LOUVAIN DRUG RESEARCH INSTITUTE

REPORT 2019

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https://uclouvain.be/en/research-institutes/ldri

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UCLouvain

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FOREWORD

The general objective of the **Louvain Drug Research Institute (LDRI)** is to develop fundamental and/or applied cutting-edge research, in the field of drugs, with a focus on personalized treatment, innovative therapies, and regenerative medicine. Overall research activities are developed from target identification & validation to clinical practice through hit identification/hit to lead, preclinical evaluation, pharmaceutics, clinical assays and optimization of clinical practice.

The LDRI is located on the Health Sciences Campus of the *Université catholique de Louvain* (UCLouvain) in Brussels. Active collaboration is established with UCLouvain-related University Hospitals (*Cliniques Universitaires St Luc* –located within walking distance of the LDRI, and *CHU UCL-Namur*).

The LDRI was officially created in 2010. At that time, it included most researchers previously working at the "School of Pharmacy". Since then, the LDRI increased its external visibility and promoted human and financial resources to strengthen its scientific output. The research quality conducted at the LDRI is illustrated by a constant increase both in the number and in the ranking of the published papers in well-recognized international journals. According to the prestigious QS World University Ranking 2018, our research activities in "Pharmacy and Pharmacology" are recognized in the Top 51-100 Universities over the world, the UCLouvain being ranked as the first French-speaking University in Belgium, and the second at a national level after the KULeuven. In 2019, six researchers of the LDRI were among the highly cited researchers worldwide. LDRI researchers were successful in getting highly competitive grants, namely from European Research Council, WELBIO, or Excellence of Science in Belgium.

"Bridging sciences for better health" is the LDRI's motto. The LDRI is proud of the diversity and wealth of its research. The modest size of the institute (22 Principal Investigators (PI), around 160 members in total) creates a convivial atmosphere and optimal conditions to share common objectives in terms of quality of science and well-being. The LDRI members work together in this **multidisciplinary Institute**, to cover original research topics related to Microbes, Inflammation, Cancer, Ageing and Chronic Diseases, via the implementation of New Technologies. The research activities range from the design or identification of a new drug (and the discovery of new targets) to its optimal use. Our research involves *in silico*, *in vitro* (membranes and cells) approaches, *in vivo* pre-clinical models (small animals) and clinical studies.

The LDRI is organized in seven functional research groups led by highly motivated scientists, who are involved in international networks, and are regularly invited as speakers in international congresses. The Institute is hosting two technology platforms - with outstanding equipment and expertise in small molecules Mass Spectrometry (MASSMET platform) and Magnetic Resonance (NEST platform).

The LDRI is also a wealthy niche for the education of young researchers. In order to stimulate the interdisciplinary approach, research seminars with reputed experts, as well as data club -presented by young researchers enrolled in the Doctoral School in Biomedical and Pharmaceutical Sciences-, are organized weekly. We are also happy to welcome a continuous influx of doctoral students and post-docs with a large proportion of international fellows.

The LDRI ensures the continuous dissemination of knowledge to the scientific community, and offers its expertise to Public Health Authorities and pharmaceutical, chemical and biotechnologies industries. Two spin-off companies were recently issued from LDRI's members activities. Thanks to our internationally competitive research, our involvement in creating and fostering new knowledge with a direct impact on healthcare, and our ambition to develop efficient partnerships with industry and society, the activities of the LDRI closely meet the ambitions of the "League of European Research Universities". We promote a research approach in line with the UCLouvain priorities and research policies of international, federal and funding bodies (Horizon Europe, European Research Council, FNRS/FRS, Regional funding). The LDRI is open to external evaluation by the international reputed experts and welcomed its Scientific Advisory Board in 2019, who gave fruitful advices to establish LDRI future strategy.

In this report, we present a brief overview of our objectives and mission statements as well as a detailed description of research groups and platforms. The human resources, funding, and the scientific output of the LDRI are mostly based on data collected in 2019 or upon the last 3 years. All the data were collected using the University's official databases. We wish to thank the members of LDRI who have greatly contributed to the data collection and redaction of this report.

We hope that this report will give you a clear vision of the Louvain Drug Research Institute, and will encourage you to work with us, in the future. Enjoy the reading!

Nathalie Delzenne,

President of the LDRI

Raphaël Frédérick, Giulio Muccioli and Françoise Van Bambeke,

Vice-Presidents of the LDRI

Abbreviations:

ADDB: Advanced Drug Delivery and Biomaterials

ARC: *Action de Recherche Concertée (Collaborative research funding by UCLouvain)*

BPBL: Bioanalysis and Pharmacology of Bioactive Lipids

CLIP: Clinical Pharmacy

CMFA: Medicinal Chemistry

FACM: Pharmacologie cellulaire et moléculaire

FNRS: *Fonds National de la Recherche Scientifique*

FRIA: Fund for Research Training in Industry and Agriculture

FRS: Fonds de la Recherche Scientifique

FSR: *Fonds spécial de la recherche*

FTE: Full Time Equivalent

GNOS: Pharmacognosy

IF: Impact Factor

MNUT: Metabolism and Nutrition

PI: Principal Investigator

PMGK: Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics

REMA: Biomedical Magnetic Resonance

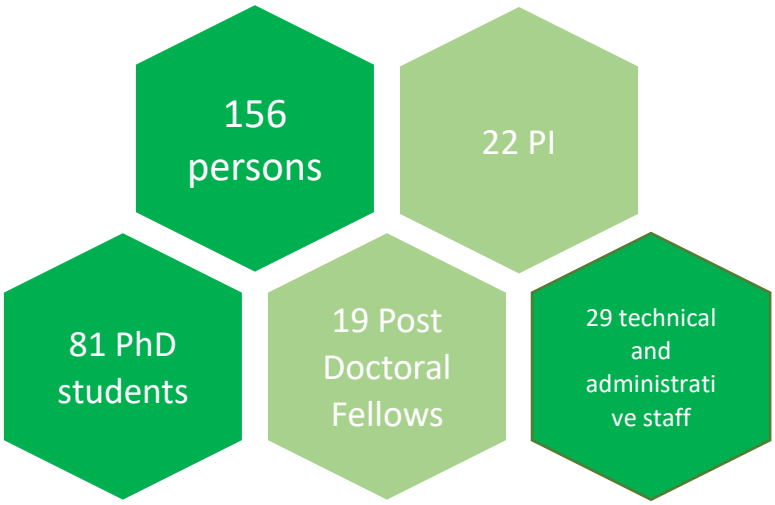
SME: Small and Medium Entreprise

TFAR: Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization

Support the LDRI in its development and actions:

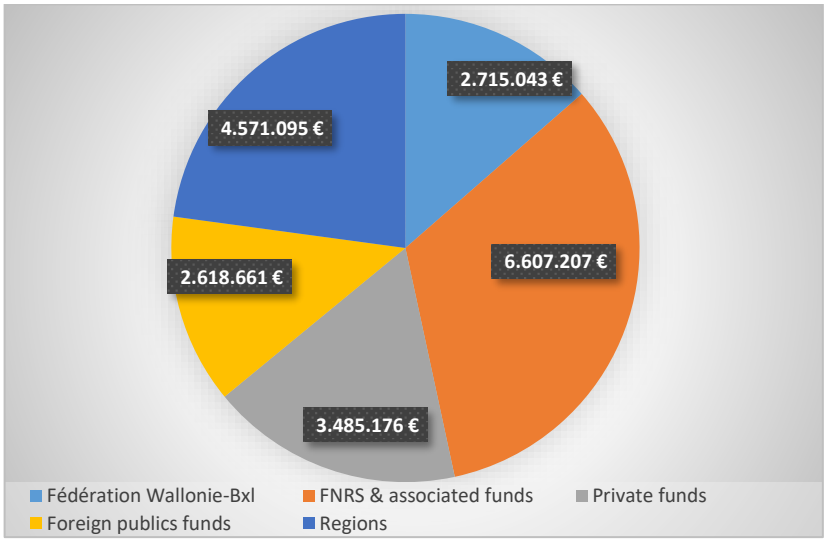
Account Number: BE29 2710 3664 0164 / BIC: GEBABEBB with the communication **"don LDRI 13.21100.001"**. You can add a specific team, person or research project.

LDRI – 2019 HIGHLIGHTS

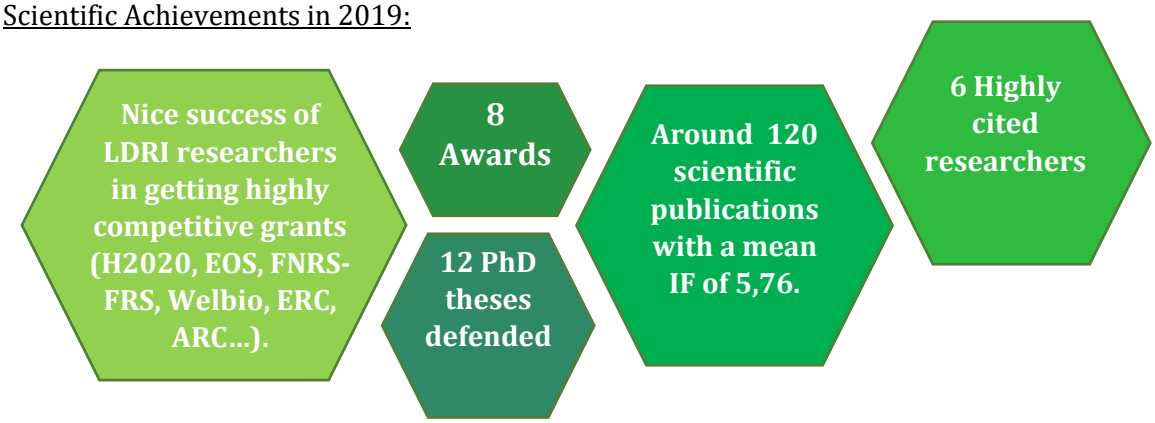


Funding:

Sources of funding of the LDRI (Global amount in EUR for projects running in 2019)

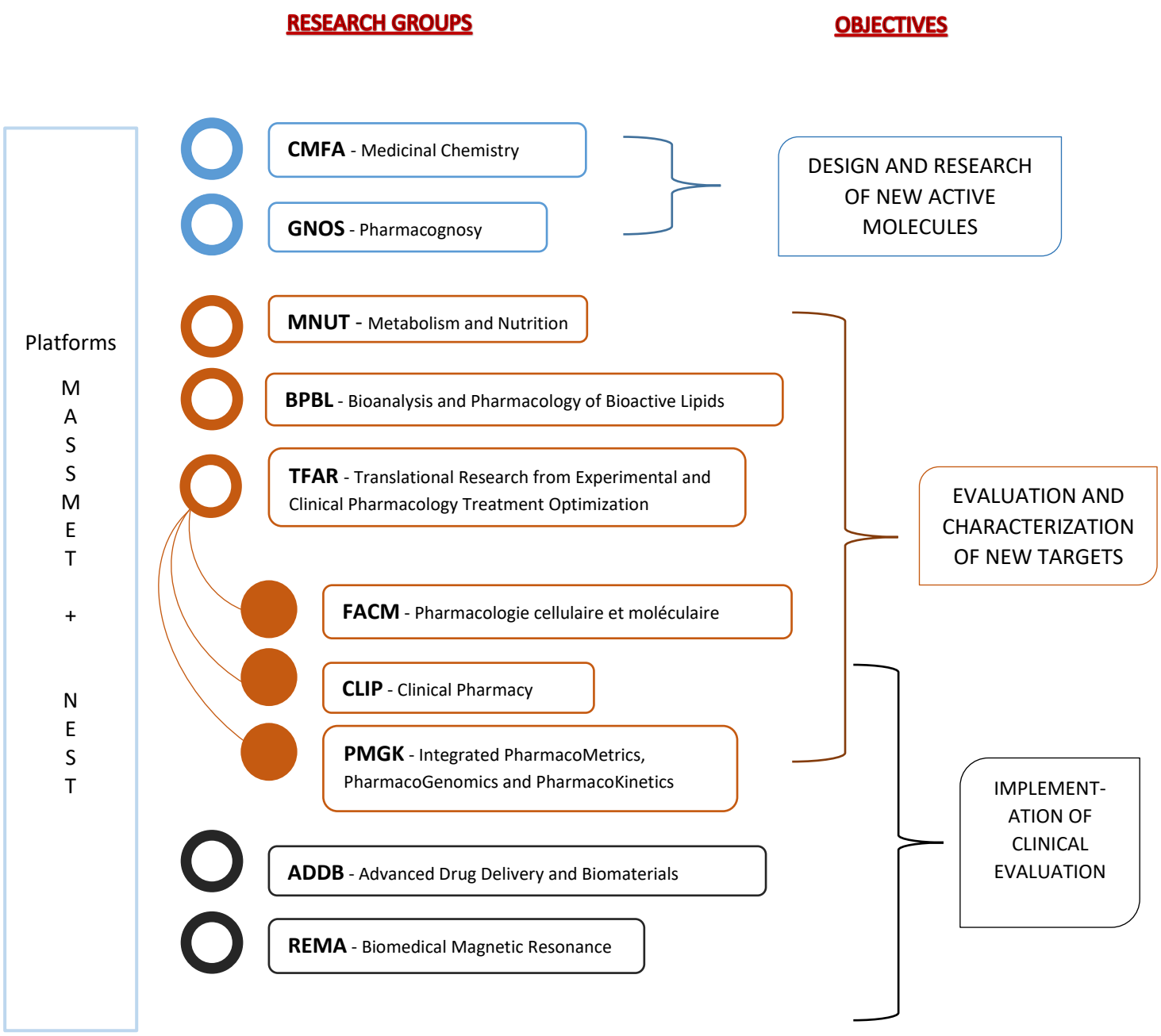


Scientific Achievements in 2019:



SECTION I – LDRI GENERAL PRESENTATION

I. Objectives and mission statements
II. Research fields and groups
III. Decision-making and management
IV. Human resources
V. Fundings
VI. Scientific output


RESEARCH GROUPS
OBJECTIVES
Platforms
M A S S M E T + N E S T
CMFA - Medicinal Chemistry
GNOS - Pharmacognosy
DESIGN AND RESEARCH OF NEW ACTIVE MOLECULES
MNUT - Metabolism and Nutrition
BPBL - Bioanalysis and Pharmacology of Bioactive Lipids
TFAR - Translational Research from Experimental and Clinical Pharmacology Treatment Optimization
FACM - Pharmacologie cellulaire et moléculaire
CLIP - Clinical Pharmacy
PMGK - Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics
EVALUATION AND CHARACTERIZATION OF NEW TARGETS
ADDB - Advanced Drug Delivery and Biomaterials
REMA - Biomedical Magnetic Resonance
IMPLEMENT-ATION OF CLINICAL EVALUATION

I. Objectives and mission statements

The general objective of the Louvain Drug Research Institute (LDRI) is to develop a cutting-edge translational research in the field of drugs, within the Health Sciences Sector of the Université catholique de Louvain.

The research themes encompass the characterization of novel drug targets, the discovery and the conception of new active molecules, the study of their pharmacological profile, their metabolism and toxicity, their formulation, and the optimization of their use. These research projects are supported by two technological platforms with high standard analytical tools in the field of mass spectrometry (MASSMET) and pre-clinical magnetic resonance (NEST).

Research Excellence conducted at the Louvain Drug Research Institute must ensure the following:

- Publications in well recognized international journals and / or patents,
- Training of young researchers,
- Dissemination of knowledge to the scientific community,
- Expertise for public authorities' health and /or pharmaceutical, chemical and biotechnological industries.

II. Research fields and groups

Overall, research activities are developed by seven research groups that share their expertise to develop original projects related to Microbes and Health, Inflammation, Cancer, Elderly and Metabolic Diseases, as well as Advanced Technology. The main aspects of a drug development are:

1) Design and research of new active molecules. It involves 2 research groups: Medicinal Chemistry (CMFA, developing the expertise on rational based-synthesis of new compounds) and Pharmacognosy (GNOS, specialised in the extraction and identification of new bioactive molecules isolated from plants).

2) The research on the evaluation and characterisation of new targets is performed by three entities:

i) Metabolism and Nutrition (MNUT), covering metabolomics, integrative physiology and nutrition for therapeutic innovation related to microbiome;

(ii.) Bioanalysis and Pharmacology of Bioactive Lipids (BPBL), which focuses on bioactive lipids in disease;

(iii) Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR), that includes FACM (pharmacology), PMGK (pharmacometrics), CLIP (clinical pharmacy) research groups, gathering their expertise from bench to bedside to propose innovative and safe therapeutic approaches.

3) The implementation and clinical evaluation are covered by the following research groups:

(i) Population Pharmacokinetics and Pharmacometrics (TFAR/PMGK);

(ii) Clinical Pharmacy (TFAR/CLIP), which evaluates the quality of use in medicine and clinical practice;

(iii) Advanced Drug Delivery and Biomaterials (ADDB), specialized in drug delivery systems and biomaterials as a means to improve therapeutic outcomes of drugs;

(iv) Biomedical Magnetic Resonance (REMA) that develops innovative tools using magnetic resonance with applications mainly in oncology.

(v) Metabolism and Nutrition (MNUT) group that elaborates the proof of concept of innovative nutritional approaches in clinical intervention studies.

Therefore, all major aspects of the drug are covered from its design to its optimal use.

III. Decision-making and management

The LDRI Management Committee is currently made up of a President (Nathalie Delzenne) and three Vice-Presidents (Raphaël Frédérick, Giulio Muccioli and Françoise Van Bambeke) re-elected (second mandate) by the LDRI Council in 2019.

N. Delzenne

F. Van Bambeke

R. Frédérick

G. Muccioli

The Board and the Council are involved in the functioning of the Institute and are responsible for all major decisions concerning the LDRI.

The Board is made up of the President and Vice-Presidents of the Institute as well as elected representatives from the academic (2), scientific (2) and administrative and technical staffs (2).

The Council is composed of the permanent scientific and academic LDRI members and representatives of the scientific (3), administrative and technical staff (2). The roles of the Council, Board and President, as well as the mode of elections, are described in a recent revision of internal rules, in accordance with the general rules of the University.

An International Scientific Council of five well-known researchers, broadly covering the research areas of the Institute, provides advices on research and recruitment strategy. The scientific board has been renewed for 3 years in 2017: C. Hughes (Primary care Pharmacy) (Queen's University, Belfast, Ireland), D. Crommelin (Drug delivery and Pharmceutical sciences) (Dutch Top Institute Pharma, Leiden; University of Utrecht, The Netherlands), J.L. Veuthey (Analytical Chemistry) (Université de Genève, Switzerland), P. Ferré (metabolism, pathophysiology and molecular biology) (Inserm-Université Pierre et Marie Curie, Paris, France) and P. Herdewyn (Medicinal Chemistry) (KULeuven, Belgium).

Prof. C. Hughes (UK)

Prof. D. Crommelin (NL)

Prof. J.L. Veuthey (CH)

Prof. P. Ferré (FR)

Prof. P. Herdewyn (BE)

IV. Human Resources

The **total staff** of the LDRI in December 2019 was 146 full members (affected to LDRI as main entity) and 10 affiliated members. It represents 156 persons involved in LDRI's activities, with a proportion of 40% men and 60% women.

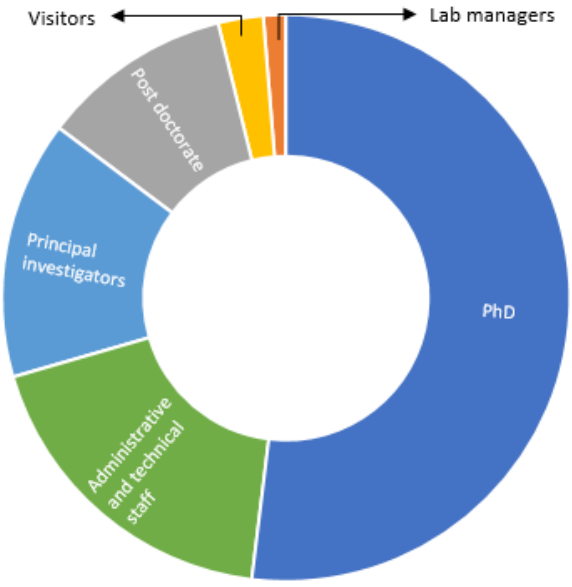
Academic staff:

Due to part-time contracts, the 22 Principal Investigators stand for 21 FTE (Full Time Equivalent). Among them, 7 FTE senior researchers are paid by the FRS-FNRS.

Scientific staff:

16 post-doctoral fellows and 78 PhD students, including 19 teaching assistants, are mainly affected to the LDRI. In addition, several PhD students and post-doctoral fellows are affiliated to the LDRI, since they are co-supervised by LDRI members, having their main affiliation in other Institutes or Universities.

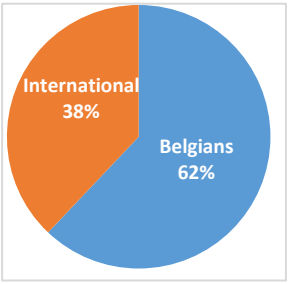
The technical and administrative staff affected to the LDRI represents 29 persons.



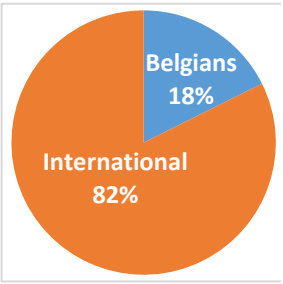
Proportion of the different categories of the staff (includes affected and affiliated members).

The LDRI is attracting numerous **international researchers**, which proportion is presented in orange in the graphs below. 38% of the PhD students and 82 % of the post-doctoral fellows are coming from abroad.

PhD students



Post-doctoral fellows



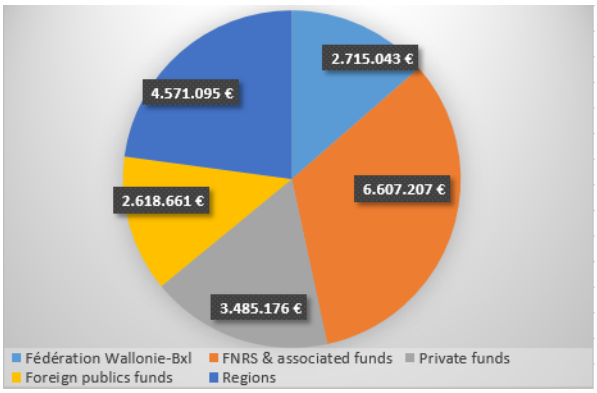
The background of the scientists is also quite diverse reflecting the multidisciplinary research performed within the Institute. The PhD students within the LDRI's research groups have trained as pharmacists, bioengineers, engineers, chemists, biologists, physicists, MD or masters in biomedical sciences.

