

## ACTIVITY REPORT



2007

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# THE BOARD OF ADMINISTRATION

## MEMBERS OF THE BOARD OF ADMINISTRATION

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Mr. René ZIMMER



## THE CHAIRMAN'S MESSAGE

Dear Reader,

The annual report of the research activities at the research centre for health CRP-Santé of the Grand Duchy of Luxembourg for 2007 is now available.

I would like to thank all members of staff and other employees in the CRP for their input into our research during 2007. I would also like to thank all the organizations and companies who have contributed to our research, either by collaboration in the research program itself or through the provision of funding or other kind of partnership. I look forward to further

fruitful relationships during the coming years.

We are thus very delighted to present in this annual report the activities carried out during 2007 by the staff of our research centre for health CRP-Santé.

The main challenge during 2007 was, after a period of important changes in the center's structure, to consolidate the research activities programs and to build or reinforce cooperation relations with potential partners.

The overall goal is to create a strong research center able to reach its targets in the future, which means, coordinating and organizing quality research in health with the clearly

identified goal to improve medical treatment and the public health sector. We are very confident that we are going to reach our objectives because over the last several years, our centre is responding at a scientific level to high international quality standards. That's why the CRP-Santé has succeeded in building confidence among its partners.

This year, it will be an honour for me to celebrate with all the members, friends and partners of the CRP-Santé its 20th anniversary. A review over 20 years of research activities carried out in the centre will underline its importance for a dynamic health sector.

At the same time, the centre will sign a three-year cooperation agreement with the government, which will continue to increase spending for research and innovation up to 1% of GNP. In the frame of this cooperation agreement, the CRP-Santé has clearly defined its research areas and priorities and officially confirmed its commitment to quality.

The signature of this agreement means for us and tremendously important and big step toward future because it will provide us the

necessary funding for growth and will insure the financial viability of our research programs. Any research centre needs growth in order to achieve critical mass in its selected fields of activities, this means growth in terms of research activities and consequently in terms of employment.

There is a new challenge on the horizon we will have to face. Making adequate infrastructure investments in order to be able to carry out all these above-mentioned projects and activities and to cover the scientists needs under the best possible conditions. I am very confident that together and with the support from our governments, we will reach this objective too.

Dear Reader, together with the members of the board of administration I am looking forward to deepening and widening the excellent collaboration with all the scientific and administrative collaborators of the research centre for health CRP-Santé of the Grand Duchy of Luxembourg.

Frank GANSEN

# II BOARD OF MANAGEMENT



## THE BOARD OF MANAGEMENT'S MESSAGE

The past year was again very busy for the Centre de Recherche Public de la Santé (CRP-Santé). Building on changes initiated in 2006, we were able to improve further our organisational performance and the quality of our research. In 2007, our researchers published 49 scientific articles in peer-reviewed journals and a large number of reports on public health issues. They actively contributed research results in multiple international conferences. Three applications for patents were filed and a couple of students successfully completed their PhD training during the past year. In order to confirm our strategic research orientations, a new independent evaluation of our activities by our external scientific advisory board is foreseen in 2008.

2007 was the year of the preparation and negotiation of our first performance contract with government for the years 2008 to 2010. It was a stimulating exercise which allowed

us to develop a clear vision and mission for CRP-Santé and translate these into goals and key performance indicators at a corporate level. However, we still need to deploy the corporate performance objectives downwards into a cascade of integrated departmental and individual goals. In a very natural way, this will be the first step for the introduction of a formal performance management system in our organisation. No doubt, working under a performance contract with government represents a paradigm change for the public research sector. CRP-Santé is looking forward to this new challenge, as it guarantees autonomy and development, and fosters innovation.

In order to achieve high level performance, our main asset is human resources. CRP-Santé is committed to invest significantly in its human capital over the next years. In 2007, we adhered – as a first organisation in Luxembourg – to the European Charter of Researchers, which defines the rights, but also the obligations of researchers. Favouring fair and equal treatment, we abolished the differences in salaries between permanent work contracts and those limited in time, allowing now people with limited contracts to have competitive salaries, too. In order to adapt to the Bolo-

gna process, we designed specific careers schemes for research engineers and other persons with a master diploma. And finally, we initiated a large consultation process with employees and their delegation to understand better their needs and aspirations. This process will result in further improvements in human resources management in 2008.

In 2007, the organisational integration of CRP-Santé progressed in major ways. First, collaboration contracts with the Centre Hospitalier de Luxembourg (CHL) were signed for the many laboratories – now being part of CRP-Santé – which were initially founded by physicians from CHL. A collaboration contract for the department of immunology was also concluded with the Laboratoire National de Santé (LNS). These contracts clarify the relationship between the three partner organisations, enhancing their collaborative potential, and allowing CRP-Santé to build a clear corporate identity.

The second major change, stimulated by our self-assessment in preparation for the performance contract and following a recommendation by our scientific advisory board in 2006, was to group our research units into five thematic research departments. These departments have now a critical mass in terms of human resources, enhance collabo-

rations between research units and researchers and help focusing our work on promising thematic subjects. First steps have also been taken to integrate more efficiently the technical and administrative support functions and the research activities. This effort will continue over the next years. In addition, our management structure was adapted in a way that it ensures clear leadership, but always based on a consensual decision-making process involving the entire board of management.

The year 2007 was also marked by moving to a new location. First, we were able to find new space for our administrative and public health departments nearby our provisional laboratory building. And then, as a consequence of our organisational growth, we initiated the extension of our research building providing additional laboratory space for our research groups in early 2008. Plans for a final CRP-Santé building have also been activated again.

In 2008, we shall proudly celebrate the 20th anniversary of our organisation, and at the same time we fully recognise that new stimulating challenges still lie ahead of CRP-Santé.

The Board of Management,

Daniel CARDAO

Marie-Lise LAIR

Jean-Claude SCHMIT

III SCIENTIFIC ADVISORY BOARD



Prof. Jules HOFFMANN, President

Distinguished Class Research Director at CNRS and Group Leader (since 1990), Member of the Board of Administrators of CNRS

President of the French National Academy of Sciences for 2007 / 2008

Director of the CNRS Research Unit 9022 "Immune Response and Development in Insects" (1978-2005)

Director of the Institute of Molecular and Cellular Biology, CNRS, Strasbourg (1993-2005)

SCIENTIFIC ADVISORY BOARD MEMBERS 2006 / 2007

Prof. Jean-François BACH  
"Secrétaire perpétuel" of the French National Academy of Sciences, *France*

Prof. Monique CAPRON  
Research Director - INSERM U167 at the "Institut Pasteur de Lille", *France*  
Member of the French National Academy of Sciences

Prof. Jean-Luc IMLER  
Professor of cell biology at the University Louis Pasteur, *Strasbourg, France*

Prof. Peter LICHTER  
Group Leader – Division of Molecular Genetics at the DKFZ, *Heidelberg, Germany*

Prof. Robert MACKEL  
Rockefeller University, *New York, USA*

Prof. Anders SOENNERBORG  
Department of Medicine at Huddinge University Hospital  
Karolinska Institute, *Stockholm, Sweden*

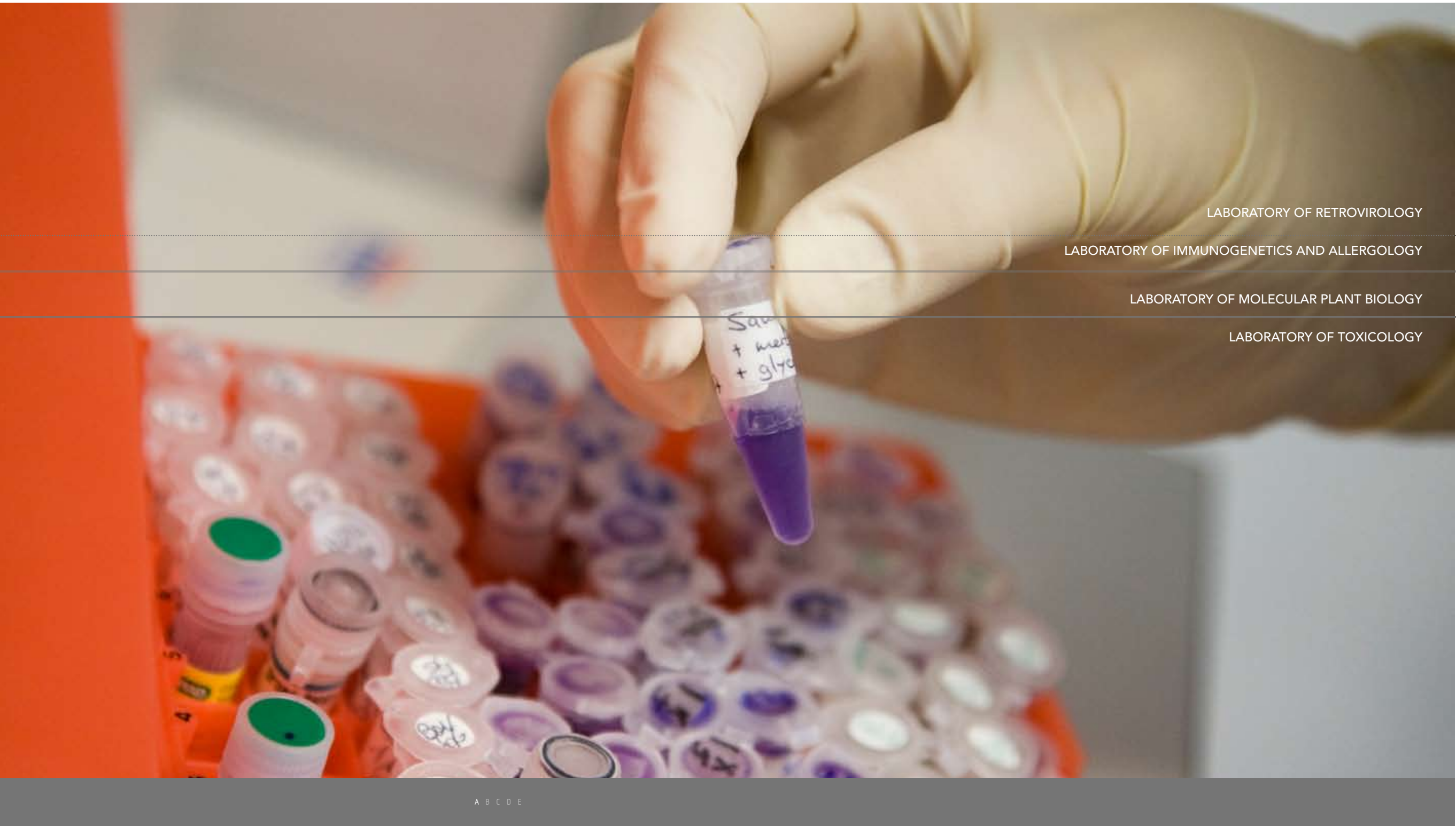
Prof. Alain-Jacques VALLERON  
Leader of the Public Health Unit at the Saint-Antoine Hospital  
Director of the Doctoral School "Santé Publique et Sciences de l'Information" at the "Université Paris 6"  
Member of the French National Academy of Sciences, *France*

Prof. Andreas M. ZEIHNER  
Director Cardiology Hospital, University Hospital, *Frankfurt/Main, Germany*

RESEARCH DEPARTMENTS

DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY

- DEPARTMENT OF IMMUNOLOGY
- DEPARTMENT OF ONCOLOGY
- DEPARTMENT OF CARDIOVASCULAR DISEASES
- DEPARTMENT OF PUBLIC HEALTH



LABORATORY OF RETROVIROLOGY

LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

LABORATORY OF MOLECULAR PLANT BIOLOGY

LABORATORY OF TOXICOLOGY

A



## / LABORATORY OF RETROVIROLOGY

Head of the laboratory:

Jean-Claude SCHMIT, MD, PhD



Presently, the staff of the laboratory is as follows:

Carole DEVAUX, PhD, Associate Head of the Laboratory, Researcher  
Sabrina DEROO, PhD, Head of the Research Group Immuno-Virology, Researcher  
François ROMAN, MD, PhD, Head of the Research Group Clinical Virology (till 2007), Researcher  
Danielle PEREZ-BERCOFF, PhD, Head of Research Group Clinical Virology (from 2008 on), Researcher  
Sylvie DELHALLE, PhD, Researcher in Immuno-Virology, Researcher  
Robert HEMMER, MD, Researcher in Clinical Virology, Researcher  
Vic ARENDT, MD, Collaboration with African countries, Researcher  
Jean-Yves SERVAIS, Technician  
Cécile MASQUELIER, Engineer  
Aurélie FISCHER, Technician  
Daniel STRUCK, Biologist  
Anne-Marie TERNES, Bioinformatician  
Jean-Marc PLESSERIA, Technician  
Nadia BEAUPAIN, Technician  
Manuel COUNSON, Technician  
Morgane LEMAIRE, Technician  
Julie MATHU, Technician  
Franky BAATZ, PhD Student  
Cyrille LEJCZAK, PhD Student  
Jean-Claude KARASI, PhD Student  
Hélène AGOSTINIS, Secretary

External collaborators (Fondation Recherche sur le SiDA) :

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



## HISTORY OF THE LABORATORY

The Retrovirology Laboratory was founded in 1989 by governmental decision as the national reference laboratory for HIV and has started its activities by end of 1991. Its activities have been progressively integrated into CRP-Santé.

Chronic viral infections caused by HIV are an ever-growing public health problem in Luxembourg. More than 700 patients infected with HIV have been followed in the Centre Hospitalier of Luxembourg (CHL) during the past decade. The laboratory is working in close relation with the National Department of Infectious Diseases to provide highly specialized technical support for the clinical follow-up of these patients. The available technologies focus on diagnosis, viral load quantification, sequencing and viral culture. More specialized assays can also be performed upon request, when specific questions are addressed concerning the disease evolution or the treatment response of a patient. The behaviour of clinical HIV-1 isolates can be documented *in vitro*, in the presence of antiretroviral drugs, to evaluate the so-called phenotypic resistance. In addition, polymorphisms of HIV-1 co-receptor genes are screened using various technologies (RFLP, sequencing, real-time PCR).

Besides these routine activities, the Retrovirology Laboratory has developed clinically oriented research since its beginnings. The members of the retrovirology laboratory are either employees of CRP-Santé, the AIDS Research Foundation (Fondation Recherche sur le SiDA), or they are employed by the CHL and contribute part time to research.

Our first research projects focused on genotypic detection of HIV-1 drug resistance mutations

and phenotypic drug resistance tests. Antiviral treatment resistance is a major consequence of the high variability of HIV-1. Polymorphisms displayed by several HIV-1 variants may be consensus sequences that could act as resistance-associated mutations, a phenomenon referred to as “primary resistance”. The resistance known as “secondary” corresponds to mutations which come after treatment. The first publication of the laboratory described several drug resistance mutations as predictors of phenotypic zidovudine (AZT) resistance in 1997. We performed then several studies to estimate the frequency of primary, transmitted and secondary resistance in treatment naïve patients and patients failing antiretroviral therapy in the reverse transcriptase and protease genes. Combinations of mutations were shown to predict phenotypic resistance and were associated with virological and clinical outcomes of the patient. At that time, these results were suggested that sequence-based genotyping was critical to optimize treatment monitoring before emergence of broadly cross-resistant virus.

At the request of the Directorate of Health (Ministry of Health), the role of the Retrovirology laboratory has also been to describe the HIV epidemic at a national level. Therefore, we conducted several molecular epidemiological studies to estimate the distribution of HIV-1 subtypes in Luxembourg. We evaluated then the effectiveness of highly active antiretroviral treatment as well as the prevalence of secondary resistance –associated mutations and polymorphisms before the onset of the treatment in the Luxembourg HIV cohort.

We built a robust PostgreSQL database system that allows for reliable and systematic integration of clinical and virological data encoded by two data managers.

Sophisticated queries can be made upon request to the database manager. An alignment tool has been implemented in the database for HIV-1 that allows for automatic detection of resistance mutations for HIV-1 reverse transcriptase and protease, as well as subtype. The laboratory edits annually a report on the molecular evolution of HIV in Luxembourg.

Anti-HIV treatment resistance appears in infected patients with all drugs classes: nucleosidic and non-nucleosidic retro-transcriptase inhibitors, protease inhibitors, integrase inhibitors and entry inhibitors. Our studies have highlighted the need for an international surveillance program on HIV drug resistance.

Since 2002, the laboratory has extended its expertise in phenotypic tests by developing its own “in-house” recombinant virus assays for protease inhibitors and entry inhibitors susceptibility. Using this technique, we have developed a sensitive tool for measuring both viral co-receptor usage (tropism determination) for initiation and monitoring of the treatment with specific co-receptors inhibitors and entry inhibitors resistance such as T-20.

Finally, the role of the Retrovirology Laboratory is also to be a partner for developing countries, favouring knowledge and technology transfer. Our first collaborations with African countries began in 2002 with Rwanda. Dried blood spots on filter paper facilitate the collection, transport and storage of blood samples in resource limited poor settings. We developed in 2004 a rapid and simple DNA extraction procedure from dried blood spot as well as new PCR assays to detect HIV-infection in children born to HIV-infected mothers. This reliable detection method allows us to determinate HIV-1 diagnosis in children in several African countries in collaboration with Médecins Sans Frontières (MSF). Since 2004, we have performed several research projects in Rwanda to describe the HIV-1 subtype distribution and resistance to antiretroviral therapy as well as genetic variability of the non-B subtype viruses predominant in this country. Our studies were the first to describe the baseline situation before the launch of large antiretroviral therapy programs in Rwanda. In addition, we have identified in 2007 a novel 24-base pair deletion in the coding region of CCR5 in the Rwandese population (hCCR5Δ24).

One of the major topics studied in the Laboratory of Retrovirology is the entry of the Human Immunodeficiency Virus (HIV) in the host cell. The virus enters the host cell by binding its envelope to the host cell receptor CD4 and the co-receptors CXCR4 and CCR5. These interactions are an attractive target to develop new drug candidates, in particular the co-re-

ceptors CXCR4 and CCR5, that belong to the G protein coupled receptors (GPCR). More than half of the commercially available drugs modulate the activity of GPCR (drugs against allergy, migraine, hypertension, asthma...).

The phage display technology was introduced in the laboratory in 2001 to study the entry mechanisms of the virus. Firstly, *phage displayed peptide libraries* were used to identify specific sequences that potentially inhibit the virus entry.

Phage displayed peptide libraries consist of large collections of bacteriophage displaying a vast number of unique peptide sequences expressed at the surface of the phage. These libraries are powerful tools for the elucidation of protein-protein interactions and the characterization of receptor-ligand interactions.

Screening strategies with phage displayed peptide libraries on whole cells were developed to identify peptides that interact with the chemokine receptors CCR5 or CXCR4 in their native conformation.

In 2003 the laboratory started to develop novel phage libraries derived from *human antibody repertoires* in close collaboration with the Belgian company AlgoNomics (Ghent, Belgium). The aim was to develop a methodology to minimize the size of an antibody to the level of peptides while retaining its specificity. Phage libraries were engineered displaying the complete repertoires of the heavy chain complementarity determining region 3 (HCDR3) of the antibody. The strength of these phage libraries resided in their potential to be developed as small therapeutic molecules starting from natural products such as immunoglobulins.

As with most pathogens, HIV induces a polyclonal antibody response to different viral proteins but the immune response fails to control the infection. However, some patients after long term exposure to the virus develop broadly neutralizing antibodies. Since 2005 we study the humoral immune responses in HIV patients using both types of phage libraries based on peptides and HCDR3 fragments. These studies are extremely helpful to discover new broadly neutralizing antibody fragments that could be developed as new therapeutic molecules and to develop multi-epitope prophylactic vaccines.



## CURRENT KEY OBJECTIVES

The aims of the laboratory were not fully defined at the beginnings in 1989. Since 2003, clear objectives have been set. Besides clinically oriented research programs, the Retrovirology Laboratory have developed fundamental and applied research in the field of chronic viral infections (HIV, HBV, HCV). The activities of the laboratory is subdivided into two research units, which interact closely: immuno-virology and clinical virology.

### 1 IMMUNO-VIROLOGY RESEARCH UNIT

The research topic of the immuno-virology subunit is the role of the humoral immune response in the context of HIV-1 infection. One of the main objectives of the unit is to identify new human antibody fragments directed against HIV-1. Human chemokine receptors, mainly CCR5 and CXCR4, act as HIV coreceptors and play a critical role in the binding and fusion steps of the viral replication cycle. Several new antiretroviral drugs-so-called entry or fusion inhibitors, directed against HIV coreceptors or HIV envelope subunits, have shown potent antiviral activity and are currently evaluated in clinical trials.

We have developed two different approaches to identify peptides that interact with the chemokine receptors: phage displayed peptide libraries and structurally more complex phage libraries. The phage display technology will allow us to study the fundamental aspects of HIV entry into the cell.



Currently our research is focused on the study of the host and viral factors implicated in the virus entry to unravel their complex interplay and to potentially identify new diagnostic and therapeutic lead compounds.

The mode of interaction between the viral envelope proteins and the co-receptors CCR5 and CXCR4 is not completely clear. The exact epitopes and the viral and host parameters that influence these interactions are not yet deciphered. The identification of antibodies, antibody fragments and peptides that interact with viral proteins and/or co-receptors could lead to a better understanding of these interactions. Moreover, these molecules could act as potent entry inhibitors.

To study the entry mechanisms, the state of the art phage display technologies available in the laboratory will be used. The innovative HCDR3 libraries offer exciting prospects in the development of novel immunotherapeutics and immunodiagnostics. This technology allows the effective and efficient search or screen wherein HCDR3 loops display significant binding to the viral envelope or the co-receptors CXCR4 and CCR5. The HCDR3 fragments offer the advantages of being small peptides derived from natural products that can be more easily manufactured as peptidomimetic. Our research will contribute to a better understanding of the closely guarded conserved structures of the envelope proteins and the epitopes implicated in HIV entry and the design of innovative entry inhibitors.

The research unit also studies the signal transduction pathways of the newly identified ligands of the co-receptors CXCR4 and CCR5. These studies are performed in collaboration with Dr. J.L. Galzi of the Institute Gilbert Laustriat, UMR7175 (Strasbourg, France), who is specialized in the study of G protein coupled receptors. The impact of inhibiting the interactions of the chemokine receptor CXCR4 is not only restricted to HIV but is much larger and could affect positively the outcome of diseases such as cancer, leukaemia and rheumatoid arthritis. In this regard, the development of antagonists of CXCR4 holds promising therapeutic applications. To date relatively few small molecule CXCR4 antagonists are known. There is a considerable need to identify new potent and selective CXCR4 inhibitors. Therefore, knowledge on the mechanisms of interaction of CXCR4 is of key importance to understand the physiological role of the receptor and to design specific small surrogate ligands interfering with the role of CXCR4 in different disease settings.

A second key objective is the study of the humoral immune responses during HIV infection to obtain a better understanding of the impact of the infection on the B cell immune repertoire and to characterize the role of neutralizing and non-neutralizing antibodies in different disease progressions.

During the 20 years of the HIV epidemic, only five human monoclonal antibodies with broad neutralizing activity were described, demonstrating the difficulties and the challenges to identify and to generate human antibodies that neutralize the virus efficiently. The antibody phage display technology allows retrieving the rare HIV neutralizing antibody fragments that cannot be identified by classical methods. Amplification of the immune repertoires of HIV patients in different disease progressions will provide more knowledge on the impact of the infection on the B cell repertoires and will allow the isolation of potentially neutralizing antibody fragments. These antibody fragments could be the lead compounds for the development of new therapeutic molecules.

One of the major applications of the phage display technology is the identification of epitopes of antibodies. Phage displayed peptide libraries will be used in this context to identify immunogenic epitopes of the virus. These epitopes could be useful for the development of new immunogens for the development of prophylactic vaccines.

## 2 CLINICAL VIROLOGY RESEARCH UNIT

The laboratory is an important collaborator within several European HIV and HCV networks (EuroSiDA, EuroHIV, SPREAD/EuropeHIVResistance, VIRGIL). The main objectives of these studies are to improve knowledge on molecular epidemiology, transmission, prevalence and factors facilitating the emergence and spread of drug resistant HIV-1.

The HCV epidemic is about 4-times larger than infections with HIV and represents a major public health challenge. Therefore, we extended our database system to HCV but also to HBV cases. HIV and HCV are extremely variable RNA viruses, which has tremendous implications on molecular epidemiology and antiviral treatment. The pathogenic potential of these viruses can be affected by such variability, as it is the case for sensitivity to antiviral drugs.

These past years, bioinformatics and phylogenetics expertise has been implemented in the laboratory to develop complex integrated clinical and virological databases for chronic infectious diseases. The main objectives of the clinical virology unit are (i) to describe the transmission networks and the dynamics of epidemics caused by HIV and HCV in Luxembourg, (ii) to evaluate primary transmitted and secondary resistance to antiviral drugs (HIV-1, HIV-2, HCV, HBV), (iii) to estimate the clinical relevance of viral and host factors involved in HIV-1 entry.

In addition, the clinical virology research unit is a partner for developing countries to favour knowledge and technology transfer in such countries (network ARTA, MSF, collaboration with the Laboratoire National de Référence of Kigali, Rwanda).

Ultimately, the main goal of the research programs of the Retrovirology Laboratory is to improve the management of HIV and HCV infections.

Our expertise in viral culture as well as our biosafety P3 level confinement place us in a leading position to be a partner of biotechnology and pharmaceutical industry for the development of anti-HIV drugs in Luxembourg.

## ONGOING PROJECTS AND MAIN RESULTS

### 1 IMMUNO-VIROLOGY RESEARCH UNIT

Phage libraries displaying HCDR3 repertoires of healthy donors were engineered and we demonstrated the feasibility to reduce the size of the antibodies to the level of the peptide while conserving the antibody specificity. We developed a rational strategy for efficient searching of target-specific high affinity binders from non-immunized HCDR3 phage libraries. The proof of concept of this technology was provided by isolating highly specific HCDR3 sequences from these libraries displaying nanomolar affinities. Our findings strongly underlined the value of HCDR3 libraries as a source of 'biologically randomized' sequences from which potent and target-specific binders can be retrieved (Deroo et al., 2008).

To study the impact of the HIV infection on the diversity, the complexity and the evolution of the B cell immune repertoire, we compared the length distributions of HCDR3 fragments in healthy donors and in HIV infected patients progressing slowly in their infection.

A reduced or biased repertoire complexity was observed for HIV infected patients in comparison to the B cell immune repertoire of healthy donors. These findings need to be confirmed by analysis on larger panels of HIV infected patients. These findings were presented at the First International symposium on Genetic and Immune Correlates of HIV Infection and Vaccine-Induced Immunity, 10-13 June 2007, Budapest, Hungary and at the 15th Conference on Retroviruses and Opportunistic Infections, 2-6 February 2008, Boston, MA, USA.

Phage-chemokines expressing the natural ligands of the co-receptor CCR5 were engineered

as research tools to study CCR5 mediated interactions. Preliminary results indicate that the RANTES-expressing phage acts as an agonist of CCR5. Phage chemokines are a low-cost alternative to purified recombinant proteins to monitor protein-receptor interactions. These results were presented at the First International symposium on Genetic and Immune Correlates of HIV Infection and Vaccine-Induced Immunity, 10-13 June 2007, Budapest, Hungary.

## 2 CLINICAL VIROLOGY RESEARCH UNIT

The main achievements in the area of molecular epidemiology and antiviral resistance during year 2007 were:

- **Epidemiological follow-up of HIV infection in Luxembourg:** a dramatic increase of the incidence of HIV-1 unique recombinant forms (URF) has been observed in Luxembourg since 2001 where 19% of the newly diagnosed patients have been infected with URF. In this context, we have identified a new B/F1 recombinant in 21 patients who reported sexual transmission over a short time interval (2003-2006). We have confirmed the inedited nature of this new URF by near full-length genome characterization of the 21 clinical strains. We have analyzed the epidemic history of the variant by co-estimating the evolutionary rate and growth rate using Bayesian coalescent methods. While the URF B/F1 strains were not characterized by a higher evolutionary rate as compared to the founder B and F1 strains, our Bayesian estimates of population dynamics revealed a strong initial growth phase of the recombinant form. This rapid population growth may reflect an elevated transmission fitness of this variant and explain its aggressive behaviour.
- **Epidemiological follow-up of HCV infection in Luxembourg:** we analyzed the HCV genotype distribution and transmission risk factors in a population of 802 unselected patients in Luxembourg. Genotype 1 was predominant (53.4%) followed by genotype 3 (33%), 4 (8.3%), 2 (4.6%) and 5 (0.6%). The repartition is similar to other European countries, with one of the highest European prevalence rates of genotype 3 which is attributed to intravenous drug usage (IVDU). Like in other countries from European Union, IVDU is the driving force of HCV transmission in Luxembourg. Therefore, we have developed in-house sequencing technologies for NS3 and NS5B genes of HCV to detect specific mutations that may explain the efficiency of genotype 3 transmission in Luxembourg.
- **European surveillance network for vigilance against viral resistance (Virgil):** our participation in the Virgil network has been finalized with the insertion of the clinical, epidemiological and virological data of 42 patients from Luxembourg in the Virgil database. This database allows a global approach for monitoring and ultimately improving the management of antiviral drug resistance in HCV treated patients, leading to outstanding practices and standards in Europe.

The main achievements in the area of HIV-1 entry and HIV-1 entry inhibition were:

- **Development of a HIV-1 coreceptor usage assay and a gp41-recombinant virus assay:** novel HIV entry inhibitors blocking viral co-receptors are being developed. Rapid, easy to handle phenotypic HIV-1 testing for co-receptor usage (tropism) determination before treatment onset as well as for drug resistance monitoring throughout treatment follow-up has therefore become crucial for optimizing the therapeutic strategy. To improve the sensitivity of such phenotypic tools, we developed two env recombinant virus assays using the *Gaussia* Luciferase as reporter: one full-env-based assay for viral co-receptor usage determination and one gp41 T20-resistance assay. The full-length env-based tropism assay was validated on various reference strains as well as primary B and non-B recombinant viruses. The T-20 resistance assay was validated comparing susceptibility to T-20 of a HXB2-derived mutant harbouring two mutations in gp41. Our first results indicate that these assays should provide a sensitive useful tool for measuring both viral co-receptor usage and T-20 resistance with clinical samples.

The main achievements in the area of collaboration with African countries were:

- **Impact of the hCCR5Δ24 deletion on CCR5 expression:** we have identified a novel 24-base pair deletion in the coding region of CCR5 in the Rwandese population (hCCR5Δ24). This deletion has not been detected in a Caucasian cohort of 500 people in Luxembourg. We have tested *in vitro* the effect of hCCR5Δ24 on CCR5 expression using flow cytometry. We have shown that HEK-293 cells transfected with an hCCR5Δ24 plasmid did not express CCR5 in the cell surface whereas they expressed mRNA coding for CCR5 and intracellular CCR5 protein. Our data may suggest a major role of hCCR5Δ24 in HIV infectivity in African populations.
- **Effectiveness of antiretroviral therapy in breastfeeding mothers to prevent post-natal vertical transmission in Rwanda (AMATA study):** Vertical transmission through breastfeeding remains a major problem in limited resources countries. We have compared triple antiretroviral therapy given to breastfeeding mothers with formula feeding for prevention of post-natal mother-to-child transmission. 572 women were enrolled. 528 children have been born as of March 1, 2007. Among these, 6 children were infected with HIV (1.4%) at birth and, among those tested so far, none has become infected through breastfeeding under antiretroviral therapy. No significant difference in morbidity and mortality was observed between the two groups. Our results suggest that breastfeeding under triple antiretroviral therapy in children born to HIV-1 infected mothers is a safe way of avoiding HIV-1 infection in the baby while keeping the benefits of breastfeeding and avoiding the stigmatisation and risks of artificial feeding.
- **Collaborations with MSF:** treatment efficacy was evaluated using dried plasma spots technology on 160 patients from Burkina Faso and 24 children from Liberia. Resistance testing has also been realized in case of treatment failure for 33 patients in Mozambique.

During 2007, we had five presentations at international congresses and published four manuscripts in peer-reviewed journals.

## COLLABORATIONS

The research projects of the Retrovirology Laboratory have received the support of international collaborations:

- SME: Algonomics/Ablynx (Ghent), Advanced Biological Laboratories (Luxembourg) and TherapyEdge (USA).
- University of Strasbourg: UMR 7175, Département Récepteurs et Protéines Membranaires, Pr. J.L. Galzi.
- University of Louvain: Département de Virologie, Pr. P. Goubau.
- University of Liège: Centre de Référence SIDA, Pr. M. Moutschen.

The Retrovirology Laboratory is participating in several international network/studies:

- Belgian AIDS Reference Laboratory Network.
- SPREAD/EuropeHIVResistance (6 EU-FP): the laboratory was in charge of the database construction and management (initially 17, now 32 European countries).
- VIRGIL (6 EU-FP): the laboratory is providing clinical, epidemiological and virological data in the Virgil database Surveil (30 European countries).
- EuroSiDA: the laboratory is providing samples, sequencing results and information.
- EuroHIV: the laboratory is providing statistics on the HIV Luxembourg epidemic.
- INSIGHT (Clinical network financed by NIH): the laboratory gives technical support for clinical trials.

- ART-A (African collaboration): the laboratory is involved in the development of a sequence edition software.

The laboratory is working in collaboration with other laboratories from the CRP-Santé:

- Laboratoire de Biologie Moléculaire, d’Analyse Génique et de Modélisation (Transcriptomic Core Facility), Dr Laurent Vallar: use of the microarray platform, Nanodrop ® and the Agilent 2100 bioanalyzer ® in several research projects.
- Flow Cytometry Core Facility, Dr René Brons: use of the flow cytometer and cell sorter in several research projects.
- Laboratoire StraLux, unité Biologie Moléculaire Végétale, Dr André Steinmetz: screening of Traditional Chinese Medecine herbs for anti-HIV activity.
- Laboratoire National de Santé, Laboratoire de Toxicologie, Dr Serge Schneider: measurement of antiretroviral drugs in plasma.

