08

ACTIVITY REPORT 08

CENTRE DE RECHERCHE
PUBLIC DE LA SANTÉ

crp SANTÉ
CENTRE DE RECHERCHE PUBLIC

08

CONTENTS
ANNUAL REPORT
CENTRE DE RECHERCHE
PUBLIC DE LA SANTÉ

ADMINISTRATION BOARD

CRP-SANTÉ
CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

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

ADMINISTRATION BOARD
CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

Dear Reader,

I am delighted to submit as Chairman the report for 2008 on the operations and activities of the research centre for health CRP-Santé of the Grand Duchy of Luxembourg.

In doing so, I would like to pay tribute to all members of staff and other employees in the CRP and thank them for their input into our research during 2008, a period which was very important for CRP-Santé.

On April 28, we had the great pleasure to celebrate together the 20th anniversary of CRP-Santé.

The importance of this event was accentuated by the fact that the members of the board and the directorate were granted an audience by the Grand-Duc of Luxembourg, His Royal Highness Henri of Luxembourg. The ceremonial was also attended by the Minister of Research and by the Minister of Health.

This event underlined that CRP-Santé is still a young, even a very young institution in the field of research. As we do not have a past with decades of experiences from which we could have learned lessons for our own future, it has been thus the right choice to strengthen during the last two years the discussion around the Center's overall research policy and to stress the need to identify some few future key research fields and to build up the scientific critical mass needed in order to be competitive at international level.

Thanks to this, CRP-Santé will now be able to act as a key partner of the collaboration in biomedical research launched by the Gouvernement with some outstanding research institutions in the United States where building up an Integrated BioBank and research programs on biomarkers will be among the main issues in the first stage of this new partnership.

If on the one hand, such activities are very attractive for local and foreign researchers, one has to consider on the other hand, that the financial needs in order to implement such ambitious projects are huge. But in this context, we are all pleased with the Government's policy regarding research and innovation because, despite of the financial and economic crisis we have to face, it will continue to increase spending for research human resources, equipment an infrastructure allowing to offer excellent working conditions and to attract high level senior researcher from abroad. We are indeed also very pleased with the recent announcement that the foundation stone of CRP-Santé's new building will be laid very soon an its construction should be finished in 2011. Thus, in the near future we will be able to offer to our staff even better working conditions than we already do today.

Since this year, our research activities have been carried out under a performance contract with the Government. This means i.e. that CRP-Santé's projects have to respect some criteria which have been set up together with the Ministry for Research. Among these criteria, I would like to underline that, besides the research quality measured on behalf of the number and the impact factor of scientific publications, a new emphasis will be placed on the notion of return on investment. This means that besides the aim to promote excellence in research projects, we will also have to build up an evaluation tool in order to identify projects likely to produce results economic or commercial potential.

Dear Reader, as a conclusion, I would like to underline that I am very confident for the future. CRP-Santé has placed in its recent past the cornerstones needed in order to face the challenges of the future. Personally I am very much looking forward to this future.

Frank Gansen

THE MANAGEMENT BOARD'S MESSAGE

MANAGEMENT BOARD
CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

For CRP-Santé, the year 2008 was the first year under a performance contract with the Government. Establishing performance contracts for public research institutions had emerged as a key conclusion from the OECD review on research in Luxembourg a couple of years ago, and the Government, represented by the MCESR and in close collaboration with the research institutes, has straightforwardly driven their implementation. This first performance contract generated a high level of excitement at CRP-Santé in anticipation of a more mature relationship with the Government, but also some degree of anxiety. However, after the first year we are confident to say that the experience was overall positive and, due to a huge team effort by its employees, CRP-Santé was able to fulfil most of its key performance indicators with success. As a research institute our most important performance indicator relates to scientific publications: we were able to increase the number of peer-reviewed publications by 21% and their mean impact factor – reflecting their scientific value – by 75% in only one year. We are looking forward to deepen our compliance with the performance contract over the next two years and to suggest further improvements – taking advantage of our organisational learning capabilities – for the second contract starting in 2011.

Our independent scientific advisory board reviews are an additional, more qualitative mechanism for performance evaluation. As in 2005 and 2006, the research work of CRP-Santé was positively evaluated in 2008 by an external panel of experts composed of twelve renowned researchers from six European countries. For CRP-Santé this independent audit is essential, as it allows situating our activities in an international context and helps to set new challenging targets for continuous improvement. This time it also triggered a more profound strategic reflection which will result in some short-term adjustments and, more importantly, should help to inform and shape the next performance contract.

At an operational level, the management team had chosen to put special emphasis on external communication during the past year. As a public institution, we strongly believe that the taxpayer has the right to be informed on the projects and results on which public money is spent. Therefore, we took the opportunity of the 20th anniversary of CRP-Santé to broadly communicate about our work in more than a dozen conferences targeted at the general public. We also completely redesigned our website and largely communicated about our work in collaboration with the local press. We are committed to continuing this communication effort in 2009 and will add a focus on internal communication, too. Internal communication is essential for the effective management of a complex knowledge creating organisation such as CRP-Santé and contributes significantly to human resources satisfaction. As the first Luxembourg organisation signing the European Charter for Researchers in 2007, we took the engagement to improve the career development opportunities of our employees. Part of this has been realised in 2008 by the revision of our scientific careers and associated compensations. Researchers can now access different careers based on their competences and level of commitment to the organisation. In addition, our human resources department has intensively collaborated with European instances to prepare the implementation of other key points from the Charter.

The year 2008 also saw the start of our new clinical and epidemiological investigation department (CIEC). CRP-Santé is convinced that clinical and epidemiological research and translational medicine will become increasingly important over the next years and the CIEC gives us the practical instrument to be a national leader in these fields. In collaboration with its partners, CIEC has started the first high quality clinical research projects, complying strictly with ethical and data-protection guidelines and bringing together the needs of pharmaceutical/biotech companies, medical community and diseased people. In order to achieve its mission, it interfaces closely with the hospitals, the national ethics and data-protection committees and the newly created "Integrated BioBank of Luxembourg" (IBBL).

Finally, the biomedical research environment of Luxembourg was profoundly modified in 2008 after the Government announced in June a large collaboration effort of the Luxembourg research institutions with researchers from three outstanding US institutions (i.e. TGen Foundation in Phoenix, Arizona and the Fred Hutchinson Cancer Center and the Institute for System Biology, both in Seattle, Washington). On the Luxembourg side, CRP-Santé, together with its national partners, is a main actor in this exciting endeavour. More specifically, CRP-Santé is one of the four co-founders of the Integrated BioBank of Luxembourg, and its Oncology Department pursues a collaborative project on biomarkers in lung cancer together with the research group of the 2001 Nobel Prize winner Lee Hartwell from Seattle. Our Centre for Health Studies is associated with the health economics part of the project.

In conclusion, we believe that 2008 has been a pivotal year for CRP-Santé, allowing the institution to continue its internal reorganisation and improvements whilst opening exciting new opportunities at both national and international level.

The Management Board,

Marie-Lise Lair **Jean-Claude Schmit** **Daniel Cardao**

THE INTEGRATED BIOBANK OF LUXEMBOURG

IBBL

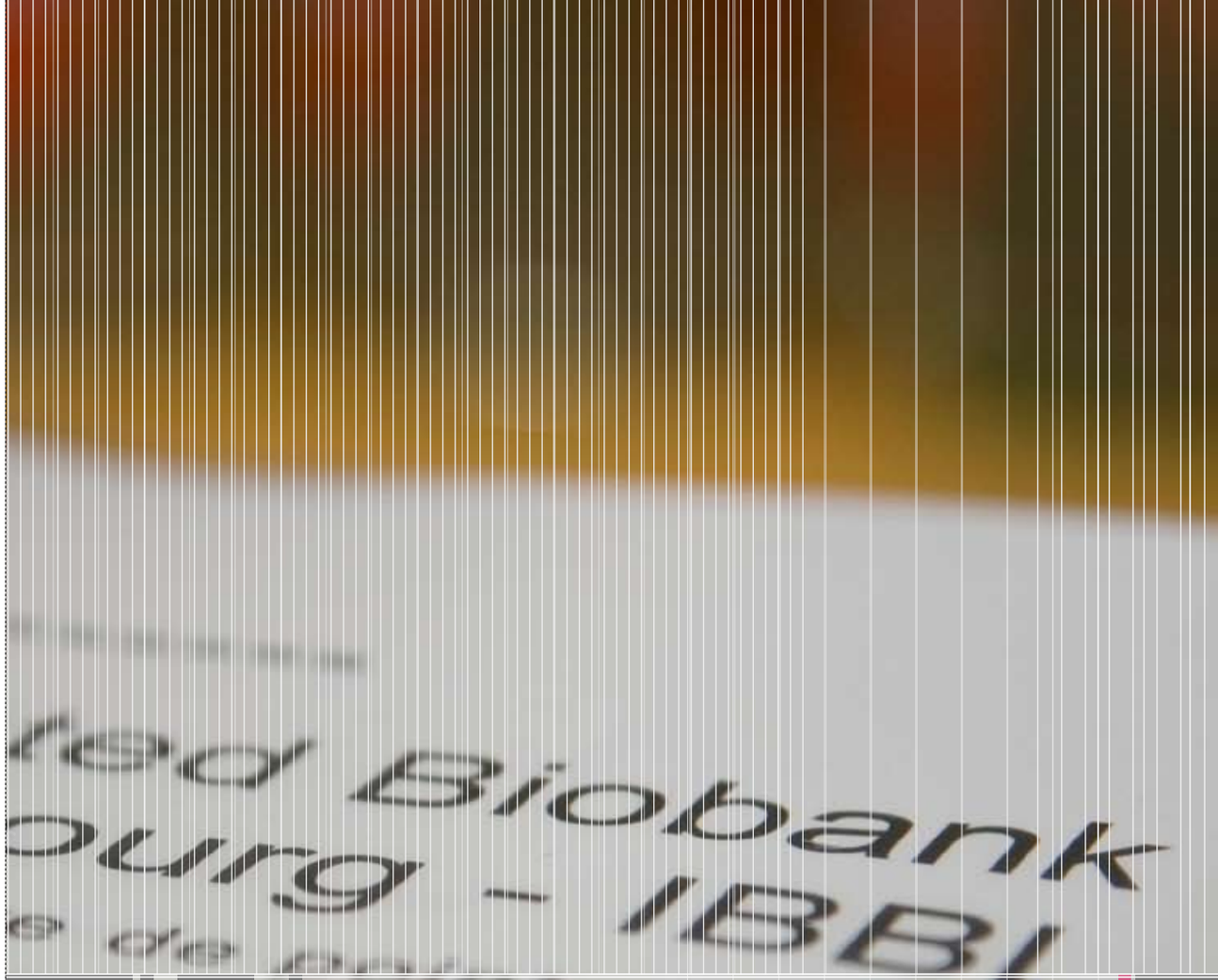
Collaboration with outstanding US scientists gives new momentum for biomedical research in Luxembourg: CRP-Santé is one of the founders of the Integrated BioBank of Luxembourg (IBBL)

The Government of Luxembourg decided in June 2008 to invest more than 150 million Euros to foster biomedical research and development in our country. Three projects will be realised over the next five years. CRP-Santé is a partner in all of them.

First, an institute for systems biology will be established at the University of Luxembourg in collaboration with the Institute of System Biology in Seattle, headed by Dr Leroy Hood. Amongst others, this institute will support training in life sciences in Luxembourg, thus helping to supply the local recruitment market with highly qualified researchers.

Second, a state-of-the-art BioBank called IBBL (Integrated BioBank of Luxembourg) will be established in close partnership with the non-for-profit Translational Genomics Research Institute (TGen) in Phoenix, Arizona. TGen is led by Dr Jeffrey Trent, an internationally renowned researcher and former leader of the human genome project at the National Institute of Health (NIH). Together with the University of Luxembourg and the two other public research centers, CRP-Santé is one of the founders of IBBL, which will be located on the premises of CRP-Santé and will collaborate closely with CRP-Santé's new clinical investigation center (CIEC), the national and regional hospitals and the pathology department at Laboratoire National de la Santé. While starting its activities, IBBL has already gained international attention and entered collaborations with the European network BBMRI (Biobanking and Biomolecular Resources Research Infrastructure) and the international consortium P3G (Public Population Project in Genomics). IBBL was able to attract Dr Robert Hewitt, currently head of the tissue repository at the National University Hospital in Singapore and president of the international consortium of biobanking ISBER (International Society for Biological and Environmental Repositories). Dr Hewitt will start his activities as CEO of IBBL during summer 2009.

Finally, the third part of the programme is related to the discovery of biomarkers for lung cancer in collaboration with the Fred Hutchinson Cancer Research Center in Seattle, led by Nobel Prize winner Dr Leland Hartwell. Lung cancer is still one of the most deadly diseases and biomarkers would be of invaluable help for early diagnosis and treatment guidance. The biomarker discovery programme will be conducted together with the oncology unit of CRP-Santé and will develop skills in clinical proteomics and health economics in Luxembourg.



SCIENTIFIC ADVISORY BOARD

COMPOSITION 2008/ 2009

Professor Jules Hoffmann
CNRS, 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
Hombourg, Germany

Professor Eric Van den Neste
Cliniques Universitaires St.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

CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

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

Prof.	Albert, Jan	Karolinska Institute
Dr	Arenzana - Seisdedos, Fernando	Institut Pasteur
Dr	Benfenati, Emilio	Istituto di Ricerche Farmacologiche "Mario Negri"
Prof.	Böhm, Michael	Universität des Saarlandes
Prof.	Bouchard, Stephane	Université du Québec en Outaouais
Prof.	Bron, Dominique	Institut Jules Bordet
Prof.	Capron, Monique	Institut Pasteur de Lille
Prof.	Carlberg, Carsten	Université du Luxembourg
Dr	Dadoun, Frédéric	CHU Nord - Marseille
Prof.	Deckmyn, Hans	Katholieke Universiteit Leuven
Dr	Demeulemeester, René	INPES
Prof.	Dunlop, Tom	University of Kuopio
Dr	Fiszman, Marc	INSERM
Prof.	Gärtner, Barbara	Universität des Saarlandes
Prof.	Goubeau, Patrick	Université Catholique de Louvain
Prof.	Griffioen, Arjan	University Hospital Maastricht
Prof.	Hemmer, Wolfgang	FAZ Florisdorf Allergy Center
Prof.	Jouvent, Roland	Hôpital La Salpêtrière
Dr	Kleine-Tebbe, Jörg	Allergie- und Asthma Zentrum Westend
Prof.	Klys, Malgorzata	Institute of Forensic Medicine Jagiellonia University
Dr	Losch, Serge	Ministère de l'Agriculture
Prof.	Louvard, Daniel	Institut Curie
Prof.	MacGowan, Guy	Freeman Hospital
Prof.	Meyer, Jobst	Universität Trier
Prof.	Möller, Manfred	Universität des Saarlandes
Prof.	Moutschen, Michel	Centre Hospitalier Universitaire de Liège
Prof.	Müller, Ulrich	Spital Bern
Prof.	Nielsen, Lars	Staten Serum Institute
Prof.	Panagiotakos, Demosthenes	Harokopio University
Prof.	Pancino, Gianfranco	Institut Pasteur
Prof.	Pauli, Gabrielle	Nouvel Hôpital Civil Strasbourg
Prof.	Ringelstein, E.	Universitätsklinik Münster
Prof.	Sinski, Edward	University of Warsaw
Prof.	Sitkovsky, Michail	New England Inflammation and Tissue Protection Institute
Prof.	Sönnerborg, Anders	Karolinska Institute
Dr	Staub, Christian	Centre Médical Universitaire - Genève
Prof.	Swynghedauw, Bernard	Hôpital Lariboisière
Prof.	Tennant, Alan	University of Leeds
Prof.	Van Riet, Ivan	Vrije Universiteit Brussel
Prof.	Van Troys, Marleen	Ghent University
Prof.	Vieths, Stefan	Paul Ehrlich Institut
Prof.	Wehland, Jürgen	Helmholtz Zentrum für Infektionsforschung
Prof.	Westphal, Manfred	Universitätsklinikum Hamburg Eppendorf
Dr	Wüst, Stefan	Universität Trier

RESEARCH DEPARTMENTS
DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

LABORATORY OF RETROVIROLOGY

HEAD OF LABORATORY:
Jean-Claude SCHMIT, MD, PhD

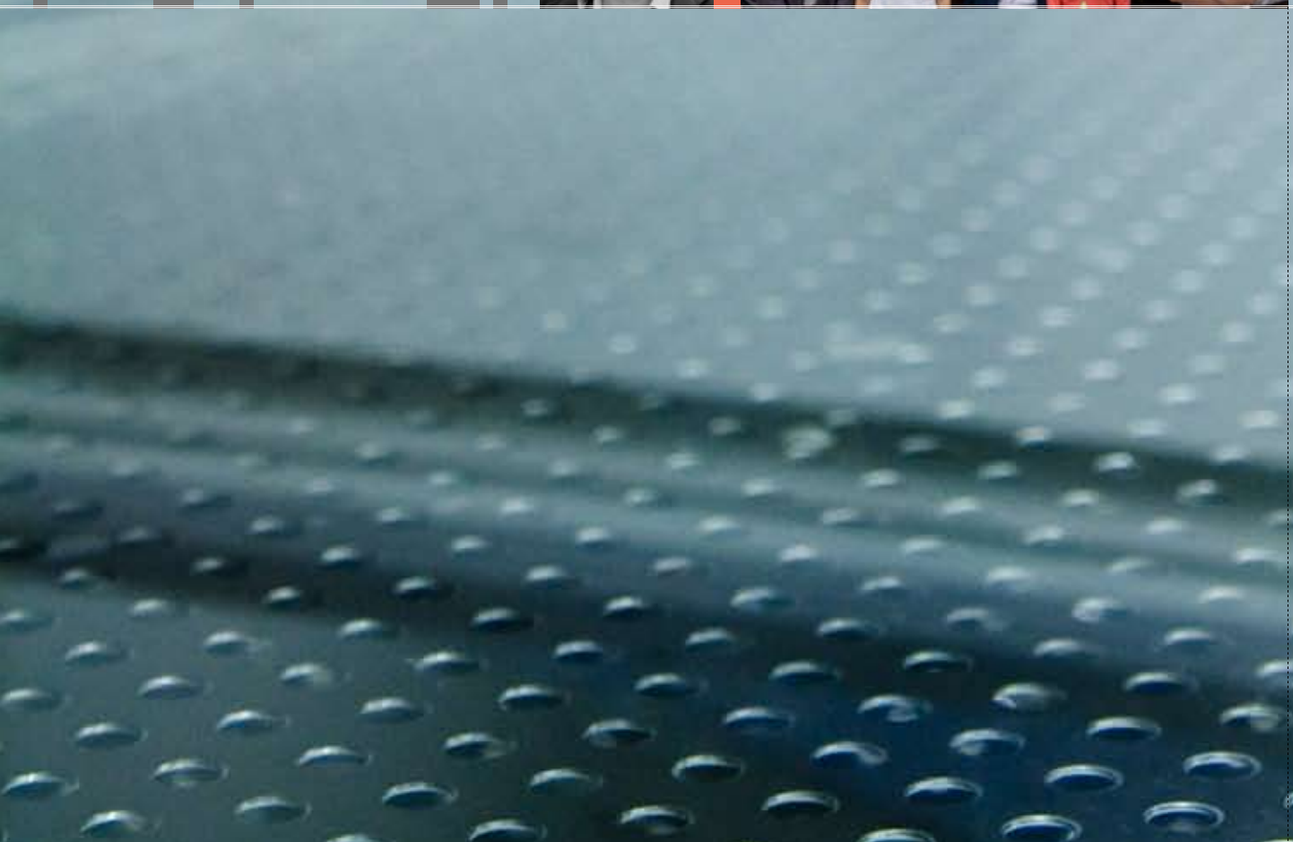
ASSOCIATE HEAD OF LABORATORY:
Carole DEVAUX, PhD

PRESENTLY THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Hélène AGOSTINIS, Administrative Assistant
Vic ARENDT, MD, Collaboration with African countries, Researcher
Franky BAATZ, PhD Student
Nadia BEAUPAIN, Technician
Andy CHEVIGNE, PhD Researcher
Manuel COUNSON, Technician
Sylvie DELHALLE, PhD, Researcher in Immuno-Virology
Sabrina DEROO, PhD, Team-leader of the Research Group Immuno-Virology, Researcher
Aurélie FISCHER, Engineer
Alain GRAS, PhD Student
Robert HEMMER, MD, Researcher in Clinical Virology
Gilles ISERENTANT, Engineer
Jean-Claude KARASI, PhD Student
Cyrille LEJCZAK, Research Assistant
Morgane LEMAIRE, Technician
Cécile MASQUELIER, Engineer
Julie MATHU, Technician
Danielle PEREZ-BERCOFF, PhD, Team-leader of Research Group Clinical Virology, Researcher
Sandrine PERUCHON, PhD, Researcher
Jean-Marc PLESSERIA, Technician
Jean-Yves SERVAIS, Engineer
Daniel STRUCK, Bioinformatician
Anne-Marie TERNES, Bioinformatician
Charlène VERSCHUEREN, Technician

EXTERNAL COLLABORATORS
(FONDATION RECHERCHE SUR LE SIDA):

Thérèse BAURITH, Data Manager
Valérie ETIENNE, Data Manager
Karin HAWOTTE, Technician
Christine LAMBERT, Engineer
Samiha REGAIA, Technician
Thérèse STAUB, MD, Clinical Trials



LABORATORY OF RETROVIROLOGY

The Laboratory of Retrovirology was founded in 1989 by a governmental decision as the national reference laboratory for HIV. HIV is an ever-growing public health problem in Luxembourg. More than 700 patients infected with HIV have been admitted to the Centre Hospitalier de Luxembourg (CHL) over the past decade. The laboratory is working in close relationship with the National Department of Infectious Diseases to provide highly specialized technical support for the clinical follow-up of these patients. At the same time, the Laboratory of Retrovirology developed clinically oriented research and provided relevant epidemiological data in the field of chronic viral infections (HIV and HCV) in 2008. The activities of the laboratory are organised in two closely integrated units, immuno-virology and clinical virology.

OBJECTIVES OF THE LABORATORY OF RETROVIROLOGY

IMMUNO-VIROLOGY RESEARCH UNIT (DR SABRINA DEROO, PHD)

One of the objectives of the Immuno-Virology 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 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.

CLINICAL VIROLOGY RESEARCH UNIT (DR DANIELLE PEREZ-BERCOFF, PHD)

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),
III. clinical implications of viral and host factors involved in HIV-1 entry,
IV. collaborations with African countries.

ONGOING PROJECTS

IMMUNO-VIROLOGY RESEARCH UNIT

We focused our efforts on identifying new ligands of the main co-receptors of HIV-1, the G protein coupled chemokine receptors CCR5 and CXCR4. Antibody fragment libraries were used for screening on ECL2. These libraries were engineered in collaboration with the Belgian company Algonomics and are based on the monovalent display of the heavy chain complementarity determining region 3 (HCDR3). We have constructed immune HCDR3 phage libraries from PBMC of long-term non-progressing HIV patients (LTNP). Both naïve and LTNP HCDR3 libraries were screened against ECL2: 149 and 40 positive clones were retrieved from the immune and naïve libraries, respectively. The 149 positive clones displayed 10 different HCDR3 sequences of which one sequence represented 85% of the sequences. The sequence diversity of the naïve library was higher and 22 different HCDR3 sequences were identified. Finally, 4 HCDR3 sequences from the naïve library were revealed ECL2-specific in dose responses assays and two synthetic peptides corresponding to the HCDR3 sequences, in cyclic and linear format, inhibited the HIV fusion event between CCR5+/CD4+ cells and cells expressing an R5 envelope with an IC50 of 125 and 148 mM respectively (patent filing and manuscript in preparation). The inhibition of fusion on laboratory and primary R5 isolates viruses will be assessed. The physiological activity of these peptides on the CCR5 receptor will be tested to determine whether these peptides act as agonists or antagonists. Their competitive effect with RANTES and other CCR5 agonists/antagonists will be studied to understand the mode of inhibition of these peptides. For the CXCR4 receptor, analogous experiments were performed on different peptides corresponding to the three extracellular loops and the N-terminus of CXCR4. The screening of linear and cyclic peptide libraries was not successful. Screening on CXCR4 derived peptides with constrained peptides expressed in fusion with the phage protein VIII is ongoing, as well as screening strategies using competitive elution with the natural ligand CXCL12.

CLINICAL VIROLOGY RESEARCH UNIT

EPIDEMIOLOGICAL FOLLOW-UP OF HIV INFECTION IN LUXEMBOURG

A dramatic increase of the incidence of HIV-1 recombinant forms has been observed in Luxembourg since 2003 where 35.5% of the newly diagnosed patients were infected with unique recombinant forms (URF) or circulating recombinant forms (CRF). We have identified a new B/F1 recombinant (CRF42) in 21 patients who mostly reported heterosexual transmission over a short time interval (2003-2006). We have confirmed the inedited nature of this new CRF by near full-length genome characterization. The demographic history of the epidemic was described using a non-parametric Bayesian skyline plot. The origin of the most recent common ancestor (MRCA) was estimated to be the beginning of 2002. While the CRF42 strains were not characterized by a high evolutionary rate, the exponential phase of the logistic growth happened in a very short time period of approximately 5 months, which may reflect an elevated transmission fitness. Based on growth experiments, we have shown that CRF42 did not exhibit replication advantage to parental B or F1 strains in lymphocytes. The transmission fitness of the variant from dendritic to quiescent T cells is currently under investigation (manuscript in preparation).

PRIMARY TRANSMITTED AND SECONDARY RESISTANCE TO ANTIRETROVIRAL THERAPY

We are analyzing Integrase determinants/polymorphisms involved in resistance and selection in HIV-1 and HIV-2 viral strains in 147 baseline sequences and 100 sequences from highly HAART-experienced patients failing to respond to NRTI treatment. In collaboration with the University of Kaiserslautern in Germany, we will characterize resistance patterns of minority species of 25 patients hosting viral strains with resistance mutations to at least one NRTI and/or NNRTI and/or PI prior to any treatment using ultra-deep sequencing with the Roche 454 technology.

DEVELOPMENT OF A HIV-1 ENV- AND A GP41-RECOMBINANT VIRUS ASSAY (RVA)

New molecules acting as Fusion Inhibitors (FI) or as Entry Inhibitors (EI) are being developed. The FI Enfuvirtide (ENF or T-20) and the CCR5 antagonist Maraviroc have recently been licensed for clinical use. New reliable, easy-to-use phenotypic tools are needed for optimizing therapeutic follow-up in clinic. Resistance to T-20 is governed by changes in the HR1 and HR2 regions of gp41. There is accumulating evidence that other Env domains also play a role on susceptibility/resistance to T-20. We therefore engineered 2 recombinant virus assays (RVA) targeting only HR1/HR2 or the full Env of HIV-1 based on the NL4-3 reference clone tagged with a Firefly luciferase reporter gene. Both RVAs have been validated using reference clones and experiments comparing susceptibility to T-20 of patient-derived samples using both RVAs are ongoing in collaboration with the Department of Virology of the Medical Center of Utrecht (manuscript in preparation). The Env RVA is also being validated to monitor viral tropism and sensitivity/resistance to Maraviroc. We are currently assessing its sensitivity to detect minority species in different HIV-1 subtypes. We intend to investigate the susceptibility to infection of distinct primary target CD4+ T-cell subsets with patient-derived Env quasispecies.

COLLABORATIONS WITH AFRICAN COUNTRIES

We have previously described a novel 24-base-pair deletion (hCCR5D24) in the first extracellular loop (ECL1) of CCR5 in the Rwandese population (Masquelier et al., 2007). We further investigated the prevalence of the deletion in several Rwandese cohorts. In Rwanda, 12 individuals were heterozygous for hCCR5D24: 2/386 (0.52%, 95%CI: -0.20;1.23) individuals from the general population, 2/572 (0.35%, 95%CI: -0.13;0.83) HIV+ against 6/605 (0.99%, 95%CI: 0.20;1.78, p = 0.078) HIV- Exposed Uninfected partners from serodiscordant couples and 2/83 (2.41%, 95%CI: -0.89;5.71, p = 0.078 vs HIV + members) Long-Term Survivors. The deletion was not detected in 500 HIV- and 300 HIV+ Caucasians in Luxembourg. In parallel, HEK-293T cells, HeLa-CD4 cells and PBMCs were transfected with N-terminus HA-tagged WT- or D24-hCCR5. Intracellular and extracellular CCR5 expression was measured by flow cytometry and confocal immunofluorescence microscopy using several anti-HA-tag and anti-CCR5 antibodies targeting the N-terminal and ECL1/ECL2 regions. Our results suggest that the hCCR5D24 deletion affects CCR5 conformation at the membrane of HEK293T cells and CCR5 addressing at the surface of HeLa-CD4 cells and PBMCs. These findings might explain the high prevalence of the heterozygous deletion in Rwandese EU and LTS individuals. Potential effects of the 24-basepair deletion in HIV fusion and CCR5 function are currently under investigation using HEK-293T cells (manuscript in preparation). In Rwanda, we have also initiated one project to describe HIV-1 genetic variability and resistance drug mutation patterns in naïve-treatment and treated patients in 8 health centers. In 2008, 1523 people have been enrolled in the study with a mean treatment duration of 2.8 years. 130 patients presented therapeutic failure and viral RNA will be sequenced with the Viroseq genotyping system. The functional role of several mutations highlighted in subtype A and C from Rwanda will be assessed in phenotypic assays. We will develop one PR and one RT RVA with an appropriate backbone A. These geno-phenotypic data may lead to the development of an algorithm for non-B drug resistance interpretation in collaboration with Euresist. The ART-A project (Affordable Resistance Tests for Africa) brings together 5 public and private institutions under the coordination of PharmAccess, Amsterdam, to develop an affordable, robust and accessible resistance test customised for Africa that takes into account the local circulating strains of HIV (mainly subtype C). The Laboratory of Retrovirology is currently developing a sequence editing, alignment and interpretation software to process the data that will be generated.

KEY RESULTS

IMMUNO-VIROLOGY RESEARCH UNIT

We developed a rational strategy for efficient searching of target-specific high affinity binders from naïve HCDR3 libraries displaying the IgM and IgG repertoire of healthy donors. The proof of concept of this technology was provided by the identification of high affinity binders (KD of 7.6 nM) to two different targets including an anti-hemagglutinin antibody (Deroo et al., 2008). Our findings strongly underlined the value of the HCDR3 libraries as a source of biologically randomized sequences. Screening on enzymatic and protein targets with the HCDR3 libraries is ongoing. The first results are promising but further investigations to confirm the specificity of the identified HCDR3 sequences is required (manuscript in preparation).