V. Fundings

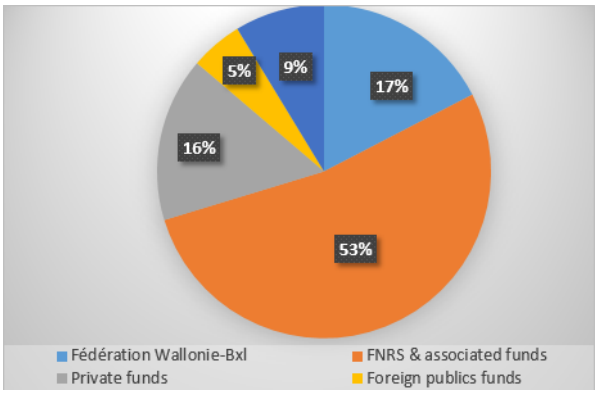
The LDRI personal is mainly financed by internal incomes. Indeed, the Fédération Wallonie Bruxelles (UCLouvain) guarantees the salary of the academic staff (except FNRS PI's), of the scientific staff working as assistant, and of part of the technical and administrative staff, as well as the availability and maintenance of the buildings. The UCLouvain is also supporting research activities through the implication of the central administration, and through the attribution of grants (FSR, ARC…) upon project evaluation. The LDRI receives an annual fee from the Health Sector (about 106.000€ in 2019), calculated upon criteria based on the number of academic and scientific staff involved in research activities.

Therefore, the members of the LDRI are very active in obtaining **financial incomes from third parties** (as illustrated below), which are essential to cover the needs for a competitive scientific outcome.

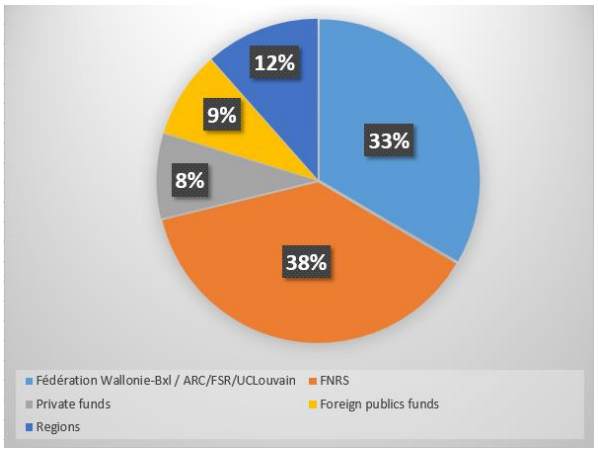
Sources of funding of the LDRI as (Global amount in EUR for projects running in 2019)



Sources of Funding of the LDRI (as percentage of the number of projects running in 2019)



Sources of funding of the LDRI staff in 2019*



** This graph includes full and affiliated members.*

The main sources of staff funding (see graphic below) are the FNRS (38%), UCLouvain/Fédération Wallonie Bruxelles (33%), the Belgian regions (12%), foreign public funds (9%) and private funds (8%). The FNRS pays the salary of 7 permanent principal investigators, 3 postdoctoral researchers, and 18 PhD students (including FRIA - Fund for Research Training in Industry and Agriculture - and Televie doctoral grants). 19 teaching assistants are paid by UCLouvain, and spend 50% of their time for research (50% in teaching activities).

VI. Scientific Output

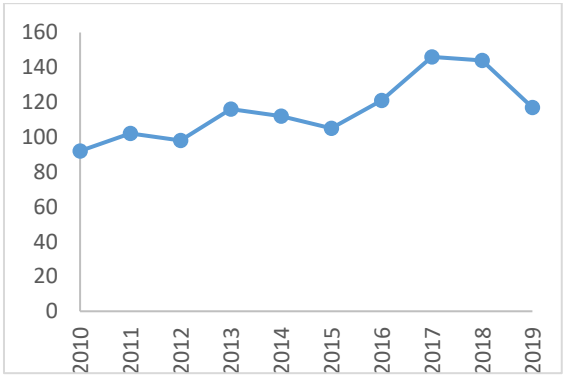
The scientific output of the LDRI can be appreciated by well-cited publications in recognized international journals and through the training of young researchers (illustrated by the high number of PhD and post-docs per PI). The members of LDRI are also involved in missions devoted to the dissemination of their knowledge to the scientific community and society, to official expertise for Public Health Authorities, pharmaceutical/chemical/ biotechnological companies and/as evaluators for research agencies at the national and international level.

Publications

(https://uclouvain.be/en/research-institutes/ldri/ldri-publications.html)

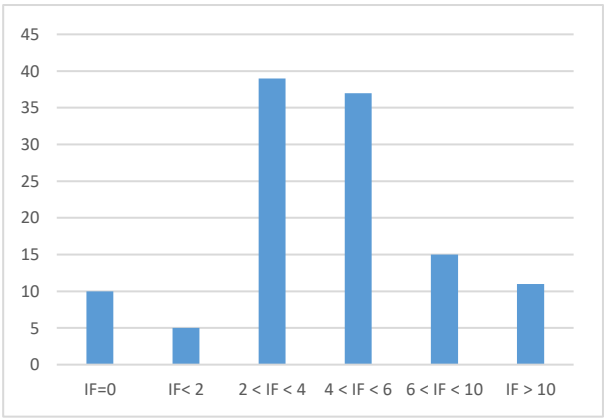
Altogether, the research groups of the LDRI published 407 scientific articles in international journals or book chapters during the last three years (2017-2019).

Evolution over the time of the number of annual publications published by the LDRI research groups



The mean Impact Factor (IF) of the publications during the period 2010-2019 was 4.78 (including review papers and educational papers). In 2019, this mean IF is **5,76**.

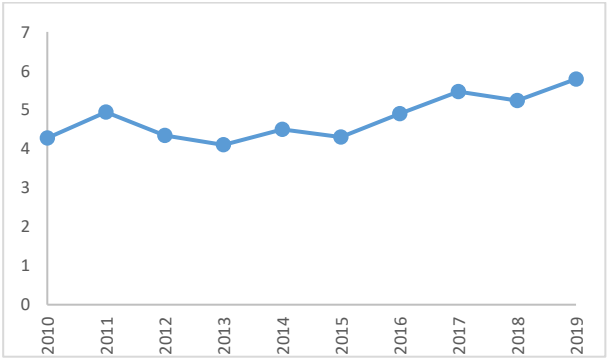
The distribution of publications per impact factor is shown hereafter.



(Papers with IF=0 are book chapters, educational papers or papers published in recent journals without IF, data 2019)

Since the creation of the institute in 2010, there is an increase in the number of publications as well as a mean impact factor above 5.

Evolution over the time of the mean impact factor of original papers published by the LDRI research groups



In addition to these publications, two patents have been published by LDRI members in 2019.

Training in research

81 PhD students are currently supervised by the PIs of the LDRI. Most of these PhD students are enrolled in the Doctoral School of Biomedical and Pharmaceutical Sciences.

Moreover, all the PIs of the LDRI are promotors of Master Degree theses in Biomedical Sciences, Pharmacy, Biology (among others) and of Bachelor's degree dissertations (technicians, dieteticians,...).

Seminars-Symposia

Researchers and other professionals having an interest for the scientific areas related to drugs attend the LDRI seminars (at least once a week). Their subjects alternate between presentations by junior scientists from the Institute and by senior scientists (from the Institute, from other Institutes within the University, from other Universities in Belgium or abroad, from the Industry).

The PhD day is organised by the scientific staff once a year, allowing PhD's and postdoctoral fellows to exchange in a friendly atmosphere.

Expertise

All the principal investigators of the LDRI are recognised for their expertise by the Public Health Authorities and/or pharmaceutical, chemical and bio-technological industries, and/or research agencies. They participate as (co) leaders or members of:

- Superior Health Council (Belgium), Belgian Nutrition Society (Belgium)

- Royal Academy of Medicine (5 members)

- European Medicines Agency (EMA)

- Public Health Institute

- Federal Agency for Medicines and Health Products

- Federal Agency for Nuclear Control

- French National Research Agencies (ANR and HCERES)

- Fonds Wetenschappelijk Onderzoek (FWO)

(*The list is illustrative rather than exhaustive*).

Collaborative projects with the industry

Many collaborative projects are ongoing with regional and international industries and SME's.

KEY AWARDS 2017-2019

2019

Bastiancich Chiara (ADDB): Prix de thèse « formulation galénique » de l'académie nationale de pharmacie (France)

Beloqui Ana (ADDB): Prix Paul Van de Velde. Amount: 2.500 €

Vandermeulen Gaelle (ADDB): Prix Cornélis-Lebègue, Académie royale de Médecine de Belgique (Belgium)

Sarah Pötgens (MNUT): Best oral communication Award at the 9th annual meeting of the Belgian Nutrition Society.

Nathalie M Delzenne (MNUT): Sir Cuthbertson Lecture- selected upon outstanding contribution in nutrition research. European society for clinical nutrition and metabolism (ESPEN).

Audrey Neyrinck (MNUT): Prize of the Belgian Society of Clinical Nutrition.

Patrice D. Cani (MNUT): Prize Paris MATCH of the National TV RTBF "Matière grise) for "The ability of popularization Science work"

Patrice D. Cani (MNUT): Award for Excellence in Biomedical Research and Creativity from the joint scientific committee of the Academies of Medicine of Belgium and France, and the Council of the JA DeSève Research Chair.

Highly cited researchers: Véronique Préat, Fabienne Danhier, Patrice Cani, Nathalie Delzenne, Audrey Neyrinck and Amandine Everard.

2018

Bénédicte Jordan (REMA): Fonds Maisin. Amount: 22 000 €

Bernard Gallez (REMA): Fonds Maisin. Amount: 21 000 €

Raphaël Frédérick (CMFA): Fonds Maisin. Amount: 21 000 €

Laure Bindels (MNUT): Pharmabiotics Young Investigator. Amount: 10 000 €

Giulio Muccioli (BPBL): Prix Eugene de Somer. Amount: 7 000 €

Laure Elens (PMGK): Victor Armstrong Young Investigator. Amount: 1 000 €

Séverine Henrard (CLIP): Award of the Belgian Society for Gerontology and Geriatrics for the best poster presentation at the 28th autumn meeting (Liège, Belgium).

Highly cited researchers: Véronique Préat, Fabienne Danhier, Patrice Cani and Nathalie Delzenne

2017

Patrice Cani (MNUT): Belgian Endocrine Society Lecture Award. Amount: 4 000€

Anne des Rieux (ADDB): Grant of Fondation Charcot. Amount: 30 000 €

Laure Elens (PMGK): Theophile Gluge Award (Académie Royale de Belgique). Amount: 1 000 €

Raphaël Frédérick (CMFA): Prix Paul Van de Velde. Amount: 2 500 €

Huber Plovier (MNUT): Danone Institute Award. Amount: 2 500 €

Anne Spinewine (CLIP): Healthy Ageing Award (ASTRAZENECA Foundation). Amount: 25 000 €

Françoise Van Bambeke (FACM) and Dr. Hussein Chalhoub: Fonds Forton (Fondation Roi Baudouin). Amount: 200 000 €

Gaëlle Vandermeulen (ADDB): Prix Depelchin. Amount: 1 500€

Highly cited researcher: Fabienne Danhier, Veronique Préat

PLENARY LECTURES by EXTERNAL SPEAKERS 2019

Johann MIGNOLET
Louvain Institute of Biomolecular Science and Technology (IBST), UCLouvain, Louvain-la-Neuve
"Mobilization of microflora commensals and their weapons for therapy"

Prof Stanislas GORIELY
ULB
"Key role of AU-Rich element (ARE)-mediated mRNA decay in Inflammation and Cancer"

Lucas MORIN
Aging Research Center, Karolinska Institutet, Stockholm, Sweden
"Too much, too late? Patterns of drug prescribing for older adults near the end of life"

Géraldine LALOUX
de Duve Institute, UCLouvain, Woluwe
"How to thrive inside your prey: revealing the cell biology of a bacterial predator"

Jean-Marie RAQUEZ
Matériaux Polymères et Composites, Umons
"Polymeric and Composite Materials implemented towards biomedical realm."

Terry RISS
Promega Corporation
How to choose a cell-health assay: "Are my cells dead?" to "How did my cells die?"

Pr. Vincenzo CERULLO
Head of Drug Research Program, Faculty of Pharmacy, University of Helsinki, Helsinki Finland
"Dressing viruses in tumor's clothing: welcome to the new era of therapeutic cancer vaccines"

James PHILIPS
University College of London (UK)
"Nervous system tissue engineering and regenerative medicine"

Prof. Véronique MIRON
Edinburgh University (UK)
"The rise and fall of microglia driving central nervous system remyelination"

Dr Marta PUTRINS
University of Tartu, Estonia
"Fluorescent bioreporters for detecting bacterial physiological state"

Prof. Jesse JANSEN
University of Sydney
"Too much medicine and not enough conversation: reducing medication burden in older people by supporting communication and decision making"

Dr Philippe JACQUMIN
MnS, Dinant
"Potential of PK/PD modelling and simulation in drug development through concrete examples"

Prof. Florence GAZEAU
CNRS, Université Paris Diderot
"Engineering extracellular vesicles for therapeutic applications"

Dr Jean-Michel BRUNEL
Faculté de pharmacie, Aix-Marseille Université, France
"Antibiotic adjuvants: Make antibiotics great again"

Dr Pierre-Marie ALLARD
EPGL - UniGe - Geneva
"Computational strategies for the exploration of specialized metabolomes: applications in drug discovery"

Prof. Alain MUSELLI
Université de Corse, Lab. Chimie des Produits Naturels
"Huiles essentielles de Corse et du bassin Méditerranéen: Caractérisation moléculaire, Chimiovariabilité, Activités biologiques"

Dr Bella B MANSHIAN
Department of Imaging and Pathology, KU Leuven
"Nanomaterials for therapeutic and optical imaging applications"

Murali C. KRISHNA
National Cancer Institute
"Molecular Imaging of the tumor microenvironment. Imaging Biomarkers to guide treatment and monitor response"

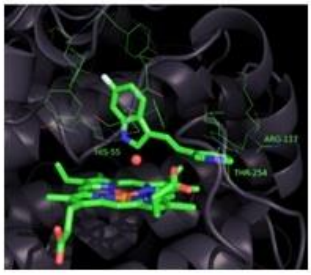
SECTION II – RESEARCH GROUP PRESENTATION

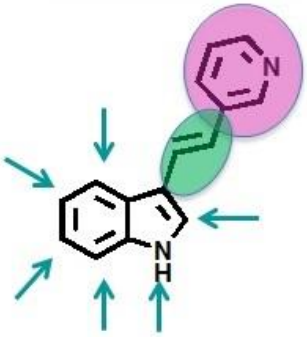
I. Medicinal Chemistry (CMFA)
II. Pharmacognosy (GNOS)
III. Metabolism and Nutrition (MNUT)
IV. Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)
V. Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR)
VI. Advanced Drug Delivery and Biomaterials (ADDB)
VII. Biomedical Magnetic Resonance (REMA)

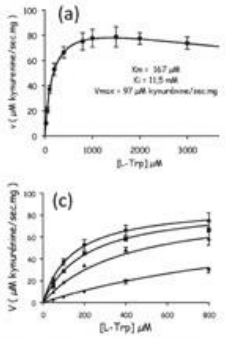
MEDICINAL CHEMISTRY (CMFA)

« Towards the discovery of innovative pharmacological tools and drugs »

Hit Identification → **Hit-to-lead Optimization** → **Pharmacological Evaluation**







In silico studies
Structure-based design

Chemical synthesis
Structural analysis

In vitro and *in vivo* assays

Screening

PhysChem properties

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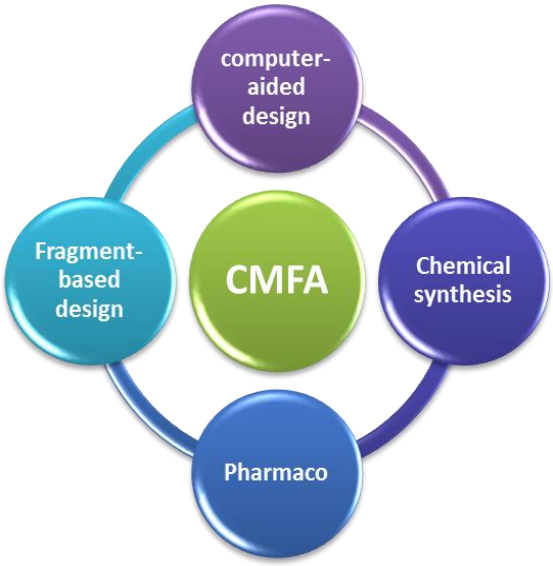
The discovery of new innovative medicines is a priority for human health. It is in this context that the Medicinal Chemistry Research Group (CMFA) is pursuing its research activities

To reach this ambitious objective, two complementary strategies are used:

The first is driven by a pharmacological approach aiming at designing new bioactive molecules mainly interacting with a physiological system of neurotransmission: the endocannabinoid system. This system consists of several proteins regulating the signaling of endogenous lipid compounds, i.e. the endocannabinoids. Several targets emerge from this system: endocannabinoid biosynthesis enzymes, GPCR receptors, nuclear receptors and endocannabinoid degradation enzymes.

The second complementary approach is driven by a chemical approach of drug design and aims at discovering new chemical entities following a computationally-assisted strategy. This strategy involves the initial discovery of hits via virtual screening and fragment-based inspired methodology. These hits are then optimized by rational drug design to generate new pharmacological tools or drugs.

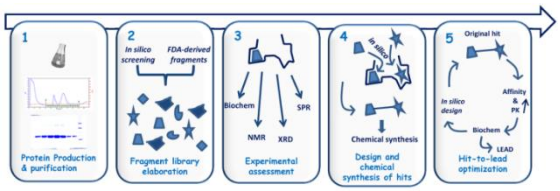


RESEARCH RESULTS

Fragment-based drug design

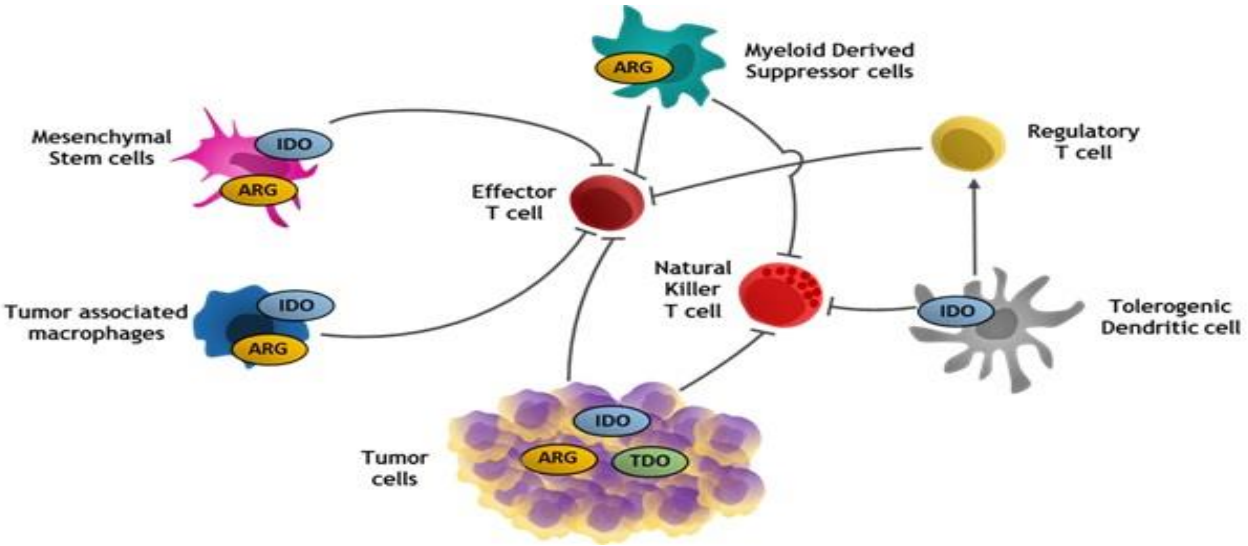
In medicinal chemistry, one of the big challenges remains the discovery of an original hit that can be easily tuned into a lead and then in a drug candidate. In this regard, our aim is to develop an innovative fragment-to-lead strategy for high-quality lead identification. This computationally-assisted approach involves the initial discovery of low-molecular weight molecules called fragments. Owing to their small-size, fragments are more likely to reach key pockets within a protein active site, and, once their interaction within the active site is clearly understood, they represent a unique possibility of designing a promising hit compound in an efficient way.

The originality of this approach resides in the compilation of available experimental information about structural motifs recognized by the target, and their use to guide the selection of fragments from large chemical databases using a computationally-assisted screening. The interaction of chosen fragments with the target protein can then be experimentally assessed by means of biochemical and biophysical techniques, such as NMR, surface plasmon resonance (SPR), mass spectrometry (MS) and/or X-ray diffraction (XRD). Once the binding experimentally confirmed, rational drug design can start and the selected fragments can be finely tuned to provide an original hit. This original computationally-assisted fragment-to-lead strategy offers the prospect of a more efficient approach to drug discovery – resulting in the generation of high-quality leads with a better chance of success in future development.



Anticancer immunotherapy

Tryptophan catabolism is an important mechanism of peripheral immune tolerance contributing to tumoral immune resistance, and indoleamine 2,3-dioxygenases (IDO and TDO) inhibition is a promising strategy for anticancer drug development. IDO and TDO are unrelated heme-containing enzymes catalyzing the oxidative cleavage of the indole ring of L-tryptophan (L-Trp), the first and rate-limiting step along the kynurenine pathway. The implication of IDO in the phenomenon of tumoral immune resistance is the focus of intense researches and the enzyme is now recognized as a validated target for anti-cancer therapy. Therefore, a

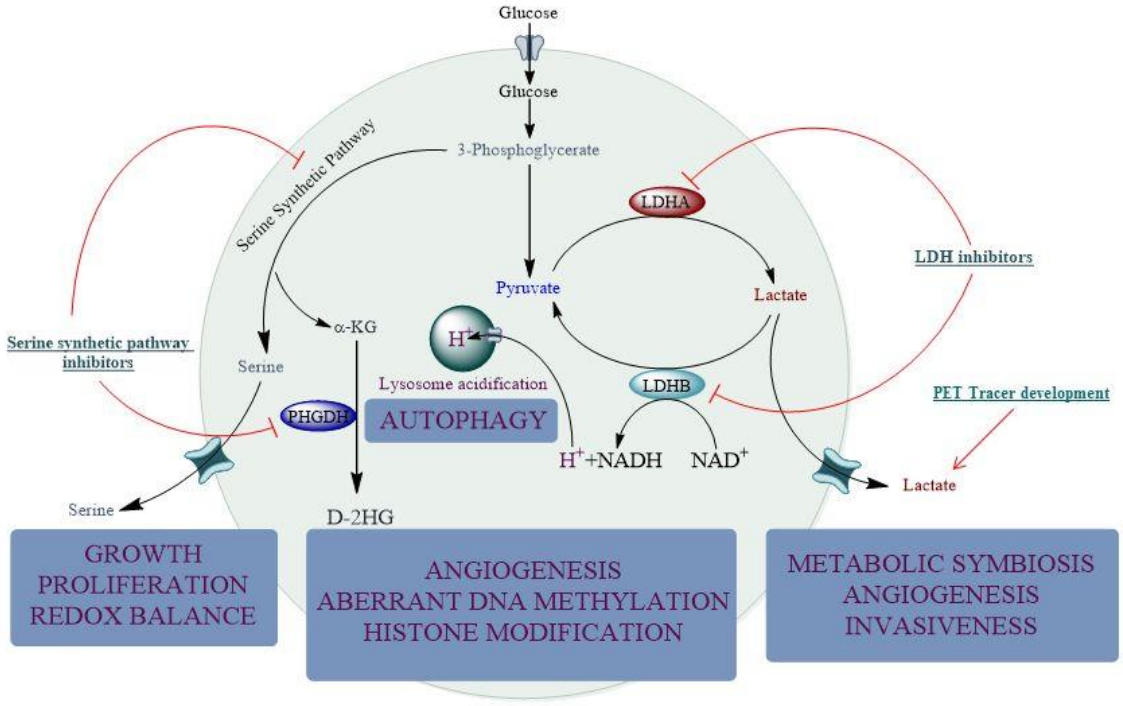


number of groups, including us, are actively searching for novel original IDO inhibitors. In contrast, the effect of TDO expression on the immune response has only been recently investigated in detail. Indeed, we showed in collaboration with the group of Prof Van den Eynde that TDO was effectively overexpressed by a number of human tumors and that this expression prevented rejection of tumor cells. We designed a novel TDO inhibitor and proved, in a preclinical model, the concept that TDO inhibition promotes tumoral immune rejection. Interestingly, blocking both TDO and IDO to improve the efficacy of cancer immunotherapy would be complementary: in a series of 104 human tumor lines of various histological types, we showed that 20 tumors expressed only TDO, 17 only IDO and 16 expressed both enzymes. Therefore, targeting both IDO and TDO would allow reaching 51% of tumors instead of either 32% or 35% with a compound inhibiting IDO or TDO alone, respectively. The design of IDO, TDO or dual IDO/TDO inhibitors is thus of major importance. Interestingly, our fragment-based drug design strategy recently provided promising results for the discovery of new IDO. These preliminary data are very encouraging to pursue the search for new anticancer agents through a fragment-based drug design strategy.

