANCY	France
RYCHEN Guido	ENSAIA - ECOLE NATIONALE SUPERIEURE D'AGRONOMIE ET DES INDUSTRIES ALIMENTAIRES - VANDOEUVRE LES NANCY	France
SINSKI Edward	INSTITUTE OF ZOOLOGY - WARSAW	Poland
SITKOVSKY Michail	NORTHEASTERN UNIVERSITY - BOSTON	United States of America
SÖNNERBORG Anders	KAROLINSKA INSTITUTET - STOCKHOLM	Sweden
STAUB Christian	INSTITUT UNIVERSITAIRE DE MEDECINE LEGALE - GENÈVE	Switzerland
SWOBODA Ines	MEDICAL UNIVERSITY OF VIENNA - VIENNA GENERAL HOSPITALI, AKH - VIENNA	Austria
SWYNGHEDAUW Bernard	HÔPITAL LARIBOISIERE - PARIS	France
TELENTI Amato	INSTITUT DE MICROBIOLOGIE IMUL - LAUSANNE	Switzerland
TENNANT Alan	LEEDS INSTITUTE OF MOLECULAR MEDICINE - LEEDS	United Kingdom
VALLERON Alain	INSERM UNITE DE SANTE PUBLIQUE - PARIS	France
VAN DEN NESTE Eric	CLINIQUES UNIVERSITAIRES SAINT LUC - BRUXELLES	Belgium
VAN RIET Ivan	VRIJE UNIVERSITEIT BRUSSEL	Belgium
VAN TROYS Marleen	GHENT UNIVERSITY - FAC. GENEESKUNDE	Belgium
VIVIER Eric	CENTRE D'IMMUNOLOGIE INSERM-CNRS UNIVERSITE DE MARSEILLE LUMINY	France
WEHLAND Jürgen	GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG mbH - HELMHOLTZ CENTRE FOR INFECTION - BRAUNSCHWEIG	Germany
WERFEL Thomas	MEDIZINISCHE HOCHSCHULE HANNOVER - MHH	Germany
WESTPHAL Manfred	UNIVERSITAETSKLINIKUM HAMBURG-EPPENDORF	Germany
WITTCHEN Hans-Ulrich	TECHNISCHE UNIVERSITÄT DRESDEN	Germany
WÜST Stefan	UNIVERSITÄT TRIER	Germany
ZHAO Zhongming	VANDERBILT UNIVERSITY MEDICAL CENTER NASHVILLE	United States of America
ZOULIM Fabien	INSERM INSTITUT NATIONAL DE SANTE ET RECHERCHE MEDICALE - LYON	France





DEPARTMENT OF CARDIOVASCULAR DISEASES

#### **HEAD OF LABORATORY**

Dr. Daniel WAGNER MD, PhD

#### ASSOCIATE HEAD OF LABORATORY

Dr. Yvan DEVAUX, PhD

# LABORATORY OF CARDIOVASCULAR RESEARCH



Complete list of collaborators available on www.crp-sante.lu

### OBJECTIVES OF THE LABORATORY OF CARDIOVASCULAR RESEARCH

The number of deaths from cardiovascular pathologies continuously increasing in modern countries. Cardiovascular diseases represent the number one cause of morbidity and mortality in Luxembourg. Despite the benefit achieved by modern techniques such as mechanical reperfusion therapy, the development of heart failure following myocardial infarction still largely contributes to these deaths. Profiting from a tight collaboration with the "Centre Hospitalier de Luxembourg" (CHL), the "Institut National de Chirurgie Cardiague et de Cardiologie Interventionnelle" (INCCI), the team of the Laboratory of Cardiovascular Research of CRP-Santé is pursuing its research activities aiming at understanding the mechanisms responsible for the development of heart failure. Keeping in mind the importance of the discovery of new therapeutic and prognostic targets, the activities of the group tackle the fields of translational medicine and systems biology of the disease.

DEPARTMENT OF CARDIOVASCULAR DISEASES

# LABORATORY OF CARDIOVASCULAR RESEARCH

#### ONGOING PROJECTS

Seven projects were running in the laboratory at the end of 2009, which can be separated in two main research axis: the study of the effects of adenosine on left ventricular remodelling and the development of heart failure (therapeutic approach of the disease), and the identification of new biomarkers of clinical outcome after myocardial infarction (prognostic approach).

#### ADENOSINE AND HEART FAILURE PROJECT

Influence of adenosine on left ventricular remodelling and the development of heart failure post myocardial infarction.

Acronym: ADO-HF

Contract numbers: REC 20060903 (EPC project), REC 20070104 (Fibros project), FNR 20070407 (Running mouse project), REC 20080903 (Angiogenesis project), REC 20090710 (Chemotaxis project).

Grant period: 01/2007 – 06/2012
Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Fonds National de la Recherche (FNR), Luxembourg.

#### Project summary:

Cardioprotective properties adenosine are known but its role in the development of heart failure after myocardial infarction is poorly characterized. It appears adenosine modulates all four main biological processes that set the stage for the development of left ventricular remodelling and heart failure: inflammation, turnover of the extracellular matrix, angiogenesis and cell death. We have designed several research projects that each targets one of these processes. We are now studying the effects of adenosine on the production of collagen by cardiac fibroblasts, on the formation of new blood vessels, or on the recruitment of endothelial progenitors. We are also testing the hypothesis that adenosine may be involved in the protection from heart failure provided by physical activity.



#### People involved:

Benjamin HAAS, Bernadette LENERS, Carine PEREZ, Christelle NICOLAS, Daniel WAGNER, Emilie LIEFFRIG, Frédérique LÉONARD, Isabelle ERNENS, Magali ROLLAND-TURNER, Mélanie BOUSQUENAUD, Mélanie KIRCHMEYER, Sarah-Lena PUHL, Yvan DEVAUX

#### Associated PhD projects:

- 1. Frédérique LEONARD
- Title: Effects of adenosine on the biology of endothelial progenitor cells
- **Start date:** 01/11/2007
- Financial support: AFR grant from Fonds National de la Recherche (FNR).
- 2. Mélanie BOUSQUENAUD
- Title: Effects of adenosine on chemotaxis. Implication for left ventricular remodelling
- Start date: 01/01/2009
- Financial support: AFR grant from Fonds National de la Recherche (FNR).
- 3. Sarah-Lena PUHL
- Title: Exercise training and ventricular remodelling in the

- mouse: effects of adenosine and matrix metalloproteinases
- **Start date:** 01/06/2008
- Financial support: AFR grant from Fonds National de la Recherche (FNR).

#### 2. BIOMARKER PROJECT

Identification of new biomarkers of the development of heart failure after myocardial infarction

Acronym: BIOMARKER

**Contract numbers:** REC 20061201 (LUCKY project), FNR 20070408 (SNP project), FNR 20080306 (Prot'heart project).

Grant period: 01/2007 – 12/2010

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Fonds National de la Recherche (FNR), Luxembourg.

#### Project summary:

Identification of patients at risk of developing heart failure after myocardial infarction is a research priority for our laboratory as heart failure is potentially preventable. Started in 2006, the national myocardial infarction registry now accounts over 500 patients. From this cohort, we have designed several projects to identify potential new

biomarkers of heart failure. Our view of the systems biology motivated us to combine several types of data such as transcriptomic biosignatures of blood cells using whole-genome microarrays, proteomic analysis of plasma proteins, single nucleotide polymorphisms, and very diverse bioinformatics tools like networks of protein-protein interactions, in the final goal to discover new prognostic biomarkers of heart failure.

#### People involved:

Céline JEANTY, Céline YVORRA, Daniel WAGNER, Francisco AZUAJE, Loredana JACOBS, Lu ZHANG, Malou GLOESENER, Mélanie VAUSORT, Sophie RODIUS, Yvan DEVAUX

#### Associated PhD project:

Benjamin HAAS

- Title: Identification of new biomarkers of heart failure by proteomic analysis of plasma proteins
- Start date: 01/11/2008
- Financial support: AFR grant from Fonds National de la Recherche (FNR).

#### **KEY RESULTS**

- I. Through the FNR SNP project, we have designed three potential peptide inhibitors of MMP9 that are currently under testing in our laboratory.
- **II.** An international patent on MMP-9 as a prognostic marker of heart failure has been accepted.
- III. Three patents have been submitted: (1) VEGFB as a prognostic marker of heart failure (US provisional application), (2) Use of an adenosine A2a receptor agonist and A3 receptor antagonist to prevent heart failure after infarction (PCT international phase), and (3) Diagnostic marker and platform for drug design in myocardial infarction and heart failure (PCT international phase).

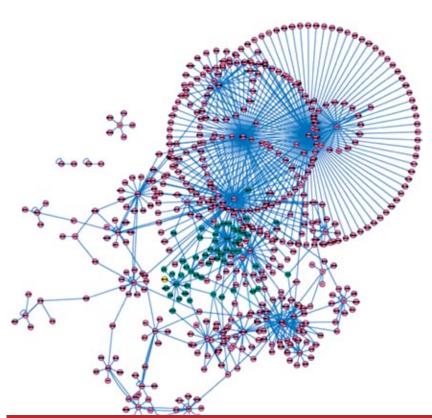
#### **COLLABORATIONS**

#### NATIONAL

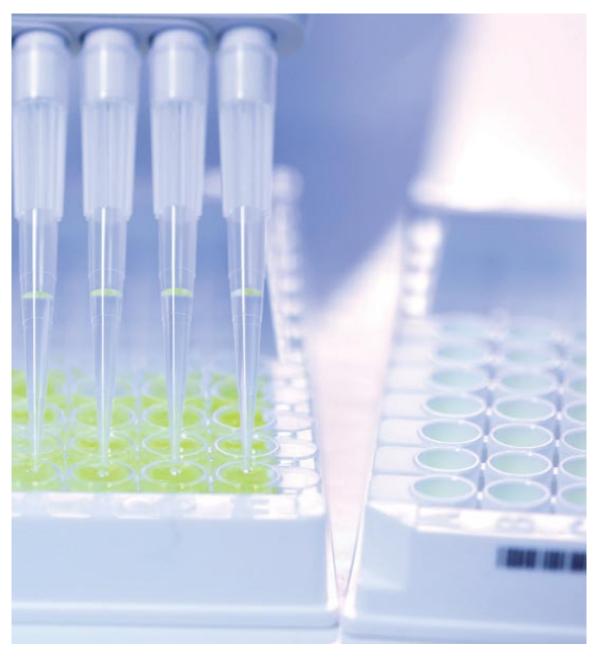
- Centre Hospitalier de Luxembourg (CHL): Département de Cardiologie, Service d'Hémato-Cancérologie, Service de Réanimation Médicale.
- Institut National de Chirurgie Cardiaque et de Cardiologie Interventionnelle (INCCI)
- CRP-Santé: Flow Cytometry: N.H.C. Brons
- In Luxembourg: Department EVA, CRP-Gabriel Lippmann; Laboratoires Réunis, Junglinster.

#### INTERNATIONAL

- Departments of Cardiology and Internal Medicine, University of Homburg/Saar, and University of Cologne, Germany.
- Department of Cardiovascular Sciences, University of Leicester, UK.
- Centre Hospitalier et Universitaire (Departments of Anaesthesia and Intensive Care, Nancyclotep),
   Faculty of Sciences (Laboratory of Crystallography), Nancy, France.
- Department of Internal Medicine,
   Philadelphia Hospital and Jefferson
   University, USA.
- Center for Vascular Biology and Cardiovascular Division,
   Department of Medicine, University of Minnesota, Minneapolis, USA.
- Center for Heart Failure Research and Department of Cardiology, Maastricht, The Netherlands.



Protein-protein interaction network of angiogenesis in human myocardial infarction



#### **2009 PUBLICATIONS**

- 1. Fiona Browne, Haiying Wang, Huiru Zheng and Francisco Azuaje. GRIP: A web-based system for constructing Gold Standard datasets for protein-protein interaction prediction. Source Code for Biology and Medicine 2009,4:2.
- 2. Dan Longrois, Michaela Agavriloai, Yvan Devaux, Paul-Michel Mertes. Comments on methodological problems related to the use of biomarkers in clinical practice and research. Annales Françaises d'Anesthésie et de Réanimation 2009;28:473-481.
- 3. Francisco Azuaje, Yvan Devaux and Daniel Wagner. Computational biology for cardiovascular biomarker discovery. *Briefings in Bioinformatics* 2009;10(4):367-77.
- 4. Francisco Azuaje, Yvan Devaux, and Daniel Wagner. Challenges and Standards in Reporting Diagnostic and Prognostic Biomarker Studies, *Clinical and Translational Science* 2009;2(2):156-161.







#### **HEAD OF LABORATORY**

Dr. Catalina ILIESCU, MD, PhD (until October 2009)

Dr. Manon GANTENBEIN, PhD (interim october 2009 - March 2010)

Dr. Anna CHIOTI, MD (from April 2010)

DEPARTMENT OF CLINICAL AND EPIDEMIOLOGICAL INVESTIGATIONS

# CLINICAL AND EPIDEMIOLOGICAL INVESTIGATION CENTER (CIEC)



Complete list of collaborators available on www.crp-sante.lu

#### **OBJECTIVES OF THE CIEC**

Early September 2009, the CIEC has celebrated its first birthday. On that occasion the first Clinical Research Day has been organized in Luxembourg, bringing together national and international experts in clinical research.

The main objectives of the CIEC are to promote clinical research and to assist and support clinical research projects in Luxembourg. Therefore the CIEC may provide any kind of logistic support to hospitals or physicians interested in clinical trials. The CIEC is also a contact partner for the pharmaceutical industries interested in conducting clinical trials in Luxembourg.

In addition, the CIEC is aiming at developing a national clinical research network including clinicians and physicians in various medical fields in order to react in a timely manner to demands emanating from potential sponsors.

Furthermore the CIEC is an infrastructure intended to prepare and realize, partially or totally, clinical research projects based on patients or healthy volunteers, in the respect of Good Clinical Practice (ICH-GCP) and Quality Assurance. The CIEC is therefore also an opportunity for internal and external research teams to consolidate fundamental and experimental findings by a clinical research study in healthy volunteers or patients.

Finally, the CIEC is committed to:

- Scientific value and authenticity of collected data
- Traceability of its actions
- Security of the patients, respect of the person and confidentiality of patient data

DEPARTMENT OF CLINICAL AND EPIDEMIOLOGICAL INVESTIGATIONS

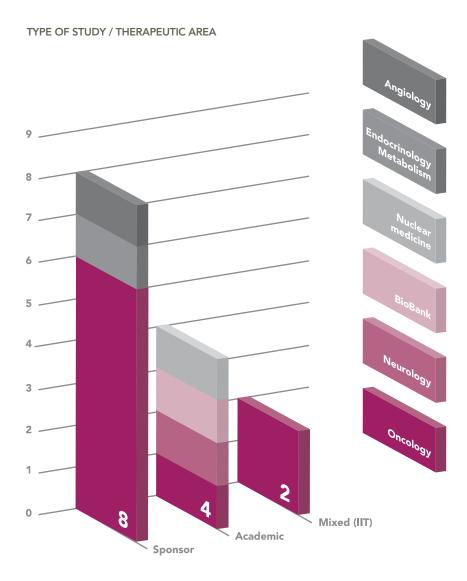
# CLINICAL AND EPIDEMIOLOGICAL INVESTIGATION CENTER (CIEC)

SAFETY OF THE PATIENTS	STUDY CONCEPTION AND PREPARATION	Hypothesis	1 question = 1 clinical research project	
		Study Design	Scientific and medical validity     Methodology     Costs	al months
		Essential Documents	<ul> <li>Protocol</li> <li>Investigator's brochure</li> <li>Patient Information letter and inform consent form</li> <li>Patient questionnaires</li> <li>Case Report Form</li> </ul>	1 to several
		Health Authorities	<ul> <li>Local Health Authorities (Direction de la Santé Division de la Pharmacie et des Médicaments)</li> <li>Ethic Commitee (CNER)</li> <li>CNPD notification / autorisation</li> <li>Insurance</li> </ul>	2 to 3 months
	DURING THE STUDY	Study Logistics	<ul> <li>Premises and staff</li> <li>Recruitment of investigators/sites</li> <li>Management of study drug</li> <li>Management of samples</li> <li>Case report form</li> <li>Create randomization system</li> </ul>	
		Study Conduct	<ul> <li>Clinical Team motivation and support</li> <li>Support of patient recruitment</li> <li>Support during patient visits</li> <li>Monitoring visits</li> </ul>	to several years
		Data Management	Data collection     Confidentiality      Data Validation and Credibility     (assure data is integer, authentic, accurate, contemporaneous, true)	1 tc
		Steering Comitee	Report on study status     Protocol deviations	
	END OF STUDY	Statistical Analysis Results	<ul> <li>Final report</li> <li>Publications</li> <li>Presentations</li> </ul>	2 to 6 months

#### ONGOING PROJECTS

Title	Acronym	Study period	Financial support	Study Status	Patient included vs. committed
A non interventional study to follow and evaluate patients with advanced non-small cell lung cancer (NSCLC) who are treated in a second line setting with Erlotinib in an "real life" clinical setting.	TEAM	August 2008 - December 2010	Sponsor	Ongoing	8 / 10
Randomized multicentric Phase II study of prolonged adjuvant Temozolomide or "stop and go" in glioblastoma patients.	PATSGO	September 2008 - July 2010	Academic	Ongoing	2 patients included
A non-interventional observational post authorisation safety study of subjects treated with lenalidomide.	PASS	April 2009 - April 2014	Sponsor	In preparation	3-5 per site 2 sites planned
Multicenter, randomized, parallel group efficacy and safety study for the prevention of venous thromboembolism in hospitalized medically ill patients comparing rivaroxaban with enoxaparin.	MAGELLAN	March 2009 - June 2010	Sponsor	Ongoing	5 / 16
An open-label expanded access study of Lapatinib and Capecitabine therapy in subjects with ErbB2 over-expressing locally advanced or metastatic breast cancer.	LAPATINIB	September 2008 - June 2010	Sponsor	End of study	23 included
Biomarker discovery and validation in lung cancer.	IBBL 0001	September 2009 - September 2012	Academic	Ongoing	11 / 150
Prospective evaluation of small molecule EGFR-1 tyrosine kinase inhibition as a first-line treatment in patients with advanced non-small cell lung cancer (NSCLC) harbouring a mutant EGFR gene.	FIELT	January 2009 - November 2010	Academic	Ongoing	10 / 10 screened (1 positive mutation included)
A multinational, multicenter, post-authorization, prospective observational cohort study to assess the profile of vildagliptin and the fixed-dose combination of vildagliptin/metformin relative to comparator oral anti-diabetic drugs in patients with type 2 diabetes in a real-world setting.	EDGE	September 2009 - May 2014	Sponsor	Ongoing	200 planned
Current practice of Aranesp in the management of haemoglobin levels: an observational international cancer evaluation.	CHOICE	September 2009 - June 2010	Sponsor	End of study	4 / 10
A phase III, randomized, double-blind, placebo-controlled multicentre study of ASA404 in combination with docetaxel in second-line treatment of patients with advanced or metastasic (stage IIIb/IV) non-small cell lung cancer (NSCLC).	CASA 404	May 2009 - January 2011	Sponsor	In preparation	10 planned
A randomized double-blind placebo-controlled study to evaluate the long-term safety and efficacy of Darbepoetin Alfa administered at 500 ug once every 3 weeks in anaemic subjects with advanced stage non-small cell lung cancer receiving multicycle chemotherapy.	ARANESP 782	January 2010 - January 2017	Sponsor	In preparation	7 patients per site / 2 sites planned
Study of the prevalence and treatment of anaemia (≤ 12 g/dl) in cancer patients treated with chemotherapy and / or radiotherapy or treatment follow-up in the Grand Duchy of Luxemburg.	ANEMIA	June 2009 - June 2010	CRP-Santé via MESR*	Ongoing	57 / 90
Nutrition and physical activity in patients with cerebrovascular disease.	ALVINA	January 2009 - February 2012	CRP-Santé via MESR*	Ongoing	34 / 100
Single center pilot study of the clinical evaluation of the use of 18FNa in the nuclear medicine department in a 2nd diagnostic intention in orthopedic pathologies.	FNa18	January 2009 - March 2012	CRP-Santé via MESR*	In preparation	50-100 patients planned

<sup>\*</sup> Ministère de l'Enseignement Supérieur et de la Recherche



#### **KEY RESULTS**

**I.** 145 patients enrolled in total in 14 trials covering 5 different therapeutic areas.

#### II. 3 ongoing academic trials:

A public health study (nutrition and cardiovascular disease) in collaboration with the Neurology department of the "Centre Hospitalier de Luxembourg" (CHL), counting 27 patients who have been followed up over a period of 4 months.

Furthermore the CIEC is supporting the IBBL (Integrated BioBank of Luxembourg) in a Lung Cancer Study in association with the CHL in means of patients' clinical data as well as tissue, saliva and blood collections. The data and samples of 9 patients have been collected so far.

Finally a single centre radio-diagnostic study using FNa18 in orthopaedic diseases is in preparation in collaboration with CHL.

- **III.** 5 clinical trials sponsored by the pharmaceutical industry were initiated in Luxembourg: 3 oncology studies, 1 cardiovascular and 1 endocrinology trial, all ongoing and recruiting.
- IV. The coordination and logistic of 5 ongoing clinical trials at the CHL was taken over by the CIEC, including 2 Investigator Initiated Trials (IIT) run in collaboration with Belgian University hospitals.
- V. In order to ensure the quality of the clinical trials performed, the CIEC is working according to ICH-GCP and standard operating procedures (SOPs). So far 60% of these SOPs have been compiled and validated.

VI. The CIEC is striving to ensure respect of patient rights and patient data privacy, while offering the opportunity to access new and innovative therapeutic approaches that would not be accessible otherwise. The CIEC personnel is trained and dedicated to offer the best patient information and support all along the clinical trial process.



#### COLLABORATIONS

#### NATIONAL

- SLMG (Société Luxembourgeoise de Médecine Générale)
- CRP Henri Tudor Department CR SANTEC
- Integrated BioBank of Luxembourg (IBBL)
- CIEC collaborates to the MEMOVIE project run by the Centre for Health Studies (CES) and receives support from the methodological platform (CES) within the framework of the ALVINA Nutrition study.

Furthermore 5 major hospitals are actually involved in clinical research projects in Luxembourg via the CIEC:

- Centre Hospitalier du Kirchberg (CHK)
- Centre Hospitalier de Luxembourg (CHL)
- Centre Hospitalier Emile Mayrisch (CHFM)
- Centre Hospitalier du Nord (Hôpital St. Louis, Ettelbruck)
- Zithaklinik

Additionally, the CIEC works in close collaboration with the National Center for Radiotherapy:

■ Centre François Baclesse (CFB)

#### INTERNATIONAL

- Translational Genomics Research Institute (TGen), Phoenix
- Amgen Belgium
- Bayer SA-NV
- Celgene BeLux
- GlaxoSmithKline
- N.V. Novartis Pharma S.A
- NV Roche SA
- Université Catholique de Louvain
   Oncologie Médicale, Centre du Cancer, Cliniques Universitaires
   St. Luc
- Oncologie Médicale Centre d'Oncologie, Hôpital Académique-Université Libre de Bruxelles

CIEC is a member of the European Forum for Good Clinical Practice (EFGCP)

#### 2009 PUBLICATIONS

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## LABORATORY OF IMMUNOLOGY

DEPARTMENT OF IMMUNOLOGY



**HEAD OF LABORATORY**Prof. Claude P. MULLER, MD, M.S.

Complete list of collaborators available on www.crp-sante.lu

## OBJECTIVES OF THE LABORATORY OF IMMUNOLOGY

## 1.1 SURVEILLANCE OF INFECTIOUS DISEASES CAUSED BY VIRUSES

The main purpose of these studies is to contribute to the understanding of the geographic distribution, genetic variants (including new variants and genotypes), the variability and the natural history of viruses. These studies provide answers that are important, in the case of human and zoonotic viruses, for public health and virus outbreak control. In the case of veterinary viruses, the main beneficiaries of this research are the farmers, consumers in need of high quality proteins, the veterinary services and animal welfare. These collaborative projects with resourcepoor countries include important components, such as (i) training of laboratory staff, academic staff, and of students working towards their degrees; (ii) upgrading of laboratory capacity; (iii) providing expertise in laboratory surveillance; (iv) and academic teaching. In addition, department of Immunology scientists are in demand

## 1.2 DEVELOPMENT OF VACCINATION STRATEGIES

The experience acquired with the development of measles vaccine strategies has been further applied to explore immune-prophylactic strategies against carcinogens; the approach is based on innovative conjugate vaccines that induce antibodies to influence the pharmacokinetic and pharmacodynamic of carcinogens. One PhD student has graduated in 2009 and 1 is in progress.

## 1.3 PSYCHOENDOCRINE IMMUNOLOGY OF STRESS

The department is also a department of the Graduate School for Psychobiology of the University of Trier. Six different departments collaborate to investigate stress related aspects from molecules to cognate functions. Within this academic collaboration, we contribute our expertise in immunology, molecular biology and cell biology, to investigate on a cellular level control mechanism of the main stress response system, the hypothalamic-pituitary-adrenal (HPA) axis and nuclear receptor functions in immune cells and beyond. According to EU and WHO estimates, stress represents the single most important cause of disease, causing costs as high as 3-4 % of the European gross national product. Many of these diseases are related to infections and aberrant immune reactions. In addition, stress affects social behaviour, mood, learning and memory. In the framework of these collaborations, we are part of the International Research Training Group (IRTG) «Psychoneuroendocrinology of stress: from molecules and genes to affect and cognition » a partnership with the Leiden/Amsterdam Center for Drug Research and Leiden University Medical Center. Four PhD students graduated in 2008/9, 4 PhD students of the department are currently enrolled in the IRTG Graduate School.