### KEY PUBLICATIONS OF THE LABORATORY

- HIV-1 subtypes in Luxembourg, 1983-2000. S. Deroo, I. Robert, E. Fontaine, C. Lambert, J.M. Plesséria, V. Arendt, T. Staub, R. Hemmer, F. Schneider and J.C. Schmit. AIDS 2002; 16: 2461-7 (IF: 5.983).
- Uncommon mutations at residue positions critical for enfuvirtide (T-20) resistance in enfuvirtide naïve patients infected with subtype B and non-B virus. F. Roman, D. Gonzalez, C. Lambert, S. Deroo, A. Fischer, T. Baurith, T. Staub, R. Boulmé, V. Arendt, F. Schneider, R. Hemmer, J.C. Schmit. J Acquir Immune Defic Syndr 2003; 33(2): 134-9 (IF:3.681).
- Simple DNA extraction method for dried blood spots and comparison of two PCR assays for diagnosis of vertical human immunodeficiency virus type 1 transmission in Rwanda. A. Fischer, C. Lejczak, C. Lambert, J. Servais, N. Makombe, J. Russine, T. Staub, R. Hemmer, F. Schneider, J.C. Schmit, V. Arendt. J Clin Microbiol 2004;42(1):16-20 (IF: 3.489).
- Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. A.M.J. Wensing, D.A.M.C. van de Vijver, G. Agarano, B. Asjo, C. Balotta, E. Boeri, R. Camacho, M.L. Chaix, E. Op de Coul, A. De Luca, I. Maljkovic, C. de Mendoza, I. Derdelinckx, Z. Grossman, O. Hamouda, A. Hatzakis, A. Hoepelman, R. Hemmer, A. Horban, K. Korn, C. Kuecherer, T. Leitner, C. Loveday, E. Macrae. C. Nielsen, V. Ormaasen, L. Perrin, D. Paraskevis, E. Puchhammer, L. Ruiz, M. Salminen, J.C. Schmit, F. Schneider, R. Schuurman, V. Soriano, G. Stanczak, M. Stanojevic, A.M. Vandamme, K. Van Laethem, M. Violin, S. Yerly, M. Zazzi and C.A.B. Boucher. J Inf Dis 2005;192:958-66 (IF: 4.95).
- A novel 24-base pair deletion in the coding region of CCR5 in an African population. C. Masquelier, J.Y. Servais, F. Roman, J.C.Schmit and V. Arendt. AIDS 2007;21(1):111-3 (IF:5.84).

### PUBLICATIONS 2007

- Masquelier C, Servais JY, Rusanganwa E, Roman F, Havuga E, Servais J, Tuyizere S, Omes C, Karasi JC, Courteille O, Karita E, Allen S, Schmit JC, Arendt V. A novel 24-base pair deletion in the coding region of CCR5 in an African population. AIDS 2007, 21:111-113.
- Helm M, Walter H, Ehret R, Schmit JC, Kurowski M, Knechten H, Korn K, Braun P, Schmidt B. Differences of nine drug resistance interpretation systems in predicting short-term therapy outcomes of treatment-experienced HIV-1 infected patients: a retrospective observational cohort study. Eur J Med Res 2007, 12(6):231-42.
- 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.
- 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, in press.





## / LABORATORY OF IMMUNOGENETICS AND ALLERGOLOGY

Head of the laboratory:

François HENTGES, MD



Presently, the staff of the laboratory is as follows:

Biomolecular, immune and biochemical characterisation of animal allergens:

Christiane HILGER, PhD, Senior Scientist, Project Supervisor  
Cathy LEONARD, PhD, Project Manager  
Annette KUEHN, PhD, Project Manager  
Kyra SWIONTEK, Engineer  
Stéphanie KLER, Engineer  
Caroline DAVRIL, Technician  
Olivia DOMINGUEZ, Technician  
Marie-Paule PARISSOT, Technician

Innate immunity at the cellular level:

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



## HISTORY OF THE LABORATORY

The history of the research unit can be divided in a period of research activities existing before financial support came from the CRP-Santé. Then there is a long period of some 15-17 years of regular but modest funding which allowed research activities to grow while relying on the infrastructure and the work of the routine laboratory of Immunology and Allergology at the CHL. The last five years are characterized by an important increase in research activities made possible by a financial support becoming substantial. During this period took place a regrouping of the laboratory of Immunogenetics and Allergology (LIGA) with different other CRP-Santé research units at the BAM I modular building 84, val Fleuri.

### 1 PRE-CRP ERA

By the mid 80ties it had been possible to establish in the laboratory of Immunology and Allergology at the CHL besides the routine work, some small scale research activities. These non routine analyses were developed to answer specific patient-related questions that required the use of new techniques. Funding was provided by the Fondation Recherche Cancer et Sang. Depending on the subject, support was given by the Laboratory of Biochemistry of the CHL and by the Laboratoire National de Santé. Among the first new techniques established in that way were the cultivation of primary tumour cells and the production of hybridomas to generate monoclonal antibodies.

## 2 CRP THE FIRST 15 YEARS

With the creation of the CRP-Santé it became possible to obtain funding for periods of 2 or 3 years for 1 or generally 2 persons. Thus some continuity in the research activities was assured for the duration of the grant. Establishment and application of new techniques to solve patient related problems continued to be major goals. Some of these techniques or the developments thereof were later transferred to the routine laboratory. Using the technique of *monoclonal antibody production* several projects were realized. Indeed the very first project funded by the CRP-Santé was about the generation of monoclonal antibodies to high- molecular- weight kininogen (HMWK) an essential molecule implicated in the blood clotting and the contact system pathways. The produced monoclonal antibodies allowed monitoring HMWK activation and bradykinin generation in cases of acquired C1 esterase inhibitor deficiency, a topic the laboratory worked on at that time (1).

It is interesting to note that very recently these same monoclonal antibodies were instrumental in documenting contact phase activation in a new type of angioedema linked to a mutation in Hageman Factor. The technique of monoclonal antibody production was further used for quite different purposes. Monoclonal antibodies were developed for the specific and discriminative detection of several mammalian serum albumins but also for the localisation of LIM proteins in sunflower pollen and young plants (collaboration A. Steinmetz, Strasbourg). Recently a modified technique was used to generate monoclonal antibodies for the discriminative detection and tracing of fish parvalbumin, the major fish allergen.

Very different technical developments were needed before performing projects using *immunogenetic and later genetic markers* in disease-associated studies. It was with the help of a CRP-Santé grant that our lab first used in 90-91 generic PCR-techniques to amplify informative exons of the HLA genes and determined the specific HLA alleles at the genetic level by hybridizing the blotted PCR-products with specific oligonucleotides. Although genetic typing of HLA alleles has changed several times since then, this was a mile stone in HLA typing and Immunogenetics for the routine laboratory to which these techniques were transferred.

Further grant applications were made to perform together with interested clinical units at the CHL a series of epidemiological studies addressing the question of HLA association with immune mediated diseases like Insulin Dependent Diabetes Mellitus or for instance disease progression in patients infected with human immunodeficiency type 1 virus (2).

A very different set of genetic markers was somewhat later used to test for their association with neurological diseases like Parkinson's disease or psychiatric diseases as bipolar affective disorder and personality trait disorders.

With the acquisition of *powerful molecular biology techniques* managed by Dr. Hilger the laboratory became capable of realizing technically highly skilled projects like the production of chimaeric monoclonal antibodies and thereafter the cloning and recombinant production of hitherto undefined and unknown allergens. The first allergen to be cloned by the lab was cat serum albumin. It was possible to show that cat allergic persons who had IgE antibodies to cat serum albumin often had cross-reactive IgE antibodies to dog serum albumin but also porcine serum albumin and sometimes bovine serum albumin (3). This was the starting point to a still ongoing in depth thematic analysis of the humoral and cellular immune response to cat serum albumin especially as to what the role of regulatory T cells and possible autoimmune aspects are concerned. This question deems important when considering the fact that 80 % of amino-acid sequences between different mammalian serum albumins are identical and therefore should not be able to induce an immune and hence allergic response.

The next animal allergen family our lab got hands on was when we realized that the cloned allergen that almost killed a patient while eating fried frog legs was a parvalbumin, which was interesting because the major fish allergens are also parvalbumins It was further possible to show that fish allergic patients can have allergic cross-reactions when consuming meat of

frog origin (4). Fish parvalbumins have since then been chosen as the target molecules and parvalbumin genes the target DNA in an ongoing FNR on food safety.

Lipocalins are an important family of small secreted proteins binding a variety of small ligands. With the exception of Fel d1, the major cat allergen, and the serum albumins they represent the major allergen family of pets and domestic animals. After finding that the cloned major allergen of the pigeon tick, *Argas reflexus*, is a histamine binding lipocalin, the laboratory has become interested in cloning the hitherto unknown allergens in rodents responsible for allergies to laboratory animals but also to pets.

## 3 CRP THE LAST 5 YEARS

Thanks to the political commitment of the Luxembourg Government, to foster research in Luxembourg the situation of our laboratory has very markedly improved during the last few years. This was on one side realized directly through the CRP-Santé by increasing its support for research projects along defined and agreed priorities. A very important push came also from the Fonds National de la Recherche whose research programs have a very profound and positive impact on the biomedical research in Luxembourg.

It is only through these major efforts that we can now focus research with enough power on already existing research themes and develop new ones for instance in immune regulation and cellular innate immunity. The current key objectives of our laboratory are summarized in the following lines.

### CURRENT KEY OBJECTIVES

Over the last decades allergic diseases have become a major health problem for hundreds of millions of people in developed 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 LIA aims at performing high quality basic research focussing on the:**

- Characterisation at the protein and DNA-level of 3 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.
- Understanding some general principles of adaptive and innate immunity through investigation on natural killer cells and regulatory T cells.
- Analysing interactions between the nervous system and the immune system by studying the effects of several neurotrophins on natural killer cells.
- Study 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 sensitisation to food and respiratory allergens.



- Development of protein and DNA reagents to detect and trace animal allergens and proteins of animal origin.
- Test in mouse models, modified recombinant allergens susceptible for future treatment of allergic patients.
- Technology transfer to the Laboratory of Immunology and Allergology of the CHL in molecular biology techniques.

## ONGOING PROJECTS

### 1 BIOMOLECULAR, IMMUNE AND BIOCHEMICAL CHARACTERISATION OF ANIMAL ALLERGENS.

During the recent years the laboratory has cloned and characterized several proteins belonging to the 3 major animal allergen families: lipocalins, serum albumins, and parvalbumins. Over the next years the lab will extend its biomolecular and cloning activities of allergens, develop the analysis of the regulatory immune response to selected allergens and finish the biochemical characterisation of selected allergen molecules.

The projects are:

- Further characterisation of guinea pig and rabbit allergens and patent deposition. (Project 1a).  
Biochemical and pharmacological characterisation of Arg r 1, the major allergen responsible for pigeon tick anaphylaxis (project 1b).
- Production of genomic and molecular tools for the tracing of parvalbumins of fish, mammalian, or bird origin (project 2).  
Cloning of new fish allergens belonging to a new protein family and possible patent deposition.
- 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. (project 3).

### 2 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, and (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 with around 25 cases described worldwide until now. It is 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 (project 4).

## SCIENTIFIC RESULTS

### 1 ALLERGEN CHARACTERISATION AND CLONING

- **The lipocalin family:** the LIA has recently cloned and expressed as recombinant proteins two lipocalin allergens from the guinea-pig and determined the protein sequence of 2 other lipocalin allergens. During 2007 the cDNA sequences of 2 further allergens, one of guinea-pig origin and one of rabbit origin have been unravelled; both belong to the lipocalin family of animal allergens. Together with the previously cloned allergens the LIGA possesses now the greatest number of cloned animal allergens available. These results have not yet been published, awaiting patent application. (Project 1)

Furthermore it was possible to identify the nature of an allergen implicated in a case of allergic reaction to chicken meat.

- **Parvalbumins:** in the frame of a collaborative FNR project on food safety the laboratory works on food and allergen tracing using animal parvalbumins as tracers (protein and DNA level). During the first part of the project the laboratory has purified or produced by recombinant technology the beta 1 and 2 parvalbumins of eleven fish species. Monoclonal antibodies detecting specifically the parvalbumins of four commonly consumed fish species have been produced. Oligonucleotide primers have been designed and used in allele-specific PCR reactions to detect correctly these same species among a series of unknown fish probes. The material for this tracing was given by a renowned German Institute on food safety. (Project 2)

A case of unexpected anaphylactic reaction provoked by the ingestion of fish gelatine has been worked out.

### 2 IMMUNE RESPONSE TO ALLERGENS AND T REGULATORY CELLS: THE SERUM ALBUMIN PROTEIN FAMILY

This project aims at defining T cell epitopes recognized by natural T regulatory and induced T regulatory cells on cat serum albumin. Overlapping peptides mapping the CSA molecule or mouse serum albumin have been used for the study of the cellular proliferation and cytokine secretion pattern of natural and induced T regulatory cells in the BALB/c mouse (*T regs and allergens Project 3*).

A new autoimmune phenotype has been described and investigated in a patient found to have a special CD 45 A splice variant.

### 3 INNATE IMMUNITY AT THE CELLULAR LEVEL

In the NK cell and neurotrophin field, we have obtained three types of knockout (KO) mouse models useful as important genetic controls for our studies, by initiating international collaborations: neurturin KO mice, GFR $\alpha$ 1 conditional KO mice and GFR $\alpha$ 2 KO mice. The three KO strains are on the C57BL/6 (B6) genetic background.

The phenotype of neurturin KO mice is in no way different from that of wild type B6 animals, regarding a panel of activating and inhibitory NK cell receptors. They are also not different in terms of cytotoxic activity, cytokine production and proliferation in response to interleukin 2 (an NK cell growth and stimulation factor). Moreover, the addition of exogenous neurturin has no influence on the phenotype or the functional properties of B6 mice. Similar studies are planned in the near future with the other KO strains.

The expression of GFR $\alpha$ 2, a part of the neurturin receptor, by mouse NK cells could be confirmed by RT-PCR, but not yet by flow cytometry, maybe because of the inefficiency of

available antibodies. In contrast, GFR $\alpha$ 1, a part of the GDNF receptor, was shown by flow cytometry to be expressed by 30 – 50 % of resting and activated B6 mouse NK cells. The same was true for the high affinity NGF receptor, TrkA.

This molecule was also demonstrated in mouse NK cells by RT-PCR. Mouse NK cells were also positive for NGF, as assessed by RT-PCR. Regarding the expression of TrkA, but not that of GFR $\delta$ 1, it appeared that the percentage of positive NK cells was different according to the various maturational stages of NK cell differentiation, which could give a clue to its functional role.

A comparative flow cytometry study of NK cells between normal and allergic human patients was started.

A new human case of MHC class I deficiency was identified by our subgroup. It was a young female patient with a very low cell surface expression (<10 % of normal) of MHC class I molecules. MHC class I serotyping was negative. Different techniques revealed that the deficiency of the patient was due to a homozygous TAP1 gene mutation. Her CD8+ T cell number was very low as expected. She had an unusual NK cell subtype distribution with a higher than normal percentage of CD56<sup>bright</sup> NK cells. NK cells over-expressed the inhibitory receptors ILT2 and NK-G2A, a feature that had previously been identified in other TAP-deficient patients. The patient also expressed the receptor KIR2DL5 whose function is currently unknown. Further phenotypic and functional NK cell studies are in progress.

## KEY PUBLICATIONS OF THE LABORATORY

- Hentges F, Humbel R, Dicato M, Hemmer R, Kuntziger H. Acquired C1 esterase-inhibitor deficiency: case report with emphasis on complement and kallikrein activation during two patterns of clinical manifestations. J Allergy Clin Immunol 1986; 78:860-7.
- Hentges F, Hoffmann A, Oliveira de Araujo F, Hemmer R. Prolonged clinically asymptomatic evolution after HIV-1 infection is marked by the absence of complement C4 null alleles t the MHC .Clin Exp.Immunol. 1992; 88:237-242.
- Hilger C, Kohnen M, Grigioni F, Lehnert C, Hentges F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. Allergy. 1997; 52:179-87.
- Hilger C, Thill L, Grigioni C, Falagiani P, Ferrara A, Romano C, Stewens W, Hentges F. IgE antibodies of fish allergic patients cross-react with frog parvalbumin. Allergy 2004; 59:653-60.
- Hilger C, Bessot JC, Hutt N, Grigioni F, De Blay F, Pauli G, Hentges F. IgE- mediated anaphylaxis caused by bites of the pigeon tick Argas reflexus: cloning and expression of the major allergen Arg r1. J Allergy Clin Immunol 2005; 115:617-22.
- Zimmer J, Poli A, Andres E, Hanau D, Brons NH, Hentges F. Reduced cytokine-mediated up-regulation of HLA-DR in TAP-deficient fibroblasts. Immunol Lett. 2006; 107:109-118.

## PUBLICATIONS 2007

### 1 ORIGINAL PUBLICATIONS DIRECTLY LINKED TO THE PROGRAM OF THE LAB

- Zimmer J, Bausinger H, Andr  s E, Donato L, Hanau D, Hentges F, Moretta A, de la Salle H. Phenotypic studies of natural killer cell subsets in human transporter associate with antigen processing deficiency. PLoS ONE. 2007 Oct 17;2(10):e1033.
- Zimmer J, Michel T, Andr  s E, Hentges F Up-regulation of NKG2D ligands by AML cells to increase sensitivity to NK cells: The tumour might strike back Comment on "Differentiation-promoting drugs up-regulate NKG2D ligand expression and enhance the susceptibility of acute myeloid leukemia cells to natural killer cell-mediated lysis" by Rohner et al. Leuk Res. 2007 Oct 5.
- Zimmer J, Andr  s E, Hentges F Transporter associated with antigen processing deficiency: an additional condition associated with bronchiectasis. Intern Med J. 2007 Mar;37(3):208-9; author reply 209-10.
- Ralainirina N, Poli A, Michel T, Poos L, Andr  s E, Hentges F, Zimmer J. Control of NK cell functions by CD4+CD25+ regulatory T cells. J Leukoc Biol. 2007 Jan; 81(1):144-53. Epub 2006 Sep 7.

- Ralainirina N, Poli A, Michel T, Poos L, Andres E, Hentges F, Zimmer J. Control of natural killer (NK) cell functions by CD4+CD25+ regulatory T cells. J Leukoc Biol. 2007; 81:144-53.

## 2 OTHER PUBLICATION

- Hentges F, Kockhans Bieda MC. Recensement des pollens et des spores atmosph  riques. Minist  re de la Sant  . Station d'A  robiologie Luxembourg-Ville. Bulletin de l'ann  e 2006.

## COLLABORATIONS WITH OTHER CRP-SANT   LABORATORIES

The LIA unit has close collaborations in the field of food safety and food allergen characterisation with:

- the laboratory on plant molecular biology of Dr Andr   Steinmetz
- the microarray platform directed by Dr Laurent Vallar
- the laboratory on food safety of Dr Gilbert Morris at the LNS

The LIA unit has developed collaboration in the field of functional immunology (definition of T cell epitopes on allergens with overlapping peptides)

- with the Immunology Department of Prof. Claude Muller at the LNS

## NATIONAL COLLABORATIONS/PARTNERS

- Laboratory of Immunology and Allergology, CHL
- Laboratory of Biochemistry, CHL

## INTERNATIONAL COLLABORATIONS/PARTNERS

The LIA has ongoing collaborations with the following units and laboratories for clinical, as well as functional studies of allergens:

- Pr. Gabrielle Pauli and F. de Blay from the Pneumology department at the University of Strasbourg
- Dr. Guido Paesen from the CEH in Oxford
- Pr. Rita Bernhardt, Saarland University

Collaboration 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 Fran  ais du Sang – Alsace, Strasbourg, France

## HIGHER EDUCATION AND TRAINING

- The LIA unit participates in the master programme “Physiopathologie Cellulaire et Moléculaire” of the University Louis Pasteur of Strasbourg.
- Dr. Jacques Zimmer has qualified in 2006 at the University Louis Pasteur of Strasbourg to guide research activities (HDR or Habilitation à Diriger des Recherches).



## / LABORATORY OF PLANT MOLECULAR BIOLOGY

Head of the laboratory:

André STEINMETZ, PhD



Presently, the staff of the laboratory is as follows:

Clément THOMAS, PhD, Research Scientist, Project Leader Actin Cytoskeleton Research  
Ning WANG, MD, Research Scientist, Project Leader TCM Research  
Céline HOFFMANN, PhD, Research Scientist, in charge of the Confocal Microscopy Plateform  
Monika DIETERLE, PhD, Research Scientist  
Cécile HUSTIN, PhD, Research Scientist  
Flora MOREAU, Lab Technician  
Katrín NEUMANN, Lab Technician  
Sabrina GATTI, PhD Student  
Jessica PAPUGA, PhD Student



## HISTORY OF THE LABORATORY

Since its creation in 2004 the Plant Molecular Biology (PMB) Unit has been closely associated with the Laboratory of ImmunoGenetics-Allergology (LIGA). A common objective of the two laboratories was to intensify research on allergens of animal and plant origin at CRP-Santé. They have been collaborating since in FNR-funded projects in the domain of Food Safety (SECAL I and SECAL II), focusing on the development of molecular probes for the tracing of allergenic material derived from plant and animal species.

The laboratory also sustained the original research on plant LIM proteins initiated in 1990 in André Steinmetz's former laboratory at the Plant Molecular Biology Institute in Strasbourg (France). With the recent results demonstrating that plant LIM proteins function as regulators of the actin cytoskeleton the laboratory has acquired a good level of expertise in actin cytoskeleton research and aspires to reinforce this competence in the years to come. Confocal microscopy has made and will continue to make important contributions in this and other cell biology-based projects. Céline Hoffmann, a staff member of the laboratory, is in charge of the confocal microscopy platform set up at CRP-Santé in 2004.

The proximity to biomedical research groups provided a unique opportunity to foster additional collaborations and the development of more interdisciplinary research. As an example, this proximity, as well as the worldwide growing interest of the scientific community in Traditional Chinese Medicine (TCM), was at the origin of new collaborative research projects at CRP-Santé where initial tests in cellular systems have been performed since 2006.





## CURRENT KEY OBJECTIVES

### 1 PLANT ALLERGENS AND FOOD SAFETY

Recent research 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 three other local laboratories (F. Hentges, LIGA/CRP-Santé; L. Vallar, Microarray Center/CRP-Santé; G. Moris, Division of Food Control/LNS), in FNR-funded projects aiming to develop new molecular tools for unambiguous tracing and identification of allergenic components of animal or plant origin in food products. Several major allergenic plants including several cereals, tree nuts, soybean and peanut are targeted.

### 2 FUNCTIONS AND REGULATION OF THE PLANT ACTIN CYTOSKELETON

The actin cytoskeleton plays important roles in the life of eucaryotic cells. These roles may vary in part between cell types and are determined by a set of proteins that have the ability to bind actin (Actin Binding Proteins or ABPs). Binding of these proteins can control the actin polymerization and depolymerization processes, the stability of actin filaments, or the assembly into higher order structures such as cables or networks. Physiological consequences of the remodeling of actin cytoskeleton structure are changes in cell morphology or cell motility. ABPs with associated motor functions are implicated in intracellular transport and in cell contraction (e.g. muscles).

One of the key objectives of our actin cytoskeleton research is to better understand the mechanisms by which actin-binding proteins regulate actin cytoskeleton structure and dynamics in plants. We particularly focus on the formation of actin networks and bundles, which are structures common to animal and plant cells, and on their biological roles.

Another key objective is to understand how the actin cytoskeleton communicates with the nucleus. Many signals, including mechanical ones, are perceived and/or, at least transmitted via the actin cytoskeleton to the nucleus where gene expression is modified to generate the appropriate cell response(s).

To address these two important biological issues, LIM proteins represent ideal working models.

On the one hand, they display an autonomous ability to modify the actin cytoskeleton structure and dynamics, notably through their actin filament stabilizing and bundling activities. Their level of expression can be manipulated using transgenesis and mutagenesis approaches to conduct functional studies. On the other hand, LIM proteins are good candidates for linking cytoskeleton and nuclear activities since they localize to both sites. Importantly, at least two LIM proteins have been shown to directly bind to specific DNA sequences. Last but not least, plant LIM proteins have structurally related counterparts in vertebrates that play important roles in muscle development and maintenance. A comparative analysis of these proteins in plants and animals is expected to provide a better insight into their functions and may help uncover underlying common molecular mechanisms such as the control of actin-binding and cytoskeleton-nuclear crosstalks.

### 3 CHINESE MEDICINAL HERBS

Herbs have been used in Traditional Chinese Medicine for several hundred generations, and this generation-old traditional knowledge has culminated in the identification of the anti-malarial drug artemisine; this "re-discovery" is comparable in importance to the discovery of penicillin used to fight bacterial infections. Not only artemisine, but many other modern pharmaceuticals have been derived from TCM herbs.

The objective of the PMB Unit is to help establish the CRP-Santé as a dedicated partner in the study of the molecular basis of Traditional Chinese Medicine. In international collaborative projects we aim to identify novel active compounds present in Chinese medicinal herb extracts and to determine their cellular and molecular targets.

It is expected that this research will lead to the discovery of novel plant-derived pharmaceuticals to be used in the treatment of modern societal diseases including cancer as well as cardiovascular and retroviral-induced diseases.



## ONGOING PROJECTS AND MAIN RESULTS

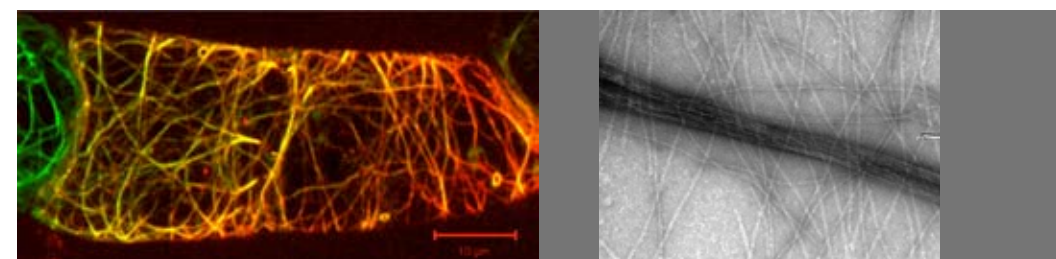
### 1 ALLERGENS AND FOOD TRACING

Food testing for the presence of material derived from allergenic plants requires sensitive and unambiguous assays that are able to detect inter-species differences in the molecular structure of cell components. The most appropriate of these components is the DNA, and more specifically the highly variable noncoding regions. The laboratory exploits differences at the level of the nucleotide sequence as well as intron lengths of a marker gene to develop oligonucleotide sets to be used in PCR-based tests. Such sets have been designed for maize, rice, wheat, barley, soybean, peanut and sesame.

Allergens, which are often abundant proteins in edible parts of a number of plant species, can also be directly detected in food products using specific antibodies. To elaborate such protein-based tests the laboratory is developing monoclonal antibodies against Lipid Transfer Proteins which are potent allergens in the above-mentioned species.

### 2 LIM PROTEIN RESEARCH

Recent research at the PMB Unit had identified plant LIM domain proteins as novel actin bundlers (Thomas *et al.*, 2006). To understand the mechanism by which these proteins assemble and cross-link actin filaments, a mutant analysis has been performed which identified the individual LIM domains as being directly involved in this cross-linking (Thomas *et al.*, 2007). Cross-linking activities require the presence of two actin-binding sites per cross-linking unit. This can be achieved either by dimerization of single-actin-binding-domain proteins or by monomeric polypeptides containing two actin binding sites. Supporting the latter hypothesis, the laboratory has no evidence yet for dimerization of individual LIM domains. The identification of the actin-binding motifs in the LIM domains is under investigation.



Actin cytoskeleton of a tobacco cell visualized by rhodamin-phalloidin staining and the actin-binding protein WLIM1 fused to GFP (confocal microscopy observation)

Actin cable induced by the tobacco WLIM1 protein in vitro (electron micrograph)

The presence of plant LIM proteins in the nucleus as well as in the cytoplasm (Mundel *et al.*, 2000), and recent observations that plant LIM proteins can bind *in vitro* to specific DNA sequence motifs corroborate an earlier hypothesis that these proteins could participate in gene expression. Interestingly, the animal counterparts of plant LIM proteins, namely the Cysteine-Rich-Proteins (or CRPs), were not found to date to bind DNA, but they were shown to participate in transcription of muscle-specific genes by binding specific transcription factors. A target sequence for the



tobacco protein WLIM2 has been identified in the promoter region of a number of plant genes (Sabrina Gatti, PhD thesis), and the use of deletion mutants generated by site-directed mutagenesis has also identified the LIM domains as being responsible for DNA binding. It remains to be seen if this binding also occurs in the live cell and how it affects expression of the target genes. This raises the question regarding the identity of LIM protein-controlled genes and of direct interacting partners. Other open issues include the roles of plant LIM proteins in nuclear functions other than transcription: for instance, one of the tobacco proteins was detected in the nucleolus in a small fraction of cells (Sabrina Gatti, PhD thesis) and therefore likely also participates in a nucleolar activity.

### 3 CHINESE MEDICINAL HERBS

Following a call for proposals in the 7th Framework Programme of the EC in February 2007, the laboratory has participated, together with three other CRP-Santé laboratories (Neuro-Oncology, Retrovirology and Cardiovascular Research), in the setup of a Sino-European Consortium whose aims are to study the molecular basis of Traditional Chinese Medicine by identifying the cellular and molecular targets of active components present in Chinese medicinal herb extracts.

The nomination of Ning Wang, staff member of the PMB Unit, on the Scientific and Advisory Board of the above-mentioned Consortium and other international TCM-Research Committees provides unique opportunities for participations in international collaborative projects in the future.

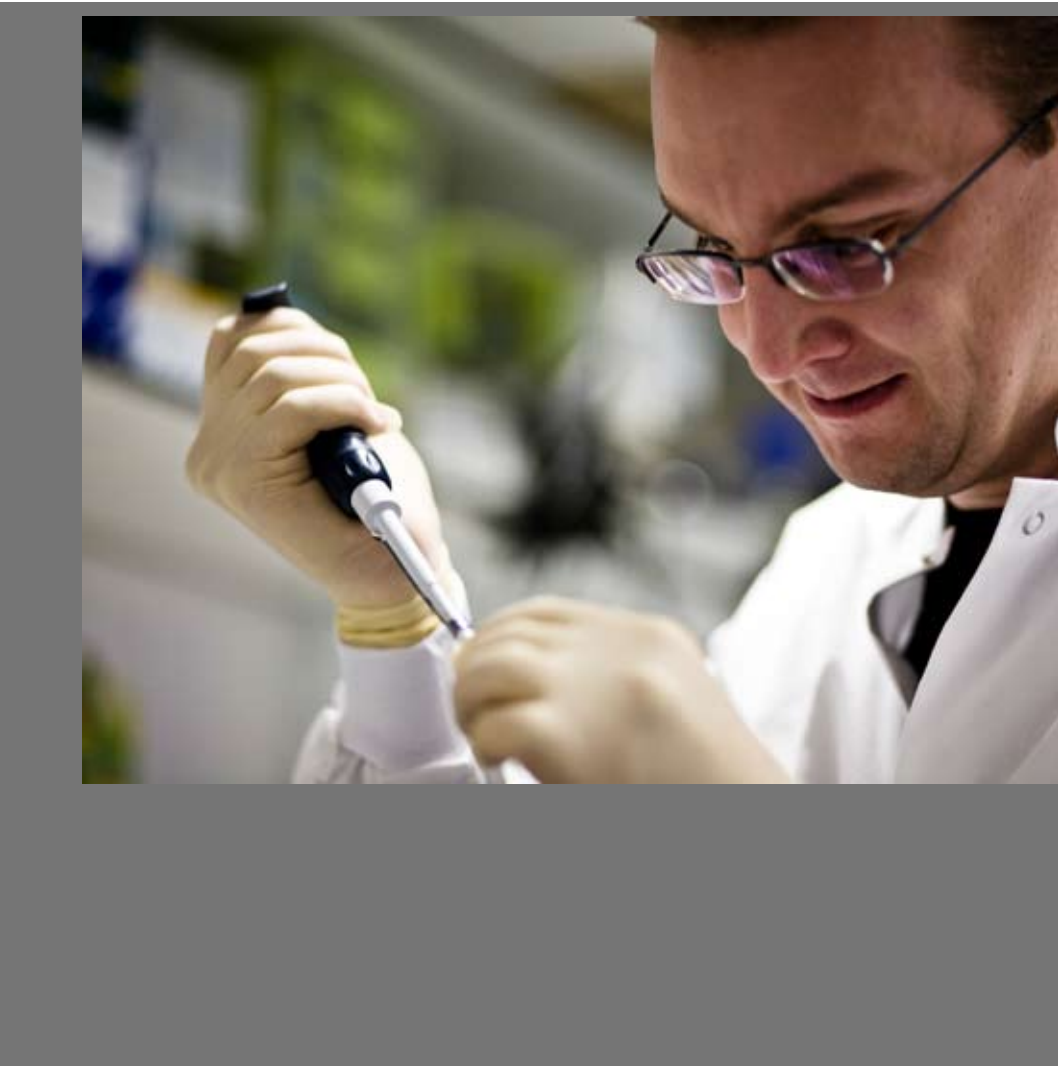
Such a collaboration, also involving the Gade Institute of the University of Bergen (Norway), the Modern Research Center for Traditional Chinese Medicine, SMMU Shanghai (China), and the Institute of Medicinal Plant Development (IMPLAD) Beijing (China), has been initiated recently. In this study, 40 purified secondary metabolites belonging to different chemical families (steroid derivatives, alkaloids, terpenoids), some of which with demonstrated anti-cancer activities, provided by SMMU will be tested by a joint NORLUX-PMB team at CRP-Santé in monolayer cancer cell and spheroid cultures. A comparative transcript analysis will be performed on treated and untreated cells at the Gade Institute in Bergen and the primary targets of the various compounds as well as the pathways affected will be identified. Active components will be tested in animals for anticancer activities at IMPLAD, Beijing.