Tumor metabolism

The last ten years have witnessed an increased regain of interest for tumor metabolism. Recent advances in this field have shed light on how tumors fuel rapid growth by preferentially engaging biosynthetic pathways. Although cellular metabolic pathways are rich pickings for drug targets, pinpointing enzymes that critically contribute to tumor metabolism is key to establish a therapeutic window since most of metabolic enzymes also play important roles in normal tissues.

1. PHGDH (3-phosphoglycerate dehydrogenase) and PSAT1 (phosphoserine aminotransferase-1) represent ideal targets for new anticancer strategies. These enzymes catalyze the first and second steps in the serine biosynthetic pathway, respectively. This pathway diverts a relatively small fraction of 3-phosphoglycerate from glycolysis to



generate serine as well as equimolar amounts of NADH and α-ketoglutarate (αKG). Interestingly, two simultaneous recent reports have recently identified the serine pathway as a vital source of αKG to fuel the TCA cycle in a variety of tumor cells. These two studies further documented that serine supplement could not rescue tumor cells in which PHGDH and PSAT1 genes were knocked down, thereby identifying the serine pathway as a process providing malignant cells with critical amounts of its intermediary synthetic products, αKG and possibly NADH, instead of the end product, serine (that may also be taken up from the extracellular fluid). In good agreement with the above statement on the rationale to identify specific targets to tackle tumor metabolism, this latter observation indicates that serine deficiency in healthy tissues and possible disorders associated with the inhibition of either PHGDH or PSAT1 could be treated by exogenous serine supplement, whereas treatment with such inhibitory compounds could take advantage of the strict addiction of tumors to the by-products resulting from PHGDH and PSAT1 activation.

In this project, we aim to understand the role of the serine pathway in tumor progression and in particular to develop pharmacological tools to evaluate the extent of tumor addiction to this metabolic path and their therapeutic potential by exploring potential side effects on healthy tissues. To this end, novel innovative pharmacological inhibitors of PHGDH and PSAT1, the two main enzymes of the serine pathway (see Figure 1), were designed and chemically synthesized. These compounds are currently optimized by chemical modifications and are expected to help deciphering the exact roles of these enzymes in cancer progression and insights on their physiological roles (that could represent limitations to the clinical use of such inhibitors).

2. Tumor cells are also characterized by a remarkable metabolic plasticity allowing them to survive and proliferate in hypoxic and extracellular acidic environments. In tumor cells, this plasticity allows the coexistence and coordination of several metabolic phenotypes, leading to an optimal use or resources. Hypoxic cells uses glucose that is metabolized by anaerobic glycolysis. Lactate is secreted and diffuses, and can be subsequently used by oxygenated tumor cells as a preferred energetic source to glucose. The lactate oxidative pathway requires the entrance of lactate in oxidative cells via a process that is mainly facilitated by the Monocarboxylate Transporter MCT1 and the oxidation of lactate to pyruvate by the lactate dehydrogenase B (LDHB). The pyruvate can then fueled the Krebs cycle and NADH uses the malate-aspartate shuttle to directly fuel the mitochondrial respiration chain. The oxidative use of lactate in the oxygenated tumor compartment therefore optimizes the availability of glucose for cells of the hypoxic compartment, thus constituting a unique metabolic cooperation. If the use of lactate by oxidative cells is a proven fact, the advantage it gives them remains largely unknown. A first series of studies showed that lactate can act as a pro-angiogenic agent. This signaling activity also depends on the oxidation of lactate to pyruvate by LDHB, allowing pyruvate to inhibit enzymes of the prolylhydroxylase family and activate the hypoxia-inducible transcription factor factor-1 (HIF-1) independently of hypoxia. In addition, a recent collaborative led with the team of P. Sonveaux (IREC) has shown that the oxidative use of lactate promotes autophagy, ie, a process of degradation and recycling of proteins and organelles requiring formation of specialized structures, autophagosomes, and their fusion with lysosomes. To promote autophagy, LDHB physically interacts with V-ATPase, a proton pump located on the surface of lysosomes, which it feeds with the protons produced during the lactate + NAD^+ $\rightleftharpoons$ pyruvate + NADH + H^+ reaction. This observation seems important to us as autophagy participates in tumor progression by recycling damaged proteins and

organelles when cancer cells are exposed to oxidative stress, and because it provides cells with energy substrates under metabolic stress conditions.

All these observations suggest that LDHB may be a new target in cancer therapy. However, there is currently no specific inhibitor of this enzyme, and the consequences of systemic inhibition of LDHB activity remain largely unknown.

In this project our aim is thus to develop and validate a peptide inhibitor and a non-peptide inhibitor to selectively inhibit tetramerization of LDHB. Our strategy will involve the use of Protein-Protein Interaction Inhibitor (PPI) identification methods that is, a highly multidisciplinary approach involving molecular modeling studies (identification of "Hot Spots"), biochemical studies (in vitro and in vivo inhibition of LDHB tetramerization, selectivity study) and biophysical studies (nuclear magnetic resonance analysis of ligand-LDHB interaction). To achieve the goal of a selective inhibition of LDHB, we will use an innovative strategy targeting the tetramerization site of LDHB rather than the active site of the enzyme.

Ferroptosis

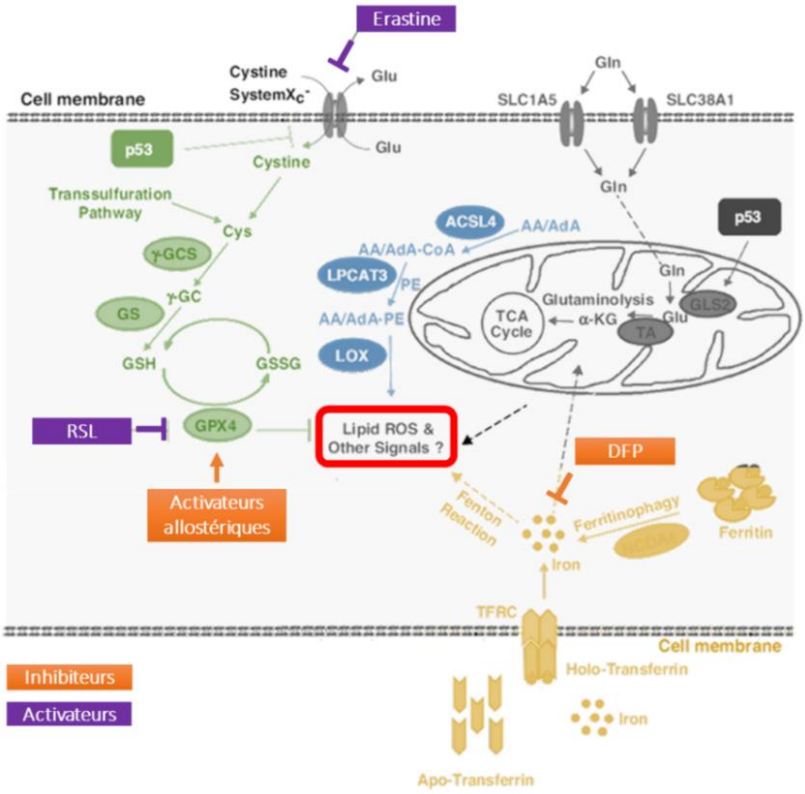
Regulated cell death is necessary for tissue homeostasis, embryonic development, immunity and other biological processes. Nevertheless, dysregulated cell death leads to pathological developments especially in neurodegenerative diseases. On the other hand, cancer cells tend to avoid death in order to enhance proliferation.

In the past decade, multiple ways of regulated cell death were identified and characterized. Among them, Ferroptosis was discovered through the study of a genotype selective antitumor agent, erastin, a small molecule able to kill cancer cells without presenting any morphological or biochemical features of known cell death pathways. Instead, ferroptotic dying cells show a mitochondrial disruption associated with high levels of free intracellular iron and lipid hydroperoxides.

Although the exact mechanism leading to cell death is not yet fully understood, this lipid peroxidation phenomenon initiates the process and is considered as the main hallmark of ferroptosis (see Fig). Free intracellular iron plays a crucial role in redox mechanism of ferroptosis by catalyzing Fenton reaction (see Fig, yellow pathway). Thus, an increase in free iron levels mediated by a dysregulation of its transporting or stocking directly contributes to the accumulation of lipid peroxides.

Once those lipid hydroperoxides are formed, only the reducing action of the glutathione peroxidase 4 (GPX4) is able to prevent their damaging outcome (see Fig, green pathway). Because GPX4 uses Glutathione (GSH) as cofactor, a depletion of the pseudo-tripeptide and/or cysteine, the limiting amino acid in the GSH synthesis, causes an increase in lipid peroxides levels involved in the cell death initiation.

Interestingly, erastin induces ferroptosis by inhibiting the Xc- antiporter. Ras synthetic lethal compounds (RSL) are other pharmacological tools currently used to induce ferroptosis by directly inhibiting GPX4. On the contrary, ferroptosis can be prevented by several different ways. For example, allosteric activators of GPX4 were identified to enhance the oxidative stress controlling activity of the peroxidase. It is also possible to inhibit ferroptosis by decreasing the intracellular free iron levels of iron with metal chelators such as deferiprone.



Abbreviations :

AA arachidonic acid
AdA, adrenic acid
ACSL4, *acyl-CoA synthetase long-chain family member 4*
CoA, Coenzyme A
PE, phosphatidyl-ethanolamine
γ-GCS, γ-glutamylcysteine synthetase
GPX4, glutathione peroxidase 4
GSH, reduced glutathione
α-KG, α-ketoglutarate
LPCAT3, lysophosphatidylcholine acyl-transferase 3
LOX, lipoxygenase.

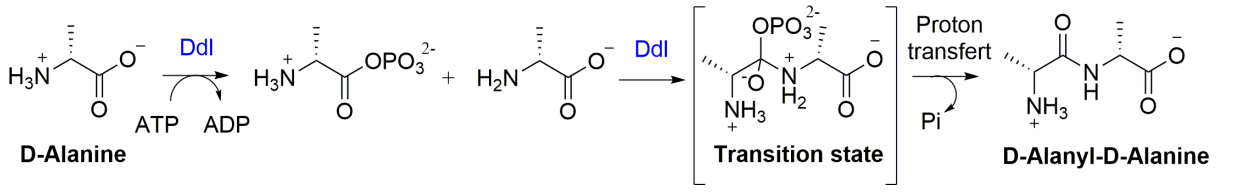
More recently, a genome-wide recessive genetic screen has pointed to an essential role for Acsl4 in ferroptosis. This gene codes for the acyl-CoA synthetase long chain family member 4 (ACSL4) which catalyzes the ligation of a Coenzyme A (CoA) on a long chain polyunsaturated fatty acid (see Fig, blue pathway). By preferentially activating arachidonic and adrenic acids, ACSL4 shapes cellular lipid composition and dictates ferroptosis. Indeed, part of the acyl-CoA formed are esterified into membrane phospholipids by LPCAT3 where they can be oxidized (for example by the lipoxygenases) and then contribute to the lipid peroxides pool necessary for the initiation of the cell death. It was shown that the extinction of ACSL4 or its inhibition by glitazones was enough to prevent ferroptosis pharmacologically induced.

Ferroptosis was studied in few experimental Ferroptosis was studied in few experimental models. Nevertheless, this regulated cell death seems to have a real therapeutic interest. Indeed, several studies have highlighted the potential benefit of inducing ferroptosis in cancer therapy. For example, it was shown that sorafenib, a multi-kinase inhibitor mainly used in renal carcinoma, induces ferroptosis in cancer cells by disrupting system Xc-. Simultaneously, ferroptosis studies have widened their scope and it seems particularly relevant that preventing ferroptis is promising therapeutic strategy in a neurodegenerative context. This idea is supported by the FAIRPARK-II project, a clinical assay that study the role of DFP in the neurodegeneration slow-down in Parkinson's disease.

In this new project, we are thus developing novel pharmacological tools to study ferroptosis and particularly ACSL4 inhibitors.

Antibiotics

As the phenomenon of antibiotic resistance is dramatically increasing these days, the search for new therapeutic targets less



vulnerable to these resistance mechanisms appears as a real need. The cell wall of bacteria and the enzymes that are involved in its synthesis are prime targets for many antibiotics, which inhibit the late stages of peptidoglycan biosynthetic pathway. But the resistance phenomena have revealed the high flexibility in this assembly pathway, and the need to target other enzymes acting on earlier steps of peptidoglycan synthesis. D-alanyl-D-alanine ligase (Ddl) is of particular interest as it utilizes a substrate (D-alanine) which is specific for bacterial peptidoglycan biosynthesis and essential for bacterial growth.

In this work, we aim at designing novel DD-ligases inhibitors. Previous works in our group have highlighted a novel hit (S89) characterized with thiosemicarbazide motif. First, analogues of S89 were synthesized. Indeed, the thiosemicarbazide family is very promising due to its low half maximal effective concentration (EC50) and its good antibacterial activity. These compounds will be evaluated on recombinant protein Ddl-His6 produced and purified in our group. This study will provide initial structure-activity relationships (SAR) and thus help understanding the structure requirements to achieve a high DD-ligases inhibition. Then, novel hits will be identified through a fragment-based strategy. To this end, an in-house library of 280 diverse fragments will be first assessed. Finally, the more potent fragments will undergo a structure guided optimization to design potent DD-ligases inhibitors.

pH Low Insertion Peptides (pHLIP) - drug conjugates as a novel tumor targeting strategy

Many diseases such as cancer (solid tumors), ischemia, stroke or infection lead to the development of local hypoxia and acidosis. Acidosis results from enhanced glycolytic flux which produces lactate and H+ ions (but also in tumors from hydration of CO2 which represents another source of H+ ions). Protons are intensively pumped out of the cells to keep intracellular pH near neutral. However, due to the poor blood circulation in diseased tissues, H+ ions accumulate in extracellular spaces. Consequently, a reverse membrane pH gradient is formed, the extracellular pH (pHe) being lower than the intracellular pH (pHi) (contrary to normal tissues). Therefore, extracellular acidity might serve as a general marker for detecting and targeting ischemic tissues and tumors. However, since the bulk extracellular pH in these diseased tissues is only 0.5–0.8 pH units lower than the extracellular pH in healthy tissue, this strategy remains particularly challenging. Several pH-sensitive imaging and drug delivery systems have actually been envisioned as diagnostic or therapeutic modalities specifically triggered by the acidic tumor microenvironment. Among these are the pH Low Insertion Peptides (pHLIP) family derived from the bacteriorhodopsin C helix. This family represents a unique class of water-soluble membrane polypeptides which were found to insert across a membrane to form a stable transmembrane α-helix. According to pH conditions, pHLIP can adopt three particular states: (i) at neutral and high pH, without the presence of a lipid bilayer, the pHLIP is soluble, (ii) in the presence of a lipid bilayer under the same pH conditions (neutral of high), the pHLIP is monomeric and unstructured, and binds to the membrane in a random coil

conformation, and (iii) in an acidic environment, the transmembrane part (TM) of the polypeptide folds into α-helix and inserts across the membrane.

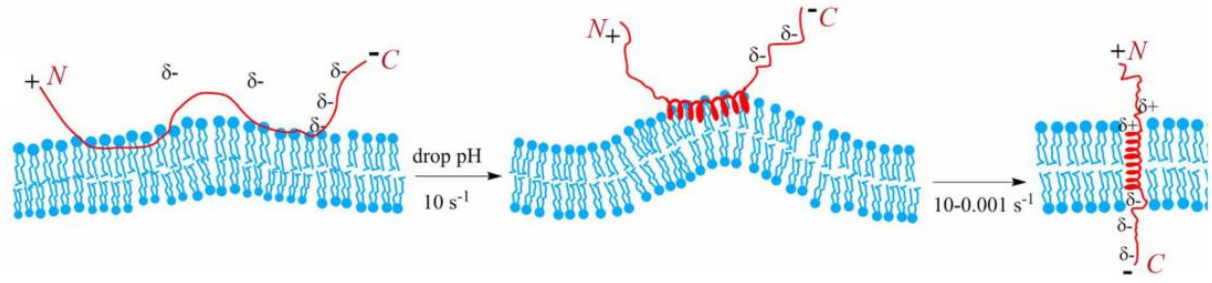
In the last years, pHLIP's were investigated in various fields and for instance combined with fluorescent dyes in order to target different disorders. In vivo studies were performed to target tumors, ischemic myocardium, the sites of inflammatory arthritis and infections. More recently, the very first examples of pHLIP linked to chemotherapeutic agents were published: paclitaxel, doxorubicine and monomethyl auristatin F.

In our research project we will study, develop and apply the pHLIP technology in two promising fields of cancer therapy: tumor lipid metabolism and the response of T cells to tumor microenvironment acidification. For the latter, we will focus on indoleamine-2,3-dioxygenases (IDO and TDO), two enzymes that have been shown to be account for tumoral immune escape. In the context of tumor metabolism, other therapeutic targets already studied in our labs will be selected: lactate dehydrogenase (LDH) and phosphoglycerate dehydrogenase (PHGDH). Finally, we will also focus on Glutaminase 1 (GLS1), an enzyme involved in glutamine pathway largely employed by cancer cells to proliferate, as well as on diacylglycerol O-acyltransferase 1 (DGAT1) and adipose triglyceride lipase (ATGL) that are involved in lipid droplet formation (commonly used by tumor cells for lipid storage). These targets were chosen because of existing expertise and models in our labs, including cellular and biochemical assays able to evaluate the inhibitory effects of dedicated drug candidates. Importantly, for these different targets, known (commercial or proprietary) reference inhibitors are already available and will be used for optimization using the pHLIP technology.

Cannabinoid system modulators

The aim of this work is to design new bioactive molecules interacting with a physiological system of neurotransmission: the endocannabinoid system. This system consists in several proteins regulating the signaling of endogenous lipid compounds, i.e. the endocannabinoids. Several targets emerge from this system: endocannabinoid biosynthesis enzymes, GPCR receptors, nuclear receptors and endocannabinoid degradation enzymes.

Ongoing research involves the development of selective inhibitors for the three main enzymes involved in the degradation of the endocannabinoids, namely the fatty acid amide hydrolase (type I), the monoacylglycerollipase, the N-acylethanolamine Acid Amidase. Several inhibitors of endocannabinoids metabolism have been discovered. The main achievement was the synthesis of the first inhibitor of the N-acylethanolamine Acid Amidase. Regarding monoacylglycerollipase (MGL), the search was focused on selective inhibitors, disulfiram has been identified as MGL inhibitor with a high selectivity profile regarding fatty acid amide hydrolase (type I).



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Didier LAMBERT

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Raphaël FREDERICK

Kozlova A, Frédérick R. Current state on tryptophan 2,3-dioxygenase inhibitors: a patent review (2019) Expert Opin Ther Pat. 29(1):11-23

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Ameryckx, A., Thabault, L., Pochet, L., Leimanis, S., Poupaert, J.H., Wouters, J., Joris, B., Van Bambeke, F., Frédérick, R. 1-(2-Hydroxybenzoyl)-thiosemicarbazides are promising antimicrobial agents targeting D-alanine-D-alanine ligase in bacterio. Eur. J. Med. Chem. (2018) 159, 324-338

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THESIS DEFENDED IN 2019

Spillier Quentin: "Mechanistic study of PHGDH enzyme inhibition: towards the development of new anticancer drugs".
Director: Raphaël Frédérick; Co-director: Olivier Feron

THESES IN PROGRESS

Ameryckx Alice: "Design and synthesis of DD-ligases inhibitors: Peptidoglycan intracellular biosynthesis as antibiotics target".
Director: Raphaël Frédérick; Co-director: Françoise Van Bambeke

Brustenga Chiara: "LDH disruptors for anticancer therapy"
Director: Raphaël Frédérick; co-director: Pierre Sonveaux

Deskeuvre Marine: "pH Low Insertion Peptides (pHLIP) - drug conjugates as a novel tumor targeting strategy: design, chemical synthesis, biophysical characterization and in vitro evaluation."
Director: Raphaël Frédérick; Co-director: Olivier Feron

Kozlova Arina: "Towards the discovery of dual inhibitors of IDO and TDO, two promising targets for anticancer immunotherapy".
Director: Raphaël Frédérick; Co-director: Benoit Van den Eynde

Marteau Romain: "Development of ACSL4's selective ligands: new tools to target ferroptosis".
Director: Raphaël Frédérick; Co-director: Séverine Ravez (Univ. lille2, France)

Prévost Julien: "Design and synthesis of Arg1 inhibitors for anticancer immunotherapy".
Director: Raphaël Frédérick

Thabault Léopold: "Development and validation of a new anticancer strategy targeting the Lactate Dehydrogenase B (LDHB) with innovative tetramerization site inhibitors."
Director: Raphaël Frédérick; Co-director: Pierre Sonveaux

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Pharmacognosy *implies multidisciplinary studies to identify new drug candidates (pure compounds or extracts) or new leads from natural origin and control their quality.*

Our laboratory, created in 1996, chose to focus on plants used in traditional medicine to:

1. *Evaluate the activities of crude extracts from traditional medicinal plants and obtain data to support their traditional uses, their indications and analyse potential toxicities.*
2. *Isolate and identify bioactive compounds which could constitute new prototypes for drug development*
3. *Analyse the possible targets and identify structure-activity relationships*
4. *Control their quality to limit adulterations and standardise treatments.*

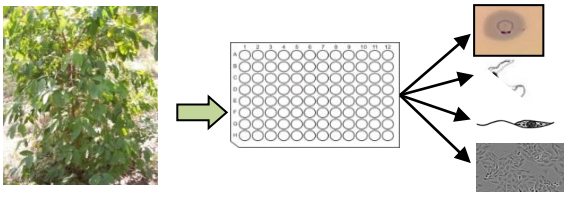
To allow these researches, we developed an expertise in extraction, purification, structure determination of compounds from complex matrices and development of quantification validated methods, while most of the pharmacological experiments are realised in collaboration with teams having expertise in the selected biological activities.