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as experts and consultants of the WHO, FAO, World Bank, UNDP and EU for the accreditation of laboratories, for supporting local scientists during viral outbreaks, to provide expertadvice to national or sub-national Governments and Ministries for establishing or upgrading outbreak surveillance systems. Virus variants are further investigated with respect to their detectability, cross-protection by existing vaccines, drug resistance mutations, and pathogenicity. One PhD student has graduated in 2008/9 and 7 are in progress.



LNSI host the WHO Reference Laboratory Network meeting with the Minister of Health M. Di Bartolomeo.

#### 1.4 TEACHING AND TRAINING

Our institute offers a teaching and training programme for PhD students and undergraduate students (e.g. master students). The students are trained in the department and obtain their degree from a foreign university. In 2003, the Head of the department was nominated to the Chair of Immunology of the University of Trier and Associate Professor of the University of Saarland, from 1995-2003 the Head of the department was Associate Professor at the University of Tübingen. In 2002 the department became associated with the Graduate School of Biology and Environmental Sciences (BIOSE) of the University of Nancy. The PhD students of the department enrol predominantly in Trier, Nancy and Homburg. The department also serves as a very active WHO training centre. The European Summer School of the department hosts an increasing number of students from European universities for 2-4 months research training periods. In 2009 the start of the 50th doctoral student was celebrated with a scientific symposium with the Nobel Laureate Prof. Harald zur Hausen as a guest speaker.

#### **ONGOING PROJECTS**

#### GENETIC AND PHENOTYPIC DIVERSITY OF VIRUS

## 1.1 Measles (ref 2, 5, 15, 16, 21, 23, 25)

As a WHO regional reference laboratory for Europe for Measles and Rubella (M/R) the department is involved in laboratory surveillance and quality control activities for the National M/R WHO Laboratory Network. In 2009, the identification of alternative sequencing windows for measles virus genotyping and for international tracing of transmission of viruses as well as during outbreaks, the evaluation and quality control of new ELISA kits for measles IgM testing within the WHO Network were some of the activities. An invited commentary on the current progress towards measles elimination in WHO-EURO was published in The Lancet. The innate immunity of a panel of measles viruses showed that wildtype strains differ in the innate immune response that they induce and in their susceptibility to effector molecules of the innate immune response.

### 1.2 Other fever rash viruses (Rubella, Parvovirus) (ref 4, 7, 25)

As a WHO regional reference laboratory for the European Region for Rubella we developed a single-tube multiplex TaqMan assay for measles and rubella diagnosis for the WHO laboratory network. In collaboration with the WHO office in Bosnia and Herzegovina a rubella outbreak was investigated in the country. A comprehensive phylogenetic analysis of rubella virus strains involved in congenital rubella infections in France during the past 15 years was investigated. A rubella seroprevalence study among pregnant women in Burkina Faso was conducted together with the National M/R laboratory in the country to support public health decision makers. In the framework of WHO rash fever disease surveillance, parvovirus B19 strains were genetically characterized and shifts in their worldwide distribution were observed.

#### 1.3 Hep B (ref 14, 17, 19)

Hepatitis B virus (HBV) is highly endemic in Africa as well as Asia. In sub-Saharan Africa we found that genotype E is highly prevalent but has a low genetic diversity, suggesting that it has only recently spread in the West-African general population. Our study

from Haiti showed that the genotype was only introduced within the last 200 years after the transatlantic slave trade had come to an end. Other genotypes are found in East-Africa, but in no African country we detected such a genotype and genetic diversity such as in Rwanda, including a new subgenotype A7.

Also in Asia a variety of sub-/genotypes of HBV co-circulate. In Lao People's Democratic Republic, where we defined a new genotype I, many patients were found to be mixed-infected with different HBV variants, leading to a surprisingly large number of recombination events within these patients.

## 1.4 Human Influenza (ref 1, 9, 11, 12, 20)

1.4.1 Surveillance activities. The department monitors the emergence and spread of new influenza viruses. We provided the expertise to ensure the detection and molecular characterization of human influenza A of swine origin (A/H1N1) early 2009 in Luxembourg. These methods were later applied in laboratories of African collaborators. We conducted the first comprehensive serological study in Europe, measuring the antibody response against swine origin A/ H1N1 on 211 Luxembourgers with professional swine contact. Our results suggest that a higher proportion of

individuals with contact to swine had pre-existing neutralising antibodies against pandemic A/H1N1 virus than non-exposed controls. The same was true for classical swine influenza (H1N1). More swine contacts responded to the classical swine influenza (H1N1) that circulated 10 years earlier in Europe than to the recent pandemic A/H1N1 virus that emerged in 2009.

1.4.2 Drug development. In light of the increasing resistance of influenza viruses to current antiviral drugs, we explored new approaches to treat influenza. While viral proteins tend to accumulate mutations, cellular proteins do not. More than 300 cellular proteins have been found to be involved in influenza A virus replication. To circumvent the development of drug resistance of viruses by accumulation of mutations, we target selected cellular proteins. We have demonstrated that cellular proteins important for entry of the virus into the cell, block the replication of different subtypes of wildtype viruses. These cellular proteins are not susceptible to mutations. Thus, targeting cellular proteins critical for viral replication could be a strategy to overcome drug resistance.

**1.4.3 Pathogenicity.** NS1 is a nonstructural protein of Influenza A virus. Its major role is the inhibition of the host immune reaction, mainly by limiting the interferon response of the infected organism. The NS1 protein is known to prevent maturation of host (but not viral) pre-mRNAs by blocking polyadenylation. Moreover, NS1 enhances translation of mostly viral mRNA, thereby promoting virus spread. We showed the differential effect of NS1 proteins from three influenza strains (pandemic, highly pathogenic and low pathogenic) on polyadenylation and translation and could make a link to the differential virulence of the three strains. Moreover, we were able to identify a mutation leading to low inhibition of polyadenylation (F103Y), as well as several amino acids which could be involved in translation enhancement and which will be studied further.

#### 1.5 Avian Influenza (ref 8)

Although wild birds are the major reservoir of low pathogenic avian influenza (LPAI) viruses and migratory flyways connect wildlife from Northern and Southern hemisphere, the presence and persistence of avian influenza in African birds is poorly understood. As part of the surveillance for highly pathogenic avian influenza H5N1 viruses in Africa, we monitored avian influenza in wild and domestic birds in two different locations in Nigeria. We found LPAI H5N2 viruses in wild ducks in North-Eastern Nigeria. Full genome analyses revealed that these LPAI H5N2 viruses were reassortant viruses with genes from the Eurasian gene pool in wild birds, suggesting an introduction in Africa by migratory birds.

## 1.6 Newcastle Disease virus (NDV) (ref 22)

During our avian disease surveillance program in Western African countries, several NDV strains, obtained between 2002 and 2007 from different poultry species in Nigeria, Niger, Burkina Faso and Cameroon were phylogenetically analysed. Interestingly some highly pathogenic strains formed 3 new clusters within lineage 5. Their high genetic diversity and their presence in non-commercial farms in three Sub-Saharan different countries suggest that these new sublineages represent the indigenous West-African NDV strains. It also suggests that wild birds, in contact with non-commercial poultry, could be a wild reservoir for these endemic strains. This hypothesis is currently under investigation.



Nobel Prize Laureate Prof. H. zur Hausen gives the keynote lecture at the Symposium organized on the occasion of the 50th doctoral student of LNSI

## 1.7 Infectious Bronchitis Virus (IBV) (ref 10)

Between 2002 and 2007, more than 1000 chickens from commercial farms, live bird markets and backyard farms in Nigeria and Niger were monitored for infectious bronchitis virus (IBV). Phylogenetic analysis of spike 1 gene (S1) sequences, which plays a major role in antigenicity, revealed a new genotype of this coronavirus that we named "IBADAN". S1 sequence analyses also identified several amino acids which may play a role in IBV antigenicity. The antigenic relatedness of IBADAN strains with strains of other serotypes suggested that IBADAN also represents a new serotype. These findings are of interest in light of the recent outbreak of SARS coronavirus since they demonstrate that the full genetic diversity of avian coronaviruses is not yet fully understood.

#### 1.8 Tick-born pathogens

Lyme borreliosis is the most commonly reported tick-borne infection in Europe with infection rates of ticks ranging from 2-58%. In Luxembourg we found a mean infection rate of 15.7% in ticks, with higher infection rates in 2008 (18.4%) than in 2007 (11.3%). Differences in the prevalence of Borrelia species were also observed on a regional level. Other tickborne pathogens like Rickettsia sp., Anaplasma sp. Bartonella sp. and Babesia sp. have lower prevalence rates from 0.3 to 6.4%. Although single cases of FSME occurred in neighbouring parts of Germany, Luxembourg so far seems to be free of this virus. Ticks from Moldova, Bulgaria, Belarus, Cameroon and Nigeria are currently being analysed.



#### 1.9 Norovirus

Seven different Norovirus genotypes were identified in gastroenteritis patients from Luxembourg between October 2008 and June 2009 (GI.2, GII.2, GII.3, GII.4, GII.6, GII.12 and GII.14). The large majority of strains belonged to GII.4 (~80%) which was further subdivided in several distinct lineages, including 2 new variants. Our data thus showed that multiple variants of NoV were co-circulating in Luxembourg during the study period.

#### 2. VACCINATION STRATEGY AGAINST CHEMICAL CARCINOGENESIS (REF 6, 13, 18, 26)

The objective of this project (TOBAVAC II) is to explore the possibility to develop an immune prophylactic strategy against chemical carcinogenesis. Immunogens based on benzo(a)pyrene conjugated to carrier proteins licensed as vaccines in humans have been developed that induce antibodies that attenuate detrimental effects of carcinogens in vitro and vivo. As direct measurements of effects of carcinogens at low concentrations that are environmentally relevant often do not provide experimental read-outs, surrogate models were developed to assess the protective effect of B[a] P-specific antibodies. The capacity of specific antibodies to reduce the risk of chemical carcinogenesis was demonstrated by their effect on B[a]P

uptake, metabolism, redistribution and excretion in mice and by modulation of B[a]P induced adverse effects such as immunotoxicity and neurotoxicity.

# 3. NEUROENDOCRINE IMMUNOLOGY (REF 27)

The glucocorticoid receptor (GR) is responsible for the homeostasis of many cell and organ functions including most inflammatory and immune processes, in particular during stress. The GR is found in most tissues and mediates the critical feedback loop of the hypothalamus-pituitary axis (HPA) between the brain and peripheral tissues including the immune system. As the primary stress response mediator, the GR is implicated in many diseases. By some estimates of the EU, stress is responsible for up to 60% of all days lost to disease. The objective of this project is to investigate the transcriptional control of the glucocorticoid receptor (GR) and its activity on a molecular level and to understand the interaction of the central nervous system with the immune system.



levels are transcriptionally controlled through the highly variable 5' gene region. This region codes for 11 alternative untranslated first exons. We have shown that seven of these exons are located within a CpG island immediately upstream of exon 2. We have identified upstream of each one of these alternative exon 1s promoter region, and showed that each of these exons 1s have their own promoters. We have further shown that the function of these promoters is highly controlled by epigenetic methylation. Analysis of the 128 CpG dinucleotides contained within the 5 promoters known to be active in blood lymphocytes revealed stochastic and individual methylation patterns: the majority of CpG dinucleotides were methylated at levels >25% in at least one donor. The majority of evolutionarily conserved, and confirmed active transcription factor binding sites within these promoters contain methylatable CpG sites, suggesting that methylation orchestrates alternative first exon usage, silencing, and controls expression of the GR in a tissue specific fashion. In collaboration with the Leiden-Amsterdam Center for Drug Research (LACDR) we performed the first study giving an overview of GR transcript expression in multiple areas of the limbic system in the healthy and depressed human brain. Even if distribution patterns throughout all

analysed brain regions were similar, we observed significant differences in 5'- and 3'-transcripts between control subjects and Major Depressive Disease (MDD) patients. NGFI-A, the transcription factor of exon 1F was down-regulated in the hippocampus of MDD patients; concomitantly exon 1F expression was reduced. Promoter 1F was uniformly unmethylated throughout all regions in MDD and control subjects, suggesting that in MDD, unlike in childhood abuse victims, lower 1F transcript levels are not linked to promoter methylation but to a dearth of NGFI-A. Thus, in MDD there is a similar but distinct pathomechanism differing from that of abuse victims, explaining the clinical similarity of both conditions and that susceptibility to depression can be either predisposed by early trauma or developed independent of such a condition. Thus our results showed that changes in expression levels are not due to epigenetic methylation of GR promoters. This would suggest that epigenetic control of the GR is limited to peripheral glucocorticoid target tissues, rather than central tissues controlling GC levels.

Recently, we have started to investigate the distal GR promoter (promoter 1A), some 24kbp upstream of the 7 CpG island promoters. This alternative exon 1A is of particular interest since it is thought to encode the membrane GR at least in mice. We investigate the membrane-associated GR also at a protein level.

In collaboration with the University of Trier and the CIEC (CRP-Santé) we investigate the effect of ultradian and stress-induced cortisol release on GR target gene response in humans. Stress is induced by a standardised stress protocol, the Trier Social Stress Test (TSST). We observed highly diverse gene- and donor-dependent kinetic responses of the GR-target genes. Transcription of some GR target genes (GILZ, FKBP) was induced approximately 100 minutes after initiation of the stress protocol, and a second class of genes (such as SDPR) showed up regulation some 150-200 minutes after the cortisol peak.

#### 4. PROTEOMICS (REF 3)

combined **Proteomics** with bioinformatics pathway analysis identified IFN type I and type II signalling pathways as principal targets of immunomodulatory effects of cortisol on LPS-activated macrophages. New isoforms of cortisol-sensitive IFNinducible proteins were found such as for MX1 and SYWC. Proteomic profiling of non-genomic effects of cortisol in a rat model for acute stress revealed new insights in the early stages of a stress response. Nuclear translocated proteins identified belong mainly to transcriptional control and mRNA splicing/processing suggesting the preparation of the cell for a forthcoming transcription.

## 5. PERINATAL PROGRAMMING OF THE IMMUNE SYSTEM

Perinatal programming refers to the concept that foetal or early postnatal environmental factors, such as nutrition, stress or infectious disease, have a considerable longterm impact on adult phenotype and disease susceptibility. Although initial studies focused on perinatal programming in cardiovascular or nervous systems, it has become clear that early environment may influence a large range of diseases and conditions, including disorders of the immune system. Recent evidence indicates that epigenetic mechanisms, such as methylation of CpG dinucleotides, are involved in the memorization of earlylife events. The objective of this project is to study the role of DNA methylation in immune cells and animal models, and to identify genes responsible for early programming of the immune system.

This project is partially based on our earlier observations of highly individual methylation patterns of glucocorticoid receptor promoters in human peripheral blood lymphocytes. These studies were the first to demonstrate the highly diverse methylation patterns of a gene that plays an important role in the regulation of the immune system. Here we expand these observations to other genes involved in the immune response and investigate how such individual methylation patterns are generated and what they mean with respect to individual differences in immune response. The effects of different compounds on DNA methyltransferase (DNMT) expression and total genomic methylation in human immune cell lines and freshly explanted mouse lymphocytes or human PBMCs are investigated, before comparing DNA methylation



between stimulated and unstimulated cells using methylation-sensitive restriction fingerprinting. In parallel, we investigate DNMT expression in mice of different ages and after stimulation with molecules that modulate DNA methylation. Based on these results, the transcriptome of mice exposed or not to an adequate perinatal stressor will be compared in adulthood by microarray analysis. Interindividual variability in methylation of candidate

genes from literature and of the identified genes will be analyzed in peripheral blood mononuclear cells (PBMCs) of healthy human individuals, and finally, the effects of other stressors, such as viral infection or vaccination, on promoter methylation of the identified genes will be analyzed in mice. Our results contribute to the understanding of perinatal events, such as vaccination or viral infections, on adult immune function and disease

susceptibility. In addition, knowledge of methylation patterns that influence adult disease susceptibility may become an important tool for the individual management of disease susceptibilities by predicting a risk factor that is potentially accessible to manipulation.



LNSI hosts the autumn school of the International Research Training Group in collaboration with the University of Trier and the Deutsche Forschungsgemeinschaft

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#### **HEAD OF LABORATORY**

Dr. Guy BERCHEM, MD

#### ASSOCIATE HEAD OF LABORATORY

Dr. Eric VAN DYCK, PhD

**DEPARTMENT OF ONCOLOGY** 

# LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY



Complete list of collaborators available on www.crp-sante.lu

#### OBJECTIVES OF THE LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

The LHCE conducts cancer research at the interface of basic and clinical research. Our studies focus on the molecular mechanisms that govern cell death and the cellular resistance to chemotherapy in haematological and non-haematological cancers. Using appropriate cell-based models, we try to identify promising therapy strategies, including combination strategies, for extending patient survival. Recently, biomarker discovery studies in patient fluid samples have been implemented in the laboratory in order to improve early diagnostic and treatment efficacy.

The molecular mechanisms that underlie the cellular response to chemotherapy are investigated at the genetic, biochemical, transcriptomic and proteomic level, using in vivo

#### **ONGOING PROJECTS**

#### **BIOMARKERS PROJECT**

 Discovery and validation of new plasma biomarkers in lung cancer and their health economic importance

Acronym: ppm project Grant period: 2009-2011 Financial support: CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Luxembourg

#### Project summary:

The incidence and mortality rates of lung cancer make this disease the first cancer-related cause of death in Luxembourg, with a major impact on people's life and our public health system. More effective molecular diagnostics are required, as are technologies capable of defining patient risk, identifying early disease

 Detection of microRNAs in vesicles isolated from plasma of B chronic lymphocytic leukaemia (B-CLL) patients

Contract number: F5/20/5-MCF/DD

grant number 7.4512.09 **Grant period:** 2009-2011 **Financial support:** Télévie 2009

#### Project summary:

Chronic lymphocytic leukaemia (CLL) remains an incurable hematologic disorder, in great part for lack of early detection tools. Analysis of plasma biomarkers offers a noninvasive diagnostic method for cancer detection. Recent reports have highlighted the high potential of circulating microRNAs (miRNAs) as plasma biomarkers for improved detection of malignancies. The aim of this project is to investigate plasma miRNAs as potential biomarkers for the early stages of CLL.

#### People involved:

Guy BERCHEM, Manon BOSSELER, David J GALAS, Etienne MOUSSAY, Maria Pires PACHECCO, Valérie PALISSOT, Sandrine PIERSON, Kris VAN MOER, Kai WANG

 Study of genetic aberrations during cancer in bronchial neoplasia and early stages of oncogenesis

Contract number: F5/20/5-MCF/DD grant number 7.4502.07
Grant period: 2006-2010
Financial support: Fondation
Luxembourgeoise contre le cancer and Télévie 2007

#### Project summary:

A better understanding of the sequence of events involved in lung neoplasia and their underlying molecular mechanisms is necessary to improve disease management. This project aims to improve early diagnosis and detection of highrisk patients with no histological modifications, and to identify early diagnostic biomarkers. Our approach uses a whole genome microarray approach associated with comparative genomic hybridization array analysis, to compare gene expression patterns of normal-appearing mucosa (identified by fluorescence bronchoscopy) in different lung cancer risk groups (heavy smokers without previous lung cancer, lung cancer patients, non-smokers).

#### DEPARTMENT OF ONCOLOGY

# THE LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

and in vitro studies of patient cells, as well as cell lines. In this context, the laboratory has developed novel methodologies to characterize abnormal cells. In accordance with its translational biomedical objectives, the LHCE also aims to initiate clinical trials and improve the personalization of therapy.

onset and discriminating between related pathologies. This project aims to identify potential lung cancer biomarkers using innovative proteomic analyses of plasma as well as lung tissues.

#### People involved:

Guy BERCHEM, Olivia ROLAND, Ester GASPERINI, Etienne MOUSSAY



#### People involved:

Guy BERCHEM, Manon BOSSELER, Microarray center staff headed by Laurent VALLAR, Valérie PALISSOT, Maria Pires PACHECCO, Marc SCHLESSER

#### CELL DEATH/RESISTANCE PROJECT

 Anti-cancer activity of Histone deacetylase inhibitors, alone or in combination, on B-cell chronic lymphocytic leukaemia

Contract number: F5/20/5-MCF/DD grant number 7.4536.07
Grant period: 2006-2010
Financial support: Télévie 2007

#### **Project summary:**

The anti-cancer activity of Histone Deacetylase (HDAC) inhibitors in different hematologic malignancies has been demonstrated in clinical trials. Several data support the use of HDAC inhibitors in association with other chemotherapeutic drugs. In order to provide more rational combination therapies, we explore the molecular basis of the cytotoxicity of HDACs. The apoptotic cell death pathways induced by a combination of the class 1 HDAC inhibitor MGCD0103 (MethylGene Inc) and classical CLL drugs are investigated.

#### People involved:

Guy BERCHEM, Victoria EL KHOURY, Maria Pires PACHECCO, Kris VAN MOER  Implication of the hTopoIII alpha in telomere maintenance in leukaemia

Acronym: Totecan
Contract number: FNR/
BIOSAN/07/22

**Grant period:** 11.2007- 11.2009 Financial support: Fonds National de la Recherche (FNR)

#### Project summary:

Telomeres consist of repetitive G-rich DNA sequences that protect the chromosome ends. The maintenance of telomere length in human tumour involves the telomerase, a specialized enzyme translated from the hTERT gene, or a recombination mechanism observed in about 15% of tumours. These tumours display a heterogeneous telomere length, maintained by a recombinogenic mechanism involving a complex formed of the Topoisomerase III alpha (Topo IIIα), the RecQ-like helicase BLM and the telomeric protein TRF2. As Topoisomerase I and II are targets of numerous and essential anticancer drugs, the aim of this project is to study the relevance of the Topo III $\alpha$ as a potential target of anticancer treatment and especially in Chronic Lymphoid Leukaemia (CLL) and Chronic Myeloid Leukaemia patients (CML).

#### People involved:

Thomas WENNER, Guy BERCHEM, Mario DICATO, Valérie PALISSOT, Laetitia CHAMBEAU, Brigitte METZGER, Wim AMMERLAAN

## 3. Multiple Myeloma subpopulation characterization

Contract number: F5/20/5-MCF/DD grant number 7.4537.07 Grant period: 01.2008-Financial support: Télévie 2007

#### Project summary:

The quantification of plasma cells in bone marrow represents an essential diagnostic test. Whereas multiparameter flow cytometry is widely used in hematologic neoplasia, this immunophenotyping technique is currently not exploited for the clinical diagnosis of Multiple Myeloma (MM), being limited instead to some specific cases. For instance, multiparameter flow cytometry allows discrimination between normal plasma cells and malignant ones, based on differential expression of CD56 and CD19 cell markers. We are trying to increase the use of this technique by developing 12-colour flow cytometry to detect cell subpopulations out of full blood and bone marrow samples. Potential markers have been identified that are being investigated in our laboratory for a better discrimination of MM subpopulations and identification of their roles in disease progression and in clinical outcome.

#### People involved:

Nassera AOUALI, Guy BERCHEM, Manon BOSSELER, René BRONS, Valérie PALISSOT, Sandrine PIERSON



 Cellular and molecular mechanisms involved in sensitivity of Multiple Myeloma to new therapeutic agents

#### Contract number:

REC-LHCE-20071005

Grant period: 04.2008-03.2011 Financial support: CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Luxembourg

#### **Project summary:**

In order to evaluate the anti-neoplastic properties of new drug combinations on selected cell populations in multiple myeloma (MM), valproic acid (VPA) and PPARy agonists (clinically used in diabetes) have been tested on MM cell lines. While VPA is already known to have anticancer activity on different cancer types including MM, we show that PPARy agonists potentiate the cytotoxic effect of VPA on various MM cell lines. Importantly, this potentiation is also observed in MM patient cells treated in vitro. The molecular and cellular mechanisms underlying this potentiation are being studied.

#### People involved:

Nassera AOUALI, Guy BERCHEM, Valérie PALISSOT, Sandrine PIERSON, Maria Pires PACHECCO 5. Proteomic study of multiple myeloma cell stress response

#### Contract number:

F5/20/5-MCF/DD grant number 7.4532.08

**Grant period:** 01.2009-03.2010 **Financial support:** Télévie 2008

#### **Project summary:**

Plasma cells are rare in normal bone marrow (BM) of adults, making up about 0.4% of the marrow cell population. This percentage is modified in diseases observed before and after Multiple Myeloma (MM), where plasma cells compose up to 1-10% in BM in MGUS or Smoldering myeloma, at least 10% in bone in MM and more than 20% in the blood of Plasma Cell Leukaemia. This project aims to implement and develop a proteomic methodology to study protein profiles of malignant plasma cells isolated from bone marrow and blood MM patient samples. The proteins implicated in stress response are investigated to identify new therapeutic targets as well as biomarkers for disease stage.