The circulating IgA population of LTNP was used to identify new HIV-specific epitopes/mimotopes that could lead to the development of a multi-epitope vaccine eliciting protective IgA responses at mucosal entry sites. Screenings of different phage libraries on IgA from LTNP allowed us to obtain 3 mimotopes reacting with the IgA of at least 2 LTNP. To test the HIV specificity of these LTNP- specific IgA mimotopes, large panels of HIV positive and negative sera need to be tested. Therefore, microarray experiments allowing the high throughput serum analysis of large panels of sera with a minimal use of biological material are under development.

Our first experiments results showed that

1. phage-displayed inserts are recognised by specific antibodies when phage are spotted on nitrocellulose-coated glass slides,
2. IgA purified or in human plasma spotted on slides are specifically recognised by an anti-human IgA antibody, and
3. IgA specific phage clones obtained after screening are recognised by their corresponding IgA in microarray experiments comparable to the ELISA experiments.

These results demonstrate the feasibility of a high throughput serum microarray analysis using phage-displayed inserts as antigen (manuscript in preparation). The analysis of the HIV specificity of all clones issued from the different IgA screening strategies in the microarray assay on large panels of HIV positive and negative sera is ongoing. Immunogens based on the HIV-specific mimotopes identified in these studies will be engineered and their ability to elicit HIV-neutralizing antibodies will be studied in mice.

CLINICAL VIROLOGY RESEARCH UNIT

DESCRIPTION OF THE HIV-2 EPIDEMIC IN BELGIUM AND LUXEMBOURG

In collaboration with the AIDS Reference Laboratory of the Catholic University of Louvain, clinical data of about 65 HIV-2 infected patients were collected. The effect of ARV therapy on viral load and CD4 counts were analyzed and protease (PR) and reverse transcriptase (RT) genes from ARV-naïve and treated patients were sequenced (Ruelle J et al., 2008). Twenty patients were treated with 25 different ARV combinations in a total of 34 regimens, and 6 months after the start of ARV therapy, only one third achieved viral load suppression. All of these successful regimens bar one contained protease inhibitors (PI) and better virologic and immunological results were achieved with PI-containing regimens. The analysis of HIV-2 specific mutations selected during therapy showed for the first time transmission of resistant HIV-2 viruses in Belgium and Luxembourg.

EPIDEMIOLOGICAL FOLLOW-UP OF HIV-1 AND HCV INFECTION IN LUXEMBOURG

Our laboratory has participated in several international surveillance programs on HIV and HCV drug resistance, including Spread/Europe HIV Resistance, EuroHIV, EuroSida, Virgil and Euresist (Strock P et al., 2008, Rosen-Zvi M et al., 2008, Altmann A et al., 2008).

CHARACTERIZATION OF THE IMPACT OF A RARE HIV-1 PROTEASE SUBSTITUTION (G48E) ON HIV-1 REPLICATION CAPACITY IN VITRO

We observed an unusual glycine-to-glutamate substitution at PR residue position 48 (G48E) in an African patient infected with a subtype A1 HIV-1 strain failing a saquinavir (SQV)-containing regimen. Phenotypic analysis of protease inhibitor (PI) susceptibility showed that the G48E site-directed mutant was slightly resistant to SQV. The G48E and G48E/V82A site-directed mutants were associated with a decrease in fitness, whereas a reversion to the wild type at position 48 was observed in vitro. Growth competition experiments performed in the department of Virology of the Cleveland Clinic Foundation showed that the replicative fitness of the G48E virus was reduced to 55% compared with the parental NL4-3 virus. A molecular dynamics simulation approach documented that the G48E mutant interacted with PI resistance mutations and with polymorphisms specific to subtype A1 present in the patient's virus (Zimmer et al., 2008).

EFFECTIVENESS OF ANTIRETROVIRAL THERAPY IN BREASTFEEDING MOTHERS TO PREVENT POST-NATAL VERTICAL TRANSMISSION IN RWANDA

Vertical transmission through breastfeeding remains a major problem in limited-resource countries. HAART with efavirenz (EFV) in HIV-positive pregnant women during the last trimester of pregnancy and for 6 months after delivery was evaluated in 13 women and their infant. In collaboration with the Laboratory of Toxicology, we have measured EFV concentrations in maternal plasma, breast milk and in newborns' plasma. After 6 months of breastfeeding, no child had been infected with HIV and all had good psychomotor and growth development (Schneider S et al., 2008).

TECHNOLOGY TRANSFER TO AFRICAN COUNTRIES

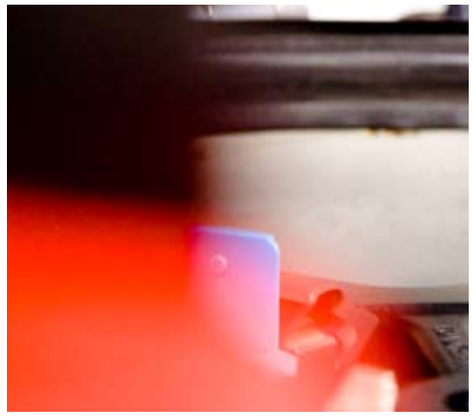
In collaboration with Médecins Sans Frontières (MSF), ARV treatment efficacy and resistance testing was evaluated using dried plasma-spot technology on 150 patients from Mozambique (Maldonado F et al., 2008). HIV diagnostic tests have also been performed on 100 children from Guinea. Our laboratory has trained two Rwandese technicians in the ViroSeq HIV-1 genotyping system and implemented the technology in the National Reference Laboratory of Rwanda.
In 2008, the Laboratory of Retrovirology had 3 oral and 2 poster presentations at international congress.

COLLABORATIONS

- **PMEs:** Algonomics (Ghent), ABL (Luxembourg), Institut für Immunologie und Genetik (Kaiserslaütern), Dr Margarete Fisher-Bosch Institut of Clinical Pharmacology (Stuttgart)
- University of Strasbourg (Pr. J.L. Galzi), University of Louvain (Pr. P. Goubau), University of Liège (Pr. M. Moutschen, Pr V. Bours, Pr L. Wehenkel), University of Utrecht (Dr M Nijhuis, Dr A.M. Wensing)
- Belgian AIDS reference laboratories
- **European networks:** SPREAD/EHR , Virgil , EuroSiDA, EuroHIV, INSIGHT (NIH), Euresist
- **Collaborations with Africa:** Network ART-A, MSF, The Rwanda –Zambia HIV Research Group (Emory University, Atlanta), The National Reference Laboratory of Rwanda, LuxDevelopment.

PUBLICATIONS 2008

- Roman F, Hawotte K, Struck D, Ternes AM, Servais JY, Arendt V, Hoffman P, Hemmer R, Staub T, Seguin-Devaux C, Schmit JC. ***Hepatitis C Virus genotypes distribution and transmission risk factors in Luxembourg from 1991 to 2006.*** World J Gastroenterol 2008, 14(8): 1237-1243.
- Ruelle JP, Roman F, Vandenbroucke AT, Lambert C, Fransen K, Echahidi F, Pierard D, Verhofstede C, Van Laethem K, Delforge ML, Vaira D, Schmit JC, Goubau P. ***Transmitted drug resistance, selection of resistance mutations and moderate antiretroviral efficacy in HIV-2: analysis of the HIV-2 Belgium and Luxembourg database.*** BMC Infect Dis 2008, 8(1): 21- 26.
- Deroo S, Fischer A, Beaupain N, Counson M, Boutonnet N, Pletinckx J, Loverix S, Beirnaert E, De Haard H, Schmit JC, Lasters I. ***Non-immunized natural human heavy chain CDR3 repertoires allow the isolation of high affinity peptides mimicking a human influenza hemagglutinin epitope.*** Mol Immunol 2008, 45(5):1366-73.
- Zimmer JM, Roman F, Lambert C, Jonckheer A, Vazquez A, Plesséria JM, Servais JY, Covens K, Weber J, Van Laethem K, Schmit JC, Vandamme AM, Quinones-Mateu ME, De Maeyer M. ***Impact on replicative fitness of the G48E substitution in the protease of human immunodeficiency virus type 1: an in vitro and in silico evaluation.*** J Acquir Immune Defic Syndr 2008, 48(3):255-62.
- ***Efavirenz in human breast milk, mothers' and newborns' plasma.*** Schneider S, Peltier A, Gras A, Arendt V, Karasi-Omes C, Mujawamariwa A, Ndimubanzi PC, Ndayisaba G, Wennig R. J Acquir Immune Defic Syndr 2008, 48(4):450-4.
- ***Selecting anti-HIV therapies based on a variety of genomic and clinical factors.*** Rosen-Zvi M, Altmann A, Prosperi M, Aharoni E, Neuvirth H, Sönnerborg A, Schülter E, Struck D, Peres Y, Incardona F, Kaiser R, Zazzi M and Lengauer T. Bioinformatics 2008, 24(13):i399-i406.
- ***Comparison of classifier fusion methods for predicting response to anti HIV-1 therapy.*** Altmann A, Rosen-Zvi M, Prosperi M, Aharoni E, Neuvirth H, Schülter E, Büch J, Struck D, Peres Y, Incardona F, Sönnerborg A , Kaiser R, Zazzi M and Lengauer T. PLoS ONE 2008, 3(10):e3470.
- ***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, in press.
- Strock P, Mossong J, Hawotte K and Arendt V. ***Access to Treatment of Hepatitis C in Prison Inmates.*** Dig Dis Sci 2008, in press.



RESEARCH DEPARTMENTS

DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

HEAD OF LABORATORY:

François HENTGES, MD

PRESENTLY THE STAFF OF THE LABORATORY IS AS FOLLOWS:

BIOMOLECULAR, IMMUNE AND BIOCHEMICAL CHARACTERISATION OF ANIMAL ALLERGENS:

Caroline DAVRIL, Technician
Olivia DOMINGUEZ, Technician
Thorsten GRAFF, Technician
Christiane HILGER, PhD, Project Supervisor
Stéphanie KLER, Engineer
Annette KUEHN, PhD, Project Manager
Cathy LEONARD, PhD, Project Manager
Tania SCHUMACHER, Technician
Kyra SWIONTEK, Engineer

INNATE IMMUNITY AT THE CELLULAR LEVEL:

Tatiana MICHEL, PhD (post-doc)
Aurélie POLI, Engineer
Natacha RALAINIRINA, Doctoral Student
Maude THERESINE, Technician
Jacques ZIMMER, MD, PhD, Project Supervisor

LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

Allergic diseases are a major health problem for hundreds of millions of people in developed countries, and increasingly so in developing countries. It is the strategy 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.
In its scientific strategies the LIGA aims at performing high quality basic research focusing on the:

- Characterisation 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.
- Analysing 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.

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 of the CHL in molecular biology techniques.

ONGOING PROJECTS

BIOMOLECULAR, IMMUNE AND BIOCHEMICAL CHARACTERISATION OF ANIMAL ALLERGENS

The laboratory has cloned and characterized an important number of proteins belonging to the 3 major animal allergen families: lipocalins, serum albumins, and parvalbumins. Over the next years the lab will continue the cloning activities of new allergens, extend the analysis of the immune and regulatory immune response to selected allergens, develop the biochemical characterisation of selected allergen molecules. Running projects are:
Further characterisation at the DNA and protein level of guinea pig and rabbit allergen families.
Biochemical and pharmacological characterisation of Arg r 1, the major allergen responsible for pigeon tick anaphylaxis.
Production of genomic and molecular tools for the tracing of parvalbumins of fish, mammalian, or bird origin. Cloning of new fish allergens.
Analysis in an in-vitro BALB/c mouse system of the major T-cell epitopes recognized by natural and induced T regulatory cells against cat serum albumin, cat Fel d1, and cod parvalbumin.

INNATE IMMUNITY AT THE CELLULAR LEVEL

The laboratory works on natural killer (NK) cells, neurotrophins and the interactions between them in the context of normal individuals (humans and mice) compared to allergic ones. The aim is to know

I. if NK cells express the receptors for the neurotrophins nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and neurturin,
II. if NK cells themselves produce these factors,
III. if neurotrophins modulate the phenotypic and functional properties of NK cells,
IV. if some of these parameters are different between normal and allergic humans and mice.

Another part of the activities deals with the study of human major histocompatibility complex class I (MHC class I) deficiency. This is a rare disease mostly due to homozygous mutations in the transporter associated with antigen processing (TAP) and is characterized clinically by chronic bacterial infections of the upper and lower respiratory tract and by granulomatous debilitating skin lesions. NK cells from these patients are anergic due to the absence of "educating" MHC class I molecules.

KEY RESULTS

ALLERGEN CHARACTERISATION AND CLONING

THE LIPOCALIN FAMILY

New allergens were purified from guinea pig salivary glands and identified by N-terminal sequencing. One of them has been cloned. Its recombinant protein is recognized by IgE in allergic patients. It belongs to the lipocalin family and has 49% identity with horse lipocalin Equ c 1. A patent concerning 3 guinea pig allergens has been deposited. Protein purification from rabbit fur has yielded N-terminal sequence data of two new allergenic molecules. One of them belongs to the lipocalin family and has a high sequence identity (48%) to one of the new guinea pig allergens. Both cDNAs are currently being expressed in E. coli for characterization with patient sera.
Our laboratory has previously characterized Arg r 1, the major allergen from Argas reflexus, a soft tick feeding on pigeons. We have expressed Arg r 1 in different E. coli strains and compared the recombinant to the native molecule by circular dichroism spectroscopy. IgE reactivity to rArg r 1 will be analysed in an epidemiological collaboratative study using a microarray system.

THE PARVALBUMIN FAMILY (FNR PROJECT)

In the framework of a collaborative project on food safety (SECAL I-II) using animal parvalbumins as tracers (at the DNA and protein level), the laboratory has characterized, produced as recombinant molecules, a panel of purified and standardized recombinant fish parvalbumins from which several have been newly identified. Hybridoma cell lines secreting species-specific monoclonal antibodies to parvalbumins were produced. Using species-specific PCRs based on parvalbumin gene sequences, unknown fish samples from an international reference institute have been successfully identified. The competences and the data gained in the framework of the food safety project allowed the development of spin-of activities in matters of food allergy. Purified native and recombinant parvalbumins were used in quantitative ELISA assays. IgE-antibody profiling of a series of fish-allergic subjects was performed. The recombinant fish parvalbumins are being tested in a microarray system in an international collaboration to valorize them for allergy diagnostic purposes.. The laboratory has published and shown for the first time that fish gelatin in processed food can be responsible for severe anaphylaxis. In a collaborative study, the case of a fish-allergic patient monosensitized to pangasius and tilapia was investigated and submitted for publication.

IMMUNE RESPONSE TO SERUM ALBUMIN FAMILY REGULATORY T CELLS

This projects aims at defining T-cell epitopes recognized by induced and natural T-regulatory cells on cat serum albumin. Cat (CSA) and mouse (MSA) serum albumins and overlapping peptides have been used to study cellular proliferation and cytokine secretion pattern in the BALB/c mouse via an in-vitro myeloid dendritic cell - T-cell assay. T-cell proliferation, cytokine secretion pattern analysed in the culture supernatant or by Elispot technique (IL-10, IL-2, IL-5, and IFN-γ) has shown that the same major T-cell epitopes play an essential role in different T-cell responses Th1, Th2, Th17 and induced T-regulatory cells.

INVESTIGATIONS ON ALLERGIC AND IMMUNE DISEASES

ANGIOEDEMA WITH ACTIVATION OF THE CONTACT SYSTEM

Taking advantage of the work done in a previous CRP-project, we could show and publish for the first time that activation of the contact system and cleavage of high molecular weight kininogen occurs during angioedema attacks in women on oestrogen-containing contraceptives.

CD45RA PERSISTENCE ON MEMORY T CELLS

A female patient suffering from several autoimmune traits was investigated for an underlying immune deregulation. She was found to have a major shift of the naïve/memory T-cell profile, as all T cells had the CD45RA surface marker. Further genetic investigation confirmed the presence of a C77G mutation on exon 4 of the CD45RA gene of this woman.

INNATE IMMUNITY AT THE CELLULAR LEVEL

NEUROTROPHINS AND NK CELLS

Three neurotrophins were investigated: nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) and neurturin (NTN). Regarding NGF, we could show in the C57BL/6 mouse strain that NK cells express TrkA, the high affinity receptor for NGF, but not the pan-neurotrophin receptor p75NTR. The expression can be seen by RT-PCR, flow cytometry and confocal microscopy. It is strongest on IL2-activated NK cells (100 % positive and high density of expression), whereas resting NK cells have a lower density of expression and the receptor can only be found on approximately 20 % of the cells. Concerning GDNF and neurturin in murine NK, we have shown by RT-PCR that NK cells express GFRα-2, the receptor of the neurotrophin NTN and the co-receptor RET.

ROLE OF NEUROTROPHINS IN A MURINE MODEL OF ASTHMA (FNR PROJECT)

A murine model of asthma was developed. Allergen-specific IgG1, IgG2 and IgE were measured in serum, cellular composition of broncho-alveolar lavage and lung tissue inflammation were analysed by immuno-histology. Comparison of airway inflammation in NTN KO mice versus WT mice has shown that NTN KO mice have a stronger immune response to ovalbumin than wild-type mice.

NK CELLS AND HUMAN TAP DEFICIENCY

In the field of human TAP deficiencies, we could show that symptomatic patients have a higher than normal percentage of CD56bright NK cells, but that this increased percentage is also found in other patients with unrelated diseases (respiratory insufficiency, pneumonia, vasculitis). We published a new case of TAP1 deficiency with typical clinical and biological features, including the expansion of CD56bright NK cells.

RAT NK CELL PURIFICATION

A new efficient method of negative selection of rat NK cells was developed. The purified NK cells' cytotoxic activity towards glioblastoma cell lines was developed for collaborative purposes with the University of Bergen.

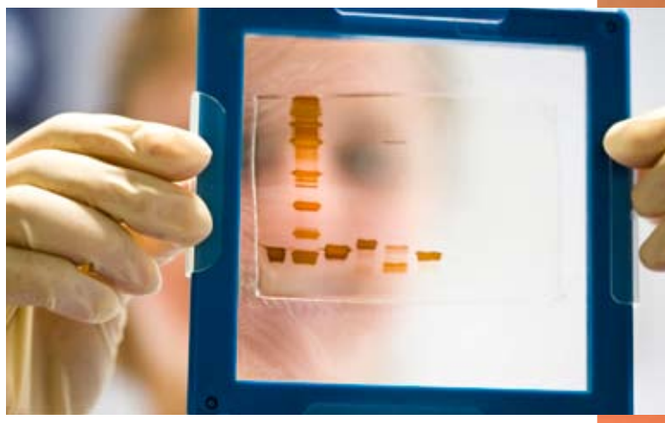
COLLABORATIONS

On a national level the LIGA had close collaborations with:

- The CRP-Santé microarray platform directed by Dr Laurent Vallar
- The national Unit of Immunology and Allergology at the CHL
- On an international level the LIGA has ongoing collaborations with the following units and laboratories for clinical, as well as functional, studies of allergens:
- Prof. F. de Blay, Unité de pneumologie, allergologie et de pathologie respiratoire de l'environnement. University of Strasbourg
- Dr Guido Paesen, CEH in Oxford
- Prof. Rita Bernhardt, Saarland University
- International collaborating partners in the NK cell and MHC class I deficiency fields are:
- Pr. Matti Airaksinen, Neuroscience Center, University of Helsinki, Finland
- Pr. Jeffrey Milbrandt, Washington University School of Medicine, St. Louis, USA
- Dr Henri de la Salle, INSERM U725, Etablissement Français du Sang – Alsace, Strasbourg, France

PUBLICATIONS 2008

- Hentges F, Hilger C, Kohnen M, Gilson G. ***Angioedema and estrogen-dependent angioedema with activation of the contact system.*** Journal of Allergy and Clinical Immunology 2009;123:262.
- Kuehn A, Hilger C, Hentges F. ***Anaphylaxis provoked by ingestion of marshmallows containing fish gelatine.*** Journal of Allergy and Clinical Immunology (in press).
- Villa-Forte A, De la Salle H, Fricker D, Hentges F, Zimmer J. ***HLA class I deficiency syndrome mimicking Wegener's granulomatosis.*** Arthritis and Rheumatism 2008, 58, 2579-2582.
- Zimmer J, Hentges F, Andres E. ***Eltrombopag in thrombocytopenia.*** The New England Journal of Medicine 2008, 358, 1072.
- Zimmer J, Michel T., Andres E, and Hentges F. ***Up-regulation of NKG2D ligands by AML cells to increase sensitivity to NK cells: the tumour might strike back.*** Leukaemia Research 2008, 32, 676-677.
- Zimmer J, Andrès E, Hentges F. ***NK cells and Treg cells: A fascinating dance cheek to cheek.*** Eur J Immunol 2008, 38, 2942-2945.



HEAD OF LABORATORY:
André STEINMETZ, DSc

RESEARCH DEPARTMENTS
DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

LABORATORY OF PLANT MOLECULAR BIOLOGY

PRESENTLY THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Monika DIETERLE, PhD, Researcher
Sabrina GATTI (PhD defense in April 2008, now at Uni Basel, Switzerland), PhD Student
Céline HOFFMANN, PhD, Researcher
Cécile HUSTIN, PhD, Researcher
Danièle MOES, PhD, Researcher
Flora MOREAU, Technician
Jessica PAPUGA, PhD Student
Katrin STRIEMKE, Technician
Stéphane THOLL, PhD Student
Clément THOMAS, PhD, Researcher
Ning WANG, MD, PhD, Researcher

LABORATORY OF PLANT MOLECULAR BIOLOGY

Research objectives of the Plant Molecular Biology Unit are: (i) the elucidation of the functions of actin cytoskeleton-associated proteins, with a particular focus on LIM domain proteins; (ii) the development of molecular probes for detection of allergenic plant material in food products; (iii) the isolation and identification of bioactive compounds from Chinese medicinal herbs and the study of their molecular targets and activities.

ONGOING PROJECTS

THE ACTIN CYTOSKELETON AND ITS ASSOCIATED PROTEINS

The actin cytoskeleton is a complex intracellular system of dynamic filaments that supports various central biological processes in higher eukaryotes, including cell division, growth, contraction, motility and defense against pathogens. Deregulation of its dynamics and/or organization can have dramatic consequences on human health, such as myopathy and cancer development. The PMB unit carries out basic research that aims at deciphering the actin cytoskeleton functions and their regulation. It particularly focuses on actin regulatory protein families that are conserved in animals and plants, since those can be expected to participate in fundamental cellular actin-based processes. Comparative analyses in both kingdoms should provide a better insight into functions of actin regulatory proteins and uncover common molecular pathways involved in actin cytoskeleton remodeling.
In the past few years the PMB unit has focused its main activities on small actin cytoskeleton-associated proteins that are found in both vertebrates and plants: the two-LIM domain-containing (LIM) proteins (also called cysteine-rich proteins or CRPs). CRP defects in mice cause severe damage, including cardiomyopathy, cardiohypertrophy, heart failure, as well as abnormalities in thymus development. In addition, many observations strongly suggest that CRPs display tumor suppressive properties and are therefore of high interest in biomedical research. However, the precise mode of action of CRPs remains largely elusive. In 2006, the PMB unit established that the plant counterparts of the mammalian CRPs define a novel class of actin filament crosslinking proteins responsible for the assembly of thick cytoplasmic actin bundles (Plant Cell, 2006, 18:2194-2206). In 2007, the PMB unit investigated the underlying bundling mechanism and demonstrated that the two LIM domains present in LIM proteins are autonomous, actin-binding and -bundling modules that cooperate to achieve optimal activities (J Biol Chem, 2007, 282: 33599-33608). Studies carried out in 2008 aimed at further characterizing the biological roles of plant LIM proteins. Exhaustive analyses including all the LIM family members of the model plant Arabidopsis thaliana (thale cress) revealed the existence of two subfamilies that exhibit different expression patterns and modes of regulation. In addition, two novel and promising research axes emerged: (i) there is evidence that, in addition to their cytoplasmic actin regulatory functions, LIM proteins act in the nucleus as (possibly direct) regulators of gene expression; (ii), several observations suggest that LIM protein localization and functions are modulated by mechanical stress. A current working hypothesis is that LIM proteins function as mechanosensors which respond to mechanical cues by increasing actin cytoskeleton rigidity and turning on/off appropriate sets of genes. This is consistent with the concept that some human CRPs function as cytoprotective factors, possibly by reinforcing actin stress fibers, during stress responses.

MOLECULAR PROBES FOR FOOD TRACING

Recent observations revealed that people have become more sensitive to allergens over the last 25 years. Most of these allergies are caused by airborne material such as pollen, but many severe allergies are caused by ingested fresh as well as processed food. Hence there is increasing pressure for clear labeling of food products containing allergenic ingredients (EU Directive 2003/89/EC).
The laboratory participates, together with the Laboratory of ImmunoGenetics and Allergology, the MicroArray Center (both CRP-Santé), and the Division of Food Control (LNS), in FNR-funded projects aiming at developing novel molecular tools for unambiguous tracing and identification of allergenic components of animal or plant origin in food products. Several major allergenic plants, including cereals, tree nuts, soybean and peanut are targeted.

BIOACTIVE COMPONENTS FROM CHINESE MEDICINAL HERBS

Plants produce numerous small metabolites, many of which target complex biomacromolecules such as proteins in the human body. Quite a few of these components - or derivatives thereof - are presently used as medications to treat human diseases. Chinese herbal medicine has identified many plant extracts that have been used effectively in Traditional Chinese Medicine over hundreds of generations. Investigating the downstream effects induced by activity changes of the molecular targets of specific active components can therefore significantly contribute to a better understanding of the complexity of molecular interactions in living systems like cells or even organisms.
The Plant Molecular Biology Laboratory has recently initiated, together with the NorLux Laboratory of Neuro-Oncology, an international collaboration on the isolation and identification of Chinese medicinal herbal components that affect specific pathways or programs disturbed in cancer cells. Such effects include, for instance, restoration of normal cell cycle control (arrest of uncontrolled cell division), and loss of invasive characteristics (by restoring cell adhesion and reducing cell mobility). The molecular targets and the underlying mechanisms will be investigated using transcriptomics, proteomics, as well as additional molecular and cell biology tools. The other partners in this project, which is supported by the International Research Scientist Exchange Scheme (IRSES) of FP7, are the Gade Institute of the University of Bergen (Norway), the Modern Research Center of Traditional Chinese Medicine (Shanghai), and the Institute of Medicinal Plant Development (Beijing).

KEY RESULTS

THE ACTIN CYTOSKELETON AND ITS ASSOCIATED PROTEINS

Most of the previous data regarding the actin-associated properties of plant LIM proteins result from experiments conducted with the tobacco WLIM1 protein. However, plants contain several LIM proteins (six in Arabidopsis), raising questions as to the reasons of this apparent redundancy. Detailed expression patterns of Arabidopsis LIM genes have been determined in transgenic plants expressing the beta-glucuronidase (GUS) reporter gene under the control of each LIM promoter. Data confirm and extend previous analyses: three LIM genes (PLIMs) are abundantly and almost exclusively expressed in the pollen grains, whereas the three others (WLIMs) are more widely expressed in non-reproductive tissues. The six Arabidopsis LIM proteins have been produced in E. coli and their actin-associated activities evaluated by a series of in-vitro assays. All the recombinant proteins exhibit, in certain conditions, a similar capacity to bind, stabilize and bundle actin filaments. Different factors have been assessed for their ability to inhibit or enhance these activities. Whereas none of the potential regulators has significant effects on WLIM activities, an elevation of pH and/or calcium specifically inhibits PLIM activities. These important results may explain the asymmetric distribution of actin bundles observed in pollen tubes, which are characterized by a highly polarized mode of growth. Indeed, within the pollen tube

shank, where the pH and calcium concentration are maintained relatively low, "activated" PLIM proteins would trigger the formation of the thick actin bundles required for the myosin-dependent transport of vesicles to the actively growing region. At the tip, both pH and calcium reach high values, conditions which would affect PLIM protein activities and therefore prevent formation of actin bundles and delivery of vesicles to the precise sites of growth. Regulation by calcium of (some) vertebrate CRPs is highly conceivable as they are strongly expressed in muscle cells whose contractile activity is controlled by calcium concentration oscillations. Regulation of vertebrate CRP actin-associated activities is currently investigated in the lab.

A regulatory domain responding to pH and calcium ions has been identified in PLIM proteins. Truncated versions of PLIM proteins lacking this domain retain the full actin-binding, -stabilizing and -bundling activities but are no longer sensitive to pH and calcium concentration variations. Domain swap experiments showed that the translocation of this regulatory domain into a WLIM protein makes the latter responsive to pH and calcium.

Evidence that LIM proteins display nuclear functions originates from their previously reported nuclear localization. Focusing on the DNA-binding capacity of the protein WLIM2, we demonstrated its specific binding to cis-elements of a promoter in vitro, which depends on the functionality of each of the two LIM domains of the WLIM2 protein. Besides, our results also suggest a role of the C-terminus of the the protein in regulating the interaction between the WLIM2 protein and the targeted promoter. In a recent collaboration with the Technical University of Munich, we confirmed these data in vivo using the transient protoplast system. This experimental approach allowed us to observe a specific WLIM2-induced activation of the target promoter in vivo. In addition to further characterizing these promoter-binding and activation properties of the WLIM2 protein, we plan, on a broader scale and together with the Microarray Center at CRP-Santé, to perform microarray analyses to determine which genes are up/down regulated in Arabidopsis seedlings over-expressing LIM proteins, as well as in LIM knock-out plants.

Recent observations suggest that LIM proteins may also participate in the response of the actin cytoskeleton to mechanical cues. Indeed, transgenic cells ectopically expressing a GFP fusion of the WLIM1 protein exhibit only a weak actin cytoskeleton labelling when placed in an open-cell growth chamber. When these cells are transferred between slide and coverslip (treatment that may be considered as a mechanical stress), the fluorescent protein massively accumulates at the actin cytoskeleton and significantly increases the thickness of the bundles. Interestingly, the nuclear fraction of WLIM1-GFP observed for cells in the open chamber was also remobilized to the cytoplasmic actin cytoskeleton, supporting the hypothesis of a cytoskeleton-nuclear LIM crosstalk. We observed a similar behavior of WLIM1 in centrifuged and agar-embedded cells, supporting the idea that mechanical stress is the inducing signal. We are currently developing appropriate tools to more accurately investigate the links between the mechanical cues, LIM protein subcellular localization and actin cytoskeleton responses. In agreement with our data, vertebrate CRPs have been suggested to function as biosensors of the cell physiological status in response to mechanical stress. Importantly, other animal-specific LIM proteins, e.g. zyxin, have been found to participate in actin cytoskeleton reinforcement upon mechanical stress. The absence of homologues of these proteins in plants gives our research a strategic advantage to further decipher plant LIM and vertebrate CRP biological functions.