Our future researches will mainly focus on antiparasitic and antimicrobial activities for which a majority of available drugs are natural substances or derivatives, as well as immunostimulating and anti-inflammatory drugs.

1/ CRUDE EXTRACTS AND PURE COMPOUNDS EVALUATIONS

Plants used in traditional medicine in different countries are obtained through research collaborations (Marocco, Benin, Congo Democratic Republic, Rwanda, Madagascar, Mauritius in Africa, Vietnam in Asia, Peru, Bolivia and Brazil in South America). The first step is the selection on an ethnopharmacological basis and a literature survey. Different extracts are prepared and pharmacologically evaluated according to their traditional use(s). Several properties are analysed in our lab or in collaboration with other teams who developed suitable pharmacological tests (LDRI, other UCL or Belgian partners): in the last years we mainly focused on antimicrobial and antiparasitic activities, but two new projects were developed dealing with immunostimulant and anti-inflammatory activities.

Crude extracts are first evaluated by *in vitro* tests and their cytoxicity assessed on cancer and non-cancer cell lines.

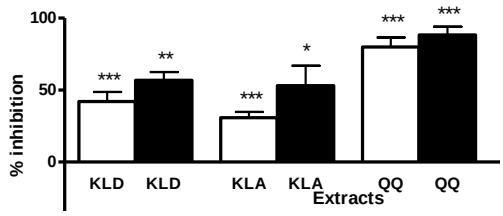


Keetia leucantha

The originality of our works is that we do not just realise screenings. The most promising extracts are also tested *in vivo* to assess their activity and eventual toxicity. The mode of administration is chosen according to the nature of the extract but most of them are given by oral route.

Several extracts possessing biological activities at low concentrations *in vitro* were identified (cfr publications).

The activities of the most interesting ones as well as purified compounds were also analysed *in vivo*. Acute and sub-acute toxicity tests are realised on rodents or using zebrafish (collaboration with Prof. Frédérich, University of Liège). Results indicate that extracts of *Croton zambesicus, Ajuga iva* and *Marrubium vulgare* showed, *in vivo*, antihypertensive properties but some extracts of *Croton zambesicus* also showed toxicities. Extracts from i.e. *Keetia leucantha Vitellaria paradoxa* and *Acanthospermum hispidum* as well as isolated triterpenic esters proved to have antimalarial activities on mice infected by *Plasmodium berghei.*



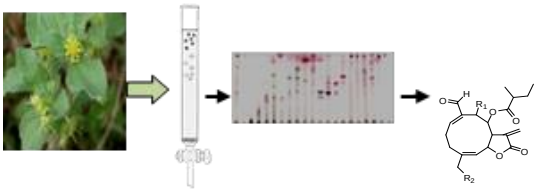
Efficient extracts and pure compounds on mice infected with *Trypanosoma brucei* were also identified and their highest tolerated dose determined.

Our works on antimicrobial plants allowed us to identify some promising plant extracts and natural compounds reducing the resistance of methicillin resistant *Staphylococcus aureus in vitro* (collaboration with F. Van Bambeke) and *in vivo* (collaboration with Prof. Niset, San Diego, USA). Other collaborations allow us to identify extracts improving fish resistance to microbial infection (collaboration with P. Kestomont, UNamur) or possessing antihelminthic properties for cattle (collaboration with UAC Benin).

Other extracts were shown to reduce the cytokines production of LPS activated mcrophages (collaboration with Prof. Muccioli).

2/ ISOLATION AND STRUCTURE IDENTIFICATION OF BIOACTIVE NATURAL COMPOUNDS

Plant extracts having interesting *in vitro* and/or *in vivo* activities are subject to bio- and chimio- guided fractionations to identify active components which could constitute new leads for further developments.



Acanthospermum hispidum

Fractions obtained by different chromatographic methods are evaluated and active ones analysed by LC-MS to identify well known compounds (based on retention times and MS^n spectra, collaboration with MASSMET platform) and determine those which should be further purified (unidentified substances). The use of molecular networks will also help identifying known compounds. Structural identification is based on UV, IR, SM, 1D and 2DNMR spectra.

In addition to known compounds, we identified several new molecules which are found for the first time in plants. Among them, we can point out diterpenes isolated from *Croton zambesicus*. Some of these diterpenes have been shown by our team to possess cytotoxic and pro-apoptotic properties but others relax significantly rat aorta contracted by KCl. Comparison of the cytotoxic and vasorelaxant activities of isolated molecules and synthetic analogues indicates that both effects are not linked. We can also cite several promising specific antiparasitic terpenic derivatives isolated form *Keetia leucantha*, *Ocimum basilicum*, *Vitellaria paradoxa* or *Cymbopogon* species or essential oil components from Vietnamese plants.

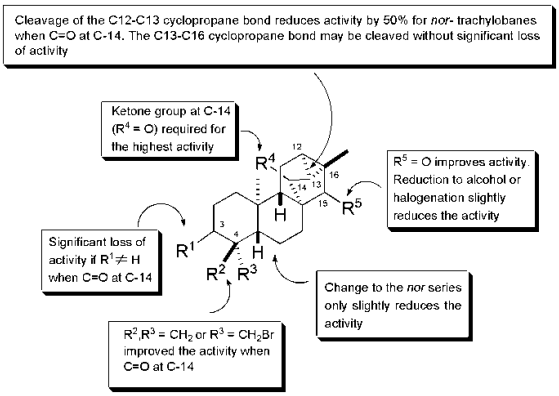
Identifications of antimalarial compounds is also guided by supervised metabolomics studies of crude extracts (collaboration with Prof. Choi, Leiden) while collaborations with LIST (Luxembourg Institute of Science and Technology, Dr André) allowed us to identify and produce potential bioactive compounds from in vitro plant cell cultures.

3/ IDENTIFICATION OF TARGET(S) AND STRUCTURE-ACTIVITY RELATIONSHIPS

Once structures are identified, we realise further experiments in collaboration with specialised teams to determine their targets and modes of actions and compare their activities with related natural or (semi)-synthetic compounds to assess structure-activity relationships.

We also analysed the possible targets for crude extracts. For example the activity of an extract of *Keetia leucantha* on different forms of trypanosomas showed a possible effect on glycolysis. We also proved the inhibiting effect of *Pterocarpus erinaceus* extracts on γ-secretase, an enzymatic complex responsible for A-Beta formation, and the effect of *Croton zambesicus* or *Marrubium vulgare* extracts on voltage dependent calcium channels.

Researches on pure isolated compounds allowed us to determine some structure-activity relationships for the vasorelaxant effect of trachylobanes diterpenes (collaboration with N. Morel, IREC). Targets were identified as voltage dependent calcium channels.



Structure-activity relationships for the vasorelaxant activity of trachylobanes

Alkaloids inhibiting topoisomerase I were identified in *Cassytha filiformis*. Synthetic derivatives were prepared in Spain and were also shown to possess antimalarial properties with a high selectivity index. Structure-activity relationships have been studied.

In the antiparasitic domain, we identified several antitrypanosomal terpenic compounds, some of them inhibiting trypanosomal GAPDH activity, a key enzyme of glycolysis, a process vital for trypanosoma development during its human cycle. We also collaborate with the teams of Prof. J. Palermo (University of Buenos Aires), Profs. J. Poupaert and R. Frédérick (LDRI-CMFA) and Profs. G. Acrombessi and F. Gbaguidi (UAC-Bénin) for the evaluation of the antiparasitic activities of (semi)synthetic compounds and establishment of structure-activity relationships. Some semi-synthetic compounds showed very promising antiplamsodial *in vitro* activity, in the same range as artemisinin.

The physico-chemical interactions of natural saponins with cholesterol and biological membranes were studied in collaboration with the team of M.P. Mingeot (TFAR-FACM/LDRI) and new results were obtained which could explain several activities of this class of compounds. We also analyse with Prof. Mingeot the interaction of terpenic

compounds with parasites membrane models.

4/ QUALITY CONTROL AND ANALYTICAL VALIDATED METHODS DEVELOPMENT

The last part of our research is to develop and validate analytical methods to identify and quantify natural compounds in complex media (crude extracts, cells, biological fluids…).

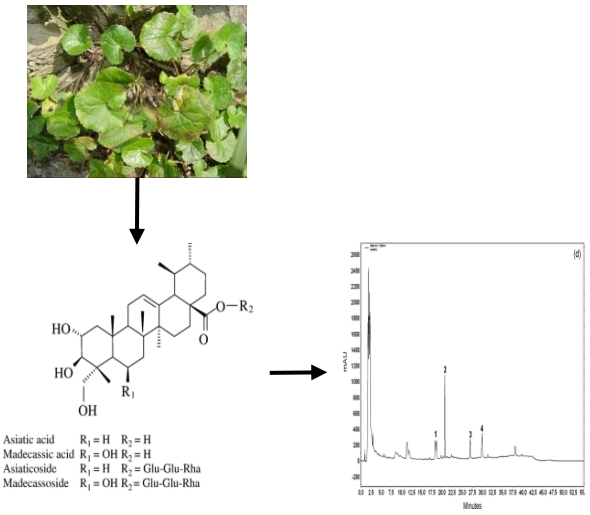
Analytical methods are useful:

- To control the quality of plant preparations

- To increase the yields and/or the quality of productions by studying the effects of growth/cultivating/harvesting conditions on the active molecules contents of plants.

- To analyse the mode of action, resorption and/or metabolisation of natural substances or derivatives

- To find methods to eliminate toxic compounds and find less toxic accessions.



Methods to identify by LC-MS and quantify several types of bio active molecules by GC-FID, GC-MS, LC-UV or LC-MS in crude extracts (particularly alkaloids, mono-, di-, triterpenes, steroids, rotenoids and flavonoids) were developed and validated in collaboration, for LC-MS, with MASSMET platform. We also developed a model to predict and analyse metabolic stability, identify metabolites from pure compounds and quantify anti-angiogenic hemi-synthethic products in blood.

The laboratory is also officially agreed (by the Federal Agency for Medicine and Health Products) for the quality control of drugs.

SELECTED PUBLICATIONS
Joëlle Quetin-Leclercq

Frere R.T., Bero J., Beaufay, C., Selegato A., Coquerio A., Choi Y.H., Quetin-Leclercq J. Identification of antiplasmodial triterpenes from *Keetia* species using NMR-based metabolic profiling. Metabolomics (2019) 15 (3), 27, 1-11

Beaufay C., Henry, G., Streel C., Bony E., Herent MF., Bero J., Quetin-Leclercq J. Optimization and validation of extraction and quantification methods of antimalarial triterpenic esters in *Keetia leucantha* plant and plasma. J. Chrom B (2019) 1104, 109-118

Le TB, Beaufay C, Pham T.A., Nghiem DT, Mingeot-Leclercq MP, Quetin-Leclercq J. Evaluation of the antitrypanosomal activity of Vietnamese essential oils, with emphasis on *Curcuma lonoga* L. and its components. Molecules. (2019) ;24(6),1158, 1-13

Beaufay C, Hérent MF, Quetin-Leclercq J, Bero J. *In vivo* anti-malarial activity and toxicity studies of triterpenic esters isolated form *Keetia leucantha* and crude extracts. Malar J. (2017) Oct 10; 16(1):406-413.

Catteau L., Reichmann NT, Olson,J. Pinho MG, Nizet V.,Van Bambeke F., Quetin-Leclercq J. Synergy between Ursolic and Oleanolic Acids from *Vitellaria paradoxa* Leaf Extract and β-Lactams against Methicillin-Resistant *Staphylococcus aureus*: In Vitro and In Vivo Activity and Underlying Mechanisms. Molecules. (2017) Dec 16;22(12).

Bero J., Beaufay C., Hannaert V., Hérent M.F., Michels P.A., Quetin-Leclercq J. Anti-trypanosomal compounds from the essential oil and extracts of *Keetia leucantha* leaves with inhibitor activity on Trypanosoma brucei glyceraldehyde-3-phosphate dehydrogenase. Phytomedicine (2013), 20: 270-274.

THESIS DEFENDED IN 2019

Le Than Binh: "Anti-parasitic activities of essential oil of plants used in traditional medicine in Vietnam and study of interaction of their constituents with membranes"**.**
Directors**:** Joëlle Quetin-Leclercq and Marie-Paule Mingeot

THESES IN PROGRESS

Hafiz Abdul Khaliq: Evaluation of the potential of Pakistan's plants used in traditional medicine for the treatment of inflammatory bowel diseases and identification of their active molecules
Directors: Joëlle Quetin-Leclercq ; Giulio Muccioli

Schioppa Laura: Study of the potential of triterpenic esters and derivatives as antiparasitic agents
Director: Joëlle Quetin-Leclercq

Toukourou Habib :** "Etude de l'innocuité et des potentialités d'huiles essentielles pour le traitement d'infections respiratoires".
Directors: Joëlle Quetin-Leclercq, Fernand Gbaguidi.

Malapert Anne-Sophie: "*Carpolobia lutea:* interest of molecular networks to identify active molecules from an antiparasitic African medicinal plant"
Director: Joëlle Quetin-Leclercq

Esaie Tchetan:** "Phytochemical studies and evaluation of the anthelmintic properties of extracts and molecules isolated from plants used in veterinary traditional medicine in Benin"
Directors: Joëlle Quetin-Leclercq, Fernand Gbaguidi, Pascal Olunlade

*** « Mixed » doctorate – Defensis at the UAC (Université d'Abomey-Calavi) –Experimental work done in part in Belgium*

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Our research group proposes an approach based on integrative physiology, metabolism and nutrition, to investigate the role of the gut microbiota in the development of metabolic and behavioral disorders associated with obesity and cardiometabolic risk, alcohol dependence, cancer development and cachexia. In collaborative projects, we also evaluate the implication of the gut microbiota in xenobiotic metabolism and wound healing.

We mostly focus on nutrients targeting the gut microbiota, such as carbohydrates which escape the digestion (e.g., prebiotics) plant-derived compounds (e.g., polyphenols) or lipids. We also isolate and characterize novel bacteria considered as next-generation beneficial microbes (e.g., Akkermansia muciniphila, Dysosmobacter welbionis).

Omics and targeted approaches are used for the evaluation of gut endocrine and barrier functions and repair (wound healing), endocannabinnoid system, organ (liver, muscle, brain, adipose tissue) dysfunctions.

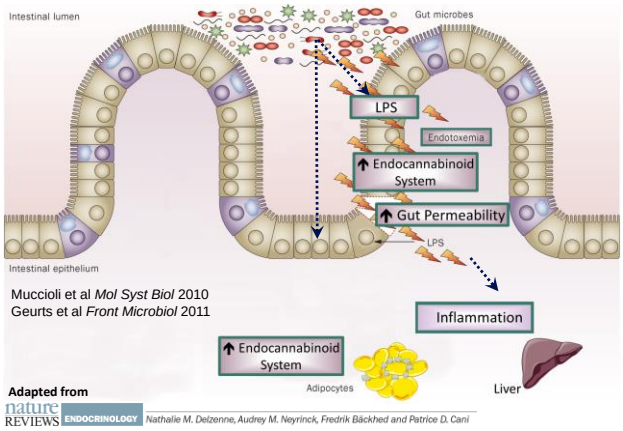
Experimental animal models (through genetic, pharmacologic, surgical or nutritional manipulation) and a panel of biomarkers and techniques have been developed in order to assess the molecular mechanism underlying the "metabolic bridge" built by the gut microbiota between the gastro-intestinal tract and key organs involved in the control of energy metabolism (brain, liver, adipose tissues, muscle).

On one side, specific *in vitro* models, such as "Precision-Cut Liver Slices (PCLS)" and mouse adipose explants, have been implemented to study the contribution of tissue-fixed macrophages and other cell types in the metabolic response to nutrients, drugs and microbial compounds. We also developed intestinal organoids and use reporter cell lines and genetic deletion in cancer cell lines to investigate the presence and role of key microbial-related proteins.

On the other side, the integrative physiology of the different metabolic systems (including the microbial one) is studied through *in vivo* experiments in live animals, using biochemical, surgical interventions, molecular, (meta)genomic and metabolomics approaches in biological fluids and tissues.

Finally, nutritional intervention studies and cohort studies are also performed in humans, in collaboration with colleagues at the St Luc University Hospital, University Hospital Gent and University Hospital Leuven, as well as with colleagues from abroad.

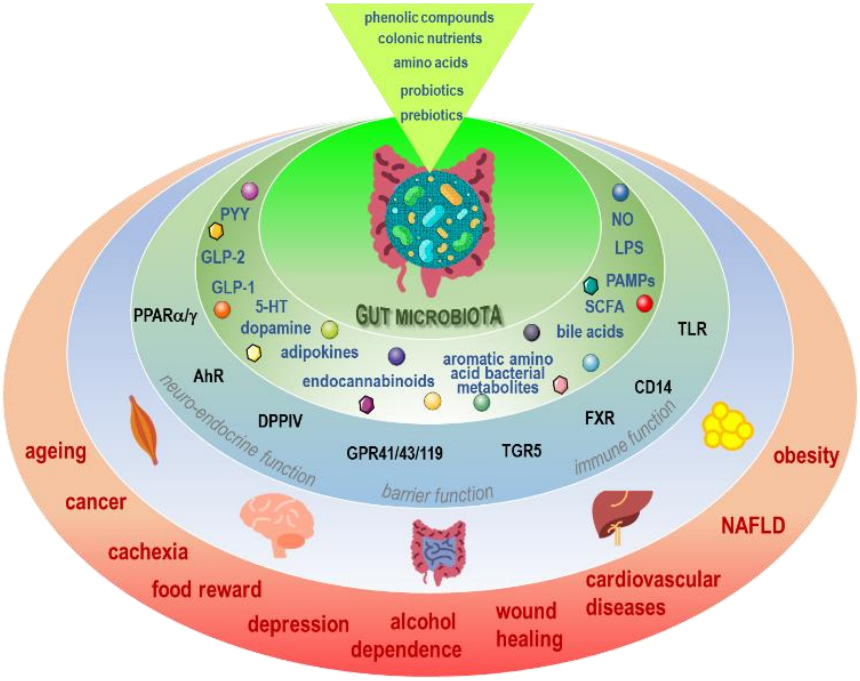
A decade ago, one of our breakthroughs has been the identification of the role of the endocannabinoid system and its interaction with the gut microbiota in the development of adipose tissue and metabolic inflammation associated with obesity, insulin resistance and type 2 diabetes.



To this aim, specific animal models of tissue specific (i.e., gut, liver, adipose), genetic deletions of genes involved in the host-bacteria interaction or in the synthesis of endocannabinoids have been or are currently developed and studied. In addition, both *in vivo* and *in vitro* models are proposed to analyse the modulation of metabolic, oxidative, and inflammatory stresses by nutrients, ingredients and/or pharmacological compounds.

1) CURRENT RESEARCH ACTIVITIES

1. to develop experimental models mimicking metabolic and behavioural disturbances occurring during obesity, cancer development, addiction;
2. to evaluate the implication and therapeutic interest of the gut microbiota and associated microbial metabolites in the occurrence of tmetabolic and behavioral disorders and the progression of cancer;
3. to investigate the role of the gut microbiota in the control of food intake, food reward, alcohol dependence, and depression;
4. to investigate the role of the endocannabinoid system and of specific receptors responding to gut microbial components or metabolites;
5. to decipher the role of the innate immune system in the development of obesity, inflammation, insulin resistance, oxidative stress, type 2 diabetes, hepatic steatosis, or behaviour in mice;
6. to evaluate the involvement of key gut functions (endocrine, immune, endothelial, barrier functions) alterations in the occurrence of behavioural and metabolic disorders associated to obesity, alcohol consumption and cancer progression.
7. to develop specific surgical interventions in mice and techniques of real-time imagery (i.e., mouse colonoscopy), in order to evaluate the role of the mucosal microbiota on wound healing.
8. to evaluate how drugs such as immunosuppressive agents can affect the gut microbiota and how conversely the gut microbiota can affect the pharmacokinetics and pharmacodynamics of such drugs.



Some of these metabolic alterations are modulated by the gut microbiota through specific bacteria-derived compounds such as pathogen-associated molecular patterns (PAMPs). Among them, we have identified the key role played by the lipopolysaccharides (LPS) in the onset of metabolic inflammation and glucose homeostasis disorders in the context of obesity and associated disorders, as well as in the inflammation linked to alcohol dependence in humans. The alteration of the gut barrier is one important cause of the translocation of bacterial elements (e.g., LPS, peptidoglycans) and metabolites which promote inflammation and metabolic disorders occurring in nutritional or behavioural disorders (diabetes and obesity, cancer cachexia, alcohol dependence…) (For reviews, Cani et al Nature Metabolism 2019; Delzenne et al Proc. Nutr. Soc. 2019; Cani Gut 2018 ; Bindels et al, Clin Nutr Exp 2016 ; Delzenne et al, Diabetologia 2015)

A link exists between the composition of the gut microbiota – that is profoundly modified in genetic (*ob/ob*) and dietary models of obesity – and the control of body weight, insulin secretion/response, inflammation and appetite. The gut microbiota may also be involved in the hepatic steatosis and vascular disorders induced by nutritional deficiency in essential polyunsaturated fatty acids, as well as in the occurrence of cachexia and inflammation linked to systemic cancer development. Non digestible carbohydrates such as inulin-type fructans are defined as prebiotics since they are highly fermented by certain bacterial species and thereby improve host health. We have tested the influence of several non-digestible dietary carbohydrates (e.g., fructans, cereal subfractions, and/or glucans derivatives, pectooligocaccharides…) and polyphenolic compounds on gut microbiota composition, activity and systemic metabolism.