### RELEVANT PUBLICATIONS

- Thomas C, Dieterle M, Gatti S, Hoffmann C, Moreau F, Papuga J and Steinmetz A (2008). Actin bundling via LIM domains. *Plant Signaling & Behavior*, in press.
- Xu L, Zhao Z, Dong A, Soubigou-Taconnat L, Renou JP, Steinmetz A and Shen WH (2008). Di- and tri- but not mono-methylation on histone H3 lysine 36 marks active transcription in *Arabidopsis thaliana*. *Molecular and Cellular Biology* 28, 1348-1360.
- Thomas C, Moreau F, Dieterle M, Hofmann C, Gatti S, Hoffmann C, Van Troys M, Ampe C and Steinmetz A (2007). The LIM domains of WLIM1 define a new class of actin bundling modules. *Journal of Biological Chemistry* 282, 33599-33608.
- Thomas C, Hoffmann C, Gatti S and Steinmetz A (2007). LIM proteins, a novel class of actin cytoskeleton organizers in plants. *Plant Signaling & Behavior* 2, 99-100.
- Liu S, Yu Y, Ruan Y, Meyer D, Wolff M, Xu L, Wang N, Steinmetz A and Shen WH (2007). Plant SET- and RING-associated domain proteins in heterochromatinization. *Plant Journal* 52, 914-926.
- Thomas C, Hoffmann C, Dieterle M, Van Troys M, Ampe C and Steinmetz A (2006). Tobacco WLIM1 is a novel F-actin binding protein involved in actin cytoskeleton remodeling. *Plant Cell* 18, 2194-2206.
- Lazarescu E, Friedt W, Horn R and Steinmetz A (2006). Expression analysis of the sunflower SF21 gene family reveals multiple alternative and organ-specific splicing of transcripts. *Gene* 374, 77-86.

### COLLABORATIONS:

Christophe AMPE and Marleen VAN TROYS, University of Ghent (Belgium)  
Christophe RITZENTHALER and Christina HOFMANN, IBMP Strasbourg (France)  
Wen-Hui SHEN and Lin XU (PhD student), IBMP Strasbourg (France)  
Wolfgang FRIEDT and Eduard LAZARESCU, University of Giessen (Germany)



## / LABORATORY OF TOXICOLOGY

Head of the laboratory:

Robert WENNIG, MD, PhD



Presently, the staff of the laboratory is as follows:

Brice APPENZELLER, PhD, Research Scientist

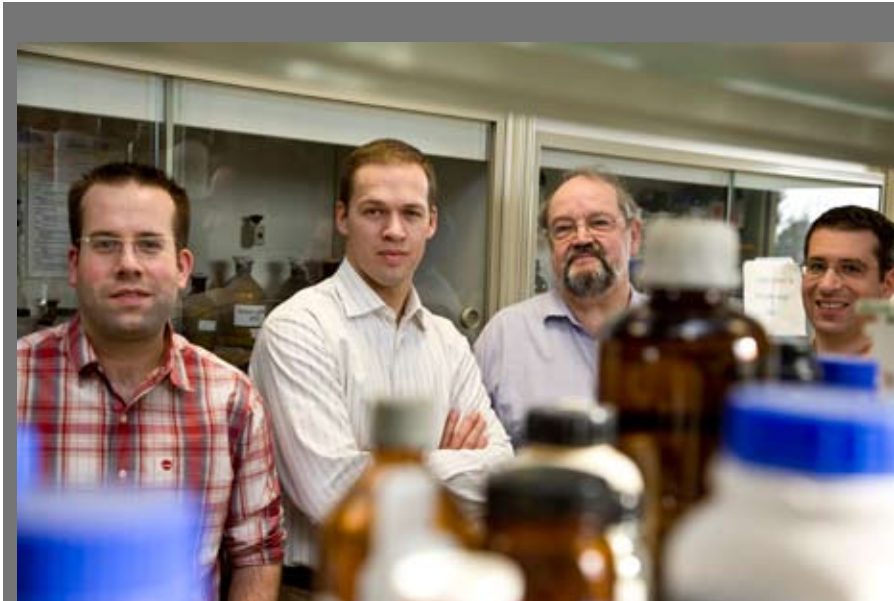
Marc SCHUMAN, PhD, Research Scientist

Liliane MARTINS, PhD Student

Claude SCHUMMER PhD Student

Michel YEGLES, PhD, LNS associated Research Scientist

Serge SCHNEIDER, PhD, LNS associated Research Scientist



### HISTORY OF THE LABORATORY

The Laboratory of Toxicology, which is part of the Laboratoire National de Santé (LNS), started its activity in 1978, with Pr. R. Wennig as head of department. In 1995, a CRP-Santé research group associated to the LNS was created in the laboratory.

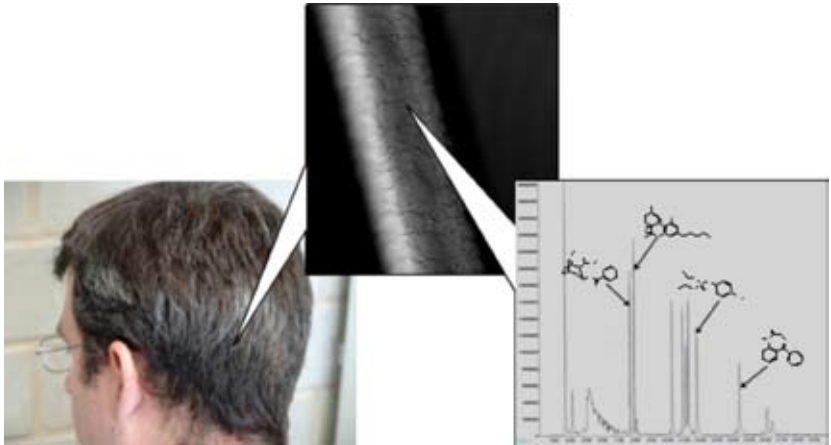
This time marked an increase in research activity with a first research project (1995-1997) mainly focused on the use of hair as a matrix for analytical toxicology.

**The use of hair for the detection of chemicals represented a revolutionary approach in the field of analytical toxicology at two main levels:**

- Firstly, the persistence of chemicals in hair allowed the detection of ingested toxicants long after they have been excreted from the body and are no more detectable in classical matrices such as urine or blood. As a consequence, "detection windows" are increased from hours or days up to months or years.
- Secondly, the detection of chemicals accumulated in hair represented the possibility to have a direct marker (the molecule itself) of a chronic exposure to substances, instead of indirect biomarkers such as modified proteins/enzymes that were previously used as diagnostic tools.

The first research topics concerned the detection of medicinal and illicit drugs and found applications in clinical and medico-legal domains for the assessment of toxicomaniac behaviour or to prove rapes under the influence of psychoactive substances for example.

New opportunities provided by hair analysis incited several laboratories in the world to develop competences in this specialised domain. This led to the creation of the international Society of Hair Testing (SoHT) in 1995, in which the laboratory of Luxembourg was involved as co-founder and board member. So far, the Laboratory of Toxicology is still a key player in the SoHT by participating to advisory and scientific committees and presenting several research results at SoHT meetings.



Macroscopic, microscopic, and molecular aspects of hair

- During the period from 1998 to 2001, the laboratory undertook research in order to assess the relevance of physico-chemical techniques for analytical toxicology issues.

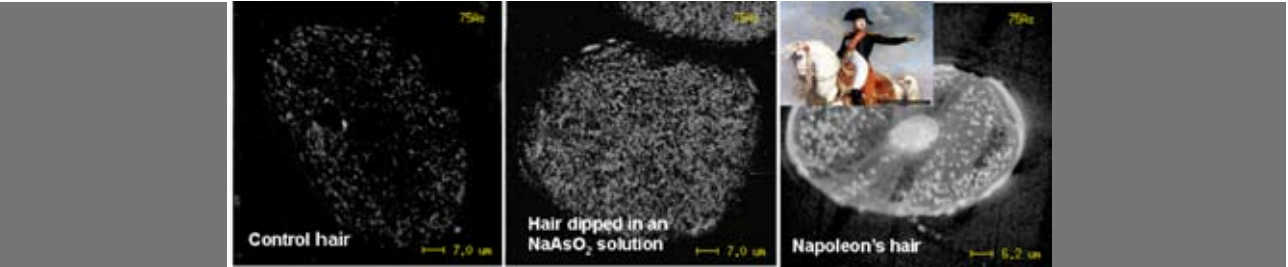
The application of techniques such as ToF-SIMS (time of flight secondary ion mass spectrometry) classically devoted to surface and material analysis, to the analysis of human specimens was initiated in order to gain in sensitivity and to detect trace levels of molecules present in hair.

The application of techniques such as ToF-SIMS (time of flight secondary ion mass spectrometry) classically devoted to surface and material analysis, was evaluated for the detection of trace levels molecules present in hair.

In parallel, a large part of the research activity was focused on the study of new biomarkers for the diagnosis of chronic alcohol abuse, by different complementary approaches: the determination of carbohydrate deficient transferrin (a seric protein), and the determination of ethyl glucuronide (a direct metabolite of ethanol) in hair. These new biomarkers displayed quite higher sensitivity and specificity than classical markers that were previously used in the field (mean corpuscular volume, gamma glutamyl transferases...) and found immediate interest among physicians in clinical settings and in withdrawal programmes.

At the same time, the laboratory initiated reflexions on the use of sweat and saliva as potential matrices for toxicological analysis. Experiments performed with these fluids demonstrated the possibility of detecting several medicinal and illicit drugs through complementary "detection windows", which contributed to an improvement in the therapeutic monitoring of patients.

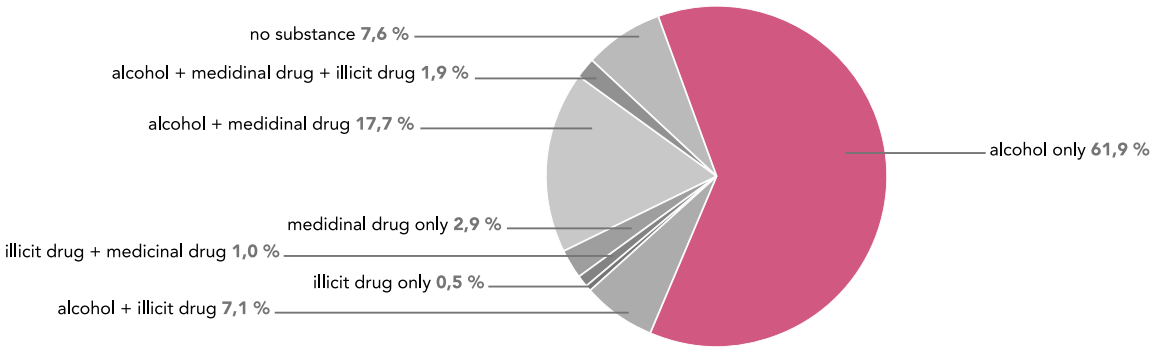
- During the 2002-2004 period, further research involving the use of material sciences techniques for toxicological issues was performed. Among the several studies published on the application of physico-chemical techniques to analytical toxicology must be noticed the work aimed at imaging arsenic traces in hair by nano-SIMS, and particularly the famous contribution provided by the Laboratory of Toxicology to the controversial debate on the possible poisoning of the Emperor Napoleon Bonaparte.



Nano-SIMS imaging of arsenic in a control hair specimen, a control hair spike with soluble arsenic, and a hair specimen from Napoleon Bonaparte.

This period was also marked by the application of analytical tools developed in previous research projects to public health issues such as driving safety. Indeed, epidemiological studies conducted on traffic offenders gave relevant information on the importance of chronic alcohol abuse among drivers in Luxembourg. Results also demonstrated that beside the alcohol problem, the incidence of illicit drugs and above all medicinal psychoactive drugs (anxiolytics, antidepressants, hypnotics...) was a critical problem observed among drivers in the GDL.

Owing to their important socio-economical impact, these studies were taken up by several newspapers in the GDL. Such findings are likely to inform authorities about particular public health issues and to help institutions in decisional processes.



Overview of psychoactive substances detected in apprehended drivers during 2001 and 2002 in the GDL.

- Since 2004, the Laboratory of Toxicology keeps on developing new methodologies based on the analysis of alternative matrices, in order to improve the therapeutic monitoring of patients and the identification of hazardous behaviour associated to drugs consumption.

In parallel, an enduring effort is performed for the application of methodologies previously developed to public health issues:

On a local scale, the laboratory collaborated to programmes such as *Jugend an Drogenh  lfe*, aimed at identifying hazardous drugs consumption among drug addicts in treatment by saliva analysis. Such a contribution represents a considerable support for physicians in the assessment of patient health, and gives a further advantage for the success of withdrawal programme.

At the international level, the laboratory participates in humanitarian actions developed to help African countries. As an example, the AMATA collaboration with Rwanda was developed to assess the transfer of antiretroviral drugs from HIV positive mother under treatment to child by breastfeeding, in order to assess side effects and child protection from contamination.

More recently, the Laboratory of Toxicology extended its research activity to questions related to the impact on human health of exposure to environmental pollutants (due to transports, industry, agriculture ...). Indeed, consequences of such exposure reach several levels including cancer development, neuropathies, reproductive disorders, allergic symptoms and defects in immune system functions.

In that regard, experience acquired in clinical and medico-legal domains in the use of the different human matrices is valorised in the biomonitoring of human exposure to pollutions. Information obtained from biomonitoring is thought to help identifying exposed populations and exposure routes in order to take action to preserve people's health.

## CURRENT KEY OBJECTIVES

Our laboratory has acquired valuable skills in the development of highly sensitive analytical methodologies for the detection and quantification of xenobiotics in classic and more importantly, in alternative matrices, especially hair, sweat and oral fluids.

Some of the developed techniques are now routinely used in our laboratory in diagnosis and therapeutic monitoring of patients needing a regular and precise medical treatment (alcohol withdrawal, methadone substitution programme, tri-therapeutic treatment of HIV-positive patients, etc.).

### Current and future R&D activities:

- Application of scientific advances, derived from internal and external work, to public health problems related to toxicology (drug addiction, chronic alcohol abuse, therapeutic drug monitoring, ...).
- Research on new biomarkers related to chronic intoxication.
- Study of biomolecular dynamics (proteins, endogen molecules...) to identify fundamental mechanisms responsible for pathologies and determine research routes for treatment, and to develop new diagnostic tools.
- Development of methodologies based on hair analysis for the biomonitoring of human exposure to environmental pollutants.
- Development of analytical methods to detect xenobiotics in sweat, in order to perform monitoring of therapeutic treatment / drugs consumption / exposure to toxics and pollutants in non-invasive way.
- Improvement of therapeutic monitoring in order to adapt treatments to individual metabolic specificities.
- Assessment of drug consumption tendencies in the general population by analysis of waste water.

The laboratory disposes of a large analytical platform including gas chromatography coupled with tandem mass spectrometry (GC-MS/MS), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), high pressure liquide chromatography (HPLC) with UV detection and capillary electrophoresis (CE-UV).

## MAIN RESULTS

Our research efforts are mainly valorised in public health domains such as medical follow-up or diagnosis. Analytical developments and research results are continuously integrated into the routine analytical services of the Toxicology Division of the National Health Laboratory (LNS), which offers its services to general practitioners, hospital services and legal services.

The Laboratory of Toxicology keeps close links with medical teams of the Centre Hospitalier de Luxembourg (CHL). Analytical competencies of the laboratory are then regularly requested to support CHL and other local hospitals on punctual atypical questions.

Through the organisation (locally) and participation (internationally) at scientific conferences, our research results are communicated to directly interested professionals (medical staff, scientists...).

In addition, the laboratory actively participates in higher education by accepting interns and doctoral students, giving lectures and practicals in analytical and organic chemistry.

## NATIONAL AND INTERNATIONAL COLLABORATIONS

### 1 MARKERS OF CHRONIC ALCOHOLISM IN HAIR:

- Forensic Medicine Institute, Humbolt University (Pr. Pragst, Berlin)
- Therapeutic Center of Useldange (P. Neuberg, Useldange)
- Institut Universitaire de Médecine Légale, Laboratoire de Toxicologie et de Chimie Forensiques (Dr. M. Augsburger)
- Lehrstuhl Tierphysiologie, Universität Bayreuth (Dr. F. Wiens)

### 2 ANALYTICAL METHODOLOGY ON ANTI-RETROVIRAL DRUGS:

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

### 3 DETERMINATION OF VARIOUS DRUGS AND BENZODIAZEPINES IN SALIVA:

- Methadone substitution programme (Dr. De Winter J-P., Dr. Staut W.)

### 4 DETECTION OF AMPHETAMINES AND CONGENERS IN HAIR:

- Federal Public Service Justice, National Institute of Criminalistics and Criminology (NICC), Brussels, Belgium (Dr. Samyn N)

### 5 BIOMOLECULAR DYNAMICS OF SERUM PROTEIN:

- Laboratoire d'Immunologie CRP-Santé, Luxembourg (Pr. C. Muller)



6 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)
- Centre d’Etudes en Santé (CES, CRP-Santé)

7 IDENTIFICATION AND QUANTIFICATION OF DRUGS AND THEIR METABOLITES IN SURFACE AND WASTEWATER. ESTIMATION AND MONITORING OF DRUG CONSUMPTION IN THE GRAND DUCHY OF LUXEMBOURG.

- Syndicat Intercommunal de Dépollution des Eaux Résiduaire de l’Ouest (SIDERO)
- Syndicat Intercommunal de Dépollution des Eaux Résiduaire du Nord (SIDEN)

- Kupper J., Schuman M., Wennig R., Gorber U., Mittelholzer A., Artho R., Meyer S., Kupferschmidt H. and Naegeli H. 2007. Cyanide poisoning and increased thiocyanate levels in cows after feeding the soft hulls of apricot kernels. *The Veterinary Record*. In press.
- Wurst M.F., Yegles M., Alling C., Aradottir S., Dierkes J., Wiesbeck G., Halter C., Pragst F., Auwärter V. 2007. Measurement of direct ethanol metabolites in a case of a former driving under influence (DUI) of alcohol offender, now claiming abstinence. *International Journal of Legal Medicine*. In press.
- Schummer C., Appenzeller B.M.R., Wennig R. Quantitative determination of ethyl glucuronide in sweat. Submitted to *Therapeutic Drug Monitoring*.
- Appenzeller B.M.R., Schuman M., Wennig R. Was a child poisoned by ethanol? Discrimination between ante-mortem consumption and post-mortem formation. Submitted to *International Journal of Legal Medicine*.
- Schneider S., Peltier A., Gras A., Karasi – Omes C., Arendt V., Mujawamariwa A., Ndimubanzi Cyaga P., Wennig R. Efavirenz in human breast milk, mothers’ and newborns’ plasma. Submitted to *JAIDS*.
- Wurst F.M., Dürsteler-MacFarland K.M., Pragst F., Auwärter V., Yegles M., Halter C., Weinmann W., Ergovic T., Wiesbeck G. Assessment of alcohol use among methadone maintenance patients by direct ethanol metabolites and self-reports. Submitted to *Alcoholism: Clinical and Experimental Research*.

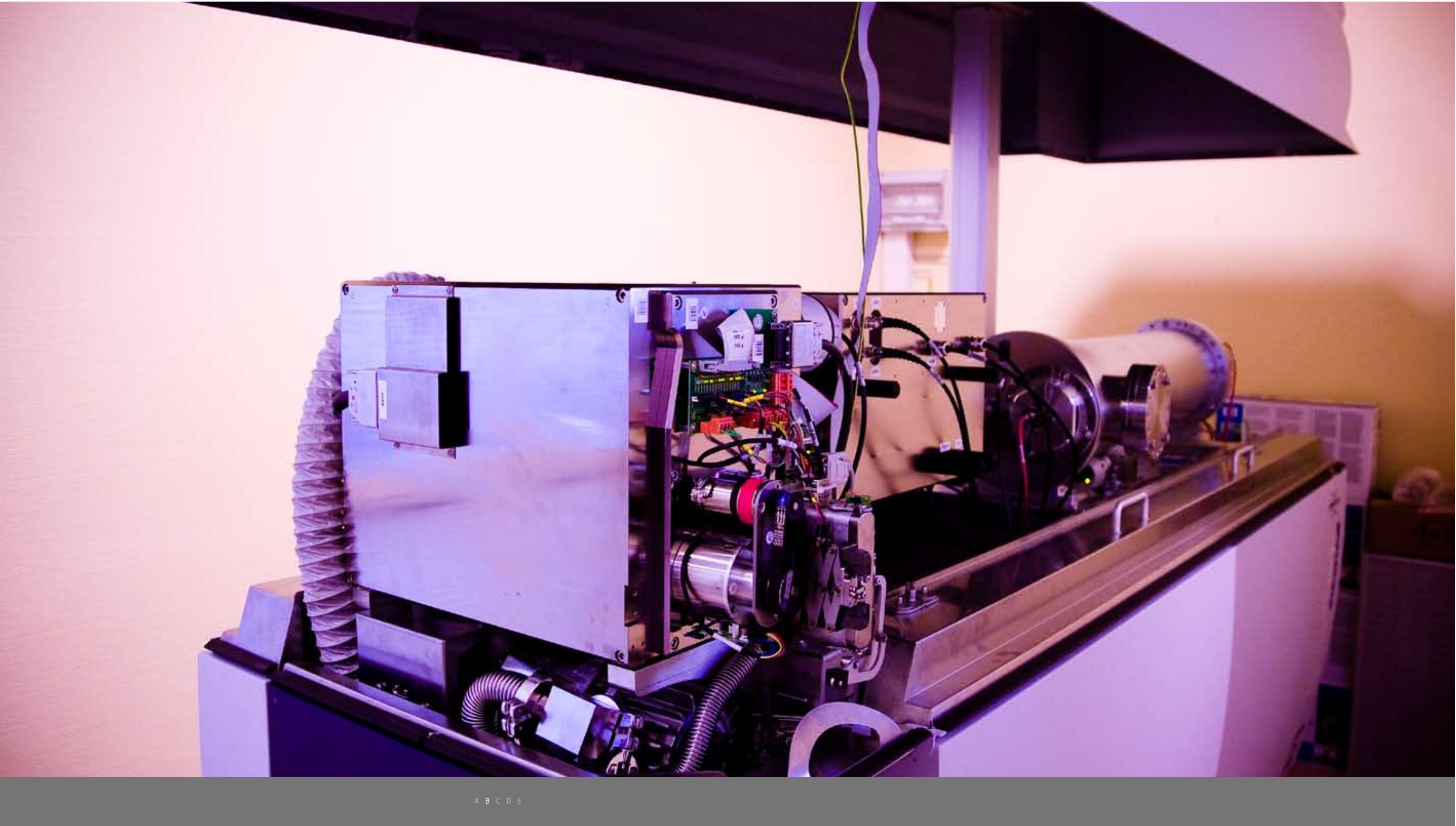
PUBLICATIONS 2007

- Appenzeller B.M.R., Agirman R., Neuberg P., Yegles M., Wennig R. 2007. Segmental determination of ethyl glucuronide in hair: a pilot study. *Forensic Science International*. 173(2-3): 87-92.
- Appenzeller B.M.R., Schummer C. Boura Rodrigues, Wennig R. 2007. Determination of the volume of sweat accumulated in a sweat-patch using sodium and potassium as internal reference. *Journal of Chromatography B*. 852(1-2): 333-337.
- Appenzeller B.M.R., Schuman M., Yegles M., Wennig R. 2007. Ehtyl glucuronide determination in white and pigmented hair. *Alcohol and Alcoholism*. 42(4): 326-327.
- Frascht M., Schneider S., Schuman M., Wennig R. 2007. Formation of scopolamine from N-butylscopolammonium bromide in cigarettes. *Journal of Analytical Toxicology*. 31(4)220-223.
- Lemmer P., Schneider S., Mühe A., Wennig R. Quantification of lorazepam and lormetazepam in human breast milk using GC-MS in the negative chemical ionization mode. *Journal of Analytical Toxicology*. 31(4): 224-226.
- Martins L., Yegles M., Samyn N., Ramaekers J.G., Wennig R. 2007. Time-resolved hair analysis of MDMA enantiomers by GC/MS-NCI. *Forensic Science International*. 172(2-3):150-5.
- Yegles M.and Wennig R. 2007. “Opioids testing in hair”, in Analytical and pratical aspects drug testing in hair, P. Kintz ed CRC Press Taylor & Francis (Boca Raton, London, New-York). 73-95, 2007.
- Pragst F.and Yegles M. 2007. “Alcohol markers in hair” in Analytical and pratical aspects drug testing in hair, P. Kintz ed CRC Press Taylor & Francis (Boca Raton, London, New-York). 287-324.
- Martins L., Yegles M., Wennig R. Simultaneous enantioselective quantification of methadone and of 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine in oral fluid using capillary electrophoresis. *Journal of Chromatography B*. In press (online 12 Nov).
- Martins L., Yegles M., Thieme D., Wennig R. Influence of bleaching on the enantiomeric disposition of amphetamine-type stimulants in hair. *Forensic Science International*. In press (online Nov 12).
- Schneider S., Ait-m-bark Z., Schummer C., Lemmer P., Yegles M., Appenzeller B., Wennig R.. 2007. Determination of fentanyl in sweat and hair of a patient using transdermal patches. *Journal of Analytical Toxicology*. In press.
- Pragst F. and Yegles M. 2007. Determination of Fatty Acid Ethyl Esters (FAEE) and Ethyl Glucuronide (EtG) in Hair – a Reliable Way for Retrospective Detection of Alcohol Abuse During Pregnancy. *Therapeutic Drug Monitoring*. In press.
- Kelso E., Sundström Poromaa I., Weinmann W., Pragst F., Yegles M., Wurst F.M. 2007. Measurement of Direct Ethanol Metabolites suggest higher rate of alcohol use among pregnant women, than found with the AUDIT – a pilot study in a population-based sample of Swedish women. *American journal of obstetrics and gynecology*. In press.
- Wennig R.and Verstraete A. “Drugs and driving” in Handbook of Analytical separations vol III - Forensic Science, M. Bogusz ed Elsevier Amsterdam. In press.



RESEARCH DEPARTMENTS

- DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY
- DEPARTMENT OF IMMUNOLOGY
- DEPARTMENT OF ONCOLOGY
- DEPARTMENT OF CARDIOVASCULAR DISEASES
- DEPARTMENT OF PUBLIC HEALTH



/ DEPARTMENT OF IMMUNOLOGY

Head of the laboratory:

Claude P. MULLER, MD, MS



Presently, the staff of the laboratory is as follows:

Ulla MULLER, Administration  
Carole WEIS, Administration  
Vitor LOPES, Technical Core Facility  
Nathalie RODENBOUR, Technical Core Facility

Virology:

Jacques KREMER, PhD  
Judith HÜBSCHEN, PhD  
Marianne DUCATEZ, PhD  
Denis KAINOV, PhD  
Christophe OLINGER, PhD Student  
Julia KESSLER, PhD Student  
Nancy GERLOFF, PhD Student  
Anna REYE, PhD Student  
Iris ANDERNACH, PhD Student  
Sebastien DE LANDTSHEER, Technician  
Emilie CHARPENTIER, Technician  
Aurélien SAUSY, Technician  
Patrick MBAH, Visiting Scientist  
Edith NKWEMBE, Visiting Scientist  
Joseph FORBI, Visiting Scientist  
Bello ALKALI, Visiting Scientist  
Burul CHOPOBAEVA, Visiting Scientist  
Kalia KASYMBEKOVA, Visiting Scientist  
Bart TUMMERS, Trainee as MS Student  
Veronique VENARD, Sabbatical

Psychoneuroimmunology:

Jonathan TURNER, PhD  
Joana MACEDO, PhD Student  
Andrea SCHOTE, PhD Student  
Simone ALT, PhD Student  
Lei CAO, PhD Student  
Laetitia PELASCINI, Technician  
Caroline MOUTON, Trainee and Summer Student

Massspectrometry and Proteomics:

Fred FACK, PhD  
Patrick PIRROTTE, PhD Student  
Anja BILLING, PhD Student  
Dominique REVETS, Technician

Peptide and hapten chemistry:

Emmanuel PRODHOMME, PhD

Vaccinology :

Nathalie Grova, PhD  
Fabienne Bouche, PhD  
Stéphanie Willieme, Technician  
Sophie Farinelle, Technician  
Tom Bechet, PhD Student  
Mario Schellenberger, PhD Student  
Lei Cao, Trainee as MS Student



HISTORY OF THE LABORATORY

The Department of Immunology is a department of the Laboratoire National de Santé (LNSI) and the CRP-Santé. The department was de facto created in 1992. Since then it has gradually expanded its scientific activities with the support of competitive research grants from national (CRP-Santé, Ministère de la Recherche, Ministère de la Santé, Ministère de la Coopération, Fonds National de la Recherche, Foundations) and international sources (the European Union DGXII and DGXIII, the World Health Organisation, diagnostic and vaccine industry). By the end of 2001, a contract was signed between the LNS and the CRP-Santé, which serves as a basis for the joint operation of the Department. This contract has been updated in 2006 and completed by a specific contract in 2007.

Academically, the department is affiliated with:

- the Graduate School of Psychobiology of the University of Trier, where the head of department holds the chair of immunology, and the International Research Training Group (IRTG) jointly established with the University of Trier and the University of Leiden
- the Medical Faculty in Homburg/Saar of the University of Saarbrücken (HOD: Associate Professor of Experimental Medicine)
- the Graduate School of Biology and Environmental Sciences of the University of Nancy
- the University of Ibadan, Nigeria (HOD: Associate Visiting Professor)



Training of Ph.D., M.D. students and laboratory staff of the WHO Laboratory Network and from resource-poor countries are important activities.

As a WHO Collaborating Center and WHO European Reference Center for Measles and Rubella the Department has a high profile with the WHO and within the WHO Laboratory Network. The department serves as consultants to International Organisations such as WHO (HOD: member of WHO Steering Committee for Measles), the World Bank and the European Union 3-7th Framework Programme, scientific departments and companies.

In 1993, the group had a staff of four. Today, the department of Immunology has grown to a staff of about 30 scientists, engineers, technicians, Ph.D. and M.D students, as well as undergraduate students and support staff. The department is one of the most productive biomedical research groups in Luxembourg, with about 14-16 peer-reviewed international scientific publications per year (18 in 2007, see Section 6), and 50-60 annual contributions to scientific conferences and invited oral presentations per year.

### THE CUMULATIVE OUT-PUT 1991- 2007:

- 128 publications in peer reviewed international scientific journals
- 293 contributions to congresses
- 146 invited conferences
- 1 analytical product commercialised
- 3 international patents
- 14 PhD graduated + 13 in progress
- 19 MD graduated + 1 in progress

Theses for University Degrees (Diploma, DESS, etc.) and doctoral thesis of which important parts are done at the department of Immunology: 31 finished (16 in progress).

The activities range from basic research to contract R&D for the diagnostic and vaccine industry in areas that are important for public health and relate in some ways to the immunology of viruses, vaccines and the neuroendocrine system.

### HIGHLIGHTS 1992-2007:

- **1992.** The beginning of the Laboratory of Immunology within the labs of the Department of Microbiology of the LNS
- **01/92.** The first research project with the CRP-Santé
- **04/93.** The first European Union BIO-TECHNOLOGY project
- **03/95.** Sabin Prize for Vaccinology of the Sabin Foundation, Connecticut, USA and SmithKline Beecham, Rixsensart, Belgium
- **06/95.** Defense of first of so far 19 M.D. thesis prepared in the department (total 19 plus 1 in preparation)
- **12/95.** HOD appointed Professor at the University of Tübingen
- **03/96.** The department moves into own laboratories

- **06/96.** Förderpreis der Stiftung zur Förderung der Deutsch-Luxemburgischen Zusammenarbeit auf dem Gebiet der Wissenschaften, Deutsche Bank, S.A., Luxemburg
- **07/97.** Defense of first of so far 14 Ph.D. thesis (P. Fournier); 13 more are in preparation
- **08/96.** Submission of first of so far three patents
- **02/98.** The department is nominated one of four WHO Collaborative Centers for Measles
- **02/98.** The first field study in molecular epidemiology in West Africa (Nigeria)
- **12/98.** Signing of the first contract with the Industrial INNOVATION Programme, DGXIII, EU
- **02/99.** Contract with WHO for development of reference/research reagents
- **03/99.** HOD appointed Temporary Scientific Advisor to the WHO Measles Programme
- **04/99.** HOD appointed Visiting Professor of Immunology at the University of Ibadan, Nigeria
- **06/99.** First research contract with diagnostic industry
- **11/99.** Extension of laboratory space for contract research
- **11/99.** First research contract with the vaccine industry
- **2001.** Project EU-Demonstration 970273, coordinated by CP Muller, wins special recognition at the BIOTECHNICA 2001 in Hannover among several hundred projects funded by the Fourth Framework Programme
- **03/02.** Association with the *Ecole Doctorale* BIOSE of the University of Nancy
- **12/2002-2007.** Member of the WHO Steering Committee for Measles. This 7-member committee advises the WHO on strategies for world-wide control of measles
- **10/2002-2005.** Member of the Board of the Fraunhofer Centre for Molecular Biotechnology and Vaccinology, Philadelphia, USA
- **12/02.** Accreditation as National Reference Laboratory for Measles and Rubella by the Ministry of Health and the World Health Organisation (WHO)
- **03/03.** Accreditation as Regional Reference Laboratory for M/R of the WHO for 24 countries of the European Region.
- **07/03.** VIRIM, a Network of Excellence in Viral Immunology, coordinated by the Department was awarded the Innovation/Network Award by the Ministers and Presidents of the 7th Sommet of the Greater Region SarLorLux (including Rheinland-Pfalz and Wallonie)
- **12/03.** Dr. B. Oyefolu is awarded for the best PhD thesis in 2003 by the Government of Nigeria. Critical parts of the thesis were done in the Department of Immunology, Luxembourg
- **08/03.** M. Mulders Ph.D., scientist at the Department is invited to join the WHO European Regional Office in Copenhagen, DK
- **06/03.** HOD is appointed Associate Professor of Experimental Medicine of the University of Saarland (Homburg), Germany
- **08/05.** Invitation of HOD by the Government of Guangxi Province (China) as an external expert to advise the provincial surveillance programmes for avian influenza and SARS
- **Since 2005** Department scientists serve as WHO-evaluators of National Reference Laboratories in the EURO Region, including NIS states

- **02/06.** Rapid intervention of Department scientists in Nigeria to establish the first laboratory to diagnose H5N1, 2 weeks after bird flu was first reported from Africa. Results were published in *NATURE* as MF Ducatez, CM Olinger, AA Owoade, S De Landsheer, W Ammerlaan, HGM Niesters, ADME Osterhaus, RA Fouchier, CP Muller. Avian flu: multiple introductions of H5N1 in Nigeria. *Nature* 442, 37, 2006
- **04/06.** Interview published by *NATURE* on H5N1 in Nigeria (D. Butler. H5N1, from the front lines: Nigeria. CP Muller. *Nature* 440,726-727, 2006)
- **03/06.** Rapid intervention of Department scientists in Burkina Faso to establish the first laboratory to diagnose H5N1, 1 month after bird flu was first reported from the country
- **2006 and 2007.** Laboratory surveillance of AIV in Luxembourg. Perfect score in EU-wide test panel in all categories
- **Since 2005** department scientists serve as invited expert for AIV, SARS in Guangxi Province, China, EU 7th framework, FAO (Nigeria), Oyo State Government (Nigeria), Kyrgystan, Bosnia-Herzegovina and the Federal Government of Nigeria (after the first human case of AIV emergence in the country)
- **04/07.** Publication in *Emerging Infectious Diseases* of the first findings on avian influenza virus (AIV) in wild birds in Africa (Ducatez et al 2007)
- **06/07.** The University of Trier, the University of Leiden, The Netherlands and the Department of Immunology have jointly established the International Research Training Group (IRTG) "Psychoneuroendocrinology of stress : from molecules and genes to affect and cognition". IRTGs are prestigious international graduate schools funded by the DFG, Germany
- **06/07.** CP Muller was invited as *Helen C. Levitt Visiting Professor* to the Mayo Clinic, Rochester, USA
- **07/07.** Appointment of CP Muller by the World Bank as expert for the reorganisation of the AIV National Reference Laboratory in Bishkek and Osh, Kyrgystan
- **11/07.** The department inaugurates its new P3 laboratory facilities

## CURRENT KEY OBJECTIVES

### 1 IMMUNOLOGY, GENETIC/ANTIGENETIC DIVERSITY OF VIRUSES

- To identify new variants of human and veterinary viruses and to understand their genetic and antigenetic diversity, consequences for surveillance and their virus-host interactions.
- To study public health issues related to infectious diseases in humans (measles virus, rubella virus, parvovirus, hepatitis viruses, influenza virus and other respiratory viruses, tick-borne pathogens).
- To study public health and animal welfare issues related to veterinary viruses, in particular avian viruses including avian influenza virus.