MOLECULAR PROBES FOR FOOD TRACING

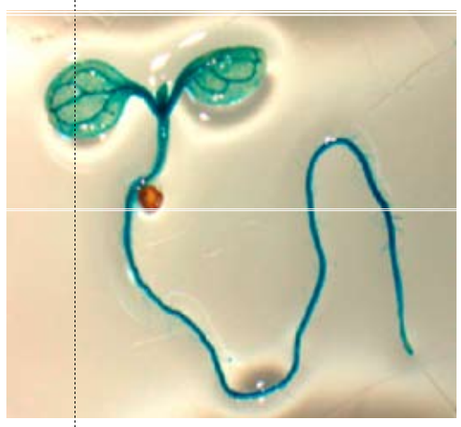
To identify species-specific DNA sequences to be used in the tracing and identification of the plant species targeted in the project, genomic DNA sequences corresponding to members of a selected conserved gene family were amplified using appropriate primer pairs and sequenced. A comparative sequence analysis allowed us to identify unique nucleotide sequences which have been used to amplify DNA fragments of specific lengths in a species-dependent manner. Probes allowing unambiguous identification of six nut species were developed in 2008.

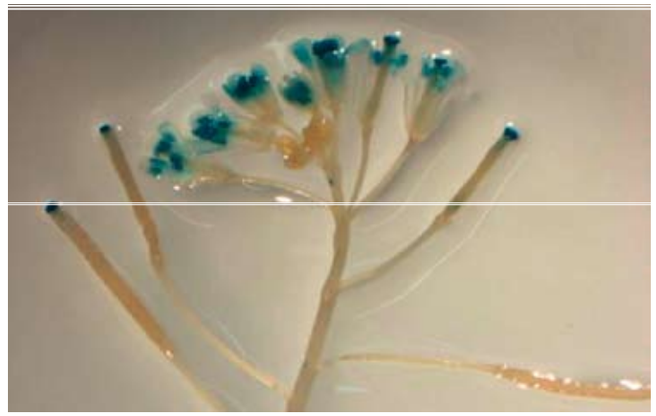
COLLABORATIONS

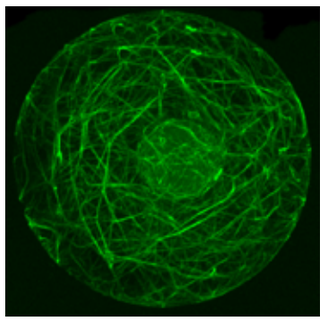
- Institute of Plant Molecular Biology (IBMP), Strasbourg (France)
- Profs. Dr Christophe AMPE and Dr Marleen VAN TROYS, University of Ghent (Belgium)
- Prof. Dr Erwin GRILL, Lehrstuhl für Botanik, Technical University, Munich

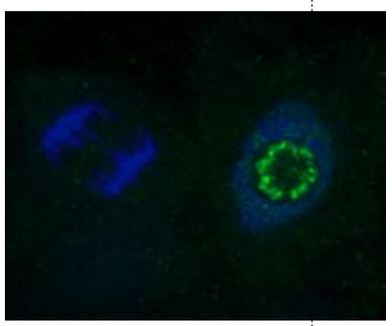
PUBLICATIONS 2008

- Moes, D., Himmelbach, A., Korte, A., Haberer, G., and Grill, E. (2008). ***Nuclear localization of the mutant protein phosphatase abi1 is required for insensitivity towards ABA responses in Arabidopsis.*** Plant J. 54, 806-819
- Thomas C, Dieterle M, Gatti S, Hoffmann C, Moreau F, Papuga J, Steinmetz A. 2008. ***Actin bundling via LIM domains.*** Plant Signaling & Behavior 3(5):320-321.









HEAD OF LABORATORY:
Robert WENNIG, MD, PhD

RESEARCH DEPARTMENTS
DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

LABORATORY OF TOXICOLOGY

PRESENTLY THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Brice APPENZELLER, PhD, Researcher, Project Leader
Guillaume SALQUEBRE, Technician
Serge SCHNEIDER, PhD (LNS Associated Researcher)
Claude SCHUMMER, PhD Student
Michel YEGLES, PhD (LNS Associated Researcher)

LABORATORY OF TOXICOLOGY

The laboratory has acquired valuable skills in the development of highly sensitive analytical methodologies for the detection and quantification of xenobiotics in human matrices such as classic biological fluids (blood and urine) and, more importantly, in "alternative" matrices with a special focus on human hair.
Some of the developed techniques are now used in routine analysis, in diagnosis and therapeutic monitoring of patients needing a regular and specific medical treatment (alcohol withdrawal, methadone substitution programme, tri-therapeutic treatment of HIV-positive patients, etc.), and in large-scale studies on public-health issues such as alcohol consumption, tobacco smoking and assessment of human exposure to chemicals responsible for occupationally- and environmentally-induced diseases.
The laboratory disposes of a large analytical platform including gas chromatography coupled with mass spectrometry (GC-MS) or tandem mass spectrometry (GC-MS/MS), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), high-pressure liquid chromatography (HPLC) with UV detection and capillary electrophoresis (CE-UV).

OBJECTIVES OF THE LABORATORY OF TOXICOLOGY

In the framework of clinical and forensic analytical toxicology, investigations primarily orientated to therapeutic drug monitoring, acute intoxication cases and drug-related death cases allowed the laboratory to develop specializations in the use of alternative matrices (including sweat, oral fluids, maternal milk, and with a special focus on hair).
The many advances obtained in such contexts provided additional information on anterior and chronic consumption/exposure and developed the knowledge on incorporation pathways of xenobiotics in biological matrices and on toxicological significance of the observed results. A part of the current research activity is still devoted to punctual investigations performed on clinical or forensic issues requiring thorough analytical development. In parallel, analytical competences in the diagnosis of chronic consumption or abuse, or exposure to substances (alcohol, tobacco, etc…), are now exploited through large-scale studies, involving extended populations, in order to perform epidemiological approaches for the relationships with associated public health issues. Aside from these aspects, the background of the Laboratory of Toxicology in the analysis of atypical biological matrices was also applied to studies oriented to humanitarian assistance through collaboration with non-governmental organizations.
Research activities performed on developments in alternative matrices analyses, in a thorough study of clinical and forensic cases and in improvement in diagnostic tools, led to several publications over the few past years.
The research activity of the laboratory is currently extended to the biomonitoring of human exposure to pollutants associated with occupationally- and environmentally-induced diseases. Since health problems generally associated with environmental pollutants are reported to be associated with chronic exposure to low levels of chemicals, hair appears to be the most suitable matrix in providing detection windows encompassing several months. Hence, the methodologies currently developed are mainly based on the use of this matrix.

ONGOING PROJECTS

ASSESSMENT OF HUMAN EXPOSURE TO ORGANIC POLLUTANTS USING HAIR ANALYSIS

Staff members involved:
Brice Appenzeller (Project leader / researcher), Claude Schummer (PhD student)

Collaboration with the University Louis Pasteur (Strasbourg, France)

Beginning: January 2008

The aim of this project is to assess the usefulness of hair as a suitable matrix for the monitoring of human exposure to organic pollutants, with a special focus on polycyclic aromatic hydrocarbons (PAHs) and on pesticides, which are among the most critical pollutants to which humans are chronically exposed in European countries with highly developed agricultural and transport activities. The consequences of such exposure on human health can reach several levels, including cancer development, neuropathies, allergic symptoms and defects in immune-system functions resulting in an increase in infectious diseases.
The aim of biomonitoring is to detect and identify exposures to chemicals in order to preserve individuals from further contamination prior to the occurrence of affection. In this regard, hair presents many advantages, such as facilitated sampling and extended window of detection. Moreover, the previous works performed in medico-legal and clinical context demonstrated that hair analysis can provide a time-frame of the exposure or consumption of substances. The present work first aims at setting up detection methods based on gas chromatography coupled with tandem mass spectrometry detection (GC/MS-MS) and with mass spectrometry in negative chemical ionisation mode (GC/MS-NCI), and liquid chromatography coupled with tandem mass spectrometry detection (LC/MS-MS). Thereafter, methods are applied to the analysis of specimens from subjects living in Luxembourg, selected according to parameters such as living area (urban vs rural), occupation (vineyard workers, people exposed to vehicle exhausts, etc.), or lifestyle habits, in order to correlate results obtained from hair analyses to these parameters and to obtain reference values for exposed populations. Later, analysis performed on the general population of the Grand Duchy of Luxembourg shall 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.

HAIR ANALYSIS FOR THE ASSESSMENT OF CHILDREN EXPOSURE TO INDOOR PESTICIDES

Staff members involved:
Brice Appenzeller (Project leader / researcher), Guillaume Salquèbre (technician)

Collaboration with the University Louis Pasteur (Strasbourg, France)

Financial support:
Afsset - Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (French agency of environmental and occupational sanitary security)

Beginning: March 2008

Recent reports alerted French authorities to high levels of biocides detected in the indoor atmosphere of 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.
In order to accurately determine the levels of exposure and the prevalence of such problems, the Afsset mandated the Laboratory of Toxicology to assess the suitability of hair analysis for the determination of children exposure to indoor pesticides. The project aims at developing analytical methodologies for the determination of selected compounds (including organochlorinated, phosphorilated compounds, pyrethrinoids and polychlorophenols) and applying the methodology to the analysis of specimens from a French-children cohort. 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).
In order to optimize the analytical development (extraction / purification steps), the study includes a preliminary step using an animal model (rats). This step, currently in progress, consists of repeated administration of low doses of pesticides to rats during a defined period in order to simulate a chronic exposure. Doses administrated to animals correspond to the levels of exposure generally reported for humans to indoor pesticides.
This part of the work will provide specimens (rat's hairs) containing the molecules to be further analyzed in human hair, which will help to optimize extraction steps, assess the recovery from hair and the importance of parameters such as the influence of pigmentation (in multi-colored hair animals).

ORISCAV-LUX (OBSERVATION DES RISQUES ET DE LA SANTÉ CARDIOVASCULAIRE AU LUXEMBOURG) (OBSERVATION OF RISKS AND CARDIO-VASCULAR HEALTH IN LUXEMBOURG)

Main investigator:
Centre d'Etudes en Santé – CRP-Santé

Staff members involved as collaborators:
Brice Appenzeller (researcher), N.N. (technician)

Beginning: End of 2007

The Department Centre d'Etudes en Santé (Center of Health Studies) of the CRP-Santé initiated a project aimed at assessing different parameters potentially associated to cardiovascular diseases among about a thousand volunteers randomly selected in the population living in Luxembourg. Among the different parameters investigated were the alcohol consumption and smoking habits of the volunteers, which were initially assessed based on self-declaration in questionnaires.
In order to counteract such pitfalls as the well-known lack of reliability of self-declared alcohol consumption or passive smoking (exposure of non-smokers to cigarette smoke) which cannot be assessed in questionnaires, volunteers were also asked to provide a hair sample. The collection of specimens was initiated at the end of 2007 and is still in progress.
The alcohol consumption will be assessed by determining the ethyl glucuronide content in hair, on which the Laboratory developed a specialization during the past years. For the assessment of smoking habits (active smoking and exposure of non-smokers), the laboratory developed a method based on the detection of nicotine in hair. In order to obtain the maximum information from hair, the method was adapted to determine nicotine and hydroxy-PAH simultaneously in the same specimen. This method, based on a two-step extraction in basic and acidic conditions allowed satisfactory recovery and limits of detection for all the compounds investigated. The validation of this method included several parameters such as testing the influence of washing steps in order to take into account contamination due to external deposition of nicotine on hair, and also the influence of melanin on the incorporation of nicotine in hair. This methodology was moreover applied to the analysis of specimens from a limited population of about a hundred volunteers. The first results obtained demonstrated that this highly sensitive method, based on GC-MS/NCI after nicotine derivatization, permits the identification of active smokers, non-smokers and exposed non-smokers.

DETECTION AND QUANTIFICATION OF EFAVIRENZ (EFV) IN HUMAN BREAST MILK, MOTHERS' AND NEWBORNS' PLASMA IN PATIENTS FROM RWANDA

Staff members involved:
Gras Alain (PhD student)

Collaboration with the Laboratory of Retrovirology

Beginning: January 2007

Prevention of mother-to-child transmission of HIV-type-1 through human breast milk remains a controversially discussed topic. WHO guidelines recommend avoidance of breastfeeding and use of replacement feeding if it is "acceptable, feasible, affordable, sustainable and safe". Consequently, in industrialized countries, breastfeeding is strongly discouraged or even prohibited to HIV-positive mothers and a decrease of mother-to-child transmission below 2% has been observed. In many resource-limited countries, however, formula feeding encounters major problems, including high costs of the formula diets, reduced availability of clean drinking water, no stable supply of electricity and low acceptance among mothers and family members. In theses regions, WHO recommends exclusive breastfeeding during the first months of life and use of replacement feeding as soon as possible.
In this study, we investigated EFV concentrations in mothers' plasma, the breastfed newborns' plasma and the mothers' breast milk in a cohort of 13 HIV-positive women and their non-infected children (9 boys and 4 girls) from Rwanda. All women (HIV status 1, CD4 cell count > 350 cells/mm3) were part of the "AMATA" feasibility study and were eligible for "Highly Active Anti Retroviral Therapy" (HAART) including zidovudine (AZT), lamivudine (3TC) and efavirenz (EFV) starting from at the third trimester of gestation.
EFV has been linked to birth defects in animals and in humans in the case of exposure during the first trimester of pregnancy. Although the prevalence of abnormalities reported in the literature is low, consensus recommendation is that EFV treatment should be avoided during the first trimester of pregnancy. As a consequence, no data about treatment of newborns with EFV is available, but treatment of older children was generally well tolerated and EFV showed a sustained antiviral effect.

KEY RESULTS

During this year, the laboratory continued its activity in the development of new biomarkers for the human biomonitoring (HBM) of exposure to pollutants associated to occupationally- and environmentally-induced diseases. A new method was developed for the assessment of human chronic exposure to PAH. This method, based on the detection of hydroxylated metabolites of PAH in hair, represents a brand new tool for the HBM of exposure to PAH, which is associated to several affections and particularly cancers and respiratory diseases. Results describing the analytical development and the application of the method to the analysis of specimens from a selected cohort of volunteers allowed are currently under publication.
Results obtained from the analysis of anti-retroviral drugs in the plasma of HIV-positive mothers under treatment demonstrated significant correlations with levels observed in skim milk and infant plasma. These findings, associated with information concerning the absence of child infection and clinical side effects, were published in the JAIDS.

COLLABORATIONS

Analytical methodology on anti-retroviral drugs:

- Laboratory of Retrovirology (CRP-Sante, Dr J-C Schmit, Dr Vic Arendt)
- LuxDevelopment and ESTHER project in Rwanda (Mme C. Omes)

Assessment of Human Exposure to Organic Pollutants by Hair Analysis:

- Centre de Géochimie de la Surface de Strasbourg, UMR 7517 CNRS – Université Louis Pasteur. (Dr Millet M.)
- Agence Française de la Sécurité Sanitaire de l'Environnement et du Travail (AFSSET)
- Institut Vitivinicole, Remich. Luxembourg.
- Centre d'Etudes en Santé-CRP-Santé (Resp. M.L. Lair, Dr S. Couffignal, Dr A. Alkerwi)

PUBLICATIONS 2008

- S. Schneider, Z. Ait-m-bark, C. Schummer, P. Lemmer, M. Yegles, B. Appenzeller, R. Wennig. ***Determination of fentanyl in sweat and hair of a patient using transdermal patches.*** J Anal Toxicol, 32:260-4 (2008)
- L Martins, M Yegles , D. Thieme and R Wennig. ***Influence of bleaching on the enantio-selective disposition of amphetamine-type stimulants in hair.*** Forensic Sci Int, 176:38-41 (2008)
- J. Kupper, M. Schuman, R. Wennig, U. Gorber, A. Mittelholzer, R. Artho, S. Meyer, H. Kupferschmidt and H. Naegeli. ***Cyanide poisoning and increased thiocyanate levels in cows after feeding the soft hulls of apricot kernels.*** The Veterinary Record, 162:488-489 (2008)
- L. Martins, M. Yegles and R. Wennig. ***Simultaneous enantioselective quantification of methadone and of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine in oral fluid using capillary electrophoresis.*** J Chromatogr B 862:79-85 (2008)
- S. Schneider, A. Peltier, A. Gras, C. Karasi – Omes, V. Arendt, A. Mujawamariwa, P. Ndimubanzi Cyaga and R. Wennig. ***Efavirenz in human breast milk, mothers' and newborns' plasma.*** J Acquir Immune Defic Syndr, 48:450-454 (2008)
- B.M.R. Appenzeller, M. Schuman and R. Wennig. ***Was a child poisoned by ethanol? Discrimination between ante-mortem consumption and post-mortem formation.*** Int J Legal Med. 122:429-434 (2008)
- C. Schummer, B.M.R. Appenzeller and R. Wennig. ***Quantitative determination of ethyl glucuronide in sweat.*** Ther. Drug Monit. 30:536-539 (2008)
- C. Schummer, O. Delhomme, B.M.R. Appenzeller, R. Wennig, M. Millet. ***Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis.*** Talanta, 77:1473-1482 (2009)
- F. Pragst and M. Yegles. ***Determination of Fatty Acid Ethyl Esters (FAEE) and Ethyl Glucuronide (EtG) in Hair – a Reliable Way for Retrospective Detection of Alcohol Abuse During Pregnancy.*** Ther. Drug Monit, 30:255-263 (2008)
- FM Wurst, E. Kelso, W. Weinmann, F. Pragst, M. Yegles, I. Sundström Poromaa. ***Measurement of direct ethanol metabolites suggests higher rate of alcohol use among pregnant women than found with the AUDIT – a pilot study in a population-based sample of Swedish women.*** Am J Obstet Gynecol, 198:407.e1-5. (2008)
- FM Wurst, M Yegles, C Alling, S Aradottir, J Dierkes, GA Wiesbeck, C Halter, F Pragst, V. Auwaerter. ***Measurement of direct ethanol metabolites in a case of a former driving under the influence (DUI) of alcohol offender, now claiming abstinence, driving under the influence (DUI)*** of. Int J Legal Med. 122:235-239. (2008)
- F. Wiens, A. Zitzmann, M. Lachance, M. Yegles, FM. Wurst, D. von Holst, S. Guan and R. Spanagel. ***"The search for the missing drink: fermenting nectar and pollination of a palm by non-flying mammals"*** Proc Natl Acad Sci U S A.105:10426-10431 (2008)
- FM Wurst, KM Dürsteler-Macfarland, V Auwaerter, S Ergovic, N Thon, M Yegles, C Halter, W Weinmann, GA Wiesbeck. ***Assessment of Alcohol Use Among Methadone Maintenance Patients by Direct Ethanol Metabolites and Self-Reports.*** Alcohol Clin Exp Res. 2008 Jun 25. [Epub ahead of print]

HEAD OF LABORATORY:

Claude P. MULLER, MD, MS

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Simone ALT, PhD Student
Iris ANDERNACH, PhD Student
Tom BECHET, Research Assistant
Anja BILLING, Research Assistant
Lei CAO, PhD Student
Emilie CHARPENTIER, Technician
Sébastien DE LANDTSHEER, Technician
Fred FACK, PhD, Researcher
Sophie FARINELLE, Technician
Nancy GERLOFF, PhD Student
Judith HUBSCHEN, PhD, Researcher
Oliver HUNEWALD, IT
Monique JACOBY, PhD, Researcher
Denis KAINOV, PhD, Researcher
Julia KESSLER, PhD Student

RESEARCH DEPARTMENTS

DEPARTMENT OF IMMUNOLOGY

Jacques KREMER, PhD, Researcher
Vitor LOPES, Technical Support
Konstantin MULLER, PhD Student
Ulla MULLER, Administrative Assistant
Dominique REVETS, Technician
Anna REYE, PhD Student
Nathalie RODENBOUR, Technical Core Support
Carlos SALOMON LEIJA, Technician
Aurélie SAUSY, Technician
Mario SCHELLENBERGER, PhD Student
Chantal SNOECK, PhD Student
Slavena TRIFONOVA, PhD Student
Jonathan TURNER, PhD, Researcher
Sara VERNOCCHI, PhD Student
Carole WEIS, Secretary
Stéphanie WILLIEME-LALOUETTE, Technician

DEPARTMENT OF IMMUNOLOGY

DIVERSIFICATION

Historically, the Department of Immunology has earned an international reputation for its work on different aspects of the measles virus (MV). These included the epidemiology and molecular epidemiology of outbreaks, the immune response against the wild-type and the vaccine virus, T- and B-cell epitope mapping, as well as the development of a vaccine strategy. As a result of these studies, the Department has gained high visibility with the WHO, both as a Reference Center for Europe for Measles and Rubella (RRL, since 2004) and as a WHO Collaborating Center (since 1998), with virtually daily contacts between both institutions. Training of laboratory personnel, upgrading of selected laboratories, quality control and on-site laboratory accreditation visits, missions as temporary scientific advisors, and seconding of staff are important aspects in our function as RRL. The Department has been involved in the development and characterization of reference reagents and diagnostic procedures for measles and rubella. This has triggered worldwide partnerships of our Department with many public-health laboratories, as well as with scientific and academic laboratories. Within these partnerships, our interest was drawn to other human viruses using our acquired expertise. This leads to diversification of our scientific activities. Thus, our first non-measles paper appeared in 2000. This has now come to a "record" low of less than one third in 2008. Other human and veterinary viruses have become important and very successful fields with dozens of publications, 24 in 2008 alone. In 2008, members of the department gave more than 100 presentations at scientific meetings.

OBJECTIVES OF THE DEPARTMENT OF IMMUNOLOGY

PUBLIC-HEALTH ASPECTS

The main purpose of these studies is to contribute to understanding the geographic distribution and their genetic variants (including new variants and genotypes), their variability and their natural history. 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 are the farmers, consumers in need of high-quality proteins in particular in impoverished countries, the veterinary services and animal welfare.

CAPACITY BUILDING

These collaborative projects with resource-poor countries include important capacity-building components, such as (i) training of laboratory and academic staff, as well as students working towards their degrees, (ii) upgrading laboratory infrastructure, (iii) providing equipment, (iv) providing protocols, consumables and advice, and (v) academic teaching. In addition, immunology-department scientists are in demand 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 expert-advice to National or sub-national Governments and Ministries for establishing or upgrading outbreak surveillance systems.

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. A patent has recently been deposited. One PhD student has graduated and one is in progress.

GRADUATE-SCHOOL PSYCHOBIOLOGY

More recently, the Department also became the Dept. of Immunology 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 the 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 behavior, mood, learning and memory. In the framework of these collaborations, we are also part of the International Research Training Group "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. Three PhD students graduated in 2008, four PhD students are currently enrolled in this Graduate School.

TEACHING AND TRAINING

Our department offers a teaching and training programme for MD and PhD students and, more recently, undergraduate students (e.g. master students). The students are trained in the department and obtain their degree from a foreign university. Initially, their degree was from the University of Tübingen. In 2003, the head of the Dept. was nominated to the Chair of Immunology of the University of Trier and Associate Professor of the University of Saarland. In 2002, the Department became associated with the Graduate School of Biology and Environmental Sciences (BIOSE) of the University of Nancy. Now, students of the Department enroll predominantly in Trier, Nancy and Homburg. The Department also serves as a very active WHO training center. The European Summer School of the Department hosts an increasing number of European university students during a two to four-month research training period. More than 45 MD and PhD students are enrolled or have graduated from the Department of Immunology in collaboration with a foreign University.

ONGOING PROJECTS AND KEY RESULTS

MEASLES AND RUBELLA

The most recent activities in this area include the development of a multiplex PCR for measles and rubella RNA detection, a longitudinal study on measles-specific IgG levels in vaccinees and late convalescents exposed to wild-type MV, the evaluation of MV vaccine-potency status in Nigeria, as well as the development and validation of measles and rubella IgM tests using dried serum. The Department continues to be very active in the genetic characterization of measles and rubella viruses circulating worldwide. In recognition of its specific expertise in this area, the Department has been asked by WHO to review the temporal and geographical distribution of measles-virus genotypes in the WHO-EURO region and to contribute to the update on the global distribution of measles and rubella genotypes.The corresponding study showed that, despite the reduction of endemic measles virus circulation, importation of viruses from other continents caused prolonged circulation and large outbreaks in the WHO-EURO region after their introduction into unvaccinated and highly mobile communities. The genetic characterization of MV strains from Nigeria and the Democratic Republic of Congo identified the circulation of a MV genotype which had previously been considered to be inactive, and brought out a striking difference in the genetic diversity of MV in both countries. One of the strains identified in Nigeria has also been found in three other countries.

In collaboration with our partners from the Gabrichevsky Institute in Moscow, we also showed that Russia has been gradually moving from an endemic to an epidemic transmission pattern of MV after the Implementation of a National Measles Elimination Program in 2003. MV field isolates from Russia and Vietnam were also used to validate our previously established non-sequencing genotyping method for MV. Furthermore, we showed that MV strains with identical sequences in the WHO-recommended gene segment (C-terminus of the N-gene) could be distinguished on the basis of their complete H- and P- gene sequences. This may

become more and more important for molecular epidemiology applications as the genetic diversity of MV decreases due to enhanced measles control. For the rubella virus (RV), we recently found multiple genetic lineages co-circulating in Belarus, which formed novel genetic groups within clade 1. These results demonstrated that the previous WHO nomenclature of Rubella Virus needs to be partially revised. As a member of the WHO Nomenclature Committee, we proposed the revisions that were then published in the Weekly Epidemiological Record. The Department has also been invited to write a Book Chapter on Measles for Conn's Current Therapy as well as the report of the WHO Steering Committee on research related to measles and rubella vaccines and vaccination.

The above studies monitored the genetic diversity and the molecular epidemiology of routes of transmission. Although differences between vaccine and wild-type strains have received some scientific attention, the significance of genetic differences between wild-type viruses has not been investigated. Taking advantage of our large collection of worldwide MV isolates, we have started to investigate the interaction of genetically distinct measles viruses with the host innate immunity. Significant differences in sensitivity to type I IFN were identified in different wild-type strains (as well as vaccine strains), affecting MV replication. MV wild-type strains also differed in their capability to induce Type I IFN and this was related with their ability to produce small defective viral RNAs (diRNA). In contrast, high levels of IFN were induced by vaccine strains despite the absence of diRNAs, indicating that vaccine strains induce Type I IFN by another mechanism. Considering that MV nucleoprotein (NP) plays a prominent role in MV transcription and replication, we have started to investigate whether post-translational modification of MV NP might play a role in the generation of diRNAs. We used our proteomics platform to map posttranslational modifications of the nucleoprotein (NP). An unexpected large number of phosphorylation sites were detected in the C-terminal tail of native and recombinant NP. Other post-translational modifications are under investigation.

HEPATITIS B VIRUS

With four new subgenotypes (A3-6) in Africa and one new genotype (I) in South-East Asia, it is safe to say that the molecular epidemiology of hepatitis B virus worldwide has become another flagship of the department. In SE-Asia, detailed phylogenetic analysis of strains revealed multiple different subtypes of B and C, mixed infections as well as numerous related new strains that fulfilled the criteria of a new HBV genotype (I) with two subtypes (I1 and I2). In Asia, a high frequency (>20%) of mixed infections was found, including recombinations with this new genotype. Also, Sub-Saharan Africa suffers from an excessively high endemicity of hepatitis B virus (HBV), but until recently little was known about the prevalent genotypes. In this study, we investigated several hundred gene sequences and representative complete genomes from 15 locations in Africa. Except for Cameroon (18/22 genotype A), >85% of sequences from each location belonged to genotype E with a very low diversity (1.67%) throughout West and Central Africa. In contrast, genotype A strains were highly diverse (5.1 %) and separated into three new subtypes (A3, A4, A5). Also a triple recombination of genotypes E/D and A was found. Thus, the diversity of genotype A is higher in Africa than anywhere else; suggesting that genotype A has developed in Africa over thousands of years before spreading to other parts of the world. In contrast, the low genetic diversity of genotype E is suggestive of a short evolutionary history: it would take a maximum of only 200 years for the strain diversity of HBV/E viruses to develop from an unknown ancestor. This would explain its conspicuous absence in the New World, despite the forced immigration of slaves from West Africa, until the early nineteenth century. However, its widespread throughout Africa seems only possible in a virgin population, which is in contradiction to the distribution and genetic diversity of genotype A. Infection during infancy is mostly associated with chronic carrier status, but could hardly account for the explosive spread of virtually identical viruses in Africa. Our recent studies from Haiti and Rwanda seem to provide some clues to the above puzzle.

INFLUENZA

The genetic diversity of oseltamivir-sensitive and -resistant strains from Luxembourg (2007/08), as well as clinical and epidemiological factors associated with the emergence of the latter, were investigated. 22% of 270 Influenza A H1N1 strains showed oseltamivir resistance (ORH1N1). Both resistant and sensitive strains evolve in parallel. Deep-sequencing suggested that also sensitive strains have resistant quasi-species. The phylogenetic analysis suggested that reassortments between sensitive and resistant strains may have occurred. New techniques for the detection of the influenza virus and other respiratory viruses in resource-poor laboratories have been developed in collaboration.

AVIAN INFLUENZA

With the emergence of highly pathogenic avian influenza H5N1, we became interested in avian viruses. In parallel with the hepatitis work, the Department became active in the surveillance and molecular characterisation of avian viruses. This also very rapidly proved to become a very successful field of research: We published the first papers on avian influenza in Sub-Saharan Africa, and after HPAI H5N1 reached Africa, we published the first papers in poultry as well as in wild birds (vultures). Today, the majority of the publications of H5N1 in Africa are from the Department. Some of these publications have received world-wide attention in the media and the general press, with hundreds of newspapers world-wide reproducing our results. More recently, we have shown that 2 of the three lineages have been replaced by well-defined reassortment (ACHA/NS). Although independent reassortment events have resulted in the same gene combinations, which have not been found outside of Africa, we speculate that these reassortments may correspond to some adaption to the African environment of HPAI H5N1 viruses that came from the cold. Our surveillance studies both in poultry and in wild birds suggest that transmission of the virus to Africa is an excessively rare event, in comparison to the number of migrating birds that winter in Africa. While these surveillance studies are continuing, we also try to understand the significance of the above adaption process (e.g S145P in the HA) for viral fitness and in particular also for its pathogenicity. We also analysed LP H5N2 genomes from wild birds in Nigeria and found a mutation which may destabilize NS1 interaction with the cellular CPSF30 protein which normally occurs during HP virus infection.