#### People involved:

Thierry ARNOULD, Nassera AOUALI, Guy BERCHEM, Manon BOSSELER, Edouard DELAIVE, Amandine LEQUEUX, Valérie PALISSOT, Sandrine PIERSON, Martine RAES  The involvement of the autophagy-dependent cell survival process in the mechanism of tumour cells resistance to cytotoxic agents

Acronym: Autophagy

Contract number: REC-LHCE-

20090201

Grant period: 07.2009-07.2012 Financial support: CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Luxembourg

#### Project summary:

There is accumulating evidence that various anticancer therapies induce autophagy in tumour cells. Autophagy is a cellular catabolic degradation process whereby proteins, organelles and cytoplasm are engulfed in autophagosomes to be digested and recycled to sustain the cellular metabolism. Until recently, it was still unclear whether autophagy is a prodeath or a pro-survival mechanism. It is now definitively admitted that autophagy may play a dual role in cell survival and cell death. This project aims to investigate the role of autophagy in tumour cells resistance to different anticancer therapy including chemotherapy and immunotherapy. The present project will contribute to the development of

novel therapeutic strategies based on targeting the autophagy machinery as a resistance mechanism, which represents a significant challenge in cancer therapy.

#### People involved:

Guy BERCHEM, Manon BOSSELER, Bassam JANJI, Valérie PALISSOT, Kris VAN MOER

#### Associated PhD project:

**Title:** Study of the involvement of autophagy in the acquisition of tumour resistance to TNF alpha

Start date: December 2009

**Financial support:** BFR/AFR grant from Fonds National de la Recherche (FNR)

#### **KEY RESULTS**

- I. We have identified promising drug combinations for the treatment of CLL and Multiple Myeloma. The cell death signaling pathways elicited by these drugs have been described.
- II. Promoter analysis of the TOPIIIalpha gene has revealed significant cases of CpG island hyper-methylation in CLL patients (17 cases out of 31), compared to healthy individuals (one case of hemi-methylation case out of 10 individuals). Hyper-methylation was also observed in two haematological cell lines (MEC and MOLP) and for the ALT cell line (osteosarcoma U2OS) but not in Hela, MCF7, or SaOS cell lines.
- **III.** We have developed the first autophagy-dedicated microarray as a high throughput tool to study the regulation of autophagy genes in biological samples.
- **IV.** We have identified a role for autophagy as a novel mechanism involved in the resistance of tumour cells to immunotherapy of non-small-cell lung carcinoma.

#### **COLLABORATIONS**

#### NATIONAL

- Centre Hospitalier de Luxembourg (CHL), Service de Pneumologie, Dr. M. Schlesser
- Life science research unit,
   University of Luxembourg, Drs
   I.Behrmann, C.Haan

#### INTERNATIONAL

- Institute for Systems Biology, Seattle, USA, Drs K. Wang and D J. Galas
- Laboratoire de Biologie Cellulaire et Moléculaire de l'Institut Jules Bordet (ULB). Bruxelles, Belgique, Prof.A. Burny
- Laboratoire de Recherche en cancérologie pulmonaire, Institut Jules Bordet, Bruxelles, Belgique, Prof. Drs JP Sculier, V. Ninane, C. Mascaux
- Laboratoire de biochimie et de Biologie Moléculaire, Facultés Universitaires Notre-dame de la paix, Namur, Belgique, Drs Martine RAES, Thierry Arnould
- Unité CNRS UMR6237 MEDyC, IFR53, UFR de Pharmacie, Reims, France, Dr. H. Morjani
- Unité INSERM 753- Institut de Cancérologie Gustave Roussy, Immunologie des tumeurs humaines, Villejuif, France, Dr. S. Chouaib

#### 2009 PUBLICATIONS

The actin filament cross-linker L-plastin confers resistance to TNF- $\alpha$  in MCF-7 breast cancer cells in a phosphorylation-dependent manner. Janji B., Vallar L., Altanoury Z., Bernardin F., Vetter G., Schaffner-Reckinger E., Berchem G., Friederich E. and Chouaib S. Journal of Cellular and Molecular Medicine 2009

If the CTLs play the music, does the tumoural system call the tune? Hamaï A., Benlalam H., Meslin F., Hasmim M., Carré T, Akalay I., Janji B.,

Berchem G., Noman MZ., and Chouaïb S. Tissue Antigens 2009

Peroxisome proliferator-activated receptor y agonists potentiate the cytotoxic effect of Valproic acid in multiple myeloma cells. Aouali N., Palissot V., El-Khoury V., Moussay E., Janji B., Pierson S., Brons N. H. C., Kellner L., Bosseler M., Van Moer K. and Berchem G. British Journal of Haematology 2009





#### **HEAD OF LABORATORY**

Dr. Laurent VALLAR, PhD

**DEPARTMENT OF ONCOLOGY** 

## **MICROARRAY CENTER**



Complete list of collaborators available on www.crp-sante.lu

## OBJECTIVES OF THE MICROARRAY CENTER

From its inception in 2002, the Microarray Center actively collaborates with many national and foreign institutions to advance genomics research in areas as diverse as health, food tracing and environmental issues. Equipped with a wide range of state-of-the-art technology platforms, the facility aims to provide comprehensive, quality and reliable services for academic and private sector scientists alike. The Center offers expertise and know-how in molecular and cell biology, biostatistics and bioinformatics.

The goal of the Microarray Center is to partner all stages of genomics research projects, including:

- Assistance with experimental design and project proposal,
- RNA extraction, quality control and processing (RT-PCR, amplification),
- Custom microarray design including design of oligonucleotide probes,
- Microarray manufacturing.
   Besides traditional DNA- and oligonucleotide-based microarrays, protein arrays can also be produced using purified proteins or antibodies, as well as tissue and cell microarrays using tissue/cell paraffin blocks,
- Microarray processing and analysis.
   Whole-genome expression
   profiling experiments, as well as
   comparative genomic analysis
   (aCGH) can be conducted in a
   large set of organisms using major
   commercial microarray platforms
   (Affymetrix & Agilent),
- In-depth analysis and interpretation of microarray data,
- Assistance in publication writing.

#### DEPARTMENT OF ONCOLOGY

## **MICROARRAY CENTER**

#### **ONGOING PROJECTS**

The Microarray Center currently participates as a contracting partner in 16 projects developed by scientists from CRP-Santé and other institutions, and carries out independent research in genomics and bioinformatics.

## ALTERNATIVE SPLICING IN LUNG CANCER PROJECT:

Alternative splicing and differential gene expression in tumour and stroma compartments: study of tumour progression induced by elastin peptides in lung cancer

Acronym: ASTSTRO

Contract number: CO8/BM/05 Grant period: February 2009 –

January 2012

**Financial support:** Fonds National de la Recherche (FNR), Luxembourg

#### Project summary:

Alternative splicing (AS) of gene transcripts is a fine-tuned process which generates multiple functional variants from individual genes. In cancers, AS is significantly altered, and there is increasing indication that alternative and aberrant splice variants may have a critical impact on all major aspects of

tumour biology, including proliferation, invasion, motility, metastasis, and angiogenesis. The project aims to identify and characterise lung cancer-specific transcript variants as putative biomarkers and targets for diagnostic, prognostic, and treatment purposes. The project also focuses on the alterations in gene expression which take place following the interaction of tumour cells with their microenvironment. Special emphasis is placed on the contribution of peptides generated upon elastin degradation in cancerous lungs. We apply high throughput Affymetrix GeneChip technology to study the transcriptome of lung tumour biopsies as well as cell co-culture models that mimic tumourstroma interactions and xenografts obtained by implanting human lung tumour cells into immunodeficient mice. Analysis of gene expression and AS patterns of these samples should provide new insights into the molecular events taking place during tumour-stroma crosstalk and enable us to characterise the specific impact of elastin peptides on tumour progression.

#### People involved:

Laurent VALLAR, Christelle GHONEIM, François BERNARDIN, Petr NAZAROV, Arnaud MULLER, Tony KAOMA

#### **ACTINOME PROJECT:**

Creation of new competence in bioinformatics and of a technological platform for large-scale genomic analysis

Acronym: DNA chips

Contract number: BIOSAN/01/04/09b Grant period: February 2002 –

January 2009

**Financial support:** Fonds National de la Recherche (FNR), Luxembourg

#### Project summary:

We designed a thematic microarray called Actichip which showed robust performance as a tool to profile the expression level of about 2000 genes coding for key proteins from the actin cytoskeleton and associated regulatory pathways. Using this biochip, we analysed the transcriptome of various human normal and tumour specimens as well as cell lines, and identified several groups of genes with

significant expression patterns which are characteristic of each of the sample types which we tested. Starting from a subset of these genes, we were able to discriminate normal and tumour samples and to correctly classify them into distinct histopathological subgroups. We also built a large gene expression database by retrieving, reannotating, filtering and normalising data available in public repositories. The database currently compiles high quality expression data including 7381 experiments performed with human normal and tumour samples from 41 tissue categories. Through a metaanalysis of this data, we confirmed that a majority of the genes identified using Actichip has tissue- or cancerspecific expression pattern. The value of these genes as biomarkers should now be validated through the screening of representative panels of clinical samples.

#### People involved:

Laurent VALLAR, Nathalie NICOT, François BERNARDIN, Petr NAZAROV, Arnaud MULLER





#### **KEY RESULTS**

I. In collaboration with the Cytoskeleton and Plasticity Laboratory (E. Friederich, University of Luxembourg), the Microarray Center contributed to the characterization of the transcriptional events which occurs during epithelial to mesenchyme transition (EMT), a key process involved in carcinoma progression towards an invasive state. We performed a time-resolved transcriptome profiling of a cellular model which undergoes EMT upon inducible expression of the transcription factor Sna1 and was developed in the former Laboratory of Molecular Biology and Modelling headed by E. Friederich at CRP-Santé (Vetter G. et al., BBRC 2009).

II. A novel role for the actin bundling protein L-plastin as a cell protector against TNF- $\alpha$  cytotoxicity was described by the Laboratory of Experimental Hematology (Dr. G. Berchem, CRP-Santé). We contributed to this study by revealing the overexpression of L-plastin in TNF- $\alpha$ -resistant breast adenocarcinoma MCF-7 cells through a gene expression analysis using Actichip microarrays.

III. Members of the Microarray Center participated with poster presentations in scientific conferences (BBC'09, Liège; LuciLinx, Luxembourg).

IV. On March 5, 2009, the Microarray Center organized a seminar on Affymetrix technology which attracted more than 80 participants from Luxembourg and the Greater Region.

**V.** On November 3, 2009, the Microarray Center organized a two-week training session on applied statistics which met a great success within the community.

VI. Loïc Couderc, a graduate student from the University of Bordeaux, successfully completed his Master's degree in bioinformatics after having conducted his research project for six months in the laboratory.

VII. In an effort to upgrade their skills, members of the Microarray Center participated in several workshops and training courses related to Affymetrix technology, array data analysis or data management for systems biology (EMBL, Heidelberg; EMBO, Torino; ERASysBio, Tenerife).

VIII. The Affymetrix GeneChip instrument system was successfully implemented in the laboratory. With this technology supported by a robust IT infrastructure, the Microarray Center now offers a wide range of solutions for in-depth analysis of the genome, transcriptome and epigenome.

#### **COLLABORATIONS**

#### **NATIONAL**

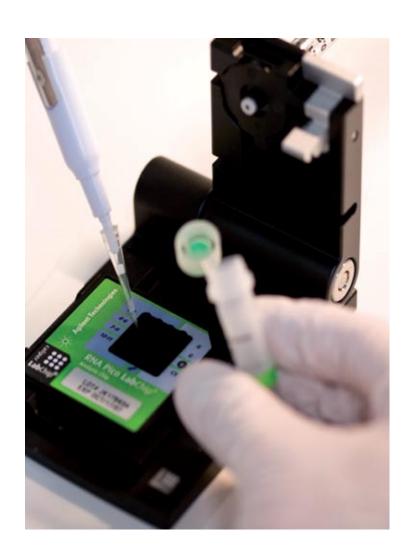
- CRP-Santé, Laboratory of Experimental Hemato-Oncology, Dr. G. Berchem
- CRP-Santé, Norlux Neuro-Oncology Laboratory, Dr. S. Niclou
- CRP-Santé, Laboratory of Immunogenetics and Allergology, Dr. F. Hentges
- CRP-Santé, Laboratory of Retrovirology (Dr. JC. Schmit)
- CRP-Santé, Department of Immunology (Prof. CP Muller)
- Integrated Biobank of Luxembourg (IBBL)
- Department of environment and agro-biotechnology, CRP-Gabriel Lippmann (Dr. L. Hoffmann)
- Life sciences research unit,
   University of Luxembourg (8
   collaborators including Profs
   I. Behrmann, E. Friederich, P.
   Heuschling, T. Sauter, Drs L.
   Grandbarbe, T. Heurtaux, S. Kreis,
   E. Morga)

#### 2009 PUBLICATIONS

- Janji B, Vallar L, Al-Tanoury Z, Bernardin F, Vetter G, Schaffner-Reckinger E, Berchem G, Friederich E, Chouaib S. The actin filament cross-linker L-plastin confers resistance to TNF-alpha in MCF-7 breast cancer cells in a phosphorylation-dependent manner. J Cell Mol Med. 2009 Oct 3. Epub ahead of print.
- Vetter G, Le Béchec A, Muller J, Muller A, Moes M, Yatskou M, Al Tanoury Z, Poch O, Vallar L, Friederich E. Time-resolved analysis of transcriptional events during SNAI1-triggered epithelial to mesenchymal transition. Biochem Biophys Res Commun. 2009 Aug 7;385(4):485-91. Epub 2009 May 12.

#### INTERNATIONAL

- Institut de génétique et de biologie moléculaire et cellulaire (IGBMC), Strasbourg, France (Dr. O. Poch)
- Institut de la vision, Paris, France (Dr. T. Leveillard)
- Institut de génétique moléculaire, CNRS UMR5535-IFR122, Montpellier, France (Dr. CH Lecellier)
- Institut de Génétique humaine, CNRS UPR1142, Montpellier, France (Dr. A. Saumet)
- UMR CNRS 6237 MEDyC, Reims, France (4 collaborators including Drs. S. Brézillon, S. Dedieu, L. Duca, S. Pasco)
- Institut Gustave Roussy, Villejuif, France (Dr. S. Chouaib)
- Cancéropôle du Grand Est,
   Strasbourg, France (Prof. P. Oudet)
- Institute of Oncology, Université d'Umea, Sweden (Drs. Hakan Hedman and M. Johansson)
- Translational Genomics Institute (TGen), USA (Dr. M. Barrett)







#### **HEAD OF LABORATORY**

Dr. Simone P. NICLOU, PhD

#### ASSOCIATE HEAD OF LABORATORY

Prof. Rolf BJERKVIG, PhD

**DEPARTMENT OF ONCOLOGY** 

## NORLUX NEURO-ONCOLOGY LABORATORY



Complete list of collaborators available on www.crp-sante.lu

## OBJECTIVES OF THE NORLUX NEURO-ONCOLOGY LABORATORY

The aim of the research unit is to understand the biological mechanisms underlying the initiation and progression of malignant gliomas and to identify new molecular targets against brain tumours. Based on patient tumour material, the laboratory has developed appropriate animal models to study glioma development in vivo. Using these models we address the identity of cancer-initiating cells, tumour-host interaction, angiogenesis and tumour cell metabolism. We also apply cell micro-encapsulation technology for the delivery of therapeutic proteins in brain diseases.

#### **DEPARTMENT OF ONCOLOGY**

## NORLUX NEURO-ONCOLOGY LABORATORY

#### **ONGOING PROJECTS**

#### 1. Cancer Stem Cell Project

Role of Cancer Stem Cells in Brain

Tumour Initiation and Progression Acronym: CaStemCell Contract number: REC 070602 Grant period: January 2008 - June 2011 Financial support: CRP-Santé / Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

#### Project summary:

Recent studies have described stemlike cancer cells in several tumours including malignant glioma. This project aims to identify such cells in glioblastoma and characterize them functionally and phenotypically. Using glioma xenografts in GFP-expressing immunodeficient mice, we are able to separate tumour cells from the host cell compartment and characterize subpopulations of cancer cells. The overall aim of this project is to identify molecular processes involved in tumour initiation and characterize key molecules involved in tumourhost interactions. The project will also provide novel insight into the current cancer stem cell controversy.

#### People involved:

Associated PhD project:

Anna GOLEBIEWSKA, Sébastien BOUGNAUD, Anaïs OUDIN, Virginie BAUS, Amandine BERNARD

Role of the tumour microenvironment on invasive and angiogenesis-dependent growth patterns of brain tumours PhD student: Sébastien BOUGNAUD Start date: January 2010 Financial support: AFR grant from Fonds National de la Recherche (FNR)

#### 2. Encapsulation project

Application of Cell Microencapsulation Technology to the Treatment of Brain Disorders
Acronym: ENCAPS

Contract number: C08/BM/11 Grant period: January 2009 - January

2011

**Financial support:** FNR\_CORE, Fonds National de la Recherche

(FNR), Luxembourg

#### Project summary:

The limited passage of drugs through the blood brain barrier and the short half-life of locally injected therapeutic molecules are major hurdles for efficient delivery of therapeutic compounds to the diseased brain. Cell-based delivery systems, e.g. cell micro-encapsulation devices, provide continuous delivery of the biologically active compound in situ and are a promising strategy for therapeutic applications in the brain. In this project we apply cell encapsulation technology to deliver growth-inhibitory and anti-angiogenic proteins in tumour-bearing mice.

#### People involved:

Jo K. UTVIK, Mikael JOHANSSON, Frédéric MAYER, Anaïs OUDIN

#### 3. GBM Targets

Identifying Molecular Targets on Human Glioblastoma
Acronym: GBM\_Targets
Contract number: C08/BM/12
Grant period: April 2009-April 2011
Financial support: FNR\_CORE, Fonds
National de la Recherche (FNR),
Luxembourg

#### Project summary:

Glioblastoma is a highly heterogeneous cancer both at the genetic

and phenotypic level. This diversity needs to be taken into account in the design of novel therapeutic strategies. In this project we apply high throughput screening methods, including transcriptomics and whole genome shRNA libraries, to identify novel molecular targets against malignant glioma. In addition we use array comparative genomic hybridization (aCGH) to define the genetic profile of glioblastoma samples and to correlate this with their phenotypic appearance.

#### People involved:

Rolf BJERKVIG, Daniel STIEBER, Virginie BAUS

#### 4. Glioma Angiogenesis

Mechanisms of anti-angiogenic treatment in malignant gliomas Acronym: ANGIO Contract number: (follow-up of

Angiotargeting Project)
Financial support: CRP-Santé /
Ministère de l'Enseignement
Supérieur et de la Recherche (MESR)

#### Project summary:

Glioblastoma is a highly vascularised tumour and its aggressive growth depends on the supply of nutrients and oxygen from newly generated blood vessels. The importance of

'tumour angiogenesis' has fuelled the emergence of therapeutic strategies based on anti-angiogenic properties. Although initial clinical studies with anti-angiogenic compounds for glioblastoma are promising, such treatment may enhance the invasive properties of tumour cells. Here we investigate the effects of anti-VEGF treatment in a highly angiogenic glioma model, using noninvasive imaging techniques such as magnetic resonance imaging (MRI) correlated with detailed molecular analysis including gene expression, phospho-protein and metabolomics approaches.

#### People involved:

Olivier KEUNEN, Fred FACK, Siti Aminah ABDUL RAHIM, Anaïs OUDIN, Amandine BERNARD

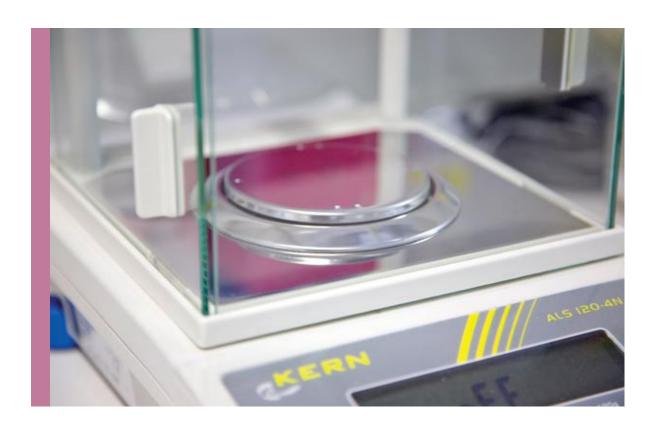
#### Associated PhD project:

**Title:** Functional Validation of Novel Biomarkers Involved in Invasive and Angiogenic Properties of Brain Tumours

PhD student: Siti Aminah ABDUL

RAHIM

Start date: January 2010
Financial support: AFR grant from
Fonds National de la Recherche (FNR)



#### **KEY RESULTS**

**I.** In collaboration and co-supervision with the University of Luxembourg, two PhD student projects started this year with financial support from the FNR (AFR grants).

II. Our first FNR financed project within the BIOSAN-Provie programme was successfully completed this year. The manuscript (Garcia et al.) has been accepted for publication. Two novel research projects financed within the FNR CORE programme set off in 2009

III. The EU 6<sup>th</sup> FP funded Angiotargeting project has come to an end in April 2009. As a result of this project, a state-of-the-art quantitative proteomics analysis on glioma xenografts, based on peptide labelling and tandem mass spectrometry (MALDI TOF/TOF), has been published in Molecular and Cellular Proteomics (Rajcevic et al., 2009).

IV. In collaboration with the University of Bergen, Norway, new competences have been developed in Magnetic Resonance Imaging (MRI) on small animals. These have been implemented in the analysis of anti-angiogenic treatment in malignant gliomas and are greatly contributing to the development of improved protocols/software for MRI applications.

V. Novel results regarding antiangiogenic therapy in malignant glioma have been presented at several major international conferences including the World Congress for Neuro-Oncology in Yokohama, Japan (May 2009), the Meeting of the British Neuro-Oncology Society (June 2009) and the Scandinavian Neuro-Oncology Meeting in Helsinki (October 2009). The manuscript describing this work is currently in preparation (Keunen et al.)

VI. The laboratory was the local organizer for the Spring 2009 Workshop of the COST 865 Action on Bioencapsulation: Microcapsule property assessment. April 24-25, 2009. Luxembourg, Luxembourg (50-60 participants). With the kind support of the FNR.



#### NATIONAL

- Centre Hospitalier de Luxembourg (CHL): Neurosurgery Department Dr. F. Hertel
- CRP-Santé, Microarray Center, Dr.
   L. Vallar
- CRP-Santé, Flow Cytometry: N.H.C.
   Brons
- University of Luxembourg: Prof. P. Heuschling, Prof. E. Friederich
- Integrated Biobank of Luxembourg (IBBL), Luxembourg

#### INTERNATIONAL

- University of Bergen, Norway, Molecular Imaging Center: Prof. F. Thorsen
- University of Umeå, Umeå, Sweden: Dr. H. Hedman (Radiation Oncology) and Dr. H. Antti (Department of Biological Chemistry)
- Translational Genomics Research Institute (TGen), Oncology Division, Brain Tumour Unit, Phoenix, Arizona: Dr. M. Berens
- University of Texas M. D. Anderson Cancer Center, Department of Neuro-Oncology, Houston, Texas: Prof. V.A. Levin
- Institut National Polytechnique de Lorraine (INPL), Lipidomix Laboratory, Nancy, France: Dr. T. Pillot
- Netherlands Institute for Neurosciences, NeuroRegeneration Department, Amsterdam, NL: Prof. J. Verhaagen
- University of Groningen, Medical Biology, Groningen, NL: Prof. P. de Vos
- Vrije Universiteit (VU) Cancer Center, Amsterdam, Netherlands, Onco-Proteomics Facility: Dr. C.
   Jimenez
- Angiotargeting Consortium, EU Integrated Project 6th FP (11 partners). Website: www.uib.no/ med/angiotargeting
- COST Action 865: Bioencapsulation multiscale interaction analysis (coordinator: Prof. D. Poncelet, France). Website: http://cost865. bioencapsulation.net





#### **2009 PUBLICATIONS**

- Bjerkvig R, Johansson M, Miletic H, Niclou SP. Cancer stem cells and angiogenesis. Semin Cancer Biol. 2009 Oct;19(5):279-84. Epub 2009 Oct 7.
- 2. Rajcevic U, Petersen K, Knol JC, Loos M, Bougnaud S, Klychnikov O, Li KW, Pham TV, Wang J, Miletic H, Peng Z, Bjerkvig R, Jimenez CR, Niclou SP. iTRAQ-based proteomics profiling reveals increased metabolic activity and cellular cross-talk in angiogenic compared with invasive glioblastoma phenotype. Mol Cell Proteomics. 2009 Nov;8(11):2595-612. Epub 2009 Aug 12.
- 3. Miletic H, Niclou SP, Johansson M, Bjerkvig R. Anti-VEGF therapies for malignant glioma: treatment effects and escape mechanisms. Expert Opin Ther Targets. 2009 Apr;13(4):455-68. Review.
- Rajcevic U, Niclou SP, Jimenez CR. Proteomics strategies for target identification and biomarker discovery in cancer. Front Biosci. 2009 Jan1;14:3292-303. Review.

- 5. Utvik JK & Niclou SP. Treatment of neurodegenerative diseases (Parkinson's, Huntington's and Alzheimer's' diseases) with cell encapsulation technology. In: The Bioartificial Pancreas and other Biohybrid Therapies. Eds. Hallé JP, de Vos P, Rosenberg L. Transworld Research Network 2009.
- 6. Niclou SP & Bjerkvig R. Treatment of brain tumours with microencapsulated cell therapy. In: The Bioartificial Pancreas and other Biohybrid Therapies. Eds. Hallé JP, de Vos P, Rosenberg L. Transworld Research Network 2009.
- 7. Garcia P, Youssef I, Utvik JK, Florent-Béchard S, Hanse M, Barthelemy V, Armand-Malaplate C, Kriem B, Stenger C, Koziel V, Jean-Luc Olivier, Escanye MC, Allouche A, Desbène C, Yen FT, Bjerkvig R, Oster T, Niclou SP and Pillot T. CNTF cell based delivery prevents synaptic impairment and improves memory in mouse models of Alzheimer's disease (in press).