### 2 THE IMMUNE SYSTEM, THE NEUROENDOCRINE SYSTEM AND STRESS

- To understand the effect of stress response mediators on the immune system, and the interaction between the immune system and the neuroendocrine system
- To understand the crosstalk between nuclear receptors and immune receptors; transcriptional and translational regulation of nuclear receptors

### 3 IMMUNOPROPHYLACTIC STRATEGIES

- To develop immune-preventive strategies against infectious agents and against environmental risk factors, such as carcinogens. The strategies are based on novel conjugate vaccines, peptides and recombinant designer proteins based on T and B cell epitopes.

## ONGOING PROJECTS AND MAIN RESULTS

### 1 IMMUNOLOGY, GENETIC/ANTIGENETIC DIVERSITY OF VIRUSES

- An important task of the department of Immunology is the surveillance of infectious diseases and molecular characterisation of human and veterinary viruses world-wide, including in particular also avian influenza. In collaboration with partners in Sub-Saharan Africa, (Nigeria, Burkina Faso, Democratic Republic of the Congo, Cameroon, Central African Republic, Mali, Benin, Niger) South-East-Asia (Vietnam, China, Laos and Thailand) and the New Independent States (i.e. the former Soviet Republics such as Russia, Belarus, Kyrgyzstan). The programmes are tailored towards the needs of resource-poor countries and offer anything from technical protocols to training, reagents, consumables, equipment, laboratory infrastructure and technical advice to enhance the laboratory surveillance capacity for outbreak investigation and control. Our interventions combine laboratory surveillance with capacity building and training. We have provided technical and scientific support (Lagos, Ibadan, Abuja, Nigeria; Niamey, Niger; Bobo Dioulasso, Burkina Faso; Bafousam, Cameroon) and rehabilitated existing laboratories (Nigeria, Burkina Faso).

Over the past 10 years we have trained more than 30 laboratory and scientific personnel from 10 countries in molecular detection and characterisation of viruses. More than 1000 students and staff from West-African universities have attended the Course on Molecular and Cellular Immunology given by Prof. Muller since 11 years in Ibadan Nigeria. Both human and veterinary viral diseases are targeted. We also serve as a WHO training centre.

The infectious disease team can be deployed rapidly in response to outbreaks or to intervene in disaster situations that are prone to facilitate such outbreaks. Specific interventions of the Avian Influenza Response Team included Ibadan, Nigeria 2006, Burkina Faso 2006, Niger 2007, Bosnia Herzegovina 2007. On-site Laboratory Accreditation visits of National and Subnational Reference Laboratories in Bosnia-Herzegovina, Macedonia, Blagoveshensk (Russia), Bishkek (Kyrgyzstan), Banya-Luka, Sarajevo. Infectious Disease Programme evaluation: Russia 2005, Blagoveshensk (WHO), China 2005, Nanning (Chinese CDC, Guangxi Province). Government Consulting (avian influenza): Provincial Government Guangxi, China, Special Disease Unit, Abuja, Nigeria; Ministry of Agriculture, Bishkek, Kyrgyzstan. Outbreak investigation included Nigeria 1998, 2002, 2006, 2007, Burkina Faso 2001, 2006, Democratic Republic of Congo (DRC) 2002, Romania 2006, Serbia 2007, Bosnia Herzegovina 2007.

- **Measles and Rubella:** the Department of Immunology has earned an international reputation for its work on morbilliviruses, in particular, issues related to the goal of WHO to control and eliminate measles world-wide by optimized vaccination strategies (Muller et al. 2001; Mulders et al. 2001). The Department was appointed in 1998 one of world-wide four WHO Collaborating Centers for Measles. In 2004 the Department became WHO Reference Center for the European Region for Measles and Rubella with responsibilities for 23 countries including the Mediterranean countries, the Balkan States and others. Department scientists are called upon to investigate measles outbreaks (e.g. Blagoveshensk, Russia; Sarajevo, Bosnia Herzegovina), Department staff (Dr. M Mulders) has been seconded to support the WHO EURO office in Copenhagen, Denmark. Since 2002 until the end CP Muller served as a member of the WHO Steering Committee for Research related to Measles (Muller et al. 2007).

This 7-member committee advised the WHO on strategies for world-wide control of measles.

The committee has been retired in 2007, when the morbidity and mortality of measles had come down significantly. Department scientists serve as Temporary Scientific Advisors to different ad hoc WHO Committees and peer-review research projects submitted to WHO. Joint research projects on new diagnostic tests and the training of personnel of the WHO Laboratory Network are other areas of collaboration. The Department regularly also contributes to technical reports and publications (WHO anonymous 2007, 2006, Kremer et al. in press) under the WHO label and has published a number of books chapters in reference books on measles (Muller and Mulders 2002; Muller 2001; Muller and Putz 2005; Muller and Kremer 2007).

Scientifically, the Department has been involved in surveillance and molecular characterization of measles (Rota et al. 2006, Mulders et al. 2001) and rubella (Huebschen et al. 2007) in Europe (Schierup et al. 2005, Hanses et al. 2000, Kremer et al. in press), New Independent States, Asia (Kremer et al. 2007, Shulga et al. 2006, Kremer and Muller 2003) and West-Africa (Mulders et al. 2003). Three of the 5 new genotypes of measles have been discovered by us in Nigeria (B3), Nepal (D6, Truong et al. 2001) and Indonesia (G2, deSwart et al. 2000).

Issues related to the immune-targets (epitopes) of the humoral response (Ertl et al. 2003; Bouche et al. 2002, Muller 2001, Klingele et al. 2000), the waning of measles/rubella immunity in infants (Hartter et al. 2000) and adults (Kremer et al. 2006a, 2006b, Oyedele et al. 2005), the epidemiology of measles (Oyefolu et al. 2007; Mossong et al. 2003, Mossong and Muller 2000), the development and testing of new protocols for the WHO laboratory network (Kremer et al. 2005, Kremer et al. 2004, Hartter et al. 2000), the management of measles cases (Kremer and Muller, 2007; Grancher et al. 2004).

- **Hepatitis B Virus:** genetic variants of Hepatitis B virus have been investigated in NIS (Olinger et al. in press), Africa and SE-Asia. Sub-Saharan Africa suffers from an excessively high endemicity of hepatitis B virus (Mulders et al. 2004), but until recently little was known about the prevalent genotypes. We investigated extensively HBV strains (Olinger et al. 2007), sequences from 15 locations in Mali (Maïga et al. 2005; 2003), Burkina Faso, Togo, Benin, Nigeria (Odemuyiwa et al. 2001), Cameroon, Democratic Republic of Congo and Central African Republic (Bekondi et al. 2007). 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 (Mulders et al. 2004, Olinger et al. 2007). 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 (Olinger et al. 2006). 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 (Mulders et al. 2004). 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 (Mulders et al. 2004; Olinger et al. 2007).

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. A new genotyping technique was developed in collaboration with China for resource-poor countries (Zhang et al. 2007; Pei-qiong et al. in press).

- **Avian Influenza in birds and humans:** the AIV team is responsible for the surveillance of AIV in Luxembourg. No H5N1 or low pathogenic AIV has been found in Luxembourg so far. In the EU test panel the Department had a perfect score in all categories.

The Luxembourg-Nigerian Poultry Virus Surveillance Network showed in the first seroepidemiological study in Sub-Saharan Africa that low pathogenic AIV is not common in African poultry (Owoade et al. 2006).

With the emerging highly pathogenic avian influenza (HPAI) H5N1, the Department has set up with the support of the Ministry of Cooperation and the Ministry of Health the AIV response team for rapid deployment. When H5N1 was reported for the first time from Sub-Saharan Africa in February 2006, the team responded to an urgent request by FAO and the University of Ibadan to set-up a diagnostic laboratory. Five days later the AIR-Team landed in Lagos with a ton of equipment, diagnostic reagents, containment equipment, protective gear to set up the first laboratory for molecular detection of HPAI H5N1 (Butler, *Nature* 440, 726-727, 2006). The team showed that, surprisingly, several lineages of HPAI H5N1 were introduced simultaneously into the country. This finding was also published in *Nature* (Ducatez et al. Avian flu: multiple introductions of H5N1 in Nigeria. *Nature* 442, 37, 2006b; Ducatez et al. 2006a, 2007a) and was reported worldwide by most major news agencies and > 300 of the most important newspapers including Washington Post, New York Times, Le Monde etc.

In April 2006, the team responded to a similar request from Burkina Faso and Niger and organised a training course in Burkina attended by laboratory staff from both countries.

These investigations lead to the first reports of AIV in wild-birds in Sub-Saharan Africa: Vultures can serve as conspicuous sentinel animals similar to swans in Europe and can be vectors of AIV transmission (Ducatez et al. 2007b). In collaboration with the University of Hong Kong, we investigated synergies of AIV with other poultry viruses and the role of live bird markets (Ducatez et al. 2006c).

Laboratory staff also from several other countries including Kyrgyzstan (World Bank Programme) and Egypt was trained in our Department in AIV laboratory surveillance. Department scientists served as invited expert for AIV and SARS for the Guangxi Province, China, the EU 7th framework, FAO, Oyo State Government, Federal Government of Nigeria, the Ministry of Agriculture of Kyrgyzstan. Peer-reviewing of AIV projects, manuscripts and programmes is steadily increasing. The Department is a partner and workpackage coordinator of COST B28 (BSL3 and BSL4 pathogens).

- **Other avian/poultry viruses:** in many developing countries diseases in poultry are a major obstacle to the production of high quality protein for human consumption. Since about 8 years the Department collaborates with veterinary laboratories in West Africa and Asia to set up surveillance capacity and to train staff and students in laboratory surveillance of avian viruses. The first seroepidemiological study of avian viruses in commercial poultry in Sub-Saharan Africa was conducted between 2001-2004 (Owoade et al. 2006, 2004a; Ducatez et al. 2005, 2006c). The techniques for the detection and molecular characterisation have been established for a number of viruses including avian influenza (see Section 4.6), infectious bursal disease virus (IBDV, Owoade et al. 2004b), infectious bronchitis virus (IBV), chicken anaemia virus (CAV, Ducatez et al. 2006d), infectious laryngotracheitis virus (ILT), avian metapneumovirus (aMPV), coronaviruses, Avian Paramyxovirus (NDV), and others.





For many of these viruses we demonstrated for the first time the presence and the economic relevance of these viruses in Africa as well as in China (Ducatez et al. 2006c) and investigated the role of live bird markets in the spread of viruses (e.g. CAV in China, Ducatez et al. in press). A new very virulent IBDV and a new serotype of IBV were detected.

- **Other animal viruses:** department scientists published the first molecular identification and characteristics of African Swine Fever in West Africa (Odemuyiwa et al. 2000). The seroprevalence of antibodies against canine distemper in Luxembourg foxes was investigated (Damien et al. 2002). In collaboration with the Naturmuseum several studies in wildlife have been completed (Frantz et al. 2004, 2006).
- **Other human viruses:** these include the surveillance and molecular characterisation of respiratory viruses, parvovirus, tick-borne pathogens and norovirus. A collaboration with the Laboratory of Retrovirology on the development of a new recombinant virus system for the study of HIV-1 entry and inhibition has recently been published (Roman et al. 2006).
- **Tick-borne viruses.** Ticks are well-known to transmit a number of pathogens to humans. The Department has initiated a national survey in Luxembourg to investigate the prevalence of human pathogens in ticks including *Anaplasma* sp., *Bartonella* sp., *Borrelia burgdorferi* s.l., *Ehrlichia* sp., *Rickettsia* sp., *Babesia* sp. *Coxiella* sp., *Francisella* sp., TBE virus. Ticks from pet animals (cats, dogs), from wild animals (deer, hare, etc. ...) can also be submitted for testing by the general public by hunters, bird ringers, farmers etc. Doctors can submit ticks-removed from their patients.
- **Veterinary viruses**
- **Avian viruses**
- **Noroviruses (NoVs)** are a main cause of infectious gastroenteritis and the most common foodborne virus in developed countries causing complications in infants, the elderly or immuno-depressed persons. The objective of this project is to develop surveillance program in Luxembourg in humans, food and the environment.

## 2 THE IMMUNE SYSTEM, THE NEUROENDOCRINE SYSTEM AND STRESS

Stress is currently considered to be one of the most important causes of disease and invalidity within the European Union. Together with the University of Leiden and the Graduate School of Psychobiology of the University of Trier the Department of Immunology has established the multidisciplinary International Research Training Group (IRTG) "Psychoneuroendocrinology of stress : from molecules and genes to affect and cognition" to investigate stress-related health

issues. The objective of the IRTG is to identify new links between psychological function and biological stress mechanisms. Our research focuses on the identification and characterisation of molecular aspects of stress response mediators such as corticosteroids, and nuclear receptors in health and disease (e.g. fibromyalgia, Macedo et al. 2007), in immune cells (Schote et al. 2007). In some of these studies a comprehensive proteomics approach is applied (Billing et al. 2007).

Recent work of the Department has investigated the transcriptional control of the human glucocorticoid receptor (GR). We have shown that the CpG island upstream of the GR is highly structured and organised into multiple alternative first exons probably each with its own promoter region. The exon 1 remains untranslated and without effect on the structure of the GR (Turner et al. 2006; Turner and Muller 2005). Also a new transcript splice variant of the GR was identified (Turner et al. 2007). To understand the role of the alternative first exons these will be investigated in differentiating immune cells.

## 3 IMMUNOPROPHYLACTIC STRATEGIES

- Immune prophylactic strategy against chemical carcinogenesis (TOBAVAC).

The objective is the development of novel prophylactic immune strategies based on conjugate vaccines to protect against detrimental effects caused by environmental carcinogens and tobacco associated chemical carcinogenesis (e.g. polycyclic aromatic hydrocarbons, nitrosamines).

### Vaccines against low molecular weight antigens, novel strategies and applications:

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 (De Buck et al. 2005b). These results demonstrate that with the current understanding of the pharmacokinetic/dynamic (De Buck et al. 2005a) and of mechanisms of chemical carcinogenesis of benzo[a]pyrene (BaP) and NNK, an immunoprophylactic approach against chemical carcinogenesis is warranted (De Buck and Muller 2005).

- Vaccine candidates have been developed using BaP and NNK derivatives conjugated to immunogenic carrier proteins. For this purpose a novel synthesis of an amino derivative has been developed, which is the basis of a European patent currently pending (Prodhomme et al. 2007, patent "Immunoprophylactic approach against NNK using N-nitrosamine specific antibodies"). Small laboratory animals have been vaccinated to develop monoclonal antibodies and to investigate how antibodies can influence detrimental effects of BaP on new surrogate markers like weight gain, behaviour, specific learning and memory deficits, anxiety and NMDA receptor regulation (Grova et al. 2007; Grova et al. in press; Grova et al. 2005), BaP associated immune toxicity, and the modulation of enzyme expression by BaP.
- Development of a PRE-vaccine to protect against measles (PREMAVAC).

Infants are partially protected against measles by transplacentally acquired maternal antibodies. However, many children lose these antibodies within the first months after birth (Harterter et al. 2000). As a result they become susceptible to measles before they can be immunized with the current live-attenuated vaccine recommended only at 9-15 months of age (Muller 2001a, Putz and Muller 2003). The goal of the PREMAVAC project is to develop a pre-vaccine to protect against measles, which is compatible with current immunization programs to close the susceptibility-gap in young infant (Putz and Muller 2003).

Strategies are based on sequential B cell epitopes (Putz et al. 2003b, Putz et al. 2005) delivered as synthetic peptides (Muller and Putz 2005; Putz et al. 2004a) constrained by e.g. chemical scaffolds (Bodé et al. 2007, Halassy et al. 2006a) or included in permutational polyepitopes expressed in eukaryotic systems (Theisen et al. 2000) including plants (Bouche et al. 2005; Bouche et al. 2003; Muller et al. 2003; Marquet-Blouin et al. 2003). We have shown that these

epitopes protect in mice against a lethal challenge of measles virus (Putz et al 2004a), that they are not recognized by maternal antibodies (El Kasmi et al. 2000) and that the immune response is not suppressed by passive pre-existing MV-antibodies (Putz et al. 2003a, 2004, El Kasmi and Muller 2001). The strategies based on proprietary technology are developed in our Department (patent “Detection of measles virus specific antibodies using recombinant measles virus hemagglutinin protein” 97 11 5107.1).

4 PROTEOMICS AND MASS SPECTROMETRY PLATFORM

This platform is fully equipped with state-of-the-art instrumentation for proteomics, including protein isolation, 2-D DIGE technology for the separation and differential protein expression profiling, SD-Multi-fluorescence scanner (Typhoon), automated protein digester, protein identification by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF) with post-source decay capability, LC-electrospray mass spectrometry (Iontrap). Proteins are identified on the basis of their peptide mass finger print and sequencing after post-source decay (PSD). The platform can also detect post-translational modification such as phosphorylation, glycosylation etc. These techniques have been used to characterise hapten conjugated carrier proteins (Prodhomme et al. 2007), to investigate the effect of cortisol in differentiating macrophages (Billing et al. 2007) and post-translational modifications of viral proteins. The platform is available on the basis of scientific collaborations.

In another project applications and limitations of Secondary Ion Mass Spectrometry (SIMS) on a high resolution NanoSIMS instrument are explored (Pirotte et al. 2006).

KEY PUBLICATIONS OF THE LABORATORY

1 VIRAL IMMUNOLOGY

- MF Ducatez, CM Olinger, AA Owoade, S De Landsheer, W Ammerlaan, HGM Niesters, ADME Osterhaus, RA Fouchier, CP Muller. Avian flu: multiple introductions of H5N1 in Nigeria. Nature 442, 37, 2006.
- D. Butler. H5N1, From the front lines: Nigeria, CP Muller. Nature, 440,726-727, 2006.
- MN. Mulders, V Venard, M Njayou, AP. Edorh, AOB. Oyefolu, MO. Kehinde, JJ. Muyembe Tamfum, YK. Nèbie, I. Maïga, W. Ammerlaan, F. Fack, SA. Omilabu, A. le Faou, CP. Muller. Low Genetic Diversity despite Hyperendemicity of Hepatitis B Virus Genotype E throughout West Africa. Journal of Infectious Disease 190, 400-408, 2004.
- RL De Swart, PME Wertheim-van Dillen, RS van Binnendijk, CP Muller, J Frenkel, ADME Osterhaus. Measles in a Dutch hospital introduced by an immunocompromised infant from Indonesia infected with a new virus genotype. Lancet 355, 201-202, 2000.
- CP Muller, S Huiss, F Schneider. Secondary immune responses in parents of children with recent measles. Lancet 1996ii, 1379-1380, 1996.

2 PSYCHONEUROIMMUNOLOGY

- JD Turner and CP Muller. Structure of the glucocorticoid receptor (NR3C1) gene 5' untranslated region: identification, and tissue distribution of multiple new human exon 1. J Mol Endo. 35, 283-92, 2005.
- JD Turner, AB Schote, JA Macedo, LPL Pelascini, CP Muller. Tissue specific glucocorticoid receptor expression, a role for alternative first exon usage? Biochem Pharmacol 72, 1529-37, 2006.

3 IMMUNSTRATEGIES

- SS De Buck, P Augustijns, CP Muller. Specific antibody modulates absorptive transport and metabolic activation of benzo[a]pyrene across Caco-2 monolayers. J Pharmacol Exp Ther 313, 640-6, 2005.

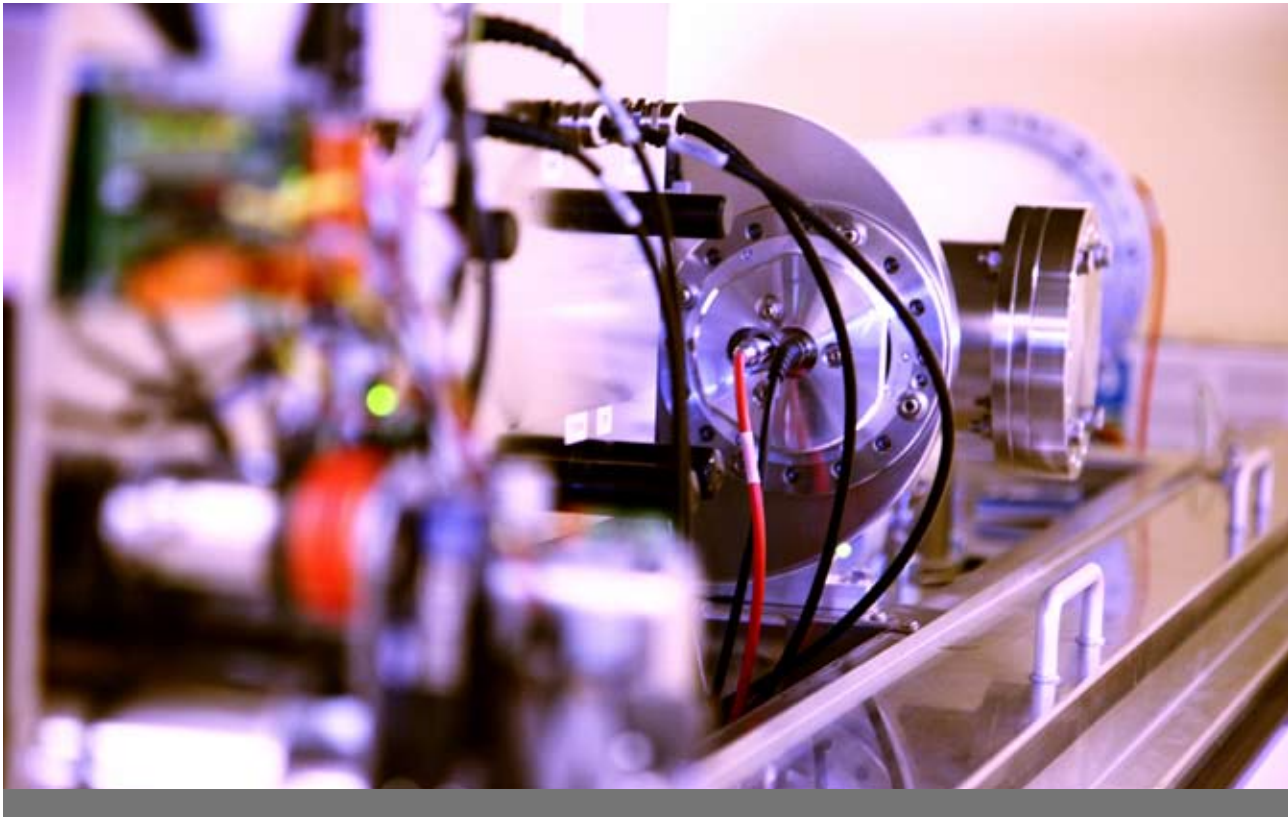
- SS De Buck, FB Bouche, A Brandenburger, CP Muller. Modulation of metabolism and adverse effects of benzo[a]pyrene by specific antibody: a novel host factor in environmental carcinogenesis? Carcinogenesis 26, 835-44, 2005.
- FB Bouche, A Steinmetz, Y. Yanagi, CP Muller. Induction of broadly neutralizing antibodies against measles virus mutants using a polypeptide vaccine strategy. Vaccine 23, 2074-7, 2005.
- MM. Pütz, J. Hoebeke, W. Ammerlaan, S. Schneider, CP. Muller. Functional fine-mapping and molecular modeling of a conserved loop epitope of the measles virus hemagglutinin protein. European Journal of Biochemistry 270, 1515-1527, 2003.
- CP Muller, W Ammerlaan, B Fleckenstein, S Krauss, H Kalbacher, F Schneider, G Jung, KH Wiesmüller. Activation of T cells by the ragged tail of MHC class II-presented peptides of the measles virus fusion protein. Int Immunol 8, 445-456, 1996.



PUBLICATIONS 2007

- JR Kremer and CP Muller. Measles – Rubella. Conn’s Current Therapy. Invited Book Chapter. Rakel and Bope, Saunders Elsevier p 143-144, 2007.
- C Bekondi, CM Olinger, N Boua, A Talarmin, CP Muller, A Le Faou, and V Venard. Central African Republic is part of the West-African hepatitis B virus genotype E crescent. J Clin Virol. 40, 31-37, 2007.
- MF Ducatez, Z Tarnagda, MC Tahita, A Sow, S De Landtsheer, BZ Londt, IH Brown, ADME Osterhaus, RA Fouchier, JB Ouedraogo, CP Muller. Genetic characterization of HPAI (H5N1) viruses from poultry and wild vultures, Burkina Faso. Emerg Infect Dis 13, 611-613, 2007.
- MF Ducatez, CM Olinger, AA Owoade, Z Tarnagda, MC Tahita, A Sow, S De Landtsheer, W Ammerlaan, JB Ouedraogo, ADME Osterhaus, RA Fouchier, CP Muller. Molecular and antigenic evolution and geographical spread of H5N1 highly pathogenic avian influenza viruses in western Africa. J Gen Virol 88, 2297-306, 2007.
- JM Hübschen, M Yermalovich, G Semeiko, E Semailovich, E Blatun S De Landtsheer, CP Muller. Co-circulation of multiple rubella virus strains in Belarus forming novel genetic groups within clade 1. J Gen Virol 88, 1960-6, 2007.
- CP Muller, JR Kremer, JM Best, I Dourado, H Triki, S Reef. Reducing global disease burden of measles and rubella: Report of the WHO Steering Committee on research related to measles and rubella vaccines and vaccination, 2005. Vaccine 25, 1-9, 2007.
- R Zhang, Y Deng, CP Muller, Zhi-Ying Ou, Li Ma, Min Wang, Pei-Qiong Lia, Yun-Shao He. Determination of Hepatitis B Virus Genotype by Flow-through Reverse Dot Blot. J Clin Virol 39, 94-100, 2007.
- JR Kremer, GH Nguyen, SV Shulga, PH Nguyen, UT Nguyen, NT Tikhonova, CP Muller. Genotyping of recent Measles Virus strains from Russia and Vietnam by nucleotide specific multiplex PCR. J Med Virol. 79, 987-94, 2007.
- CM Olinger, B Weber, JA Otegbayo, W Ammerlaan, N van der Taelem-Brulé, CP Muller. Hepatitis B virus genotype E surface antigen detection with different immunoassays and diagnostic impact of mutations in the preS/S gene. Med Microbiol & Immunol 196, 247-252, 2007.
- World Health Organization. Anonymus. Update of standard nomenclature for wild-type rubella viruses, Wkly Epidemiol Rec 82:216-222, 2007.
- AOB Oyefolu, AC Nwaeke, KO Akinyemi, Olumuyiwa B. Salu, CP Muller and SA Omilabu. Evaluation of vaccine cold chain system and potency status of measles vaccine administered in Lagos State, Nigeria. Afric J Clin Exp Microbiol 8, 1-7, 2007.

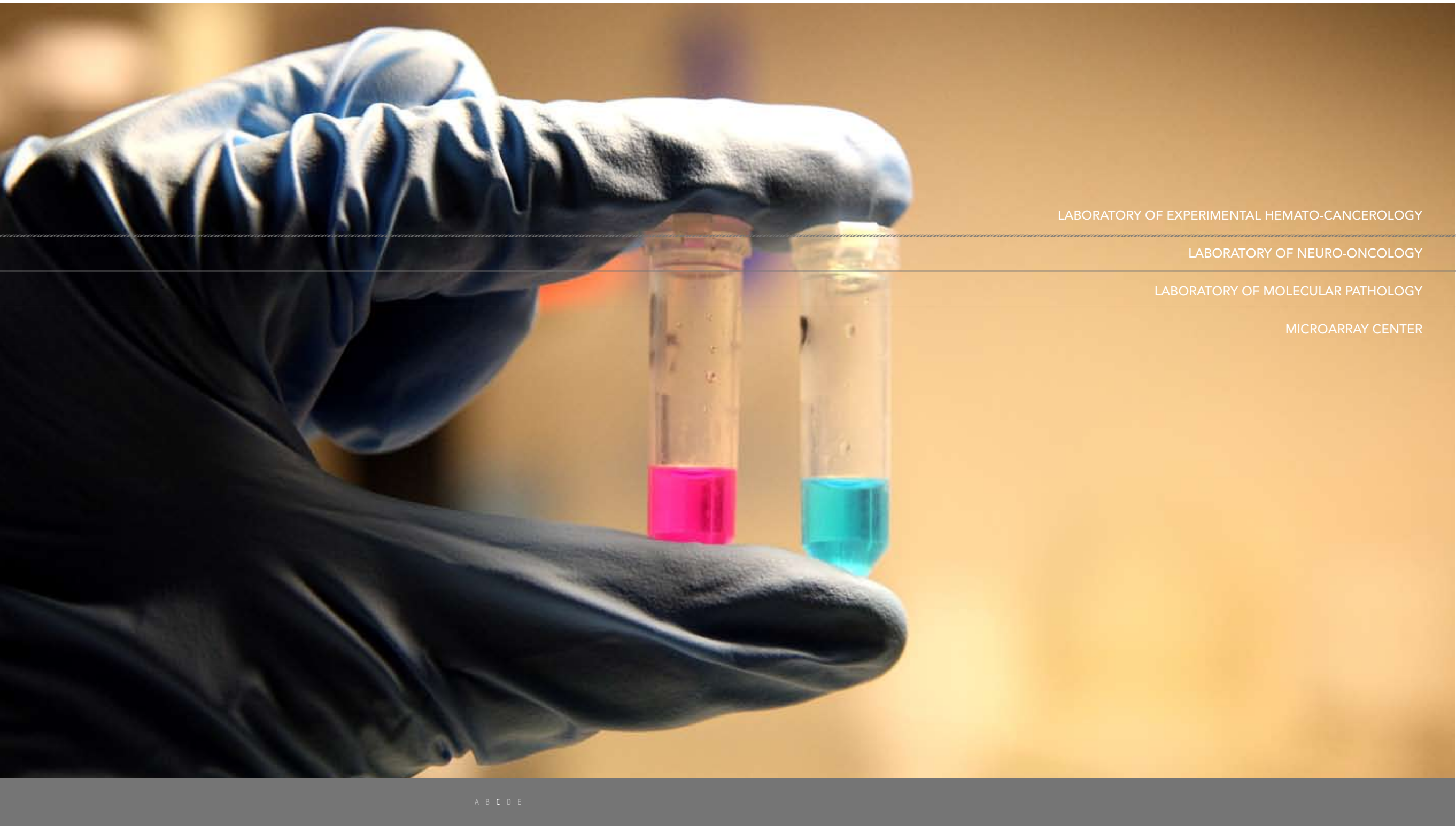
- Li Pei-qiong, Jun Zhang, CP Muller, Jing-xian Chen, Zi-feng Yang, Ren Zhang, Juan Li, Yun-shao He. Development of a Multiplex Real-Time Polymerase Chain Reaction Assay for Influenza Virus Type A including H5 and H9 Subtypes. *Diagn Micr Infect Dis*. *In press*.
- CM Olinger, NV Lazouskaya, VF Eremin, CP Muller. Multiple genotypes and subtypes of hepatitis B and C viruses in Belarus: similarities with Russia and West European influences. *Clin Microbiol Infect*. *In press*.
- MF Ducatez, H Chen, Y Guan, CP Muller. High chicken anemia virus (CAV) prevalence in South Eastern Chinese live birds markets: molecular epidemiology of strains under highly purifying selection. *Avian Dis*. *In press*.
- JR Kremer, KE Brown, L Jin, S Santibanez,...CP Muller, MN Mulders. High genetic diversity of Measles Virus, World Health Organization European Region, 2005-2006. *Emerg. Infect. Dis*. *In press*.
- JM Hübschen, JR Kremer, S De Landtsheer, CP Muller. A multiplex TaqMan assay for the detection of measles and rubella virus. *J Virol Meth*. *In press*.
- EJF Prodhomme, C Ensich, FB Bouche, T Kaminski, P Seck, G Kirsch and CP Muller. Synthesis of 4-[2-Amino-ethyl(nitrosamino)]-1-pyridin-3-yl-butan-1-one, a new NNK hapten for the induction of N-nitrosamine specific antibodies. *Bioconj Chem* 18, 2045-2053, 2007.
- CA Bodé, CP Muller, A Maddier. Validation of a solid phase-bound steroid scaffold for the synthesis of novel cyclic peptidosteroids. *J Pept Sci* 13, 702-708, 2007.
- N Grova, A Valley, JD Turner, A Morel, CP Muller & H Schroeder. Modulation of behavior and NMDA-R 1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. *Neurotoxicology* 28, 630-36, 2007.
- N Grova, H Schroeder, E Prodhomme, S Farinelle, A Valley, CP Muller. Low and high subchronic doses of Benzo[a]pyrene (B[a]P) chronically administrated to the mice differentially affect anxiety-related behavior and regional expression of N-methyl-D-aspartate (NMDA) receptor genes. *Chemosphere*. *In press*.
- AM Billing, F Fack, J Renaut, CM Olinger, AB Schote, JD Turner, CP Muller. Proteomic analysis of the cortisol-mediated stress response in THP-1 monocytes using DIGE technology. *J of Mass Spectrometry* 42, 1433-1444, 2007.
- JA Macedo, J Hesse, JD Turner, W Ammerlaan, AC Gierens, DH Hellhammer and CP Muller. Adhesion molecules and cytokine expression in fibromyalgia patients: increased L-selectin on monocytes and neutrophils. *J Neuroimmunology* 188, 159-166, 2007.
- JD Turner, AB Schote, M Keipes and CP Muller. A new transcript splice variant of the human glucocorticoid receptor: Identification and tissue distribution of hGRΔ313-338, an alternative exon 2 transactivation domain isoform. *Annals NY Acad Scie* 1095, 334-341, 2007.
- AB Schote, JD Turner, J Schiltz, CP Muller. Nuclear receptors in human immune cells: expression and correlations. *Mol Immunol* 44, 1436-45, 2007.