Our experimental data suggest their potential to improve metabolic disorders associated with obesity. In rodents, changing the gut microbiota composition using fructans reduces food intake, improves glucose homeostasis and steatosis, and decreases fat mass development, these events being clearly related to the modulation of endogenous gut peptides production. Indeed, changing the microbiota with dietary prebiotics administration leads to an increase in the differentiation of stem cells into endocrine L cells in the proximal colon of rats, and therefore promotes the production of glucagon-like peptide-1 and 2 (GLP-1 and GLP-2) in this organ. The relevance of the GLP-1 in the improvement of metabolic disorders is shown through experiments performed in mice lacking functional GLP-1 receptor: those mice are resistant to the beneficial effect of fructans on obesity and glucose metabolism. In addition, the GLP-2 is known to improve gut barrier function, here we found that the endogenous production of GLP-2 is a key event responsible for the reduced gut permeability observed upon severe obesity and type 2 diabetes. High-throughput molecular analysis of bacterial 16S rRNA gene allowed to point out novel interesting bacteria (*Bifidobacteria*, *Akkermansia muciniphila*, *Roseburia* spp., *Lactobacillus* spp., …) or yeast (*Saccharomyces boulardii*) in the control of host metabolic status, adiposity and immunity.

2) OVERVIEW OF THE RECENT RESULTS

a) *In the context of cardiometabolic disorders*

Akkermansia muciniphila

In 2013, we have identified *Akkermansia muciniphila* as a key bacterium involved in the control of the gut barrier function and host metabolism (Everard et al PNAS 2013 and patents). We demonstrated that *A. muciniphila*, a mucin-degrading bacterium that resides in the mucus layer and abundantly colonizes it, negatively correlates with body weight and is decreased under high-fat diet. Moreover, daily administration of *A. muciniphila* to high-fat-diet-induced obese mice for 4 weeks improves metabolic profile, by decreasing weight gain, restoring mucus layer thickness, antimicrobial peptides production and counteracting metabolic endotoxemia and insulin resistance. We discovered that the fatty acids composition may also strongly contribute to the modulation of the abundance of *A. muciniphila*. We found that mice fed with a saturated fatty acid diet (lard-enriched diet) exhibited a significant decrease in *Akkermansia muciniphila*, whereas omega 3 fatty acids (fish oil-enriched diet) dramatically increased *Akkermansia muciniphila* in the gut. This effect was associated with a better gut barrier function and decreased adipose tissue inflammation, a phenomenon that can be transferred to germ-free recipient mice (Caesar et al. Cell Metabolism 2015).

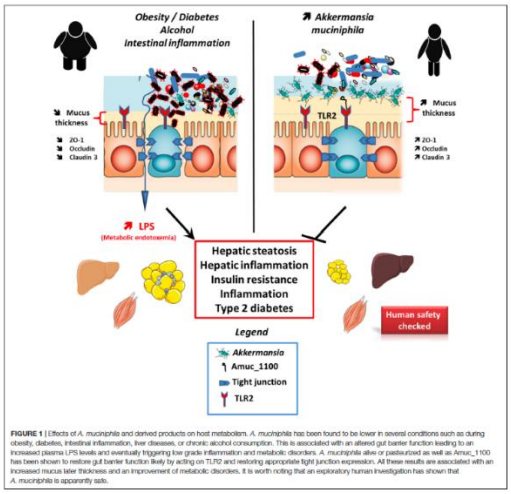
Besides obesity and diabetes, aging is also linked with *A. muciniphila* (Schneeberger et al Sci Reports 2015). Indeed, the intestinal levels of this bacterium declined with age upon a normal diet feeding. We found that high-fat diet feeding strongly influenced adipose tissue profile and intestinal microbiota in a way that mimicked aging, or at least older mice. In the same set of experiments, we found by using multifactorial analysis that these changes in *A. muciniphila* were robustly linked with the expression of lipid metabolism and inflammation markers in adipose tissue, as well as several blood markers (i.e., glucose, insulin, triglycerides, leptin) (Schneeberger et al Sci Reports 2015).

In accordance with the data obtained in rodents, we show in obese humans, that in the basal state, the abundance of *Akkermansia muciniphila* is inversely related to fasting plasma glucose levels, visceral fat accumulation, and adipocyte diameter in subcutaneous adipose tissue (Dao et al., 2016). More precisely, subjects with higher *Akkermansia muciniphila* abundance have a lower fasting glucose, triglycerides and lower body composition. In addition, upon caloric restriction, obese individuals with higher baseline *Akkermansia muciniphila* displayed greater improved insulin sensitivity markers and other cardiometabolic risk factors (Dao et al., GUT 2016), whereas upon gastric bypass *Akkermansia muciniphila* is dramatically increased (Dao et al. Am J Phyisol Endocrinol Metab. 2019 . Thus, all these data suggest that *A. muciniphila* is of interest **and merits further investigation in humans.** However, the classic growth requirements for the culture of *Akkermansia muciniphila* and its oxygen sensitivity render this bacterium unsuitable for human investigations and putative therapeutic opportunities. Therefore, the sensitivity of *Akkermansia muciniphila* to oxygen and the presence of animal-derived compounds in its growth medium currently limit the development of translational approaches for human medicine.

The team of Prof. Cani have contributed to solve these critical issues by developing synthetic medium compatible with human administration (collaboration with Prof. Willem de Vos). We demonstrated that *Akkermansia muciniphila* cultured on this media retains its efficacy (Plovier et al,

Nature Medicine 2017 and patent pending). Unexpectedly, pasteurizing *Akkermansia muciniphila* in order to stabilize the bacterium without destroying it, enhanced its capacity to reduce fat mass development, insulin resistance and dyslipidemia in mice.

These improvements were notably associated with a modulation of the host urinary metabolomics profile and higher intestinal energy excretion upon pasteurized *Akkermansia muciniphila* treatment. Then we wanted to understand why *Akkermansia muciniphila* behaved differently when live and pasteurized. By combining genomic and proteomic analyses of *Akkermansia muciniphila* our collaborators identified proteins encoded by a specific Type IV pili gene cluster in fractions enriched for outer membrane proteins (Plovier et al Nature Medicine 2017). Among these, Amuc_1100 was one of the most abundant. To test this hypothesis, we showed that a His-tagged Amuc_1100 produced in *E. coli* completely reproduced the beneficial effects of pasteurized *Akkermansia muciniphila* (see figure) (Plovier et al Nature Medicine 2017 and patents pending). Interestingly, this protein also remains active after heating to 70°C (pasteurization). In a molecular point of view, we demonstrated that Amuc_1100 interacts with TLR-2 and improves the gut barrier (see figure).



Patrice D. Cani and Willem M. de Vos, Frontiers in Microbiology 2017

Finally, we also developed the production of *Akkermansia muciniphila* at a large scale in order to test the safety and efficacy of the bacterium on parameters associated with cardiometabolic risks factors. The study Microbes4U© has been published in July 2019. The major aim was to evaluate the safety and tolerability of *Akkermansia muciniphila* in individuals with excess body weight by supplementing them with different doses of live *Akkermansia muciniphila* (Akk Synthetic - 10^{10}) or pasteurized *Akkermansia muciniphila* (Akk Pasteurized - 10^{10}). This study published in Nature Medicine (Depommier et al. 2019), showed that administration of live or pasteurized bacteria grown on the synthetic medium is safe in humans and also improves numerous cardiometabolic risks factors, including insulin sensitivity, insulinemia, inflammation, liver enzymes, cholesterol but also markers of reinforced gut barrier (Depommier et al. Nature Medicine 2019).

These findings provide support for the use of different preparations of *A. muciniphila* as dietary supplements to target human cardiometabolic risk factors associated with obesity. Based on all these results Prof. Cani has co-founded the spinoff company "A-Manisa Biotech SA" in 2016 and accomplished in 2018 a series A capitalizing A-Mansia at 22 Million euros. The company is devoted to develop a food supplement and a drug based on *Akkermansia* and on other derived compounds.

Besides *Akkermansia muciniphila* Prof. Cani and his team have isolated several novel bacteria including one novel genus/species/strain. The bacterium is called *Dysosmobacter welbionis* in reference to the project WELBIO which is supporting this innovative research since 2012 (Le Roy et al IJSEM 2019, and patent pending). The metabolic effects of these novel bacteria are currently under investigation in the laboratory.

The innate immunity

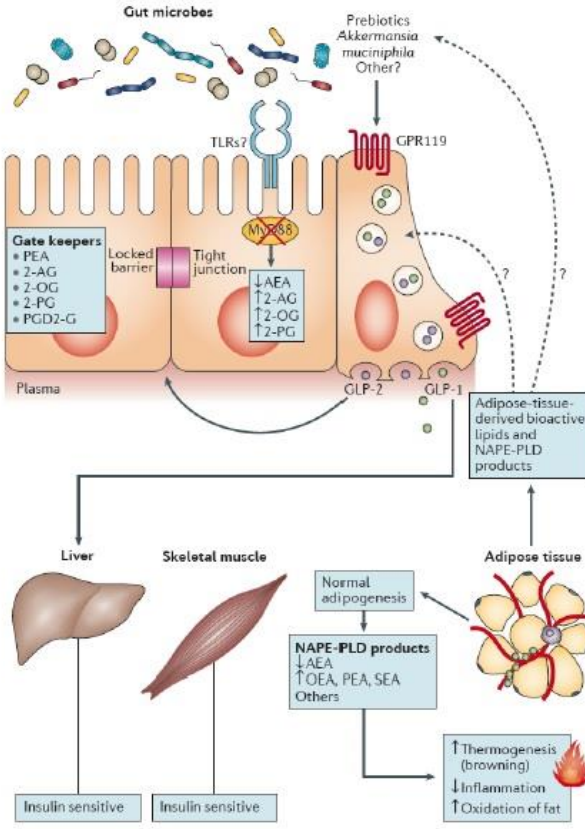
In 2014, we found that a link between the innate immune system from intestinal cells (i.e., the protein MyD88) and energy homeostasis. More precisely, we found that modifying the response of the immune system by deactivating the protein MyD88 in the intestinal cells delay the development of type 2 diabetes induced by a high fat diet, reduces the development of fat mass, reduces the deleterious inflammation observed during obesity and reinforced the gut barrier thereby preventing the leakage of unsuitable bacterial compounds from the intestine to the organism. More importantly, we found that it is experimentally possible, through this modification of the immune system, to induce body weight loss and therefore to have a therapeutic effect despite the fact that the animals were already obese and diabetic. Surprisingly, we found that it is possible to partially protects against obesity and diabetes by transferring (i.e., grafting) the gut microbiota from these mice to axenic mice (i.e., germ free) (Everard et al. Nature Communications 2014). By investigating the role of Myd88 deletion in the hepatocyte and host metabolism, we discovered that hepatic MyD88 is a key factor controlling the onset of glucose intolerance and liver inflammation (Duparc et al, GUT 2017). In a second study, we found that hepatic Myd88 is a key actor controlling the synthesis of different biacotive lipids such as oxysterols and eventually controls the endogenous production of bile acids and related factors (Lefort et al. Am J Physiol Endocrinol Metab 2019).

The endocannabibnoid system

We have previously identified that the endocannabinoid system links the gut microbiota to adipogenesis in both physiological and pathological situations such as obesity and type 2 diabetes. Our data pointed out that targeting specifically the endocannabinoid system tone in the adipose tissue may contribute to change host-microbiota interactions (for review Cani et al Nature Reviews Endocrinology 2016). In 2015, we published data showing that deleting *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) in the adipose tissue (tissue specific deletion) induces obesity in normal diet-fed mice by promoting fat mass development, insulin resistance and inflammation. This key enzyme is involved in anandamide and NAE biosynthesis. We discovered that the deletion of NAPE-PLD in adipocytes induces also a decreased thermogenic programme (i.e., browning/beiging) in adipose tissue. Importantly, we found that NAPE-PLD deletion in adipose tissue induced a profound shift in the gut microbiota composition and activity. By transferring the microbiota from mice in which the adipose tissue NAPE-PLD was deleted into germ-free recipient mice replicated the overall phenotype (Geurts et al. Nature Communications 2015).

Actually, we discovered that mice harbouring and inducible intestinal epithelial cell (IEC)-specific deletion of NAPE-PLD ($Napepld^{\Delta IEC}$) were hyperphagic upon first exposure to a high-fat diet and developed exacerbated diet-induced obesity and hepatic steatosis. Among the mechanisms, we found that these mice displayed a defect in hypothalamic Pomc neurons and alterations in intestinal and plasma NAE and 2-acylglycerols. After long-term HFD exposure, $Napepld^{\Delta IEC}$ mice presented a lower energy expenditure. The increased hepatic lipid storage was associated with higher lipid absorption in the gut and in the liver. Moreover, $Napepld^{\Delta IEC}$ mice displayed altered gut microbiota composition. Strikingly, treatment with *Akkermansia muciniphila*, a bacterium influencing NAE, endocannabinoids and related mediators, partly counteracted the effects of the deletion. These results suggest that intestinal NAPE-PLD is a key sensor in nutritional adaptation to fat intake, gut to brain axis and energy homeostasis and thereby constitute a novel target to tackle obesity and related disorders (Everard*, Plovier*, Rastelli* et al Nature Communications 2019).

Taken together, these findings indicate that bioactive lipids produced by the NAPE-PLD contribute to changes in the gut microbiota even at distance of the organ targeted (e.g., the adipose tissue). These changes then participate in the altered metabolic disorders observed following NAPE-PLD deletion. These results provide strong support for the crosstalk between the gut microbiota and the endocannabinoid system as a potent mediator.



Patrice D. Cani, Hubert Plovier, Matthias Van Hul, Lucie Geurts, Nathalie M. Delzenne, Céline Druart and Amandine Everard. Nature Reviews Endocrinology 2016

Innovation in prebiotic effects

Recently, several prebiotics have been tested in original mice models of endothelial dysfunction and gluten-induced obesity. Those data revealed that the improvement of the endothelial dysfunction by fructans and chitin glucans is associated with specific changes in microbiota and increased intestinal production of nitric oxide release. Among microbial metabolites, change in bile acid profiling by inulin-type fructans support their potent contribution to the improvement of gut endocrine and vascular functions (Catry et al Gut 2018, Neyrinck et al Sci Report 2019). Arabinoxylo- and fructo-oligosaccharides were able to improve gluten induced obesity and metabolic disorders, by driving intestinal and microbial gluten cleavage (Olivares et al Mol Nutr Food Res 2019). These data confirm that behind the effect of prebiotics in the caeco-colon, those nutrients are also able to change the digestion of other nutrients in the upper part of the gut, as we have previously shown for dietary lipids and disaccharides (Suriano, Bindels et al Sci Report 2017; Neyrinck et al, J Functional Food 2018; Hiel et al Nutrient 2018). (In the context of a European project (MyNewGut, http://www.mynewgut.eu/), we have also highlighted a potential interest of amino-acid microbial metabolites to counteract hepatic inflammation and of microbial conjugated polyunsaturated fatty acids to improve lipid metabolism (Beaumont et al FASEB J 2018, Pachikian et al Plos One 2018, Delzenne et al Clin Nutr and Proc Nutr Soc 2019), thereby extending the concept of prebiotics and related bioactives.

Other classes of food products have been evaluated in term of microbiota modulation in preclinical models of nutritional disorders. Among them, green tea - rhubarb- curcuma- or pomegranate- extracts, as well as spirulina counteract inflammation associated with nutritional disorders. We have also collaborated to the demonstration of the interest of milk polar lipids in obesity

(Millard et al Mol Nutr Food Res 2019). Some effects (those of green tea) seem independent on changes in microbiota composition, whereas we could point out interesting bacterial metabolites produced from phenolic compounds, as potential drivers of anti-inflammatory effect (Neyrinck et al Plos One 2013, Mol Nutr Food Res 2017, J Nutr Biochem 2017, Nutrients 2017).

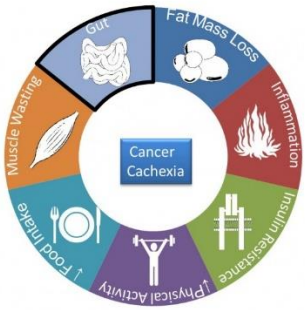
Our current projects will evaluate – by using untargeted and targeted metabolomics- the relevance of microbial cometabolites in the changes in behavior (depression, social behavior) in the models of mice transferred with the gut microbiota from obese or alcohol-dependent human volunteers.

In 2019, we also published the first data related to inulin-intervention studies programmed in the context of the Food4gut project (www.food4gut). We have demonstrated in a food-based intervention in healthy volunteers that daily eating of locally produced- inulin rich vegetables was able to exert specific and reversible changes in the gut microbiota, and modulates food-related behavior, leading namely to a decrease in envy to eat sugar or fat (Hiel et al Am J Clin Nutr 2019). This fits with the observation of the contribution of inulin to the improvement of sweet taste perception in mice (Bernard et al Nutrients 2019).

b) In the context of cancer cachexia

Our research team focusses on the development of new therapeutic pharmacological and nutritional tools based on the gut microbiota-host crosstalk in several pathological contexts. Our work highlights the importance of the gut and its microbes to target cancer cachexia, paving the way to new therapeutic opportunities (Bindels & Thissen, Clin Nutr Exp 2016; Pötgens et al, Curr Opin Nutr Metab Care 2018). Our work has clearly led to a better consideration of the potential of the gut microbiota in cancer cachexia (Argiles et al, Nat Rev Endocrinol 2018; Dzutsev et al, Annu Rev Immunol 2017).

Cancer cachexia is a complex multi-organ syndrome characterized by body weight loss, weakness, muscle atrophy and fat depletion (Fearon et al, Lancet Oncol 2011). Importantly, fat depletion may precedes muscle wasting in cancer cachexia and preserving fat mass can spare muscle mass (Das et al, Science 2011). Paradoxically, accumulation of ectopic fat in the liver was found in rodent models of cancer cachexia and in cachectic patients (Berriel et al, Hepatology 2008). Clinically, cachexia results in increased morbidity and mortality rates as well as reduced tolerance to anti-cancer treatments (Farkas et al, JCSM 2013). Currently, limited therapeutic options exist for this important medical challenge and new approaches to tackle this syndrome, including innovative and scientifically relevant nutritional and pharmacological tools, are needed (Fearon et al, Nat Rev Clin Oncol 2013). In this context, targeting the gut microbiota represents an exciting opportunity for this public health issue.



Links between gut microbiota and cancer have been studied for years (Schwabe & Jobin, Nat Rev Cancer 2013). Our research over the last ten years has evidenced the existence of a crosstalk between the gut, the microbes its harbors and metabolic alterations occurring during cancer.

First, we showed in 2012 that restoring the lactobacilli levels through the administration of lactobacilli counteracted muscle atrophy

and decreased systemic inflammation in a mouse model of leukemia and cachexia (Bindels et al, Plos ONE 2011).

Second, we highlighted a common microbial signature (characterized mainly by an increase in Enterobacteriaceae) in preclinical models of cancer cachexia, in strong association with some cachectic features (Bindels et al, Plos ONE 2015; Bindels et al, The ISME J 2016. This microbial signature was not due to the anorexia observed in the last stage of the disease (Bindels et al, Plos ONE 2011; Bindels et al, The ISME J 2016. More recently, we have highlighted that *Klebsiella oxytoca* was the Enterobacteriaceae species that was fostered in cancer cachexia. We evidenced a mechanism of emergence for this bacteria similar to the one described for the bloom of Enterobacteriaceae during antibiotics consumption. This framework includes a reduction in Treg cells in the intestine, together with a glycolytic switch and a host-derived production of nitrate (Pötgens et al, Sci Rep 2018).

Third, we found drastic changes in the gut permeability and intestinal morphology of cachectic mice. Such changes were strongly correlated with the cachectic features. These alterations occurred independently of anorexia and were driven by interleukin 6. Gut dysfunction was found to be resistant to treatments with an anti-inflammatory bacterium (*Faecalibacterium prausnitzii*) or with gut peptides involved in intestinal cell renewal (teduglutide, a glucagon-like peptide 2 analogue) (Bindels et al, Oncotarget 2018). We also demonstrated that *K. oxytoca* behaves as a gut pathobiont contributing to intestinal dysfunction in cachectic mice (Pötgens et al, Sci Rep 208).

Last but not least, we reported several times that nutritional interventions targeting the microbiota, such as prebiotics or probiotics, decreased cancer progression, reduced morbidity and fat mass loss, and/or increased survival of cachectic mice with leukemia (Bindels et al, the ISME J 2016; Bindels et al, Plos ONE 2015; Bindels et al, Br J Cancer 2013). Our data highlight propionate, a short-chain fatty acid produced through the fermentation of prebiotics, as a potential mediator of this anti-cancer effect observed in leukemic mice with cachexia. Indeed, administration of inulin-type fructans (a well-known prebiotic) increased portal levels of propionate which is able to control the proliferation of leukemic cells (Bindels et al, Br J Cancer 2013). We investigated in this context the potential implication of FFAR2, a G-protein-coupled receptor which binds propionate and whose activation reduces cancer cell proliferation (Bindels et al, Br J Cancer 2013; Bindels, Dewulf & Delzenne, Trends Pharmacol 2013). Among others, our work indicates that a modulation of Ffar2 expression through nutritional microbiota-targeting tools may constitute an attractive therapeutic approach to tackle leukaemia progression in humans (Bindels et al, Br J Cancer 2017).

Altogether, our studies reveal a previously unexpected link between cancer, cachexia and the gut microbiota. However, the exact mechanisms underlying this crosstalk remain elusive and constitute the topic of research of the newly established team of Prof Bindels. To achieve such goal, her team is using targeted and untargeted metabolomics analyses (recent implementation of H^1-NMR metabolomics) using the NEST and MASSMET platforms. These data will be integrated with targeted microbial metagenomics and transcriptomics to highlight new pathways involved in this crosstalk.

c) *In the context of food intake and food reward*

Food intake, appetite and satiety are mainly integrated at the level of hypothalamic neuronal circuits. Importantly, energy balance is also controlled by hedonic/reward brain systems encoded by the neuronal network of the mesolimbic dopaminergic system. Hedonic properties of food stimulate feeding and some food substances (e.g., sugars, sweeteners, salt, and lipids) are more prompt to be involved in these addictive processes. These effects are mediated by abrupt dopamine increases in the brain reward system. This mesocorticolimbic system encodes for the three psychological component of reward: liking, wanting and learning.

During obesity, this gut-to-brain axis is altered at the level of the hedonic responses to food intake, leading to an abnormal increase in energy consumption. Moreover, the concept of the implication of the gut microbiota in the gut-to-brain axis to control food intake emerged over these last years, however the mechanisms still remain incompletely known and the roles of the gut microbiota in the regulation of hedonic/reward aspects of food intake are completely unknown.

Therefore, it is of utmost importance to fill in this gap to better understand the alterations of the gut-to-brain axis to control food intake during obesity and the implication of the gut microbiota in that context.

The originality of this work is to investigate how gut microbes are able to control hedonic and reward system in healthy conditions as well as in the physiopathology of obesity.