**DEPARTMENT OF PUBLIC HEALTH** 

## CENTRE FOR HEALTH STUDIES

**HEAD OF UNIT**Marie-Lise LAIR

ASSOCIATE HEAD OF UNIT Dr. Sophie COUFFIGNAL, MD



Complete list of collaborators available on www.crp-sante.lu

#### DEPARTMENT OF PUBLIC HEALTH

## CENTRE FOR HEALTH STUDIES

## OBJECTIVES OF THE CENTRE FOR HEALTH STUDIES

The objective of the Centre for Health Studies is to become a benchmark centre for public authorities in the field of epidemiological studies concerning the general population and assessing public health programmes, so as to offer useful information for defining health policies and financing health services.

At the same time, the development of public health research is a priority objective in order to contribute to the development of knowledge in this field, particularly in terms of researching the determining factors and biomarkers that have a favourable influence on health.

The research themes of the Centre for Health Studies are the diseases prevalent in Europe and in Luxembourg:

- Cardio-cerebrovascular diseases and their risk factors: diabetes, obesity, the metabolic syndrome, nutrition and physical activity,
- Cancer,
- Neuro-degenerative diseases during the ageing process,
- Mental health disorders.

Measuring the prevalence and incidence of pathologies and their risk factors, measuring whether healthcare and resources meet the standards required for the population's health needs, identifying vulnerable populations due to major risk factors for their health, and equal access to healthcare are our research objectives.

#### **ONGOING PROJECTS**

Monitoring perinatal health in Luxembourg: The SUSANA register Project Manager: Aline LECOMTE Project sponsored and financed by the Ministère de la Santé

In order to respond to the European Peristat and EuroNeoNet indicators, in collaboration with the Health Directorate, the SUSANA register (which stands for "Monitoring the health of the mother and child at birth") was implemented in all maternity units nationwide. Data collection was carried out using Diane software. In parallel with this, the 2001-2002-2003 report on perinatal health in Luxembourg was published in 2009.

The mental health of young people in Luxembourg

**Project Manager:** Véronique LOUAZEL

**Project sponsored and financed** by the Ministère de la Santé

The objective is to identify ways of promoting mental health, prevention, and treating the mental disorders experienced by young people, as well as issues encountered by professionals, parents and children. Following an inventory carried out in 2008 among actors, workshops were held with them in 2009, in collaboration with the Health Directorate, in order to draw up a report concerning recommendations for public authorities, which will be published in 2010.

HBSC Survey (Health Behaviour School-Aged Children) Project Manager: Sophie COUFFIGNAL, MD Project sponsored and financed by the Ministère de la Santé

In 2009, a national report was prepared concerning the consumption of alcohol, tobacco, drugs, and concerning the polysubstance dependence of young people aged 12 to 18 who attended school in Luxembourg in 2006. The report will be published in 2010.

OSPEL (Obesity and overweightness in children and adolescents in Luxembourg) Project Manager: Hanène SAMOUDA Research project sponsored by the



Ministère de la Santé and co financed by the Ministère de la Santé and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

This research project is aimed at developing reliable, non-invasive methods suitable for children in order to diagnose obesity and predict the risks of complications associated with it, as well as to compare the effectiveness of two healthcare models for obese children, 205 children were included in the study, which enabled obese or overweight children to be identified who were already presenting with a metabolic syndrome, arterial hypertension, or diabetes. improvement of anthropomorphic indicators was measured 4 months after the study commenced. The oneyear results are expected in 2010.

ORISCAV-Lu (Observation of the risk factors for cardio-vascular diseases in Luxembourg) Project Manager: Alaa AL KERWI

Research project sponsored by the Ministère de la Santé and co financed by the Ministère de la Santé and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

1,432 participants representative of the population residing in Luxembourg

were recruited and benefited from an in-depth study of their state of health and their risk factors, as well as from biological examinations and anthropometric measurements. The prevalence of high blood pressure, treated and untreated diabetes, treated and untreated dyslipidemia, and risk factors (tobacco, alcohol, physical activity) in the general population was measured. nutritional habits of the resident population were identified for the first time and compared with the recommendations of the national health plan in Luxembourg.

NESCAV (Nutrition, Environment and Cardio-Vascular Health) Project Manager: Marie-Lise LAIR Project financed by INTERREG and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

The objective of this project, which lasts 3 years, is to map the cardiovascular risks of the cross-border population using the same standardized validated tool, identify nutritional behavioural patterns, and to look for potential links between pollutants and cardiovascular risks. Secondly, the level of the patient's own control of the cardiovascular risk factors is studied.

Report on cardiovascular health in Luxembourg

**Project Manager:** Nathalie REMOVILLE

Project sponsored and financed by the Ministère de la Santé

A report on cardio-vascular health in Luxembourg was prepared in 2009 and will be published in 2010. It summarizes the epidemiological data and takes stock of the programmes and initiatives implemented by the public authorities and professionals, as well as taking stock of available resources.

DIABETES (The state of diabetes in Luxembourg based on medical administrative data)

DIABECOLUX (Modelling complications related to diabetes based on medical administrative data) DIABETES Project Manager:

Valéry BOCQUET

Study sponsored and financed by the Ministère de la Santé

### Associated DIABECOLUX research project: Laurence RENARD.

An analysis of medical administrative data from the National Health Office concerning patients treated for diabetes between 2000 and 2006 enabled conclusions to be drawn



concerning the rate of prevalence of diabetes treated in the population covered by health insurance and its development, identification of the number and types of complications treated annually, and comparison of the types of healthcare given with international recommendations. The results published in 2010 will provide the basis for the preparation of a concerted pilot healthcare programme by the Ministry of Health and the National Health Office.

EDUDORA<sup>2</sup> (Therapeutic education of adults and adolescents who are suffering from diabetes or are obese) Project Manager: Marie-Lise LAIR Project financed by INTERREG and the Ministère de l'Enseignement

Supérieur et de la Recherche (MESR) The objective of this 3-year project, which started in June 2009, is to draw up a cross-border inventory of the practices and tools in the field of therapeutic education, identify the needs of patients, families and professionals for successful therapeutic education, and to propose recommendations to the public authorities aimed at reinforcing therapeutic observance.

Living in Luxembourg after a cerebral vascular accident (stroke): the repercussions for the family and quality of life. Equal access to healthcare and social resources

#### Project Manager:

Sophie COUFFIGNAL in collaboration with the University.

**Research project financed by** the Fonds National de la Recherche (FNR)

The objectives of the study are to establish the profile of patients suffering from strokes in Luxembourg, to study healthcare in a hospital environment, measure the individual, family and social repercussions of strokes, and to measure satisfaction regarding the services and resources used. The initial results will be published in 2010.

EUROLIGHT: The prevalence of headaches and measuring the socioeconomic impact of the condition in 11 European countries:

Project Manager: Colette ANDRÉE Research project co financed by the Public Health Executive Agency and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

The objective is to measure the prevalence of headaches in each of the participating European countries, and to measure the socio-economic impact and the personal and family repercussions of the condition, by using a standardized, validated questionnaire. The initial results for Luxembourg are currently being published. The results for the other countries are expected for 2010.

MémoVie (Prospective evaluation of the neuropsychological, biological and sub-clinical characteristics of the Mild Cognitive Impairment stage) Project Manager: Magali PERQUIN Research project conducted in partnership with the University of Luxembourg, financed by the Fonds National de la Recherche (FNR)

A cohort of elderly people was established. They benefited from an indepth neuropsychological evaluation, biological examinations, and a medical examination in the case of cognitive disorders. Monitoring after one year had elapsed was carried out. The initial results will be published in 2010.

PPSM (Prevention and promotion of mental health)

**Project Manager:** Laurence FOND-HARMANT

**Project financed by** INTERREG and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

The objective of this 3-year project, which started in May 2009, is to create a cross-border alliance for mental health prevention and promotion, reinforcing links between professional actors, patients, and their families. It is also aimed at evaluating regional pilot projects in order to adopt a position on whether it is useful to deploy them within other regions. Luxembourg chose to study the link between school dropouts and mental problems.

Measuring the satisfaction of people residing in home for dependent people

Project Manager: Marie-Lise LAIR Project sponsored and financed by the Ministère de la Sécurité Sociale

The objective of this project is to measure the satisfaction of people residing in home for dependent people about their accommodation conditions, their quality of life, and respect for their basic rights. This study will continue in 2010. The results will be published in 2010.

#### Pilot project for setting up a national injuries register in Luxembourg

Project Manager: Dritan BEJKO
Project sponsored and financed by
the Ministère de la Santé

**Pilot institution:** The Luxembourg Hospital Centre.

In 2009, a feasibility study concerning the setup of a national injuries register was conducted with the Luxembourg Hospital Centre. All of the various injuries entered in this institution over a period ranging from 15 October to 31 December 2009 are being collected. A feasibility report concerning deployment on other hospitals will be presented to the Ministry of Health in 2010.

#### Clinical epidemiology projects:

In 2009, collaborative efforts with clinicians were devoted to preparing the studies design and/or statistical analysis of the clinical data collected:

- Dr. Nico Diederich's project:
   Frequency and evolution of non-motor signs in early Parkinson's disease: a prospective case-control study.
- Dr. Martine Goergen's study on genetic factors in patients operated for morbid obesity
- Dr. Charles Delagardelle's project:
   Training patients with heart failure
- Preparations for the VERTIGO project and memory problems with Dr Alexandre Bisdorff

Assignment of healthcare staff according to healthcare needs and the workloads related to them

Project Manager: Marie-Lise LAIR

Studies sponsored and financed by the National Health Office.

The work involves developing the methodologies required for taking stock of the healthcare needs of patients in the various hospital departments, proposing assignment standards, organizing data collection, developing collection tools, validating the data collected, processing that data, calculating the allocation levels required for each hospital and department, and publishing the ratios for the hospitals and the National Health Office.

## Water management in Luxembourg Project Manager:

Nicolas SAUVAGEOT

Project sponsored and financed by the Water Management Administrative Authority

The project consisted of determining measurement uncertainties regarding bacteriological analysis methods for the Water Management Administrative Authority in Luxembourg and developing tools enabling professionals to carry out their uncertainty measurements in the future.

#### **KEY RESULTS**

#### I. PERINATAL HEALTH

The Centre for Health Studies, working in close collaboration with the Health Directorate, published the 2001-2002-2003 Report on perinatal health in Luxembourg, highlighting potential improvements to the SUSANA monitoring system.

#### II. HBSC INTERNATIONAL REPORT

Thanks to the work carried out in 2006-2007-2008 by the Centre for Health Studies, in partnership with the Health Directorate and the Ministry of Education, it was possible for the first time to compare health behavioural patterns of young people in Luxembourg with those of other countries, according to the same methodology, and include the details in the international report published in 2009.

### III. THREE INTERREG PROJECTS ACCEPTED

In 2009, three INTERREG projects concerning public health were started, two of which were conducted under the leadership of the Centre for Health Studies. They relate to the relationship between cardiovascular risk factors, nutrition and the environment and their impact on mental health promotion and prevention, as well as on the education of adults and adolescents who are diabetic or obese.

#### IV. ENJEUX SANTÉ PUBLICATION: HEALTHCARE FOR FOREIGNERS RESIDING IN LUXEMBOURG

Thanks to the data collected by the Social Security Medical Control Administrative Authority as part of requests for authorizations for healthcare abroad, it was possible for the first time to identify the development of this healthcare consumption in order to make new service offers available in Luxembourg such as radiotherapy, heart surgery, and fertility treatments.

#### V. HEALTH PORTAL

On 22 April 2009, the Health Portal (www.sante.lu) was launched. The Centre for Health Studies ensured its development and manages it on behalf of the Ministry of Health. The Health Portal enables citizens residing in Luxembourg and professionals to find all relevant information relating to the health system.

#### VI. 2009 ALASS PRIZE

Laurence Renard, a PhD student, was awarded the prize for excellence for the presentation of her work at the Annual Congress of the Latin Association for Health Systems Analysis, regarding the use of medical administrative databases for identifying states of health, and the application of this to diabetic nephropathy.



#### **COLLABORATIONS**

#### **NATIONAL**

- Ministère de la Santé, Direction de la Santé
- Ministère de la Sécurité Sociale, Inspection Générale de la Sécurité Sociale, Cellule d'Evaluation et d'Orientation de l'Assurance Dépendance, Administration du contrôle médical de la sécurité sociale
- Caisse Nationale de Santé
- Hôpitaux, et médecins cliniciens
- Université de Luxembourg, Département INSIDE (Pr Dieter Ferring) et UR Sciences de la vie, Unité Neuro-Inflammation (Pr Heuschling).
- Centre d'Etudes de Populations, de Pauvreté et de Politiques Socio-Economiques
- Centre des Technologies de l'Information de l'Etat
- Association Luxembourgeoise du Diabète
- Association Luxembourgeoise de Cardiologie

#### INTERNATIONAL

- Organisation Mondiale de la Santé,
   Dr. Piero Olliaro
- Université Libre de Bruxelles,
   Ecole de Santé Publique, Pr Alain
   Leveque
- Université de Liège, Ecole de Santé Publique (Pr Adelin Albert, Pr Michèle Guillaume), APES (Chantal Vandoorne)
- Centre Hospitalier Universitaire de Liège, Pr André Scheen, Pr Jean-Pierre Bourguignon, Pr Marc Ansseau
- Institut Wallon de Santé Mentale et Plate forme de concertation psychiatrique de la province de Luxembourg
- Centre Psychothérapeutique de Nancy, Dr. Demogeot
- Associations de patients: ESPOIR 54, Migraine Action Association, Association Espanola de Pacientes con Cefalea, Migraine Association of Ireland, Nederlandse Vereniging van Hoofdpijnpatienten
- Université Victor Segalen, Bordeaux 2, ISPED, Pr Rachid Salmi, Pr Jean-François Dartigues
- Observatoire Régional de la Santé et des Affaires Sociales de Lorraine

- LORDIAMN en Lorraine, Pr Olivier Ziegler
- Centre Hospitalier Universitaire de Nancy, CIC, Pr Faïez Zannad
- Centre Hospitalier Universitaire de Nice, Pr Michel Lantéri-Minet
- Université de la Sarre, CHU de Homburg, Pr Michael Böhm, Pr Ulrich Laufs
- SHG Saarland Heilstatten Gmbh
- Universitätsklinikum Essen, Pr Hans-Christoph Diener
- General Hospital Bamherzige Brüder Linz, Pr Christian Lampl
- Equipe de Recherche
   Opérationnelle en Santé de Montréal, Pr Charles Tilquin
- European Headache Alliance et World Headache Alliance
- Kauno Medicinos Universitetas, Lithuanie
- Clinico universitario de Valencia
- Migraine Clinic of London
- St, Olavs Hospital of Trondheim, Norvège
- Centro Italiano di Ricerche Neurologiche Applicate, Italie

#### 2009 PUBLICATIONS

- Surveillance de la santé
  périnatale au Luxembourg
  2001-2003, Rapport pour le
  Ministère de la Santé, Alkerwi
  A.,Couffignal S.,Rott C.,Wibrin
  G., Lair M.L., Differding-Gengler
  M.,Guedes E.,Molitor D.,Schanck
  J.M., Weber G., Wagener Y.
- 2. Inequalities in young people's health, Rapport International HBSC, collaboration à la réalisation pour le Luxembourg: Couffignal S. Vaillant M., Lair M.L., Wagener Y., Brochmann C., Weber G., Kafai A.
- Bocquet V., Smit J., Couffignal S., Lair M.L. « Evolution des soins des résidents luxembourgeois à l'étranger entre 1998 et 2007 » Enjeux Santé, 2009-3
- 4. Jemel B., Schuller A.M., Paulos C., Namer I., Dooms G., Pull C., Danion J.M., Foucher J.
  "Characterising the Spatio-Temporal Dynamics of the Neural Events occurring prior to ans up to overt recognition of Famous Faces". Journal of Cognitive Neurosciences
- Diederich NJ, Paolini V. Vaillant M. "Slow wave sleep and dopaminergic treatment in Parkinson's disease: a polysomnographic study" Acta neurologica Scandinavica 2009
- 6. Diederich NJ., Pieri V., Hipp G., Rufra O., Blyth S., Vaillant M. "Discriminative power of different non-motors signs in early Parkinson's disease. A case-control study" Movement Disorders 2009
- Alkerwi A., Boutsen M., Vaillant M., Barre J., Lair M.L., Albert A., Guillaume M., Dramaix M. "Alcohol consumption and the prevalence of metabolic syndrome: a metaanalysis of observational studies" Atherosclerosis 2009, 204 (2): 624-635

- 8. Bisdorff A., Andrée C., Vaillant M., Sandor P.S. "Headache associated dizziness in a headache population: prevalence and impact" Cephalalgia 2009
- Vaillant M., Andrée C., Barre
  J., Removille N., Tassorelli C.,
  Lanteri.Minet M., Lampl C., Ruiz
  de la Torre E., Lainez JM., Lair
  M.L. et al: "Development and
  validation of the EUROLIGHT
  questionnaire to evaluate the
  burden of headaches, migraine
  and chronic daily headaches in
  Europe" Cephalalgia 2009
- 10. Brasseur P., Agnamey P, Gaye O., Cisse M., Badiane M., Vaillant M., Taylor WR., Olliaro P." Dosing accuracy of artesunate and amodiaquine as treatment for falciparum malaria in Casamance, Senegal" Trop Med Int Health 2009 14(1):79-87
- 11. Navaratnaam V., Ramanathan S., Wahab MS, Siew Hua G., Mansor SM, Kiechel J.R., Vaillant M., Taylor WR, Olliaro P."Tolerability and pharmacokinetics of nonfixed and fixed combinations of artesunate and amodiaquine in Malaysian healthy normal volunteers" European journal of clinical pharmacology 2009
- 12. Frisch A., Seil R., Urhausen A., Croisier J.L., Lair M.L., Theisen D. "Analysis of sex-specific injury patterns and risk factors in young high-level athletes" Scandinavian journal of Medicine and Science in Sports 200
- 13. Lair, M.L., Cornez, J.P., Wibrin, G., Rott, C. Dotation en personnel soignant sur base des soins requis dans les unités d'hospitalisation, rapport 2009 pour la Caisse Nationale de Santé

- 14. Lair, M.L., Cornez, J.P., Wibrin, G., Rott, C. Dotation en personnel soignant sur base des activités dans les policliniques chimiothérapiques, rapport 2009 pour la Caisse Nationale de Santé
- 15. Lair, M.L., Cornez, J.P., Andrianne D., Rott, C. Dotation en personnel soignant sur base des activités dans les policliniques endoscopiques, rapport 2009 pour la Caisse Nationale de santé
- 16. Lair, M.L. Dotation en personnel soignant sur base des activités dans les services de dialyse, rapport 2009 pour la Caisse Nationale de Santé
- Cornez J.P. Développement du module Euronéonet du logiciel DIANE.





NATIONAL DRUG COORDINATOR

Alain ORIGER

**DEPARTMENT OF PUBLIC HEALTH** 

## EUROPEAN MONITORING CENTRE FOR DRUGS AND DRUG ADDICTION LUXEMBOURG FOCAL POINT



Complete list of collaborators available on www.crp-sante.lu

### OBJECTIVES OF THE NATIONAL EMCDDA FOCAL POINT

#### AT EUROPEAN LEVEL:

The National Focal Point (NFP) provides the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) with reliable and comparable data on the drugs phenomenon in Luxembourg.

The main objective is to meet the EMCDDA's annual requirements and to contribute to the achievement of the European decentralized agency's work programme. The following core issues are part of the NFPs yearly contributions:

- Prevalence and patterns of use among the general population
- Problem drug use
- Treatment demand
- Drug-related deaths and mortality among drug users
- Drug-related infectious diseases

#### DEPARTMENT OF PUBLIC HEALTH

## EUROPEAN MONITORING CENTRE FOR DRUGS AND DRUG ADDICTION LUXEMBOURG FOCAL POINT

- Crime, markets and supply data
- Public expenditures dedicated to the fight against drugs
- Action on new drugs and emerging trends

#### AT NATIONAL LEVEL:

The priorities of the NFP are the collection and the compilation of any relevant information on drugs and drug addiction. Over the past ten years, the Focal Point has consolidated national and international cooperation networks and allows promoting and initiating research activities in the field of illicit drug use and its consequences.

#### ONGOING PROJECTS/WORK

RELIS : Réseau Luxembourgeois d'Information sur les Stupéfiants

Acronym: RELIS Grant period:

January 2009 – December 2009 **Financial support:** Activities of the National Focal Point are co-financed by the EMCDDA and the Ministère de la Santé

#### Project summary:

RELIS (abbreviation of "Réseau Luxembourgeois d'Information sur les Stupéfiants" - "Luxembourgish Information Network on Drugs") consists of a network of data sources, standardized data collection tools, a centralized database and a routine data processing protocol. It has been launched in 1994 and is presently involving all specialized drug treatment services as well as psychiatric units of general hospitals, prison and judicial police specialized in the fight against drugs. It serves the epidemiological surveillance of problem drug use at the national level and provides sound evidence for policy making and action planning. Due to its multiple data sources, RELIS also provides valuable data for further research activities such as prevalence studies or studies on health correlates of drug abuse.

#### People involved:

Céline DIEDERICH, Sofia LOPES DA COSTA, David MARCIC, Alain ORIGER, Pascale STRAUS

Serial multi-methods drug prevalence study 1997 – 2007 Estimation de la prévalence nationale de l'usage problématique de drogues a risque élevé et d'acquisition illicite - Etude sérielle multi-méthodes 1997 – 2000 – 2003 – 2007

Acronym: PREVAL2009
Grant period: January 2009 –

December 2009

**Financial support:** Activities of the National Focal Point are co-financed by the EMCDDA and the Ministère de la Santé

#### Project summary:

The PREVAL2009 study had two primary objectives. It is the second serial update of multi-methods prevalence data (2003 and 2007) on PDUs at national level. Furthermore, it allowed to analyse the evolution of PDU prevalence between 1997 and 2007, since applied methodologies and data sources referred to during the same period are highly comparable. The following methods were applied: Case finding (CF), capture-recapture on 2, 3 and 4 sources (CR 2,3,4), truncated Poisson model associated to Zelterman's and Chao's estimators (tPm), and four different multiplier methods using data from law enforcement sources, drug mortality registers (D1,2,3) and treatment agencies (T).

#### People involved:

Céline DIEDERICH, Sofia LOPES DA COSTA, David MARCIC, Alain ORIGER, Pascale STRAUS

#### **KEY RESULTS**

- 1. Operational routine problem drug use surveillance.
- 2. Updated and serial national drug prevalence figures.

#### **COLLABORATIONS**

#### NATIONAL

 Treatment centres: Centre thérapeutique de Manternach (CTM – CHNP), Fondation Jugendan Drogenhëllef (JDH), Tox-In (CNDS), Service thérapeutique Solidarité Jeunes (Jongenheem asbl), Centre Emmanuel asbl.

Psychiatric services of general hospitals: Centre Hospitalier Neuro-psychiatrique (CHNP), Centre Hospitalier du Luxembourg (CHL), Hôpital Kirchberg (HK), Zitha Klinik (ZK), Centre Hospitalier du Nord (CHN), Centre Hospitalier Emile Mayrisch (CHEM)

Judicial Institutions : Judicial Police (section stupéfiants), Centre pénitentiaire de Schrassig (Programme Tox)

#### INTERNATIONAL

- FMCDDA
- REITOX national Focal Points
   Council of Europe (Pompidou Group)

#### **2009 PUBLICATIONS**

Origer A. L'état du phénomène de la drogue au Grand-duché de Luxembourg. 2009, PF OEDT – CRP-Sante. Luxembourg.

Origer A. Estimation de la prévalence nationale de l'usage problématique de drogues à risque élevé et d'acquisition illicite - Etude sérielle multi-méthodes 1997 – 2000 – 2003 – 2007, Séries de recherche 6. 2009. PF OEDT – CRPSanté. Luxembourg.







**HEAD OF LABORATORY** 

Prof. Charles PULL, MD, MA

**DEPARTMENT OF PUBLIC HEALTH** 

## LABORATORY OF EMOTIONAL DISORDERS



Complete list of collaborators available on www.crp-sante.lu

#### **OBJECTIVES OF THE LABORATORY** OF EMOTIONAL DISORDERS

The Laboratory of Emotional Disorders (LATE) carries out studies on emotions with a particular focus on anxiety and depression. It has strong links with the Clinique des Emotions of the "Centre Hospitalier de Luxembourg" (CHL) which focuses on the treatment of patients suffering from anxiety and other emotional disorders.