NEWCASTLE DISEASE VIRUS

Before the emergence of HPAI H5N1, this avian paramyxovirus represented the most important threat to the poultry industry. In the framework of our avian virus surveillance program in Western Africa, (Nigeria, Niger, Burkina Faso and Cameroon) five different genetic lineages were detected (lineages 1 to 5). All viruses belonging to lineages 1-4 were mostly vaccine strains. In backyard farms, live bird markets and wild birds, three new highly pathogenic clusters of lineage 5 were found. Their high genetic diversity and their presence in non-commercial farms in three different Sub-Saharan countries suggest that these sublineages represent the indigenous NDV variants from West Africa. We are looking for a reservoir of these viruses in wild birds from the same regions.

OTHER AVIAN VIRUSES

We have investigated the prevalence, spread and the genetic variants of six other avian viruses in African and Chinese poultry, as well as in wild birds. For most of these viruses, we reported the virus for the first time from Sub-Saharan Africa, characterized the prevalent strains and provided estimates of their prevalence within commercial farms and or life-bird-markets. Similar studies were done in South-China, in order to investigate whether there are viral infections that promote avian influenza. In this context, we have first-described two new highly virulent subtypes of Infectious Bursal Disease Virus. We have also found a new Infectious Bronchitis Virus serotype, amongst others.

TICK-BORNE PATHOGENS

Since a number of years, there has been an increasing debate about the prevalence in Luxembourg of Lyme borreliosis, the most common tick-borne infection in Europe (2-58%) as well as other tick-borne pathogens. The need for a tick study in Luxembourg became even more urgent with the first detection of FSME in the Saarland. Accordingly, the response of the public and the media to this study was overwhelming. We found an overall infection rate of 20% in ticks with geographic differences between 5% to 27%. Anaplasma sp. was detected in 1.13% of field collected ticks and in 27.5% of ticks collected from hosts. Since the majority of Anaplasma-positive ticks were engorged females feeding on deer, we suggest that the hosts were reservoir hosts for Anaplasma. Other analysed tick-borne pathogens are Rickettsia sp. (8%), Bartonella sp. (2.3%), TBEV (0%), and Francisella sp. (0%). This project has now been extended to Moldavia, Belarus, Bulgaria, and other countries.

VACCINATION STRATEGY AGAINST CARCINOGENS

The general objective of the TOBAVAC project is to explore prophylactic immune strategy based on carcinogen-specific antibodies induced by NNK- and B[a]P- hapten conjugates, to lower the risks of chemical carcinogenesis. Chemical carcinogenesis in humans is the result of many years of exposure of susceptible tissues to very low concentrations of carcinogens. TOBAVAC I has shown that these "pathophysiological" concentrations can be matched by the 10- to 300-fold higher molar concentrations of specific antibodies found after the induction of a specific immune response in vivo. However, most in-vitro and in-vivo animal models conveniently use concentrations of the low-molecular weight carcinogens that are much higher and can normally not be matched by equimolar concentrations of high-molecular weight antibodies. Thus, one of the most important challenges of the TOBAVAC I and II are the necessity to use and develop in-vivo and in-vitro models based on low concentrations of carcinogens that can be matched by molar excesses of antibody concentrations that give measurable, surrogate read-outs in short-term or long-term experiments.

The First Phase of TOBAVAC (TOBAVAC I) showed that both active- and passive-specific antibodies protect cells by a number of expected and unexpected mechanisms against adverse effects of carcinogens. TOBAVAC I demonstrated that with the current understanding of the pharmocokinetic/dynamic, and of mechanisms of chemical carcinogenesis of the ubiquitous prototype carcinogen B[a]P and the potent pulmonary tobacco carcinogen NNK, an immunoprophylactic approach against chemical carcinogenesis is absolutely warranted. A patent application based on these results has been submitted.

On the basis of these results, TOBAVAC II further investigates how antibodies can influence detrimental effects of carcinogens. We explore how antibodies can favorably influence in-vitro and in-vivo cellular mechanisms that lead to cancer development. If during TOBAVAC I the focus was more on in-vitro studies, TOBAVAC II emphasizes more in-vivo aspects. As direct measurements of effects of carcinogens at the low concentrations that are environmentally relevant often do not provide experimental read-outs, surrogate models are used to some extent to assess the protective effect of NNK and B[a]P-specific antibodies. The capacity of specific antibodies to reduce the risk of chemical carcinogenesis is currently investigated by several approaches including:

I. The influences of B[a]P- and NNK-specific antibodies on mechanisms that lead to genotoxicity and carcinogenesis (such as prevent tumour cell proliferation induced by NNK receptor ligation, prevent the induction of enzymes involved in chemical carcinogenesis, prevent the down-regulation of enzymes that prevent carcinogenesis and the modulation of adduct formation and adduct-induced tumour formation in highly susceptible transgenic mice).

II. The modulation of BaP uptake, metabolism, redistribution and excretion by specific antibodies in animal models.

III. The reversal effects of specific antibodies on new surrogate markers of B[a]P and NNK at low concentrations (such as the effects of low doses of B[a]P on the behavior of adult and young mice).

NEUROENDOCRINE IMMUNOLOGY

ALTERNATIVE FIRST EXON 1S OF GR

We focus on the Hypothalamic-Pituitary-Adrenal (HPA) system as one of the most important stress response mediators, and its role in human stress and immune response mechanisms. The key HPA mediators are cortisol and it's ubiquitously expressed and tightly controlled receptor (GR). We investigate the gene structure, transcriptional, translational and post-translational regulation of the expression of the GR and other nuclear receptors within cells of the immune system and beyond. We have described an exceptionally complex gene structure with multiple untranslated alternative first exons in the CpG island upstream of the first translated exon 2. While this was one of the first descriptions of a gene with such a complex first exon structure, it is now recognized that many genes have similar structures.

PROMOTORS OF FIRST EXONS

Transcriptional control mechanisms operating via the differential use of multiple first exons (1C-1H) were identified. These studies showed that the GR also has an unusually complex promoter structure: GR transcription is controlled through 9 promoters, each associated with an alternative TSS, 7 of which are found in an upstream CpG island. This transcript variability does not alter the protein produced, since the ATG translation start codon is in exon 2. Whether there may be an association between first exon usage and C-terminal GR splice variants requires further attention. We hypothesised that TSS usage, resulting in differential GR tissue expression profiles, depends on short, immediately upstream proximal promoter regions within the CpG island. In order to prove that the GR alternative first exons depend upon independent promoters immediately upstream of the independent transcription start sites, seven promoter regions (pr1D, 1J, 1E, 1B, 1F, 1C and 1H) from the CpG island associated first exons were cloned into luciferase and GFP reporter constructs. Transient transfections of multiple cell lines (e.g. MCF7, HEK293T, and A549) were performed to measure their promoter activity. These data show that the first exons depend on the promoter immediately upstream. This work is currently ongoing to confirm the correlation between the native promoter activity, as measured by the specific GR variant levels, and the activity of the proposed promoter region in the reporter gene construct.

HPA, GR AND IMMUNE REGULATION IN DISEASE

Disregulation of alternative first exon expression presents an interesting hypothesis to explain HPA axis disfunctions. Fibromyalgia and major depression disorder are two diseases in which this may play a role. In fibromyalgia patients we observed lower GR and MR (mineralcorticoid receptor, the high affinity glucocorticoid receptor) levels, coupled with lower baseline cortisol levels. These observations translated into lower GR target gene expression levels, as well as into minor disturbances in the innate immune system. In major depression, the glucocorticoid-mediated feedback regulation of the HPA axis is impaired probably due to a down-regulation of the number of GRs or their reduced function. Several studies have assessed GR expression in patients with major depression, primarily in peripheral cell types including fibroblasts and immune cells, but none of these studies looked at the role of the different GR mRNA transcripts and alternative exon 1 usage. Alterations in the genetic structure of the GR could be another possibility for GR resistance in major depression. Also, epigenetic modifications may have profound effects on tissue-specific gene expression and contribute to individual differences in vulnerability to stressors. We were able to show in the above reporter gene assays that in-vitro methylation of CpG island promotors had profound effects on their activity, effectively shutting them down. While methylation patterns were highly individualized, we did not observe any systematic difference in methylation patterns between fibromyalgia patients and normal controls.

GR PROMOTOR METHYLATION

Methylation levels of the individual proximal promoter regions were investigated in immune cells within five of the seven CpG island promoters upstream of exon 1s; we showed that essentially all methylatable CpG positions had at least a low level of methylation in at least a few individuals. Thus, methylation patterns of GR promotors were highly variable between individuals. The rodent literature suggests that this epigenetic control of promoter activity corresponds to perinatal conditioning of immune cells to stress responses. Thus, perinatal conditioning of alternative GR promotors associated with CpG islands may have a profound and lasting epigenetic conditioning effect on adult immune responses.

PROTEOMICS OF GR RESPONSE GENES OF MONOCYTES/MACROPHAGES

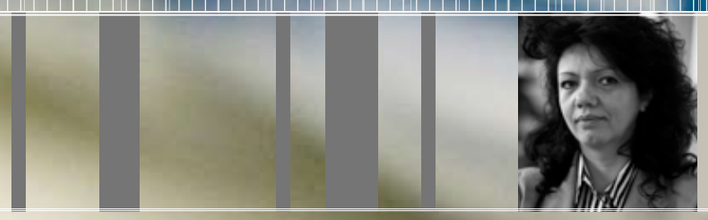
In addition to the transcriptional control of the GR, we are also interested in the downstream activities after GR ligation. The effects of cortisol on differentiating monocytes and macrophages were studied using the human THP-1 cell line and a 2D DIGE (fluorescence difference gel electrophoresis) approach. More than 50 cortisol-modulated proteins were identified by MALDI-TOF MS, belonging to five functional groups: cytoskeleton, chaperones, immune response, metabolism and transcription/translation. For several of these proteins, new isoforms and posttranslational modifications have been identified and were further characterized in human PBMCs. Rapid non-genomic effects within minutes after GR ligation are still poorly understood. Using a restraint stress in-vivo model in rats, we currently investigate rapid changes in protein expression in the cytosol and nucleus of thymus cells using the above proteomics approach. These studies already allowed us to follow initiating processes involved in GR-specific regulation.

A NUCLEAR RECEPTOR SPLICE VARIANT WITHOUT DNA-BINDING DOMAINE (DBD)

COUP-TFII Splice the GR is part of the larger nuclear receptor (NR) family. It is known to have many and important interactions, such as heterodimerisation, with other NRs, which have profound but so far poorly understood effects on GR activity. However, the expression of nuclear receptors in immune cells at this point had not been mapped. We investigated expression levels of most NRs in the different immune cells in human peripheral blood. Several of the NR, including COUP-TF. appeared to be co-regulated in the different immune cells. In order to shed light on the regulatory mechanisms, we investigated the 5' region of the COUP-TFII gene. We were surprised to identify a new splice variant of COUP-TFII lacked a DNA Binding Domain (DBD). This is indeed the first report of a typical nuclear receptor without this critical domain. Since the remaining LBD of Coup-TFII is mostly involved in repression of genes via protein-protein interactions, we suggest that Coup-TFIIΔ acts mainly as decoy, or repressor of the classical form without DNA binding capacity and direct transcriptional activity. Localisation studies with GFP-Coup-TFII and Coup-TFIIΔ fusion proteins showed that, unlike Coup-TFII, Coup-TFIIΔ is present also in the cytoplasm, suggesting that the isoform acts as an atypical NR, shuttling between both compartments and silencing gene transcription. Coup-TFIIΔ was highly expressed in the brain, but absent in immune cells, suggesting a role in tissue-specific gene regulation of GR as proposed for other atypical NRs.
Similarly, whilst sequencing the GR from fibromyalgic subject, we observed a new splice variant (hGRΔ313-338) with a deletion on the N-terminal transactivation domain.

PUBLICATIONS 2008

- JR Kremer, CP Muller. Measles in Europe – there is room for improvement. Invited Commentary. Lancet . In press.
- JM Hübschen, J Mugabo, CA Peltier, JC Karasi, A Sausy, V Arendt , CP Muller. ***Four different non-E genotypes of hepatitis B virus indicate that Rwanda is east of the African genotype A/E divide. J Med Virol. In press.***
- SV Shulga, PA Rota, JR Kremer, MA Naumova, CP Muller, NT Tikhonova, EV Lopareva, TA Mamaeva, OV Tsvirkun, MN Mulders, GY Lipskaya AG Gerasimova. ***Genetic variability of wild-type measles viruses, circulating in the Russian Federation during Implementation of the National Measles Elimination Program 2003–2007.*** Clin Microbiol Infect. In press.
- MA Yermalovich, GV Semeiko, EO Samoilovich, JM Hübschen, CP Muller. ***Molecular epidemiology of parvovirus B19 infection in Belarus.*** Voprosy Virusologii. In press.
- F Fack, J Kessler, P Pirrotte, J Kremer, D Revets, W Ammerlaan CP Muller. ***Detection of differentially modified pathogen proteins by Western Blot after 2-D gel electrophoresis and identification by Maldi-Tof-Tof.*** COST Booklet. In press.
- CJ Snoeck, MF Ducatez, AA Owoade, OO Faleke, BR Alkali, MC Tahita, Z Tarnagda, JB Ouedraogo, I Maikano, PO Mbah, JR Kremer, CP Muller. ***Newcastle disease virus in West Africa: new virulent strains identified in non-commercial farms.*** Archives of Virology. Epub ahead of print. 2008.
- JR Kremer and CP Muller. Measles – Rubeola. ***Conn's Current Therapy. Invited Book Chapter. Rakel and Bope, Saunders Elsevier.*** In press.
- JD Turner, LPL Pelascini, JA Macedo, CP Muller. ***Highly individual methylation patterns of alternative glucocorticoid receptor promoters suggest individualised epigenetic regulatory mechanisms.***Nucleic Acids Research 36, 7207-7218, 2008.
- MA Yermalovich, GV Semeiko, EO Samoilovich, VA Matveev, NV Proschaeva, JM Hübschen, CP Muller. ***Acute parvovirus infection in Belarus [Russian].*** Zdravookhranenie 11, 13-18, 2008.
- JM Hübschen, IE Andernach, CP Muller. ***Hepatitis B Virus genotype E variability in Africa.*** J Clin Virol 43, 376-389, 2008.
- AA Owoade, MF Ducatez, JM Hübschen, A Sausy, H Chen, Y Guan, CP Muller. ***Avian metapneumovirus subtype A in China and subtypes A and B in Nigeria.*** Avian Diseases 52, 502-506, 2008.
- AA Owoade, NA Gerloff, MF Ducatez, JO Taiwo, JR Kremer, CP Muller. ***Replacement of sublineages of Avian Influenza (H5N1) by reassortments, Sub-Saharan Africa.*** EID 14, 1731-1735, 2008.
- CM Olinger, P Jutavijittum, JM Hübschen, A Yousukh, B Samountry, T Thammavong, K Toriyama, CP Muller. ***Possible new Hepatitis virus genotype, Southeast Asia.*** EID 14, 1777-1780, 2008.
- PQ Li, ZF Yang, JX Chen, CP Muller, J Zhang, DF Wang, R Zhang, YS He. ***Simultaneous detection of different respiratory virus by a multiplex reverse transcription polymerase chain reaction combined with flow-through reverse dot blotting assay.*** Diagn Microbiol Infect Dis 62, 44-51, 2008.
- N Grova, H Schroeder, S Farinelle, E Prodhomme, A Valley, CP Muller. ***Sub-acute administration of benzo[a]pyrene (B[a]P) reduces anxiety-related behavior in adult mice and modulates regional expression of N-methyl-d-aspartate (NMDA) receptors genes in relevant brain regions.*** Chemospere 73, 5295-5302, 2008.
- C Bekondi, CM Olinger, N Boua, A Talarmin, V Venard, CP Muller, A Le Faou. ***Characterization of Hepatitis B virus strains from the Central African Republic: Preliminary results.*** Pathol Biol 56, 310-313, 2008.
- JA Macedo, J Hesse, JD Turner, J Meyer, DH Hellhammer, CP Muller. ***Glucocorticoid sensitivity in fibromyalgia patients: decreased expression of corticosteroid receptors and glucocorticoid-induced leucine zipper.*** Psychoneuroendocrinology. 33, 799-809, 2008.
- B Bankamp, EN Lopareva, JR Kremer, Y Tian, MS Clemens, R Patel, AL Fowlkes, JR Kessler, CP Muller, WJ Bellini, PA Rota. ***Genetic variability and mRNA editing frequencies of the phosphoprotein genes of wild-type measles viruses.*** Virus Research 2, 298-306, 2008.
- PQ Li, J Zhang, CP Muller, JX Chen, ZF Yang, R Zhang, J Li, YS He. ***Development of a multiplex real-time polymerase chain reaction for Influenza Virus Type A including H5 and H9 subtypes.*** Diagn Microbiol Infect Dis 61, 192-7, 2008.
- MF Ducatez, H Chen, Y Guan, CP Muller. ***Molecular epidemiology of chicken anemia virus (CAV) in southeastern Chinese live birds markets.*** Avian Diseases 52, 68-73, 2008.
- CM Olinger, NV Lazouskaya, VF Eremin, CP Muller. ***Multiple genotypes and subtypes of hepatitis B and C viruses in Belarus: similarities with Russia and western European influences.*** Clin Microbiol Infect. 14, 575-81, 2008.
- KY Nébié, CM Olinger, E Kafando, H Dahourou, S Diallo, Y Kientega, Y Domo, K Kienou, S Ouattara, I Sawadogo, L Ky, CP Muller. ***Faible niveau de connaissances des donneurs de sang au Burkina Faso; une entrave potentielle à la sécurité transfusionnelle. Lack of knowledge of blood donors in Burkina Faso (West Africa); potential obstacle to transfusion security.*** Transfusion Clinique et Biologique 14, 446-452, 2008.
- JM Hübschen, JR Kremer, S De Landtsheer, CP Muller. ***A multiplex TaqMan assay for the detection of measles and rubella virus.*** J Virol Meth 149, 246-250, 2008.
- JR Kremer, KE Brown, L Jin, S Santibanez, SV Shulga, Y Aboudy, IV Demchyshyna, S Djemileva, JE Echevarria, DF Featherstone, M Hukic, K Johansen, B Litwinska, E Lopareva, E Lupulescu, A Mentis, Z Mihneva, MM Mosquera, M Muscat, MA Naumova, J Nedeljkovic, LS Nekrasova, F Magurano, C Fortuna, HR de Andrade, JL Richard, A Robo, PA Rota, EO Samoilovich, I Sarv, GV Semeiko, N Shugayev, ES Utegenova, R van Binnendijk, L Vinner, D Waku-Kouomou, TF Wild, DW Brown, A Mankertz, CP Muller, MN Mulders. ***High genetic diversity of Measles Virus, World Health Organization European Region, 2005-2006.*** EID 14,107-114.



HEAD OF CIEC:
Catalina ILIESCU, MD, PhD

RESEARCH DEPARTMENTS

THE CLINICAL INVESTIGATION AND EPIDEMIOLOGICAL CENTER

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Roxanne BATUTU, Research Nurse
Pascale KAUPP, Pharmacist – Quality Assurance Clinical Trials
Myriam MENSTER, Clinical research associate
Karine MOSSELMANS, Research Nurse
Telma VELEZ, Research Nurse

THE CLINICAL INVESTIGATION AND EPIDEMIOLOGICAL CENTER

Clinical Investigation and Epidemiological Center (CIEC) is the new clinical research unit based in CRP-Santé. CIEC started its activities in September 2008. CIEC is a clinical research unit fosuing on the realisation of clinical trials and academic clinical research projects in all medical fields.

OBJECTIVES OF THE CIEC

The purpose of the CIEC unit is to promote academic clinical research and clinical trials sponsored by biotechnology, pharmaceutical drug industry and medical device companies.
CIEC aims at being the interface between the fundamental and clinical research (translational research) to produce new scientific and medical knowledge. It respects ethical and legal standards, Good Clinical Practices (GCP), ICH (International Conference of Harmonization) and quality assurance.
In order to succeed in this ambitious endavour, CIEC develops a high-quality clinical research network at a national level, inserting it in a wider international context of collaboration.
CIEC provides logistic help at all stages of a clinical research project: conception, drafting of the protocol, generation of the clinical report forms (CRF), monitoring, management and traceability of blood and tissue samples (in collaboration with IBBL), management of the administrative and the financial documents of the study, data analysis and protection and final report writing and presentation.
CIEC ensures communication between the different project collaborators, organises working party activities and develops international meetings.

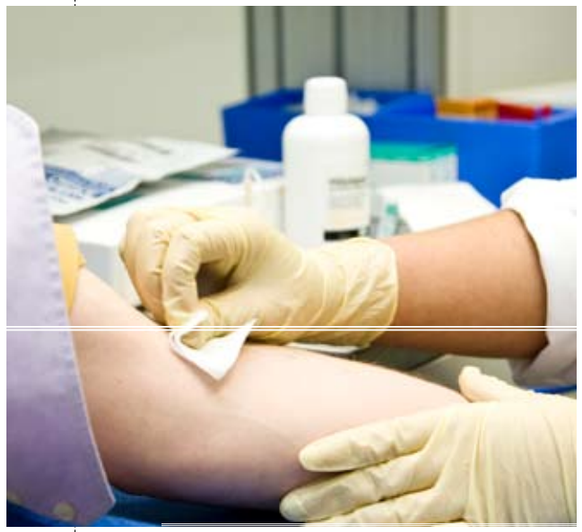
ONGOING PROJECTS

CIEC's first projects are in the fields of hemato-oncology (five clinical trials in close collaboration with Centre Hospitalier in Luxembourg), thrombosis (one clinical trial, currently awaiting authorities' approval) and neurological diseases (one clinical trial). CIEC also offers academic support and logistic help for the development of new clinical research projects in Luxembourg hospitals and GPs' offices.

KEY RESULTS

CIEC internal achievements:

- Our research team (staff recruitment) is composed of a clinical research physician, investigators from Luxembourg hospitals, a pharmacist with training in quality assurance, three research nurses, two clinical research associates and a secretary.
- Since its creation, CIEC has set up: the opening of two consultation boxes for patients, a room for taking blood samples and the acquisition of medical equipment.
- Prepared standard operating procedures (POS) in order to respect quality assurance and ethical issues.
- Acquired state-of-the art logistic and administrative research software for clinical trials (EMEA and FDA-approved). The ultimate goals: clinical data application and quality assurance of the monitoring and the follow-up of clinical trials; assume labelling and traceability of blood samples according to GCP, ICH and Organisation for Economic Cooperation and Development requirements.
- On December 7, CIEC published the first newsletter of our clinical research unit and the CIEC website page went online.



COLLABORATIONS

CIEC networking achievements:

- Individual contacts and meetings with 12 hospital directors in Luxembourg
- Intense national networking with the objective to cover main medical speciality fields and epidemiology.
- Collaboration with the Centre for Health Studies for epidemiological studies.
- Ambitious collaboration efforts and strategic partnerships involving the Integrated Biobank of Luxembourg (IBBL) and the US partners (Tgen Foundation) in the field of personalized medicine: active participation and contribution to the the first clinical research project (lung cancer project).
- Collaborations with the Clinical Investigation Center in Nancy (Prof. Faiez Zannad and his team).

HEAD OF LABORATORY:
Guy BERCHEM, MD

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Wim AMMERLAAN, Technician Cytoflow Core Facility
Nasséra AOUALI, PhD, Researcher, Project Leader
Manon BOSSELER, Technician
René BRONS, Engineer, Cytoflow Core Facility Supervisor
Laetitia CHAMBEAU, Technician - Télévie/RCMS
Victoria EL KHOURY, PhD, Researcher - Télévie
Bassam JANJI, PhD, Researcher, Project Leader
Laurent KELLNER, Technician
Audrey LEMASSON, Secretary
Brigitte METZGER, Technician RCMS

RESEARCH DEPARTMENTS
DEPARTMENT OF ONCOLOGY

LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

Etienne MOUSSAY, PhD, Researcher - Télévie
Tony Kaoma MUKENDI, Bioinformatician
Valérie PALISSOT, PhD, Researcher and Lab Supervisor
Marc PAULY, PhD, Researcher, Project Leader - RCMS
Maria PIRES PACCHECO, Technician - Télévie
Sandrine PIERSON, Engineer
Kris VAN MOER, Technician - Télévie
Thomas WENNER, PhD, Researcher, Project Leader - Télévie/RCMS

LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

Established in 2003, the Laboratory of Experimental Hemato-Oncology, under the direction of Dr Guy BERCHEM MD, forms the link between the clinical and research worlds. Its research activity is focused on the study of cell death and resistance to chemotherapy in hematological and non-hematological cancers. In 2008, the laboratory was composed of 14 persons, with two of them dedicated to Flow cytometry.

OBJECTIVES OF THE LABORATORY OF EXPERIMENTAL HEMATO-ONCOLOGY

The LHCE studies cell death signalling pathways induced by drugs on patient cells in vitro , in vivo and on cellular models. Molecular mechanisms that regulate the effect of chemotherapeutic compounds are studied at genetic, transcriptomic and proteomic levels in order to identify new potential therapeutic targets and to improve personalization of therapy.

ONGOING PROJECTS AND KEY RESULTS

MOLECULAR SIGNATURE OF SENSITIVITY VS RESISTANCE TO FLUDARABINE IN B CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) PATIENTS IN VIVO

The human B-chronic lymphocytic leukemia (B-CLL) is the most common hematological malignancy in Western countries, representing 22-30% of all leukemia cases.1 The B-CLL is characterized by the accumulation in the blood of a high number of monoclonal CD5+ CD19+ CD23+ B lymphocytes mainly arrested in the G0/G1 phase of the cell cycle. These B cells do not fulfill their immunological role anymore and are not able to progress either towards final differentiation nor to programmed cell death. Their accumulation in peripheral blood, in the bone marrow and in certain organs leads to the death of the patient by a progressive failure of the immune and hematopoietic systems.

Although B-CLL is still considered incurable, many drugs can be used to prolong progression-free survival of patients. Due to their potential to kill non-dividing cells, purine nucleoside analogs such as fludarabine (9-b-D-arabinofuranosyl-2-fluoroadenine) are often used in first-line B-CLL therapy. Mechanisms underlying the toxic effects of fludarabine have been studied in vitro in proliferating cells and quiescent human lymphocytes. Fludarabine induces respectively apoptosis in a cell cycle-dependent manner and inhibits DNA repair inducing irreversible damages. In vitro studies, however, do not take into consideration parameters like pharmacokinetics and cell microenvironment which represent critical factors for treatment outcome. Although fludarabine has been known for twenty years to have a considerable activity against CLL cells, its mode of action at the molecular level in vivo is only poorly understood.

In the present study, we investigated the in vivo response to fludarabine, aiming at elucidating the cell death mechanisms in sensitive cells and at identifying predictor genes capable of predicting a poor clinical response to treatment.

For this purpose, we performed a whole-genome expression profiling up to 9 days of treatment. Statistical analysis and hierarchical clustering led to identifying characteristic gene expression profiles of sensitivity or resistance to fludarabine in vivo. In addition, we investigated the expression of genes implicated in apoptosis, cell cycle regulation, and in the response of the B cells to the fludarabine-induced DNA damage. We compared the gene expression profiles with the cytogenetic status of the patients determined by Comparative Genomic Hybridization (CGH) array analysis.

Statistical analysis and clustering of cDNA microarray data helped us to identify gene expression signatures of in vivo sensitivity and/or resistance to fludarabine of B-CLL patients. Most of the genes regulated in vivo in sensitive patients could be classified with gene ontology software as part of the cell death mechanisms, and cell cycle regulation. The data obtained increased the actual knowledge about fludarabine mechanisms in vivo. Interestingly, p53 independent pathways underlying action of this drug have been identified. In resistant patients, DNA damage and repair and nucleotide metabolism were predominantly regulated. Taken together, our data show significant distinct profiles between sensitive and resistant patients and strongly argue in favor of the establishment of targeted PCR on 4 genes (DCLRE1A, PCNA, SULF2, and MYC) before and after one day of treatment as a reliable tool to determine early the outcome of the therapy of B-CLL patients by fludarabine. Moreover, we identified genomic abnormalities associated with resistance to fludarabine that would require further attention, could represent new targets for therapy in B-CLL, and could be important for the choice of an adapted chemotherapy.

STUDY OF GENETIC ABERRATIONS DURING CANCER IN BRONCHIAL NEOPLASIA AND EARLY STAGES OF ONCOGENESIS

Using a whole genome microarray approach associated with comparative genomic hybridization array analysis, gene expression is compared between normal appearing mucosa (identified by fluorescence bronchoscopy) of different lung cancer risk group patients. Heavy smokers without previous lung cancer are compared to lung cancer patients and to non-smokers. This project aims at improving early diagnosis and detection of high-risk patients with no histological modifications, and identifiing early diagnostic biomarkers. Better understanding of molecular mechanisms and their timing in lung neoplasia could considerably improve disease management.

This study is performed in collaboration with the Laboratoire de Recherche en cancérologie pulmonaire, Institut Jules Bordet, Bruxelles, Belgium and the Service de Pneumologie of the Centre Hospitalier in Luxembourg.

Banking of samples has been followed in 2008 and the bank is now constituted with three biopsies of each patient in previously defined groups. The transcriptome will be evaluated on an affymetrix system. This project will be continued and associated with the Fred Hutchinson Tgen project which will evaluate the proteome in lung cancer.

STUDY OF THE EFFECT OF THE HDAC INHIBITORS MGCD-0103 AND VPA ON B-CLL AND MM CELLS ALONE OR IN COMBINATION WITH PPAR GAMMA AGONIST

In this study, we have investigated the effect of histone deacetylase inhibitors (HDACi), valproic acid (VPA) and MGCD-0103 (Pharmion, Ireland), drugs clinically used in the treatment of epilepsy and undergoing several phase II clinical trials in solid malignancy and hematological diseases (e.g. B-CLL, B-cell lymphoma) respectively. The HDACi have well-described anti-proliferative properties on different types of cancer cell lines. In our laboratory, we have been testing these drugs on different multiple myeloma and B-CLL cell lines as well as on cells selected from patients to determinate the potential activity of these 2 drugs in vitro.