In order to proof a causal link between gut microbiota and alterations of hedonic response to food intake associated with obesity, we use gut microbiota transplantation. Preliminary data suggest that transferring the gut microbiota from high-fat diet-induced obese mice into control diet fed mice is enough to alter the dopaminergic signalling in the striatum of the mice in a similar way to alterations observed during obesity such as reduction of D2 receptor. Moreover, these alterations of dopaminergic signalling are associated with alteration of psychological component of reward such as liking. Indeed mice transplanted with the gut microbiota from high-fat diet-induced obese mice present a reduction of the high-fat high-sugar diet consumption in comparison to mice transplanted with the gut microbiota from control fed mice. Altogether these data suggest for the first time the implication of the gut microbiota into the alteration of hedonic regulation of food intake during obesity. These preliminary data need to be confirmed and we will investigate the mechanisms involved in these interactions between the gut microbiota and the hedonic regulation of food intake during obesity.

d) *Clinical trials*

Clinical trials are essential to evaluate the translational potential of our findings issued from preclinical models. For this reason, several clinical trials have been launched by the MNUT team in close collaboration with clinicians, whose names and affiliations are details on the related websites. Three of them are described below: the **MicroAML**, the **FOOD4GUT**, the **FIBERTAG**, the **Gut2Brain_ClinX** and the **Microbes4U©** studies. MNUT PI are also involved as collaborators for other international studies. To evaluate the translational value of the experimental work linking the gut microbiota to cancer cachexia, we have recently launched the **MicroAML study**. The MicroAML study aims to evaluate the composition and activity of the gut microbiota in patients with acute myeloid leukemia. Information related to appetite, food habits, body composition and muscle strength as well as biological samples are collected before any chemotherapy (additional details available online at https://uclouvain.be/fr/node/43424). First results are expected for 2020.

FOOD4GUT is a multidisciplinary and inter-university project that aims to demonstrate that changing the microbiota via supplementation with inulin-type fructans accompanied by dietary advice promoting the consumption of vegetables rich in these colonic nutrients, may modulate obesity. We have recently shown that dietary intervention with vegetables naturally rich in fructans for two weeks can selectively modulate the gut microbiota and improved appetite and food intake behaviour (Hiel, Bindels et al Am J Clin Nutr 2019). A simple-blind placebo-controlled randomized multi-center trial has been conducted to highlight the interest for the health of vegetables in inulin-type fructans in obese adults. More information is available on the website of the FOOD4GUT project (Food4Gut project, lead by N. Delzenne, clinical coordination UCLouvain J.P. Thissen - https://sites.uclouvain.be/FOOD4GUT).

Our data obtained in alcohol dependent patients allowed to point out that the gut microbiota characteristics (and related gut barrier) are factors driving the depression and related behaviour in alcohol-dependent patients (Leclercq et al PNAS 2014). An intervention study (**Gut2Brain_ClinX** study) with inulin-type fructans has been initiated in 2018 in a cohort of alcohol-dependent patients of St Luc Hospital (under the supervision of Ph de Timary and P. Starkel), in order to try modulating the gut microbiota to counteract gut dysbiosis and related social and mental disorders. The intervention study has been completed in December 2019.

The **FiberTAG** project aims at establishing a set of biomarkers linking dietary fiber (DF) intake and gut-microbiota related health effect, by using existing cohorts of healthy or overweight populations, as well as in intervention studies with dietary fibers (chitin glutan), programmed in healthy volunteers and in patients at risk for cardiometabolic health.

The **Microbes4U** study investigates the effects of an oral administration of Akkermansia muciniphila on metabolic disorders associated with overweight and obesity. After the inclusion according to specific criteria, the volunteers were randomly assigned into 4 different groups: one placebo group, two others groups receiving live ***Akkermansia muciniphila*** 10^9 cells/day or 10^{10} cells/day and the last group receiving pasteurized ***Akkermansia muciniphila***. The supplementation lasts for 3 months. Different parameters were recorded such as anthropometric parameters, lipid and glucose metabolic markers, and inflammation, the study is now published in Depommier et al. Nature Medicine 2019.

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Patrice D. CANI

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AWARDS 2018-2019

Laure Bindels
Pharmabiotics Young Investigators Award 2018

Sarah Pötgens
Best oral communication Award at the 9th annual meeting of the Belgian Nutrition Society 2019

Patrice D. Cani, Nathalie M. Delzenne, Amandine Everard, Audrey Neyrinck,
Highly cited researcher 2019, Clarivate analytics Web of Sciences

Nathalie M Delzenne: Sir Cuthbertson Lecture- selected upon outstanding contribution in nutrition research- ESPEN 2019.

Audrey Neyrinck
Prize of the Belgian Society of Clinical Nutrition 2019 (the best abstract selected at ESPEN 2018)

Patrice D. Cani
Prize Paris MATCH of the National TV RTBF "Matière grise) for "The ability of popularization Science work"

Patrice D. Cani
Award for Excellence in Biomedical Research and Creativity from the joint scientific committee of the Academies of Medicine of Belgium and France, and the Council of the JA DeSève Research Chair

THESIS DEFENDED IN 2019

Sophie Hiel: "Interest of native inulin in the modulation of dysbiosis and metabolic alterations: experimental approach and nutritional interventions in humans".
Director: Nathalie M. Delzenne and Jean-Paul Thissen

THESES IN PROGRESS

Clara Depommier: "Evaluation of the impact of Akkermansia muciniphila on the metabolic syndrome: pre-clinical and clinical investigations".
Directors: Patrice D. Cani and Amandine Everard

Charlotte Lefort: "Interactions between the hepatic endocannabinoid system and gut microbes: impact on the metabolic syndrome."
Director: Patrice D. Cani.

Marialetizia Rastelli: "Gut to brain axis and energy metabolism: impact of the gut microbiota and intestinal bioactive lipids".
Director: Patrice D. Cani.

Florian Gourgue: "Obesity and breast cancer, role of the adipokine apelin in the tumor progression and response to anti-cancer therapy."
Directors: Bénédicte Jordan and Patrice D. Cani.

Radu Bachmann: "Impact of the gut microbiota on colorectal surgery."
Directors: Patrice D. Cani and Alex Kartheuser

Sarah Pötgens: "Using NMR metabolomics to unravel the pathways underlying the gut microbiota-host crosstalk in cancer cachexia".
Director: Laure B. Bindels

Morgane Thibaut: "Evaluation of the role of bile acids in cancer cachexia".
Director: Laure B. Bindels

Alexandra Degraeve: "Tacrolimus pharmacokinetic pathway and microbiota: study of the complex bidirectional partnership for explaining metabolic variability and modulations".
Directors: Laure Elens and Laure B. Bindels

Justine Gillard: "Role of bile acids in the pathogenesis of the non-alcoholic steatohepatitis in foz/foz mice".
Directors: Isabelle Leclercq and Laure B. Bindels

Camille Amadieu: "Gut brain interactions in the context of alcohol-dependence"
Directors: Nathalie M. Delzenne, Philippe de Timary, and Sophie Leclercq

Caner Yalek: «Impact of bioactive lipids on tumor cell metabolism and cancer progression: novel insight from the gut microbiota"
Directors: Bénédicte Jordan and Patrice D. Cani.

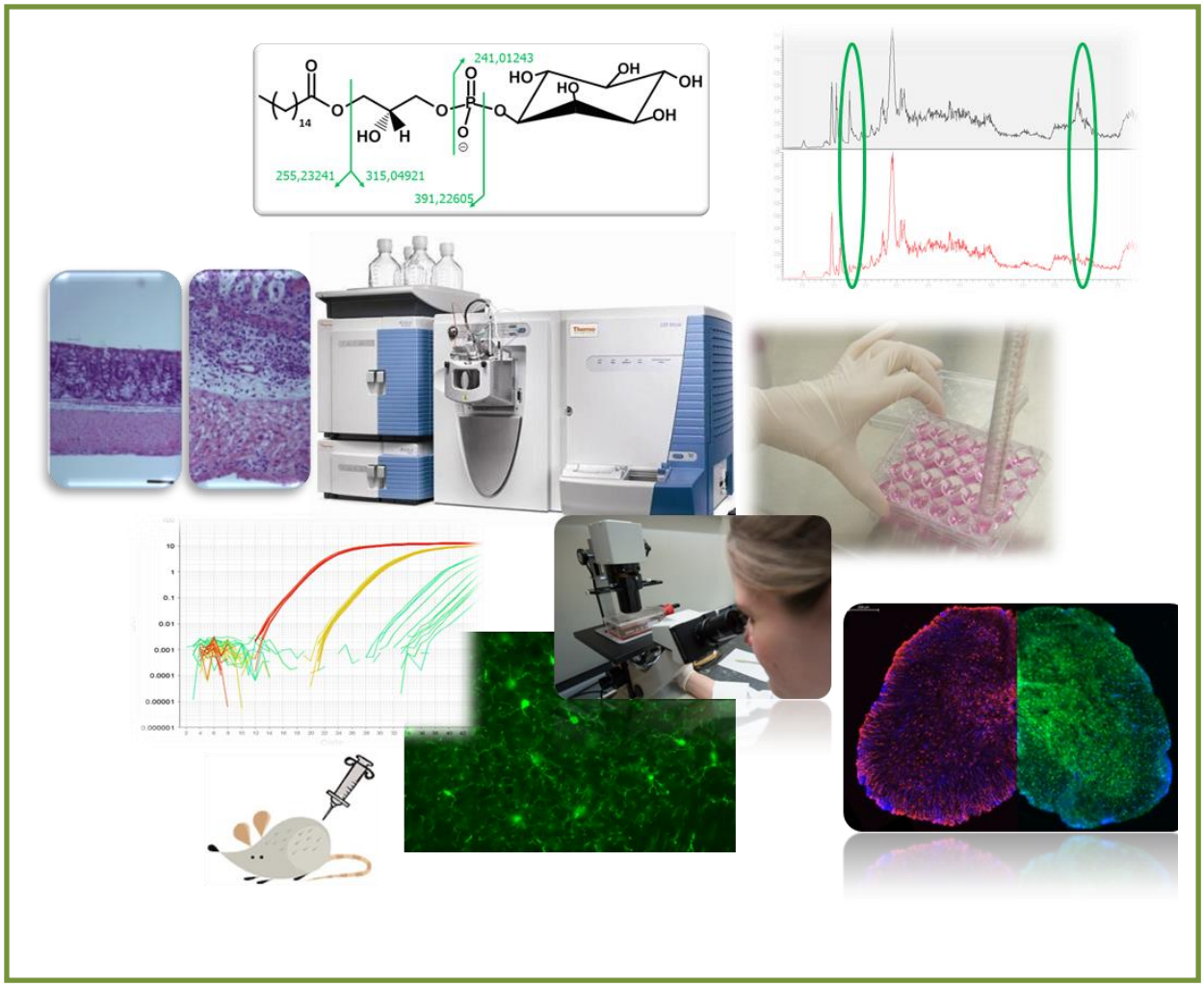
Alice de Wouters: « Roles of gut microbes in the gut-to-brain axis controlling hedonic/reward responses to food intake in physiological condition and in the pathology of obesity. »
Directors: Amandine Everard and Patrice D. Cani

Paola Paone: **"**Studying and modulating mucosal-gut microbiota interface: impact on the pathophysiology of obesity, diabetes and cardiometabolic disorders**."**
Director: Patrice D. Cani

Emilie Moens de Hase: **"**Investigation of the effects of newly discovered bacteria Dysosmobacter welbionis on metabolism and inflammation**."**
Director: Patrice D. Cani

Philippe Stevens: **"**Microbiome and tumor immune micro-environment: characterization and interaction during progression and treatment of advanced and metastatic colorectal cancer**."**
Directors: Marc Van den Eynde and Patrice D. Cani

Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)



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Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)

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Our group is interested in understanding the roles of lipid mediators both in physiological and pathological situations mainly related to inflammation.

*Lipids are essential constituents of biological membranes and control numerous cellular activities. An increasing number of lipids are shown to possess biological activities, thus behaving as transmitters or mediators. A large proportion of these "**bioactive lipids**" act by binding to and activating their own receptors, and have their levels tightly regulated by specific enzymes. The endocannabinoids and the oxysterols, two of our major research interests, are prime examples of such bioactive lipid signaling systems.*

We investigate the role of bioactive lipids (1) by setting up mass spectrometry-based methods allowing the quantification of their endogenous levels and (2) by interrogating the role of selected bioactive lipids in cellular and in-vivo models of inflammation-related diseases.

The overall aim of the group is to identify novel lipid-related therapeutic targets amenable to pharmacological modulation.

1) OVERVIEW

Bioactive lipids are important molecular mediators in inflammatory settings. As inflammation, and especially chronic inflammation, are important drivers of many chronic disorders, investigating the role of lipid mediators in inflammation could have a strong impact. Thus, our group aims to identify novel lipid mediators and lipid-related targets (i.e. receptors and enzymes) in inflammatory settings. Bioactive lipids are selected either based on reported effects or following their identification in lipidomics studies performed in our laboratory. The effects of these bioactive lipids are assessed, in vitro, ex vivo and in vivo, to determine their potential impact on inflammation (*Figure 1*). Once interesting lipids are selected and their effects identified, we turn to the identification of potential means to control their effects in vivo, for example by using agonists or antagonists of their receptors, or interfering with their metabolic pathways using pharmacological tools. A key aspect of our research strategy is to integrate the information gathered by quantifying the lipids and the information obtained by assessing their effects in our models. Over the years, this strategy allowed us to put forth several lipids and enzymes as important mediators of inflammation.

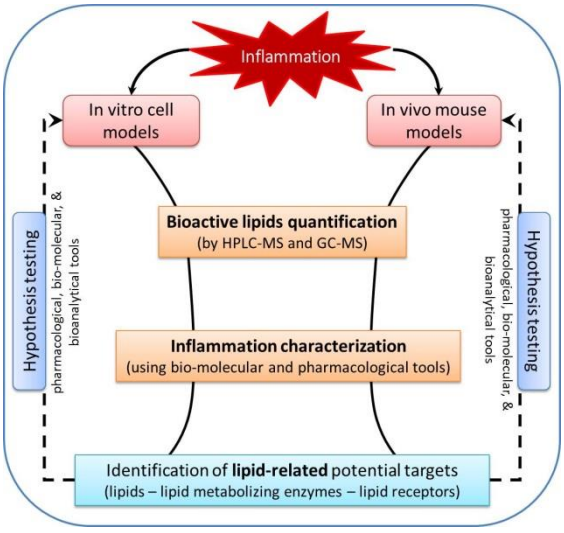


Figure 1. BPBL research group's strategy overview

a) Lipid quantification methods

The biological activities of most lipid mediators are controlled by the balance between their production and degradation. Because most lipids have multiple metabolic pathways, measuring the expression or activities of the enzymes involved is often not enough to appreciate fully the overall activity of a lipid system. It is therefore

crucial to quantify their endogenous levels. Thus, our group develops analytical methods to help understand the involvement of bioactive lipids in pathophysiological settings. We routinely use HPLC-MS methods allowing the relative quantification of a large number of lipids in a single run using an LTQ-Orbitrap (e.g. Guillemot-Legris et al. J. Neuroinfl., 2016). We also use validated methods for the absolute quantification of lipids of particular interest in our work (e.g. oxysterols, bile acids, endocannabinoids, …). (Mutemberezi et al. Anal Bioanal Chem, 2016), (Masquelier et al. J Pharm Biomed Anal., 2016). Moreover, using a Xevo-TQS tandem quadrupole, we are developing quantification methods for challenging lipids due to their low abundance, as well as more general methods allowing the quantification of phospholipids and lysophospholipds. Indeed the latter will be useful to quantify "bioactive" lysophospholipid species but also to characterize the changes in cell membrane composition.

b) Inflammation models

Over the years, we have implemented in our laboratory several in vivo and in vitro models to help us study inflammation. We developed a recognized expertise in studying colon inflammation using models of inflammatory bowel diseases (IBD). These acute (e.g. DSS, TNBS, oxazolone) and chronic (e.g. cycles of DSS) models allow us to study the effect of modulating bioactive lipid levels on the evolution of colitis (e.g. Alhouayek et al. FASEB J 2011; Alhouayek et al. FASEB J 2015; Guillemot-Legris et al. J. Crohns Colitis 2019). Other examples of models currently used in our research group are the models of lung inflammation (LPS-induced inflammation, house dust mite-induced inflammation), of multiple sclerosis (EAE model in mice), and of inflammatory pain (carrageenan, LPS, capsaicin) (e.g. Bottemanne et al. FASEB J 2019; Buisseret et al. BBA Mol Cell Biol Lipids 2019; Mutemeberezi et al. J. Neuroinflammation 2018). Besides the in vivo models, we rely also on in vitro models such as primary macrophages (alveolar and peritoneal) and neutrophils, primary glial cells, as well as tissue explants. In addition, our expanding network of clinical collaborations helps us improve the translational potential of our findings.

2) RESEARCH RESULTS

a) Endocannabinoids and related lipids

We and others have shown that several endocannabinoids and related lipids play an important role in inflammation.

We have shown that increasing 2-AG levels via MAGL inhibition reduces colitis in a partially CB_1- and CB_2-dependent manner (Alhouayek et al. FASEB J., 2011). We also showed that inhibition of ABHD6 increases 2-AG levels in some tissues, and has pronounced anti-inflammatory effects in vivo (Alhouayek et al. PNAS, 2013). We also demonstrated in vitro that ABHD6 inhibition in activated macrophages allows for the production of PGD_2-G, a bioactive lipid derived from 2-AG (*see Figure 2*), that has potent anti-inflammatory effects (Alhouayek et al. PNAS, 2013).

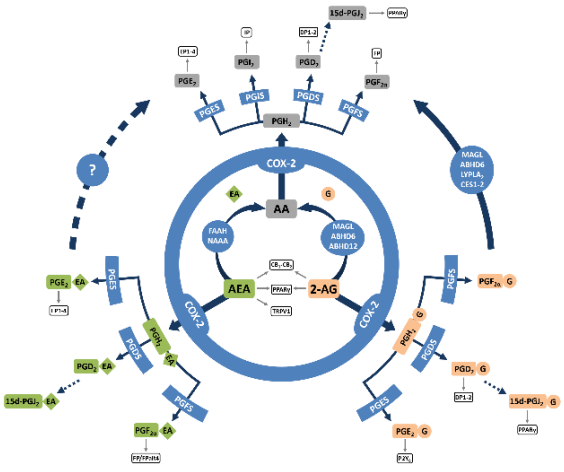


Figure 2: The two main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are part of a large network of bioactive lipids (Buisseret et al., Trends Mol. Med., 2019).

Based on our previous results, we recently investigated the effect of ABHD6 inhibition in the context of acute lung inflammation (ALI).

We showed that the ABHD6 inhibitor WWL70 was able to strongly decrease all the hallmarks of lung inflammation (including neutrophil infiltration, cytokine secretion, and protein extravasation) induced by intratracheal administration of LPS, a model of acute lung injury. As macrophages and neutrophils are key cells in ALI, we also studied ABHD6 inhibition on primary alveolar macrophages and neutrophils to explore their potential implication in the effects observed in vivo. (Bottemanne et al. FASEB J, 2019).

Demonstrating that PGD_2-G has anti-inflammatory properties (Alhouayek et al. PNAS, 2013) opened several research projects in our research group. For instance we recently reported that administration of PGD_2-G to mice having DSS-induced colitis allows for a strong reduction of the major hallmarks of the disease. Moreover, we could put forth the DP1 receptor as one of the receptors mediating the effects of PGD_2-G in the colon (Alhouayek et al. FASEB J., 2018).

Because inflammation can lead to painful sensations, we asked whether PGD_2-G could reduce pain in an inflammatory pain model. We found that PGD_2-G decreased hyperalgesia and edema in carrageenan-induced inflammatory pain, leading to a faster recovery. PGD_2-G also decreased carrageenan-induced inflammatory markers in the paw as well as inflammatory cell recruitment. The effects of PGD_2-G were independent from metabolite formation (PGD_2 and 15d-PGJ_2-G) or DP1 receptor activation in this model (Buisseret et al. BBA Mol. Cell Biol. Bipids, 2019).

Besides PGD_2-G, our continuing efforts will contribute to highlight further the interest of modulating endocannabinoids and related lipids' levels in inflammatory situations. For instance, we are actively collaborating with Dr Makriyannis and Dr Malamas (Northeastern University, Boston) on the characterization of the role of NAAA in inflammation. Our first works focused on colitis and macrophages (Alhouayek et al. FASEB J, 2015; Alhouayek et al. BBA lipids, 2017). We are now studying the interest of inhibiting this enzyme in the context of neuroinflammation and multiple sclerosis (EAE mice model).

b) Oxysterols

Oxysterols are now considered as important lipid mediators, beyond their role in controlling lipid metabolism (Guillemot-Legris et al., Trends Mol. Med., 2016; Mutemeberezi et al., Prog. Lipid Res. 2016).

We recently reported that colitis profoundly affects oxysterol levels, both in mice models and in human patients suffering from Crohn's disease and ulcerative colitis. For some oxysterols we also found a link between the changes in oxysterol levels and alterations in the expression of key metabolic enzymes (e.g. cyp3A4) (Guillemot-Legris et al., J. Crohns Colitis, 2019). These compelling data were obtained thanks to a close collaboration with gastroenterologists from CHU UCL Namur and especially Dr Rahier. Because we are convinced that reporting lipid level alterations is a key step but hardy a goal *per se*, we are now investigating further the properties of oxysterols in colitis models. For instance, we reported already that the administration of 4β-hydroxycholesterol worsens the impact of DSS-induced colitis (Guillemot-Legris et al., J. Crohns Colitis, 2019).

In another series of experiments, using mice models of diet-induced obesity, we found that obesity profoundly affects the levels of oxysterols in numerous tissues (Guillemot-Legris et al. Sci. Rep., 2016). We are now investigating the consequences of these alterations on the development of obesity.

In parallel, we are studying the effect of neuroinflammation on oxysterols and the

potential effect of oxysterols on these models. Using in vitro models of primary glial cells, we found pronounced changes in oxysterol levels upon their activation with lipopolysaccharides (LPS). Moreover, several oxysterols were able to decrease LPS-induced activation of these primary glial cells (Mutemberezi et al. J. Neuroinfl., 2018).

Together these data are of interest when considering the phenomenon of obesity-induced neuroinflammation. Indeed, we (Guillemot-Legris et al. J. Neuroinfl., 2016) and others have shown that obesity leads to neuroinflammation as we recently reviewed (Guillemot-Legris et al. Trends Neurosci., 2017). An exciting hypothesis is that changes in oxysterol levels might represent a potential explanation for the changes in inflammatory status found in the central and peripheral nervous system during obesity development.

Interestingly, the consequences of obesity on post-operative pain remain poorly explored. In a recent paper, we showed that obesity affects the resolution of post-operative pain induced by hind paw incision and actually leads to a chronic pain state in mice. In this context, we found that following hind paw incision, high fat diet prolonged glial cell activation in the spinal cord. It also altered the expression of neurotrophins and increased inflammatory and endoplasmic reticulum stress markers in both central and peripheral nervous systems. Moreover, we show that a dietary intervention, leading to weight reduction and decreased inflammation, was able to restore normal pain sensitivity in mice suffering from chronic pain for more than 10 weeks. Thus our data support the notion that obesity is responsible for pain chronicization (Guillemot-Legris et al. Brain Behav Immun, 2018).