The LATE focuses on the study and treatment of anxiety, depression and other emotions that define or accompany anxiety disorders, depressive disorders and eating disorders. It specializes in the use of virtual reality technology to assess emotions and of virtual reality exposure therapy to treat emotional disorders.

the effects of exposure to anxiety provoking virtual environments are compared to the effects elicited by exposure to the same environments in imagination. The main objective of the project is a randomized controlled trial (RCT) designed to compare the efficiency of traditional cognitive behaviour therapy and virtual reality exposure therapy (VRET) in each of these phobias.

software and hardware. In a first step,

#### People involved:

Charles PULL, Marc DAMME. Maxime LARCELET

#### 2. Psychological assessment of severe obesity project

Assessment of mental status including body image in severe obesity

Acronym: PASO

Contract number: REC-Late-

20080101

Grant period: November 2008-

October 2011

Financial support: CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR),

Luxembourg

#### Project summary:

The project intends to identify major psychological problems (including problems with body image) associated with severe obesity (BMI over 40), to study the course of these problems at follow-up after surgery and to detect psychological factors that may be involved in the short-term as well as the long-term prognosis of patients presenting for gastric by-pass surgery. All candidates are assessed prior to the surgery and one year after surgery. The assessment includes assessment of depression, anxiety, self-esteem, quality of life, handicap, body dissatisfaction and the presence of eating disorders such as binge eating disorders. In addition, the project includes the assessment of body image by questionnaires and by exposure to different body shapes and sizes using virtual reality.

#### People involved:

Charles PULL. Gloria AGUAYO. Maxime LARCELET

#### DEPARTMENT OF PUBLIC HEALTH

## LABORATORY OF **EMOTIONAL DISORDERS**

#### ONGOING PROJECTS

#### 1. Specific phobias project

Assessment and treatment of specific phobias using virtual reality Acronym: ATSP-VR

Contract number: REC-LATE-

20080102

Grant period: November 2009-

October 2011

Financial support: CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Luxembourg

#### Project summary:

The project aims to explore the potential of virtual reality exposure to study and treat specific phobias such as fear of driving, fear of flying and fear of public speaking. To achieve this goal, the LATE acquires, further develops and adapts relevant specific



#### **KEY RESULTS**

#### I. Specific phobias project

Virtual reality exposure therapy or VRET has been shown to be a viable treatment option for panic disorder with agoraphobia.

Virtual environments for fear of driving a car and fear of flying have been acquired, adapted and further developed.

Equipment for measuring physiological parameters of anxiety including heart rate, skin conductance, muscle tension and blood oxygen saturation has been acquired and tested.

## II. Psychological assessment of severe obesity project

Patients with severe obesity have abnormal rates of depression, anxiety, handicap and dissatisfaction with their body size and low rates of self-esteem and quality of life.

Patients with severe obesity have problems with their body image and they have a tendency to overestimate their weight which is linked to depression.

#### **COLLABORATIONS**

#### **NATIONAL**

The LATE is working in close collaboration with the Centre for Health Studies at the CRP-Santé. In particular, the work on obesity is done in the framework of a global project initiated by the CES on obesity.

The LATE is also working in close collaboration with the Clinique des Emotions of the Centre Hospitalier de Luxembourg. A major part of the assessment in all the current projects is done by psychometricians (L.Seven, P. Pereira, C. Arendt and S. Bachim). All treatments included in the projects are done by the psychotherapists of the day hospital (MC Pull, F Münster, L. Wouters, P Pereira and F Pezzan)

The LATE is working in close collaboration with the Unité polyvalente de l'obésité or UPO (Coordinator F. Dadoun) of the Centre Hospitalier de Luxembourg.

#### INTERNATIONAL

The LATE has close connections with the team of S. Bouchard at the Université du Québec en Outaouais in Gatineau, Quebec, Canada for the project on virtual reality in specific phobias.

The LATE has close connections with the team of C. Botella at the Universitat Jaume I, Castellon, Spain for the project on virtual reality in obesity.

#### 2009 PUBLICATIONS

- 1. Pull P. TCC et classification des troubles mentaux. In J. Cottraux (ed): TCC et Neurosciences. Masson, 3-28, 2009
- 2. Pull C. Current empirical status of acceptance and commitment therapy. Current opinion in Psychiatry. 55-60, 2009
- 3. Pull C, Pelissolo A, Zaoui M, Pull-Erpelding MC, Wouters L, Aguayo G, Larcelet M, Genouilhac V,Duinat A, Znaid F, De Mey Guillard C, Panagiotaki P,, Fanget F, Mollard E, Gaillard S, Gueyffier F, Roche S, Ecochrad R, Berthoz A, Jouvent R and Cottraux J. A Randomized Controlled Study of Virtual Reality Exposure Therapy and Cognitive-Behaviour Therapy in Panic Disorder with Agoraphobia. Abstract of an oral presentation at Cybertherapy 14. CyberPsychology & Behavior, 12, 5, 582, 647, 2009
- 4. Aguayo G, Dammé M, Arendt C, Bachim S, Larcelet M, Vaillant M and Pull C. Body image in obese candidates for bariatric surgery. Abstract of an oral presentation at Cybertherapy 14. CyberPsychology & Behavior, 12, 5, 582, 2009
- 5. Dammé M, Schuller A, Schilbach L and Pull C. (2009). The perception of social interactions by subjects with social phobia: A study with virtual characters. Abstract of an oral presentation at Cybertherapy 14. CyberPsychology & Behavior, 12, 5, 602-603, 2009
- 6. Aguayo G., Pull C., Michel G., Goergen M., Azagra J-S. Factors leading patients with severe obesity to present for gastric bypass surgery. Abstract of a poster presented at the European Congress on Obesity (ECO2009). Obesity Facts, Vol. 2, suppl. 2, 254, 2009.
- 7. Aguayo G., Pull C., Michel G., Goergen M., Azagra J-S. Psychological changes 1 year after gastric bypass surgery in 84 severe obese patients. Abstract of a poster presented at the European Congress on Obesity. Obesity Facts, Vol. 2, suppl. 2, 253, 2009.





# **DEPARTMENT OF PUBLIC HEALTH**

# **HEAD OF LABORATORY**

Prof. Daniel THEISEN, PhD

# ASSOCIATE HEADS OF LABORATORY

Prof. Dietrich PAPE, MD, PhD
Prof. Romain SEIL, MD, PhD
Prof. Axel URHAUSEN, MD, PhD

# SPORTS MEDICINE RESEARCH LABORATORY



Complete list of collaborators available on www.crp-sante.lu

# OBJECTIVES OF THE SPORTS MEDICINE RESEARCH LABORATORY

The general aim of the Sports Medicine Research Laboratory is to develop and stimulate high-level research in the field of sports medicine. According to the World Health Organization, sports medicine investigates the impact of movement, training and sports, as well as inactivity, on healthy and ill individuals of all ages to provide decisive information for prevention, therapy, rehabilitation and sports. The current main focus of the Sports Medicine Research Laboratory is on sports injury prevention and recovery/ rehabilitation ("return-to-play").

New technologies and procedures applicable to rehabilitation are being developed, but are not yet integrated into full research projects. On a medium-term basis two other sectors will be developed: healthy ageing and chronic disease management.

risk factors, introduce prevention initiatives and verify their effectiveness by repeating step 1. The system under development is an internetbased platform, by which relevant data pertaining to training volume, subjective training intensity and, if applicable, sports injury characteristics are collected. This platform allows for real-time, individualized injury surveillance based on objective risk factors pertaining to the athlete's training and injury characteristics. Additionally, post-hoc injury statistics can be generated to guide general prevention initiatives.

# People involved:

Daniel THEISEN, Hélène AGOSTINIS, Anne FRISCH, Thierry WINDAL, David MARCIC

# Associated PhD project:

**Title:** Injury prevention in youth sports

(A. Frisch)

**Start date:** Sept. 2006 **Financial support:** None

# 2. Running-related injuries

Injury risk factors of novice and experienced runners preparing for a marathon race: a prospective study Acronym: RRI

Acronym: Kr

**Financial support**: This project is financially supported by the Ministère de l'Enseignement Supérieur et de la Recherche (MESR), an AFR grant will be solicited at the Fonds National de la Recherche (FNR)

# Project summary:

Running is one of the best strategies to prevent cardio-respiratory affections, metabolic syndrome and obesity. However, running also induces running-related injury (RRI), which, as a consequence, could discourage an active life-style. Especially novice runners seem to be at an increased risk for RRI compared to more experienced runners. The major aim of this project is to compare RRIs in novice and experienced runners and to test if they have different risk factor profiles. The findings of this project will provide practical recommendations and valuable feedback to runners of all performance levels as well as to trainers and coaches involved in longdistance running training.

# People involved:

Joakim GENIN, Daniel THEISEN, David MARCIC

**DEPARTMENT OF PUBLIC HEALTH** 

# SPORTS MEDICINE RESEARCH LABORATORY

# ONGOING PROJECTS

INJURY PREVENTION IN YOUTH SPORTS

# 1. Injury prevention in youth sports

Acronym: EVARI Contract number: NA Grant period: 2006 Financial support:

This project is financially supported by the Département Ministériel des Sports, the Comité Olympique et Sportif Luxembourgeois and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

# **Project summary:**

The aim of this project is to develop and implement an injury surveillance system at the national high school "Sportlycée". Injury surveillance is approached with a systematic, 4-step procedure: establish the extent of the sports injury problem, identify

### **KEY RESULTS**

- I. The research group has gained invaluable experience with sports injury surveillance and has developed a working methodology based on the state-of-the-art scientific literature (Frisch et al, 2009a, 2009b).
- II. Sports injury surveillance at the "Sportlycée" during the school years 2007-2008 and 2008-2009 has defined the extent of the problem and identified key risk factors that can now be targeted by active prevention measures.
- **III.** The internet-based platform makes it possible to perform online monitoring and injury prevention of the athletes from the "Sportlycée". This methodology is currently being applied in the framework of the RRI-project and shows strong potential in other sport contexts.

### **COLLABORATIONS**

### **NATIONAL**

- Departement Ministériel des Sports
- Centre Médical Olympique Luxembourgeois,
   Centre de l'Appareil Locomoteur, de Médecine du Sport et de Prévention du CHL
- Comité Olympique et Sportif Luxembourgeois
- Société Luxembourgeoise de Médecine du Sport
- Société Luxembourgeoise de Kinésithérapie du Sport
- Société Luxembourgeoise de Recherche en Orthopédie et en Médecine du Sport

# INTERNATIONAL

- Université de Liège (prof. Jean-Louis Croisier)
- Universität des Saarlandes (prof. Tim Meyer)
- Universitaetsklinikum des Saarlandes (prof. Henning Mardy)

# 2009 PUBLICATIONS

- Frisch A, Croisier JL, Urhausen A, Seil R & Theisen D (2009a). Injuries, risk factors and prevention initiatives in youth sport. Br Med Bull, Idp034 [pii], 10.1093/bmb/ldp034.
- 2. Frisch A, Seil R, Urhausen A, Croisier JL, Lair ML & Theisen D (2009b). Analysis of sex-specific injury patterns and risk factors in young high-level athletes. Scand J Med Sci Sports, 19: 834-41.
- 3. Theisen D, Nührenbörger C & Frisch A (2009). Sportverletzungen des Kniegelenks im Kindesalter. Arthroskopie, 22: 8-13.
- 4. Lorbach O, Wilmes P, Theisen D, Maas S, Zerbe T, Seil R, Kohn D (2009). Reliability of a non-invasive device to objectively measure tibial rotation. Knee Surg Sports Traumatol Arthrosc, 17:920-6.







DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

# LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY



**HEAD OF LABORATORY** 

Dr. François HENTGES, MD

Complete list of collaborators available on www.crp-sante.lu

# OBJECTIVES OF THE LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

Allergic and immune diseases are a major health problem for hundreds of millions of people worldwide. It is the objective of the Laboratory of Immunogenetics and Allergology (LIGA) to strengthen its skills in applied research, to develop competitive basic research and to create an optimal environment for improved diagnosis, prevention and treatment of patients with allergic and immune-mediated diseases. Research is being performed in close collaboration with the national unit of Immunology and Allergology at the CHL.

Secondly the laboratory applies its research potential at improving patient diagnosis and care. This implies:

- Development of reagents for in vitro detection of patient sensitization to food and respiratory allergens.
- Development of protein and DNA reagents to detect and trace animal allergens and proteins of animal origin.
- Technology transfer to the laboratory of Immunology and Allergology at the CHL in molecular biology techniques.

### **ONGOING PROJECTS**

BIOMOLECULAR, IMMUNE AND BIOCHEMICAL CHARACTERIZATION OF ANIMAL ALLERGENS

 Biomolecular, immune and biochemical characterization of lipocalins

Acronym: Animal allergens Contract number: REC-LIGA -20060702 and REC-LIGA-20081101 Grant period:

July 2007-April 2009 May 2009-October 2012 **Financial support:** Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

# Project summary:

Conventional animal allergen extracts are prepared from pelt, hair, dander, saliva, serum, urine. Although much work has been done on the characterization of these native allergen extracts, they are difficult to standardize as to what the content of major and minor allergens are concerned. The major goals of cloning and characterization of allergens are the definition of their biochemical as well as immune properties and the use of recombinant allergens for diagnostic applications

Lipocalins represent an important family of mammalian respiratory allergens. The focus of the present project is the isolation and characterization of lipocalins from the guinea pig and the rabbit.

# People involved:

Christiane HILGER, Kyra SWIONTEK, Stéphanie KLER

DEPARTMENT OF VIROLOGY, ALLERGOLOGY, AND IMMUNITY

# LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

In its scientific strategies the LIGA aims at performing high quality basic research focusing on the:

- Characterization at the protein and DNA-level of three major animal allergen families: lipocalins, serum albumins and parvalbumins.
- Analysis of the cellular immune response, including cellular regulatory mechanisms to selected allergens belonging to these protein families.
- Analyzing interactions between the nervous system and the immune system by studying the effects of several neurotrophins on natural killer cells.
- Study of the functional cellular modification found in TAP deficiency.

# 2. Further characterization of the Argas reflexus tick lipocalin Arg r 1

Acronym: Tick lipocalin Contract number: REC-LIGA-

20061002

Grant period: September 2007-

July 2010

Financial support: Ministère de l'Enseignement Supérieur et de la

Recherche (MESR)

# **Project summary:**

During the last decade severe anaphylactic reactions provoked by pigeon tick bites have been reported from France, Italy, Germany and Poland. Diagnosis of pigeon tick allergy relies on a careful clinical history. Diagnostic confirmation is hampered by the fact that no commercial test is available. The cDNA coding for the major allergen of Argas reflexus, Arg r 1, has been isolated in our laboratory. The aim of this project is firstly to develop the diagnostic potential of the recombinant allergen and secondly to analyze its immune properties and elucidate its biological function.

# People involved:

Christiane HILGER, Delphine LENTZ

3. Specific detection of food allergens from animal and plant origin: Molecular characterization for application in clinical diagnosis.

Acronym: Allergens

Contract number: FNR/SECAL/07/06

Grant period:

April 2008- March 2010

Financial support: Fonds National de la Recherche (FNR) and Ministère de l'Enseignement Supérieur et de la Recherche (MSER)

# Project summary:

This is a follow-up project in the FNR SECAL program and comprises an animal and a plant component. In the animal component the project intends to further characterize the previously produced recombinant  $\beta$  parvalbumin isoforms from cod, salmon, tuna, mackerel and in addition characterize an allergen previously detected in cod. For tracing purposes it is foreseen to develop specific oligonucleotide probes and monoclonal antibodies specifically directed at herring, redfish, tuna and trout parvalbumin.

### People involved:

François HENTGES, Christiane HILGER, Annette KUEHN, Tania SCHEUERMAN, Thorsten GRAF

4. T cell epitopes recognized by natural and induced T regulatory cells on 3 major allergens: Cat Fel d 1, cat serum albumin, cod parvalbumin. Analysis in the BALB/c mouse.

Acronym: T cell epitopes Contract number: REC-LIGA-

20070118 Grant period:

March 2007 – September 2009 Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

# Project summary:

T cell epitopes of allergens, when processed and presented by dendritic cells to T cells in the context of accessory signals, push the further differentiation of naïve and trigger the immune response of memory T cells. This project aims to identify, in a BALB/c mouse model, the T cell epitopes on 3 allergens (cat Fel d 1, cat serum albumin and cod parvalbumin) recognized by induced and natural regulatory T cells. The analysis of T cell proliferation via an in vitro myeloid dendritic cell- T cell assay, as well as the profile of cytokines secreted is used to identify the role of the major epitopes of these allergens in inducing Th1, Th2, Th17 and induced Treg cells.

# People involved:

François HENTGES, Cathy LEONARD, Caroline DAVRIL, Olivia DOMINGUES

5. Challenging memory Th2-cell plasticity with TLR ligands 9, 4 and cat allergens.

Acronym: Th2-cell plasticity Contract number: REC-LIGA Grant period: December 2009 –

November 2012

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

# Project summary:

CD4+ T helper (Th) cells comprise four main lineages: Th1, Th2, Th17 and T regulatory cells, according to their function and pattern of cytokine secretion. It is becoming increasingly clear that Th cell subtypes are heterogeneous and have more

plasticity than originally thought. In this project, mice will be immunized with cat allergens in presence of Al (OH)3 or Flagellin in order to induce a Th2 type immune response. Splenic memory T cells will be challenged ex vivo with CpG and LPS to induce a shift from the Th2 type to Tr1 and Th1 cells. Different stages of change in Th subtype will be characterized and gene expression profiling will be performed to identify the cellular pathways involved in the shift in vivo and in vitro in a mouse system before being analyzed for human cells in vitro.

# People involved:

François HENTGES, Cathy LEONARD, Caroline DAVRIL, Olivia DOMINGUES

# INNATE IMMUNITY AT THE **CELLULAR LEVEL**

# 1. Asthma, Natural Killer Cells and **Nerve Growth Factor**

Acronym: NK and NGF Contract number: REC-LIGA-

20090104

Grant period: 4.2009 - 9.2011 Financial support: Ministère de l'Enseignement Supérieur et de la

Recherche (MESR)

# Project summary:

Neurotrophins, crucial growth and survival factors of the nervous system, can also act on immune cells and are produced by some immune cells. We focus on the neurotrophin Nerve Growth Factor (NGF) and its receptors TrkA (high affinity) and p75NTR (low affinity) in the context of NK cell biology. We could show for the first time that mouse NK cells express TrkA but not p75NTR. Initially present on 20% of NK cells (and differentially on certain NK cell subsets), TrkA is progressively upregulated with time on activated NK cells. TrkA+ NK cells are equally distributed among Ly49+ compared to Ly49- subpopulations. We are currently studying the effects of NGF on the functional properties of NK cells (cytotoxicity and cytokine production).

# People involved:

Aurélie POLI, Jacques ZIMMER, Natacha RALAINIRINA

# Associated PhD project:

Title: Regulation of NK Cells by the

Nerve Growth Factor **Start date:** 2006

**Financial support:** Fonds National de la Recherche (FNR) (AFR grant)

# Investigation of Natural Killer Cells in Human and Mouse TAP Deficiency

Acronym: NK and TAP Contract number: REC-LIGA-

20081202

Grant period: September 2009 -

August 2012

**Financial support:** Ministère de l'Enseignement Supérieur et de la

Recherche (MESR)

# Project summary:

TAP deficiency is a rare disease characterized by a very low cell surface expression of MHC class I molecules. Clinically, the patients present recurrent infections of the respiratory tract and granulomatous skin ulcers. Their NK cells are usually hyporesponsive, as a normal NK cell education is not possible in an environment nearly devoid of MHC class I molecules. Nevertheless, we recently described several patients with cytotoxic NK cells.

We are investigating NK cells from patients and mice as a model of "missed" NK cell education.

and more generally what this model can tell us about NK cell education. We are also building up techniques supposed to allow a very detailed phenotypic NK cell characterization by flow cytometry.

# People involved:

Aurélie POLI, Jacques ZIMMER

# Associated PhD project:

**Title:** Investigation of Natural Killer Cells in mouse TAP Deficiency **Financial support:** An application for an AFR Grant from the FNR has been made; the decision is still pending.

3. Investigation of the Role(s) of the neurotrophins GDNF and Neurturin in allergic Asthma in Mouse Models

Acronym: GDNF and Neurturin in

mouse Asthma

**Contract number:** FNR-LIGA-20070403 or FNR/BIOSAN/07/21

Grant period:

January 2008-December 2009
Financial support: Fonds National de

la Recherche (FNR)

# Project summary:

Murine models help in elucidating the pathogenesis of allergic asthma and the evaluation of new therapeutic strategies. By using a C57BL/6 mouse model, we observed the influence of two neurotrophins, GDNF and Neurturin (NTN), in airway inflammation. In our model, we compared the inflammatory response in NTN-KO mice and in GFRalpha2-KO mice with the wild-type mice.

# People involved:

Jacques ZIMMER, Maud THERESINE, Tatiana MICHEL

### **KEY RESULTS**

# Biomolecular, immune and biochemical characterization of animal allergens

I. Several allergens of the guinea pig have been isolated and characterized in the frame of the lipocalin project. Four allergens have been cloned and the proteins were expressed in E. coli. Sera from guinea pig allergic patients were used to evaluate the diagnostic potential of those allergens. Each of the recombinant proteins was recognized by more than 50 % of the patient sera. These are the first recombinant allergens available for diagnosis of guinea pig allergy. A PCT application covering the 4 allergens has been filed at the European Patent Office. Title: Novel Caviidae allergens and uses thereof.

In a collaborative study with the University of Strasbourg, porcine and chicken hemoglobin as well as porcine and chicken serum albumin could be shown to be allergens responsible for professional and domestic allergic cross-sensitization.

II. A fish species-specific PCR assay oligonucleotide using primers designed for specific parvalbumin amplification has been standardized and submitted for publication. Mouse antisera raised specifically against different fish parvalbumins have allowed to measure the parvalbumin content in 8 commonly consumed fish species in native and processed form. Concentrations of parvalbumin, the major fish allergen varied from several- to hundred-fold between fish species. This finding is of major clinical relevance. IgE sensitization to chicken α parvalbumin has been described in a case of allergy to chicken.

III. The type of cytokines secreted by T cells when stimulated by the major Fel d 1 epitopes or CSA epitopes showed that although some peptides have a dominant roles in inducing immune reactions with mixed Th1-, Th2-, Th17- and induced T reg-type orientation, the role of (TLR) ligands like LPS, CpG or Flagellin in changing the profile of the immune responses was found to be important. The phenotype of responding T cells seems to be far less rigid than previously assumed. This effect of TLR-ligands on T cell plasticity will be further analyzed.

# Innate Immunity at the cellular level

I. In the context of the TAP project, we were the first to describe a patient with cytotoxic NK cells before activation. In the meantime, among four new patients, two additional ones displayed a significant NK cell cytotoxicity at baseline. Interestingly, all the "cytotoxic" patients expressed the NK cell receptor KIR2DL5, at higher percentages and higher expression levels than normal donors. The ligand of this receptor is still unknown.

II. In the experimental mouse asthma model we have shown an increase of Th2 cytokines, an increased infiltration of eosinophils in airways and lung tissue and airway hyper-reactivity in NTN-KO mice compared to wild-type mice. Furthermore, we observed mRNA expression of GFRalpha2, the NTN receptor, in different types of cells present in lung and lymph node tissues. Our aims are to determine the source and the regulation of NTN and its receptor in the airways.



### **COLLABORATIONS**

# **NATIONAL**

- CRP-Santé, Microarray Center: Dr. L. Vallar
- The National Unit of Immunology and Allergology at the CHL
- The Proteomics Platform of the CRP-Gabriel Lippmann

# INTERNATIONAL

- Département de Pneumologie at the University of Strasbourg,
   Prof. F. de Blay and Prof. G. Pauli
- Center for Clinical and Experimental Allergology, Rome -Dr. Adriano Mari
- Department of Biochemistry,
   Saarland University Prof. Rita
   Bernhardt
- CEH in Oxford, Dr. Guido Paesen
- Department of Veterinary
   Medecine, University of Namur Dr.
   Claire Diederich
- Neuroscience Center, University of Helsinki, Finland, Prof. Matti Airaksinen
- University of Barcelona, Spain, Dr. Carlos Vilches
- University of Southampton, UK, Prof. Stephan Gadola
- Charité Universitätsmedizin, Berlin, Germany - Dr. Katja Kotsch,
- Department of Immunology and Allergology, University of Antwerp, Belgium - Dr. Didier Ebo
- Max-Planck-Institute, Freiburg, Germany - Dr. Marinus Lamers
- Division of Molecular and Cellular Science, University of Nottingham, UK - Dr. Franco Falcone

### 2009 PUBLICATIONS

- A. Kuehn, C. Hilger, F Hentges. Anaphylaxis provoked by ingestion of marshmallows containing fish gelatin. Journal of Allergy and Clinical Immunology 2009;123:708-709
- 2. F. Hentges, C. Hilger, M. Kohnen, G. Gilson. Angioedema and estrogendependent angioedema with activation of the contact system. Journal of Allergy and Clinical Immunology 2009;123: 262-264
- A. Kuehn, C Lehners, C Hilger, F Hentges. Food allergy to chicken meat with IgE reactivity to muscle α -parvalbumin. Allergy 2009; 64:1557-1558.
- **4.** F. Hentges. Allergènes de poissons et crustacés. Revue Française d'Allergologie 2009; 49:156-159.
- A. Poli, T. Michel, M. Thérésine, E. Andrès, F. Hentges, J. Zimmer. CD56bright natural killer (NK) cells. An important NK cell subset. Immunology 2009; 126:458-465.
- 6. D. von Bubnoff, E. Andrès, F. Hentges, T. Bieber, T. Michel, J. Zimmer. Natural killer (NK) cells in atopic and autoimmune diseases of the skin. Journal of Allergy and Clinical Immunology 2010; 125:60-68.