The anti-tumor activity of MGCD-0103 (kindly provided by Pharmion/MethylGene Inc.), an orally active molecule belonging to the benzamides class of HDAC inhibitors, was demonstrated on the growth of EHEB, MEC-1 and JVM-3 human B-CLL cell lines after 48 hour-incubation. We also confirmed the potential anticancer activity of MGCD-0103 against B-CLL cells in vitro on PBMC (peripheral blood mononuclear cells) from 13 CLL patients at a micromolar range. To investigate the mechanisms leading to the cytotoxicity of MGCD-0103, different pathways are under investigation (caspases, reactive oxygen species). This drug has been used alone or in combination with a Bcl-2 inhibitor, in chronic lymphocytic leukemia (CLL). MGCD-0103 induced cell death of PBMC from 13 CLL patients, with a marked difference in sensitivity among patients analyzed. The apoptotic effect of MGCD-0103 was characterized by the presence of Annexin V-positive cells. Although discrepancies were observed among patients, the results suggest that MGCD-0103 induces apoptosis by a caspase-dependent manner in B-CLL cells. A cleavage Bcl-2 family protein of Bax was observed as concomitant with proteolysis of calpain. The apoptosis was enhanced when MGCD-0103 was associated to the Bcl-2 inhibitor, HA14-1. Therefore, inhibition of Bcl-2 protein seems a promising therapeutic strategy for combining with MGCD-0103.

In order to evaluate the anti-neoplastic properties of new drug combinations in multiple myeloma (MM), valproic acid (VPA) and PPARγ agonists (clinically used in diabetes) have been tested on MM cell lines. While VPA is already known to have anticancer properties on different cancer types and in MM, we show that PPARγ agonists potentiate the cytotoxic effect of VPA on different multiple myeloma cell lines. Mechanisms underlying this potentiation were cell cycle arrest and caspase-dependent apoptosis. Down-regulation of key proteins like cyclin D1, P-Rb and cyclin A and cleavage of caspase 8 and PARP are clearly involved in the cell cycle-related and apoptotic mechanisms. The potentiation of VPA effect by PPARγ agonist is mediated by a higher acetylation level of histones H3 and H4 than induced by HDAC inhibitors alone. This potentiation was also observed in MM patient cells treated in vitro. IC50 values of VPA were in the same range as the cell-line concentrations. Besides, in favor of the use of VPA, the cytotoxic effect of this drug was lower in PBMC of healthy donors with an increase of IC50 in drug combinations.
Proteomic studies on MOLP cell line revealed 14 modulated proteins in cotreament VPA/pioglitazone as compared with control. Up to now, we focused our investigations on the acetylation of promoters of transcription factor IRF4 and SND1 coactivator, two proteins which are modulated by VPA. The data obtained by 2D gel and Maldi TOF have been confirmed using western blot. Using ingenuity software to identify the network that implicates all the proteins modulated by the different treatments, common targets have been identified and checked by RT-PCR and WB. Therefore, PCNA seems to be a key element of the cell death induced by these drugs.

ANALYSIS OF THE INVOLVEMENT OF AUTOPHAGY AS A NON-APOPTOTIC CELL DEATH MECHANISM

We have previously shown that the apoptosis-independent cell death mechanism is activated in multiple myeloma cells treated with HDAC inhibitor, valproic acid. These data support the hypothesis that the development of therapeutic strategies based on restoring an apoptosis-dependent cell death in tumor cells, does not seem to be a general characteristic to overcome resistance to cell death. New approaches that explore apoptosis-independent cell death mechanisms have recently become the subject of investigations. Thus, understanding the molecular mechanisms of apoptosis-independent cell death is crucial, because it could determine how best to develop combination therapies to regulate these two mechanisms by anticancer agents.
Accumulating evidence demonstrates that various anticancer therapies induce autophagy in tumor 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. Whether autophagy is a pro-death or a pro-survival mechanism remained unclear until recently. It is now definitively admitted that autophagy may play a dual role in cell survival and cell death.
Our project aims at investigating the involvement of the autophagic cell survival process in the mechanism of tumor resistance to cytotoxic agents. To address this question we used an MCF-7 subline (1001 cells) displaying a defect in the ceramide generation pathway. It has been proposed that this defect could confer resistance to the cytotoxic action of TNF-α in 1001 resistant cells. Indeed, preliminary results demonstrated that the autophagy execution protein Beclin-1 and the autophagosome marker LC3 proteins are overexpressed, while the anti-autophagic and anti-apoptotic protein Bcl-2 is downregulated in 1001 resistant cells. TNF-treatment of 1001 cells was able to activate the formation of numerous autophagosomes in a beclin-1 dependent manner. Together, these data provide evidence that the TNF-resistant and autophagic competent 1001 cells are an excellent model to investigate the role of autophagy as a chemotherapy resistance mechanism.
As drugs that potentially modulate autophagy are increasingly being used in clinical trials (Paclitaxel and hydroxychloroquine) and screens are being carried out for new drugs that can modulate autophagy for therapeutic purposes, we are in the process of developing a high throughput tool that could be used, not only for research, but also for diagnosis and pharmacogenomics.

FLOW CYTOMETRY PLATFORM

In accordance with its core platform assignment, this facility is now implicated in different research projects with international and Luxembourgish laboratories. It offers a broad range of flow cytometry techniques. This facility assists the researcher in choosing the best possible solutions for their investigations which require flow cytometry. The equipments (BD FACS Canto and BD FACS Aria) are upgraded and new methodologies are implemented in order to stay at state-of-the-art level. An annual cytometry conference is organized in Luxembourg and creates interactions between clinical and research flow cytometry users.

COLLABORATIONS

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 Pr A. Burny
- Laboratoire de Recherche en cancérologie pulmonaire, Institut Jules Bordet, Bruxelles, Belgique Pr Drs JP Sculier, V. Ninane, C. Mascaux
- Laboratoire de biochimie et de Biologie Moléculaire, Facultés Universitaires Notre-dame de la paix, Namur, Belgique Dr Martine RAES
- 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

International

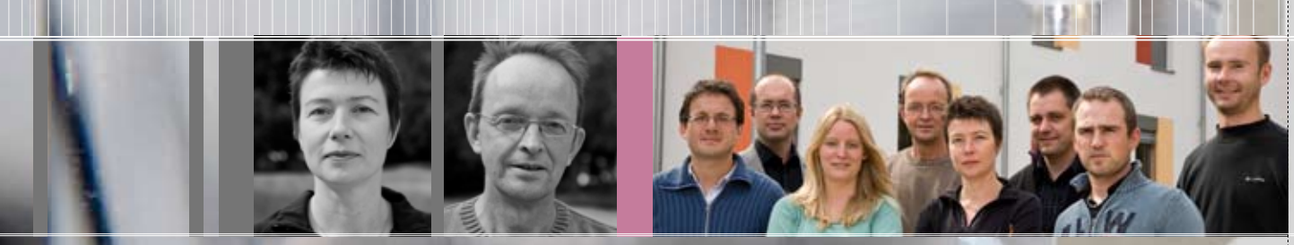
- Service de Pneumologie, Centre Hospitalier de Luxembourg, Luxembourg Dr M. Schlesser
- Laboratoire de Recherche sur le Cancer et les Maladies du Sang, Fondation RCMS, Luxembourg. Dr T.Wenner, Dr M. Pauly.

PUBLICATIONS 2008

- ***Resistance of tumor cells to cytotoxic T Lymphocytes involves Rho-GTPase and FAK activation.*** Abouzahr-Rifai S., Hamelin J., Boukerche H., Janji B., Hasmim M., Jalil A., Mami-Chouaib F., Bertoglio J., and Chouaib S. J Biol Chem, 2008 Nov 14;283(46):31665-72
- ***Valproate synergizes with nucleoside analogues to induce apoptosis of B-chronic lymphocytic leukaemia cells.*** AB Bouzar, M Boxus, J Defoiche, G Berchem, D Macallan, R Pettengel, F Willis, A Burny, L Lagneaux, D Bron, B Chatelain, C Chatelain, and L Willems British Journal of Haematology, 2009 Jan;144(1):41-52

International conferences

- ***Potentiation histone deacetylase inhibitor effect by a PPAR gamma on multiple myeloma cancer.*** N. Aouali, 1V. Palissot, 1E. Moussay, V El-Khoury, 1B.Janji, S Pierson, NHC Brons, M Bosseler, K Van Moer, G Berchem (Poster) Apoptosis Word 2008 from mechanisms to applications, January 23-26, 2008, Luxembourg.
- ***Autophagy-dependent survival pathway as a means to escape TNF-induced cell death.*** Janji B., Moussay E., El-Khoury V., Aouali N., Palissot V, Chouaib S. and Berchem G. Apoptosis Word 2008 from mechanisms to applications, January 23-26, 2008, Luxembourg
- ***Molecular mechanisms of breast cancer cells resistance to cell death inducing agent (invited speaker).*** Janji B. International Medical Conference University of Aleppo (IMCUA), May 4-7, 2008, Aleppo, Syria.
- ***Identification of an in-vivo molecular signature of sensitivity vs resistance to fludarabine in B chronic lymphocytic leukemia patients.*** Moussay E., El-Khoury V., Aouali N., Van Moer K., Leners B., Bernardin F., Muller A., Nazarov P., Yatskou M., Vallar L., Palissot V., Berchem G.13th Congress of the European Hematology Association, Copenhagen, Denmark, June 12–15, 2008. Hematologica 2008 93: 1-571.
- ***Critical role of the actin-binding protein L-plastin in tumor resistance to TNF-alpha-mediated cell death.*** Janji B., Vallar L., Bernardin F., Berchem G., Palissot V., Al-Tanoury Z., Freiderich E. and Chouaib S. (Poster) American Association for Cancer Research (AACR) Annual Meeting 2008, April 12-16, 2008, San Diego CA, USA.



HEAD OF LABORATORY:
Simone NICLOU, PhD

ASSOCIATE HEAD OF LABORATORY:
Rolf BJERKVIG, PhD

RESEARCH DEPARTMENTS
DEPARTMENT OF ONCOLOGY

NORLUX NEURO-ONCOLOGY LABORATORY

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Siti Aminah ABDUL RAHIM, MD, PhD Student
Vanessa BARTHELEMY, Technician
Virginie BAUS-TALKO, Technician
Sébastien BOUGNAUD, PhD Student
Chantal COURTOIS, Assistant Animal Facility
Claude DANZEISEN, MD, neurosurgeon in training, PhD Student
Anna GOLEBIEWSKA, PhD, Researcher
Mikael JOHANSSON, MD, PhD, Visiting Scientist
Olivier KEUNEN, Research Engineer
Anaïs OUDIN, Research Engineer
Stéphanie SALLAI, Assistant Animal Facility
Jo Kristian UTVIK, PhD, Researcher

NORLUX NEURO-ONCOLOGY LABORATORY

The NorLux Neuro-Oncology Laboratory carries out biomedical research on malignant brain tumors. The laboratory at CRP-Santé has strong links to its partner laboratory at the University of Bergen, Norway, defined by a formal agreement between the two institutions. This fosters productive collaborative research, scientific competence building and staff exchange between the two institutions.

OBJECTIVES OF THE NORLUX NEURO-ONCOLOGY LABORATORY

The goal of the research unit is to understand the biological mechanisms underlying the initiation and progression of malignant brain tumors and to identify new molecular targets to be used in cell and gene therapeutic approaches against brain tumors.

The laboratory has developed appropriate animal models to study brain tumour development in vivo. Such models are invaluable tools to investigate the interaction of tumour cells with their micro-environment and to characterize cancer-initiating cells in brain tumors. We apply high throughput proteomics techniques for biomarker discovery in brain cancer and we validate these targets in brain-tumor biopsies and functional bioassays. We also develop cell micro-encapsulation technology for the application of cell and gene therapy in the brain. This innovative technology is applicable to the treatment of different brain diseases including cancer and neurodegenerative disorders.

ONGOING PROJECTS

CANCER STEM-CELL PROJECT

Title: Role of Cancer Stem Cells in Brain Tumour Initiation and Progression

Acronym: Cancer Stem Cell

Contract number: REC 070602

Grant period: January 2008 - December 2010

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

Project summary:
Recent studies have described stem-like cancer cells in several tumours including malignant glioma. This project aims to identify and characterize such cells in Glioblastoma and determine putative stem-cell specific biomarkers. We have previously set up a brain tumour model in GFP-expressing immunodeficient mice that closely recapitulates human Glioblastoma growth. Using this model, we characterize cancer-initiating cells from human brain tumours in vitro and in vivo. Next, we separate the tumour from the host cell compartment to study tumour-host interactions. The overall aim of this project is to identify molecular processes involved in tumour formation and characterize key molecules involved in the initial steps of tumour initiation and progression. The project will also provide novel insight into the current cancer stem-cell controversy.

People involved:
Simone Niclou & Rolf Bjerkvig, project leaders
Anna Golebiewska, postdoctoral researcher
Vanessa Barthelemy, technician
Mikael Johansson, visiting scientist

ANGIOTARGETING PROJECT

Title: Targeting Tumour-Vascular /Matrix Interactions

Acronym: Angiotargeting

Website: www.uib.no/med/angiotargeting

Contract number: EU 504743

Grant period: November 2004 - April 2009

Financial support: Integrated Project, EU 6th Framework Programme

Project summary:
Solid tumour growth depends on a continuous supply of nutrients supplied from blood vessels generated within the tumour. This process termed 'tumour angiogenesis' is regulated by a number of complex factors involving both tumour and host cells. The importance of the tumour blood supply has fuelled research into target molecules with anti-angiogenic properties. Here we study the molecular mechanisms underlying the change from non-angiogenic to angiogenic growth in solid human tumours, a phenomenon called the 'angiogenic switch'. This project aims at identifying novel target molecules involved in this phenomenon through the use of high throughput proteomics technologies.

People involved:
Simone Niclou, project leader
Uros Rajcevic, postdoctoral researcher
Sébastien Bougnaud, internship
Siti Aminah Abdul Rahim, PhD student
Virginie Baus-Talko, technician

Associated PhD project:

Title: Functional Validation of Novel Biomarkers Involved in Invasive and Angiogenic Properties of Brain Tumors PhD project of Siti Aminah Abdul Rahim, in cooperation with the University of Luxembourg, Prof. Evelyne Friederich.

Start date: October 2008.

Financial support: BFR/AFR grant from FNR.

ALZHEIMER'S DISEASE PROJECT

Title: Functional validation of a new therapeutic strategy to prevent neurodegeneration and subsequent cognitive impairments in mouse models of Alzheimer's disease

Acronym: Micro-encapsulation

Contract number: FNR 06/04/02

Grant period: February 2007 - January 2009

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

Project summary:
The aim of this project is to test the hypothesis that in situ production of endogenous neuroprotective factors (Ciliary Neurotrophic Factor, CNTF) from encapsulated recombinant cells prevents soluble amyloid β oligomer-induced neurodegeneration and cognitive impairment. Behavioral studies in short-term and long-term mouse models of Alzheimer's disease show that neuroprotection from locally-implanted encapsulated cell factories significantly reduces the cognitive decline in these mice. The present project opens up new avenues for the use of micro-encapsulation technology as an innovative strategy in the treatment of brain diseases. A follow up project will start in 2009 (see Project 4).

People involved:
Simone Niclou, project leader
Jo Kristian Utvik, postdoctoral researcher
Ihsen Youssef, postdoctoral researcher (with INPL, Nancy)
Pierre Garcia, PhD student (with INPL, Nancy)
Anaïs Oudin, Master student

Associated PhD project:

Title: Validation fonctionnelle d'une nouvelle stratégie thérapeutique prévenant la neurodégénerescence et les déficits cognitifs associés dans des modèles murins de la maladie d'Alzheimer. PhD project of Pierre Garcia, in cooperation with the University of Nancy (INPL), Dr Thierry Pillot.

Start date: May 2007.

Financial support: BFR from MCESR.

ENCAPSULATION PROJECT

Title: Application of Cell Microencapsulation Technology to the Treatment of Brain Disorders

Acronym: ENCAPS

Contract number: C08/BM/11

Grant period: February 2009 - January 2011

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

Project summary:
Delivery of therapeutic compounds to the brain is a challenge for the treatment of brain diseases, including brain tumours and neurodegenerative diseases. The limited passage of drugs through the blood brain barrier and the short half-life of locally-injected therapeutic molecules are major hurdles. 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. The encapsulation of the cells in naturally occurring hydrogels (e.g. alginate-based gels) prevents the immune system from destroying the transplanted cells, which allows the use of non-autologous cells for cell therapy. The aim of this project is to optimize the micro-encapsulation technology for its application in the treatment of brain diseases.

People involved:
Simone Niclou, project leader
This project will start in 2009.

GBM TARGET PROJECT

Title: Identifying Molecular Targets on Human Glioblastoma

Acronym: GBM targets

Contract number: C08/BM/12

Grant period: April 2009 - March 2011

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

Project summary:

Based on recent findings in our laboratory, our hypothesis is that during tumour growth and progression, within a heterogenous tumour cell population, there are cells that are highly adapted to a particular micro-environment (niche). Such cells can easily trigger angiogenesis and are tumorigenic in a niche that favours angiogenesis, but they do not display cancer stem-cell markers. We propose that these cells are hypermethylated reflecting a selective gene expression adapted to a specific niche. However, within tumours there are also hypomethylated, highly infiltrative cancer cells. Such cells share several properties with normal stem cells and can, as normal stem cells, utilize more of their genetic machinery to migrate into and adapt to new niches. This application is aimed at defining the molecular make up of niche-restricted cells versus infiltrating cells which we have shown to display stem-like properties.

People involved:

Rolf Bjerkvig, project leader
This project will start in 2009.

KEY RESULTS

I. A novel immunodeficient mouse model ubiquitously expressing the green fluorescent protein (GFP) has been characterized. Its utility to study tumor vasularization and tumor host interactions has been demonstrated (Niclou et al., FASEB J 2008).

II. Based on state-of-the-art high throughput technologies, two large-scale quantitative proteomics analyses have been carried out on brain and colorectal cancer samples. We applied a peptide labeling approach, followed by tandem mass spectrometry (MALDI TOF/TOF), as well as a label-free quantitation approach based on spectral counting (Rajcevic et al., Front. Biosci. 2009, and manuscript in preparation).

III. Cell encapsulation-based neuroprotective peptide delivery to the mouse brain leads to considerable improvement in the cognitive performance in mouse models of Alzheimer's disease (manuscript in preparation). These data demonstrate the therapeutic efficacy of neuroprotection to combat neurodegenerative diseases and the applicability of local drug delivery via encapsulated recombinant cells.

IV. In 2008, the laboratory successfully received peer-reviewed funding for two new projects within the FNR CORE Program (C08/BM/11: 650'000€ and C08/BM/12: 504'000€). These projects will start in 2009.

V. On October 30, 2008, Norlux organised a Workshop on Brain Diseases with national and international specialists, which has attracted more than 70 participants from the scientific and medical arena.

VI. Norlux has acquired an in vivo imaging station (IVIS Lumina) for bioluminescence and fluorescence imaging in living mice. In addition, application of high-resolution magnetic resonance imaging (MRI) on laboratory animals has started in collaboration with the Molecular Imaging Center (MIC) in Bergen.

VII. Norlux members have actively participated with oral or Poster communications in several international scientific conferences (i.e. EANO meeting, Barcelona; HUPO meeting, Amsterdam; SNO meeting, Las Vegas; BRG conference, Dublin).

VIII. The laboratory proposed workshops for science-interested children of the Science Club, Luxembourg.

COLLABORATIONS

National

- Neurosurgery Department Centre Hospitalier Luxembourg, Neurosurgery Department Clinique Zitha
- Core Facility Flowcytometry, CRP-Santé: NHC Brons
- Microarray Center, CRP-Santé: Dr L. Vallar
- University of Luxembourg: Prof. P. Heuschling, Prof. E. Friederich

International

- University of Bergen, Norway, Department of Biomedicine: Prof. PØ. Enger
- University of Bergen, Norway, Molecular Imaging Center: Prof. F. Thorsen
- University of Bergen, Bergen Center for Computational Science: Dr K. Petersen and Prof. I. Jonassen
- Institut National Polytechnique de Lorraine (INPL), Lipidomix Laboratory, Nancy, France: Dr T. Pillot and Dr T. Oster
- 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
- Translational Genomics Research Institute (TGen), Brain tumor Unit, Phoenix, Arizona: Dr M. Berens

PUBLICATIONS 2008

- Niclou SP, Danzeisen C, Eikesdal HP, Wiig H, Brons NH, Poli AM, Svendsen A, Torsvik A, Enger PØ, Terzis JA, Bjerkvig R. ***A novel eGFP-expressing immunodeficient mouse model to study tumor-host interactions.*** FASEB J. 2008 Sep; 22(9):3120-8. Epub 2008 May 21.
- Johansson U, Rasmusson I, Niclou SP, Forslund N, Gustavsson L, Nilsson B, Korsgren O, Magnusson PU. ***Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization.*** Diabetes. 2008 Sep; 57(9):2393-401. Epub 2008 Jun 2.
- Wang J, Sakariassen PØ, Tsinkalovsky O, Immervoll H, Bøe SO, Svendsen A, Prestegarden L, Røsland G, Thorsen F, Stuhr L, Molven A, Bjerkvig R, Enger PØ. ***CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells.*** Int J Cancer. 2008 Feb 15;1 22(4):761-8.
- Rajcevic U, Niclou SP and Jimenez CR. ***Proteomics strategies for target identification and biomarker discovery in cancer.*** Frontiers in Bioscience. 2009 Jan 1; 14: 3292-3303.
- Niclou SP & Bjerkvig R. ***Treatment of brain tumors with micro-encapsulated cell therapy. Book chapter in: The Bioartificial Pancreas and other Biohybrid Therapies.*** 2009. Eds. Hallé, de Vos, Rosenberg. Transworld Research Network.
- Utvik JK & Niclou SP. ***Recombinant cell microencapsulation for treating neurodegenerative diseases in the ageing brain. Book chapter in: The Bioartificial Pancreas and other Biohybrid Therapies. 2009.*** Eds. Hallé, de Vos, Rosenberg. Transworld Research Network.

Additional publications (not involving CRP-Santé)

- Thorsen F, Jirak D, Wang J, Sykova E, Bjerkvig R, Enger PØ, van der Kogel A,Hajek M. ***Two distinct tumor phenotypes isolated from glioblastomas show different MRS characteristics.*** NMR Biomed. 2008 Oct;21(8):830-8.
- Prestegarden L, Misra A, Ware ML, Yeh RF, Bjerkvig R, Feuerstein BG. ***Amplifying small amounts of tumor DNA allows detection of DNA copy number abberations with array-CGH.*** Biotechniques. 2008 Jun;44(7):Piii-Pvi.
- Johannessen TC, Bjerkvig R, Tysnes BB. ***DNA repair and cancer stem-like cells, potential partners in glioma drug resistance?*** Cancer Treat Rev. 2008 Oct;34(6):558-67. Epub 2008 May 22. Review.
- Chekenya M, Krakstad C, Svendsen A, Netland IA, Staalesen V, Tysnes BB, Selheim F, Wang J, Sakariassen PØ, Sandal T, Lønning PE, Flatmark T, Enger PØ, Bjerkvig R, Sioud M, Stallcup WB. ***The progenitor cell marker NG2/MPG promotes chemoresistance by activation of integrin-dependent PI3K/Akt signaling.*** Oncogene. 2008 Sep 4;27(39):5182-94. Epub 2008 May 12.
- Huszthy PC, Goplen D, Thorsen F, Immervoll H, Wang J, Gutermann A, Miletic H, Bjerkvig R. ***Oncolytic herpes simplex virus type-1 therapy in a highly infiltrative animal model of human glioblastoma.*** Clin Cancer Res. 2008 Mar 1;14(5):1571-80.
- Grudic A, Jul-Larsen A, Haring SJ, Wold MS, Lønning PE, Bjerkvig R, Bøe SO. ***Replication protein A prevents accumulation of single-stranded telomeric DNA in cells that use alternative lengthening of telomeres.*** Nucleic Acids Res. 2007;35(21):7267-78. Epub 2007 Oct 24.

RESEARCH DEPARTMENTS

DEPARTMENT OF ONCOLOGY

LABORATORY OF MOLECULAR PATHOLOGY

HEAD OF LABORATORY:
Jos EVEN, MD

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:
Nadine NEY, Engineer

LABORATORY OF MOLECULAR PATHOLOGY

The unit was established in September 2007 at the LNS (Laboratoire National de Santé/National Health Laboratory) where it closely collaborates with the Pathology division of the LNS, the only pathology laboratory in Luxembourg. It functions as an interface between clinicians, pathologists and researchers and helps to set up routine molecular skills for the pathology division.

OBJECTIVES

The current objectives are

- the assessment of loss of PTEN expression as an early marker for metastatic progression of prostate cancer according to the working hypothesis published by Schmitz et al. Int J Cancer. 2007
- the optimization of techniques for DNA extraction for genetic tests in paraffin-embedded tumor specimens
- the use of the Laser Microdissection Microscope (LMD) in collaborative research projects

ONGOING PROJECTS AND KEY RESULTS

LOSS OF PTEN EXPRESSION: A PROGNOSTIC MARKER IN PROSTATE CANCER?

Given the problems of incontinence and impotence that are often the consequence of surgical removal of prostate tumors, reliable prognostic markers are needed to distinguish between slow- and fast-evolving prostate tumors at the time of first diagnosis. The paper by Schmitz et al. suggested "Complete loss of PTEN expression as a potential early prognostic marker for prostate cancer metastasis". This result was in good agreement with current views and animal models involving the anti-oncogene PTEN in prostate and other tumors, but needed to be confirmed by testing more samples in a more restricted patient population.
Therefore, 154 prostate carcinomas selected according to age <65, PSA< 10, and Gleason score <4 were obtained from the Pathology division at LNS. They were analyzed for PTEN expression by IHC (immuno- histo-chemistry) using a previously characterized anti-PTEN rabbit monoclonal antibody. 99 (63.5%) were found to be PTEN+, 24 (15.4%) PTEN- and 33 (21.2%) PTEN mixed. The analysis of these results with respect to survival rates, age distribution, the presence of metastases, the presence of another tumor derived from a different tissue did not yield results in favor of the working hypothesis. For instance, survival rates within the PTEN+, PTEN– and PTEN mixed populations were found to be very similar with PTEN- tumors supposed to be more aggressive.
The data available so far do not confirm that PTEN alone could be used a prognostic marker in prostate cancer.

HPV (HUMAN PAPILLOMA VIRUS) IN CARCINOMAS OF THE PENIS

HPV is known to be associated with some carcinomas of the penis. This pilot project was initiated to optimize techniques for DNA extraction from paraffin-embedded tumor specimens and sequence detection in this material. One of the main goals was to assess the quality of the tissue blocks archived at the LNS by the Pathology Laboratory and documented in the national RMT (Registre Morphologique des Tumeurs).
Specimens from 64 penile squamous cell carcinomas were obtained between 1980 and 2006 by the Pathology Laboratory of LNS. Three different commercially available HPV detection tests were used. Results were obtained with DNA from 26 out of 34 formalin-fixed specimens collected after 1992. The more recently obtained tumors yielded the best results. Among the 26 samples, 14 were HPV positive, 13 were high risk (9 HPV16, 3 HPV33 and 1 HPV52) and one contained HPV11, a low-risk type associated with condylomas. Thus, in this small series, 50% were positive for high-risk HPV DNA. In other studies, the prevalence of HPV in penile cancers varies between 15% and 71%. Such variations may be due to the sensitivity of the detection methods and the selection of the tumor types.
The sizes of the DNAs detected decrease with the age of the specimen. As in other studies with penile tumors, HPV16 was found to be predominant (>60%). HPV16-derived PCR fragments were sequenced: three belonged to the European prototype HPV16 (EP-T350) and five belonged to the European variant (E-G350). Sequence variations, so far not listed in public databases, were found in two patients.
This study on HPV in penile squamous-cell carcinomas showed that tumor blocks archived since 1992 can be used for DNA work using PCR-based techniques and set the stage for future work on other tumors and especially for a project on the molecular epidemiology of HPV in cervix carcinomas.

COLLABORATIONS

- LNS RMT (Registre Morphologique des Tumeurs) Dr R Scheiden
- LNS Pathology Dr U Knolle, Dr W Dippel
- CHL (Centre Hospitalier Luxembourg) Urology, Dr S Lamy
- Institute for Biomedical Aging Research, Innsbruck, Dr W Zwerschke

RESEARCH DEPARTMENTS
DEPARTMENT OF ONCOLOGY

MICROARRAY CENTER

HEAD OF LABORATORY:

Laurent VALLAR, PhD

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

François BERNARDIN, Technician
Arnaud MULLER, Engineer, Bioinformatician
Petr NAZAROV, PhD, Biostatistician
Nathalie NICOT, Engineer, Molecular

MICROARRAY CENTER

The Microarray Center (MC) includes research scientists and technical staff with expertise in cell and molecular biology, biostatistics, bioinformatics and computer science. Established in 2002, the facility actively collaborates with many national and foreign institutions to advance genomic research in areas as diverse as health, food tracing and environmental issues.
The Microarray Center offers personalized services for genomic studies based on a wide spectrum of commercial and custom-designed microarrays, and high performance instrumentation and software. The Center also supports users with complex statistical data analysis, functional data analysis, oligonucleotide design, and more specific bioinformatics requests. Analyses are carried out according to the standard operational procedures recommended by the international Microarray and Gene Expression Data (MGED) society. To guarantee the quality, comparability and exchangeability of the results, analyses run at the Microarray Center are carried out according to state-of-the-art and highly standardized procedures that comply with international guidelines (MIAME, MAQC).

OBJECTIVES OF THE MICROARRAY CENTER

The Microarray Center is dedicated to provide researchers from academic and industrial laboratories access to a comprehensive microarray analysis service. The Center offers integrated services for microarray-based studies that can be specifically tailored to users' needs, including:

- Assistance with experimental design and general analysis approaches.
- RNA extraction, quality control and processing (RT-PCR, amplification).
- Custom microarray design including the design of oligonucleotide probes.
- Microarray manufacturing. Besides traditional DNA- and oligonucleotide-based microarrays, protein arrays can be also 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 can be conducted in a large set of organisms using major commercial microarray platforms (Affymetrix & Agilent).
- Microarray data quality check.
- Biostatistical data analysis.
- Bioinformatics analysis and data mining.
- MIAME-supportive storage of gene expression data.
- Assistance in publication writing.

The MC has a full set of equipment allowing the handling, preparation and analysis of large series of samples, as well as the manufacture of microarrays. All instruments are based on the latest high-performance technologies and are highly flexible, allowing for fully customized s regarding the needs of the users. The MC uses several specialised software based on high-level computational and statistical techniques for in-depth analysis and interpretation of microarray data.