IIII RESEARCH DEPARTMENTS

- ~~DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY~~
- ~~DEPARTMENT OF IMMUNOLOGY~~
- DEPARTMENT OF ONCOLOGY
- ~~DEPARTMENT OF CARDIOVASCULAR DISEASES~~
- ~~DEPARTMENT OF PUBLIC HEALTH~~



A B C D E



## / LABORATORY OF EXPERIMENTAL HEMATO-CANCEROLOGY

Head of the laboratory:

Guy Berchem, MD



Presently, the staff of the laboratory is as follows:

Valérie PALISSOT, PhD, Researcher  
Nassera AOUALI, PhD, Researcher  
Bassam JANJI, PhD, Researcher  
Victoria El KHOURY, PhD, Researcher  
Etienne MOUSSAY, PhD, Researcher  
René BRONS (FACS Core Facility)  
Laurent KELLNER, Engineer  
Manon BOSSELER, Technician  
Julie JACQUEMIN, Technician  
Sandrine PIERSON, Engineer  
Kris Van MOER, Technician  
Maria Pires PACCHECO, Technician  
Wim AMMERLAAN (FACS Core Facility)



## HISTORY OF THE LABORATORY

The Laboratory of Experimental Hemato-Cancerology (LHCE) was established in 2003 as one of the first basic cancer research laboratories in Luxembourg. The development of the Laboratory and its program in experimental cancer research was made possible by the BIOSAN program of Fonds National de la Recherche (FNR). Expanded facilities were provided by the laboratory of "Fondation Recherche Cancer et Maladies du sang". LHCE integrated into the CRP building in September 2004.

Under the direction of Dr Guy BERCHEM MD, Hemato-Oncologist at the Centre Hospitalier in Luxembourg, LHCE makes a link between the clinical world and research.

Since its establishment, LHCE focused its research activity on the study of two haematological diseases, multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL) aiming at a better understanding of the mechanisms of cell death and at developing new treatment strategies in these diseases.

Evolution of staff and projects :

| Year | Full time equivalent employee | Projects   |
|------|-------------------------------|--|
| 2003 | 1.5                           | 1 FNR Biosan   |
| 2004 | 3.5                           | 1 FNR Biosan<br>1 Télévie  |
| 2005 | 4.5                           | 1 FNR Biosan<br>1 FNR Provie<br>1 Télévie projects<br>1 Core facility implementation |
| 2006 | 9                             | 4 Télévie projects<br>1 FLCC<br>Core facility cytometry development                  |
| 2007 | 11                            | 4 Télévie projects<br>1 FLCC   |
| 2008 | 12                            | 2 MESR<br>4 Télévie projects<br>1 FLCC<br>1 FNR Biosan                               |

Since 2005, the lab has grown and developed a flow-cytometry core facility which now provides an excellent service in different methods to characterize specific markers of the cells (phenotyping, study of differentiation...) and different cell phenomena (cell death, calcium flux,...). This core facility acquired last year a second flow-cytometer which has sorting capacity (FacsAria, Becton Dickinson).

The competences and technical know-how are shared with all researchers and with clinical hematology laboratories; the core facility is available for all institutions. Since three years, an annual cytometry conference is organised in Luxembourg in order to create further bonds between the different Luxembourgish cytometry users. The Luxembourg Society of Flow cytometry has also been created by Mr Rene Brons this year.

Research of LHCE is funded by Luxembourg’s ministry of higher education and research, the “Fonds National de la Recherche”, private institutions (Télévie, Fondation Luxembourgeoise contre le cancer) or private donations.

Since the first years our lab worked on the better understanding of apoptosis (programmed cell death) in a model of chronic lymphocytic leukaemia.

B-cell chronic lymphoid leukemia. (B-CLL) is the most common leukemia in the western hemisphere and is considered to be incurable. It is a clinically heterogeneous disease characterized by the accumulation of CD19/CD5 positive B lymphocytes with significant resistance to apoptosis and prolonged survival. The apoptosis disruption is attributed to several mechanisms. Chromosomal abnormalities in B–CLL have been shown to correlate with prognosis.

Although chemotherapy using nucleoside analogues such as fludarabine and cladribine (2-chlorodeoxyadenosine) have excellent activity in first line treatment of patients and can induce

remissions, their impact on long-term survival and activity in refractory disease is not clear and cures have not been consistently observed.

The cells of different patients and cultured cells were studied after treatment by chemotherapy as well as immuno-therapy. We have tried to determine the reasons why these cells become resistant to apoptosis induced by those treatments. One of our projects more specifically aimed at inducing a specific immune response in chronic lymphocytic leukaemia patients to their own leukaemia. Moreover, investigations on the potential relationship between the expression of cell surface antigens and the sensitivity of B-CLL cells to treatment by several drugs have been made in order to identify new markers for prognosis diagnosis.

Another project aimed at using the micro-array approach to detect differences in gene expression between sensitive and resistant patient cells.

Today, the first results of our different studies have been obtained: A new set of genes has been identified to discriminate sensitive and resistant patients. Clinical studies are conducted in collaboration with national and international hospitals in order to evaluate the interest of the drug combinations which the LHCE has studied *in vitro*.

The cytometry platform is really useful in the study of MM and CLL on the bone marrow and blood of patients respectively. In fact, the phenotyping analysis of bone marrow and blood cells from patients by flow cytometry in more than 10 colors permit us to detect with specific markers, the diseased cells in mixes of different populations of cells. Moreover, with the cell sorter we are able to select the desired populations of cells for further analysis.

CURRENT KEY OBJECTIVES

The LHCE aim at better understanding of cell death signaling pathways induced on patient cells *in vitro* or *in vivo* and on cellular model with new therapeutic drugs.

ONGOING PROJECTS AND MAIN RESULTS

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

The effects of fludarabine on B-CLL cells (B lymphocyte-chronic lymphocytic leukemia) have been well studied *in vitro*. However, the effects of this treatment on the molecular level are poorly understood *in vivo*. Rosenwald et al., (2004, Blood) reported a short list of genes all of them induced by p53 after 5 days of treatment in B-CLL patients. The molecular response was found to be similar to those induced by irradiation. In this study, we aimed at investigating late response to fludarabine treatment and investigate the mechanisms of cell death and the signaling pathways implicated. For this purpose, we performed a whole-genome analysis with cDNA microarrays until 9 days after the beginning of the treatment and we compared the results of the transcripts’ expression with the clinical data of the patients (blood examination, cytogenetics).

For this purpose, we purified blood B cells before fludarabine administration (day 0) and after 1, 2, and 9 days and then performed a whole-genome analysis with cDNA microarrays. An array-based Comparative Genomic Hybridization (CGH) analysis was used to determine the genetic aberrations of the B cells from CLL patients. Additionally, we compared the genomic and transcriptomic data (after real-time PCR confirmation) with the clinical data of the patients.

Statistical analysis and clustering led us to identify for each time-point tested a gene expression signature of *in vivo* sensitivity and/or resistance to fludarabine of B-CLL patients. Most of *in vivo*-regulated genes identified could be classified with gene ontology softwares as part of

the cell death mechanisms, cell cycle regulation, DNA damage and repair, kinase activity and the nucleotide metabolism. Taken together, our data show significant distinct profiles between sensitive and resistant patients and strongly argue in favor of the establishment of targeted PCR after 1-2 days of treatment as reliable tool to early determine the outcome of the therapy of B-CLL patients by fludarabine.

## 2 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

Lab activities have been extended to studies on Multiple Myeloma. Multiple myeloma (MM) is an incurable hematological disorder characterized by deregulated proliferation of terminally differentiated plasma cells. These malignant plasma cells are predominantly located in the bone marrow. Existing treatments mainly attempt to reduce the malignant cell masses and to overcome the disease-related complications. Whereas initial chemotherapeutic treatment can be successful, drug resistance often develop during disease progression, requiring the use of different drugs.

In this study, we have investigated the effect of histone deacetylase inhibitors (HDACi), the valproic acid and MGCD-0103 (Pharmion, Ireland), drugs clinically used in the treatment of epilepsy and used in phase 2 clinical trials on 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 *ex vivo*.

The anti-tumor activity of MGCD-0103 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 *ex vivo* 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) to determine the molecular mechanisms of MGCD-0103 cytotoxicity in B-cell CLL cells.

The PPAR $\gamma$  is one member of the family of nuclear receptors. As PPAR $\gamma$  agonist, the thiazolidinediones (TZD) show moderate anti-proliferative and anti-apoptotic activities on several cancer cells lines, these drugs have been combined with the HDACi. Here we provide evidence that the combination of VPA, a HDACi, and Pioglitazone, a PPAR $\gamma$  agonist induces a higher cytotoxicity effect on MM cell lines compared to that obtained with each drug alone.

To identify the mechanisms leading to the loss of cell viability we define the signaling pathway inducing apoptosis, we characterized the caspases activation on multiple myeloma cell lines.

In order to analyze if the efficiency of such combinations could be observed in patients with Multiple myeloma, we characterized and isolated subpopulations of multiple myeloma cells from bone marrow and blood of patients by flow cytometry using 12 color low cytometry. As expected, a strong cytotoxicity was observed when MM cells isolated from patients were treated with combination of VPA and Pioglitazone.

## 3 OTHER DRUGS STUDIED ON MULTIPLE MYELOMA AND B-CLL

While 5-azacytidine affected viability of EHEB, MEC-1 and JVM-3 B-CLL cell lines, it had no effect on the survival of PBMC from B-CLL patients cells. No additive or synergistic effect has been shown when combining 5-azacytidine with MGCD-0103.

However, our studies on multiple myeloma cell lines provided evidence of an interesting cytotoxic effect of 5-azacytidine when used alone or in combination with histone deacetylase inhibitors or other anticancer drugs.

## 4 PHENOTYPING AND ISOLATION OF INTERESTING POPULATIONS IN MULTIPLE MYELOMA PATIENT SAMPLES

The phenotyping analysis of bone marrow and blood cells from patients by flow cytometry in 12 colors was implemented to detect the diseased cells in mixes of different populations of cells. This 12 color technique is quite exceptional as the compensation problems become huge as soon as multiple colors are mixed. In 2007 only a few labs in the world have been able to successfully analyse cell populations with a 12 color technique. Using this technique we are now able to select the desired subpopulations out of full blood and end up with only a few thousand cells highly pure for further analysis.

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

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. It concerns patients either with preneoplastic lesions, which will be biopsied and analyzed in Brussels, as well as 4 groups of local patients which will also be biopsied and put into a tumor bank for later analysis. These four groups are: normal nonsmokers, normal smokers (G2), current lung cancer (G3) and previous lung or head and neck cancer (G4). After an initial phase of 2 years where the patient samples are collected, the samples will be analyzed using a whole genome Microarray approach or another technique which might be more appropriate at that time, for this reason, proteins and RNA will be collected and preserved separately. This study is supported by the Fondation Contre le Cancer of Luxembourg.

Up to now, optimization in sample storage, RNA extraction, amplification and labelling have been done. Samples are collected and immediately mixed in TriPure reagent, frozen in liquid nitrogen and stored at – 80 Celsius degrees .

Sample bank is constituted with 3 biopsies of each patient (4 patients in G2, 4 patients in G3, 3 patients in histological characterization.). 400 to 1000 ng of RNA are extracted from one biopsy. Purity evaluated using ratio 260nm /280nm and 260nm/230nm were in good range (superior to 1.8).

Amplification increased at least 94 times initial RNA quantity (300 ng). Labeled amplified RNA could be hybridized on 70 mers whole human genome oligo- arrays (21505 spots). Using MAIA software, 62 % spots were of good quality.

## 6 CHARACTERIZATION AND STUDY OF AUTOPHAGY INDUCTION IN A TNF- $\alpha$ SENSITIVE MCF-7 CELL LINE AND ITS WELL CHARACTERIZED TNF- $\alpha$ RESISTANT VARIANT

The observation that tumor development is frequently accompanied by apoptotic defects and the meanwhile accepted view that the vast majority of conventional anticancer agents primarily cause DNA damage to subsequently initiate the apoptotic machinery, have led to the conclusions that the capability to undergo apoptosis must contribute to the outcome of cancer therapy. However, resistance to such anticancer agents continues to be a major obstacle that limits the rate of successful outcomes for cancer patients, irrespective of tumor type. Thus, identification and validation of novel therapeutic strategies for chemorefractory tumor cells represents a significant challenge in cancer research.

One of our objectives in the laboratory is to analyze the signaling pathway involved in the acquisition of cell resistance to chemotherapeutic drugs using cell culture-based model. For this purpose, we have chosen the human breast adenocarcinoma MCF-7 cell line as a model for our study. For several decades, its use in independent laboratories worldwide has facilitated the evolution of distinct MCF-7 lineages (Gooch and Yee, 1999; Burow et al., 1998). These lineages acquire differences in their responses to the cell-death-inducing agents such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and, subsequently, differences in resistance to cell death signals. Thus, MCF-7 is an excellent in-vitro model for studying the mechanisms of chemoresistance as it relates to susceptibility to apoptosis.

In collaboration with Dr S. CHOUAIB (Institut Gustave Roussy, INSERM U487) we used the TNF- $\alpha$  sensitive MCF-7 cell line and its well characterized TNF- $\alpha$  resistant variant, 1001 clone, (Cai et al., 1997), in order to explore the mechanism by which 1001 cells acquire resistance to the cytotoxic effect of TNF- $\alpha$  (fig1).

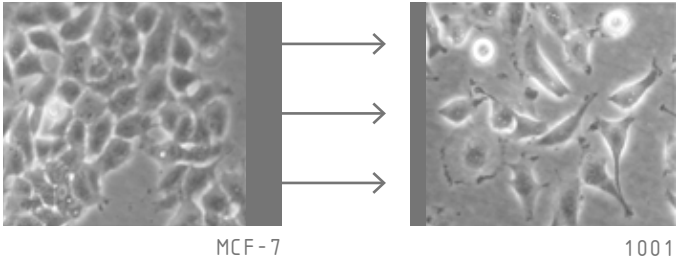


Figure 1: Cell morphology of TNF- $\alpha$ -sensitive MCF-7 and TNF- $\alpha$ -resistant 1001 by phase contrast microscopy.

We first tested whether failure of TNF- $\alpha$  to induce cell death in resistant 1001 cells was related to an overexpression of some antiapoptotic proteins. Surprisingly, lower expression level of the anti-apoptotic protein Bcl-2 was detected in 1001 compared to MCF-7 (fig 2) suggesting that the anti-apoptotic activity of Bcl-2 protein is not directly involved in the acquisition of resistance to TNF-induced cell death. Since Bcl-2 can directly inactivate the autophagy-execution protein Beclin-1 (Pattingre et al., 2005), the expression of Beclin-1 was analyzed in MCF-7 and 1001 cells (fig 2). Interestingly, the expression of Beclin-1 and other autophagy-related proteins was higher in 1001 cells compared to MCF-7 cells. The upregulated-expression of Beclin-1, together with the downregulated-expression of Bcl-2, strongly suggest that resistant 1001 cells activate the autophagy machinery.

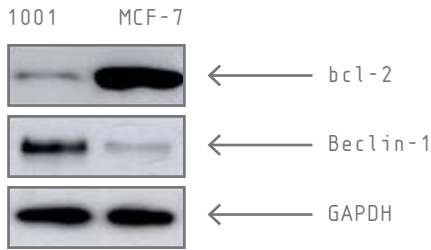


Figure 2: Expression of Bcl-2 and Beclin-1 in MCF-7 and 1001 cells analyzed by Western blot.

To prove that 1001 cells undergo autophagy we used the microtubule-associated protein 1 Light Chain 3 (LC3) as a marker of autophagy. In the absence of autophagy, LC3 is localized homogeneously in the cytoplasm; however, upon initiation of autophagy, LC3 associates with the membrane of autophagosomes (Tanida et al., 2005). We provided evidence that TNF- $\alpha$  treatment of 1001 was sufficient to induce the formation of autophagosomes in TNF- $\alpha$  resistant cells (fig 3).

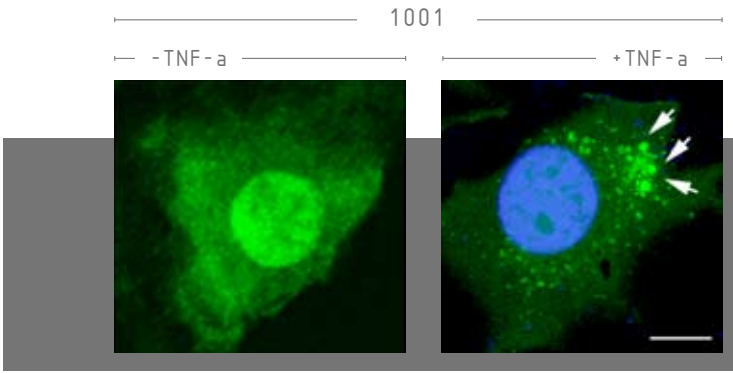


Figure 3: Immunofluorescence analysis of autophagosomes formation in 1001 cells untreated or treated with 50ng/ml TNF- $\alpha$  for 48h. Immunofluorescence was performed using an anti-LC3 primary antibody and an Alexa Fluor 488-coupled goat anti-Rabbit secondary antibody (Green). Labeled cells were analyzed by a Zeiss laser scanning confocal microscopy (LSM-510 Meta). Arrowheads indicated autophagosomes. Nucleus of TNF- $\alpha$ -treated 1001 cells was stained with DAPI (Bleu). Bar = 15  $\mu$ m.

Furthermore, time-laps microscopy performed on LC3-GFP expressing 1001 cells provides compelling evidence that these cells activated the autophagy machinery upon TNF- $\alpha$  treatment (fig 4).

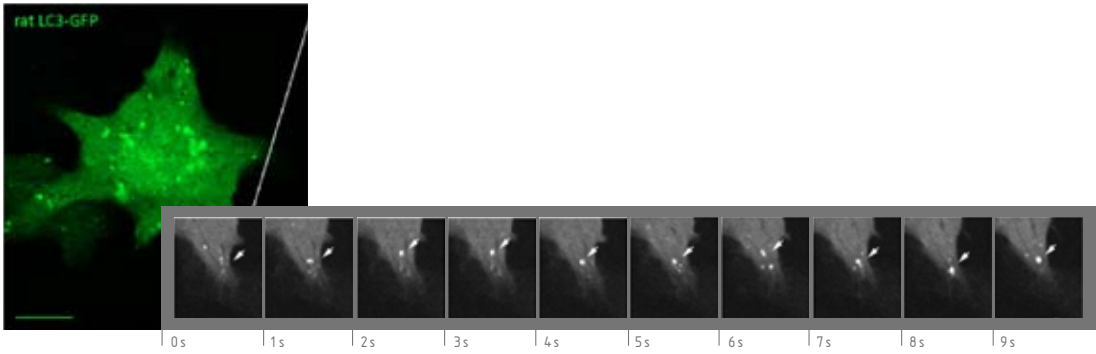


Figure 4: Real time observation of autophagosomes dynamic. rat LC3-GFP 1001 expressing cells were seeded on lab teck chambers and treated for 48 h with 50ng/ml TNF- $\alpha$ . Cells were examined by time-lapse video microscopy using laser scanning confocal microscope (LSM-510 Meta) with a X 63 oil-immersion objective and scanning module at 1024 X 1024 pixel resolution. Images of enlarged region extracted from time-lapse video at indicated time show the formation of a new autophagosome. Bar = 15  $\mu$ m.

This work demonstrates that MCF-7 cells and their TNF- $\alpha$  resistant counterpart 1001 cells displays differences in autophagy-related proteins expression. TNF-inducing autophagy in resistant 1001 cells seems to be an early adaptive response to favor cell survival rather than cell death pathway. Additional in progress experiments, using siRNA to target Beclin-1 or Bcl-2 in our cell model will enable testing of this hypothesis. Furthermore, our study underlines the potential utility to investigate the autophagy machinery activity in TNF- $\alpha$  based anti-tumor therapy.



## COLLABORATIONS

Dr CHOUAIB S. INSERM U487. Institut de Cancérologie Gustanve Roussy. F-94805 Villejuif, France.

Dr Mizushima N. Department of Physiology and Cell Biology. Tokyo Medical and Dental University, Japan.

## KEY PUBLICATIONS OF THE LABORATORY

- Chantal Schwartz, Valerie Palissot, Nassera Aouali, Severine Wack, NHC Brons, Bernadette Leners, Manon Bosseler, Guy Berchem. Valproic acid induces non apoptotic cell death mechanisms in Multiple Myeloma cell lines. Int J Oncol. 2007 Mar;30(3):573-82
- Beaujouin M, Baghdiguian S, Glondu-Lassis M, Berchem G, Liaudet-Coopman E. Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity. Oncogene. 2006 Mar 23;25(13):1967-73.
- Lebrun F, Berchem G, Delagardelle C, Beissel J, Rouy D, Wagner DR. Improvement of exercise-induced cardiac deformation after cell therapy for severe chronic ischemic heart failure. J Card Fail. 2006 Mar; 12(2):108-13.
- Palissot V, Morjani H, Belloc F, Cotteret S, Dufer J, Berchem G. From molecular characteristics to cellular events in apoptosis-resistant HL-60 cells. Int J Oncol. 2005 Mar;26(3):825-34.
- Cathepsin-D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. Berchem G, Glondu M, Gleizes M, Brouillet JP, Vignon F, Garcia M, Liaudet-Coopman E. Oncogene. 2002 Aug 29;21(38):5951-5.

## PUBLICATIONS 2007

- Chantal Schwartz, Valerie Palissot, Nassera Aouali, Severine Wack, NHC Brons, Bernadette Leners, Manon Bosseler, Guy Berchem. Valproic acid induces non apoptotic cell death mechanisms in Multiple Myeloma cell lines. Int J Oncol. 2007 Mar;30(3):573-82.
- Up regulated expression of L-plastin confer resistance to TNF- $\alpha$  mediated cell death in breast cancer cells undergoing an Epithelial-Mesenchymal Transition. Bassam Janji, Laurent Vallar, Ziad Altanoury, François Dernardin, Guy Berchem, Evelyne Freiderich & Salem Chouaib. (submitted JBC).



/ LABORATORY OF NEURO-ONCOLOGY

Head of the laboratory:

Simone NICLOU, PhD



Presently, the staff of the laboratory is as follows:

Rolf BJERKVIG, PhD, Associate Head of the Laboratory  
Uros RAJCEVIC, PhD, Researcher  
Jo Kristian UTVIK, PhD, Researcher  
Virginie BAUS-TALKO, Technician  
Vanessa BARTHELEMY, Technician



HISTORY OF THE LABORATORY

The convention to establish the NorLux laboratory was signed in the fall 2003 by CRP-Santé to further develop its prioritized areas within the fields of cancer and neurosciences. The laboratory was established by taking advantage of professional and personal contacts between academic members within CRP-Santé and the Medical Faculty, University of Bergen, Norway. A formal agreement was signed between the two institutions, which involve collaborative research, scientific competence building, and staff exchange. A complete functional laboratory was established in Luxembourg in 2005/2006 and at present, the laboratory is in an expanding phase. The NorLux laboratory has a clear vision:

- NorLux will establish a centre of excellence within the field of brain tumour research.
- NorLux will through state of the art research, build up a critical mass of competitive scientists within all the three major fields of medical research (basic science, translational research and clinic).
- NorLux will identify new therapeutic targets towards brain cancer and through the design of new treatment strategies, NorLux will be pro-active in the development of biotechnology and develop close relations to pharmaceutical industry.

The NorLux laboratory is headed by Dr. Simone Niclou and Professor Rolf Bjerkvig. In 2007 the laboratory comprised two technicians, Vanessa Barthelemy and Virginie Baus, two postdoctoral researchers, Uros Rajcevic and Jo Utvik, one PhD student, Claude Danzeisen, and a variable number of trainees.

## CURRENT KEY OBJECTIVES

The focus of the NorLux laboratory is to understand the initiation and progression of malignant brain tumors. The overall objectives are to find and validate new therapeutic targets and to utilize such targets to develop novel therapeutic strategies for brain cancer. Our laboratory has developed appropriate animal models which represent a tool to study different aspects of brain tumour development *in vivo*. Such models are invaluable for a detailed investigation of the interaction of tumour cells with their micro-environment and for the characterization of cancer stem cells (cancer-initiating cells) in brain tumors. We are using high throughput proteomics techniques to identify novel molecular targets in brain cancer and we are validating these targets in brain tumor biopsies and determine their mode of action using functional bioassays.

Thus, our research has a strong translational profile. Finally we 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 AND MAIN RESULTS

Currently the NorLux laboratory has a focus on three major research projects. A summary of the projects and their highlights in 2007 is provided below. The 'brain tumour project' (project 1) was completed in December 2007 and a new project, which represents a continuation of the latter, will start in January 2008. This project will focus on studying how tumour cells communicate with host tissues and on the process of tumour initiation, which involves stem cell research. More detailed information can be found on our website: [www.norlux.lu](http://www.norlux.lu).

### 1 BRAIN TUMOUR PROJECT

**Title:** New therapeutic principles and biological mechanisms related to brain tumour cell invasion and angiogenesis

**Acronym:** Brain tumor project

**Contract number:** SAN/03/004

**Period:** until December 2007

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

#### • Project Summary

The study of brain tumours is highly dependent on animal models that reliably recapitulate human cancer development. It is therefore a strong need for generating *in vivo* model systems that allow initiation and progression of tumours to be studied at the cellular and molecular level *in vivo*.

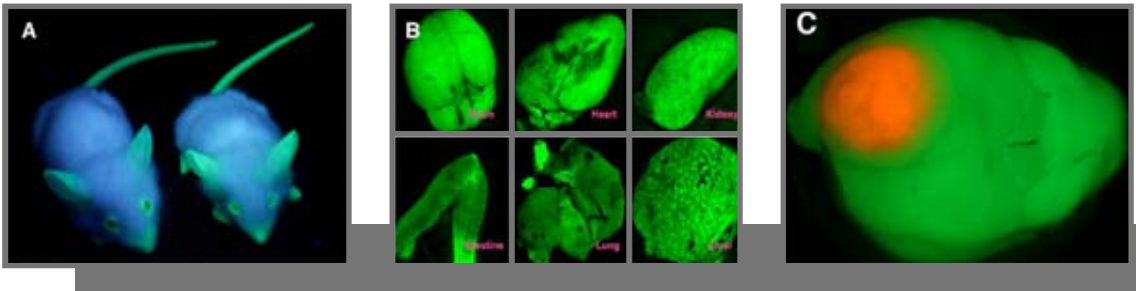
By transplantation of human glioblastoma biopsy material into the brain of immunodeficient rats, an animal model was generated in the NorLux lab in Norway that displays a highly invasive phenotype showing stem cell characteristics but no signs of angiogenesis dependent growth (Sakariassen *et al.* 2006). Through serial passages in the rat brain, the phenotype changed to a less invasive, highly angiogenic phenotype.

In the present project we have established and characterized an immunodeficient mouse model that expresses green fluorescent protein (GFP) in all nucleated cells. The brain tumor model based on human biopsy material has been adapted to the GFP expressing mouse line, thus enabling us to reliably visualize and separate the tumour - host compartments. This model will also be important for the development of new therapeutic intervention strategies targeting cancer.

#### • Main Results

An immunodeficient (NOD/Scid) mouse expressing enhanced green fluorescent protein (eGFP) has been characterized where human and mouse tumours marked with red fluorescent protein, can be established *in vivo*, both at subcutaneous and orthotopic locations (Figure 1). Using light microscopic as well as multiphoton confocal microscopy techniques, we visualize in detail the intricate colocalization of tumour and host cells *in situ*.

Moreover, using fluorescence-activated cell sorting (FACS), we were able to completely separate the host cells from the tumour cells, thus providing a system for detailed cellular and molecular analysis of tumour-host cell interactions. The fact that tumour and host cells can be reliably identified also allowed us to detect double-positive cells arising from cell fusion events or horizontal gene transfer. Similarly the model can be applied for the detection of circulating metastatic cells and for detailed studies on the vascular compartments within tumours including vasculogenic mimicry. Thus, the model described should provide significant insight into how tumour cells communicate with normal tissues.



**Figure 1:** (A) Immunodeficient eGFP expressing mice visualized under a hand held UV-lamp. (B) Internal organs showing strong eGFP expression in brain, heart, kidney, intestine, lung and liver under a fluorescence dissecting microscope. (C) Direct fluorescence of a human glioma expressing red fluorescent protein grown orthotopically in the brain of immunodeficient eGFP mice.

### 2 ANGIOTARGETING PROJECT

**Title:** Targeting Tumour-Vascular /Matrix Interactions

**Acronym:** Angiotargeting project

**Contract number:** 504743

**Period:** November 2004 - April 2009

**Financial support:** Integrated Project, EU 6th Framework Programme. Supportive grant for equipment from the MCESR, Luxembourg

**Website:** [www.uib.no/med/angiotargeting](http://www.uib.no/med/angiotargeting)

• Project Summary

Solid tumour growth depends on a continuous supply of nutrients supplied from new 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 (angiogenic switch) in solid human tumours.

This project aims at identifying novel target molecules involved in this phenomenon by applying high throughput proteomics technologies to the brain tumour animal model (Sakariassen *et al.* 2006). This has been possible through a collaboration with the Onco-Proteomics Facility of the VU Cancer Center in Amsterdam (Connie Jimenez) and a staff exchange programme for Uros Rajcevic supported by the Angiotargeting Consortium.

Insight into the differential proteome of the invasive and angiogenic tumour phenotypes will identify potential biomarkers involved in tumor angiogenesis that may represent novel drug targets to block neovascularization in tumors.

• Main Results

In addition to two dimensional in gel fluorescence electrophoresis (2D-DIGE), we have implemented a gel free proteomics approach based on isotope tags for relative and absolute quantitation and two-dimensional liquid chromatography (iTRAQ - 2D LC), followed by quantitative mass spectrometry (qMS). This has led to large and invaluable datasets of potential molecular targets and biomarkers for brain tumours.

More than 1’800 different proteins have been identified showing different expression profiles in brain tumours (Figure 2). Further validation in human brain tumour material is in progress.

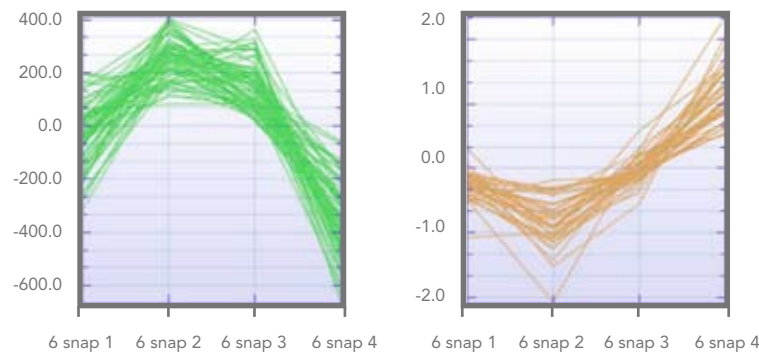


Figure 2: Two different protein expression profiles are shown, representing a subset of proteins identified in the proteomics screen on invasive versus angiogenic brain tumours. Proteins in the left panel are downregulated in the angiogenic tumour, while proteins in the right panel show increased expression in the angiogenic phenotype.

The data on differential protein expression were analyzed with specially designed software to distinguish between tumor (human) and host (rat) proteins and thus perform isoform and species specific quantitation of the peptides. In collaboration with the Bioinformatics Group of the University of Bergen (Inge Jonassen & Kjell Petersen, Angiotargeting Consortium), the data is further analysed to identify protein expression patterns and to perform comparisons with gene expression data.

3 MICRO-ENCAPSULATION 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 project  |
| Contract number:   | FNR 06/04/02   |
| Period:            | February 2007 - January 2009   |
| Financial support: | Fonds National de la Recherche (FNR), Luxembourg   |

• Project Summary

The limited passage of drugs through the blood brain barrier and the short half-life of locally injected therapeutic molecules are major hurdles in the treatment of brain diseases, including brain tumours and neurodegenerative diseases. Cell-based delivery systems, such as cell micro-encapsulation devices, provide long term 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 and its application in the treatment of brain diseases.

In collaboration with the group of Thierry Pillot (Lipidomix group, INPL, Nancy, France), encapsulated cells delivering neuroprotective peptides will be generated and implanted in the brain of murine models for Alzheimer's disease. The present project will open up new avenues for the use of micro-encapsulation technology as an innovative strategy in the treatment of neurodegeneration and brain cancer.

• Main Results

The present project was approved by the FNR in October 2006 and has started in February 2007.

Using an electrostatic bead generator we have successfully established the production of micro-encapsulated cells in the laboratory. Using a myoblast cell line stably expressing a neuroprotective factor we were able to show release from the micro-capsules and neuroprotection in neuronal cell cultures challenged with soluble oligomers of amyloid  $\beta$  peptide (A $\beta$ ). In a large scale *in vivo* experiment, mice were co-injected with soluble A $\beta$  and micro-capsules (Figure 3). The first preliminary results from the behavioral tests (Y maze and Morris water maze) indicate an improvement of the cognitive defects in the mice after neurotrophic factor treatment, suggesting a strong neuroprotective effect also *in vivo*. These experiments will be further validated at the cellular and biochemical level.