- C. Hilger, K. Swiontek, F. Hentges, C. Donnay, F. de Blay, G. Pauli. Occupational inhalant allergy to pork followed by food allergy to pork and chicken. Sensitization to hemoglobin and serum albumin. International Archives of Allergy and Immunology 2010;151:173-178
- 8. D.G. Ebo, A. Kuehn, C.H. Bridts, C. Hilger, F. Hentges, W.J. Stevens. Monosensitivity to pangasius and tilapia caused by allergens different from parvalbumin. J Investig Allergol Clin Immunol 2010; in press

# BOOKS

J. Zimmer Editor: Natural Killer Cells: at the Forefront of Modern Immunology. Springer Verlag Berlin, Heidelberg, 2010.





DEPARTMENT OF VIROLOGY, **ALLERGOLOGY AND IMMUNITY** 

# LABORATORY OF PLANT MOLECULAR BIOLOGY





**HEAD OF LABORATORY** 

Dr. André STEINMETZ, PhD

Complete list of collaborators available on www.crp-sante.lu

# OBJECTIVES OF THE LABORATORY OF PLANT MOLECULAR BIOLOGY

The Plant Molecular Biology Laboratory combines basic research in molecular and cell biology to study fundamental biological processes in plant and animal cells and to elucidate the mechanisms of action of novel bioactive components from Chinesemedicinal plants.

# **ONGOING PROJECTS**

# 1 . Identifying LIM Protein Functions in Plants

Contract number: 20070117 Grant period: April 2007-

March 2010

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

# DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

# LABORATORY OF PLANT MOLECULAR BIOLOGY

### Project summary:

The actin cytoskeleton is a complex intracellular system of dynamic filaments supporting various biological processes in higher eukaryotes, including cell division, growth, contraction, motility and defence against pathogens. In humans, deregulation of its dynamics or organization can have dramatic consequences on health, e.g. myopathies and cancers. Aims of the PMB unit are to unravel the actin cytoskeleton functions and its regulation. Particular focus is on evolutionary conserved actin regulatory proteins expected to participate in fundamental cellular actin-based processes. Comparative analyses in different fields should identify central actin regulators and uncover common pathways involved in actin cytoskeleton remodelling. By studying LIM proteins with dual cytoskeletal and nuclear functions, the unit addresses the emerging issue on cytoskeleton-nuclear cross-talks.

### People involved:

Clément THOMAS, Monike DIETERLE, Céline HOFFMANN, Danièle MOES, Flora MOREAU, Jessica PAPUGA, Stéphane THOLL

# Associated Postdoc project (D. Moes):

**Title:** Nuclear Functions of Tobacco LIM Protein NtWLIM2

**Start date:** 01.02.2008

Financial support: AFR grant TR-PDR

BFR07-100

# Associated PhD project (J. Papuga):

**Title:** LIM Proteins in the Regulation of the Actin Cytoskeleton

Start date: 01.01.2007 Financial support: AFR grant EXT-

BFR06-084 TR

# Associated PhD project (S. Tholl):

**Title:** The Actin Cytoskeleton in the Plant Cell Response to Mechanical Forces - Possible Implication of the

LIM Proteins

Start date: 01.04.2008

Financial support: AFR grant TR-PHD

BFR07-130

# 2. Chinese Medicinal Herbs: TCMCANCER

Contract number: 20080402

Start date: 2008

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR); European Commission, Framework 7 Programme: International Research Staff Exchange Scheme (IRSES)

# **Project summary:**

Chinese herbal medicine, which is an important part of Traditional Chinese Medicine (TCM), has a long tradition in the prevention and treatment diseases, including cancer. Components responsible for these therapeutic effects belong basically to four groups of plant secondary metabolites: alkaloids, terpenoids, flavonoids and steroids. In collaboration with research groups from China and Norway we are studying anti-cancer effects of novel purified components from Chinese medicinal herbs and from a number of local plants, with the aim to identify novel lead compounds for potential use in cancer therapy. The most promising candidates identified from a screening in various cancer cell lines will be selected for more in-depth in vitro investigations

including the identification of their targets in the cell membranes and the pathways affected, as well as for *in vivo* studies such as the analysis of their pharmacological properties in the mouse model.

# People involved:

Ning WANG, Rolf BJERKVIG, André STEINMETZ, Feng LI, Tiejun LI, Weihong LI, Lihua SUN, Qiong WANG, Xianwen YANG

# 3. Specific Detection of Food Allergens from Plant Origin

Contract number: FNR/SECAL/07/06 Grant period: January 2008 -

December 2009

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR); Fonds National de la Recherche (FNR)

# Project summary:

Quality control of food products as well as food safety and consumer protection requires that sensitive and reliable detection and identification tests are available. Among the most common health risks are food allergens which can cause minor symptoms, but can also cause severe to even life-threatening reactions in

sensitized persons. Among the most allergenic plant products are peanuts and tree nuts like walnuts, pistachios and cashew nuts. They are often used in the confection of cookies, snacks and chocolates. Our aim was to develop molecular tests to detect trace amounts of various nut species including peanuts in processed food.

# People involved:

Cécile HUSTIN, André STEINMETZ, Katrin NEUMANN

### **KEY RESULTS**

# 1. IDENTIFYING LIM PROTEIN FUNCTIONS IN PLANTS

A system to artificially modify and monitor the intracellular pH in live cells and to conduct confocal analyses of GFP-fusion proteins has been set up. Actin-associated activities of one specific subset of LIM proteins were shown to be regulated by pH *in vivo*. All six *Arabidopsis* CRP-like LIM proteins and the four human CRP proteins have revealed actin-binding, -stabilizing and -bundling activities, indicating a major role of these proteins in the regulation of higher-order actin structures.





The tobacco WLIM2 protein binds a specific motif of the *Arabidopsis* histone H4 gene promoter *in vitro*. In *Arabidopsis* protoplasts the protein transactivates a reporter gene placed under the control of the promoter containing this motif (in collaboration with E. Grill, Munich).

Stimulated emission depletion (STED) microscopy has revealed an accumulation of WLIM2 in yet unidentified nucleolar dotlike structures at high-resolution (in collaboration with P. Bingen, Heidelberg).

# 2. CHINESE MEDICINAL HERBS

Among 57 purified components from Chinese medicinal plants tested for anti-cancer activities in different cancer cell lines, 6 compounds (2 alkaloids and 4 terpenoids) with strong activities were found.

Ethanol extracts from 17 local plants (including 5 ferns) were also tested on different cancer cell lines. Strong cytotoxic effects were found for 6 plants.

# 3. FOOD SAFETY

PCR-based assays that allow detection of trace amounts of peanuts, cashew nuts and pistachios (down to 10 mg/kg of food material, which is below the mean threshold for peanut triggering an allergic reaction in a moderate-reacting patient) have been developed. The assays have been tested successfully on 23 different confectioneries and cookies from supermarkets.

### **COLLABORATIONS**

### **NATIONAL**

- CRP-Santé, Laboratory of ImmunoGenetics and Allergology: Dr. F. Hentges
- CRP-Santé, Microarray Center: Dr. L. Vallar
- CRP-Santé, Flow Cytometry Core Facility: N.H.C. Brons
- National Laboratory of Health, Luxembourg: Dr. G. Moris

# INTERNATIONAL

- University of Bergen, Department of Biomedicine, Bergen, Norway: Prof. R. Bjerkvig
- University of Bergen, The Gade Institute, Bergen, Norway: Prof. K.H. Kalland
- Institute of Medicinal Plant
   Development, Research Center
   for Pharmacology and Toxicology,
   Beijing, China: Prof. X.M. Liu
- Institute of Medicinal Plant Development, Resource and Conservation Research Center, Beijing, China: Prof. S.L. Chen
- Modern Research Center for Traditional Chinese Medicine, Shanghai, China: Prof. W.D. Zhang
- University of Vienna, Institute of Biomolecular Structural Chemistry, Austria: Prof. R. Konrat
- German Cancer Research
   Center, High Resolution Optical
   Microscopy, Heidelberg, Germany:
   Prof. S. Hell and P. Bingen
- TU Munich, Botany, Science Center Weihenstephan, Freising, Germany: Prof. E. Grill
- University of Zurich, Institute for Plant Biology, Zurich, Switzerland: Dr. M. Geisler
- Institut de Biologie Moléculaire des Plantes, Molecular Mechanisms of Phenotypic Plasticity, Strasbourg, France: Dr. W.H. Shen and A. Molitor

# **VISITING SCIENTISTS**

- Feng LI, MD, PhD, Beijing University of Chinese Medicine, Beijing (September 2009 - January 2010)
- Tiejun LI, Assistant Professor, MRCTCM-SMMU, Shanghai (September 2009 - November 2009)
- Lihua SUN, PhD Student, IMPLAD-PMUC, Beijing (May - August 2009)
- Qiong WANG, PhD Student,

- IMPLAD-PMUC, Beijing (May -August 2009)
- Xianwen YANG, Assistant Professor, MRCTCM-SMMU, Shanghai (September 2009 - November 2009)

# 2009 PUBLICATIONS

- 1. Yang XW, Ding Y, Li XC, Ferreira D, Shen YH, Li SM, Wang N, Zhang WD (2009). Cycloabiesesquine A, a unique sesquiterpenoid from Abies delavayi. Chemical Communications 25, 3771-3773.
- 2. Thomas C, Tholl S, Moes D, Dieterle M, Papuga J, Moreau F, Steinmetz A (2009). Actin bundling in plants. Cell Motility and Cytoskeleton 66, 940-957.
- 3. Peng B, Hu Q, Liu XM, Wang LW, Chang Q, Li JR, Tang JT, Wang N, Wang YQ (2009). Duchesnea phenolic fraction inhibits in vitro and in vivo growth of cervical cancer through induction of apoptosis and cell cycle arrest. Exp Biol Med 234, 74-83.
- 4. Tao J, Zhang P, Liu GY, Yan H, Bu XX, Ma ZJ, Wang N, Wang GM, Jia W (2009). Cytotoxicity of Chinese motherwort (YiMuCao) aqueous ethanol extract is non-apoptotic and estrogen receptor independent on human breast cancer cells. J Ethnopharmacol 122, 234-239.
- Hofmann C, Niehl A, Sambade A, Steinmetz A, Heinlein M (2009). Inhibition of TMV movement by expression of an actin-binding protein. Plant Physiology 149, 1810-1823.
- Gaudron T, Peters C, Boland E, Steinmetz A, Moris G (2009). Development of a quadruplex-realtime-PCR for screening food for genetically modified organisms. European Food Research & Technology 229, 295-305.
- 7. Berr A, Xu L, Gao J, Cognat V, Steinmetz A, Dong A, Shen WH (2009). SET DOMAIN GROUP25 encodes a histone methyltransferase and is involved in FLOWERING LOCUS C activation and repression of flowering. Plant Physiology 151, 1476-1485.





# DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

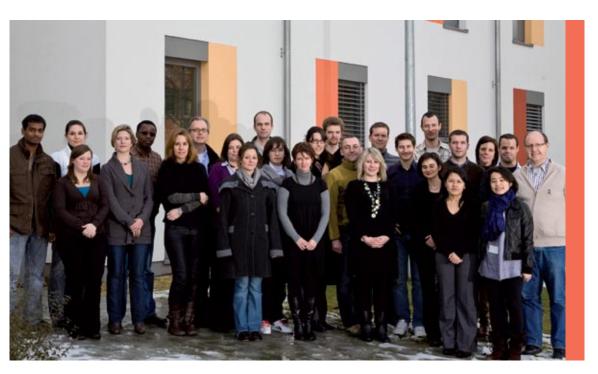
# LABORATORY OF RETROVIROLOGY

# **HEAD OF LABORATORY**

Dr. Jean-Claude SCHMIT, MD, PhD

# ASSOCIATE HEAD OF LABORATORY

Dr. Carole DEVAUX, PhD



Complete list of collaborators available on www.crp-sante.lu

# OBJECTIVES OF THE LABORATORY OF RETROVIROLOGY

# Clinical virology research unit (Danielle PEREZ-BERCOFF)

The research projects of the clinical virology unit are focused on (i) transmission networks and dynamics of epidemics caused by HIV and HCV in Luxembourg, (ii) primary transmitted and secondary resistance to antiretroviral therapy (HIV-1, HIV-2, HCV), (iii) clinical implications of viral and host factors involved in HIV-1 entry and (iv) collaborations with African countries.

# 2. Immuno-virology research unit (Sabrina DEROO)

One of the objectives of the immunovirology unit is the study of viral and host factors in virus entry to unravel the complex interplay between cellular and viral compounds and to identify diagnostic and therapeutic

# DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

# LABORATORY OF RETROVIROLOGY

lead compounds. A second objective is the study of the humoral immune responses during HIV infection to obtain a better understanding of its impact on the B cell immune repertoire and to characterize the role of the neutralizing and non-neutralizing antibodies. The identification of HIV specific epitopes/mimotopes could lead to the development of new immunogens for a multi-epitope vaccine.

### ONGOING PROJECTS

# CLINICAL VIROLOGY UNIT - HIV AND HCV IN LUXEMBOURG

"HIV and HCV in Luxembourg: molecular epidemiology and antiviral resistance" – "Viral and host factors in HIV entry and evolution"

Acronyms: "Molecular Epidemiology" and "HIV entry"

Contract numbers: REC-LRTV-20070102 and REC-LRTV-20081203

Grant periods: January 1st 2007- Dec 31st 2009 and June 1st, 2009- May 31st 2012

**Financial support:** Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

Project summaries: Chronic viral infections caused by HIV and HCV are an ever-growing public health problem. HIV and HCV are extremely variable RNA viruses, which have tremendous implications on molecular epidemiology and antiviral treatment. The objectives of these projects were to study the transmission networks and the dynamics of HIV and HCV epidemics and to evaluate primary, transmitted and secondary resistance in patients. Moreover, as novel antiretroviral drugs targeting HIV entry are available for patient care, we have studied the impact of the env genetic context on susceptibility/resistance to the fusion inhibitor Enfuvirtide.

# People involved:

Danielle PEREZ BERCOFF, Jean-Claude SCHMIT, Carole DEVAUX, Daniel STRUCK, Anne Marie TERNES, Jean-Yves SERVAIS, Christine LAMBERT, Morgane LEMAIRE

# Associated PhD project 1:

Title: Impact of the HIV-1 env genetic context on susceptibility/resistance to the fusion inhibitor Enfuvirtide.

PhD project of Franky Baatz, in collaboration with the Virology Laboratory, Dr. Monique Nijhuis and Dr. Anne Marie Wensing, UMCU Utrecht, the Netherlands,.

Start date: Sept 2006 - Financial support: AFR grant from Fonds National de la Recherche (FNR)

# Associated PhD project 2:

**Title:** HIV Env-mediated cytopathic effects in primary CD4+ T cell subsets. PhD project of Martin



Mullinge, in collaboration with the AIDS Reference Laboratory, Pr. Patrick Goubau, Université Catholique de Louvain, Bruxelles, Belgium. **Start date**: January 2009 - Financial support: AFR grant from Fonds National de la Recherche (FNR)

# Associated PhD project 3:

Title: Properties and function of the hepatitis C virus non-structural protein 2. Role and functions in vitro and in vivo. PhD project of Thomas Dentzer, in collaboration with the Laboratory of Virology and Infectious Diseases, Pr. Charles Rice, The Rockefeller University, New York, USA.

**Start date**: May 2009 - Financial support: AFR grant from Fonds National de la Recherche (FNR)

# Associated PhD project 4:

**Title:** Impact of HIV-1 subtype on transmission and infection of primary CD4+ T cells. PhD project of Eveline Santos da Silva, in collaboration with the Department of Immunology, Pr. Gilbert Faure, University of Nancy, France.

**Start date:** Oct 2009 - Financial support: AFR grant from Fonds National de la Recherche (FNR)

# 2. CLINICAL VIROLOGY UNIT - COLLABORATIONS WITH AFRICA

"HIV-1 drug resistance and nonprogressive infection in subtype A virus: genotype, phenotype and clinical relevance" and "Impact of the P450 cytochrome and ATP binding cassette (ABC) genetic polymorphisms on antiretroviral drugs concentrations of patients infected by HIV-1 in Rwanda"

Acronyms: "Resist A" and "CYP450" Contract numbers: REC-LRTV-20071004 and REC-LRTV-20090203

**Grant periods:** January 1st 2008- Dec 31st 2011 and April 1st 2009- June 30st 2012

Financial support: Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

Project summaries: Subtype A is responsible for 80% of infections in Rwanda. The objectives of the project "Resist A" are (i) to describe HIV-1 genetic variability and resistance drug mutation patterns in naïvetreatment and treated patients (ii) to evaluate the functional role of several mutations highlighted in A subtype for drug resistance in phenotypic assays using a newly developed recombinant virus assay with an appropriate backbone A. Genetic polymorphism of genes involved in drug metabolism and transport may contribute to the pharmacokinetic variability of antiretroviral agents. The main objectives of the "CYP450" project are to identify new SNPs within the CYP450 and ATP Binding Cassette genes in the Rwandese population and to correlate new haplotypes with plasma ARV concentrations and treatment response.

# People involved:

Vic ARENDT, Carole DEVAUX, Jean-Claude SCHMIT, Cécile MASQUELIER, Gilles ISERENTANT, Laurent QUENNERY

# Associated PhD project 1:

**Title:** HIV-1 subtype A in Rwanda: analysis of genotypic and phenotypic resistance to antiretroviral drugs and clinical implications. PhD project of Jean-Claude Karasi, in collaboration with the AIDS Reference Laboratory, Pr Michel Moutschen and Dr. Dolores Vaira, University of Liège, Belgium.

**Start date:** September 2007-Financial support: grant from MAE

# Associated PhD project 2:

Title: Impact of the P450 cytochrome and ATP binding cassette (ABC) genetic polymorphisms on antiretroviral drugs concentrations of patients infected by HIV-1 in Rwanda. PhD project of Alain Gras, in collaboration with the GIGA Institute, Pr Vincent Bours, University of Liège, Belgium.

**Start date**: September 2008- Financial support: BRF/AFR grant from Fonds National de la Recherche (FNR)

# 3. IMMUNO-VIROLOGY UNIT - HIV FNTRY

**Titles:** "Exploring HIV entry with human immune HCDR3 repertoires" and "Constrained peptides as small antagonists of the chemokine receptor CXCR4"

Acronyms: "HCDR3"- "PEPSAR"
Contract numbers: REC-LRTV20070115- LRV-FNR-07-01
Grant period: March 2007-March
2010- 01.09.2007-31.08.2009
Financial support: Ministère de
l'Enseignement Supérieur et de la
Recherche (MESR) – Fonds National
de la Recherche (FNR)

Project summaries: There is a growing need for virus-specific antibodies to better understand the role of the humoral immune responses and the nature of virus neutralization. The aim of the "HCDR3" project is to engineer novel antibody libraries from HIV patients to characterize their humoral immune response. These libraries will be screened to isolate antibody fragments directed against the viral proteins and co-receptor CCR5. The objectives of the "PEPSAR"

project are to identify new structurally constrained peptide ligands of the chemokine receptor CXCR4 using phage displayed peptide libraries and a simplified model of CXCR4 and to characterize their interaction in terms of agonists or antagonists in binding and functional assays.

# People involved:

Sabrina DEROO, Jean-Claude SCHMIT, Carole DEVAUX, Julie MATHU, Andy CHEVIGNE, Aurélie FISCHER

Nadia BEAUPAIN, Jean-Marc PLESSERIA, Charlène VERSCHUEREN

# 4. IMMUNO-VIROLOGY UNIT - HIV IMMUNITY

"Microarrays as tools for the phage display technology"

Acronym: "mimotope"
Contract number: REC-LRTV20070116

Grant period: October 2007-June

2010

**Financial support**: Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

Project summary: Since 90% of the overall HIV infections occur via mucosal route, a mucosal immune response would help to control viral entry. Discovery of new epitopes/ mimotopes of HIV specific IgA will help to discover immunogens that could lead to the development of a multi-epitope vaccine eliciting IgA responses at the mucosal entry sites. Our objectives are to screen phagedisplayed peptide libraries on IgA of long term non-progressors (LTNP) and HIV-infected patients to identify IgA specific epitopes/mimotopes and to develop microarray experiments to confirm the HIV specificity of the selected sequences.

# People involved:

Sylvie DELHALLE, Jean-Claude SCHMIT, Sabrina DEROO, Sandrine PERUCHON, Manuel COUNSON



### **KEY RESULTS**

- I. The SPREAD Program investigated transmitted drug resistance (TDR) among 2793 patients from September 2002 through December 2005. TDR had an overall prevalence of 8.4% and appears to be stabilizing in Europe, consistent with recent data of improved viral suppression in treated HIV-1 patients.
- II. The CATCH study performed Subtype B HIV-1 phylogeography and provided a new insight about the geographical distribution of viral lineages and the significant pathways of virus dispersal and migration across Europe.
- III. The phenotypic impact of the env genetic context on susceptibility/ resistance to Enfuvirtide was studied by comparing HR1/HR2 and env recombinant viruses of treated patients. Our results indicate that HR1/HR2 by itself accounts for most of the resistance whereas the env context rescues fitness (Franky Baatz et al, 7th European HIV Drug Resistance Workshop).
- IV. Virological and immunological responses to antiretroviral therapy were evaluated in 1706 Rwandese patients from 8 health facilities and were comparable to responses in western countries. (Karasi JC et al, oral presentation at the 7th European HIV Drug Resistance Workshop). We have observed a low proportion of drug resistance in several Rwandese mother cohorts under prophylactic HAART suggesting that maternal HAART is an effective approach to minimize acquired resistance in pregnant women.
- V. Saliva may be used as a valuable for therapeutic monitoring and compliance testing of zidovudine, lamivudine and nevirapine in Rwanda (Alain Gras et al, oral presentation at the 10th International Workshop on Clinical Pharmacology of HIV Therapy). We developed a micellar electrokinetic chromatography technique to determine zidovudine, stavudine, lamivudine and nevirapine in human plasma (Alain Gras et al, 5th European Conference on Clinical and Social Research on AIDS and Drugs).

- VI. The newly identified ligands of the coreceptor CCR5 were further characterized and at least one ligand displayed agonist activity measured in two different functional assays.
- VII. Screening on recombinant gp120 resulted in the isolation of a unique ligand displaying high specificity for gp120 derived from T tropic viruses. The antiviral activity of this ligand will be further analyzed.
- VIII. Phage displayed peptide libraries were screened on the simplified CXCR4 model and demonstrated that fully randomized peptides were too flexible to interact specifically to the CXCR4 model. Based on these insights a new phage library displaying proteins mutated chemokine was engineered. Screening of this library on the CXCR4 model resulted in the isolation of 19 chemokine mutants. Preliminary characterization of these clones indicated that 5 chemokine mutants acted as CXCR4 antagonists.
- IX. Screening campaigns resulted in the identification of 31 different phage-displayed mimotopes that could be assigned to linear or conformational motifs of HIV-1 proteins (Delhalle et al, CROI 2010). Three mimotopes corresponding to IgA epitopes from HIV-infected patients are currently tested in mice for their ability to elicit a B cell response potentially cross-reactive with HIV proteins.

### **COLLABORATIONS**

### **NATIONAL**

- Service National des Maladies Infectieuses, Centre Hospitalier de Luxembourg
- CRP-santé, Laboratoire National de Santé, Laboratoire de Toxicologie
- CRP-Santé, Immunology Laboratory, Prof. CP. Muller
- CRP-Santé, Microarray Center, Dr. L. Vallar
- CRP-Santé, Flow Cytometry: N.H.C.
   Brons
- CRP-Santé, Confocal microscopy platform

# INTERNATIONAL

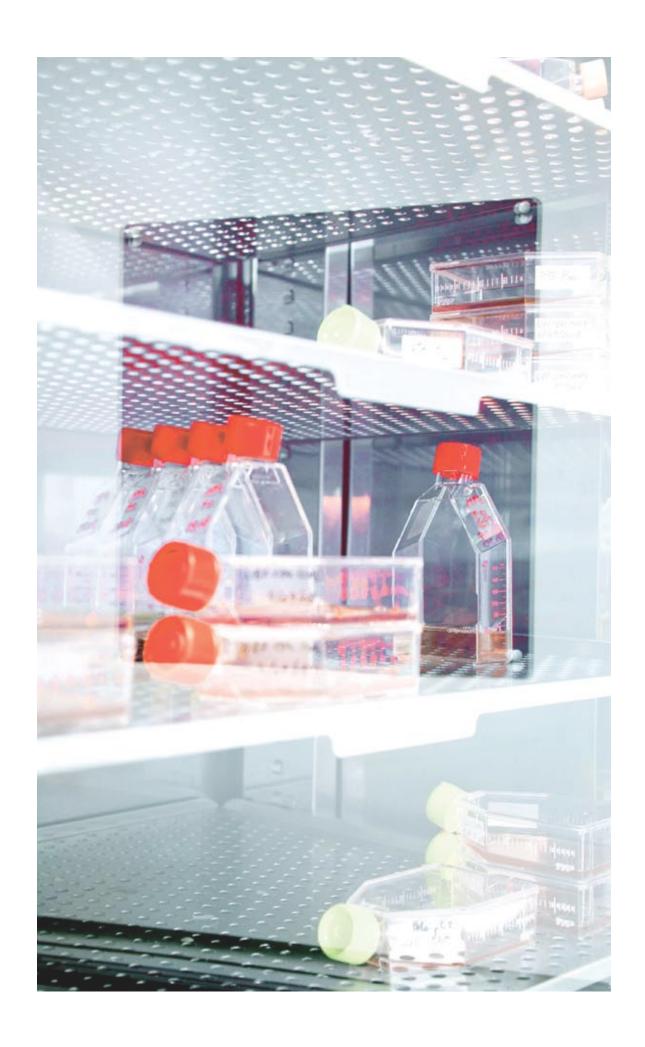
- EuroSIDA, EuroHIV, SPREAD/EHR, Euresist, Virgil, ART-A networks
- PMEs: Complix (Ghent), ABL (Luxembourg), Institut für Immunologie und Genetik (Kaiserslaütern), Dr Margarete Fisher-Bosch Institut of Clinical Pharmacology (Stuttgart)
- University Medical Center Utrecht , The Netherlands - Université Henry Poincaré Nancy I , France - Institut Gilbert Laustriat, UMR 7175, Strasbourg, France -Université Catholique de Louvain, Bruxelles, Université de Liège, Belgium - Belgian AIDS Reference laboratories.
- National Reference Laboratory and Treatment and Research on AIDS Center of Kigali, Médecins Sans Frontières, The Rwanda –Zambia HIV Research Group (Emory University, Atlanta), MONOD consortium.