ONGOING PROJECTS

The Microarray Center currently participates in projects focused on health (cancer, immunological disorders, retinal and neuro-degenerative diseases), virology, food tracing and environmental themes.
At national level:

- "Creation of new competence in bioinformatics and of a technological platform for large-scale genomic analysis" (FNR BIOSAN/01/04/09b, Coordinators: L. Vallar & E. Friederich, CRP-Santé)
- "Proteo-genomic approaches to assess molecular circuits of epithelial cancer invasion: implication of microRNAs" (FNR BIOSAN/07/12, Coord. E. Friederich, University of Luxembourg)
- "Microglial activation and differentiation: analysis of signal transduction and phenotype acquisition" (FNR BIOSAN/06/04/01, Coord. P. Heuschling, University of Luxembourg)
- "Vaccines against lowmolecular antigens: novel strategiesandapplication" (FNRBIOSAN/01/04/11, Coord. C. Muller, CRP-Santé/LNS)
- "Specific detection of food allergens from animal and plant origin: molecular characterisation for application in clinical diagnosis" (FNR SECAL/07/06, Coord. F. Hentges & A. Steinmetz, CRP-Santé)
- "Development of new genoproteomic diagnostic tools for the toxicological assessment of endocrine disruptors in food (ENDIF)" (FNR SECAL03/07/05, Coord. L. Hoffmann, CRP-Gabriel Lippmann)
- "Study of genetic aberrations during cancer in bronchial neoplasia and early stages of oncogenesis" (FLCC, Coord. G. Berchem, CRP-Santé)
- "Molecular signature of fludarabine sensitivity vs resistance in B-chronic lymphocytic leukaemia (B-cell) patients" (Télévie, Coord. G. Berchem, CRP-Santé)
- "Microarrays as tools for the phage display technology" (CRP-Santé/2008/01/16, Coord. S. Delhalle, S. Deroo & JC Schmit, CRP-Santé)
- "Janus kinase action in health and disease" (UL R1F107L01, Coord. I. Behrmann, University of Luxembourg)
- "Regulation of microRNA expression by STAT transcription factors: relevance for cancer development" (UL, Coord. S. Kreis, University of Luxembourg)

At international level:

- "Functional genomics of the retina in health and disease" (European integrated project EVIGENORET, Institut de la vision, Paris, France)
- "Gene expression signature in tumour resistance to cytotoxicity" (Institut Gustave Roussy, INSERM U 487, Villejuif, France)
- "Transcriptome regulation induced by extracellular matrix proteins (elastin, collagen, lumican)" (UMR CNRS 6237 MEDyC, Reims, France)
- "Contribution of miRNAs in acute promyolocytic leukaemia" (Institut de génétique moléculaire, UMR CNRS 5535-IFR122, Montpellier, France)

A project proposed by the MC was selected for funding by the FNR in the framework of the CORE program. Associating several partners from Luxembourg and Reims, the project will start in February 2009 and will focus on the study of the alternative splicing events occurring in lung cancers.
The MC was also invited to participate as contracting partners in two other projects starting in 2009:

- "Inhibition of Notch ligand Jagged1 on astrocytes" (CORE project, Coord. E. Morga, University of Luxembourg)
- "Investigation of oncogenic signaling pathways initiated by PDGFRs and assessment of contributions of the JAK/STAT/SOCS pathway" (UL project, Coord. S. Haan, University of Luxembourg)

KEY RESULTS

In the framework of collaboration with the Institut de génétique moléculaire, Montpellier, the MC analysed the transcriptome of Acute Promyelocytic Leukemia (APL) cell lines treated with all-trans-retinoic acid. Coupled with miRNA profiling and bioinformatics analysis followed by experimental validations, data from this study allowed the identification of a group of miRNAs found to control crucial pathways linked to leukemogenesis. The results of this study were published in Blood journal.
The MC established an optimised and standardised procedure to assess the quality of microarray images. This approach was shown to significantly increase the amount of confident and accurate data that can be extracted from microarray experiments, resulting in more meaningful biological conclusions. The results of this study were published in BMC Research Notes.
In the framework of the FNR BIOSAN/01/04/09b project, the MC upgraded the repertoire of Actichip, a thematic array that was designed

recently by the laboratory to profile the gene expression of cytoskeleton-related genes. Based on a comprehensive review of bibliography and biological databases, a list of 1611 new genes involved in the complex regulatory pathways acting on cytoskeleton was established. This list includes more particularly small GTPases from the Rho family and genes involved in pro-inflammatory pathways which play an important role both in the regulation of the cytoskeleton at the transcriptional and post-translational level and in cancer (oncogenesis, angiogenesis and invasion). Total RNAs extracted from various normal and tumour-derived human samples (cell lines and biopsies) were analysed using the new version of Actichip. Results showed that a specific expression profile was obtained for each type of sample, depending on its histopathological features. Subsets of marker genes characterising normal or cancer samples were determined. Altogether, our data indicated that the repertoire of cytoskeleton genes, even though it represents only a limited part of the global genome, is a valuable indicator of the histopathological status of biological samples. Transcriptome analysis using Actichip may therefore represent a powerful approach to classify cells and tissues.

Within the EVI-GENORET project, the MC designed and fabricated a thematic array called Retchip to investigate the transcriptome of normal and pathological retinas. Upon a validation trial, Retchip was used to study RNA samples extracted from the retina of wild-type and rd1 mice, a transgenic model of retinitis pigmentosa. Analysis of gene expression variations was performed at different time points spanning the entire retina degeneration and led to the identification of 10 genes that seem crucial in this process. These genes are currently being validated.

Within the FNR project SECAL/03/07/03, the MC designed an oligonucleotide microarray to discriminate several fish species in food samples based on the identification of orthologous genes encoding for parvalbumin. Benchmarking experiments showed that the biochip has a good level of specificity, indicating that it could be a valuable tool for food tracing. Additional tests are in progress to assess the sensitivity and reliability of the array.

In the frame of the project CRP/2008/01/16, the MC took part in the development of a phage-based microarray as a tool to serotype HIV patients. This innovative biochip was generated using a phage display library raised against HIV and showed specificity performance similar to that obtained with ELISA tests when screening various purified IgAs or plasma from HIV patients.

The MC is now home to the new Affymetrix GeneChip® platform and TMA Master microarrayer. With these high performance technologies, the Center now offers powerful solutions for expression, genotyping, functional genomics, resequencing and tissue/cell microarray experiments.

Members of the team participated in several training courses:

- "Programming language R and Bioconductor", March 2008, Strasbourg, France (A. Nazarov)
- "Ingenuity European User group meeting", April 2008, Cannes, France (M. Muller)
- "Computational and statistical aspects of microarray analysis, June 2008, Bressanone, Italy (A. Nazarov, FNR accompanying measure 08/AM2b/01)
- "Tissue and protein microarrays", EMBO course, June 2008, Dublin, Ireland (N. Nicot, EMBO grant)
- Affymetrix training course, December 2008, Luxembourg

The MC participated in training of undergraduate students (Jody Winterhalter from the ENILV, La Roche-sur-Foron, France; Anne Feltes from University of Edinburgh, UK) and received Dr D. Benouareth (University of Guelma, Algeria) as a visiting scientist.

COLLABORATIONS

The Microarray Center is integrated in a dense network of collaborations, including:

At CRP-Santé:

- Laboratory of experimental hemato-oncology (LHCE, Dr G. Berchem)
- Laboratory Norlux of neuro-oncology (LNNO, Dr S. Niclou)
- Laboratory of immuno-allergology (LIGA, Dr F. Hentges)
- Laboratory of retrovirology (LRTV, Dr JC. Schmit)
- Department of immunology (Pr C. Muller)

At national level:

- Department of environment and agro-biotechnology, CRP-Gabriel Lippmann (Dr L. Hoffmann)
- Life sciences research unit, University of Luxembourg (Profs I. Behrmann, E. Friederich, P. Heuschling, Drs L. Grandbarbe, T. Heurtaux, S. Kreis, E. Morga)

At international level:

- 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, Montpellier, France (Dr CH Lecellier)
- UMR CNRS 6237 MEDyC, Reims, France (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)

PUBLICATIONS 2008

ARTICLES IN PEER-REVIEWED JOURNALS

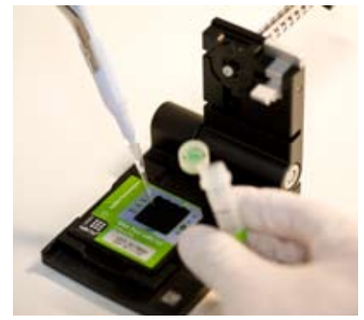
- Saumet A, Vetter G, Bouttier M, Portales-Casamar E, Wasserman WW, Maurin T, Mari B, Barbry P, Vallar L, Friederich E, Arar K, Cassinat B, Chomienne C, Lecellier CH. ***Transcriptional repression of microRNA genes by PML-RARA increases expression of key cancer proteins in acute promyelocytic leukemia***. Blood. 2009; 113(2):412-21.
- Yatskou M, Novikov E, Vetter G, Muller A, Barillot E, Vallar L, Friederich E. ***Advanced spot quality analysis in two-colour microarray experiments***. BMC Res Notes. 2008; 1:80.

PARTICIPATION AT CONFERENCES

- Janji B, Vallar L, Bernardin F, Berchem G, Palissot V, Altanoury Z, Friederich E, Chouaib S. ***Critical role of the actin-binding protein L-plastin in tumor resistance to TNF-alpha-mediated cell death***. AACR annual meeting, April 2008, San Diego.
- Moussay E, El Khoury V, Aouali N, Van Moer K, Leners B, Bernardin F, Muller A, Nazarov P, Yatskou M, Vallar L, Palissot V, Berchem G. ***Identification of an in vivo molecular signature of sensitivity vs resistance to fludarabine in B-chronic lymphocytic leukemia patients***. 13th congress of the European Haematology Association, June 12-15, 2008, Copenhagen.
- Vetter G, Saumet A, Sabbah M, Vallar L, Arar K, Gespach C, Lecellier C-H, Friederich E. ***Identification and characterisation of a microRNA involved in the regulation of the epithelial-to-mesenchyme transition (EMT) in human breast cancer cells***. 3rd RSF Functional genomics conference, October 1-4, 2008, Innsbruck.

SEMINARS

- Nazarov P. ***Simulation-based analysis in the study of complex biomolecular systems: membrane proteins and actin polymerization assays***. CRP-Santé Seminar, January 2008, Luxembourg.
- Vallar L. ***Le Microarray Center du Centre de Recherche Public de la Santé***. Faculté de Médecine, May 2008, Nancy, France.
- Vallar L. ***Transcriptome analysis of a mouse model of retinitis pigmentosa using Retchip, a thematic oligonucleotide microarray***. CRP-Santé seminar, June 2008, Luxembourg.
- Muller A. ***Bioinformatics for microarray analysis***. First LuciLinx symposium, October 2008, Luxembourg.
- Vallar L. ***A technology platform for high-throughput investigation in molecular biology***. BMR meeting, LuxInnovation, October 2008, Luxembourg.



RESEARCH DEPARTMENTS

DEPARTMENT OF CARDIOVASCULAR DISEASES

LABORATORY OF CARDIOVASCULAR RESEARCH

HEAD OF LABORATORY:

Daniel R. WAGNER, MD, PhD

ASSOCIATE HEAD OF LABORATORY:

Yvan DEVAUX, PhD

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Francisco AZUAJE, PhD, Researcher
Mélanie BOUSQUENAUD, PhD Student
Amandine DISCRET, Technician Student
Isabelle ERNENS, PhD, Researcher
Malou GLOESENER, Research Nurse
Benjamin HAAS, PhD Student
Loredana JACOBS, Research Nurse
Céline JEANTY, Technician
Mélanie KIRCHMEYER, PhD, Post-doc Researcher
Bernadette LENERS, Technician
Frédérique LEONARD, PhD Student
Emilie LIEFFRIG, Technician
Christelle NICOLAS, Technician
Aurélie ONRAET, Master Student
Carine PEREZ, Administrative Assistant
Sophie RODIUS, PhD, Post-doc Researcher
Magali ROLLAND-TURNER, PhD, Researcher
Chantal SCHWARTZ, PhD, Researcher
Mélanie VAUSORT, Engineer
Emilie VELOT, PhD Student
Céline YVORRA, Engineer

LABORATORY OF CARDIOVASCULAR RESEARCH

Created at the end of 2003, the group of the Laboratory of Cardiovascular Research presently accounts for 24 members: a director (Dr Daniel Wagner, MD-PhD), an associate director (Dr Yvan Devaux, PhD), four researchers, two engineers, four technicians, two post-docs, two research nurses, one administrative assistant, four PhD students, two master students, and one technician student. Originally located in the basement of the maternité Grande-Duchesse Charlotte – former offices of the administrative staff of the CRP-Santé – the laboratory now profits from a new experimental room and two new office spaces on the ground and second floors of the maternité. These new work spaces were kindly provided by the Centre Hospitalier du Luxembourg.
A critical feature of this research group resides in its multidisciplinary aspect. A gathering of clinicians, nurses, biochemists, molecular biologists, bioinformaticians and biostatisticians allow this group to center its activities on Translational Research.

OBJECTIVES OF THE LABORATORY OF CARDIOVASCULAR RESEARCH

Cardiovascular diseases are the number one cause of morbidity and mortality in our country with a number of deaths continuously increasing. A larger part of these deaths is consecutive to the development of heart failure following myocardial infarction. This is despite the benefit afforded by the modern techniques of reperfusion therapy, such as those performed at the Department of Cardiology of the Centre Hospitalier Municipal. Profiting from a tight collaboration between this clinical department, the Association pour la Recherche sur les Maladies Cardiovasculaires, the Institut de Chirurgie Cardiaque et de Cardiologie Interventionnelle, the team of the Laboratory of Cardiovascular Research of the CRP-Santé is pursuing its research activities aiming at a better understanding of the mechanisms responsible for the development of heart failure, always keeping in mind the discovery of new therapeutic or prognostic approaches of this disease.

ONGOING PROJECTS AND KEY RESULTS

The activities of the Laboratory are divided in two main axes. First, we sought to determine the potential usefulness of adenosine for the treatment of heart failure. Second, we aim to identify new prognostic biomarkers of heart failure.

ADENOSINE AND HEART FAILURE

One of the main causes of heart failure is the adverse structural remodeling of the heart after myocardial infarction. Matrix Metalloproteinases (MMPs), and particularly MMP-9, angiogenesis and inflammation are some of the many processes that play a key role in this remodeling. Adenosine, a nucleoside produced in massive amounts in the heart after myocardial infarction, has cardioprotective properties. We are studying a potential role of adenosine in the development of heart failure.
Following our investigations from 2006-2007 showing that adenosine inhibits the production of MMP-9 by neutrophils, the first wave of inflammatory cells recruited to the heart after myocardial infarction, we demonstrated in 2008 that adenosine enhances MMP-9 production by the second wave of cells recruited to the heart after myocardial infarction, i.e. monocytes/macrophages. These results have been protected by a provisional patent application, have been published (Velot et al. Cardiovascular Research 2008) and have contributed to a PhD thesis (Mrs Emilie Velot, doctor of the University of Nancy, December 2008).
In vitro studies started in 2007 had suggested that adenosine may possess pro-angiogenic as well as anti-inflammatory properties. In 2008, we extended these observations to animal models, thanks to two oversea collaborations (University Thomas Jefferson of Philadelphia and University of Minneapolis). Two publications are being written.

BIOMARKERS OF HEART FAILURE

Following our findings that MMP-9 may represent a powerful biomarker of the occurrence of heart failure after myocardial infarction, we started a pilot study in 2006 aiming at associating genetic variations of the MMP-9 gene with the occurrence of heart failure post myocardial infarction. This study identified several punctual mutations (or Single Nucleotide Polymorphisms) linked to heart failure. In a larger cohort of the LUCKY registry of patients with acute MI (200 patients), we then studied 4 mutations, and one of them had significant association with the development of heart failure four months after myocardial infarction. A FNR-funded research project was started in 2007 to evaluate the potential usefulness of this mutation in clinical practice. The importance of a specific mutation in the MMP-9 gene, which we think may be critical in the development of heart failure, is currently being investigated at Jefferson University, Philadelphia, in a large population from a randomized trial. This mutation is deposited in a provisional patent.
In addition, we sought to determine the potential utility of this mutation to identify new peptide inhibitors of MMP-9 activity. This project is performed through collaboration with the Laboratory of Cristallography of the University of Nancy.
Pursuing our investigations on biomarkers of heart failure and willing to extend our findings made with MMP-9, we implemented a combined approach to identify other single or groups of prognostic biomarkers of heart failure. On one hand, we analyzed the gene expression profiles of blood cells of patients from the LUCKY registry (Luxembourg Acute Myocardial Infarction) obtained by microarrays to retrieve sets of genes whose expression is linked to the development of heart failure. On the other hand, we analyzed networks of interactions between proteins known to be associated with the development of heart failure. When these approaches were combined, a set of three powerful and new biomarkers of heart failure was identified. To test the relevance of these biomarkers and their effective predictive performance, we used patients from the LUCKY registry. In addition, and to extend the number of patients available from this registry, we started collaboration with the Department of Cardiovascular Sciences at the University of Leicester (UK) to obtain samples from 400 patients with myocardial infarction.
Following these very encouraging observations, and convinced that new biomarkers of cardiovascular diseases may represent a step forward in personalising medicine, we decided to organize the first Luxembourgish Workshop on Cardiovascular Biomarkers. This FNR-funded workshop, held at the Chambre de Commerce (Kirchberg), gathered 25 international experts in the fields of biomarkers and "omics" technologies.

HIGHLIGHTS 2008

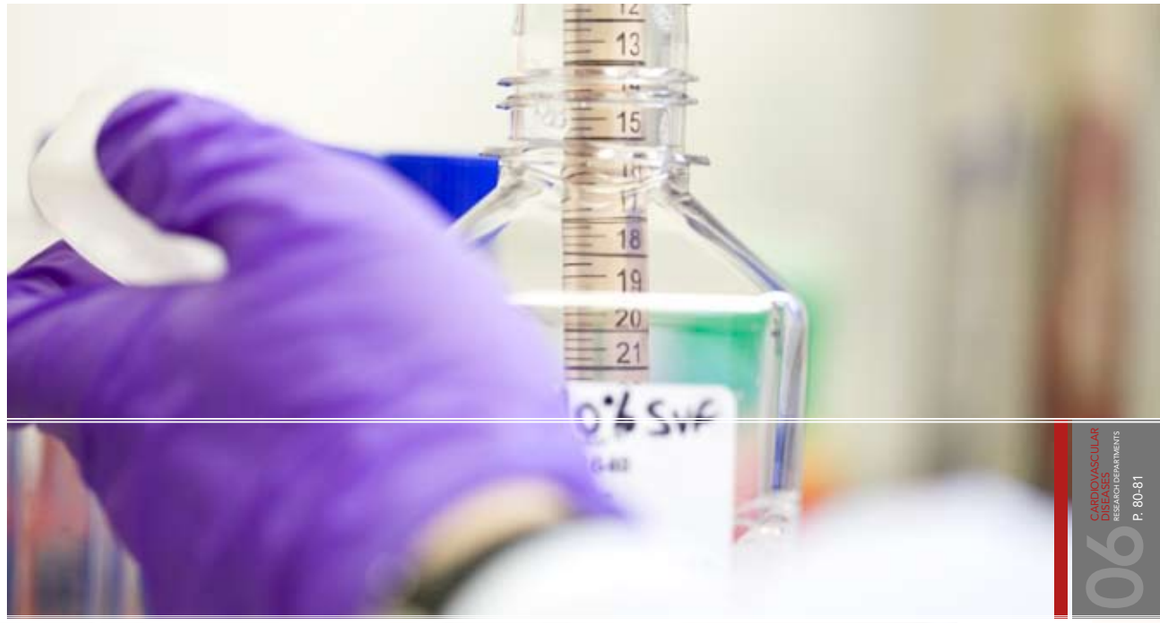
- Collaboration with a private institute to develop a potentially commercial prognostic kit
- First PhD thesis of the Laboratory
- Selection of one work on biomarkers among the 36 best presentations of the year at the world's largest cardiology meeting
- Organization of the first Luxembourgish Workshop on Cardiovascular Biomarkers.

COLLABORATIONS

- Inside the Centre Hospitalier du Luxembourg: Département de Cardiologie, Institut National de Chirurgie Cardiaque et de Cardiologie Interventionnelle (INCCI), Société pour la Recherche sur les Maladies Cardiovasculaires, Service d'Hémato-Cancérologie.
- Inside the CRP-Santé: flow cytometry core facilities.
- In Luxembourg: Department Environment and Agro-biotechnologies, CRP-Gabriel Lippmann; Laboratoires Réunis, Junglinster.
- 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 Anesthesia and Intensive Care, Nancyclotep), Faculty of Sciences (Laboratory of Cristallography), Centre d'Investigation Clinique, 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.

PUBLICATIONS 2008

- Hanriot D., Bello G., Ropars A., Seguin-Devaux C., Poitevin G., Grosjean S., Latger-Cannard V., Devaux Y., Zannad F., Regnault V., Lacolley P., Mertes P.M., Hess K., Longrois D. ***C-reactive protein induces pro- and anti-inflammatory effects, including activation of the liver X receptor alpha, on human monocytes.*** Thrombostasis Haemostasis 2008 ; 99(3): 558-569.
- Velot E, Haas B, Léonard F, Ernens I, Rolland-Turner M, Schwartz C, Longrois D, Devaux Y, Wagner DR. ***Activation of the Adenosine-A3 Receptor Stimulates Matrix Metalloproteinase-9 Secretion by Macrophages.*** Cardiovascular Research. 2008 ; 80(2): 246-254.
- Delagardelle C, Feiereisen P, Vaillant M, Gilson G, Lasar Y, Beissel J, Wagner DR. ***Reverse remodeling through exercise training is more pronounced in non-ischemic heart failure.*** Clinical Research in Cardiology. 2008 Aug 11.
- Oprea I, Vaillant M, Hesse M, Jacobs L, Beissel J, Wagner DR. ***Lucky National Registry (Luxembourg Acute Myocardial Infarction Registry). Are women much better taken care of than men?*** Bulletin de la Société des Sciences Médicales du Grand Duché de Luxembourg. 2008;(2):259-79.
- Saberin A, Wagner DR. ***Cardiac failure during myocardial infarction.*** Revue de Médecine Interne. 2008 Jun;29 Suppl 1:S3-5.
- Rouy D, Lebrun F, Berchem G, Delagardelle C, Beissel J, Wagner DR. ***Cell therapy for severe chronic heart failure: the Luxembourg experience.*** Bio-medical Materials and Engineering. 2008;18(1 Suppl):S27-31.
- Wagner DR, Devaux Y. ***Playing hide and seek with adenosine receptors.*** Clinical and Translational Science 2008 ; 1 (2): 133-135.
- Zheng H, Wang H, Azuaje F. ***Improving pattern discovery and visualization of SAGE data through poisson-based self-adaptive neural networks.*** IEEE Trans Inf Technol Biomed. 2008 Jul;12(4):459-69.
- Camargo A, Azuaje F. ***Identification of dilated cardiomyopathy signature genes through gene expression and network data integration.*** Genomics. 2008 Jun 30. [Epub ahead of print].
- Haiying Wang, Huiru Zheng, Francisco Azuaje. ***Clustering-based approaches to SAGE data mining BioData Mining 2008***, 1:5 (July 17, 2008).
- Browne F, Wang H, Zheng H and Azuaje F, ***"Computational prediction of protein interaction networks through supervised classification techniques"***, International Journal of Functional Informatics and Personalised Medicine, Vol. 1 (2), 205 – 221, 2008.



RESEARCH DEPARTMENTS
DEPARTMENT OF PUBLIC HEALTH

THE CENTRE FOR HEALTH STUDIES

HEAD OF RESEARCH UNIT:
Marie-Lise LAIR

ASSOCIATE HEAD OF RESEARCH UNIT:
Sophie COUFFIGNAL, MD, Epidemiologist, Associate Head of the CHS

PRESENTLY, THE STAFF OF THE CENTRE IS AS FOLLOWS:

Christine GAUTHIER, Secretary
Cathy PIRES, Secretary

METHODOLOGICAL PLATFORM:

Jessica BARRE, MSc, Biostatistician
Audrey BILLY, Statistics Graduate, Data Manager
Valéry BOCQUET, PhD, Doctor in Biostatistics
Sophie COUFFIGNAL, MD, Epidemiologist
Jean-Pierre CORNEZ, Computer Specialist, CNS projects
Julien JACOBS, Statistics Graduate, Data Manager
Maxime LARCELET, Computer Specialist, LATE projects
David MARCIC, Computer Specialist
Christelle ROTT, Statistics Graduate, CNS projects
Nicolas SAUVAGEOT, MSc, Biostatistician
Michel VAILLANT, MSc, Biostatistician

EPIDEMIOLOGY AND PUBLIC HEALTH UNIT:

Sophie COUFFIGNAL, MD, Epidemiologist, Unit Head
Ala'a AL KERWI, MD, Public Health Graduate, Project Leader
Graziella AMBROSET. Research Nurse
Colette ANDREE, Project Coordinator in collaboration
Dritan BEJKO, Project Assistant
Agnès COLUMEAU, Research Nurse
Frédéric DADOUN, MD, PhD, Mobility Program FNR
Marylène D'INCAU, Research Nurse
Joana GROSS, Psychologist, (maternity replacement)
Danielle HOFFMANN, Psychologist
Marie-Christine KREMER, Research Nurse
Aline LECOMTE, MSc in Public Health, Project Leader
Véronique LOUAZEL, MSc in Education, Master in Health Promotion, Project Leader
Jean-Luc LUDEWIG, Psychologist
Anabela MENEZES, Project Assistant
Magali PERQUIN, PhD, Doctor in Biomedical Engineering, Project Leader
Nathalie REMOVILLE, Scientific Collaborator, Pharmacist
Laurence RENARD, PhD Student
Hanène SAMOUDA, PhD, Doctor in Biological Anthropology, Project Leader
Anne-Marie SCHULLER, PhD, Doctor in Neuropsychology
Joël WEIS, Research Nurse
Marco ZEIMET, Research Nurse

HEALTH AND PROMOTION UNIT:

Laurence FOND-HARMANT, PhD, Doctor in Sociology

SPORTS AND HEALTH UNIT

Daniel THEISEN, PhD, Doctor in Kinesitherapy and Rehabilitation, Head of Ward Projects
Audrey BILLY, Statistics Graduate, Data Manager
Anne FRISCH, PhD Student
Thierry WINDHAL, Research Assistant, Physical Education Graduate

EUROPEAN MONITORING CENTRE FOR DRUGS AND DRUG ADDICTION:

Céline DIEDERICH, Scientific Collaborator, Psychologist (maternity replacement)
Sofia LOPES DA COSTA, Scientific Collaborator, Psychologist
Pascale STRAUS, Scientific Collaborator, Psychologist
In collaboration with Alain ORIGER (National Drug Coordinator for the Health Directorate)

SYSTEMS AND HEALTH SERVICES ANALYSIS UNIT:

Unit Head: Marie-Lise LAIR
Didier ANDRIANNE, Research Nurse
Jean-Pierre CORNEZ, Computer Specialist
Christelle ROTT, Statistics Graduate, Data Manager
Gaëtane WIBRIN, Research Nurse

HEALTH WEB PORTAL:

Project Leader: Marie-Lise LAIR
Coralie DESSENNE, Data Administrator, Filing Clerk
Sandrine LAVALLE, Editor, Communication Graduate, Sociology Graduate

EUROPEAN MEDICINES AGENCY:

Project Leader: Dr Jean-Louis ROBERT
Maura MINELLI, Secretary

THE CENTRE FOR HEALTH STUDIES

Created in 2004, the Centre for Health Studies is composed of the following units:

- Epidemiology and Public Health Unit,
- European Monitoring Centre for Drugs and Drug Addiction,
- Health Promotion Unit,
- Health Web Portal Unit,
- Sports and Health Unit,
- Systems and Health Services Analysis Unit.

In the field of epidemiology and public health, the missions of the Centre for Health Studies are to:

- assess the state of health of the inhabitants of Luxembourg in order to provide reliable information for the development of national prevention policies and a basis for comparing Luxembourg with other European countries;
- carry out research on risk factors, determinants and the development processes of certain prevalent disorders or diseases in order to contribute to the enhancement of knowledge in public health, as well as to innovations in terms of diagnosis or treatments;
- contribute to, and establish ongoing and high-quality monitoring systems for diseases prevalent in Luxembourg in order to promote the consolidation of information that is useful for monitoring the health of the population and evaluating the results of the health care provided;
- promote research in public health, especially in the area of evaluating public health policies and their related programmes ;
- contribute its methodological support to clinical research carried out by clinicians, placing at their disposal skills for study design and statistics;
- support the creation and distribution of information for the public in order to make people more responsible in managing their own health.

In the field of public health systems and services, the missions of the Centre for Health Studies are to:

- develop methodologies for allocating resources based on care needs;
- contribute to the improvement of performance in health care by seeking out those methods and technologies best adapted to the socioeconomic context of health care in Luxembourg;
- evaluate the results of treatment and care provided to people in the context of pilot projects.

OBJECTIVES OF THE CENTRE FOR HEALTH STUDIES

The goal of the Centre for Health Studies is to become, for public authorities, a reference center in terms of epidemiological studies in general population and the evaluation of programmes implemented in the field of public health, in order to offer useful information for defining health policies and for funding health care.
At the same time, the development of research on public health is a priority objective so as to contribute to the enhancement of knowledge in this field.
The research focuses of the Centre for Health Studies are the diseases prevalent in Europe and in Luxembourg:

- cardio-cerebral vascular disease, diabetes, obesity, metabolic syndrome and their pertinent risk factors: nutrition and physical activity,
- cancer,
- neuro-degenerative disorders that occur due to aging,
- mental health disorders.

Assessing the prevalence and incidence of pathologies, as well as their risk factors, constitutes the first threshold to be reached. Determining whether health care and the resources required for general public health are adequate, identifying populations that are vulnerable due to major health-related risk factors, and equal access to health care constitute additional research objectives.

The search for bio-markers or environmental determinants that can explain the origin or evolution of diseases completes our research targets, with special emphasis on lipid metabolism.

ONGOING PROJECTS

Not all of the projects and studies ongoing at the Centre for Health Studies are covered in this report. They may be found at: www.crp-sante.lu
Only a few projects have been highlighted, either because they are no longer recruiting, results have been produced or an instrument developed, or because they lead up to new projects.