These findings are of clear importance in a clinical post-operative setting and we therefore aim to further decipher the underlying mechanisms, with several bioactive lipids as potential key mediators.

In conclusion, the few examples of our current research described here clearly support the importance **of increasing our understanding of bioactive lipid signaling in inflammation to put forth novel innovative therapeutic strategies**.

SELECTED PUBLICATIONS

Bottemanne P, Paquot A, Ameraoui H, Alhouayek M, Muccioli GG. The α/β-hydrolase domain 6 inhibitor WWL70 decreases endotoxin-induced lung inflammation in mice, potential contribution of 2-arachidonoylglycerol, and lysoglycerophospholipids. FASEB J. (2019),33(6):7635-7646.

Guillemot-Legris O, Mutemberezi V, Buisseret B, Paquot A, Palmieri V, Bottemanne P, Lemaire J, Rahier JF, Alhouayek M, Muccioli GG Colitis alters oxysterol metabolism and is affected by 4β-hydroxycholesterol administration. J Crohns Colitis. (2019), 13(2):218-229

Mutemberezi V, Buisseret B, Masquelier J, Guillemot-Legris O, Alhouayek M, Muccioli GG. Oxysterol levels and metabolism in the course of neuroinflammation: insights from in vitro and in vivo models. J. Neuroinflammation (2018), 15:74

Guillemot-Legris O, Buisseret B, Mutemberezi V, Hermans E, Deumens R, Alhouayek M, Muccioli GG. Post-operative pain in mice is prolonged by diet-induced obesity and rescued by dietary intervention. Brain Behav Immun. (2018), 74:96-105

Alhouayek M., Masquelier J., Cani P.D., Lambert D.M., Muccioli G.G. Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. Proc. Natl. Acad. Sci. U.S.A. (2013), 110: 17558-17563.

THESES IN PROGRESS

Abdul Khalik Hafiz: "Evaluation of the potential of Pakistan's plants used in traditional medicine for the treatment of inflammatory bowel diseases and identification of their active molecules"
Director: Joelle Quetin-Leclercq; Co-Director: Giulio Muccioli

Ameraoui Hafsa: "Contribution to the study of oxysterols in inflammatory bowel diseases: from their quantification in patients to the study of their properties in vitro and in vivo".
Director: Giulio Muccioli

Bottemanne Pauline: "Study of the role of *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) in inflammation and models of neurodegenerative diseases".
Director: Giulio Muccioli; Co-Director: Mireille Al Houayek

Buisseret Baptiste: "Study of the effects of oxygenated derivatives of endocannabinoids in inflammation and inflammatory pain".
Director: Giulio Muccioli; Co-Director: Mireille Al Houayek

Labrak Yasmine: "Targeted nanomedicines to stimulate the differentiation of oligodendrocyte progenitor cells in the scope of multiple sclerosis".
Director: Anne des Rieux; Co-director: Giulio Muccioli

Morelle Axel: "Synthesis and pharmacological evaluation of novel β-, γ- and δ-lactames as inhibitors of Fatty Acid Amide Hydrolase"
Director: Raphael Robiette; Co-director: Giulio Muccioli

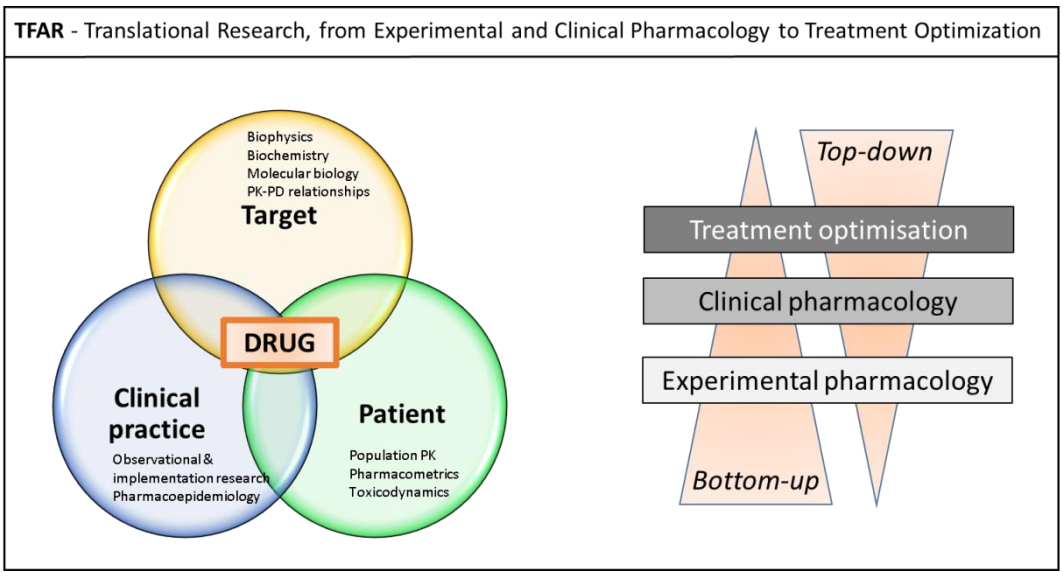
Mwema Ariane: "Nose-to-brain delivery of nanomedicines to stimulate remyelination in the scope of multiple sclerosis".
Director: Anne des Rieux; Co-director: Giulio Muccioli

Paquot Adrien: "Development and validation of an HPLC-MS method to quantify the oxygenated derivatives of the endocannabinoids"
Director: Giulio Muccioli

Roumain Martin: "Development and validation of an UPLC-MS method to quantify the oxysterols and bile acids".
Director: Giulio Muccioli

Terrasi Romano: "Development and validation of an MS/MS method to quantify phospholipids and lysophospholipids".
Director: Giulio Muccioli

Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR)



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*Pharmacological evaluation of drugs covers complementary aspects, going from **experimental pharmacology** to optimization of drug usage in **clinical practice** via a characterization of patient's specificities that could affect pharmacokinetics or pharmacodynamics (**clinical pharmacology**).*

*In this context, we conduct bidirectional **translational research**, from bench to bedside and back again, in the field of experimental, clinical pharmacology and pharmacoepidemiology, with the aim to optimizing drug treatment.*

Our common objectives are to use a deep knowledge of the molecular basis of drug action and fate (at both the cellular and the human levels) to achieve personalized pharmacokinetic and pharmacodynamic targets and implement these findings for improving quality of care. Our research focuses on high-risk medications (drugs with a narrow therapeutic window or used for severe pathologies) and/or high-risk populations (frail, immunosuppressed, or polymedicated patients).

*The main **disciplines** that are covered include: (1) in the field of experimental research: biophysics and molecular pharmacology, in vitro pharmacokinetics and pharmacodynamis (2) in the field of clinical research: population pharmacokinetics and pharmacodynamics; (3) in the field of clinical practice research: evaluative and implementation research, an pharmacoepidemiology.*

*Within TFAR, principal investigators are more specifically experts in one of these three disciplines: **FACM** (cellular and molecular pharmacology group; Marie-Paule Mingeot-Leclercq and Françoise Van Bambeke) is mainly oriented towards experimental research; **PMGK** (integrated pharmacometrics, pharmacogenomics and pharmacokinetic group; Laure Elens) towards clinical research; and CLIP (clinical pharmacy research group; Olivia Dalleur, Séverine Henrard and Anne Spinewine), towards clinical practice and implementation research.*

Some activities are unavoidably independent, but there is a clear willingness of cross-fertilization amongst us, which is operationalized through the organization of common seminars, co-supervision of translational PhD projects, submission of common grant applications and sharing logistic and technical infrastructure.

Examples of recent and ongoing translational research

The number of translational research projects within TFAR has increased over the last 5 years. In 2019, there were several ongoing projects implying eight PhD students that illustrate the type of integrative approaches existing between the groups:

- Pharmacokinetics and clinical toxicity of anti-infective drugs in specific patient populations such as patients in intensive care, HIV infected, hemodialysis patients, patients with off-label use of antibiotics (H Thirot, P Ngougni Pokem, A Bastos, G. Stillemans)
- Evaluating current practices of antibioprophylaxis in Benin to propose and then implement and evaluate strategies for a better use (AD Fiogbe, C Yehouenou, A. Dohou).
- Precision pharmacotherapy of neuroleptics in schizophrenic patients (J. Lagreula).

The next pages present the ongoing projects in each of the groups constituting TFAR.

Cellular and Molecular Pharmacology (TFAR - FACM)

Our team is studying the pharmacology of drugs, mainly anti-infective agents (antibiotics) with the aim to decipher the mechanisms responsible for their activity or their cellular toxicity, and to optimize their use in the clinics (based on a better knowledge of their pharmacodynamics and of the risks for selecting resistance). Disciplines and methodologies used involve biophysics, biochemistry, microbiology, cellular and molecular biology, and morphology.

Our main objectives are to decipher, at the molecular and cellular levels, the mechanisms of the interaction between these drugs and

- *bacteria (target cells), with the aim to progress in the understanding of their mode of action and of mechanisms of bacterial resistance;*
- *host cells, with the aim to unravel the mechanism of their transmembrane transport, and to evaluate the consequences of their cellular accumulation for activity and toxicity.*

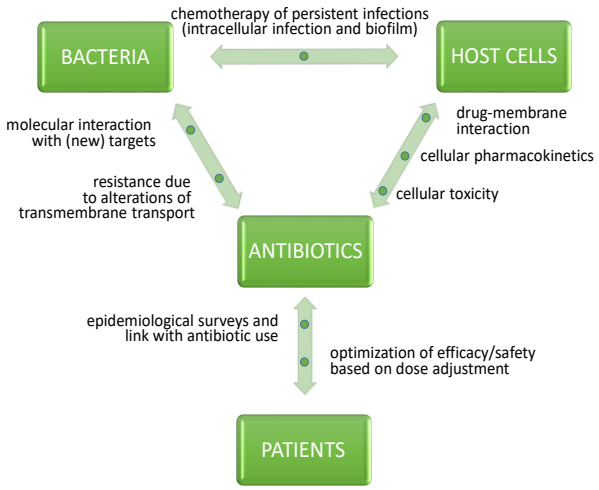
To this effect, we explore

- *at the cellular level, the cellular pharmacokinetics of antibiotics (accumulation, distribution, and efflux in eukaryotic cells), in relation with their activity against intracellular pathogens and with their capacity to cause cellular toxicity (lysosomal or mitochondrial alterations; apoptosis).*
- *at the molecular level, (i) the interaction between antibiotics and membrane lipids and consequences therof for membrane biophysical properties, (ii) the selection of resistance in vitro (with a particular interest for active efflux), and (iii) the activity of novel antibiotics acting on new, unexploited targets.*

Our experimental approaches include:

- *biophysical approaches aimed at characterizing at the molecular level the interaction between drugs and membrane lipids and at understanding how biophysics encounters cell functions (cell bacteria division, shaping/reshaping of red blood cells, e.g.);*
- *genomic and proteomic approaches aimed at evidencing the effects of drugs on the expression and function of target genes/proteins;*
- *pertinent cellular models for the study of drug pharmacokinetics (accumulation, subcellular distribution, efflux), pharmacodynamics (intracellular infection, biofilm) and cellular toxicity, which are used for exploring the mechanisms governing the interaction between host cells, drugs and bacteria, and to evaluate new molecules or new therapeutic strategies.*

In a broader context, our translational research activities include clinical trials aimed at optimizing antibiotic use (adaptation of their mode of administration or daily dosage) with the aim to increase their efficacy and/or reduce their toxicity (run in coworking with different hospitals in Belgium), and collection of clinical isolates for which we study antibiotic resistance and try to establish a potential link with the treatment received by the patient.



RESEARCH RESULTS

Over the last 5 years, we have published 80 papers, 83% of which directed related to our research dealing with anti-infective pharmacology and drug-membrane interactions (17% as reviews or book chapters and educational papers related to anti-infective pharmacology or pharmacotherapy, papers in the field of clinical pharmacy).

Our experimental research is oriented in 6 main directions. These are, however, closely linked to one another.

1) Lipid domains: a promising target for new antibiotics?

With the aim to provide a more comprehensive and biologically relevant picture of the drug-membrane interactions and how the effect of these interactions can modify the biophysical properties of the membranes in relation with pharmacological activities, most of the studies are performed by using cells (bacteria or mammalian cells) and membrane models (supported bilayers, liposomes [SUVs, LUVs; GUVs]) mimicking (i) bacterial and (ii) eukaryotic membranes. In close collaboration, we used a range of complementary methods including AFM, ^{31}P NMR, dynamic light scattering, fluorescence spectroscopy (Laurdan, DPH, TMA-DPH, DHE, calcein, octadecylrhodamine B…) and confocal and electronic microscopy.

Cardiolipin domains as a target for amphiphilic aminoglycoside derivatives?

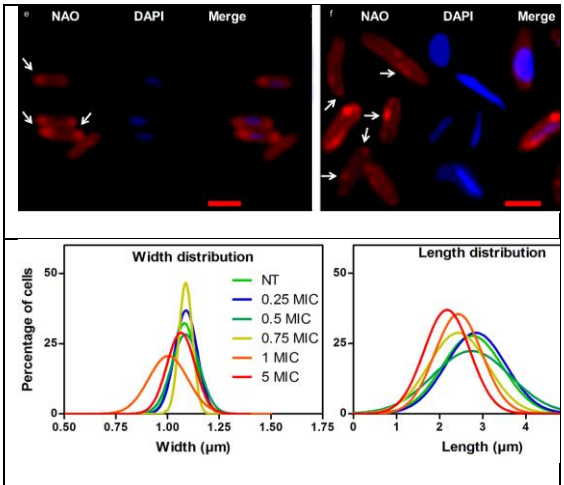
Combination of existing lipid diversity and functions with biophysics of bacterial membranes is a unique opportunity to discover new antibiotics. Bacteria (as mammalian cells) have capacity to maintain specialized zones in their membranes for fruitfully fill in their biological functions.

In the frame of our work, we focus on areas characterized by high curvature and enriched in cardiolipin, as encountered at poles and division septa of Gram-negative bacteria, with the aim to understand if and how membrane-acting antibiotics (amphiphilic neamine derivatives) might modify bacterial physiological processes.

Intensive medicinal chemistry development was performed in collaboration with Prof. JL Decout and coll. (Grenoble, F) from a group of old antibiotic drugs called aminoglycosides, which target ribosomal RNA. Molecular foundations and structure–activity relationships made on the central backbone (neamine versus neosamine), the nature of the hydrophobic tail (naphtyl, alkyl, alkyl) as well as or the position and the number of substitution on the central backbone to define optimal amphiphilicity, led to the emergence of amphiphilic antibacterial aminoglycosides. More than 80 derivatives were synthesized with very promising compounds active against Gram-positive and Gram-negative sensitive and resistant bacteria. In addition, we did not observe any emergence of resistance in P. aeruginosa treated for 35 days with amphiphilic aminoglycoside derivatves.

To decipher the molecular mechanism involved in their activity, we used both living bacteria (*P. aeruginosa*) as well as membrane model systems including LUVs (Large Unilamellar Vesicles) for membrane permeability and depolarization, GUVs (Giant Unilamellar vesicles) for confocal microscopy and lipid monolayers, for Langmuir isotherm compression. We demonstrated the interaction of the amphiphilic neamine derivatives with outer membrane's lipopolysaccharides and inner membrane's anionic phospholipids mostly cardiolipin leading to membrane permeabilization (NPN and PI assays) and depolarization (DiSC3(5) fluorescence). Targeting cardiolipin bacterial micro-domains mainly located at the cell poles, led to relocation of cardiolipin domains associated with bacterial morphological changes including a severe length decrease.

These results suggest an effect of amphiphilic aminoglycoside antibiotics on cardiolipin domains with in turn changes in the activity of proteins dependent upon cardiolipin and involved in bacterial division (FtsZ) and/or bacterial shape (MreB).



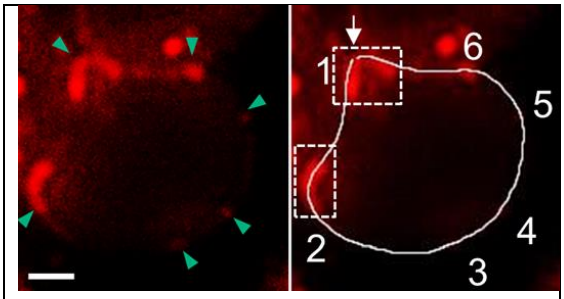
3',6-dinonyl Neamine targets P. aeruginosa microdomains of cardiolipin leading to their redistribution (top) and changes in width and length (bottom). Top: non treated bacteria (left) and treated (5 µM for 10 min at 37°C) bacteria (right); arrows indicate cardiolipin domains. (El Khoury et al, 2017, Sci. Reports)

At a glance, our results bring into light fundamental concepts which could be important in membrane-lipid therapy in which the molecular targets are the lipids and the structure they form. The role of lipids can be (i) to facilitate membrane bending and the formation of highly curved intermediates, reducing the energy barriers of fission and fusion and (ii) to recruit specialized proteins. Influencing curvature directly as well as indirectly by targeting negative intrinsic curvature of lipids or in impairing the soft mechanical behavior could be a new approach for antibiotic design.

Lipid-proteins interactions could be also critical in cell physio- and pathology of erythrocytes. In this context, we investigated (i) whether enriched lipid domains in cholesterol and sphingomyelin could contribute to function-associated cell (re)shaping, (ii) whether the seminal concept of highly ordered rafts could be refined with the presence of lipid domains exhibiting different enrichment in cholesterol and sphingomyelin and association with erythrocyte curvature areas and (iii) how differences in lipid order between domains and surrounding membrane are regulated and whether changes in order differences could participate to erythrocyte deformation and vesiculation.

For studying the first question, we probed by vital imaging the lateral distribution of cholesterol and sphingomyelin (using either specific Toxin fragments or trace insertion of BODIPY-SM) in relation with: (i) membrane biconcavity of resting red blood cells; (ii) membrane curvature changes and Ca^{2+} exchanges upon mechanical stretching of healthy red blood cells or in elliptocytes, a red blood cells model of impaired shape; and (iii) membrane vesiculation upon red blood cells aging.

We revealed the specific association of cholesterol- and sphingomyelin-enriched domains with distinct curvature areas of the erythrocyte biconcave membrane. Upon erythrocyte deformation, cholesterol-enriched domains gathered in high curvature areas. In contrast, sphingomyelin-enriched domains increased in abundance upon calcium efflux during shape restoration. Upon erythrocyte storage at 4 °C (to mimick aging), lipid domains appeared as specific vesiculation sites.



Recruitment of cholesterol-enriched domains in increased curvature areas of the red blood cells rim upon stretching. Green arrowheads indicate high curvature areas. Scale bars 2µm. Leonard et al, 2016, Sci. Reports.

The second and third questions benefit from the use of a fluorescent hydration- and membrane packing-sensitive probe, Laurdan, to determine the Generalized Polarization (GP) values of lipid domains vs the surrounding membrane. Sphingomyelin- and cholesterol- enriched domains were less ordered than the surrounding lipids in erythrocytes at resting state. Upon erythrocyte deformation (elliptocytes and stimulation of calcium exchanges) or membrane vesiculation (storage at 4°C), lipid domains became more ordered than the bulk. Upon aging and in membrane fragility diseases (spherocytosis), an increase in the difference of lipid order between domains and the surrounding lipids contributed to the initiation of domain vesiculation.

Altogether, results demonstrated the critical role of domain-bulk differential lipid order modulation for erythrocyte reshaping probably related with the pressure exerted by the cytoskeleton on the membrane.

2) Cholesterol-enriched domains and cellular toxicity

The existence of clusters of proteins and lipids and especially, the transient nanometric cholesterol- and sphingolipid-enriched domains, called rafts, are described as signaling platforms for a wide range of cellular responses to stimuli including reactive oxygen species (ROS) generation, inflammatory cytokines expression and cell death. we explored the role of cholesterol and cholesterol-enriched domains for cellular toxicity of the potential anticancer drug, the ginsenoside Rh2 and the anti-inflammatory complex budesonide: HPβCD.

Taking benefit from our previous studies investigating the mechanisms involved in nephrotoxicity induced by aminoglycoside antibiotics, we explored the capacity of new antibiotics to accumulate within the cells and to induce accumulation of undigested lipids within the lysosomes. More recently, we started to explore the mitochondrial alterations induced by oxazolidinone antibiotics.

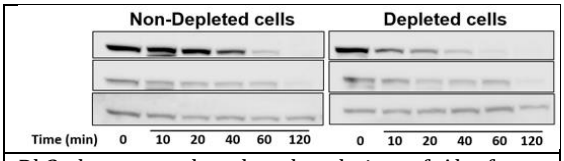
I) Cholesterol-enriched domains and cellular toxicity of anticancer drug (Ginsenoside Rh2)

Pursuing our studies on the molecular mechanism involved in necrosis and apoptosis in leukemic monocytes induced by saponins (α-hederin, a monodesmosidic triterpenoid) and especially the critical role of cholesterol and cholesterol-enriched domains, we extend to ginsenoside Rh2, a steroid saponin (protopanaxatriol) known as one of the active principles of *Panax ginseng*

root. This work is performed in close collaboration with J. Leclercq's team.

We demonstrated that membrane cholesterol could delay the activity of ginsenoside Rh2, renewing the idea that saponin cytotoxicity is ascribed to an interaction with membrane cholesterol.

The cytotoxic activity of Rh2 is accelerated in human leukemic U937 cell lines upon cholesterol depletion via the pretreatment with methyl-β-cyclodextrin, a cholesterol-sequestering agent. Mechanistically, Rh2 alters plasma membrane fluidity by compacting the hydrophobic core of lipid bilayer (DPH anisotropy) and relaxing the interfacial packaging of the polar head of phospholipids (TMA-DPH anisotropy). The treatment with Rh2 consequently conducts to the dephosphorylation of Akt and the activation of the intrinsic pathway of apoptosis (loss of mitochondrial membrane potential, caspase-9 and -3 activation).



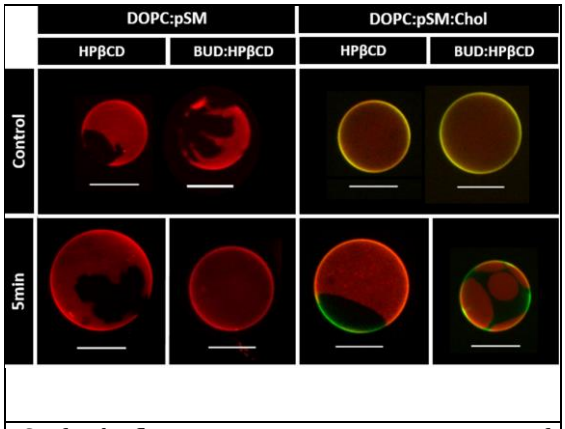
Rh2 decreases the phosphorylation of Akt faster upon cholesterol depletion. Cells depleted in cholesterol with 5 mM MβCD for 2 h, or not, were incubated for the indicated times with 60 µM Rh2 or with 0.1% DMSO (vehicle). Equal amounts of cell extracts were subjected to western-blot analysis for pAkt, Akt and β-actin protein (Verstraeten et al, 2018, Toxicol. Appl. Pharmacol.).