### 2009 PUBLICATIONS

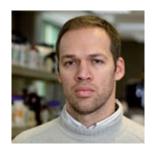
- Investigation of expert rule bases, logistic regression, and non-linear machine learning techniques for predicting response to antiretroviral treatment. Prosperi MC, Altmann A, Rosen-Zvi M, Aharoni E, Borgulya G, Bazso F, Sànnerborg A, Schülter E, Struck D, Ulivi G, Vandamme AM, Vercauteren J, Zazzi M; EuResist and Virolab study groups. Antivir Ther 2009;14(3):433-42
- 2. Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach. Paraskevis D, Pybus O, Magiorkinis G, Hatzakis A, Wensing AM, van de Vijver DA, Albert J, Angarano G, Asjö B, Balotta C, Boeri E, Camacho R, Chaix ML, Coughlan S, Costagliola D, De Luca A, de Mendoza C, Derdelinckx I, Grossman Z, Hamouda O, Hoepelman I, Horban A, Korn K, Kücherer C, Leitner T, Loveday C, Macrae E, Maljkovic-Berry I, Meyer L, Nielsen C, Op de Coul EL, Ormaasen V, Perrin L, Puchhammer-Stöckl E, Ruiz L, Salminen MO, Schmit JC, Schuurman R, Soriano V, Stanczak J, Stanojevic M, Struck D, Van Laethem K, Violin M, Yerly S, Zazzi M, Boucher CA, Vandamme SPREAD AM; Programme. Retrovirology 2009;6:49
- 3. Viraemia and HIV-1 drug resistance mutations among patients receiving antiretroviral treatment in Mozambique. Maldonado F, Biot M, Roman F, Masquelier C, Anapenge M, Bastos R, Chuquela HC, Arendt V, Schmit JC, Zachariah R. Trans R Soc Trop Med Hyg 2009;103(6):607-12.

- Interruption of antiretroviral therapy is associated with increased plasma cystatin C. Mocroft A, Wyatt C, Szczech L, Neuhaus J, El-Sadr W, Tracy R, Kuller L, Shlipak M, Angus B, Klinker H, Ross M; INSIGHT SMART Study Group. AIDS 2009;23(1):71-82.
- Exceptional genetic variability of hepatitis B virus indicates that Rwanda is east of an emerging African genotype E/A1 divide. Hübschen JM, Mugabo J, Peltier CA, Karasi JC, Sausy A, Kirpach P, Arendt V, Muller CP. J Med Virol 2009;81(3):435-40.
- Access to treatment of hepatitis C in prison inmates. Strock P, Mossong J, Hawotte K, Arendt V. Dig Dis Sci 2009;54(6):1325-30.
- 7. Validation of 2006 WHO prediction scores for true HIV infection in children less than 18 months with a positive serological HIV test. Peltier CA, Omes C, Ndimubanzi PC, Ndayisaba GF, Stulac S, Arendt V, Courteille O, Muganga N, Kayumba K, Van den Ende. J PLoS One 2009;4(4):e5312.
- 8. Breastfeeding with maternal antiretroviral therapy or formula feeding to prevent HIV postnatal mother-to-child transmission in Rwanda. Peltier CA, Ndayisaba GF, Lepage P, van Griensven J, Leroy V, Omes C, Ndimubanzi C, Courteille O and Arendt V. AIDS 2009;23(18):2415-23.

- Inactivation and coagulation biomarkers independent are predictors of the development of opportunistic disease in patients with HIV infection. Rodger AJ, Fox Z, Lundgren JD, Kuller LH, Boesecke C, Gey D, Skoutelis A, Goetz MB, Phillips AN; INSIGHT Strategies for Management of Antiretroviral Therapy (SMART) Study Group. J Infect Dis 2009;200(6):973-83.
- Transmission of drug resistant HIV is stabilizing in Europe. Vercauteren J, Wensing AM, van de Vijver DA, Albert J, Balotta C, Hamouda O, Kücherer C, Struck D, Schmit JC, Äsjo B, Bruckova M, Camacho R, Clotet B, Coughlan S, Grossman Z, Horban A, Korn K, Kostrikis L, Nielsen C, Paraskevis D, Poljak M, Puchhammer-Stockl E, Riva C, Ruiz L, Salminen M, Schuurman R, Sonnerborg A, Stanekova D, Stanojevic M, Vandamme AM, Boucher CA. J Infect Dis 2009;200(10):1503-8.
- Determinants of the hepatitis C virus non-structural protein 2 protease domain required for production of infectious virus. Dentzer TG, Lorenz IC, Evans MJ, Rice CM. J Virol 2009,83(24):12702-13.







# LABORATORY COORDINATOR

Dr. Brice APPENZELLER, PhD

DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

# LABORATORY OF TOXICOLOGY



Complete list of collaborators available on www.crp-sante.lu

# OBJECTIVES OF THE LABORATORY OF TOXICOLOGY

The goal of the research unit is to develop biomarkers for the identification of human exposure to pollutants and to understand the relationships between exposure and subsequent biological/biochemical disorders

The development of biomarkers includes research in analytical chemistry using state-of-the-art equipment (mainly based on mass spectrometry) necessary to reach low levels of molecules present in biological matrices. In order to understand the relationships between the level of exposure and the concentration of molecules in human matrices, we also work on metabolisation and incorporation mechanisms of molecules and metabolites in matrices. Disorders associated with exposure are investigated through the analysis

# DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

# LABORATORY OF TOXICOLOGY

of specimens from selected human volunteers and experimentation on animals under controlled exposure. The advances obtained are valorised through epidemiological studies involving extended cohorts in order to determine the reference levels of biomarkers for the general population and to identify over-exposed subpopulation.

### **ONGOING PROJECTS**

# Assessment of human exposure to organic pollutants using hair analysis

Contract number: REC-LNST-20070801

**Grant period:** January 2008 –

December 2011

**Financial support:** CRP-Santé via Ministère de l'Enseignement Supérieur et de la Recherche (MESR), Luxembourg

# Project summary:

Hair as a matrix for human biomonitoring (HBM) presents several advantages over classical matrices such as facilitated sampling and extended window of detection. This project aims to investigate the usefulness of hair for HBM of exposure to organic pollutants, with a special focus on polycyclic aromatic hydrocarbons (PAHs) and pesticides. The project includes analytical development mainly based on gas and liquid chromatography coupled with tandem mass spectrometry. The methods developed are applied to hair specimens from human volunteers selected from the general population of the Grand Duchy of Luxembourg in order to highlight trends (nature of molecules mainly detected, spatio-temporal mapping of pollutions), determine reference levels for the general population in Luxembourg, help to identify the main sources of pollution and highlight the most worrying concomitant human exposures.

# People involved:

Brice APPENZELLER, Claude SCHUMMER, Guillaume SALQUEBRE, Nathalie GROVA



# Hair analysis for the assessment of children exposure to indoor pesticides

Grant period: March 2008 – November 2009 Financial support: Agence Française de Sécurité Sanitaire de l'Environnement et du Travail

# Project summary:

Recent reports alerted authorities on high levels of biocides detected in indoor atmosphere in schools. Owing to their particularly active metabolism, children are frequently reported to be more sensitive than adults to health issues associated with exposure to pesticides. The project aims at developing analytical methodologies for the determination of selected compounds (including organochlorinated, phosphorilated compounds, pyrethrinoids polychlorophenols) in different biological matrices and applying the methodologies to the analysis of specimens from a cohort of French children. The analytical techniques used for this purpose are gas chromatography coupled to mass spectrometry in electronic impact and in negative chemical ionization mode (GS-MS/MS EI and NCI) and liquid chromatography coupled to mass spectrometry (LC-MS/MS).

# People involved:

Brice APPENZELLER, Guillaume SALQUEBRE

# Nutrition, environment and cardio-vascular health

Acronym: NESCAV

Grant period: January 2009 -

December 2011

Financial support: Fonds Européen de Développement Régional / Ministère de l'Enseignement Supérieur et de la Recherche (MESR)

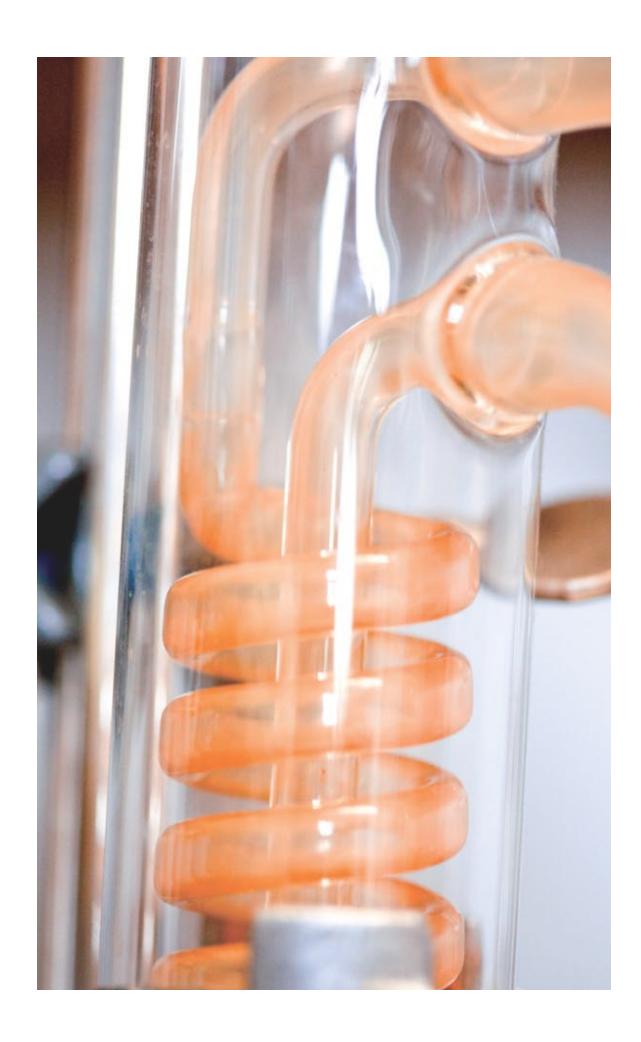
# **Project summary:**

The Centre d'Etudes en Santé (Center of Health Studies) of CRP-Santé initiated a project aimed at assessing different parameters potentially associated with cardiovascular diseases among more than three thousand volunteers randomly selected from the population living in the "Grande Region" (Luxembourg, Belgium, France and Germany). Among the different parameters which are investigated as part of this project, the Laboratory of Toxicology is in charge of investigating the volunteers' alcohol consumption, smoking habits and exposure to polycyclic aromatic hydrocarbons and pesticides as representatives of environmental and occupational exposure.

Alcohol consumption is assessed by determining ethyl glucuronide content in hair. Smoking status, including both active smoking and exposure to environmental tobacco smoke is assessed by the determination of nicotine and cotinine in hair. PAHs and pesticides exposure, also based on hair analysis, is assessed by the determination of PAHs metabolites and pesticides respectively.

# People involved:

Brice APPENZELLER, Helen KARLSSON



# **KEY RESULTS**

I. The first determination of PAH metabolites in hair was published. This represents a new relevant tool for the biomonitoring of human exposure to PAHs, particularly suitable for the assessment of chronic exposure.

# **COLLABORATIONS**

# **NATIONAL**

- CRP-Santé Centre d'Etudes en Santé (Resp. M.L. Lair, S. Couffignal MD, A. Alkerwi, MD)
- Institut Vitivinicole, Remich. Luxembourg.

# **INTERNATIONAL**

- Centre de Géochimie de la Surface de Strasbourg, UMR 7517 CNRS – Université Louis Pasteur. (Pr Millet M.)
- Agence Française de la Sécurité Sanitaire de l'Environnement et du Travail (AFSSET)

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  Appenzeller B, Schartz A, Lorang
  C, Wennig R. Internet suicide
  guidelines: report of a lifethreatening poisoning using tobacco
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- 3. Schummer C, Appenzeller BM, Millet M, Wennig R. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in human hair by gas chromatographynegative chemical ionization mass spectrometry. J Chromatogr A. 2009 Aug 7;1216(32):6012-9. Epub 2009 Jun 3.
- 4. Schummer C, Mothiron E, Appenzeller BM, Rizet AL, Wennig R, Millet M. Temporal variations of concentrations of currently used pesticides in the atmosphere of Strasbourg, France. Environ Pollut. 2009 Sep 21. [Epub ahead of print]





**HEAD OF UNIT** 

Daniel CARDAO, MBA

CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

# TECHNICAL AND ADMINISTRATIVE DEPARTMENT



Complete list of collaborators available on www.crp-sante.lu

The highlight of 2009 was the new FNR program Pearl. The CRP-Santé has introduced a new project for Clinical Proteomics to the FNR in the framework of this program. The FNR decided to finance this project in Luxembourg via CRP-Santé. The Administrative and Technical Services (ATS) was very implicated for calculating the business case which was calculated on market standards; for instance, a Discounted Free Cash Flow (DCF) and an Internal Rate of Return (IRR) have been calculated.

This exercise shows that, more and more, the projects are focused on market situations and the ATS has to provide support in setting up such business cases.

A few other highlights of 2009 were the setup of new tools as, the CRM for the Project Management Office, Document Management for the Moreover, the Purchasing unit achieved a 30% average synergies (laboratories consumables, laboratories equipment, etc) during the 2nd semester of 2009. In 2009, the ATS department has been reinforced by:

- Noémie Blaise who reinforced Health and Safety
- Florence Henry who joined as
   Executive Assistant of the CEO and
   who also assists the Communication
   Manager and the Head of
   Luxembourg Clinical Proteomics (LCP)
   Dominique Mormont who joined the
   Project Management Office
- Marc Weisen, a new purchasing manager

# HUMAN RESOURCES

Human Resources is headed by Natacha BEICHT supported by 2 collaborators acting on all aspects of human resources daily work.

In 2009 (besides the daily workload increasing steadily with the recruitment of 35 new members of staff over the year), the HR Unit has focused its attention on 4 main topics:

- Establishment of a better "welcome program" for our new staff
- Organization of various soft skills training sessions such as "English for everyone",
   12 days of "Management course" for our Senior Researchers and Administrative Head of Units.
- Finalization of our dashboards and creation of new indicators on human resources.
- Implementation of the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers.

# CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

# TECHNICAL AND ADMINISTRATIVE DEPARTMENT

Secretarial Unit, new budget layout for calculating the yearly budgets etc. These tools are used to improve support to the laboratories and to ensure a faster reactivity.

In 2009, the Management Board decided to create a new financial control. The aim is to set up internal control procedures on our accounting system.

### ONGOING PROJECTS.

- Introduction of the new AFR grant scheme for our PhD students with the promotion of 19 employment contracts between AFR beneficiaries and CRP-Santé. With this measure, the beneficiaries are entitled to full access to social security coverage throughout their research training. To cover this, we have organized training sessions on the contractual and legal obligations of an employment contract as well as information sessions on the new AFR grants in collaboration with FNR.
- Participation in the C&C Promoters' Network Community and presentation of one of our actions towards greater convergence of our HR procedures with the provisions of the European Charter: "The recruitment process at CRP-Santé".
- A specially appointed task force performed an internal audit and analysis and divided the 40 C&C principles into 4 areas (ethical and professional aspects, recruitment, working conditions & social security, training).
- In parallel, participation in the 1st Human resources strategy group in Warwick: CRP-Santé representatives exchanged opinions on their own practices with the other members of the group and in view of seeking acknowledgement of their 'HR Strategy for Researchers Incorporating the Charter & Code' by the European Commission by March 2010. Participation in the Euraxess training session in Tessaloniki must also be mentioned
- First Induction day. In collaboration with all administrative and scientific units, we chose to devote particular attention to our new employees with a presentation of our activities, a visit of our departments and explanations of our rules and corporate culture. Our goal consists in giving guidance and support to the staff at this very early stage of their collaboration with us in order to build up a constructive and positive relationship.

# FINANCE AND PURCHASING

Finance and Purchasing are headed by Jérémy KLEIN.

# Main missions of Finance:

- Manage the day-to-day financial aspects of research projects following the accounting rules of the state and agreements with partners (FNR, MESR)
- Calculate the global annual CRP-Santé budget
- Calculate the budget for new projects in collaboration with Pls
- Establish financial reports of projects for partners
- Create financial statements for the Management & the Board of Administration

### Milestones 2009:

- Implementation of a new application for the calculation of the 2010 budget
- Realization of a business case and budget in the framework of the program Pearl of the FNR
- Implementation of electronic payments via Multiline & Luxtrust to speed up financial transactions with suppliers to receive cash discount
- Implementation of the eTVA via
- Implementation of an automatic Cash Flow reporting

# Actions for 2010:

- New performance contract: calculate the budget over the 3 next years
- Implementation of an online travel fees statement
- Implementation of the stock management
- Implementation of the online intrastat statement via Luxtrust

# Purchasing

In July 2009, CRP-Santé welcomed a new collaborator as Purchasing Manager in order to create synergies among the laboratories to get better contract conditions and prices for all the Research Units. The main challenge is to aim a centralization of different contracts for a global view and to put more attention to price analysis by benchmark. The result of this strategy is to obtain a time gain for the Head of laboratories in order to cede partly the negotiations to the Purchasing Unit. Another major issue was the setting up of the cooperation with the various national partners (hospitals, university etc) in order to centralize the negotiations with different suppliers on a national level.

# FINANCIAL CONTROL

Financial Control is headed by Joseph GAUTOT.

The Financial Control unit has been created in 2009 and is reporting to the Head of the ATS. Its main role, at a general level, is to provide reasonable assurance regarding the achievement of objectives in the following fields:

- Reliability of financial reporting
- Compliance with laws, regulations and internal procedures
- Effectiveness and efficiency of operations

At a more specific level, the financial control service has put into place the Full Costs calculation model which was part of the performance contract signed between the CRP and the government for the period 2008-2010.

Besides the ongoing activities of the financial control service, its main objective for 2010 will be to put into place a monthly dashboard in order to monitor the CRP's main indicators in relation with the performance contracts assignments.

# IT

IT is headed by Patrick SCHILD.

The main achievements in 2009 have been:

- Installation of a new Citrix platform in order to improve the external accessibility and to ensure more efficiency and security.
- The storage capacity has been set to 22 TB as the volumes have significantly raised up
- The WIFI Radius has been implemented in order to have the possibility for external users to have secure internet access.
- A new backup platform has been installed to ensure a data deduplication.
- The security level is still a main concern: Actions for encrypting laptops, desktop stations and new applications have been installed (antivirus).
- In accordance to the quality assurance needs, new IT procedures have been setup.
- The internal audit, launched in 2009, was successfully completed.
   The 90 actions have been solved within 1 year.

In terms of Helpdesk, a lot of support was given to the end users, 1, 752 requests have been treated in 2009. Finally, the IT department assisted IBBL for setting up their infrastructure in collaboration with the CRP-Henri Tudor.

# TECHNICAL SUPPORT

Technical Support is headed by Laura MARTINS.

# MAIN ACHIEVEMENTS IN 2009:

- Quality procedures development for the technical service
- High curative maintenance intervention in P3 area of BAM laboratories, pressure regulators replacement, a new network of compressed air installation, depression adjustment
- Test alarm transmission via GTC of all laboratory equipment concerned
- Installation of Back up GTC (aid of the I.T. service)
- Premises in the building Thomas
   Edison, new offices and a new local
   storage for IT team
- Installation of a new UPS for IT team
- Set up of new cleaning contracts.
   Supervision of the new cleaning staff.
- Contract documents tracking for the BAM I and II, negotiation of a new maintenance contract
- Development of on-line request tool for technical assistance (help IT services)
- Recruitment of a new technician for the technical service
- Budget management on line
- Technical follow-up for the new Integrated BioBank (IBBL) building. Technical installations control concerning heating, electricity, ventilation system alarm fire and intrusion, access controls. Negotiations start for the caretaking contract, alarm monitoring and maintenance of fire and intrusion systems.
- Participation in the development project of the construction of the new building for the CRP-Santé

# PROJECT MANAGEMENT

Project Management is headed by Jo SCHROEDER.

# Some key information

In 2009 the total number of projects realized at CRP-Santé grew by 27%. This considerable increase is mainly due to the start-up of the Clinical and Epidemiological Investigation Centre (CIEC) by end of 2008. All in all, 26 new projects got started whereas 19 were finished.

Despite the decrease of the relative weight of the number of projects financed by the FNR, one has to consider the approval of the PEARL project called "Clinical Proteomics Initiative Luxembourg (CPIL)".

The Centre for Public Health Studies succeeded in launching 3 new projects financed within the framework of the FEDER program. Compared to 2008, the overall number of FP7 projects stays unchanged.

# COMMUNICATION

Communication is headed by Aurélia DERISCHEBOURG.

In 2009, the CRP-Santé Communication unit developed both an internal and external communication strategy.

The unit has improved the corporate identity as well as relationships and collaborations at national and international level through promotional events and communication tools:

- media relations,
- inter-institutional communication,
- press releases, news, flyers, posters,
- promotion of achievement and performance of the institution.

The Communication unit took actively part in improving the institution's image to the scientific community and to the public by the development of scientific seminars.

It built up its efforts regarding the internal communication strategy by promoting CRP-Santé as a centre of quality and expertise.

In 2009, the Communication unit organized following events:

# > SCIENTIFIC WORKSHOPS:

- Organization and promotion of the "Workshop Costs and Consequences of Tobacco Use", workshop realized by the Laboratory of Experimental Hemato-Oncology of CRP-Santé
- Organization and coordination of the ", Affymetrix Workshop", led by the Microarray Center
- Promotion and organization of the "Green Health: a healthy way to live from the Traditional Chinese Medicine view", workshop led by the Laboratory of Plant Molecular Biology of CRP-Santé
- Organization and coordination of the Sports Medicine Research Laboratory inauguration
- Promotion of the "Cytometry Summer course" and "Workshop on Cytometry" in relationship with Luxinnovation
- Organization and coordination of the "First Clinical Research Day" led by the Clinical and Epidemiological Investigation Center (CIEC)
- Coordination of the Bioinformatics Training session organized by the Microarray Center

# > SCIENTIFIC EVENTS:

Together, with the group for the promotion of scientific culture "Proscience", the CRP-Santé Communication unit contributed to:

- the Sciences' Festival organized by the "Fonds National de la Recherche": organization of a scientific workshop on HIV virus evolution within the world and within a host
- the "Foire de l'étudiant": in order to inform students on all potential job opportunities within sciences
- Meets@uni 2009, organized by the University of Luxembourg, to present the Public Research Centre for Health's activities to the students coming from several national and international universities.

Participation to the European Research Congress in Berlin.

# > COMMUNICATION TOOLS:

 Press conferences and press relations: Vesalius Biocapital,

- Tobacco Use, Clinical Research, Mental Health, etc.
- Flyers and invitations: Cancer, Clinical Research, Microarray
   Center, Nescav (Nutrition, Environment and Cardiovascular Health)
- Reports: Susana, Perinatal Report
- Donation campaign through flyers and newspapers.

# EH&S

EH&S is headed by Elodie Fontaine. During 2009, a new assistant joined the department in order to meet the support needs of operational units of CRP-Santé and of IBBL.

# **ACHIEVEMENTS IN 2009:**

- Design of a EH&S introduction guide for newcomers
- Update of EH&S procedures in compliance with quality assurance system
- Assistance to the new department CIEC with the implementation of safety and hygiene procedures
- Support of the planning process of the new CRP-Santé building
- Implementation of an internal pandemic influenza action plan
- Organization and conduction of EH&S training: 8 training sessions in total with four different topics (EH&S training for newcomers, first-aid refresh course, handling of liquid nitrogen, evacuation)

# **ACTIONS FOR 2010:**

- Establish an individual risk assessment system for inventory of high risk jobs
- Assist IBBL for setting up the EH&S procedures
- Improve laboratory waste management regarding separation, storage and disposal
- Organize training sessions on current safety and health at work topics

# **LEGAL**

Legal is headed by Guillaume BYK.

The Legal unit focused on different topics in 2009.

# **PUBLIC TENDERS:**

A new law regarding public procurement came into force in June 2009. It reduces the need to proceed with national public tender but will also lead to an increase for derogation request.