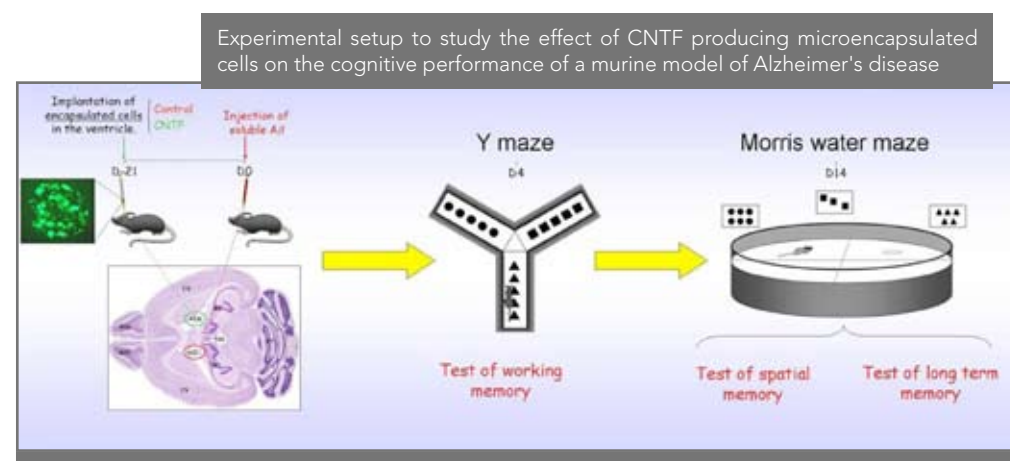


Figure 3: Workflow to test the effect of neuroprotective factors released from micro-encapsulated cells on the cognitive performance of amyloid  $\beta$  treated mice.

Jo K. Utvik was awarded a prize for the best poster at the XV International Workshop on Bioencapsulation, Vienna, Austria (September 2007). Poster title: *Delivery of neuroprotective peptides from encapsulated cells in a mouse model for neurodegenerative disease*. JK Utvik, I Youssef, B Kriem, T Oster, AJA Terzis, R Bjerkvig, T Pillot, SP Niclou.

## COLLABORATIONS

### 1 NATIONAL

- Neurosurgery Department CHL: PT. Dang, G. Matgé, G. Sandt
- Core Facility Flowcytometry, CRP-Santé: NHC. Brons
- Core Facility Confocal Microscopy, CRP-Santé: C. Hoffmann
- LIGA, CRP-Santé: A. Poli
- Mass spectrometry facility, LNS, CRP-Santé: F. Fack, D. Revets, C. Müller

### 2 INTERNATIONAL

- University of Bergen, Norway, Department of Biomedicine: PØ. Enger and F. Thorsen
- University of Bergen, Bergen Center for Computational Science: K. Petersen and I. Jonassen
- Vrije Universiteit (VU) Cancer Center, Amsterdam, Netherlands, Onco-Proteomics Facility: C. Jimenez and K. Hoekman
- Institut National Polytechnique de Lorraine (INPL), Lipidomix Laboratory, Nancy, France: T. Pillot and T. Oster
- Angiotargeting Consortium, EU Integrated Project 6th FP (11 partners): [www.uib.no/med/angiotargeting](http://www.uib.no/med/angiotargeting)
- COST Action 865: Bioencapsulation multiscale interaction analysis. (coordinator: D. Poncelet, France). Website: <http://cost865.bioencapsulation.net>

## KEY PUBLICATIONS OF THE LABORATORY

- Sakariassen PØ, Prestegarden L, Wang J, Skaftnesmo KO, Mahesparan R, Molthoff C, Sminia P, Sundlisaeter E, Misra A, Tysnes BB, Chekenya M, Peters H, Lende G, Kalland KH, Øyan AM, Petersen K, Jonassen I, van der Kogel A, Feuerstein BG, Terzis AJ, Bjerkvig R, Enger PØ. Angiogenesis-independent tumor growth mediated by stem-like cancer cells. *Proc Natl Acad Sci U S A*. 2006; 103(44):16466-71. Epub 2006 Oct 20.
- Terzis AJ, Niclou SP, Rajcevic U, Danzeisen C, Bjerkvig R. Cell therapies for glioblastoma. *Expert Opin Biol Ther*. 2006; 6(8):739-49.
- Goplen D, Wang J, Enger PØ, Tysnes BB, Terzis AJ, Laerum OD, Bjerkvig R. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. *Cancer Res*. 2006; 66(20):9895-902.
- Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. The origin of the cancer stem cell: current controversies and new insights. *Nat Rev Cancer*. 2005; 5(11):899-904 (Opinion).
- Read TA, Sorensen DR, Mahesparan R, Enger PO, Timpl R, Olsen BR, Hjelstuen MH, Haraldseth O, Bjerkvig R. *Local endostatin treatment of gliomas administered by microencapsulated producer cells*. *Nat Biotechnol*. 2001; 19(1):29-34.

## PUBLICATIONS 2007

### 1 PUBLICATIONS INVOLVING CRP-SANTÉ

- Utvik JK, Youssef I, Kriem B, Oster T, Terzis AJA, Bjerkvig R, Pillot B, Niclou SP. *Delivery of neuroprotective peptides from encapsulated cells in a mouse model for neurodegenerative disease*. Conference Proceedings. COST Action 865. XV International Workshop on Bioencapsulation in Vienna, Austria, September 2007.
- Niclou SP & Bjerkvig R. *Treatment of brain tumors with micro-encapsulated cell therapy*. Book Chapter in: *The Bioartificial Endocrine Pancreas*. Research Signpost, Ed. J-P Hallé (in press).
- Utvik JK & Niclou SP. *Recombinant cell micro-encapsulation for treating neurodegenerative diseases in the ageing brain*. Book Chapter in: *The Bioartificial Endocrine Pancreas*. Research Signpost, Ed. J-P Hallé (in press).
- Rajcevic U, Niclou SP and Jimenez CR. *Proteomics strategies for target identification and biomarker discovery in cancer*. *Frontiers in Bioscience*. Ed. R Bjerkvig (in press).

### 2 OTHER PUBLICATIONS

- Sundlisaeter E, Dicko A, Sakariassen PØ, Sondenaa K, Enger PØ, Bjerkvig R. *Lymphangiogenesis in colorectal cancer-prognostic and therapeutic aspects*. *Int J Cancer*. 2007, 121(7):1401-9. Review.
- Stuhr LE, Raa A, Oyan AM, Kalland KH, Sakariassen PO, Petersen K, Bjerkvig R, Reed RK. *Hyperoxia retards growth and induces apoptosis, changes in vascular density and gene expression in transplanted gliomas in nude rats*. *J Neurooncol*. 2007, 85(2):191-202. Epub 2007 Jun 8.
- Tysnes BB, Bjerkvig R. *Cancer initiation and progression: involvement of stem cells and the microenvironment*. *Biochim Biophys Acta*. 2007, 1775(2):283-97. Epub 2007 Feb 9. Review.
- Thorsen F, Enger PØ, Wang J, Bjerkvig R, Pedersen PH. *Human glioblastoma biopsy spheroids xenografted into the nude rat brain show growth inhibition after stereotactic radiosurgery*. *J Neurooncol*. 2007; 82(1):1-10. Epub 2006 Sep 6.
- Raa A, Stansberg C, Steen VM, Bjerkvig R, Reed RK, Stuhr LE. *Hyperoxia retards growth and induces apoptosis and loss of glands and blood vessels in DMBA-induced rat mammary tumors*. *BMC Cancer*. 2007, 30(7):23.

/ LABORATORY OF MOLECULAR PATHOLOGY

Head of the laboratory:

Jos EVEN, MD, PhD



Presently, the staff of the laboratory is as follows:

Nadine NEY, Engineer

External collaborators:

|   |                                      |
|---|--------------------------------------|
| Laboratory of Pathology   | René SCHEIDEN, MD, Pathologist, Head |
|   | Walter DIPPEL, MD, Pathologist       |
|   | Gérard GRIGNARD, Technician          |
| National Tumor Registry<br>(Régistre Morphologique des Tumeurs) | René SCHEIDEN, MD, Pathologist       |

Consultant and initiator of the project:

Nelly KIEFFER, PhD, Cell Biologist, CNRS LIA: «Posttranslational Modifications in Cancer»  
Sino-French Research Center for Life Sciences and Genomics, Rui Jin Hospital, Shanghai, China

Consultants and/or collaborators:

Carlo BOCK, MD, Oncologist, Clinique Ste Thérèse, Luxembourg  
Guy BERCHEM, MD, Oncologist, Centre Hospitalier, Luxembourg  
Stanislas LAMY, MD, Urologist, Centre Hospitalier, Luxembourg  
Joel MOSSONG, PhD, Biostatistician, Laboratoire National de Santé, Luxembourg  
Michel NATHAN, MD, Urologist, Centre Hospitalier Emile Mayrisch, Esch-sur-Alzette, Luxembourg  
Simone NICLOU, PhD, Tumor Biologist, Centre de Recherche Public Santé, Luxembourg  
François SCHNEIDER, MD, Microbiologist, Laboratoire National de Santé, Luxembourg  
Werner ZWERSCHKE, PhD, Tumor Biologist, Department for Biomedical Aging Research  
and Tyrolean Cancer Research Department, Innsbruck, Austria



HISTORY OF THE LABORATORY

In October 2001, N. Kieffer PhD (Laboratoire LBPI, Université du Luxembourg), René Scheiden, MD (Division of Pathology, LNS) and Michel Nathan MD (Département d'Urologie, CHEM) were granted a 5 year research grant by the Fonds National de la Recherche to establish a state-of-the-art research facility in molecular pathology at the interface between clinicians, pathologists and basic research scientists.

*(Title: A molecular pathology approach for prostate cancer research : Application of the laser capture microdissection technology (LCM) to the molecular investigation of patients with prostate cancer metastasis. FNR Grant Biosan 01/04/06 coordinator: N. Kieffer PhD [www.fnr.lu](http://www.fnr.lu)).*

The goal of this project was to develop new skills in molecular pathology applied to prostate cancer research in order to identify genes that are selectively up- or down-regulated during prostate cancer metastasis. Such genes could serve as prognostic markers, or alternatively, as potential targets for the development of new therapeutics. In addition, a major aspect of this FNR project was to create the basic competences for a national molecular pathology platform to be integrated into the Pathology Department of the LNS (Laboratoire National de Santé/ National Health Lab).

At the end of 2006 the major objectives to create competences in molecular biology applied to tumour tissue analysis had been achieved. In particular, the laser microdissection technology had been set up.

Scientific results of this project: identification of ILK (integrin-linked kinase) as a marker gene that allows to ascertain specific adenocarcinoma cell RNA isolation from frozen prostate biopsy tissue sections following laser microdissection, ( Kieffer et al 2005).

RNAse L gene polymorphism Arg462Gln (R462Q) is not associated with sporadic prostate cancer in the Luxembourg population.

Complete loss of PTEN expression is a possible early prognostic marker for prostate cancer metastasis; (Schmitz et al 2007).

This was by far the most important result. Given the interest in PTEN expression or lack of expression in animal prostate cancer models, human prostate cancer and other tumors the article attracted a lot of attention from the scientific community. Comments with an interview of Dr Kieffer were published by Reuters and FNR selected the project as one of its success stories (Annual report 2006 [www.fnr.lu](http://www.fnr.lu)).

In the Fall of 2007 N. Kieffer left Luxembourg to take a position in Shanghai and the project was transferred into LNS space for an additional two year period till May 2009.

## CURRENT KEY OBJECTIVES

Validation of loss of PTEN expression as an early marker for metastatic progression of prostate cancer (Schmitz et al. 2007).

Optimization of techniques for DNA extraction and genetic tests in paraffin embedded tumor specimen.

Set up new tests in oncology such as oncogene expression and/or mutations to guide bio-therapy trials.

Use the LMD microscope in collaborative studies with colleagues of the oncology department of CRP-Santé and the University of Luxembourg.

## ONGOING PROJECTS

*2007 was a gap year during which the lab was moved and set up in new space with new people.*

The validation of loss of PTEN expression as an early prognostic marker for metastatic progression of prostate cancer is presently underway in a retrospective study with samples provided by the National Tumor Registry.

Given the problems encountered with commercial anti-PTEN antibodies we are trying to generate anti-PTEN monoclonal antibodies using recombinant PTEN protein expressed in E. coli as an immunogen, in order to obtain antibodies that exhibit a high affinity for PTEN in formaldehyde-fixed tissue sections. These antibodies would be extremely useful tools in the future to investigate the loss of PTEN expression in prostate cancer as well as various other tumor types. Several hybridomas obtained from several fusions are being tested.

Molecular techniques have been optimized on archived paraffin embedded blocks of penis carcinoma fixed with Bouin (till the early 1990s) and formalin. In this project we test for the presence of HPV known to be present in about 40% of all penis carcinomas. In a collaboration with W Zwerschke in Innsbruck were looking at E7 expression in cervical carcinoma. (Ressler et al, 2007). This topic particularly relevant to recent developments in HPV detection in cervical swabs.

## PUBLICATIONS LINKED TO THE PROSTATE PROJECT

- Kieffer N, Schmitz M, Plancon S, Margue C, Huselstein F, Grignard G, Dippel W, Nathan M, Giacchi S, Scheiden R. ILK as a potential marker gene to ascertain specific adenocarcinoma cell mRNA isolation from frozen prostate biopsy tissue sections. Int J Oncol. 2005. 26:1549-1558.
- Schmitz M, Grignard G, Margue C, Mossong J, Dippel W, Giacchi S, Nathan M, Scheiden R, Capesius C, and Kieffer N. Complete loss of PTEN expression as a potential early prognostic marker for prostate cancer metastasis. Int J Cancer. 2007. 120:1284-92.

## PUBLICATION 2007

Ressler S, Scheiden R, Dreier K, Laich A, Müller-Holzner E, Pircher H, Morandell D, Stein I, Viertler HP, Santer FR, Widschwendter A, Even J, Jansen-Dürr P, Capesius C, Zwerschke W. High-risk human papillomavirus E7 oncoprotein detection in cervical squamous cell carcinoma. Clin Cancer Res. 2007.13:7067-72.

## / MICROARRAY CENTER

### Head of the laboratory:

**Laurent VALLAR**, PhD

Former head of the laboratory: **Evelyne FRIEDRICH**, PhD



### Presently, the staff of the laboratory is as follows:

**Petr NAZAROV**, PhD, (Biostatistics; replaces Dr M. Yatskou from November 13, 2007), Researcher

**Nathalie NICOT**, (Molecular Biology; replaces Dr A. Mehlen from August 1, 2007)

**Arnaud MULLER**, (Bioinformatics), Researcher Engineer

**François BERNARDIN**, (Molecular Biology), Technician



## HISTORY OF THE LABORATORY

The Microarray Center was created in early 2002 in the frame of a project from the first health care program (BIOSAN) funded by the National Research Fund. In partnership with the National Laboratory of Health and the CRP-Henri Tudor, this project aimed at implementing in Luxembourg new competence in genomics and in bioinformatics with an application in cancerology. Initially part of the laboratory of molecular biology, genomics and modelling (LBMAGM) headed by Dr E. Friederich, the Center is autonomous since September 2007 under the supervision of Dr L. Vallar and is integrated into the department of oncology of the CRP-Santé.

The team at the Microarray Center was organised with people from multiple disciplines, and includes research scientists and technical staff with expertise in cell and molecular biology, bioinformatics, statistics and computer science. The platform progressively installed a full set of equipments and software allowing the handling, the preparation and the analysis of large series of samples, as well as the manufacture of microarrays. All the instruments are based on high-performance, widely distributed technologies (to allow standardisation and exchange with other platforms). They are versatile and user-friendly systems (to allow accessibility to users with widespread interests), and are upgradable (to allow the integration of new technological developments or the implementation of new applications). At the same time, the Microarray Center installed local replica of the main biological databases (Genbank, RefSeq, SpTrembl, etc) that are necessary for microarray design and data exploration. The group also implemented a specialised database for the storage of information about actin cytoskeleton-related genes (Actinome) and a Laboratory Information Management System (LIMS) dedicated to microarray



data (BASE). With these equipments, the platform has a high flexibility, so that it can offer fully customised solution regarding the needs of the users.

Technology watch is a permanent concern on the Microarray Center to maintain updated skills and knowledge in the rapid evolving fields of the microarray technology, imaging and biological databases. Members of the team participated in number of specialised international workshops over the last years and maintain useful contacts with leading European laboratories. Knowledge valorisation was achieved through the implementation of two websites (www.microarray.lu and www.bioinformatics.lu) that give a comprehensive overview of the platform providing potential users with technical and practical information about microarray technology and access to the local resources. The group organised on a regular basis several information and training sessions for users, and drew up several protocols and tutorials that were made freely accessible to users. The microarray Center also participated in the training of doctoral and master fellows in connection with the University of Strasbourg (PhD thesis of J. Muller, master degree diploma of C. Thill).

Over the past years, the Microarray Center developed standardised protocols and tools enabling the optimisation of each step, from start to finish, of microarray-based gene expression profiling.

Standardisation of procedures was a crucial task as it is required to fully exploit microarray data notably through data sharing and comparison between laboratories. A strategy to design oligonucleotide probes with homogeneous hybridisation properties and a high specificity was validated in collaboration with the Laboratory of Biology et Structural Genomics (IGBMC, Strasbourg).

This strategy is mainly based on a home-made program named CADO4MI having the advantage, when compared with other similar existing algorithms, to combine multiple designs with the use of distinct reference biological databases (PhD thesis of J. Muller, 2006). The microarray procedure was refined and several quality controls were introduced at key steps of the process in order to improve the accuracy and reproducibility of microarray data. A comprehensive data analysis pipeline was implemented based on a suite of specialised software allowing the assessment of microarray image quality, the filtering of statistically meaningful data and the mining of gene expression profiles. The procedure was shown to be a powerful alternative to conventional approaches for the analysis of two-colour microarray experiments (M. Yatskou et al. Manuscript submitted). In partnership with the Santec group from the CRP-Henri Tudor, the Microarray Center also participated in the development of software for the analysis of microarray images.

The Microarray Center set up specific expertise in the design and the production of high quality oligonucleotide microarrays focused on definite biological theme. The group designed a dedicated microarray called Actichip for the profiling of genes related to the actin cytoskeleton. Actichip was evaluated through a careful benchmarking and showed performance equivalent to those of commercial arrays in terms of specificity, sensitivity and reproducibility (J. Muller et al. BMC Genomics 2007). The microarray is now used as a routine investigation tool in several basic and clinical research projects (see below). Controlchip is another custom array developed at the Microarray Center based on a series of 10 sense oligonucleotide probes corresponding to Arabidopsis genes spotted at various concentrations. The array allows assessing the consistency, sensitivity and reproducibility of microarray procedure through the hybridisation of a mixture of a defined copy number of the corresponding antisense RNAs synthesised *in vitro*. Several other custom arrays were successfully developed by the platform in the frame of collaborations with external laboratories including Retchip, a microarray dedicated to the study of the retina and related diseases, and specific protein- or phage-based microarrays (see below).

Tools and methodologies for transcriptome analysis implemented by the Microarray Center were validated using a biological demonstration model for tumour progression. The study focused on the modulation of gene expression occurring during a key step of epithelium-

derived tumour progression towards an invasive state, the so-called epithelial-to-mesenchyme transition (EMT). A cell model was developed by the LBMAGM based on a transfected human mammary carcinoma-derived cell line that undergoes an EMT upon the inducible expression of the transcription factor Sna1. Time-course expression profiling experiments performed at early stages of Sna1 induction (2-10h) revealed a limited number of genes which exhibited distinct expression patterns when compared to that of Sna1.

Exploration of these data, combined with functional assays, yielded novel information on the cascade of events leading to Sna1-induced EMT (G. Vetter et al. Manuscript in preparation).

Being part of the LBMAGM, a laboratory focused on the study of the actin cytoskeleton and its role in cell morphogenesis and migration, the Microarray Center naturally participates in several research projects in this field. In the recent past, the group contributed to the sequence and comparative genomics study of actin-related proteins performed at the IGBMC (J. Muller et al. *Molecular Biology of the Cell* 2005). The group took part in the development of a web tool called ARPAnno (Actin Related Proteins Annotation server) that automatically annotates protein sequences according to the ARP classification.

In collaboration with researchers and clinicians from the University and the Hospital Center of Luxembourg, the Microarray Center contributed to projects aiming at dissecting the molecular mechanisms of complex-trait pathologies like Alzheimer disease (Pr. P. Heuschling), blood and lung cancer (Dr. G. Berchem), cardio-vascular diseases (Dr. D. Wagner) or allergies (Dr. F. Hentges).

## CURRENT KEY OBJECTIVES

The Microarray Center aims at developing microarray-based collaborative projects in partnership with researchers in the academic, industrial or pharmaceutical community from Luxembourg and surrounding areas. Besides, the platform is accessible on a fee for service basis.

The Microarray Center provides know-how and expertise in transcriptome analysis based on high performance equipment and software, as well as standardised protocols and pangenomic arrays for use with human and mouse. The Center offers the possibility to design and produce custom microarrays from DNA, oligonucleotide or protein samples, and is willing to implement new microarray application that may be required for specific biomedical research. The platform also includes a bio-informatics and bio-statistics group participating in the design of specific oligonucleotide probes and in the analysis and mining of microarray data.

The Microarray Center provides a large array of equipments among which:

- **A high-precision robotic microarrayer Microgrid II** (Genomic solutions) that is dedicated to the production of microarrays through printing of cDNA; oligonucleotide or proteins on microscopy slides.
- **Microarray hybridization and washing stations** (Slidebooster 800 and Advawash, Advalytix) that improve the sensitivity and reproducibility of microarray analyses.
- **A microarray fluorescence scanner Genepix 4000 B** (Axon).
- **A high precision liquid handling robot Microlab Star** (Hamilton) that automates sample picking and dilutions and microplate reformatting.
- **A microfluidics-based platform 2100 Bioanalyzer** (Agilent Technologies) for the automated analysis of nucleic acids and protein.
- **A Server** (Sun Solaris), with 4 CPU, 4 Gb RAM and 400 Gb free hard disk to store microaray data and run local replica of a number of key databases (Genbank, Unigene, RefSeq, SpTrembl...).

- **Several specialised and high performance software** for microarray image analysis (Genepix Pro, MAIA), microarray data visualisation and statistical analysis (Acuity, Partek Genomics Suite), and microarray data mining (Ingenuity Pathways Analysis).
- **R and Bioconductor tools** for bioinformatics and computational biology.
- **A home-made program** for the design of oligonucleotide probes (CADO4MI).

## ONGOING PROJECTS AND MAIN RESULTS:

In 2007, the Microarray participated in several research projects with national and international partners:

- **Within an EU-Integrated project of the PC6 (EVI-GENORET)**, the Microarray Center designed and produced Retchip, a thematic microarray dedicated to the study of retinopathies (in collaboration with Dr. O. Poch, IGBMC, Strasbourg and Dr. T. Leveillard, INSERM U592, Paris). Retchip benchmarking showed that the array has excellent performance characteristics that make it a robust platform for transcriptome studies. Using Retchip, gene expression profiling experiments were performed with samples extracted from the retina of wild-type and rd1 mice, a transgenic model of retinal degeneration. Analysis of gene expression variations at different time points spanning the entire retina degeneration revealed several groups of genes with distinct expression patterns. Exploration of these data with bioinformatics tools is now in progress at the IGBMC and should yield important new information on the molecular mechanisms underlying retinal diseases.
- **In the frame of the FNR project BIOSAN/01/04/09**, we started the development of a new version of Actichip that will include genes implicated in cell signalling pathways linked to the actin cytoskeleton (in collaboration with Dr. E. Friederich, University of Luxembourg and Dr. O. Poch, IGBMC, Strasbourg). Actichip was used for the characterisation of a human cell line resistant to TNF $\alpha$ -induced cell death, and allowed the initial identification of L-plastin as a key player in this process (in collaboration with Dr. G. Berchem, manuscript in preparation).
- **In the context of the FNR project SECAL/03/07/05**, the Microarray Center carried out a comparative gene expression study of MCF-7 cells treated by two pesticides having known endocrine disruptor activity and frequently found in Luxembourg environment, Atrazin and PCB153. Pangenomic microarray data revealed that each of these compounds induced specific expression profiles when compared with oestradiol. The data will be validated and integrated with those resulting from proteome analysis with the aim at defining specific biomolecular signatures for the two oestrogen-like molecules (in collaboration with Pr. L. Hoffmann, CRP-GL and Pr. C. Muller, CRP-Santé/LNS).
- **Within the FNR project SECAL/03/07/03**, we designed and manufactured an oligonucleotide microarray that will be used to discriminate several fish species in food samples based on the identification of orthologous genes encoding for parvalbumin. Benchmarking experiments will be performed to determine the performance of the device in terms of sensitivity, specificity, reproducibility and accuracy (in collaboration with Dr. F. Hentges).
- **In the frame of the project CRP/2008/01/16**, the Microarray Center took part in the development of a phage-based microarray using a phage display library raised against HIV. A feasibility study was performed with control antibodies and gave promising results showing that this innovative tool could be used to screen the reactivity of patient sera. Further optimisations of the experimental procedure are in progress (in collaboration with Dr. J.C. Schmit).
- The Microarray Center participated in the statistics and bioinformatics analysis of data from the transcriptome profiling of blood leukocytes in brain-dead organ donors. The study revealed that specific genomic signatures, mostly concerning protein biosynthesis, are associated with high versus low prolactin blood levels (in collaboration with Dr. D. Wagner, manuscript submitted).

- Jean Muller and Evelyne Friederich from the laboratory participated in an interdisciplinary study (Stoezel et al., *Nature Genetics* 2006, Stoezel C et al. *Am J Hum Genet.* 2007) identifying a gene encoding a chaperonin that is mutated in the Bardet-Biedl syndrome, a disease that is characterized most prominently by a progressive retinal dystrophy, polydactyly, cognitive impairment, and renal dysplasia.

## EXTERNAL COLLABORATIONS

### 1 CRP-SANTÉ:

- Laboratory of experimental hemato-cancerology (Dr. G. Berchem).
- Laboratory of immuno-allergology (Dr. F. Hentges).
- Department of immunology (Pr. C. Muller).
- Laboratory of retrovirology (Dr. J.C. Schmit).
- Laboratory of cardio-vascular research (Dr D. Wagner).

### 2 NATIONAL:

- Department of environment and agro-biotechnology, CRP-Gabriel Lippmann (Dr. L. Hoffmann).
- Life sciences research unit, University of Luxembourg, (Pr. E. Friederich, Pr. P. Heuschling).
- Santec group, CRP-Henri Tudor (P. Plumer).

### 3 INTERNATIONAL:

- European integrated project EVI-GENORET entitled “functional genomics of the retina in health and disease” ([www.evi-genoret.org](http://www.evi-genoret.org)).
- Laboratory of Biology et structural genomics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France.
- Department of Systems Analysis, Belarusian State University, Minsk, Belarus.
- Laboratory of medical biochemistry and molecular biology, CNRS UMR 6198, Reims, France.
- Bioinformatics department, Institut Curie, Paris, France.

[ewwww.microarray.lu](http://ewwww.microarray.lu), [www.bioinformatics.lu](http://www.bioinformatics.lu)

## KEY PUBLICATIONS OF THE LABORATORY

- Muller, J., Oma, Y., Vallar, L., Friederich, E., Poch, Olivier and B. Winsor. Sequence and comparative genomics analysis of actin related Proteins in the eukaryotic phyla highlight the central role of nuclear ARPs. *Molecular Biology of the Cell* 2005, 16:5736-5748.
- Corinne Stoezel, Virginie Laurier, Erica E. Davis, Jean Muller, Suzanne Rix, José L. Badano, Carmen C. Leitch, Nabiha Salem, Eliane Chouery, Sandra Corbani, Nadine Jalk, Serge Vicaire, Pierre Sarda, Christian Hamel, Didier Lacombe, Muriel Holder, Sylvie Odent, Susan Holder, Alice S Brooks, Nursel H Elcioglu, Eduardo Da Silva, Béatrice Rossillion, Sabine Sigaudy, Thomy JL de Ravel, Richard Alan Lewis, Bruno Leheup, Alain Verloes, André Mégarbané, Olivier Poch, Dominique Bonneau, Philip Beales, Jean-Louis Mandel, Nicholas Katsanis, Hélène Dollfus. A novel gene encoding a vertebrate-specific chaperonin-like protein is a major BBS locus. *Nature Genetics* 2006, 38:521-4.

## PUBLICATIONS 2007

- Stoezel C, Muller J, Laurier V, Davis EE, Zaghoul NA, Vicaire S, Jacquelin C, Plewniak F, Leitch CC, Sarda P, Hamel C, de Ravel TJ, Lewis RA, Friederich E, Thibault C, Danse JM, Verloes A, Bonneau D, Katsanis N, Poch O, Mandel JL, Dollfus H. Identification of a novel BBS gene (BBS12) highlights the major role of a vertebrate-specific branch of chaperonin-related proteins in Bardet-Biedl syndrome. *Am J Hum Genet.* 2007;80(1):1-11.
- Jean Muller, Andre Mehlen, Guillaume Vetter, Mikalai Yatskou, Arnaud Muller, Olivier Poch, Evelyne Friederich and Laurent Vallar. Design and evaluation of Actichip, a thematic microarray for the study of the actin cytoskeleton, *BMC Genomics* 2007, 8:294. The publication fee was financed by the FNR (accompanying measure FNR/07/MA4b/09).
- Golovaty A.A., Nazarov P.V., Yatskou M.M., Apanasovich V.V., Friederich E. Simulation and analytical models for the analysis of in vitro actin polymerization. *Proceedings of the 8th International Conference “Computer Data Analysis and Modeling: Complex Stochastic Data and Systems”, v. 2, Publishing center BSU, 2007, Minsk, p. 184-187.* Petr Nazarov was granted by the FNR (accompanying measure FNR/05/MA6/17).
- Longrois D., Yatskou M., Wagner D., Mertes P. M., Devaux Y. Gene Signatures in Blood Cells at Extreme Quartiles of Prolactin in Brain-Dead Organ Donors. *Anesthesiology* 2007; 107: A1963.

RESEARCH DEPARTMENTS

- DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY
- DEPARTMENT OF IMMUNOLOGY
- DEPARTMENT OF ONCOLOGY
- DEPARTMENT OF CARDIOVASCULAR DISEASES
- DEPARTMENT OF PUBLIC HEALTH



A B C D E



## / LABORATORY OF CARDIOVASCULAR RESEARCH

Head of the laboratory:

Daniel R. WAGNER, MD, PhD



Presently, the staff of the laboratory is as follows:

Yvan DEVAUX, PhD, Associate Head  
Carine PEREZ, Administrative Assistant  
Isabelle ERNENS, PhD, Scientist  
Magali ROLLAND-TURNER, PhD, Scientist  
Chantal SCHWARTZ, PhD, Scientist  
Céline JEANTY, Technician  
Mélanie VAUSORT, Technician  
Bernadette LENERS, Technician  
Christelle NICOLAS, Technician  
Huguette LOUIS, PhD, Scientist  
Sophie RODIUS, PhD, Scientist  
Emilie VELOT, PhD Student  
Frédérique LÉONARD, PhD Student  
Benjamin HAAS, Master Student  
Céline YVORRA, Master Student  
Emilie LIEFFRIG, Technician Student

In addition, two research nurses working at the Département de Cardiologie of the Centre Hospitalier du Luxembourg are responsible for blood sampling and transfer to the laboratory for processing.



### HISTORY OF THE LABORATORY:

The Laboratory of Cardiovascular Research was the last laboratory to be created at the CRP-Santé. Starting at the end of 2003, the group first accounted four members: a director, an associate director, a scientist and a technician. In 2005, four new members joined the group: a scientist, a post-doctoral student, a PhD student and a master student. In 2006, a new technician and a master student joined the group. In 2007, the group was enriched with an administrative assistant, two scientists, two technicians, a post-doctoral student and a PhD student. Also in 2007, the laboratory hosted three master students, a license student and a technician student.

All patients suffering from acute MI and who have signed an informed consent are enrolled in the LUCKY (Luxembourg Acute Myocardial Infarction) registry maintained in the laboratory.

Until 2006, the laboratory was entirely located in the basement of the Maternité Grande-Duchesse Charlotte – the former offices of the administrative staff of the CRP-Santé. Then the laboratory was kindly provided by the Centre Hospitalier du Luxembourg with a new experimental room and a new office space in the ground floor of the Maternité.

Cardiovascular diseases are the number one cause of morbidity and mortality in our country with 1573 deaths in 2003. The majority of deaths are linked to the development of congestive heart failure (979 in 2003). Congestive heart failure (CHF) has become a disease of epidemic proportion worldwide and especially in developed countries, affecting 3% of the adult population. We estimate that in 2040, 20% of the population older than 65 years will suffer from CHF. This will have a major medical and economic impact in Luxembourg in the next few decades.



Mortality of CHF is worse than many forms of cancer with a five-year survival of less than 30%. Myocardial infarction (MI) is the leading cause of CHF.

Indeed, despite modern reperfusion therapy with thrombolysis or coronary angioplasty, approximately 30% of the patients with MI develop left ventricular remodeling and CHF.

Despite these alarming statistics, the mechanisms responsible for the occurrence of CHF are still poorly known. It is certain that a better understanding of these mechanisms constitutes the foundations for the discovery of new treatments aiming at stopping this epidemic. In this context, the activities of translational research of the laboratory are based on the study of the mechanisms setting the stage for the development of CHF.

## ONGOING PROJECTS

The laboratory is currently running the following research projects:

### 1 ADENOSINE AND MATRIX METALLOPROTEINASE -9 (MMP-9), FUNDED BY THE MCSER

One of the main causes of CHF is the adverse structural remodeling of the heart after MI. MMPs, and particularly MMP-9, play a key role in this remodeling. We have measured MMP-9 concentration in plasma from patients with acute MI from the Centre Hospitalier du Luxembourg and we have shown that MMP-9 is positively associated with the severity of CHF, therefore constituting a new biomarker for CHF. This unprecedented observation, later confirmed by other research groups, is now fully patented by our group.

Adenosine, a nucleoside produced in massive amounts in the heart after MI, has cardioprotective properties. We are studying a potential role of adenosine in the development of CHF. We have demonstrated in 2006 that adenosine inhibits the production of MMP-9 by neutrophils, the first wave of inflammatory cells recruited to the heart after MI. In 2007, we have been interested in characterizing the effect of adenosine on MMP-9 production by the second wave of cells recruited to the heart after MI, i.e. monocytes/macrophages. We have shown that, in opposition to our results obtained in neutrophils, adenosine reliably enhances MMP-9 production by macrophages.