HBSC SURVEY (HEALTH BEHAVIOR IN SCHOOL-AGED CHILDREN)

Project Leader: Dr Sophie Couffignal.
Study commissioned by the Ministry of Health.

This survey, organised by the European Division of the WHO, is a transversal epidemiological study of school children ages 11, 13, 15 and 17, which is intended to observe health and hygiene habits of young people and identify the factors that influence them. The health and hygiene habits of young people in Luxembourg were compared for the first time, using the same methodology, with those in 42 other countries in a report entitled: Inequalities in young people's health. HBSC International Report from the 2005-2006 survey. A more country-specific national report is being established.

MONITORING OF PERINATAL HEALTH IN LUXEMBOURG (SUSANA)

Project Leader: Aline Lecomte.
Study commissioned by the Ministry of Health.

A new system for monitoring perinatal health has been set up that allows Luxembourg to respond to European PERISTAT core indicators. The DIANE software program was developed and put into use in maternity hospitals.

MENTAL HEALTH ASSESSMENT OF YOUNG PEOPLE IN LUXEMBOURG

Project Leader: Véronique Louazel, with the help of Marie-Lise Lair and Joana Gross.
Study commissioned by the Ministry of Health.

The objective is to identify the types of treatment and care provided for children's mental health, as well as the problems encountered both by health care professionals and by parents and the children themselves. 68 interviews were conducted with dependent organisations on the Ministries of Health, the Family, Education and Justice. In 2009, consensus conferences will be held in order to develop proposals.

PREDICTIVE STUDY OF INJURIES AND LESIONS AMONG YOUNG, HIGH-LEVEL ATHLETES IN LUXEMBOURG

Project Leader: Daniel Theisen, Ph.D.
Study commissioned by the ministry's Department of Sports and co-funded by the Ministry of Research.

Following up on the conclusions of the retrospective study done in 2007, a prospective system for monitoring injuries of 72 pupils in Sports-Studies classes was set up in 2008. It served to demonstrate the qualitative superiority of data collected prospectively. This study will be expanded in 2009 to 200 pupils.

CHILD AND ADOLESCENT OBESITY AND OVERWEIGHT IN LUXEMBOURG (OSPEL)

Project Leader: Hanène Samouda, PhD.
Study commissioned by the Ministry of Health and co-funded by the Ministry of Research.

The objectives of this research project are to develop reliable, non-invasive methods suitable for children in order to diagnose obesity and establish predictability for the risks of complications and associated factors, as well as to compare the effectiveness of two treatment models for obese children. 193 children took part in the study. The results are due out in 2009.

OBSERVATION OF RISK FACTORS FOR CARDIOVASCULAR DISORDERS IN LUXEMBOURG (ORISCAV-LU)

Project Leader: Dr Alaa Al Kerwi.
Study commissioned by the Ministry of Health and co-funded by the Ministry of Research.

1,500 participants representative of the inhabitants of Luxembourg were recruited and provided with an in-depth investigation of their state of health and their risk factors, biological examinations and anthropometric measurements. The main results of this study are due out in 2009.
From 2009 to 2011, this study will be conducted in Lorraine, Wallonie and Sarre in the context of the NESCAV project, to be financed by INTERREG in 2009, which will enable a transborder comparison of cardiovascular risk factors.

THE STATE OF DIABETES IN LUXEMBOURG BASED ON MEDICO-ADMINISTRATIVE DATA

Project Leader: Magali Perquin, Ph.D.,
in collaboration with Véronique Louazel.

An analysis of the medico-administrative data contained in the files of the Union des Caisses de Maladie (Social Insurance) was used to determine the rate of incidence and changes in the prevalence of diabetes treated in the population covered by health insurance, to identify the type and number of complications treated annually, and to compare the treatment provided with international recommendations. Luxembourg was thus able to supply this data at the European level as part of the EUCID programme. This work has provided new opportunities for 2009: the preparation of a joint pilot treatment programme between the Ministry of Health and the National Health Insurance Agency.

LIFE IN LUXEMBOURG FOLLOWING A STROKE: IMPACT ON FAMILY AND QUALITY OF LIFE. EQUAL ACCESS TO TREATMENT AND TO SOCIAL WELFARE BENEFITS

Project Leader: Dr Sophie Couffignal,
in collaboration with the University.
Study financed by the FNR (National Research Foundation).

The objectives of this study are to establish the profile of patients suffering strokes in Luxembourg, to evaluate treatment provided at hospitals, to measure the burdens of strokes on the individual, the family and society, and to evaluate the level of satisfaction with the services and resources used. Data is currently being collected and the initial results are due out in 2009.

THE INCIDENCE OF HEADACHES AND THEIR SOCIOECONOMIC BURDEN IN 11 EUROPEAN COUNTRIES: EUROLIGHT

Project Leader: Colette Andrée.
Study co-financed by the Public Health Executive Agency and the Ministry of Research.

The goal here is to measure the incidence of migraine headaches in each of the participating European countries, to determine their socioeconomic impact and burden on the individual and their family through the use of a standard, validated questionnaire. The initial results are due out in 2009.

PREDICTIVE ASSESSMENT OF THE NEUROPSYCHOLOGICAL, BIOLOGICAL AND SUB-CLINICAL CHARACTERISTICS OF THE CONDITION OF MILD COGNITIVE IMPAIRMENT (MÉMOVIE)

Project Leader: Magali Perquin, Ph.D.
Study performed in partnership with the University of Luxembourg, financed by the FNR.

A cohort of elderly people has been made up, composed of people who do or do not have cognitive disorders. They have undergone an in-depth neuropsychological evaluation, biological examinations and a medical examination. Its continuation has been programmed. The initial results are due out in 2009.

FEASIBILITY STUDY FOR THE ESTABLISHMENT OF A NATIONAL CANCER REGISTRY IN LUXEMBOURG

Project Leader: Dr Sophie Couffignal,
in collaboration with Marie-Lise Lair.
Study commissioned by the Ministry of Health.

Luxembourg is the only country in Europe without a national cancer registry. To date, there is only a non-exhaustive tumour registry. A design has been created and submitted to the Ministry of Health, taking into account international experiences and the realities of Luxembourg.

KEY RESULTS

The Centre for Health Studies was chosen as the principal administrator of the INTERREG NESCAV project, the goal of which is to use standardised instruments to measure the prevalence of cardiovascular risk factors over the broader target region, to identify the populations at risk and to suggest to transborder public authorities areas of focus for prevention and education.
A new agreement has been signed with the Ministry of Social Security to perform an evaluation of the degree of satisfaction of patients placed in institutions.
The methodological platform of the Centre for Health Studies (biostatistics, computer processing and epidemiology) has been reinforced in order to meet the needs of clinicians and of the centre for Investigation and Clinical Epidemiology.
The Centre for Health Studies will be associated with an American Lung Cancer project to develop an activity on health economics.
A cycle of presentations on public health was organised in partnership with the University of Luxembourg.
The journal Enjeux Santé (Public Health Challenges), Luxembourg's journal on public health research and studies, was created in 2008: 2 issues were published during the year.

COLLABORATIONS

At the national level, the Centre for Health Studies collaborates primarily with those public authorities and institutions that commission studies and research work: the Ministry of Health and the Health Department, the Ministry of Social Security, the Evaluation and Orientation Division of the Assurance Dépendance (Patient Insurance Agency), the Inspectorate General of Social Security, the Ministerial Department of Sports and the National Sports Institute, and the Caisse National de Santé (Health National Agency). We have an ongoing collaboration with hospitals.

As the same time, there is a partnership between the Centre for Health Studies and the INSIDE Department (Prof. Ferring), the Life Sciences Research Unit and the Neuro-Inflammation Unit (Prof. Heuschling) at the University of Luxembourg.

Contacts have been made to plan for future collaboration with the CEPS and the CRP-Gabriel Lippmann.

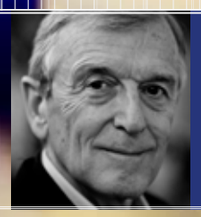
At the international level, the Centre for Health Studies has signed agreements or is collaborating with a number of specialists (Prof. J-F Dartigues - University of Bordeaux; Prof. J-L Thonnard - UCL), and hosts doctoral students from the University of Liège and the University of Paris-V. A number of staff members from the Centre for Health Studies serve as instructors at foreign universities (Nancy, ULB, Zurich, UCL).

In the context of funding provided by hospitals, ongoing projects are carried out in partnership with EROS (Health Operational Research Team) in Montreal.

PUBLICATIONS AND DEVELOPMENTS 2008

- Frisch A., Seil R., Urhausen A., Croisier J:L:, Lair M.L., Theisen D. (2008) ***Analysis of sex-specific injury patterns and risk factors in young high-level athletes. Scandinavian Journal of Medicine and Science in Sports.***
- Andrée C., Vaillant M., Rott C., Katsarava Z.,Sándor P.S. (2008) ***Development of a self-reporting questionnaire, BURMIG, to evaluate the burden of migraine.*** The Journal of Headache and Pain ISSN 1129-2369 (Print) 1129-2377 (Online).
- Stovner L.J., Andrée C. ***Impact of Headache in Europe: a review for the Eurolight project.*** The Journal of Headache and Pain. ISSN 1129-2369 (Print) 1129-2377 (Online).
- Fond-Harmant L., Tal Dia A. ***Santé publique et transfert de compétences nord-Sud: les conditions d'une approche inter-culturelle.*** Santé Publique, n°1, janvier-février 2008, ISSN: 0995-3914.
- Fond-Harmant L. ***Le développement de la recherche –action européenne en promotion de la santé mentale: Pourquoi ? Comment ?*** Revue Promotion et Education hors série 1/2008. ISSN 1025-3823.
- Oprea I.,Vaillant M.,Hesse M.,Jacobs L.,Beissel J.,Wagner D.R. ***Luxembourg Acute myocardial Registry: les femmes moins bien soignées que les hommes.*** Bulletin de la Société des Sciences Médicales 2/08.
- Delagardelle C. , Feiereisen P., Vaillant M. , Gilson G. , Lasar Y. , Beissel J. , Wagner D.R.(2008). ***Reverse remodeling through exercise training is more pronounced in non-ischemic heart failure.*** Clin Res Cardiol.
- Brasseur P., Agnamey P., Gaye O., Cisse M., Badiane M., Vaillant M., Taylor W., Olliaro P.. (2008). ***Dosing accuracy of artesunate and amodiaquine as treatment for falciparum malaria in Casamance, Senegal.*** Trop Med Int Health.
- Olliaro P., Pinoges L., Checchi F., Vaillant M., Guthmann J.P. (2008). ***Risk associated with asymptomatic parasitaemia occurring post-antimalarial treatment.*** Trop Med Int Health 13(1): 83-90.
- Sundar S., Rai M., Chakravarty J., Agarwal D., Agrawal N., Vaillant M., Olliaro P., Murray H.W. (2008). ***New treatment approach in Indian visceral leishmaniasis: Single-dose liposomal amphotericin B followed by short-course oral miltefosine.*** Clin Infect Dis.
- Mueller Y., Nguimfack A.,Cavailler P., Couffignal S.,Rwakimari J.B.,Loutan L., Chappuis F. ***Safety and effectiveness of amphotericin B deoxycholate for the treatment of visceral leishmaniasis in Uganda.*** Annual tropical medical Parasitology, 2008, 102(1):11-9.
- Louazel V.,Perquin M.,Couffignal S.,Rott C.,Michel G.,de Beaufort C., Lair M.L. ***Le diabète au Luxembourg.***

- Enjeux Santé 1/2008 ISSN (imprimé) 1998-3476 ISSN (en ligne) 1998-2484.
- D.Theisen, A.Frisch, T.Windhal,S.Couffignal,A. Urhausen,R.Seil,A.Sax,M.L.Lair. ***La recherche au service de la prévention des blessures chez les jeunes sportifs de haut niveau au Luxembourg.*** Enjeux Santé 2/2008 ISSN (imprimé)1998-3476 ISSN (en ligne) 1998-2484.
- Couffignal S., Lair M.L. ***Etude de faisabilité pour la mise en place d'un registre du cancer au Luxembourg 2008.***
- Couffignal S., Lair M.L. ***Evaluation des résultats de la prise en charge chirurgicale des personnes atteintes d'un cancer colorectal à la Clinique Sainte-Thérèse.*** 2008.
- Origer A., Lopes S. 2008 ***National Drug Report (2007 Data) New Developments, trends and in-depth information on selected issues.***
- 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 2008 pour l'Union des Caisses de Maladie.
- Lair, M.L., Cornez, J.P., Wibrin, G., Rott, C. ***Dotation en personnel soignant sur base des activités dans les blocs opératoires,*** rapport 2008 pour l'Union des Caisses de Maladie.
- Lair, M.L., Cornez, J.P., Wibrin, G., Rott, C. ***Dotation en personnel soignant sur base des activités dans les salles de réveil,*** rapport 2008 pour l'Union des Caisses de Maladie.
- Lair, M.L. ***Dotation en personnel soignant sur base des activités dans les services de dialyse,*** rapport 2008 pour l'Union des Caisses de Maladie.
- Cornez J.P. ***Développement du Logiciel Diane pour la surveillance de la santé périnatale au Luxembourg.***



RESEARCH DEPARTMENTS

DEPARTMENT OF PUBLIC HEALTH

LABORATORY OF EMOTIONAL DISORDERS

HEAD OF LABORATORY:

Charles PULL, MD, PhD

PRESENTLY, THE STAFF OF THE LABORATORY IS AS FOLLOWS:

Gloria AGUAYO, MD
Marc DAMME, MA Psychology
Maxime LARCELET, Computer Specialist

LABORATORY OF EMOTIONAL DISORDERS

The Laboratoire des Troubles Emotionnels (Laboratory of Emotional Disorders) or LATE is part of the Centre d'Etudes en Santé at the Centre de Recherche Public Santé or CRP-Santé.
It is a small unit, with a principal investigator (Pr Charles Pull), two researchers (Dr Gloria Aguayo, MD and Marc Dammé, MA), and a technician (Maxime Larcelet).

OBJECTIVES OF THE LATE

The Late is involved in the study of emotions and in the treatment of emotional disorders, with a focus on fear and sadness, anxiety disorders and major depressive disorders. Emotions are investigated using a battery of psychological tests and physiological measurements. Emotional disorders and their treatment are investigated using a number of specific questionnnaires.
The most original objective of the LATE is the use of virtual reality to study emotions and to treat emotional disorders with Virtual Reality Exposure Therapy (VRET).

ONGOING PROJECTS

TREATMENT OF SPECIFIC PHOBIAS AND SOCIAL PHOBIA BY VRET

The first project aims to explore the potential of VRET for the treatment of the fear of driving, the fear of flying and the fear of public speaking. LATE has been in contact with experts in virtual reality concerning the development of relevant software (virtual environments) and to acquire the necessary hardware and software. Part of the required material has already been purchased; the rest will be during the first six months of the project.
The study is currently going through a pilot phase in which the required equipment and software is being tested in patients and in controls. The study itself will be a randomized controlled trial (RCT) designed to compare the efficacy of traditional cognitive behavior therapy and VRET in each of the three phobias. Both the pilot study and the RCT will include the measurement and monitoring of heart rate, blood pressure, skin conductance, electrocardiogram, electromyogram, electroencephalogram, salivary cortisol, and blood oxygen saturation. Physiological parameters are measured using equipment purchased from a German company and has been tested during the past 6 months.
The project may integrate measurements from functional MRI when the neuroradiology department of the Centre Hospitalier is ready to provide this type of measurement (this should be possible in the next few years).

ASSESSMENT OF MENTAL STATUS AND BODY IMAGE IN OBESITY INCLUDING THE USE OF VR

The second project intends to use exposure to body images in VR presenting different shapes and sizes to assess the representation or image that subjects with obesity have of their bodies. The assessment of body image is part of a more global assessment of patients with morbid obesity, i.e. with a Body Mass Index or BMI over 40, presenting for bariatric surgery consisting in a gastric by-pass.
All patients presenting for this type of surgery are assessed using an extensive battery of psychometric tests in addition to a comprehensive structured interview exploring the history of the weight problem, any associated eating disorder, any attempt to lose weight through dieting, sports, medication or psychotherapy.
The aims of the assessment are 1. to identify all psychological problems (including problems with body image) associated with obesity, and 2. to identify all psychological factors (including body image) that may be involved in the short-term as well as the long-term prognosis of the surgery.

KEY RESULTS

VIRTUAL REALITY

The LATE has participated as one of three partners in the biggest study worldwide comparing the efficacy of Virtual Reality Exposure Therapy (VRET) and traditional cognitive behavioral therapy (CBT) in the treatment of Panic disorder with Agoraphobia.
The study is a randomized controlled trial comparing the efficacy of traditional CBT, VRET, and a waiting list in patients meeting DSM-IV criteria for Panic disorder with Agoraphobia. Three university hospitals participated in the study (in Luxembourg, Lyon and Paris). Patients on the waiting list were randomized to either one of the two active treatments at the end of three months. Patients were reassessed at follow-up after 3, 6 and 12 months. Patients were assessed using a number of rating scales, behavioral tests and specific cognitive tools.
The main outcome criterion was a decrease of at least 50% of the baseline Agoraphobia score on the Fear Questionnaire. Secondary outcome criteria were based on the scores obtained on scales for the assessment of anxiety, depression, quality of life and handicap. Cyber disease and sense of presence during the VR sessions were also assessed.
92 patients participated in the study. There was no significant difference in outcome between the two treatment groups, neither immediately after treatment nor at follow-up after 3, 6 and 12 months. As a consequence, VRET may be considered a viable treatment option for Panic disorder with Agoraphobia. It would be interesting to compare the efficacy of a treatment combining traditional CBT with VRET.
The main article arising from this study is being completed by Pr A. Pelissolo at the CHU Pitié Salpetrière, Paris.

OBESITY

The Late has evaluated 234 patients who were candidates for gastric by-pass surgery.
Psychiatric assessment included psychiatric examination as well as a comprehensive structured interview developed by the main investigator (C. Pull) to assess more than 100 variables including variables related to personal history and family history of obesity, risk factors for obesity, eating customs, the presence of binges and binge eating disorder (BED), efforts made to lose weight, attitudes toward bariatric surgery, and knowledge about bariatric surgery.
Psychological assessment is based upon a battery of questionnaires and rating scales including the Minnessota Multidimensional Personality Inventory (MMPI), the NEO-Personality Inventory, the screening version of the International Personality Disorders Inventory (IPDE), the Eating Disorder Inventory (EDI), the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI), the second version of the World Health Organization Disability Assessment Schedule (WHO-DAS-II) and the World Health Organization Quality of Life Assessment instrument (WHO-QuoL).
The results of the investigation obtained up to now show that 50% of the patients with morbid obesity present with moderate or severe depression and that 32% present with severe or moderate anxiety. Patients suffering a moderate or severe depression had a significantly bigger handicap and a significantly lower quality of life than patients without or with only mild depression or anxiety. Patients with a BED had more psychopathology on the whole. In particular, they were more depressed and felt more handicapped than patients without BED.

COLLABORATIONS

The LATE has two main partners.
The LATE is working in close collaboration with the Centre d'Etudes en Santé or CES (Director: Mrs Marie-Lise Lair) at the CPT-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 Psychiatric Day Hospital of the Centre Hospitalier de Luxembourg. A major part of the assesment in the VRET project as well as in the obesity project is done by psychometricians (Mrs L.Seven, Mrs C. Arendt and Mr S. Bachim) of the day hospital. All treatments included in the projects are done by the psychotherapists of the day hospital (Mrs MC Pull, F Münster, L. Wouters, P Pereira and F Pezzan.
The LATE also works in close relationship with the statistical department of the CRP-Santé (Mr M Vaillant and his team).

PUBLICATIONS 2008

- Pull C. ***New trends in the study of specific phobias.*** Current opinion in Psychiatry, 21(1):43-50, 2008.
- Pull C., Damsa, C. ***Pharmacotherapy of panic disorder.*** Neuropsychiatric Disorders and Treatment, 4(4):779-95, 2008.
- Värnik A, Kõlves K, van der Feltz-Cornelis CM, Marusic A, Oskarsson H, Palmer A, Reisch T, Scheerder G, Arensman E, Aromaa E, Giupponi G, Gusmäo R, Maxwell M, Pull C, Szekely A, Sola VP, Hegerl U. ***Suicide methods in Europe: a gender-specific analysis of countries participating in the "European Alliance Against Depression".*** Journal of Epidemiology and Community Health, 62(6):545-51, 2008.

- Hegerl U, Wittmann M, Arensman E, Van Audenhove C, Bouleau JH, Van Der Feltz-Cornelis C, Gusmao R, Kopp M, Löhr C, Maxwell M, Meise U, Mirjanic M, skarsson H, Sola VP, Pull C, Pycha R, Ricka R, Tuulari J, Värnik A, Peiffer-Gerschel T. ***The 'European Alliance Against Depression (EAAD)': a multifaceted, community-based action programme against depression and suicidality.*** World Journal of Biological Psychiatry, 9(1):51-8, 2008.
- d'Albis MA, Pull C. ***Day care hospital in psychiatry: diversity or specificity?.*** Bulletin de la Société des Sciences Médicales, (2):197-208, 2008.
- Värnik A, Kõlves K, Allik J, Arensman E, Aromaa E, van Audenhove C, Bouleau JH, Van der Feltz-Cornelis CM, Giupponi G, Gusmão R, Kopp M, Marusic A, Maxwell M, Oskarsson H, Palmer A, Pull C, Realo A, Reisch T, Schmidtke A, Sola VP, ittenburg L, Hegerl U. ***Gender issues in suicide rates, trends and methods among youths aged 15-24 in 15 European countries.*** Journal of Affective Disorders, 113(3):216-26, 2009
- ***Current status of acceptance and commitment therapy (ACT).*** Current opinion in Psychiatry, 22(1):55-60.

TECHNICAL AND ADMINISTRATIVE DEPARTMENT

CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

HEAD OF UNIT:
Daniel CARDAO, Administrative and Financial Manager
Fabienne OLINGER, Executive Assistant

The Administrative and Technical Services are composed of different departments, reporting to the Head of department:

FINANCIAL UNIT:
Joseph GAUTOT, Responsible
Jeremy KLEIN, Accountant
Pierre FOUSSE, Accountant
Patrice ROESER, Secretary

HUMAN RESOURCES UNIT:
Natacha BEICHT, Responsible
Mathieu EUSTACHE, Collaborator

TECHNICAL SUPPORT UNIT:
Laura MARTINS, Responsible

INFORMATION AND TECHNOLOGY UNIT:
Patrick SCHILD, Responsible

PURCHASING UNIT:
Alphonse CONRARDY, Responsible

SECRETARIAL UNIT:
Michelle THOMMES
Nancy PELLIZARY
Sandra MARCON
Teresa MARTINS

Reporting to the Board of Management:

EH&S UNIT:
Elodie FONTAINE, Responsible

PROJECT MANAGEMENT UNIT:
Jo SCHROEDER, Responsible

COMMUNICATION UNIT:
Aurélia DERISCHEBOURG, Responsible

LEGAL UNIT:
Guillaume BYK, Responsible

TECHNICAL AND ADMINISTRATIVE DEPARTMENT

CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

Year 2008 was very challenging for the technical and administrative department due to the growth in terms of hiring specialized people as well as also giving support to the different operational units. A lot of effort has been made to optimize administrative procedures, setting up reporting sheets in terms of human resources figures, financial figures, etc.
The main targets were as follows:

- ensure the administrative and technical support necessary for a good development of CRP-Santé structures,
- set up procedures with an excellent knowledge of the operational units,
- provide transparency of the different reporting tasks.

The technical and administrative department has been reinforced in the different units:

- a new HR collaborator, Mr. Mathieu Eustache, has been hired,
- a new technician, Mr. Mike Gehlhausen, joined the Technical Support Unit,
- accounting has been reinforced by one accountant, Mrs. Liliana Gomes,
- the secretarial department has been reinforced by a secretary-coordinator, Mrs. Michelle Thommes

The highlight of the year was based on the revival of the new CRP-Santé building. The new CRP-Santé facility project was stopped in 2005 for financial reasons. In the meantime, a work group with the Ministry of Public Constructions, architects and engineers started in 2008 to finalize the project. We expect to start the construction in 2010 by the latest.

HUMAN RESOURCES UNIT

The Human Resources unit is headed by Natacha Beicht. During 2008, a new assistant joined the department in order to increase quality support to the operational units.
Year 2008 was focused on two main topics:

- hiring specialized researchers for the operational units,
- setting up reporting sheets giving information on recruitment and selection processes, personnel transactions and evolution, administrative activities and training and development.

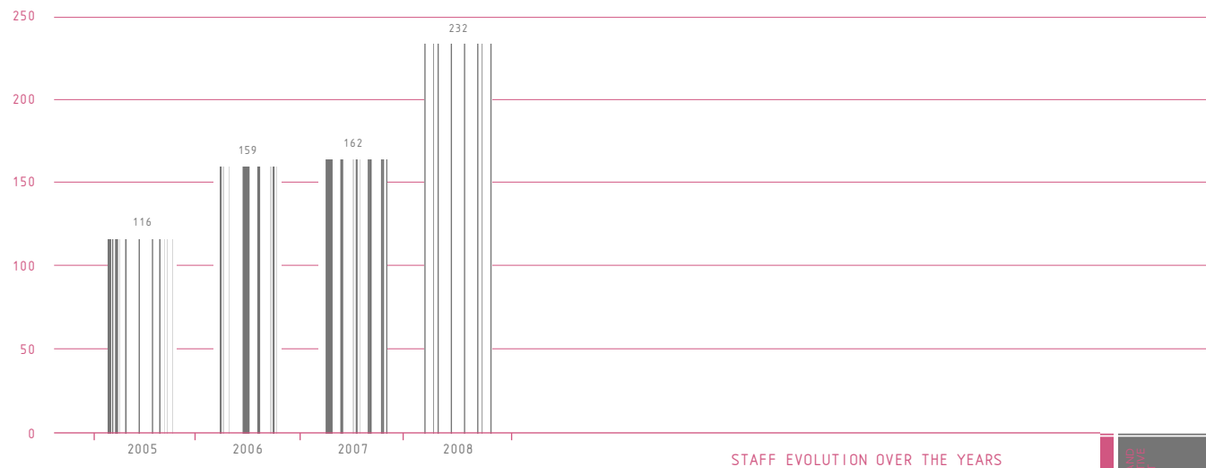
RECRUITMENT

In 2008, the HR department contributed and participated in 63 recruitments, more specifically on:

- Definition of competences (job description)
- Publications on internal and external support
- Pre selection for the research units
- Face-to-face interview and selection practices

We also noticed a significant increase in spontaneous applications; so we had to create a new database for the applications in order to manage and keep the best applicants on file. This data is dedicated to all head of research units and is constantly updated.
The recruitment process is based on the Code of Conduct for the Recruitment of Researches that was signed by the CRP-Santé in 2007. We have to add that the CRP – Santé was the first institution in Luxembourg to conform to the European Charter of Researchers.

The evolution of the headcount in 2008 was as follows:



STAFF EVOLUTION OVER THE YEARS

APPLICATION OF THE EUROPEAN CHARTER FOR RESEARCHERS

The Human Resources objective at the CRP-Santé is to set up the implementation of the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers. To achieve this target, the Human Resources Manager attends training sessions of the C&C in Brussels in order to contribute to raising awareness on the C&C within CRP-Santé.

INVENTORY OF OUR GOODWILL

CRP – Santé identified that goodwill is based on internal competences, which means our personnel "is" our permanent asset. To identify our competences, we start to review all our job descriptions and we stated that we have roughly 50 different positions in our research units which can be divided into 5 levels, such as:

I. Post Doctoral Researcher
II. Staff Researcher
III. Senior Researcher
IV. Researcher Deputy – Head of Unit
V. Researcher Head of Unit

All these categories are linked to a career path based on the new salary regulation set up at the end of 2008. This new regulation describes the different careers at CRP-Santé.

TRAINING AND DEVELOPMENT

A huge effort has been made in 2008 concerning training and development, particularly in the individual development of our employees:

I. Technical training:
   - In 2008, we had 5 Luxembourgish language groups and we also organized several individual language courses in English and German.
   - We also organized IT trainings for final users such as Word, Excel, Notes, Power Point, etc

II. Individual training:
   In 2008, we already started management training for our Heads of Units. The second step will be to fix individual development plans for all participants.

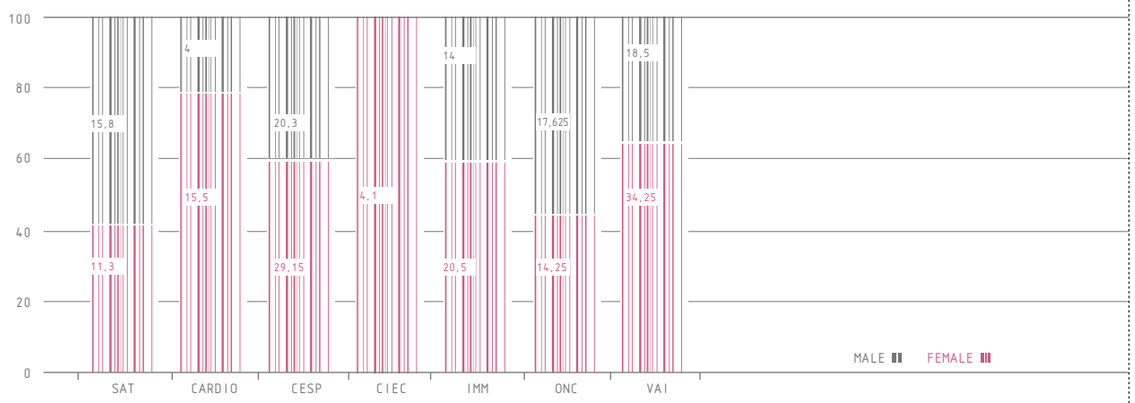
SOME KEY FIGURES

At CRP-Santé we have 22 nationalities:

FOR MORE INFORMATION

If you would like more information or clarification regarding this report, please contact us.

CRP-SANTÉ'S PERSONNEL IN CATEGORIES



HEADCOUNT BY GENDER
MALE/FEMALE REPARTION - END OF 2008 (FTE)

FINANCIAL UNIT

This group, headed by Joseph Gautot, is responsible for the financial aspects of research projects and day-to-day business concerning financial matters at CRP – Santé. This includes the preparation of all financial data and also the setting up of annual accounts, etc.
Milestones 2008

- Implementation of online financial statements allowing heads of laboratories to access the accounting data and consult in real-time the financial situation of the projects
- Implementation, as part of the CRP-Santé's ERP, of the SCM (Supply Chain Management) module allowing increased synergies between the procurement process and the accounting, as well as more accurate information about a project's real expenses at a certain time
- The CRP-Santé has taken an active role, together with KPMG and at the initiative of the Ministry of Research, in the definition of a "full costs" model which will be applied to all research projects in 2009. This topic is one of the key points of the performance contract signed with the Ministry of Research for the period 2008-2010
- The global budget for the year 2008 has reached an amount of 21, 7 MEUR, representing an increase of 35 % vs 2007.