# AGREEMENTS AND INTELLECTUAL PROPERTY:

The Legal unit reviewed 40 agreements with 15 of those for the Clinical and Epidemiological Investigation Center. It reflects the need for contractual agreement between the various parties involved in complex clinical trial. 11 Material Transfer Agreement (MTA) and Non-Disclosure Agreement (NDA) were drafted or reviewed in 2009.

In 2009, one patent application was successfully granted by the European Patent Office. CRP-Santé also seeks to protect more globally its patent portfolio and thus proceeded with filing 2 patents applications in the USA, Canada, China, Brazil, India, Japan, Russia and in Europe following their international phase (PCT). Furthermore, one provisional application was transformed into an international application (PCT).

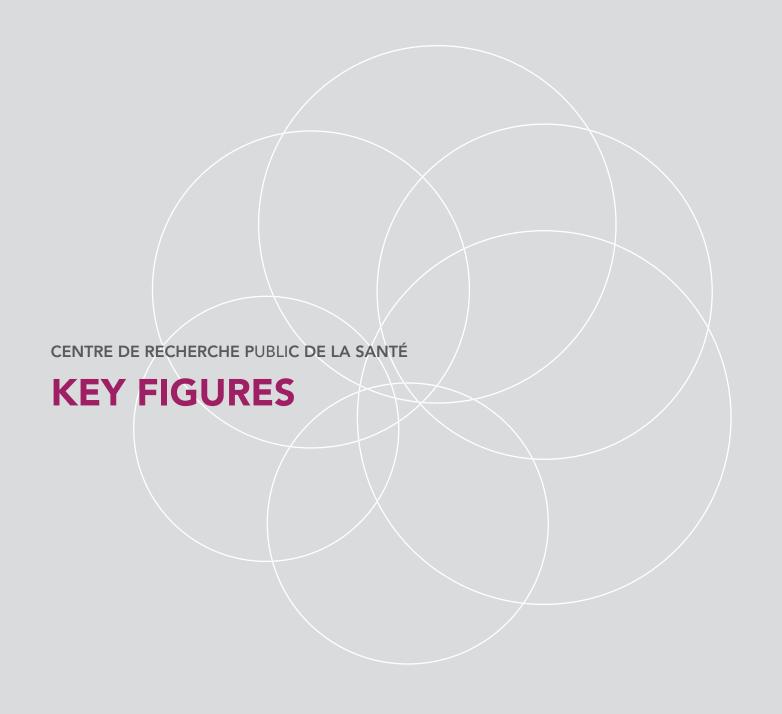
# PRIVACY AND DATA PROTECTION:

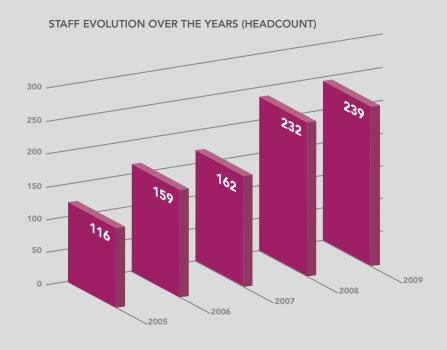
The CRP-Santé filed 4 notifications and 1 authorization (for research project involving a genetic element) to the Luxembourg Data Protection Commission.

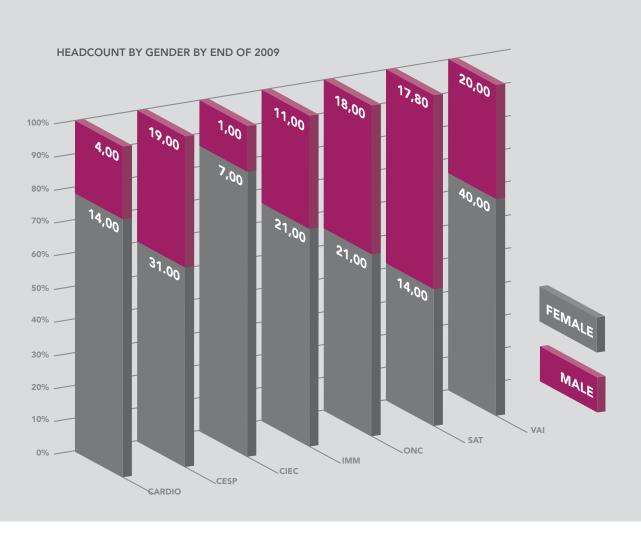
# TECHNOLOGY TRANSFER OFFICE

In 2009, CRP-Santé decided to create a new unit, Technology Transfer Office. This unit is headed by Philippe De Backer. The mission of this office is:

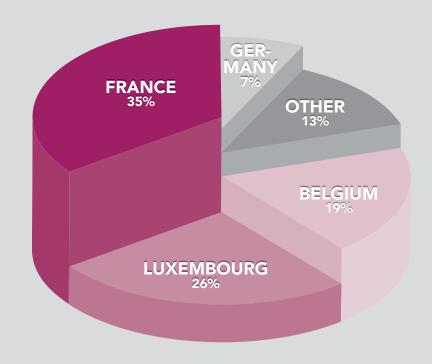
- Pro-active tracing of projects and inventions with commercial potential
- Assisting the research units in securing the necessary property rights, including patents
- Valorization of technology and IP through negotiating and executing agreements with companies and partners



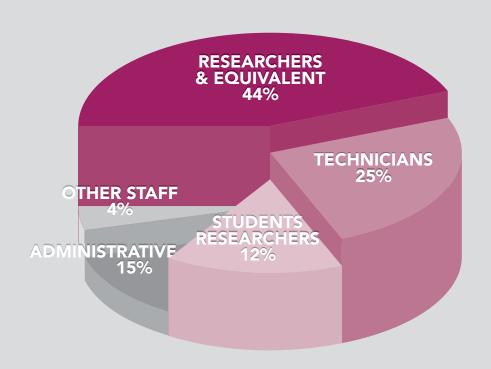


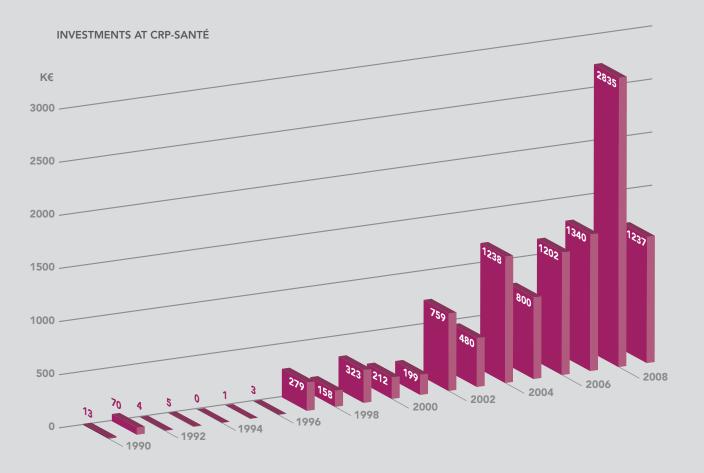


### CRP SANTÉ'S STAFF IN CATEGORIES (%)

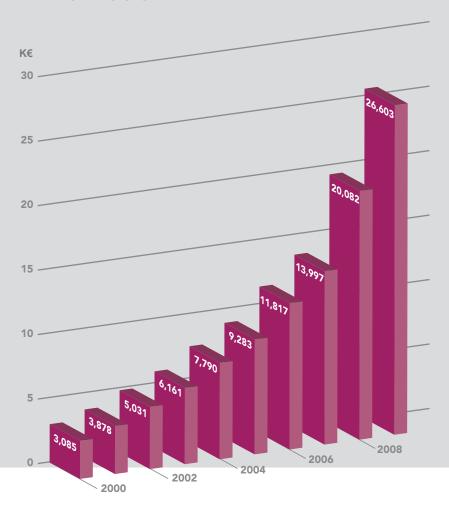


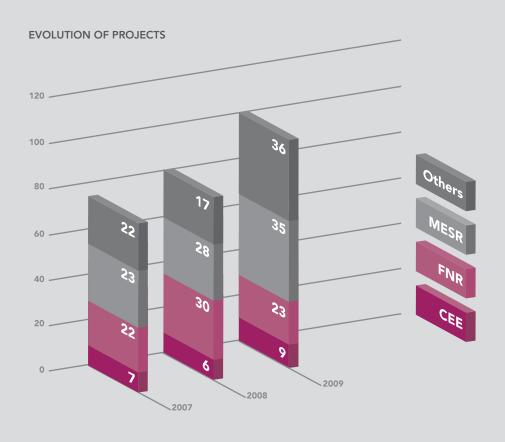
CRP SANTÉ'S PERSONNEL CAN BE SPLITTED INTO THE FOLLOWING CATEGORIES (%)





### FINANCIAL EVOLUTION





### **DECEMBER 2009 NATIONALITY**

	Albanian	0,42%
	German	7,11%
	American	0,42%
	Belgian	18,83%
2	Belarussian	0,42%
	British	0,42%
	Bulgarian	0,42%
*1	Chinese	2,09%
*	Congolese	0,42%
	French	35,15%
	Italian	1,26%
	Kenyan	0,42%
	Luxembourgish	26,36%
(*=	Malaysian	0,42%
	Dutch	0,84%
	Norwegian	0,84%
	Polish	0,84%
(1)	Portuguese	1,67%
	Rwandan	0,42%
	Swedish	0,42%
<b>©</b>	Tunisian	0,42%
print.	Venezuelan	0,42%
	GRAND TOTAL	100,00%







Au Conseil d'Administration du CRP SANTE 1A, rue Thomas Edison L-1445 STRASSEN Grant Thornton Lux Audit S.A. 83, Pafebruch L-8308 CAPELLEN (Luxembourg)

T +352 40 12 99 1 F +352 40 05 98 www.gtlux.lu

#### RAPPORT DU REVISEUR D'ENTREPRISES

#### Rapport sur les comptes annuels

Conformément au mandat reçu, nous avons effectué l'audit des comptes annuels ci-joints de l'Etablissement Centre de Recherche Public sur la Santé ou CRP-SANTE, comprenant le bilan au 31 décembre 2009 ainsi que le compte de profits et pertes pour l'exercice clos à cette date, et l'annexe contenant un résumé des principales méthodes comptables et d'autres notes explicatives.

### Responsabilité du Conseil d'Administration dans l'établissement et la présentation des comptes annuels

Le Conseil d'Administration est responsable de l'établissement et de la présentation sincère de ces comptes annuels, conformément aux obligations légales et réglementaires relatives à l'établissement et la présentation des comptes annuels en vigueur au Luxembourg. Cette responsabilité comprend: la conception, la mise en place et le suivi d'un contrôle interne relatif à l'établissement et la présentation sincère de comptes annuels ne comportant pas d'anomalies significatives, que celles-ci résultent de fraudes ou d'erreurs; le choix et l'application de méthodes comptables appropriées, ainsi que la détermination d'estimations comptables raisonnables au regard des circonstances.

#### Responsabilité du Réviseur d'entreprises

Notre responsabilité est d'exprimer une opinion sur ces comptes annuels sur la base de notre audit. Nous avons effectué notre audit selon les Normes Internationales d'Audit telles qu'adoptées par l'Institut des réviseurs d'entreprises. Ces normes requièrent de notre part de nous conformer aux règles d'éthique et de planifier et de réaliser l'audit pour obtenir une assurance raisonnable que les comptes annuels ne comportent pas d'anomalies significatives. Un audit implique la mise en oeuvre de procédures en vue de recueillir des éléments probants concernant les montants et les informations fournies dans les comptes annuels. Le choix des procédures relève du jugement du Réviseur d'entreprises, de même que l'évaluation du risque que les comptes annuels contiennent des anomalies significatives, que celles-ci résultent de fraudes ou d'erreurs. En procédant à ces évaluations du risque, le Réviseur d'entreprises prend en compte le contrôle interne en vigueur dans l'entité relatif à l'établissement et la présentation sincère des comptes annuels afin de définir des procédures d'audit appropriées en la circonstance, et non dans le but d'exprimer une opinion sur l'efficacité de celui-ci.

Un audit comporte également l'appréciation du caractère approprié des méthodes comptables retenues et le caractère raisonnable des estimations comptables faites par le Conseil d'Administration, de même que l'appréciation de la présentation d'ensemble des comptes

Nous estimons que les éléments probants recueillis sont suffisants et appropriés pour fonder notre opinion.

### Opinion

A notre avis, les comptes annuels ci-joints donnent, en conformité avec les prescriptions légales et réglementaires en vigueur au Luxembourg et avec les méthodes comptables énoncées dans l'annexe, une image fidèle du patrimoine et de la situation financière de l'Etablissement Centre de Recherche Public sur la Santé ou CRP-SANTE au 31 décembre 2009 ainsi que des résultats de l'exercice se terminant à cette date.

Luxembourg, le 10 mars 2010

Experts-Comptables
Réviseurs d'entreprises
Luxembourg Montber fron of Grant Thomson International Ltd.

Thierry REMACLE Réviseur d'Entreprises



### BILAN AU 31 DÉCEMBRE 2009

(en Euros)

ACTIF	31.12.2009	31.12.2008
A. CAPITAL SOUSCRIT NON VERSE		
B. FRAIS D'ETABLISSEMENT		
C. ACTIF IMMOBILISE		
<ol> <li>Immobilisations incorporelles</li> <li>Concessions, brevets, licences, marques, ainsi que droits et valeurs similaires</li> </ol>	162 709,42	100 844,26
a) acquis à titre onéreux b) créés par l'entreprise	162 709,42	100 844,26
II. Immobilisations corporelles	1 360 389,85	1 350 300,08
Installations techniques et machines     Autres installations, outillage et mobilier	0,00 1 360 389,85	0,00 1 350 300,08
Acomptes versés et immobilisations corporelles en cours	0,00	0,00
III. Immobilisations financières 3. Participations	1 012 394,68 1 012 394,68	1 012 394,68 1 012 394,68
o. i articipations	1 012 394,00	1 012 394,00
TOTAL DE L'ACTIF IMMOBILISE	2 535 493,95	2 463 539,02
D. ACTIF CIRCULANT		
I. Stocks	0,00	0,00
II. Créances		
Créances résultant de ventes et prestations de services     a) dont la durée résiduelle est inférieure ou égale à un an	239 529,39 239 529,39	119 003,54 119 003,54
b) dont la durée résiduelle est supérieure à un an		
2. Créances sur partenaires	1 978 621,79	1 800 644,56
<ol> <li>Créances sur des entreprises avec lesquelles la société a un lien de participation</li> </ol>	24 038,77	10 086,16
4. Autres créances	852 336,04	930 210,27
<ul> <li>a) dont la durée résiduelle est inférieure ou égale à un an</li> <li>b) dont la durée résiduelle est supérieure à un an</li> </ul>	852 336,04	930 210,27
III. Valeurs mobilières	0,00	0,00
IV. Avoirs en banque, avoirs en compte de chèques postaux, chèques	8 550 405,60	5 090 050,02
et en caisse		
TOTAL DE L'ACTIF CIRCULANT	11 644 931,59	7 949 994,55
E. COMPTES DE REGULARISATION	240 007,25	295 011,60
TOTAL DE L'ACTIF	14 420 432.79	10 708 545,17



### BILAN AU 31 DÉCEMBRE 2009

(en Euros)

(en Euros)		
PASSIF	31.12.2009	31.12.2008
C. DETTES		
Emprunts obligataires	0,00	0,00
1. Empluits obligatailes	0,00	0,00
2. Dettes envers des établissements de crédit	0,00	0,00
3. Acomptes reçus sur commandes	0,00	0,00
<ol> <li>Dettes sur achats et prestations de services</li> <li>a) dont la durée résiduelle est inférieure ou égale à un an</li> <li>b) dont la durée résiduelle est supérieure à un an</li> </ol>	1 717 267,56 1 717 267,56	2 197 839,68 2 197 839,68
5. Dettes représentées par des effets de commerce	0,00	0,00
6. Dettes envers des entreprises liées	0,00	0,00
<ol> <li>Dettes envers des entreprises avec lesquelles la société à un lien de participation</li> </ol>	0,00	0,00
<ul><li>8. Dettes fiscales et dettes au titre de la sécurité sociale</li><li>a) Dettes fiscales</li><li>b) Dettes au titre de la sécurité sociale</li></ul>	831 591,57 384 451,86 447 139,71	657 923,66 281 773,46 376 150,20
<ol> <li>Autres dettes</li> <li>a) dont la durée résiduelle est inférieure ou égale à un an</li> <li>b) dont la durée résiduelle est supérieure à un an</li> </ol>	97 520,37 80 900,37 16 620,00	159 671,59 143 051,59 16 620,00
TOTAL DES DETTES	2 646 379,50	3 015 434,93
D. COMPTES DE REGULARISATION	7 957 927,79	3 927 967,97
TOTAL DU PASSIF	14 420 432,79	10 708 545,17



### COMPTE DE PROFITS ET PERTES POUR L'EXERCICE ALLANT DU 1er JANVIER 2009 AU 31 DÉCEMBRE 2009

(en Euros)

	2009 (01.01.09-31.12.09)	2008 (01.01.08-31.12.08)
A. CHARGES		
<ol> <li>Réduction du stock de produits finis et en cours de fabrication</li> <li>a) Consommation de marchandises et de matières premières et consommables b) Autres charges externes</li> </ol>	10 497 073,83 3 482 113,56 7 014 960,27	7 116 874,58 4 097 607,07 3 019 267,51
<ul><li>3. Frais de personnel</li><li>a) Salaires et traitements</li><li>b) Charges sociales couvrant les salaires et traitements</li></ul>	14 417 775,32 12 696 402,95 1 721 372,37	11 003 970,90 9 867 024,86 1 136 946,04
a) Corrections de valeur sur frais d'établissement et sur immobilisations corporelles et incorporelles     b) Corrections de valeur sur éléments de l'actif circulant	360 110,83	190 301,71 0,00
5. Autres charges d'exploitation	1 220 579,01	1 188 536,78
Corrections de valeur sur immobilisations financières et sur valeurs mobilières faisant partie de l'actif circulant		
7. Intérêts et charges assimilées b) Autres intérêts et charges	6 356,99	4 620,50
10. Charges exceptionnelles	101 326,80	577 414,75
13. Résultat de l'exercice (bénéfice de l'exercice)	33 042,85	0,00
TOTAL DES CHARGES	26 636 265,63	20 081 719,22
TOTAL DES CHARGES  B. PRODUITS	26 636 265,63	20 081 719,22
	26 636 265,63 384 189,10	20 081 719,22 107 970,84
B. PRODUITS		,
B. PRODUITS  1. Montant net du chiffre d'affaires		, , ,
B. PRODUITS  1. Montant net du chiffre d'affaires  2. Augmentation du stock de produits finis et en cours de fabrication		, , ,
<ul> <li>B. PRODUITS</li> <li>1. Montant net du chiffre d'affaires</li> <li>2. Augmentation du stock de produits finis et en cours de fabrication</li> <li>3. Travaux effectués par l'entreprise pour elle-même et portés à l'actif</li> </ul>	384 189,10	107 970,84
B. PRODUITS  1. Montant net du chiffre d'affaires  2. Augmentation du stock de produits finis et en cours de fabrication  3. Travaux effectués par l'entreprise pour elle-même et portés à l'actif  4. Autres produits d'exploitation	384 189,10	107 970,84
B. PRODUITS  1. Montant net du chiffre d'affaires  2. Augmentation du stock de produits finis et en cours de fabrication  3. Travaux effectués par l'entreprise pour elle-même et portés à l'actif  4. Autres produits d'exploitation  5. Produits de participations	384 189,10	107 970,84
B. PRODUITS  1. Montant net du chiffre d'affaires  2. Augmentation du stock de produits finis et en cours de fabrication  3. Travaux effectués par l'entreprise pour elle-même et portés à l'actif  4. Autres produits d'exploitation  5. Produits de participations  6. Produits d'autres valeurs mobilières et de créances de l'actif immobilisé  7. Autres intérêts et produits assimilés	384 189,10 25 967 588,70	107 970,84 19 247 171,98
B. PRODUITS  1. Montant net du chiffre d'affaires  2. Augmentation du stock de produits finis et en cours de fabrication  3. Travaux effectués par l'entreprise pour elle-même et portés à l'actif  4. Autres produits d'exploitation  5. Produits de participations  6. Produits d'autres valeurs mobilières et de créances de l'actif immobilisé  7. Autres intérêts et produits assimilés b) Autres intérêts et produits assimilés	384 189,10 25 967 588,70 114 108,29	107 970,84 19 247 171,98 257 241,03





# DEPARTMENT OF CARDIOVASCULAR DISEASES

### LABORATORY OF CARDIOVASCULAR RESEARCH

Dr. Daniel Wagner c/o Maternité Grande Duchesse Charlotte 120, route d'Arlon,

L-1150 Luxembourg
Tel.: +352 26 970 300
Fax: +352 26 970 396
E-mail: wagner.daniel@chl.lu

# CLINICAL AND EPIDEMIOLOGICAL INVESTIGATIONS

### CLINICAL AND EPIDEMIOLOGICAL INVESTIGATION CENTER (CIEC)

Dr. Anna Chioti 1A, rue Thomas Edison, L-1445 Strassen Tel.: +352 26 970 800 Fax: +352 26 970 810

E-mail: anna.chioti@crp-sante.lu

## DEPARTMENT OF IMMUNOLOGY

#### LABORATORY OF IMMUNOLOGY

Prof. Dr. Claude P. Muller 20A, rue Auguste Lumière, L-1950 Luxembourg Tel.: +352 49 06 04 220 Fax: +352 49 06 86

E-mail: claude.muller@LNS.ETAT.LU

## DEPARTMENT OF ONCOLOGY

### LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

Dr. Guy Berchem CRP-Santé, BAM 84, Val Fleuri, L-1526 Luxembourg Tel.: +352 44 11 20 84 and +352 26 970 320 Fax: +352 44 12 15 E-mail: berchem.guy@chl.lu

### MICROARRAY CENTER

Dr. Laurent Vallar CRP-Santé-BAM 84, Val Fleuri, L-1526 Luxembourg Tel.: +352 26 970 281 Fax: +352 26 970 390

E-mail: laurent.vallar@crp-sante.lu

### NORLUX NEURO-ONCOLOGY LABORATORY

Dr. Simone Niclou 84, Val Fleuri, L-1526 Luxembourg Tel.: +352 26 970 273 Fax: +352 26 970 390

E-mail: simone.niclou@crp-sante.lu

## DEPARTMENT OF PUBLIC HEALTH

#### **CENTRE FOR HEALTH STUDIES**

Marie-Lise Lair CRP-Santé 1B, rue Thomas Edison, L-1445 Strassen Tel.: +352 26 970 752 Fax: +352 26 970 717

E-mail: marie-lise.lair@crp-sante.lu

### **EUROPEAN MEDICINES AGENCY**

Dr. Jean-Louis Robert CRP-Santé 1B, rue Thomas Edison, L-1445 Strassen

Tel.: +352 49 11 91 358 Fax: +352 40 43 19

E-mail: jean-louis.robert@lns.etat.lu

#### EUROPEAN MONITORING CENTRE FOR DRUGS AND DRUG ADDICTION – LUXEMBOURG FOCAL POINT

CRP-Santé
1B, rue Thomas Edison,
L-1445 Strassen
Tel.: +352 26 970 749
Fax: +352 26 970 717

E-mail: sofia.lopesdacosta@crp-sante.lu

### LABORATORY OF EMOTIONAL DISORDERS

Prof. Charles Pull
Centre Hospitalier de Luxembourg
4, rue Barblé,
L-1210 Luxembourg
Tel.: +352 44 11 27 06 and
+352 44 11 66 66
Fax: +352 44 12 47
E-mail: pull.charles@chl.lu

### SPORTS MEDICINE RESEARCH LABORATORY

Prof. Daniel Theisen
Maison Médicale - Fondation Norbert
Metz
5ème étage – 76, Rue d'Eich,
L-1460 Luxembourg
Tel.: +352 26 970 824
E-mail: daniel.theisen@crp-sante.lu

### DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

### LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

Dr. François Hentges CRP-Santé, BAM 84, Val Fleuri, L-1526 Luxembourg Tel.: +352 26 970 250 Fax: +352 26 970 390

E-mail: hentges.francois@chl.lu

### LABORATORY OF PLANT MOLECULAR BIOLOGY

Dr. André Steinmetz CRP-Santé, BAM 84, Val Fleuri, L-1526 Luxembourg Tel.: +352 26 970 251

Fax: +352 26 970 251

E-mail: andre.steinmetz@crp-sante.lu

### LABORATORY OF TOXICOLOGY

Dr. Brice Appenzeller Université de Luxembourg Bâtiment des Sciences, 162A, avenue de la Faïencerie, L-1511 Luxembourg

Tel.: +352 44 66 44 476 Fax: +352 22 13 31

E-mail: brice.appenzeller@LNS.ETAT.LU

#### LABORATORY OF RETROVIROLOGY

Dr. Jean-Claude Schmit CRP-Santé, BAM 84, Val Fleuri, L-1526 Luxembourg

Tel.: +352 26 970 224 Fax: +352 26 970 221

 $\hbox{E-mail: jc.schmit@crp-sante.lu}\\$ 

# TECHNICAL AND ADMINISTRATIVE DEPARTMENT

Daniel Cardao 1A-B, rue Thomas Edison, L-1445 Strassen

Tel.: +352 26 970 730 Fax: +352 26 970 719

E-mail: daniel.cardao@crp-sante.lu





Administration de la gestion de l'Eau

Agence Française de Sécurité Sanitaire de l'Environnement et du Travail

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