These results are actually under evaluation for a provisional patent and a publication is being written.

### 2 ADENOSINE AND ANGIOGENESIS, FUNDED BY THE MCSER

Following a MI, the deteriorated part of the heart has to be repaired. This healing involves several mechanisms among which angiogenesis, the formation of new blood vessels, plays a key role. Angiogenesis is under the control of the vascular endothelial growth factor (VEGF), a protein which stimulates the growth and multiplication of endothelial cells constituting the blood vessels. We have shown that adenosine stimulates the production of VEGF by human macrophages, thereby favoring angiogenesis.

These results are actually under evaluation for a provisional patent and a publication is being written.

### 3 ADENOSINE AND OTHER CELL TYPES INVOLVED IN CHF, FUNDED BY THE MCSER

Imbalance in myocardial extracellular matrix synthesis and degradation post-MI contributes to left ventricular remodeling and CHF. Cardiac fibroblasts and myofibroblasts are major players of extracellular matrix turnover. We hypothesize that the cardioprotective effects of adenosine on the development of heart failure are due, at least in part, to modulation of cardiac fibrosis. The aim of this project is therefore to determine the effects of adenosine on ECM turnover in human cardiac fibroblasts.

Substantial evidence indicates that cell-based therapies can repair the heart. Cell therapy involves homing of endothelial progenitor cells (EPCs) into ischemic tissue where adenosine concentrations are increased. The effects of adenosine on EPCs are poorly known. The purpose of this project is to characterize the interactions between EPCs, adenosine, MMP-9, and the clinical outcome of the patients from the LUCKY registry.

### 4 GENETIC VARIATIONS OF MMP-9, FUNDED BY THE FNR

In 2006, we performed a pilot study aiming at associating genetic variations of the MMP-9 gene with the occurrence of CHF post MI. This study identified several punctual mutations (or Single Nucleotide Polymorphism, SNP) linked to CHF. In a larger cohort of the LUCKY registry of patients with acute MI (200 patients), we studied 4 mutations and one of them had a significant association with the development of CHF four months after MI. The presence of this mutation decreases the chances to develop CHF and is therefore cardioprotective. We have started several research projects to study the impact of the potential use of this mutation in clinical practice. The importance of a specific SNP, which we think may be critical in the development of CHF, is currently being investigated at Jefferson University, Philadelphia.

These results are actually under evaluation for a provisional patent.

### 5 THE "RUNNING MICE PROJECT", FUNDED BY THE FNR

Adenosine levels are increased after muscular exercise and in patients with CHF. The activity of 5'-nucleotidase, the main enzyme controlling adenosine metabolism, is increased in the left ventricle of rats after endurance training and may play an important role in the prevention of adverse remodeling of the left ventricle. Physiological hypertrophy of the heart has been linked to cardiac insulin growth factor (IGF)-1 formation. The involvement of IGF-1 in left ventricular remodeling post MI and its relationship with the beneficial effects of adenosine are not known. Through this project, we want to test the hypothesis that the interplay between adenosine and MMPs is involved in the protection from HF provided by physical activity.

This project is partly performed at Homburg/Saar University.

### 6 TRADITIONAL CHINESE MEDICINE, A EUROPEAN UNION PROJECT

In our search for new therapeutic strategies of CHF, we wondered whether compounds from Traditional Chinese Medicine could have useful properties. We have established collaboration with the *Shanghai Innovative Research Center of Traditional Chinese Medicine* in China that will provide us with ginseng extracts. These plant extracts will be evaluated based on their effects on several mediators of angiogenesis and inflammation, both involved in left ventricular remodeling.

This project is part of a Specific International Cooperation Action (SICA) project submitted for the 7th Research Framework Program call HEALTH-2007-2.1.2-7: Traditional Chinese Medicine in post-genomic era.

## 7 IMPLEMENTATION OF THE DNA CHIPS TECHNOLOGY

Thanks to grants from multiple sources (CRP-Santé, Société pour la Recherche sur les Maladies Cardiovasculaires ...), the laboratory has set up the microarrays technology. This state-of-the-art technique allows for determination of the level of expression of thousands of genes in a single experiment. This technology is currently being applied to four research projects mainly performed in collaboration with the Centre Hospitalier et Universitaire of Nancy.

A publication on the potential biological roles of a biomarker of infection, the procalcitonin, is under submission.

## COLLABORATIONS

The Laboratory has therefore been able to undergo a rapid growth not only through the hiring of new staff members – thanks to the CRP-Santé, the Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche (MCSER) and the Fonds National pour la Recherche (FNR) – but also through the search for external collaborators. The laboratory has effectively ongoing collaborations with several departments nationwide and worldwide:

- 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'Héмато-Cancérologie.
- Inside the CRP-Santé: genomic and flow cytometry core facilities.
- Departments of Cardiology and Internal Medicine, University of Homburg/Saar, and University of Cologne, Germany.
- Centre Hospitalier et Universitaire (Departments of Anesthesia and Intensive Care, Nancyclotep), Faculty of Medicine (INSERM U684), Faculty of Sciences (Laboratory of Cristallography), Centre d'Investigation Clinique, Nancy, France.
- Department of Internal Medicine, Philadelphia Hospital and Jefferson University, USA.
- Shanghai Innovative Research Center of Traditional Chinese Medicine, China.

## KEY PUBLICATIONS OF THE LABORATORY

- Rouy D., Ernens I., Jeanty C. and Wagner D.R. Plasma Storage at –80°C Does Not Protect Matrix Metalloproteinase-9 From Degradation. *Analytical Biochemistry* 338: 294-298, 2005.
- Wagner DR, Delagardelle C, Ernens I, Rouy D, Vaillant M, Beissel J. Matrix Metalloproteinase-9 is a Marker of Ventricular Remodeling after Acute Myocardial Infarction. *Journal of Cardiac Failure*, 12(1): 66-72, 2006.
- Lebrun F, Berchem G, Delagardelle C, Beissel J, Rouy D, Wagner DR. Improvement of exercise-induced cardiac deformation after cell therapy for severe chronic ischemic heart failure. *Journal of Cardiac Failure*, 12(2): 108-113, 2006.
- Ernens I, Rouy D, Velot E, Devaux Y, Wagner DR. Adenosine inhibits matrix metalloproteinase-9 secretion by neutrophils: implication of A2a receptor and cAMP/PKA/Ca2+ pathway. *Circulation Research*, 99(6):590-597, 2006.
- Audibert G., Charpentier C., Seguin-Devaux C., Charretier P.A., Gregoire H., Devaux Y., Perrier J.F., Longrois D., Mertes P.M. Improvement of Donor Myocardial Function after Treatment of Autonomic Storm During Brain Death. *Transplantation*, 82(8): 1031-1036, 2006.

## PUBLICATIONS 2007

- Audibert G., Bousquet C., Charpentier C., Devaux Y., Mertes P.M. Hémorragie sous-arachnoïdienne : épidémiologie, prédisposition, présentation clinique. *Annales Françaises d'Anesthésie et de Réanimation*, 26(11):943-947, 2007.
- Louis H., Kakou A., Regnault V., Labat C., Bressenot A., Gao-Li J., Gardner H., Thornton S.N., Challande P., Li Z. and Lacolley P. Role of alpha-1 beta-1-integrin in arterial stiffness and angiotensin-induced arterial wall hypertrophy in mice. *American Journal of Physiology Heart and Circulatory Physiology*, 293:2597-2604, 2007.

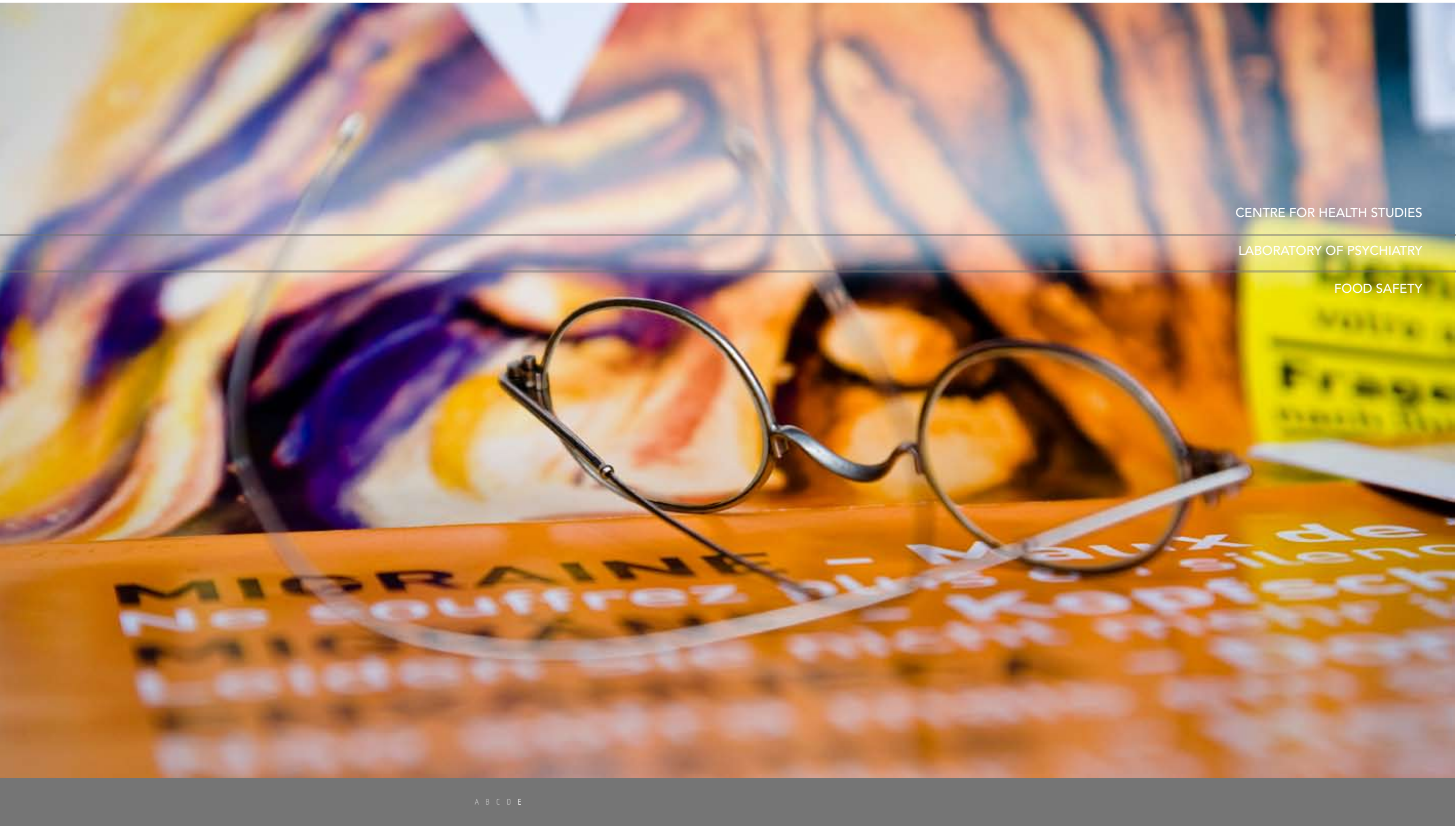
## PATENTS

- A patent on MMP-9 as a risk factor for CHF is accepted.
- A provisional patent on a mutation of the MMP-9 gene as a diagnostic marker and a platform for drug design in MI and CHF is pending.
- A provisional patent on the inhibition of MMP-9 production by a combined approach involving an A2a agonist and an A3 antagonist is pending.



RESEARCH DEPARTMENTS

- DEPARTMENT OF VIROLOGY, ALLERGOLOGY AND IMMUNITY
- DEPARTMENT OF IMMUNOLOGY
- DEPARTMENT OF ONCOLOGY
- DEPARTMENT OF CARDIOVASCULAR DISEASES
- DEPARTMENT OF PUBLIC HEALTH



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/ CENTRE FOR HEALTH STUDIES

Head of the Research Unit:

Marie-Lise LAIR



Presently, the staff of the laboratory is as follows:

Cathy PIRES, secretary  
Christine GAUTHIER, secretary

Clinical Epidemiology and Public Health Unit:

Department Head: Sophie COUFFIGNAL, MD, Epidemiologist

Epidemiology and Public Health Unit:

Alaa AL KERWI, MD, Public Health Graduate, Project Leader  
Magali PERQUIN, PhD, Doctor of Biomedical Engineering, Project Leader  
Hanène SAMOUDA, PhD, Doctor of Biological Anthropology, Project Leader  
Michel VAILLANT, MSc, Biostatistician, Project Leader  
Véronique LOUAZEL, Scientific Collaborator, MSc in Education, Master in Health Promotion, Project Leader  
Martine HENTGES, Scientific Collaborator, Psychologist  
Agnès COLUMEAU, Research Nurse  
Aline LECOMTE, Research Nurse, Public Health Graduate  
Marco ZEIMET, Research Nurse  
Jessica BARRE, Master Student in Biostatistics

Sports and Health Unit:

Daniel THEISEN, PhD, Doctor of Kinesitherapy and Rehabilitation, Head of Ward Projects  
Thierry WINDHAL, Research Assistant, Physical Education Graduate  
Anne FRISCH, PhD Student

Health Promotion Unit:

Laurence FOND-HARMANT, PhD, Doctor in Sociology

European Monitoring Centre for Drugs and Drug Addiction:  
"Focal Point":

Pascale STRAUS, Scientific Collaborator, Psychologist  
Nathalie REMOVILLE, Scientific Collaborator, Pharmacist  
Sofia LOPES, Scientific Collaborator, Psychologist  
In collaboration with Alain ORIGER (National Drug Coordinator for the Health Directorate)

European EUROLIGHT Project:

With the collaboration of Colette ANDREE, Project Coordinator  
Nathalie REMOVILLE, Scientific Collaborator, Pharmacist

Systems analysis and health services Unit:

Department Head: Marie-Lise LAIR  
Gaëtane WIBRIN, Research Nurse, UCM Projects  
Christelle ROTT, Statistics Graduate, UCM Projects  
Jean-Pierre CORNEZ, Computer Specialist, UCM Projects  
Laurence RENARD, Scientific Collaborator, MSc in Health Economics, CHEM Project

Health Web Portal:

Project Leader: Marie-Lise LAIR  
Patty MATHGEN-GEISEN, Editor, Lawyer  
Sandrine LAVALLEE, Editor, Communication Graduate, Sociology Graduate  
Coralie DESSENNE, Data Administrator, Filing Clerk





## HISTORY OF THE CENTRE FOR HEALTH STUDIES

The Centre for Health Studies was founded in 2004, a year of major reorganisation within the Public Health Research Centre. On this date, to improve management and coordination in the area, various activities in the fields of clinical epidemiology and public health were consolidated into a single Health Systems and Services Centre. The management of this new centre was entrusted to Marie-Lise Lair.

The activities of the Epidemiology Resources and Health Information Systems Centre (Centre de Ressources en Épidémiologie et Systèmes d'Informations Sanitaires - CRESIS), which was founded in 1998 to carry out epidemiological and biostatistical studies on behalf of the Health Directorate, were integrated into the Centre for Health Studies as a **Clinical Epidemiology and Public Health Department** (SECSP). Nonetheless, this department's activities have continued to develop considerably since its creation in 2004 and now extend beyond the original limits set for CRESIS.

The "Prevention, Research, European Studies and Assessments in the Health Field" department, created in 2000 within the Public Health Research Centre was formally dissolved in 2004. Its activities, which included the **Focus Point for the European Monitoring Centre for Drugs and Drug Addiction** (PF-OEDT) as well as the European Medicines Agency (EMA), were handed over to the Centre for Health Studies. Since then, the European Monitoring Centre for Drugs and Drug Addiction has been a subsection of the Clinical Epidemiology and Public Health Department. Today, the EMA's activities are linked to the Centre for Health Studies.

The **Systems Analysis and Health Services Department** (SASSS), which was created in 2001 to service the Union of Health Mutual Funds, has continued its activities in the form of a department within the Centre for Health Studies, and continues to develop them. These have been supplemented by various hospital-financed studies.

In 2006, two new wards were opened:

- The **"Sports and Health" Unit**, whose mission is to develop research in sports medicine and to carry out various studies on behalf of the Ministry of Sport. Nowadays, physical activity is recognised as a determining factor in health promotion. It is of crucial importance in our country, where lifestyles often lack sufficient exercise, which is contributing to rising levels of obesity. This research activity targets all age groups and places particular emphasis on young people and the elderly. Numerous current research projects are aimed at young sportsmen and sportswomen.
- The **"Health Promotion" Unit**, whose mission is to develop research into the assessment of health promotional schemes and health education. In fact, public authorities in all countries are now introducing numerous schemes to promote health, in the hope of preventing disease and influencing public health and its related costs. It is a question of measuring the impact of such schemes and the sustainability of their effects, as well as assessing whether those members of society most in need are helped by these schemes and determining whether discrimination in health care is reduced.

Having decided in 2006 to create a Health Web portal aimed at citizens and professionals alike, the Ministry of Health entrusted this task to the Centre for Health Studies. A team was brought together to do this, working in continuous collaboration with the Ministry of Health and e-Luxembourg.

The merging of these varied and previously autonomous activities was to be a challenge for managers and all collaborators involved in order to consolidate with success such activities in way that promoted consistent progress and efficient collaboration between projects. After a period of three years, the outcome was positive. From that point onwards, the various research and resource activities have targeted major issues in public health identified by the World Health Organisation and the European Union and which are also recognised as being in the public interest for the people of Luxembourg.

The Centre for Health Studies and the Emotional Disturbances Laboratory, along with activities linked to the European Medicines Agency (EMA) were united in 2007 to form the Department of Public Health of the Public Health Research Centre.



## MISSIONS AND OBJECTIVES

**Within the sphere of epidemiology and public health, the missions of the Centre for Health Studies are to:**

- measure the health situation of the population of Luxembourg, thus enabling relevant information to be gathered for the development of national prevention policies and to enable Luxembourg to be ranked in relation to other European countries,
- carry out research into the risk-factors, causes, and development processes of certain prevalent illnesses or disorders in order to increase the knowledge-base in public health, as well as contributing to developments in the diagnosis or treatment of disease,
- contribute to, or establish record-keeping systems or sustainable and high-quality information systems on prevalent illnesses in Luxembourg, in order to consolidate information that is useful in monitoring the general health of the population as well as to evaluate health care results,

- promote research in public health, especially in the area of health promotion and the assessment of health policies,
- provide methodological support to clinical research carried out by clinicians by providing access to study design and statistics skills,
- contribute to the development of citizen information in order to make citizens responsible for their own health care.

**Within the realm of health systems and services, the missions of the Centre for Health Studies are to:**

- develop methodologies which allow the provision of resources based on the health care needs of the population,
- contribute to the development of health care performance through research into methods and technologies adapted to the socioeconomic context of health care in Luxembourg,
- carry out assessment studies for measuring the results of patient treatment or pilot projects.

The goal of the Centre for Health Studies is to become a central point of reference for public authorities in the area of epidemiological studies and assessment of programmes established in the field of health, thus giving them access to information useful in the policy-making process.

The development of research in public health is also a priority in order to contribute to increasing the knowledge-base in this field.

## ON-GOING PROJECTS

### 1 EPIDEMIOLOGY AND PUBLIC HEALTH ACTIVITIES

#### **HBSC Survey (Health Behaviour in School-aged Children)**

*Project Leader: Dr. Sophie Couffignal*

This survey is organised in 42 countries every 4 years by the World Health Organisation's regional office for Europe and is a cross-sectional survey whose target population is school-children aged 11, 13, 15. Its objective is to monitor the health behaviours of children over time and to identify factors that influence such behaviours. The results of the 2007 study are to be published in 2008. These will enable Luxembourg to be compared to other countries for the first time.

#### **Monitoring Perinatal Health in Luxembourg**

*Project Leader: Dr. Alaa Al Kerwi*

Following certification carried out in 2006 by the Centre for Health Studies, the new perinatal health monitoring system, called SUSANA, was implemented throughout maternity wards in Luxembourg with the support of the Health Directorate and a steering committee bringing together all those involved in the project.

The report on perinatal health covering the period from 2001 to 2003 has also been completed and is to be published in March 2008.

#### **Monitoring risk-factors of cardiovascular diseases in Luxembourg (ORISCAV-Lu)**

*Project Leader: Dr. Alaa Al Kerwi*

Within the framework of INTERREG III A (Lorraine-Wallonie-Luxembourg), and the project entitled "Cross-border Cardiovascular Prevention Centre": a network of collective research and actions in cardiovascular prevention", a cross-border monitoring system was developed in 2006

with a view to establishing comparable indicators of cardiovascular health using a standardised research methodology.

In 2007, thanks to Ministry of Health funding, the Centre for Health Studies began this vast survey of a representative sample of the general population of Luxembourg. This epidemiological survey is set to continue into 2008.

The initial results of this study are expected in early 2009 and will provide professionals and public authorities with detailed information on risk-factors related to diet, physical activity, smoking and alcohol consumption. In addition, anthropometric measures and biological measurements carried out on the representative sample of the population will, for the first time, provide a national reference whose progress will be monitored over a 5 year period.

#### **Study on Obesity and Excessive Weight in Children and Adolescents in Luxembourg (OSPEL)**

*Project Leader : Hanène Samouda, PhD*

Obesity has been declared a global epidemic by the World Health Organisation and the European Union has urged all European countries to establish appropriate prevention policies. Luxembourg is not exempt from this problem. Only one model exists today for the treatment of children suffering from obesity.

**The objectives of this research are to:**

- develop non-invasive and reliable methods, specifically adapted to children, to diagnose obesity and to establish a prediction of risks of complications linked to obesity,
- compare the efficacy of two treatment models for obese children, namely the traditional model and an ambulatory care model for parents and children combining elements related to diet, physical activity and psychological support.

In 2006 and 2007, 150 children were included in the various groups. Assessment after one year was carried out for the first groups and their initial results are expected in 2008. Inclusion of a further 150 children will continue into 2008.

#### **Feasibility Study for the establishment of a Diabetes Registry in Luxembourg (DIABCARE)**

*Project Leader: Magali Perquin, PhD, in collaboration with Véronique Louazel*

Diabetes is a chronic disease and is often the root cause of very serious, debilitating and very costly complications. One of the public health objectives is to gain a better understanding of the current status of the disease in Luxembourg in the light of a significant increase in global prevalence of the disease.

In collaboration with a group of doctors and a steering committee, this study aims to establish a diabetes registry based on an annual survey to be completed by doctors treating diabetic patients over a given period of time.

The results of this feasibility study have not been deemed conclusive or representative of the status of diabetics in Luxembourg.

An analysis of admissions and discharge data gathered by the Union of Health Mutual Funds has also been carried out. This work has enabled a determination to be made of diabetes treatment among the population covered by health insurance, identification of the type and number of complications treated annually, and a comparison of care services with international recommendations.

In addition, this study has enabled Luxembourg to comply with the European EUCID indicators for the first time.

The conclusions reached by this study were presented to the Health Directorate and the Union of Health Mutual Funds, along with proposals for the clinical monitoring of Diabetes in Luxembourg, associated with an annual analysis of the Union of Health Mutual Funds' database.

*Results will be officially published in March 2008.*

**Prevalence of Migraines and Headaches and Measurement of the socioeconomic impact of the disorder in 11 European countries: EUROLIGHT**

*Project Leader: Colette Andrée*

In 2005 and 2006, a national survey on the prevalence of migraines and headaches and the socioeconomic impact of the disorder was carried out in Luxembourg. This led to the publication of two reports: one on the prevalence of the disorder and the other on its socioeconomic impact. Given the extent of the results showing the disorder's prevalence in Luxembourg and the social cost it represents, measured using the ID Migraine questionnaire (29% of the population), it was suggested that the same study be done in several other EU countries so that results could be compared.

Thus the EUROLIGHT project was born. It started in 2007 under the coordination of the Centre for Health Studies and is funded by the Public Health Executive Agency in collaboration with the Ministry of Research.

This research is carried out using the same methodology in the following countries: Luxembourg, Austria, France, Spain, Italy, Great Britain, Lithuania, the Netherlands, Germany, Norway and Ireland.

The first results are expected in 2009.

**Monitoring the drug phenomenon in Luxembourg**

*This activity is carried out in collaboration with Alain Origer, Health Directorate*

Established by Council Regulation (EEC) No. 302/93 of February 8, 1993, the EMCDDA's overall mission is to provide the Community and its Member states with objective, reliable and comparable information on the phenomenon of drugs and drug addiction on a European level.

**The tasks of the Focal Point's missions are the following:**

- national contribution to the EMCDDA annual report's results on the state of the drug phenomenon in the EU
- editing of the national report on the state of the drug problem in Luxembourg
- application and development of 5 EMCDDA epidemiological key indicators
- contribution to the European Legal Database on Drugs, the early warning system for synthetic drugs and the information system on the reduction of drug demand
- centre of excellence of epidemiological drug data

**Prevalence and spread of viral hepatitis A, B, C and of HIV in the population of problematic users of illicitly acquired drugs. Early detection, vaccination against HAV and HBV, referral and reduction of risks and damage**

*Project Leader: Nathalie Removille, in collaboration with Alain Origer, Health Directorate*

Authors included a national cross-sectional study carried out on a voluntary and anonymous basis within national prisons and outpatient and inpatient drug treatment centres and facilities. Standardised questionnaires and blood sampling were applied allowing the assessment of HIV and Hepatitis A, B, C prevalence in PDUs on the basis of serological evidence.

**This study, which was funded by the Fund to fight Illegal Drug Trafficking, showed that:**

- the prevalence of Hepatitis C within the study population ranges from 71.4% and rises to 81% among intravenous users. It was found to be most prevalent among those subjects in prison (86.3%).
- the prevalence of Hepatitis B among the population studied ranges from 21.6% and rises to 24.7% among intravenous users. This figure reaches 31.8% for those in prisons.
- 43% of the population studied has not been vaccinated against Hepatitis A.
- the global prevalence of HIV among the population studied is 2.9% and as high as 7.7 % among prisoners.

These results provide crucial information for the prevention of diseases linked to drug-use. They have facilitated the establishment of recommendations to reinforce prevention already put into action.

**Proposed assessment of neuropsychological, biological and subclinical characteristics of the Mild Cognitive Impairment stage**

*Project Leader: Magali Perquin, PhD*

The feasibility study was carried out in 2007. This enabled the research protocol to be modified and to test all the instruments on the Luxembourg population and the study organisation, in particular, within the framework of collaboration with clinicians.

The research project is to begin its field phase in 2008.

**Retrospective study on the occurrence of serious injury among young, high-level sports players in Luxembourg**

*Project leader: Daniel Theisen, PhD*

Financed by the Ministry of Sport, this study's objective was to produce an analysis of the situation in order to develop prevention policies.

The study, which was carried out on 503 players of a variety of sports between the ages of 8 and 26 (in athletics, badminton, cycling, swimming, basketball, handball, football, karate, gymnastics, tennis, triathlon, table-tennis), showed that 49% of these young people had sustained an injury during the previous 12 months. The frequency of injury averaged out at 0.69 per athlete and the global incidence was 1.16 per 1000 hours of sports activity (training and competition).

This incidence varies depending on the type of sport. The risk of sustaining an injury during competitive events is 2.26 times higher than that during training. The lower leg is involved in 58% of cases. One injury in 5 is serious enough for the athlete to require a period of rest of several weeks.

**2 ASSESSMENT ACTIVITIES**

**Mental Health in Young People**

*Project leader: Véronique Louazel*

By request of the Health Directorate, a broad observational survey on the treatment of mental health among young people in Luxembourg was developed. This involved identifying agents and their roles, identifying problems recognised by the various agents involved and describing the background and progress of the children on beginning treatment.

**Living in Luxembourg after a stroke: Impact on family and quality of life. Equal access to care and social welfare resources**

*Project leader: Dr. Sophie Couffignal, in collaboration with the University of Luxembourg*

The objectives of the study are to establish a profile of stroke patients in Luxembourg, to assess hospital care and occupational therapy, to measure the social and family impacts as well as the patient's quality of life and that of informal care givers, and to assess satisfaction with services and resources used, specific needs not met in terms of expected resources and assistance.

The feasibility study was carried out in 2007. It enabled the organisation of the study to be tested.

Initial results show that 21% of stroke patients have died. This very significant figure forced the Centre for Health Studies to make a request to the National Research Ethics Committee and the National Data Protection Committee to allow access to clinical data without the need for consent in order to reduce potential bias in this type of study. Authorisation was consequently granted. The main study begins in 2008.

**Evaluation of an experimental project for Care Insurance: Treatment within a small ward of persons suffering from neuro-dependence disorders**

*Project leader: Dr. Sophie Couffignal, in collaboration with Marie-Lise Lair*

The aim of the assessment is to show whether the use of an alternative and innovative model of service for neuro-dependent persons leads to added value for these people as well as for their treatment funding. It also aims to gather the information necessary for a possible repeat of the pilot project in Luxembourg.

The assessment phase at Time 0 has been carried out.

**Evaluation of an experimental project for Care Insurance: Use of a night nursing service**

*Project leader: Dr. Sophie Couffignal, in collaboration with Marie-Lise Lair*

The aim of the assessment is to characterise those patients requiring night nursing services, to ascertain the level of need, to measure the impact of this service on homecare for dependent patients, and to measure the cost of such a plan in order to consider possible implementation of this pilot project nationwide.

The assessment protocol was drafted in 2007.

### 3 COLLABORATIVE ACTIVITIES IN CLINICAL RESEARCH

The Centre for Health Studies has provided clinical doctors with methodological and biostatistical skills in the following studies or study proposals:

- Dr. Dirk Droste, Luxembourg Hospital Neurology Department, for the design of his project entitled "Nutrition and physical activity in patients with stroke".
- Dr. Martine Goergen, Luxembourg Hospital Surgery Department, for the design of his project entitled "The presence of obesity genes in patients operated on for severe obesity at the LHC [Lux. Hosp. Centre], and the association with results obtained in terms of weight loss".
- Dr. Charles De La Gardelle, Luxembourg Hospital Cardiology Department, on comparing models of physical therapy in patients with cardiac failure with follow-up analysis after one year.
- Dr. Charles Pull, Luxembourg Hospital Psychiatry Department, regarding factorial validity of two scales of depression.

- Dr. Nico Diederich, Luxembourg Hospital Neurology Department, for his study on non-motor signs as potential predictors of Parkinson's disease.
- Dr. Daniel Wagner, INCCI, for his study on comparisons between treatments in men and women in the event of acute myocardial infarction.
- Dr. Daniel Wagner, INCCI, for his study on treatment delays following the patient's arrival at INCCI in the event of myocardial infarction.
- Dr. Alexandre Bisdorff, Neurology Department, Emile Mayrisch Hospital, for his study on the link between dizziness and migraines.

### 4 ACTIVITIES WITHIN THE FRAMEWORK OF HEALTHCARE FUNDING

Within the framework of hospital funding, the Centre for Health Studies contributed by carrying out, among other things:

- an assessment of care costs required for hospitalised patients in each hospital by using the PRN-methodology in ambulatory chemotherapy polyclinics,
- the development of a methodology to measure endoscopic treatment, in order to calculate staffing requirements,
- the development of a methodology to measure treatment carried out in recovery rooms in order to calculate staffing requirements,
- a study for the establishment of staffing patterns in intensive care units according to care costs required for patients, in outpatient units or hospital,
- projected calculations of medical staff requirements for normal and intensive care hospitalisation units, for dialysis services, for surgical units.

This work enables the provisional calculation of staffing requirements appropriate to patients' real needs, so that available resources can be used to the patient's advantage and in the best possible way. This work is used by the Union of Health Mutual Funds and by hospitals during budget negotiations.

### 5 ACTIVITIES WITHIN THE FRAMEWORK OF HEALTH INFORMATION

In 2007, the Centre for Health Studies worked on the instruments required to improve patient information in the field of health, in particular, through:

- the validation of an education framework for Luxembourg hospitals aimed at patient education for informed consent, (in collaboration with the Luxembourg Hospital Entente),
- the development of an E-Health program in Luxembourg (delegation of Ministry of Health to the Centre for Health Studies for this project).

### 6 ACTIVITIES WITHIN THE FRAMEWORK OF QUALITY IMPROVEMENT AND HEALTHCARE RESULTS

In order to improve service quality and results within Luxembourg hospitals, the Centre for Health Studies was designated as the Union of Health Mutual Funds' consultant to suggest a way to improve the Quality Promotion model used for Luxembourg hospital funding. This concept introduces EFQM methodology and result indicators. The list of national indicators was established in 2007.



In 2007, a study carried out at the request of the Emile Mayrisch Hospital with for the development of a hospital performance measuring tool was completed. The report will be finalised in 2008.

## KEY PUBLICATIONS BY THE CENTRE FOR HEALTH STUDIES SINCE ITS CREATION

In its short history, the Centre for Health Studies has had just enough time to establish itself at a time of considerable reorganisation within the Centre for Public Health Research. Its efforts were focused on the setting-up and carrying-out of studies.

However, the search for available health information systems in Luxembourg capable of carrying out epidemiological and public health research has demonstrated the need for new databases to be created or the quality of existing ones to be improved.

As a result, the rewards for these efforts in terms of publications are only just beginning to be reaped.

The vast majority of initial publications have focused on writing national reports on epidemiological studies carried out. Certain complementary publications in specialised international journals have served to enrich the initial results.

In the field of health services, in contrast, reports on studies carried out by the Centre for Health Studies are used by the Union of Health Mutual Funds to fund hospitals.

In addition significant event was the organisation in February 2007 by the Centre for Health Studies of an International conference on research into health promotion, which has enabled the development of a special issue of the International Journal of Public Health entitled “Education and Health”, which is to be published in 50 countries in 2008. Editing of the work was entrusted to the Centre for Health Studies.