Actions for 2009

- Set up a web-based, online reporting tool for financial figures, as well as operational figures
- Review all administrative procedures and update if necessary
- Set up main dashboards reporting for financial ratios on a quarterly basis

IT UNIT

The Information Technology Unit was headed by Olivier Keunen in 2008 and, due to a professional reorientation, Olivier Keunen decided to integrate a research unit. The CRP – Santé approved this mobility and hired a new IT Manager named Patrick Schild. A transition phase of 3 months has been set up.
The main projects in 2008 have been:

- A SAN server has been installed in order to increase the storage capacity and to allow increased efficiency
- The VMWARE node was updated in order to achieve maximum reliability and availability
- The new fiber line has been installed to improve the redundancy and availability of the network between the two sites, Edison and BAM

The IT department gave assistance to the new department CIEC in installing specific software dedicated to clinical trials.
Huge efforts were made on the development of applications which are used by a lot of research units, such as newcomers' applications, antibodies, pates, etc.
Concerning the Helpdesk, a lot of support was given to the end users, with 1 789 tickets being treated in 2008. Moreover, the IT department created more than 100 users in our system (students, external, trainees, etc).
Finally, the IT department assisted IBBL in setting up their infrastructure in collaboration with the CRP Henri Tudor.

TECHNICAL SUPPORT UNIT

The Technical Support Unit, headed by Laura Martins, is composed of 2 technicians and this unit is responsible for technical issues in all buildings used by CRP – Santé.
The missions of this department are to:

- Maintain a physical environment conducive to the achievement of the CRP-Santé mission.
- Provide a professional project management service for new construction as well as for the renovation and refurbishing of existing buildings.
- Maintain and improve the functional and visual quality of indoor and outdoor spaces.

- Maintain and improve the performance of all building systems (lighting and heating, etc.).
- Carry out a preventive maintenance program to improve performance and extend the life of building equipment, furniture and interior finishes.
- Be responsible for day-to-day operations, including care of maintenance and trash collection.
- Carry out a preventive maintenance program for laboratory equipment to ensure instrument uptime, better results and increase productivity.
- Prepare and manage construction and operating budgets, which respect the overall financial plans and priorities of the service and the CRP-Santé.

PURCHASING UNIT

In 2008, the Purchasing and New Buildings Department, headed by Alphonse Conrardy, realized:

- the furnishing of the new lab-offices in the new BAM 2 building
- the refurnishing of most lab-offices in the BAM 1 building
- the interior arrangement and furnishing of the new office surfaces for the CIEC
- the furnishing for the temporary installation of the IBBL in the Edison building

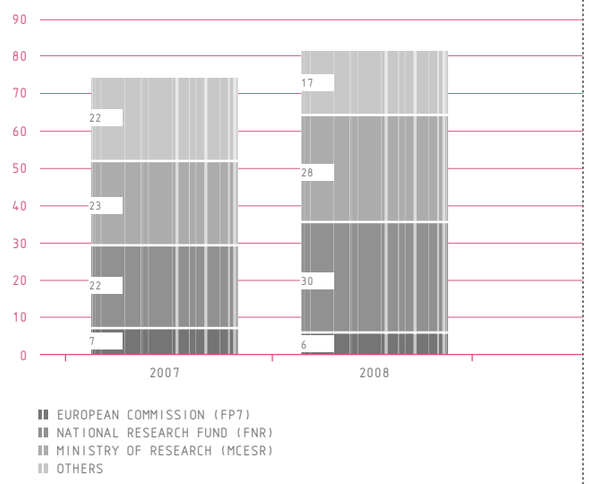
Storage space has been installed in the Edison building archives for Administration, CES and CNER. The redevelopment of the final building for the CRP-Santé has been started in collaboration with the "Administration des Bâtiments Publics", the architects and external consultants.

The new Supply Chain Management software has been adapted to the needs of CRP-Santé and was implemented on 1.1.2009. Most articles purchased during 2007 and 2008 have been entered into the database and referenced with the respective suppliers. Procedures for the supply chain and for the public tenders have been defined and implemented. Seven national and one European public tenders have been launched, and three negotiated markets were realized in 2008.

These functions are to report to the Board of Management:

PROJECT MANAGEMENT UNIT

Evolution of the projects:



SOME KEY INFORMATION

In 2008, 24 new projects were started, whereas 17 were finished. The research units of CRP-Santé submitted 8 project proposals within FNR – CORE – CALL, of which 4 were accepted for funding by the National Research Fund.
Concerning the projects financed by MCESR, 8 new projects were submitted, of which 3 were accepted after having successfully passed the external peer review process. 3 projects are still in the evaluation process and 2 projects are on stand-by for finalization.
For the FP7 programm, CRP – Santé, together with its Chinese and Norwegian partners, succeeded in obtaining a grant in the framework of FP7 – Marie Curie Actions – International Research Staff Exchange. This project will start in 2009.

COMMUNICATION UNIT

CRP-Santé created the Communication Unit in 2008. Communication is considered a strong and focused strategy orientation for the future. The activity of the Communication Unit includes:

- media relations,
- inter-institutional communication,
- publications (press releases, news, flyers, posters, etc.),
- promotion of the nature of the work, achievement and performance of the institution.

In order to support scientific research and to improve the image of the institution, the Communication Unit organized several workshops, events and seminars in 2008, in strong collaboration with different research units of the CRP-santé and other important institutions in Luxembourg.
In 2008, the unit organized the following workshops:
20th Anniversary Seminars of the CRP-Santé.
The main objectives are as follows:

- promote the CRP-Santé and its activities to the general public, in Luxembourg, Ettelbruck and Esch-sur-Alzette
- tackle important health issues by informing people about diseases: cancer, hepatitis, phobia, allergies, obesity, mental health, etc.

Other siminars:

- Organization and promotion of the "Brain Diseases: from bench to bedside" Workshop realized by Norlux Neuro-Oncology Laboratory of the CRP-Santé
- Organization and coordination of the "Cardiovascular Biomarkers" Workshop, realized by the Laboratory of Cardiovascular research, in relationship with Luxinnovation
- Promotion and organization of the "New Drug Discovery from Traditional Chinese Medicine" workshop realized by the Laboratory of Plant Molecular Biology of the CRP-Santé
- Promotion of LuciLinX Bioinformatics Symposium in relationship with Luxinnovation, and creation of the visual identity network
- Participation in the "Business Meets Research 2008" organized by Luxinnovation: presentation of three research units: Department of Immunology, Laboratory of Plant Molecular Biology and Microarray Center.

The Communication Unit took part actively in improving the institution's image to the scientific community and to the large public by the development of a new website and a new visual identity (logo, message, colour code). The unit has also created the Clinical and Epidemiological Investigation Center newsletter.
The service also supported such launching projects as "Eurolight: highlighting the impact of headache", by organizing press conferences.
It built up its efforts regarding the internal communication strategy by promoting the CRP-Santé as a center of quality and expertise.
In 2008, together, with the group for the promotion of scientific culture "Proscience", the Communication Unit of the CRP-Santé contributed to:

- the Researchers' Night organized by the Fonds National de la Recherche: organization of a scientific workshop about Cells in strong relationship with different research units of the CRP-Santé in order to promote the career of the researcher - Organization of a Science Coffee during the same event.
- The Foire de l'Etudiant: in order to inform students on the research and all the potential opportunities of Sciences
- Meets@uni 2008, organized by the University of Luxembourg, to present the activity of the Public Research Center for Health to the students coming from several national and international Universities.

EH&S UNIT

CRP-Santé actively participated in the safety training

TRAINING	PARTNERSHIP	NR. OF TRAINING SESSIONS	NR. OF PARTICIPANTS	%
EH&S training for newcomers	n.a.	5	17	48
EH&S training for subcontractors	n.a.	1	7	n.a.
First-aid course	Red Cross Luxembourg	1	13	15
First-aid refresh course	Red Cross Luxembourg	1	14	15
Fire-extinguishing course	CHL	2	20	20
Annual fire-alarm training	CHL	2	n.a.	n.a.

Based on its training program for employees and collaborators, the EH&S policy at CRP-Santé was able to reduce significantly the number of accidents related to laboratory activities. In 2008, only one minor accident was reported. In 2009, the EH&S training program will be extended to employees working in the administrative departments.

LEGAL UNIT:

The legal unit focused on different topics in 2008.

PUBLIC TENDERS:

The legal department was involved in the restructuration of the public procurement process within CRP-Santé. The new process was approved by the board of administration.

TYPE OF TENDERS	2008
European public tenders	1
National public tenders	7
National tenders	4
Derogation requests	8

AGREEMENTS AND INTELLECTUAL PROPERTY:

The legal department reviewed 17 partnership agreements and 11 material transfer agreements in 2008. In 2008, one provisional patent, 3 international patents (PCT) and one European patent were filed. With a planned cooperation agreement with a venture capital company, it is expected that CRP-Santé will proceed in 2009 with an improved assessment system to evaluate the commercial opportunities from its emerging patent portfolio.

INSURANCE TENDER:

In 2008, the legal department proceeded with a negotiated tender process in order to manage all its insurance policies with a single insurer. It also took the opportunity to add specific insurance policies for its board of administration and the activity of the clinical and epidemiological investigation center. Furthermore, 5 specific promoter insurance policies were subscribed for research projects involving human patients.

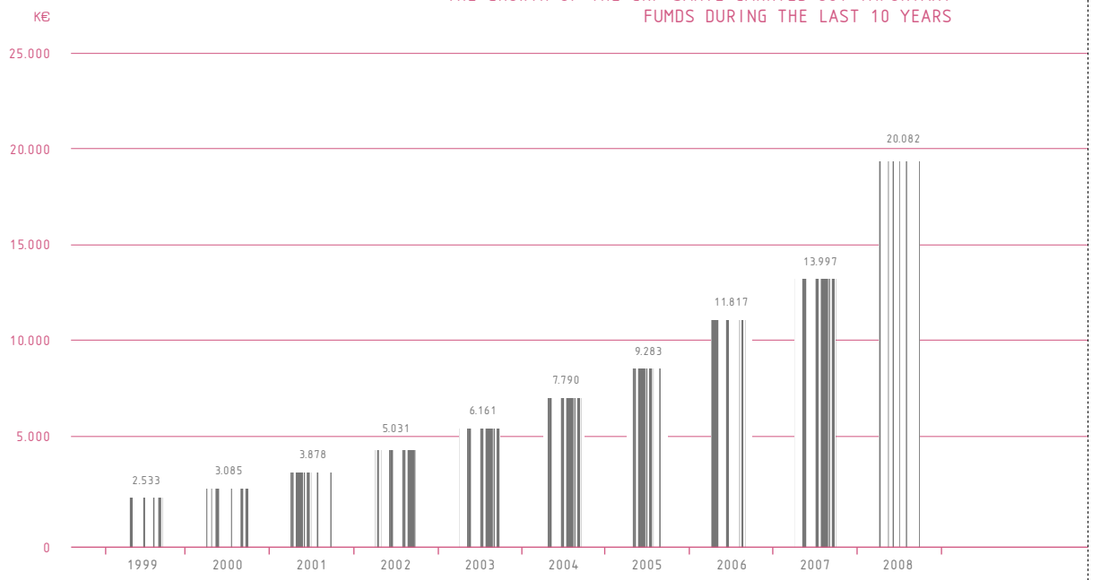
PRIVACY AND DATA PROTECTION:

The CRP-Santé filed 3 notifications and 2 authorizations (for a research project involving a genetic element) to the Luxembourg Data Protection Commission. The consent form and information letters for 3 research projects were reviewed in terms of ethical and data-protection requirement.

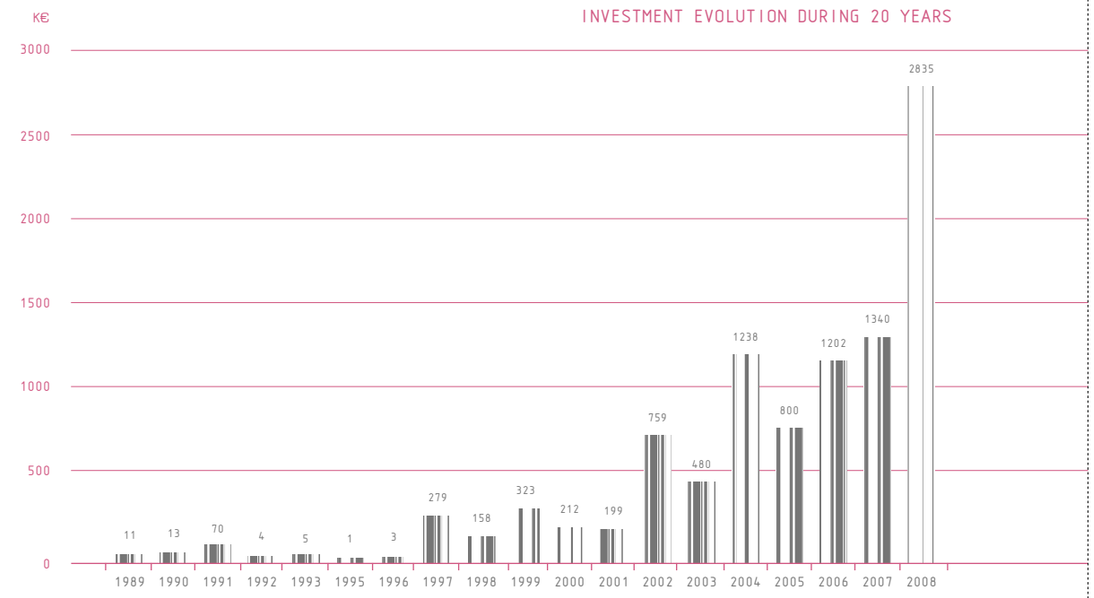
KEY FIGURES

CRP-SANTÉ
CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

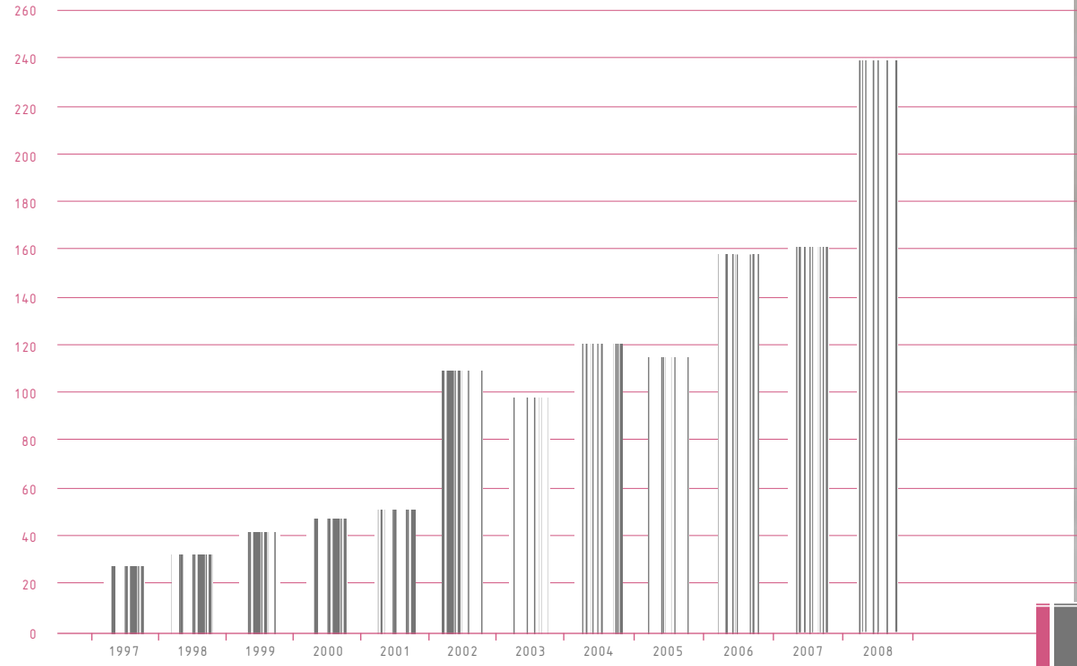
THE GROWTH OF THE CRP-SANTÉ CARRIED OUT IMPORTANT FUMDS DURING THE LAST 10 YEARS



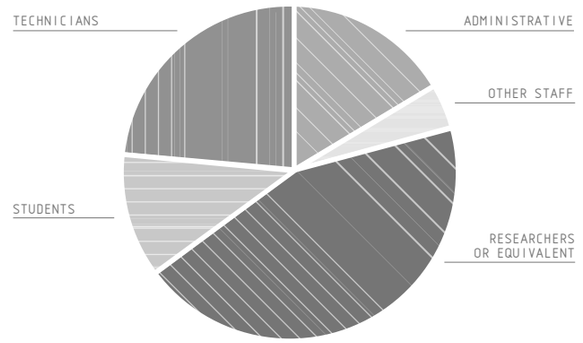
INVESTMENT EVOLUTION DURING 20 YEARS



CONCERNING THE PERSONNEL, THE HEADCOUNT INCREASED ACCORDINGLY TO THE GROWTH OF THE FUNDS



CRP-SANTÉ'S PERSONNEL CAN BE SPLITTED INTO THE FOLLOWING CATEGORIES



THE CRP-SANTÉ IS VERY DIVERSIFIED
IN TERMS OF NATIONALITIES (%)

Albania	0,4
Germany	7,0
USA	0,4
Belgium	18,4
Belarus	0,8
United Kingdom	0,4
China	0,8
Congo	0,4
France	36,1
Iran	0,4
Italy	0,8
Luxembourg	27,4
Malaysia	0,4
Netherlands	0,8
Norway	0,4
Poland	0,4
Portugal	1,7
Romania	0,4
Russia	0,4
Slovenia	0,4
Sweden	0,4
Tunisia	0,4
Venezuela	0,4

Grant Thornton
Grant Thornton Lux Audit S.A.

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

Au Conseil d'Administration du
CRP SANTE
1A, rue Thomas Edison
L-1445 STRASSEN

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 2008 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

Grant Thornton
Grant Thornton Lux Audit S.A.

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 annuels.

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 2008 ainsi que des résultats de l'exercice se terminant à cette date.

Luxembourg, le 30 mars 2009

Thierry REMACLE
Réviseur d'Entreprises
Grant Thornton Lux Audit S.A.

Experts-Comptables
Réviseurs d'entreprises
Luxembourg Member firm of Grant Thornton International Ltd Luxembourg Member

BILAN AU 31 DÉCEMBRE 2008

ACTIF (EM EUROS)

ACTIF (EN EUROS)	**31.12.2008**	**31.12.2007**
A. CAPITAL SOUSCRIT NON VERSE		
B. FRAIS D'ETABLISSEMENT		
C. ACTIF IMMOBILISE		
I. Immobilisations incorporelles	100.844,26	18.829,66
2. Concessions, brevets, licences, marques, ainsi que droits et valeurs similaires		
a) acquis à titre onéreux	100.844,26	18.829,66
b) créés par l'entreprise		
II. Immobilisations corporelles	1.350.300,08	397.867,92
2. Installations techniques et machines	0,00	24,80
3. Autres installations, outillage et mobilier	1.350.300,08	317.426,80
4. Acomptes versés et immobilisations corporelles en cours	0,00	80.416,32
III. Immobilisations financières	1.012.394,68	12.394,68
3. Participations	1.012.394,68	12.394,68
TOTAL DE L'ACTIF IMMOBILISE	**2.463.539,02**	**429.092,26**
D. ACTIF CIRCULANT		
I. Stocks	0,00	0,00
II. Créances		
1. Créances résultant de ventes et prestations de services	119.003,54	288.824,01
a) dont la durée résiduelle est inférieure ou égale à un an	119.003,54	288.824,01
b) dont la durée résiduelle est supérieure à un an		
2. Créances sur partenaires	1.800.644,56	1.289.528,89
3. Créances sur des entreprises avec lesquelles la société a un lien de participation	10.086,16	0,00
4. Autres créances	930.210,27	417.006,60
a) dont la durée résiduelle est inférieure ou égale à un an	930.210,27	417.006,60
b) dont la durée résiduelle est supérieure à un an		
III.Valeurs mobilières	0,00	0,00
IV. Avoirs en banque, avoirs en compte de chèques postaux, chèques et en caisse	5.090.050,02	5.644.880,96
TOTAL DE L'ACTIF CIRCULANT	**7.949.994,55**	**7.640.240,46**
E. COMPTES DE REGULARISATION	295.011,60	66.051,29
TOTAL DE L'ACTIF	**10.708.545,17**	**8.135.384,01**

BILAN AU 31 DÉCEMBRE 2008

PASSIF (EM EUROS)

PASSIF (EN EUROS)	**31.12.2008**	**31.12.2007**
A. CAPITAUX PROPRES		
I. Capital souscrit	99.157,41	99.157,41
IV. Réserves	0,00	0,00
V. Résultats reportés	878.058,80	235.096,82
VI. Résultats de l'exercice	-257.910,28	642.961,98
VII. Subventions d'investissement	2.451.144,34	336.281,05
TOTAL DES CAPITAUX PROPRES	3.170.450,27	1.313.497,26
B. PROVISIONS POUR RISQUES ET CHARGES		
3. Autres provisions	594.692,00	389.549,55
TOTAL DES PROVISIONS	**594.692,00**	**389.549,55**
C. DETTES		
1. Emprunts obligataires	0,00	0,00
2. Dettes envers des établissements de crédit	0,00	0,00
3. Acomptes reçus sur commandes	0,00	0,00
4. Dettes sur achats et prestations de services	2.197.839,68	1.198.912,57
a) dont la durée résiduelle est inférieure ou égale à un an	2.197.839,68	1.198.912,57
b) dont la durée résiduelle est supérieure à un an		
5. Dettes représentées par des effets de commerce	0,00	0,00
6. Dettes envers des entreprises liées	0,00	0,00
7. Dettes envers des entreprises avec lesquelles la société à un lien de participation	0,00	0,00
8. Dettes fiscales et dettes au titre de la sécurité sociale	657.923,66	464.498,87
a) Dettes fiscales	281.773,46	183.244,76
b) Dettes au titre de la sécurité sociale	376.150,20	281.254,11
9. Autres dettes	159.671,59	257.253,25
a) dont la durée résiduelle est inférieure ou égale à un an	143.051,59	240.633,25
b) dont la durée résiduelle est supérieure à un an	16.620,00	16.620,00
TOTAL DES DETTES	**3.015.434,93**	**1.920.664,69**
D. COMPTES DE REGULARISATION	3.927.967,97	4.511.672,51
TOTAL DU PASSIF	**10.708.545,17**	**8.135.384,01**

COMPTE DE PROFITS ET PERTES

POUR L'EXERCICE ALLANT DU 1 JANVIER AU 31 DÉCEMBRE 2008

	2008 (01.01.08-31.12.08)	2007 (01.01.07-31.12.07)
A. CHARGES		
1. Réduction du stock de produits finis et en cours de fabrication	7.116.874,58	4.647.599,78
2. a) Consommation de marchandises et de matières premières et consommables	4.097.607,07	2.725.940,11
b) Autres charges externes	3.019.267,51	1.921.659,67
3. Frais de personnel	11.003.970,90	8.031.687,27
a) Salaires et traitements	9.867.024,86	7.213.770,70
b) Charges sociales couvrant les salaires et traitements	1.136.946,04	817.916,57
4. a) Corrections de valeur sur frais d'établissement et sur immobilisations corporelles et incorporelles	190.301,71	77.365,76
b) Corrections de valeur sur éléments de l'actif circulant	0,00	-35.080,53
5. Autres charges d'exploitation	1.188.536,78	598.836,18
6. 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	4.620,50	2.846,33
10. Charges exceptionnelles	577.414,75	31.171,25
13. Résultat de l'exercice (bénéfice de l'exercice)	0,00	642.961,98
TOTAL DES CHARGES	20.081.719,22	13.997.388,02
B. PRODUITS		
1. Montant net du chiffre d'affaires	107.970,84	191.176,39
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	19.247.171,98	13.212.766,97
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	257.241,03	143.445,00
9. Produits exceptionnels	211.425,09	449.999,66
10. Résultat de l'exercice (perte de l'exercice)	257.910,28	0,00
TOTAL DES PRODUITS	20.081.719,22	13.997.388,02

ADDRESSES

CRP-SANTÉ
CENTRE DE RECHERCHE PUBLIC DE LA SANTÉ

ADMINISTRATIVE AND FINANCIAL DEPARTMENT

Head of the Administrative and Financial Department: **Mr. Daniel Cardao**

1A-B, rue Thomas Edison, L-1445 Strassen
T (+352) 26 970-730 | **F** (+352) 26 970 719
fabienne.olinger@crp-sante.lu

DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

Laboratory of Retrovirology
Head of Research Unit: **Dr Jean-Claude Schmit**

CRP-Santé, BAM
84, Val Fleuri, L-1526 Luxembourg
T (+352) 26 970 224 | **F** (+352) 26 970 221
e-mail: jc.schmit@crp-sante.lu

Laboratory of Immunogenetics and Allergology
Head of Research Unit: **Dr François Hentges**

CRP-Santé, BAM
84, Val Fleuri, L-1526 Luxembourg
T (+352) 26 970 250 | **F** (+352) 26 970 390
e-mail: hentges.francois@chl.lu

Laboratory of Plant Molecular Biology
Head of Research Unit: **Dr André Steinmetz**

CRP-Santé, BAM
84, Val Fleuri, L-1526 Luxembourg
T (+352) 26 970 251 | **F** (+352) 26 970 390
e-mail: andre.steinmetz@crp-sante.lu

Laboratory of Toxicology
Head of Research Unit: **Prof. Robert Wennig**

Université de Luxembourg
Bâtiment des Sciences, 162A,
avenue de la Faiencerie
T (+352) 44 66 44 476 | **F** (+352) 22 13 31
e-mail: robert.wennig@uni.lu

DEPARTMENT OF IMMUNOLOGY

Immunology Department
Head of Research Unit: **Prof. Dr. Claude P. Muller**

20A, rue Auguste Lumière, L-1950 Luxembourg
T (+352) 49 06 04 220 | **F** (+352) 49 06 86
e-mail: claude.muller@LNS.ETAT.LU

CLINICAL AND EPIDEMIOLOGICAL INVESTIGATION CENTER (CIEC)

Head of Research Unit: **Dr Catalina Iliescu**

1A rue Thomas Edison, L-1445 Strassen
T (+352) 26 970 800 | **F** (+352) 26 970 810
e-mail: catalina.iliescu@crp-sante.lu

DEPARTMENT OF ONCOLOGY

Laboratory of Experimental Hemato-Oncology
Head of Research Unit: **Dr Guy Berchem**

CRP-Santé, BAM
84, Val Fleuri, L-1526 Luxembourg
T (+352) 44 11 20 84 and (+352) 26 970 320
F (+352) 44 12 15
e-mail: berchem.guy@chl.lu

Norlux Neuro-Oncology Laboratory
Head of Research Unit: **Dr Simone Niclou**

84, Val Fleuri, L-1526 Luxembourg
T (+352) 26 970 273 | **F** (+352) 26 970 390
e-mail: simone.niclou@crp-sante.lu

Laboratory of Molecular Pathology
Head of Research Unit: **Dr Jos Even**

B.P. 1102, L-1011 Luxembourg
T (+352) 49 11 91 322 | **F** (+352) 40 42 38
e-mail: jos.even@LNS.ETAT.LU

Microarray Center
Head of Research Unit: **Dr Laurent Vallar**

CRP-Santé-BAM
84, Val Fleuri, L-1526 Luxembourg
T (+352) 26 970 281 | **F** (+352) 26 970 390
e-mail: laurent.vallar@crp-sante.lu

DEPARTMENT OF CARDIOVASCULAR DISEASES

Laboratory of Cardiovascular Research
Head of Research Unit: **Dr Daniel Wagner**

Centre Hospitalier de Luxembourg
Maternité Grande-Duchesse Charlotte
4, rue Barblé, L-1210 Luxembourg
T (+352) 26 970 303 | **F** (+352) 26 970 396
e-mail: wagner.daniel@chl.lu

DEPARTMENT OF PUBLIC HEALTH

Centre for Health Studies
Head of Research Unit: **Mrs. Marie-Lise Lair**

CRP-Santé | 1B, rue Thomas Edison
T (+352) 26 970 752 and (+352) 26 970 750
F (+352) 26 970 717
e-mail: marie-lise.lair@crp-sante.lu

Laboratory of Emotional Disorders
Head of Research Unit: **Dr. Charles Pull**

Centre Hospitalier de Luxembourg
4, rue Barblé, L-1210 Luxembourg
T (+352) 44 11 27 06 and (+352) 44 11 66 66
F (+352) 44 12 47
e-mail: pull.charles@chl.lu

LIST OF PARTNERS

PARTICIPATING IN THE FINANCING OF THE ACTIVITIES OF CRP-SANTÉ

Advanced Biological Laboratories
Agence Européenne d'Evaluation des Médicaments
Caisse nationale de Santé
Centre Hospitalier de Luxembourg
Centre Hospitalier Emile Mayrisch
Clinique Sainte Thérèse
European Commission
Département Ministériel des Sports
E-Luxembourg
Fondation Hellef fir Kriebskrank Kanner
Fondation Luxembourgeoise contre le Cancer
Fondation Recherche Cancer et Maladies du Sang
Fondation Recherche sur le SIDA
Fonds National de la Recherche
Inspection générale de la Sécurité Sociale
Laboratoire National de Santé
Laboratoires d'industries pharmaceutiques
Ministère de l'Economie
Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche
Ministère de la Santé
Ministère de la Sécurité Sociale
Ministère des Affaires Etrangères
Observatoire Européen des Drogues et Toxicomanies
Organisation Mondiale de la Santé
Pharmaccess Foundation
Private Donations
Public Health Executive Agency

PHOTOS **Miikka Heinonen,** Luxembourg (www.miikkaheinonen.com)
LAYOUT **lola,** Luxembourg (www.lola.lu)
PAPER **cover,** Phoenix Motion Xénon white 250g/m²
text, Phoenix Motion Xénon white 150g/m²
CRP-SANTÉ 1 a-b, rue Thomas Edison L-1445 Strassen, Luxembourg