## PUBLICATIONS AND DEVELOPMENTS 2007

- Malisoux L., Jamart C., Delplace K., Nielens H., Francaux M., Theisen D., Effect of long-term muscle paralysis on human single fiber mechanics, *Journal of Applied Physiology*, 2007, 102 (1): 340-9
- Malisoux L., Francaux M., Theisen D., What do single-fiber studies tell us about exercise training?, *Medicine and Science in Sports and Exercise*, 2007, 39(7):1051-1060
- Deldicque L., Theisen D., Bertrand L., Hespel P., Hue L., Francaux M., Creatinine enhances differentiation of myogenic C2C12 cells by activating both p38 and Akt/PBK pathways, *American Journal of Physiology - Cell Physiology* (accepted)
- Theisen D., Frisch A., Schaufelberger C., Conzémius R., Sax A., Seil R., Urhausen A, Lair M.L., Etude rétrospective sur l'incidence des blessures aiguës et lésions de surcharge chez les jeunes sportifs de haut niveau du GD de Luxembourg (Retrospective study on the incidence of serious injury in young, high-level GD sports players in Luxembourg), *rapport pour le Ministère des Sports*, March 2007
- Feiereisen P., Delagardelle C., Vaillant M., Lasar Y., Beissel J., Is strength training the more efficient training modality for severe chronic heart failure patients? An open, randomised, controlled versus placebo, study of strength, endurance and combined training, *Medicine and Science in sports and exercise*, November 2007
- Alkerwi A., Le syndrome métabolique: nécessité de précision scientifique, *La semaine médicale luxembourgeoise*, march 2007,306-30 : 6-7
- Mueller Y., Nguimfack A., Cavallier 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

- Removille N., Origer A., Prévalence et propagation des hépatites virales A,B,C et du HIV au sein de la population d'usagers problématiques de drogues d'acquisition illicite (prevalence and propagation of viral hepatitis A, B, C and HIV in problem users of illegal drugs), *study report*, May 2007
- Origer A., Straus P., Lopes Da Costa S., The state of the drug phenomenon in the Grand Duchy of Luxembourg (Annual report on the national network of information on drugs and drug addictions – RELIS), *report*, December 2007
- Andrée C., Rott C., Vaillant M., Couffignal S., Lair M.L., Prévalence de la migraine au Luxembourg, *rapport d'étude*, juillet 2007
- Tereanu C., Pistre F., Lair M.L., Minca D.G., Hobeau D., Soins infirmiers individualisés à la personne soignée, *Gestions Hospitalières*, novembre 2007,470 : 666-671
- 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 2007 pour l'Union des Caisses de Maladie*
- Lair M.L., Cornez J.P., Wibrin G., Rott C. Dotation en personnel soignant sur base des soins requis dans les polycliniques de chimiothérapie, *rapport 2007 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 2007 pour l'Union des Caisses de Maladie*
- Cornez J.P., Développement du logiciel DEBORA endoscopie pour le recensement des activités dans les hôpitaux, 2007



/ LABORATORY OF PSYCHIATRY

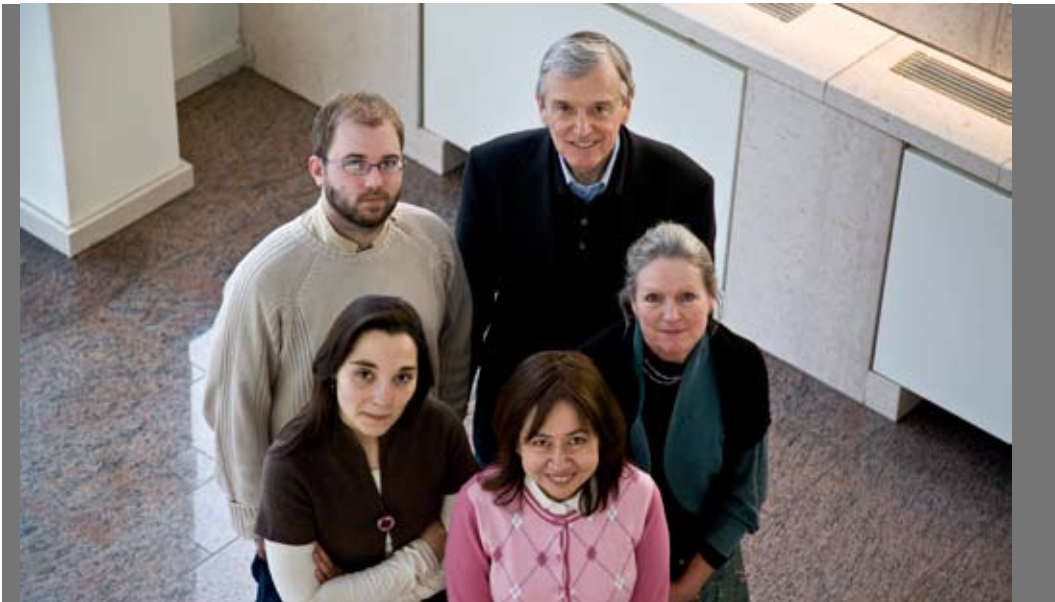
Head of the Research Unit:

Charles PULL, MD, PhD



Presently, the staff of the laboratory is as follows:

Gloria AGUAYO, MD, PhD  
Anne-Marie SCHULLER, PhD, Doctor of Neuropsychiatry  
Marc DAMME, Research Assistant



MAIN GOALS ACHIEVED IN 2007

1 EVALUATION OF THE OPERATION AND HANDICAPS OF MENTAL DISORDERS

This study was completed in November 2007. The results have been analysed and articles are being written for subsequent submission to scientific publications.

The WHO-DAS-II is a robust tool than can easily be used in evaluating the handicap associated with mental health disorders, in particular anxiety and dietary disorders.

2 NEUROPSYCHOLOGICAL ASPECTS OF AGEING IN SCHIZOPHRENIA

The data gathering stage was completed in October 2007. Results are currently being analysed. The study will be completed in March 2008, with one or two articles to be written for subsequent submission to scientific publications.

Individuals presenting schizophrenia have a tendency to develop a state of dementia more readily than control subjects, and could in fact develop dementia of a particular type.

3 EXPOSURE TO VIRTUAL REALITY AS TREATMENT FOR “PANIC ATTACKS” ASSOCIATED WITH AGORAPHOBIA

The data for this study – the most far-reaching ever conducted on this topic – have now been analysed. A number of articles are currently being written for subsequent submission to scientific publications.

Treatment through exposure to virtual reality is as effective as treatment through classic cognitive-behavioural therapies.

Scientific assessment

The laboratory was assessed on 22 September 2006 by the CRP-Santé Scientific Committee (reporters: Prof. Mackel, Rockefeller University, New York, and Prof. Valleron, Paris).

PUBLICATIONS 2007

- Pull C. Combined pharmacotherapy and cognitive-behavioural therapy for anxiety disorders. Current Opinion in Psychiatry. 2007 20(1):30-5.
- Pull C. Illusion de présence et émotions. Le traitement des troubles anxieux par exposition en réalité virtuelle. (Illusions of presence and emotions. The treatment of anxiety disorders through exposure to virtual reality). To be published in Progrès en thérapie comportementale et cognitive. La troisième vague: thérapie cognitive et émotions (Progress in cognitive-behavioural therapies. The third wave: cognitive therapy and emotions) Edited by J.Cottraux. Masson. Paris, 2007.
- Pull C. New trends in the study of specific phobia. Current Opinion in Psychiatry. Accepted for publication; to be published in 2008.

/ FOOD SAFETY

Project leader:

Gilbert MORIS, PhD



DETECTION OF PLANT SPECIES RELEVANT TO FOOD SAFETY

Using genomic DNA information from plant LIM-clones, provided by the team of Dr. André Steinmetz, our team aimed at designing PCR primers for a simultaneous detection of maize, rice, wheat, barley, peanut and soya. For that purpose, homologies in the exons were being used to design common primers for some species, which after amplification generated fragments of different lengths due to species-related intron-size differences. One of these developed PCR-systems consisted of only 3 primer pairs and was shown to enable the detection of the 6 studied species in one reaction tube. Future efforts will be focusing on the improvement of relative detection limits and of method robustness as well as on an increase of the number of covered species.

DETECTION OF GENETICALLY MODIFIED PLANTS

In the field of GMO's (genetically Modified organisms), activities were focused on developing a quadruplex-Real-Time-PCR system enabling the simultaneous detection of reference genes for maize and soya (the 2 plant species with the highest number of EU-authorized varieties) together with the most employed regulating elements in GMO's: the 35S promoter of cauliflower mosaic virus and the nopaline synthase terminator of *Agrobacterium tumefaciens*. A version of such a quadruplex-system gave satisfying results regarding sensitivity, specificity and robustness. Another quadruplex-Real-Time-PCR system aimed at detecting and quantifying 3 different transgenic maize varieties, relative to a maize reference gene.

These PCR systems are likely to represent a valuable tool in the future where high through-put analysis will be needed in order to cover the ever increasing number of EU-authorized GMO's.



||||| ATS ADMINISTRATIVE  
AND TECHNICAL SERVICES



THE EVOLUTION OVER 20 YEARS

HUMAN RESOURCES UNIT

FINANCIAL UNIT

IT UNIT

TECHNICAL SUPPORT UNIT

PURCHASING UNIT

SECRETARIAL UNIT

EH&S UNIT

PROJECT MANAGEMENT UNIT

LEGAL UNIT

## / ADMINISTRATION AND TECHNICAL SERVICES

### Head of Department:

Daniel CARDAO, Administrative and Financial Manager

Fabienne OLINGER DI MARCO, Executive Secretary



The Administrative and Technical Services are composed of different units, reporting to the Head of the ATS:

#### HUMAN RESOURCES UNIT:

Natacha BEICHT, Head of Unit

Nancy PELLIZARY, Secretary

#### FINANCIAL UNIT:

Joseph GAUTOT, Head of Unit

Jeremy KLEIN, Accounting Supervisor

Pierre FOUSSE, Accountant

Patrice ROESER, Secretary

#### INFORMATION AND TECHNOLOGY UNIT:

Olivier KEUNEN, Head of Unit

Benoît KUNZ, Computer Specialist

Frédérique LASSALLE, Computer Specialist

Pierre THEAK, Computer Specialist

#### TECHNICAL SUPPORT UNIT:

Laura MARTINS, Head of Unit

José ESPOSITO, Maintenance Technician

#### PURCHASING UNIT:

Alphonse CONRARDY, Responsible

#### SECRETARIAL UNIT:

Mireille LEYTEM, Teresa MARTINS, Sandra MARCON, Sonia MARTINS

#### EH&S UNIT:

Elodie FONTAINE, Responsible

#### PROJECT UNIT:

Jo SCHROEDER, Responsible

#### LEGAL UNIT:

Guillaume BYK, Lawyer



The main role of the ATS at the Centre de Recherche Public de la Santé (CRP-Santé), headed by Daniel CARDAO, is to relieve the research structures as much as possible of daily administrative and technical tasks. This is why the administrative staff of CRP-Santé must have an excellent knowledge of the various research structures. It goes without saying that a perfect experience in their specific field is necessary. The staff's duties are as follows:

- Perform administrative and technical duties that are necessary for proper functioning and good development of CRP-Santé structures.
- Provide quality service to the operational teams.
- Setup procedures for a lean service quality.

The team realizes basic administrative and technical duties and more specifically meets the needs of the operational research units.

In 2008, the principal challenges of the ATS department will be:

- Harmonize and strengthen administrative procedures to an effective stage.
- Setup a full cost system for a better understanding of the financial figures.
- Optimize support to the operational units.



## THE EVOLUTION OVER 20 YEARS

### A BRIEF HISTORY OF THE DEPARTMENT

Although existing at the beginning, the ATS department got shaped later in accordance with the growing demands of the research units. In 2001, the ATS department consisted of 5 staff members managing the administrative requirements of about sixty research projects and 100 employees.

Due to the growing number of projects, a new structure was set up in 2002 and the ATS department was integrated as a support department for the operational units. The ATS was then subdivided into different departments to follow the quick expansion of the centre's activities.

## HUMAN RESOURCES UNIT

The unit was created in October 2002 and is headed by Natacha Beicht. This unit's main objective is to manage all the human resources issues and to assure support to the operational units.

In 2007, the tasks of the HR department consisted of assistance and support to the research units, namely for administrative, social and legal human resources issues.

**The permanent objectives for this unit are as follows:**

- active participation on the recruitment processs
- welcome of the new recruits
- setting up training plans (languages, technical lessons etc.)

**The day-to-day business of the unit:**

- managing the personal files of the personnel
- assisting the personnel on all the HR issues
- handling of the payroll
- setting up the working agreements
- dealing with all public administrations like social security etc
- updating the database of the personnel
- communicating with other institutions like universities, other CRPs
- applying work permits for foreign students / employees

For 2007, 22 people were recruited. It's worth mentioning all the students' issues the HR unit dealt with. The CRP-Santé welcomed 53 trainees and 2 students finishing their PhD during 2007.

**A resumee database completed at the beginning of 2008 allows viewing all incoming job applications. The main actions for 2008 will be the following:**

- review the carreer system at the CRP-Santé
- setting up a new evaluation system for the personnel like a MBO
- recruit approximately 30 FTE

## FINANCIAL UNIT

The group, headed by Joseph Gautot, manages 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 2007

- 1 A budget of 15,5 million € was proposed for 2008 and approved by the board of administrators. That's an increase of 67% compared to 2007 for the Ministry of Research's contribution, representing 65 % of the global budget
- 2 Tailor-made tools that are completely integrated to the financial software got developed in order to achieve the assigned 2008 goals, namely the improvement of the general speed and the relevance of the reporting system. This assignment was partly realised in 2007 and it's to be completed in the first half of 2008
- 3 The decision of acquiring supply management software was taken in order to enable us to meet our next year's objectives.
- 4 The headcount of the financial department will be increased by one FTP to meet the growing needs of the operational units in terms of financial information.

### THE ACTIONS PLANNED FOR 2008 ARE AS FOLLOWS

- 1 Provide better management tools to heads of operational departments by the delivery of a real-time solution for the follow-up of their projects' financial results
- 2 Put in place a financial dashboard in order to achieve a proactive management of the CRP's financial resources
- 3 Speed up the global flow and processing of financial documents by the implementation of the integrated solution for the supply management, the tracking of inventories and the document management
- 4 Adapt the accounting methods in order to meet the Ministry of Research's requirements to apply "full costs" methodology to all research projects at 01/01/2009

## IT UNIT

The information technology unit, headed by Mr Olivier Keunen, manages the computing, network & telephony infrastructures of the CRP Santé. A fast growing base of over 200 users located in 3 different sites, all with very demanding and diversified needs, represents an everyday challenge to the IT team.

In 2007, the information technology unit implemented IT infrastructures for new facilities in rue Edison, the extension of the Laboratory of Immunology at the LNS, and also preparing the future extension of the BAM.

**Key IT projects completed during the course of the year included:**

- servers consolidation through virtual servers technology,
- identity management software implementation
- development of Intranet-integrated software for the labs or for improved projects management



As the CRP Santé continues its fast-paced development, the information technology unit will need to cope with:

- the booming demands for IT assistance
- the need to provide an efficient, performant, secure and yet easy to use IT infrastructure
- high standard of service quality.

The challenges ahead are many and IT is preparing for those.

## TECHNICAL SUPPORT UNIT

The main achievements of the technical unit since 2004 was the construction of the Bam I, logistic support (maintenance contracts: building and equipment, service contracts: guarding, cleaning, delivery gas, etc) was necessary to complete the building.

The department carried out modifications on certain technical equipment., It purchased equipment to improve the performance of the installations (for example: installation of a osmosor, air-conditioning of a technical room and laboratories, modification of the water supply in Bam I in order to have a better water quality, etc).

The Technical Support unit, headed by Dr. Laura Martins, provides maintenance for the technologies and facilities used at CRP-Santé. This unit is responsible for technical issues on all the buildings used by CRP-Santé, corresponding to approximately 3000m<sup>2</sup>.

The missions of this department are:

- the coordination of the maintenance and the setting up of new equipment
- the management of waste
- the logistic support of the Health and Safety department
- the coordination of the "breakdown service";
- the supervision of the work carried out by the technical personnel;
- the monitoring of the good performance and the maintenance of the technical equipment;
- the monitoring of the maintenance and service contracts;

## ACTIONS IN 2007

- 1 application on public markets for the purchasing of laboratory equipment via the budget granted by the public administration
- 2 prospection to find a new building for the Administrative services and Techniques (SAT) and the Center for Health Studies.
- 3 Negotiation of various maintenance contracts
- 4 The setting-up of BAM II building is a big challenge which is foreseen to be finished in the 1<sup>st</sup> quarter 2008

## PURCHASING UNIT

In 2007 the CRP-Santé decided to create a purchasing unit and to install a supply chain management software that is compatible with the existing business solution software and the document management software.

Buying conditions, prices and contract details will be negotiated in the future centrally by the purchasing department. The orders will still be handled in a decentralized procedure, with the advantage of a central article data base with improved corporate conditions.

The software will reduce multiple inputs in the supply chain and will increase the speed and reliability of the information flow.

## SECRETARIAL UNIT

The secretarial unit provides administrative support to the ATS and to the departments without access to personal secretaries.

As the CRP-Santé was growing so fast, the ATS have been forced to adapt to increased demand for more varied services and a huge volume of tasks.



These functions are reporting to the Board of management:

- EH&S Unit, Elodie FONTAINE
- Project Management Unit, Jo SCHROEDER
- Legal Unit, Guillaume BYK

## EH&S UNIT

As the CRP-Santé is working with a lot of chemicals and other dangerous items, the safety became a very important issue. That's why the CRP-Santé decided to set up a health and safety unit, headed by Mrs. Elodie Fontaine.

In 2007, EH&S unit set up a new data base, developed by the IT-department and accessible by intranet.

The most issue is the inventory of all chemical compounds used at the CRP-Santé. In 2007, safety auditing of all CRP services and laboratories was continued. Moreover, the safety officer was involved in the planning of BAM2 for safety matters.

In 2008, it is foreseen to develop new tools for the management of safety procedures, guidelines, safety incidents, accidents. This will lead to audit reports proposing corrective and preventive actions in order to minimize risks and to improve the overall safety behaviour in 2008. This will lead also to better focused safety training.

In 2008, EH&S service is managing the purchasing of safety equipment for the new building.

## PROJECT MANAGEMENT UNIT

This unit has been created in 2006 in order to centralize all incoming projects and to follow up their evolution; this unit is headed by Mr. Jo Schroeder.

In 2007, the project management unit set up a data base merging the most important project - related documents and other key information.

The collected data is accessible at any time via intranet by both the ATS department and the research units. This new tool was developed in-house by the IT-department.

**During 2008, the project manager will:**

- work out together with the ATS department, project management procedures and guidelines allowing a closer follow-up of projects, taking into account the full cost system;
- upgrade, together with the IT department, the above-mentioned data-base in order to offer an automated reminder system for the most relevant deadlines;
- identify, together with the ATS department, project management indicators facilitating project piloting.

## LEGAL UNIT

Created in 2004, the legal unit, headed by Mr Guillaume Byk, provides advice and resource about the legal implication of CRP-Santé's work. In 2007, it was involved in following the various patent applications arising from CRP-Santé research. There was also welcoming changes in the legal background of private data protection law that greatly facilitate CRP-Santé's day-to-day activity.

For 2008, the legal unit plans to update public procurement procedures at the CRP-Santé and the various insurance policies needed for the new clinical investigation center to be created.

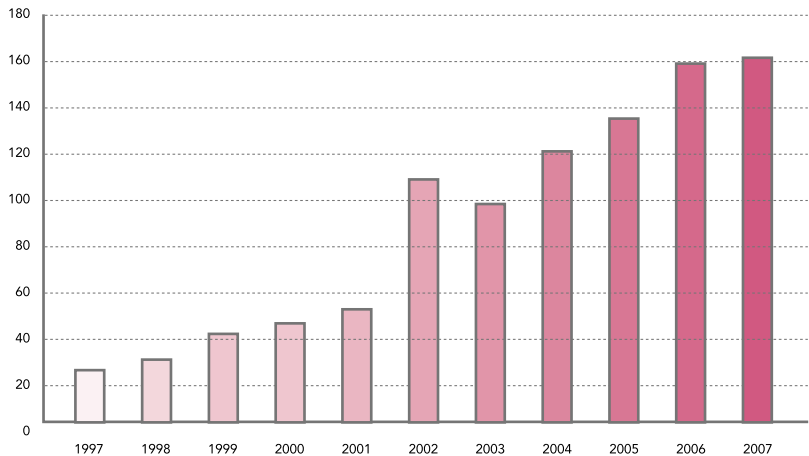
## SOME KEY FIGURES



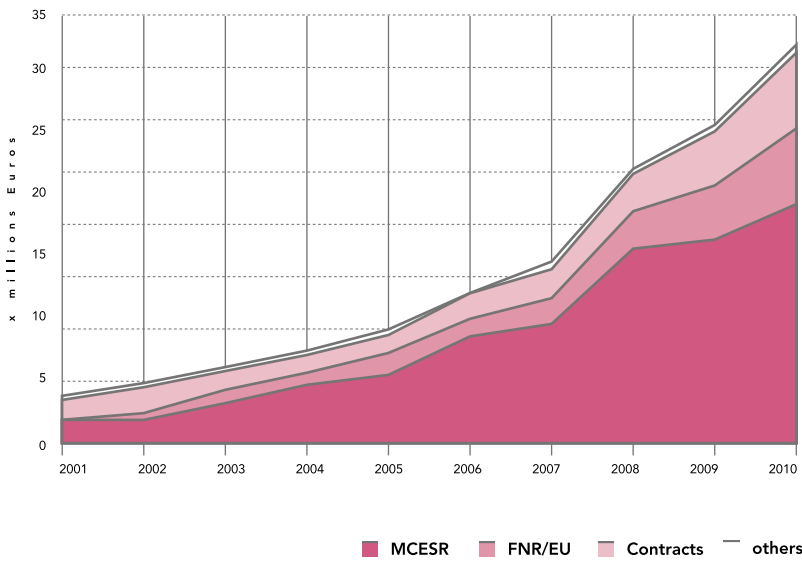
THE GROWTH OF THE CRP – SANTÉ CARRIED OUT IMPORTANT FUNDS DURING THE LAST 10 YEARS IN K€:



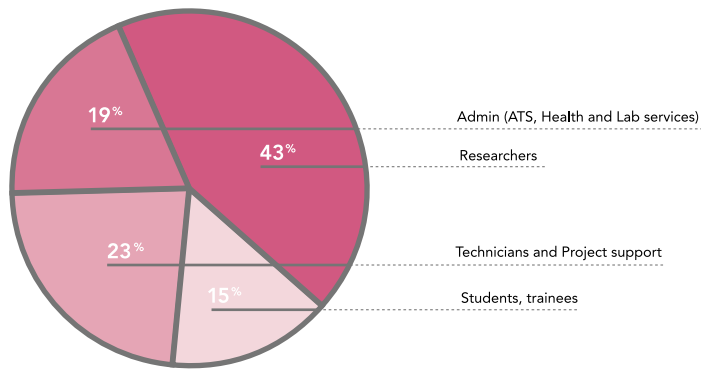
CONCERNING THE PERSONNEL, THE HEADCOUNT INCREASED ACCORDINGLY TO THE GROWTH OF THE FUNDS:



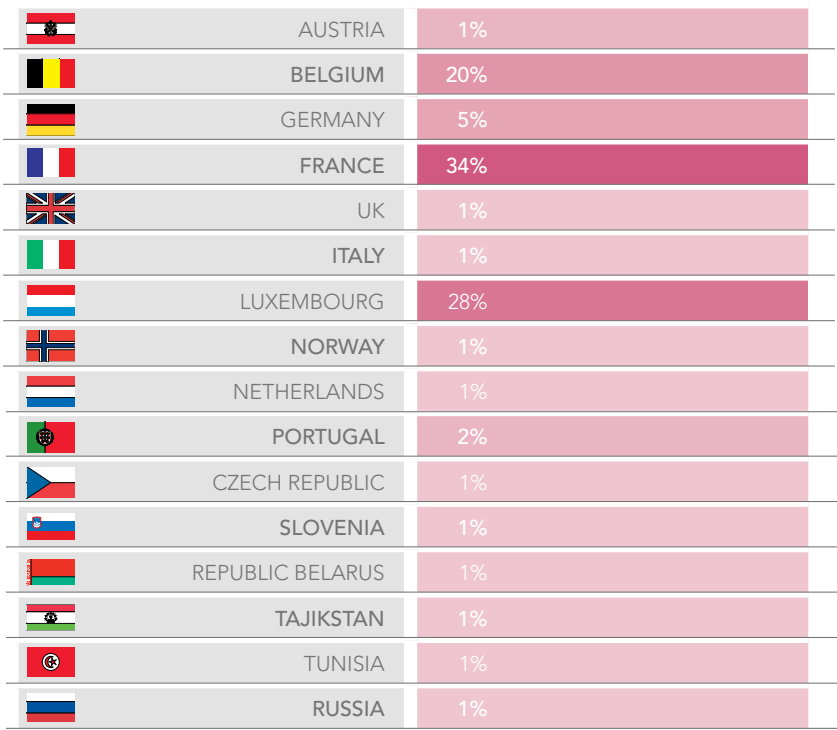
THE DIFFERENT FUNDS CAN BE DIVIDED BY DIFFERENT FUND PROVIDERS:



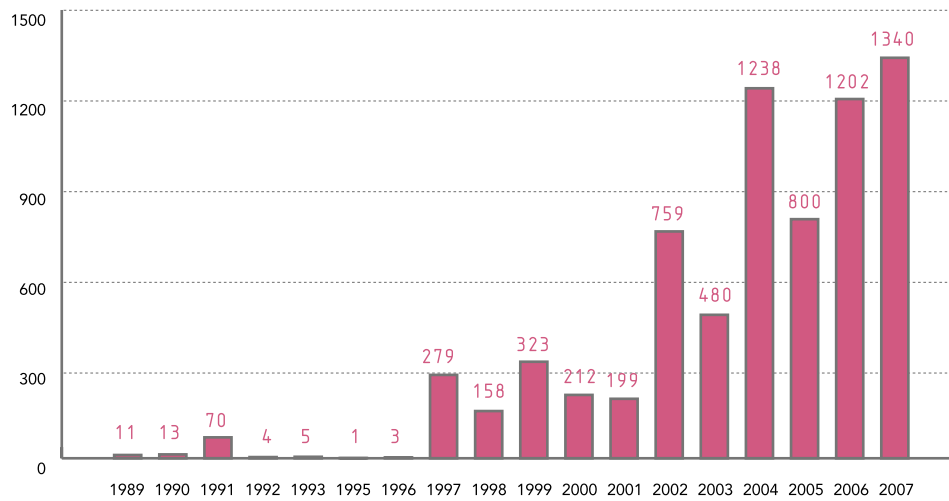
THE CRP-SANTÉ'S PERSONNEL CAN BE SPLITTED INTO THE FOLLOWING CATEGORIES:



THE CRP-SANTE IS VERY DIVERSIFIED IN TERMS OF NATIONALITIES:



THE INVESTMENTS WITHIN 20 YEARS OF EXISTENCE OF CRP-SANTÉ IN K€:





## RAPPORT DU REVISEUR D'ENTREPRISES

### Rapport sur le bilan et le compte de profits et pertes

Conformément au mandat reçu, nous avons effectué l'audit du bilan au 31 décembre 2007 et du compte de profits et pertes pour l'exercice clos à cette date (les comptes) ci-joints de l'Etablissement Centre de Recherche Public de la Santé ou CRP-SANTE.

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

Le Conseil d'Administration est responsable de l'établissement et de la présentation sincère de ces comptes, conformément aux obligations légales et réglementaires relatives à l'établissement. 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 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 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 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. Le choix des procédures relève du jugement du Réviseur d'entreprises, de même que l'évaluation du risque que les comptes 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 afin de définir des procédures d'audit appropriées en la circonstance, et non dans le but d'exprimer une opinion sur l'efficacité de celui-ci.

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

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

### Opinion

A notre avis, le bilan et le compte de profits et pertes ci-joints donnent, en conformité avec les prescriptions légales et réglementaires en vigueur au Luxembourg, une image fidèle du patrimoine et de la situation financière de l'Etablissement Centre de Recherche Public de la Santé ou CRP-SANTE au 31 décembre 2007 ainsi que des résultats de l'exercice se terminant à cette date.

Luxembourg, le 19 mars 2008



**Thierry REMACLE**  
Réviseur d'Entreprises  
Lux-Audit Révision S.à r.l.

BILAN AU 31 DÉCEMBRE 2007 (en Euros)

ACTIF

|   | 31.12.2007   | 31.12.2006   |
|---|--------------|--------------|
| A. CAPITAL SOUSCRIT NON VERSE   |              |              |
| B. FRAIS D'ETABLISSEMENT  |              |              |
| C. ACTIF IMMOBILISE   |              |              |
| I Immobilisations incorporelles   | 18 829,66    | 12 375,99    |
| 2. Concessions, brevets, licences, marques, ainsi que droits et valeurs similaires    |              |              |
| a) acquis à titre onéreux   | 18 829,66    | 12 375,99    |
| b) créés par l'entreprise   |              |              |
| II Immobilisations corporelles  | 397 867,92   | 115 612,45   |
| 2. Installations techniques et machines   | 24,80        | 725,31       |
| 3. Autres installations, outillage et mobilier  | 317 426,80   | 114 887,14   |
| 4. Acomptes versés et immobilisations corporelles en cours                            | 80 416,32    | 0,00         |
| III Immobilisations financières   | 12 394,68    | 12 394,68    |
| 3. Participations   | 12 394,68    | 12 394,68    |
| TOTAL DE L'ACTIF IMMOBILISE   | 429 092,26   | 140 383,12   |
| D. ACTIF CIRCULANT  |              |              |
| I Stocks  | 0,00         | 0,00         |
| II Créances   |              |              |
| 1. Créances résultant de ventes et prestations de services                            | 288 824,01   | 161 239,10   |
| a) dont la durée résiduelle est inférieure ou égale à un an                           | 288 824,01   | 161 239,10   |
| b) dont la durée résiduelle est supérieure à un an                                    |              |              |
| 2. Créances sur partenaires   | 1 289 528,89 | 3 166 623,49 |
| 3. Créances sur des entreprises avec lesquelles la société a un lien de participation | 0,00         | 0,00         |
| 4. Autres créances  | 417 006,60   | 205 545,51   |
| a) dont la durée résiduelle est inférieure ou égale à un an                           | 417 006,60   | 205 545,51   |
| 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        | 5 644 880,96 | 2 323 518,46 |
| TOTAL DE L'ACTIF CIRCULANT  | 7 640 240,46 | 5 856 926,56 |
| E. COMPTES DE REGULARISATION  | 66 051,29    | 39 614,38    |
| TOTAL DE L'ACTIF  | 8 135 384,01 | 6 036 924,06 |

BILAN AU 31 DÉCEMBRE 2007 (en Euros)

PASSIF

|  | 31.12.2007   | 31.12.2006   |
|--|--------------|--------------|
| A. CAPITAUX PROPRES  |              |              |
| I Capital souscrit   | 99 157,41    | 99 157,41    |
| IV Réserves  | 0,00         | 0,00         |
| V Résultats reportés   | 235 096,82   | 103 197,46   |
| VI Résultats de l'exercice   | 642 961,98   | 131 899,36   |
| VII Subventions d'investissement   | 336 281,05   | 127 982,94   |
| TOTAL DES CAPITAUX PROPRES   | 1 313 497,26 | 462 237,17   |
| B. PROVISIONS POUR RISQUES ET CHARGES  |              |              |
| 3. Autres provisions   | 389 549,55   | 482 817,01   |
| TOTAL DES PROVISIONS   | 389 549,55   | 482 817,01   |
| 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  | 1 198 912,57 | 1 261 961,86 |
| a) dont la durée résiduelle est inférieure ou égale à un an                            | 1 198 912,57 | 1 261 961,86 |
| 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 a un lien de participation | 0,00         | 0,00         |
| 8. Dettes fiscales et dettes au titre de la sécurité sociale                           | 464 498,87   | 358 770,49   |
| a) Dettes fiscales   | 183 244,76   | 139 543,90   |
| b) Dettes au titre de la sécurité sociale  | 281 254,11   | 219 226,59   |
| 9. Autres dettes   | 257 253,25   | 53 980,25    |
| a) dont la durée résiduelle est inférieure ou égale à un an                            | 240 633,25   | 53 980,25    |
| b) dont la durée résiduelle est supérieure à un an                                     | 16 620,00    | 0,00         |
| TOTAL DES DETTES   | 1 920 664,69 | 1 674 712,60 |
| D. COMPTES DE REGULARISATION   | 4 511 672,51 | 3 417 157,28 |
| TOTAL DU PASSIF  | 8 135 384,01 | 6 036 924,06 |

COMPTE DE PROFITS ET PERTES POUR L'EXERCICE  
ALLANT DU 1 JANVIER 2007 AU 31 DÉCEMBRE 2007

(en Euros)

|  | 2007<br>( 01.01.07-31.12.07 ) | 2006<br>( 01.01.06-31.12.06 ) |
|--|-------------------------------|-------------------------------|
| A. CHARGES   |                               |                               |
| 1. Réduction du stock de produits finis et en cours de fabrication   |                               |                               |
| 2. a) Consommation de marchandises et de matières premières et consommables  | 2 725 940,11                  | 2 472 584,28                  |
| b) Autres charges externes   | 1 921 659,67                  | 2 111 976,41                  |
| 3. Frais de personnel  |                               |                               |
| a) Salaires et traitements   | 7 240 817,03                  | 6 251 138,58                  |
| b) Charges sociales couvrant les salaires et traitements   | 790 870,24                    | 693 901,51                    |
| 4. a) Corrections de valeur sur frais d'établissement et sur immobilisations corporelles et incorporelles              | 77 365,76                     | 59 566,97                     |
| b) Corrections de valeur sur éléments de l'actif circulant   | -35 080,53                    | 10,04                         |
| 5. Autres charges d'exploitation   | 598 836,18                    | 60 504,27                     |
| 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  | 2 846,33                      | 35 318,91                     |
| 10. Charges exceptionnelles  | 31 171,25                     | 0,00                          |
| 13. Résultat de l'exercice (bénéfice de l'exercice)  | 642 961,98                    | 131 899,36                    |
| TOTAL DES CHARGES  | 13 997 388,02                 | 11 816 900,33                 |
| B. PRODUITS  |                               |                               |
| 1. Montant net du chiffre d'affaires   | 191 176,39                    | 90 819,19                     |
| 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  | 13 212 766,97                 | 11 562 878,00                 |
| 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   | 143 445,00                    | 43 309,31                     |
| 9. Produits exceptionnels  | 449 999,66                    | 119 893,83                    |
| 10. Résultat de l'exercice (perte de l'exercice)   | 0,00                          | 0,00                          |
| TOTAL DES PRODUITS   | 13 997 388,02                 | 11 816 900,33                 |

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