All these features are induced faster in cholesterol-depleted cells, which could be explained by faster cell accumulation of Rh2 in these conditions.

II) Cholesterol-enriched domains and cellular toxicity of antiinflammatory drug (budesonide) complexed with HPβCD

Budesonide (BUD), a poorly soluble anti-inflammatory drug, is used to treat patients suffering from asthma and COPD (Chronic Obstructive Pulmonary Disease). Hydroxypropyl-β-cyclodextrin (HPβCD), a biocompatible cyclodextrin known to interact with cholesterol, is used as a drug-solubilizing agent in pharmaceutical formulations. Budesonide administered as an inclusion complex within HPβCD (BUD:HPβCD) required a quarter of the nominal dose of the suspension formulation and significantly reduced neutrophil-induced inflammation in a COPD mouse model exceeding the effect of each molecule administered individually. This suggests the role of lipid domains enriched in cholesterol for inflammatory signaling activation.

We first showed that BUD:HPβCD induced an increase in membrane fluidity and permeability induced by BUD:HPβCD in vesicles containing cholesterol. We also demonstrated on giant unilamellar vesicles (GUVs) and lipid monolayers, the disruption of cholesterol-enriched raft-like liquid ordered domains as well as changes in lipid packing and lipid desorption from the cholesterol monolayers, respectively. Except for membrane fluidity, all these effects were enhanced when HPβCD was complexed with budesonide as compared with HPβCD.

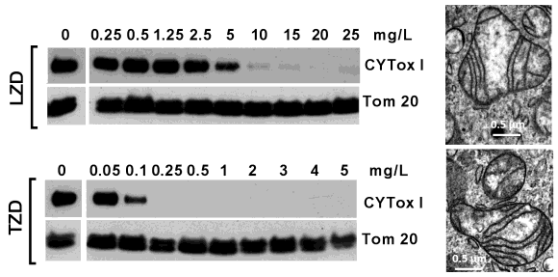


Confocal fluorescence microscopy imaging of membrane phase separation in GUVs upon incubation with BUD:HPβCD, and HPβCD. Imaging of membrane domains in GUVs composed of (left) DOPC:pSM (1:1) and (right) DOPC:pSM:Chol (1:1:3) before (top, control) and after (descending) 5, min with the BUD:HPβCD complex or HPβCD. DOPC:pSM vesicles were labeled with Rho-DOPE (red channel) to visualize the liquid disordered (ld)/solid ordered (so) phase separation in red/dark. DOPC:pSM:Chol were labelled with Rho-DOPE (red channel) and NBD-PE (green channel) to visualize the liquid disordered (ld)/liquid ordered (lo) phase separation in red/green channels, respectively. Dos Santos et al, 2017, Biochim. Biophys. Acta. Biomembranes

Since changes in biophysical membrane properties have been linked to membrane signaling including pathways involved in inflammation processes, we moved on cellular models (A549) and demonstrated that BUD:HPβCD could limit (i) hydrogen peroxide- and lipopolysaccharide-induced ROS generation, (ii) alveolar cell death mainly due to HPβCD, and (iii) CXCL8/interleukine-8 expression mainly due to BUD. Our results suggest that BUD:HPβCD would potentially be more beneficial than BUD to deal with COPD-related inflammation.

III) Mitochondrial toxicity of oxazolidinones

Oxazolidinones extert their antibacterial effect by inhibiting protein synthesis in bacteria. We evidenced a specific inhibition of the synthesis of protein encoded by the mitochondrial genome accompanied by an inhibition of the respiratory function and morphological alterations (swelling of mitochondria and disappereance of cristae). We are now exploring whether these changes may contribute to explain the thrombocytopenia and anemia reported in patients treated by these drugs. Our current data suggest that oxazolidinones prevent the maturation of platelet precursors.



Left: Influence of increasing concentrations of linezolid (LZD) and tedizolid (TZD) on CYTox I expression in HL-60 promyelocytes incubated for 120 h in the presence of increasing concentrations of these drugs. Western blots of CYTox I (protein encoded by the mitochondrial genome) and of Tom 20 (encoded by the nuclear genome) of mitochondrial protein fractions. Right: electron microscopy images of mitochondria from HL-60 cells exposed to 15 mg/L linezolid or 3 mg/L of tedizolid.

3) Pharmacokinetics and pharmaco-dynamics of antibiotics in models of persistent infections

Bacterial persistent or recurrent infections are associated with two specific lifestyles, namely intracellular survival and biofilms. We are studying antibiotic activity against these two forms of infections in relationship with antibiotic pharmacokinetics (factors determining antibiotic access to the target).

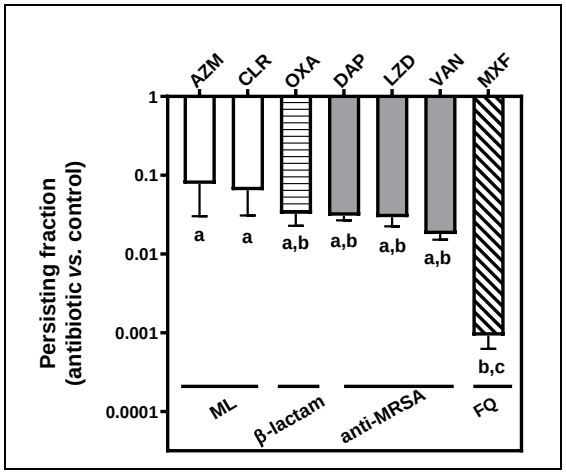
I) Cellular pharmacokinetics

We study the cellular accumulation (including the mechanisms of entry) and the subcellular localization of novel molecules in preclinical and clinical development, as a basis for further studies examining their intracellular activities in specific compartments. We try to decipher the

mechanisms for their penetration and distribution within the cells. Over the last years, we have focused our interest on new antibiotic classes, like lipoglycopeptides, ketolides, new fluoroquinolones and new oxazolidinones now present on the market. We are now examining innovative antibiotic classes acting on still unexploited targets in order to define their capacity to accumulate within the cells and then to define their interest for the treatment of intracellular infections.

II) Cellular pharmacodynamics

In parallel, we study the activity of antibiotics against intracellular bacteria sojourning in different subcellular compartments, mainly *Listeria monocytogenes* (cytosol), *Staphylococcus aureus* (phagolysosomes), and *Pseudomonas aeruginosa.* We have also extended this model to other bacterial species of medical interest. We developed an in vitro pharmacodynamic approach to compare the efficacy and the potency of the drugs. In brief, we showed that antibiotics are in general less effective but equipotent against intracellular than against extracellular bacteria, irrespective of their accumulation level. The data generated with these models have been incorporated in the dossier having led to the registration of the last antibiotics brought on the market.



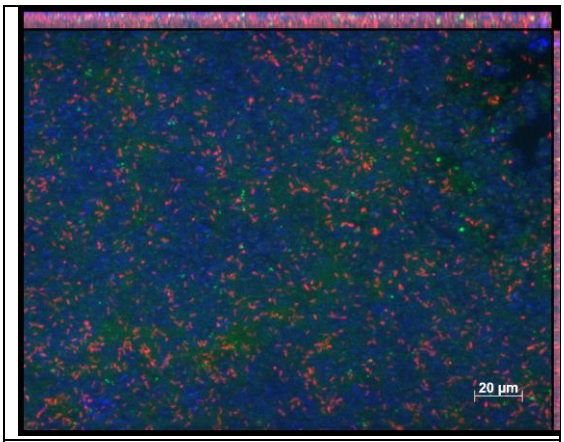
Evaluation of the intracellular persisting fraction of *S. aureus* exposed to atibiotics. For each tested antibiotic, the bar shows the ratio between the $\log_{10}$ number of CFU per milligram of protein after 24 h of incubation with 100x MIC of antibiotic and the $\log_{10}$ number of CFU per milligram of protein under control conditions. AZM, azithromycin; CLR, clarithromycin; OXA, oxacillin; DAP, daptomycin; LZD, linezolid; VAN, vancomycin; GEP, gepotidacin; MXF, moxifloxacin; ML macrolide; FQ, fluoroquinolone. Statistical analysis was performed by one-way analysis of variance with Tukey's post hoc test. Data sets with different letters are significantly different from one another ($P < 0.05$).

We are now trying to elucidate the mechanisms by which intracellular bacteria become tolerant to antibiotics. We specifally focus on trying to identify genes involved in intracellular persistence. To this effect, we are running transcriptomic analyses on intracellular *S. aureus* surviving antibiotic exposure within permissive eukaryotic cells and are chacarterizing the capacity to survie inside these cells of clinical isolates collected from persistent infections.

4) Antibiotic activity against biofilms

We developed in vitro pharmacodynamic models to evaluate the activity of antibiotics against biofilms made of *S. aureus*, *S. pneumoniae* or *P. aeruginosa*. We showed that antibiotic efficacy and relative potency are considerably reduced in biofilms as compared to planktonic cultures. With *S. aureus*, we found that biofilms made of clinical strains isolated from patients suffering from persistent infections are still more refractory to antibiotics. We could demonstrate that this was mainly due to a default of penetration of the antibiotics within these biofilms, which could attribute to the matrix composition (polysaccharide content). On these bases, we are exploring innovative strategies in order to disrupt this matrix and increase antibiotic activity.

In parallel, we have also started to develop more pertinent models of biofilms, like biofilms growing in artificial sputum medium mimicking the viscoelastic properties of the mucus found in the respiratory tract of patients suffering from cystic fibrosis, or multispecies biofilms developing on orthopedic implants.

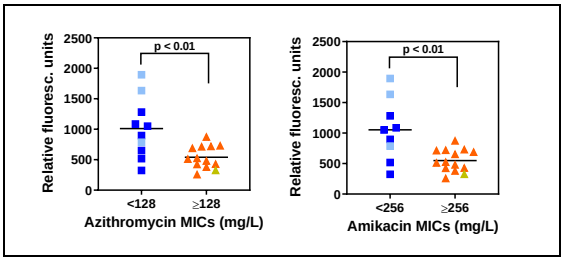


Fluorescence microscopy imaging of a multispecies biofilm made of andida albicans (glycans stained in blue), *Staphylococcus aureus* and *Escherichia coli* (stained respectively in green and red by FISH probes)

5) Antibiotic efflux and permeability resistance mechanisms

We have provioulsy demonstrated the role of active efflux as a mechanism responsible for the intrinsic resistance of *P. aeruginosa* to specific antibiotics, like temocillin, or macrolides.

We have now started to evaluate the ipact of this mechanism of resistance in *Achromobacter xylosoxidans,* a bacterial species which follows *P. aeruginosa* in the colonization of the lung of patients with cystic fibrosis (CF).



NPN (fluorescent substrate of efflux pumps) accumulation in reference strains and clinical CF isolates of *Achromobacter* as a function of their AZI/AMK MIC

We could demonstrate that efflux indeed plays a major role in the poor susceptibility of this species to commonly used antibiotics. Importantly, also we could evidence some mutations in these proteins that affect their substrate specificity.

6) Novel antibiotic targets and drug design

In a world of increasing resistance, discovery of antibiotics acting on new, unexploited targets is an important medical need. In coworking with groups active in pharmaceutical chemistry or in pharmacognosy (within the institute or outside), we evaluate the activity of new compounds and try to decipher their mode of action. In this context, we have discovered with the CMFA group new inhibitors of peptidoglycan synthesis and with the GNOS group, agents reversing resistance to β-lactams in *Staphylococcus aureus or active against Leishmania Mexicana Mexicana and Trypanosoma brucei brucei.*

In collaboration with the team of JM Bolla at the Université Aix-Marseille (France), we are also evaluating the activity of original compounds originally designed as inhibitors of efflux but showing much broader synergistic effects with antibiotics, in our models of infections, including intracellular infections and activity against strains that show resistance to other antibiotic classes or mutations in their efflux systems.

Our clinical research aims at optimizing the scheme of administration of antibiotics in terms of ease of administration, safety, and efficacy, taking into account their pharmacodynamic properties.

At the present time, we are evaluating administration by continuous infusion or

prolonged infusion of beta-lactams. More specifically, we perform pharmacokinetic studies in specific patients populations (like haemodialysis patients, critically-ill patients or children) in order to propose optimize therapeutic doses. We investigate the parameters that can affect protein binding of drugs, as only the unbound fraction is thought to be important for activity.

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THESES DEFENDED IN 2018-2019

Catteau Lucy: "Identification of natural molecules as antimicrobials or β-lactams resistance modifying agents against *Staphylococcus aureus"*
Directors: J. Quétin-Leclercq and F. Van Bambeke

Le Thanh Binh: "Anti-parasitic activity of Vietnamese essential oils and study of the mode of action of eugenol, one of their active constituents".
Directors: J. Quétin-Leclercq and Marie-Paule Mingeot-Leclercq

Milosevic Tamara: "Mitochondrial toxicity induced by oxazolidinone antibiotics: insights from studies in human cell lines and primary cells"
Director: F. Van Bambeke

Miranda Bastos Ana: "Towards optimization of temocillin exposure in haemodialysis patients: a translational approach from bedside-to-bench-to-bedside"
Directors: Françoise Van Bambeke, Anne Spinewine

Verstraeten Sandrine: "Contribution of membrane lipids to the activity of the saponin ginsenoside Rh2".
Directors: Marie-Paule Mingeot-Leclercq and Donatienne Tyteca

THESES IN PROGRESS

Ameryckx Alice: "Design and synthesis of inhibitors of DD-ligases, enzymes participating to the synthesis of peptidoglycan synthesis in the bacterial cytosol".
Directors: Raphaël Frederick, Françoise Van Bambeke

Bahiya Jules César: "Effects of a glucocorticoid-cyclodextrin complex on lipid membranes and cell response to oxidative and inflammatory stimuli".
Director: Marie-Paule Mingeot-Leclercq

Comein Audrey: "Study of the intracellular fate of Pseudomonas aeruginosa: balance between cytotoxicity and intracellular persistence"
Director: Françoise Van Bambeke

Diaz Iglesias Yvan: "Setting up a model of mixed biofilms by *P. aeruginosa* and *S. aureus* in the context of cystic fibrosis for the study of the activity of antibiotics and adjuvants acting on the matrix".
Director: Françoise Van Bambeke

Dohou Angèle: "Nosocomial infections and bacterial resistance: impact of the Belgian model of clinical pharmacy on the rational use of antibiotics in Benin".
Directors: Olivia Dalleur, Françoise Van Bambeke

Fiogbe Ariane: "Contribution of a multidisciplinary strategy for the prevention and control of care-associated infections in digestive surgery".
Directors: Olivia Dalleur, Françoise Van Bambeke

Kaur Mandeep: "The asymmetry of the outer membrane of *P. aeruginosa*: target for new amphiphilic neamine derivatives and role for membrane curvature"
Director: Marie-Paule Mingeot-Leclercq

Ngougni Pokem Perrin: "Therapeutic monitoring of beta-lactams antibiotics to improve their efficacy".
Directors: Françoise Van Bambeke, Laure Elens

Nguyen Tiep Khac: "Antibiotic resistance: mecanistic, epidemiologial and pharmacological studies. Application to the situation in Vietnam. "
Director: Françoise Van Bambeke

Peyrusson Frédéric: "Activity of new antibiotics against intracellular forms of Gram positive bacteria in relation with factors determining intracellular persistence".
Director: Françoise Van Bambeke

Poilvache Hervé; "Prosthetic joint infections: diagnostic optimisation and evaluation of innovative treatment strategies".
Directors: Olivier Cornu (IREC), Françoise Van Bambeke

Ruiz Sorribas Albert: "Targeting exopolysaccharides and their synthesis as an adjuvant therapy in the context of persistent forms of infections (biofilms) in orthopedic surgery".
Director: Françoise Van Bambeke

Thirot Hélène "Study of risks associate with the off-label use of antibiotics".
Directors: Françoise Van Bambeke, Anne Spinewine

Wang Gang "Active efflux in *Pseudomonas aeruginosa*: Role in persistent infections and pharmacological modulation".
Director: Françoise Van Bambeke

Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics (TFAR - PMGK)

The PMGK group was created in 2013 with the appointment of L. Elens as a professor in pharmacokinetics. The principal focus of this group is the development and the harmonization of precision medicine through pharmacokinetics (PK) considerations. It mainly aims at characterizing the PK behavior of drugs in humans using quantitative approaches. The research activities cover multiple fields of expertise such as Population-based PK (PopPK), Pharmacogenomics (Pgx) and PK-PD relationships, all being essential for the understanding of the fate of xenobiotics administered in humans. More specifically, the PK as well as the Pgx expertise covers vitro and in vivo approaches of drug metabolism, all indispensable and complementary to elucidate the determinants of therapeutic responses. The expertise is thus maily centered on a theme; the study of the fate of xenobiotics in the organism and the factors affecting it. Our projects cover together multiple fields of pharmacotherapy. These areas include mainly,.

- *Immunossupressants*
- *Lipid lowering drugs*
- *Anti-HIV*
- *Anticoagulants*
- *Antibiotics*

1) RESEARCH RESULTS

a) Immunosuppressants used in renal transplantation

Patient survival and graft outcome after kidney transplantation have drastically improved in recent decades, mainly because of major improvements in immuno-suppressive therapy. However, optimal immunosuppression is difficult to achieve in an individual patient. Indeed, the use of immunosuppressive drugs such as tacrolimus (Tac) is complicated by a high toxicity profile combined with a narrow therapeutic window. An important part of the variability observed in drug response is thought to be the consequence of substantial inter- but also intra-individual differences in drug PK. Some patients have relatively fast drug clearance; others exhibit a slower drug elimination rate, while some depict varying drug levels despite no dosage change. This variation in drug clearance is of importance, since it might be related to an increased risk of under- or overexposure, which can ultimately lead to a higher frequency of acute graft rejection or adverse events.

Although our previous discoveries in humans have led to personalize the initial Tac dose through new genotype-based dosage guidelines (see below, human studies), the residual unexplained PK variability is still substantial (>50%).

Animal studies

The importance of the gut microbiota for explaining the fate of Tac in the organism has been largely understudied. Not only gut microorganisms express numerous enzymes able to directly metabolize xenobiotics but also, they are able to control the host Absorption, Distribution, Metabolism and Excretion (ADME) phenotype through different processes.

Very recently (October 2018), we have started a new project combining in vitro, in vivo and clinical investigations aiming at characterizing how Tac PK and host microbiota are interrelated. The aim is thus to shed light on the mechanisms linking the gut microbiota to the Tac inter but also intra-individual PK variability.

Human studies

During the last decade, we demontrated that carriership of genetic variants in the Cytochrome P450-mediated drug metabolism is associated with a rough 30% reduction in *in vivo* metabolic activity and led to 50% lower tac dose requirements in patients. This observation led us to propose new dosage guidelines based on a validated popPK model in adult renal transplant, which can be useful in the frame of pre-emptive genotyping and dosage adjustment prior to transplantation (Figure 1).

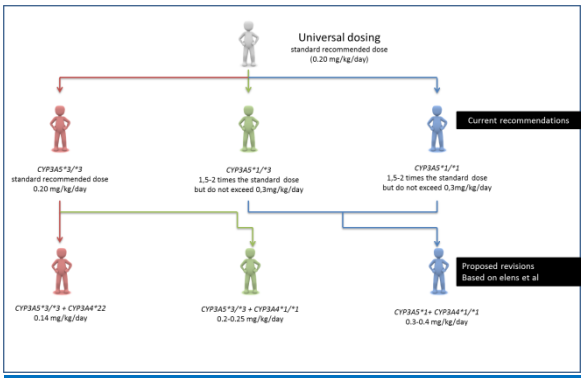

Figure 1: *New dosing guidelines for tacrolimus therapy in renal transplant recipients according to CYP3A genotype.*

As explained above, we are also starting a new clinical study investigating the potential influence of a patient's microbiome on the tac dose requirement to in fine try to unravel the reason for the PK variability unexplained by host factors. We expect that this project will lead us to validate new biomarkers of PK variabilities and help refining our dosage recommendations. Moreover, it could also be an asset for improving drug therapy, with the possible inclusion of *e.g.* antimicrobials for decreasing certain species, or probiotics in order to promote the most useful ones.

b) Statins

Since cardiovascular diseases are a real public health problem, lipid lowering medication are widely used to decrease cholesterol and triglyceride levels in the general population. There is, however, a great interindividual variation in response to therapy that is not mastered. Again, data suggest that a part of this variability might be attributed to PK differences. Atorvastatin is the world's bestselling drug of all time. However, despite this clinical success, and although doses are titrated according to cholesterol measurements, many individuals are unable to reach their respective targeted cholesterol levels. In addition, many patients suffer from side effects, and up to 10% of patients taking atorvastatin experienced muscle-related adverse drug reactions (ADRs)

In vitro investigations

The pathophysiology of statin-induced myopathy is fairly understood and local PK mechanisms determining drug cellular accumulation remain largely unexplored. To get into myocytes atorvastatin undergoes passive diffusion but also active transport. The influx protein OATP2B1 and the efflux proteins MRP1, MRP4 and are expressed at the sarcolemmal membrane of skeletal muscle fibres. We have developed recombinant HEK293 cellular models overexpressing either OATP2B1 or MRP1 (Figure 2).

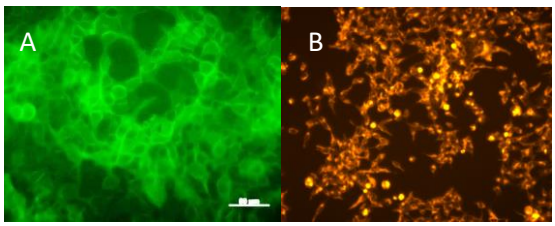

Figure 2: *Recombinant HEK293 cells overexpressing (a) MRP1 with a Green Fluorescent Protein (GFP) tag and (b) OATP2B1 with an OFP spark-red flurescent tag.*

Our data suggest that Atorvastatin is a good substrate of these 2 efflux pumps (e.g. Figure 3).

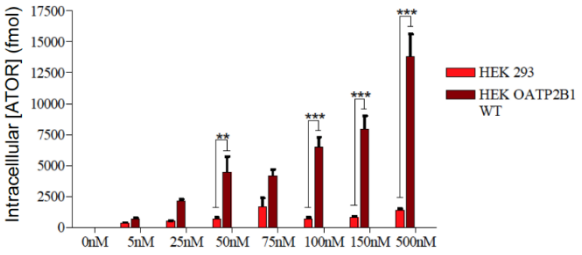

Figure 3: *Intracellular accumulation of atorvastatin in HEK293 (red) and in HEK293 stably transfected with a plasmid containing SLCO2B1 cDNA (bordeau) after 2h of incubation with increasing concentrations of atorvastatin.*

Our next move was to introduce natural genetic variations in the cDNA of those proteins (OATP2B1 or MRP1) and to analyze the functional consequences of these SNPs on the intracellular PK of atorvastatin. Indeed, we have pinpointed a natural genetic variation significantly affecting MRP1 activity towards Atorvastatin (Figure 4).

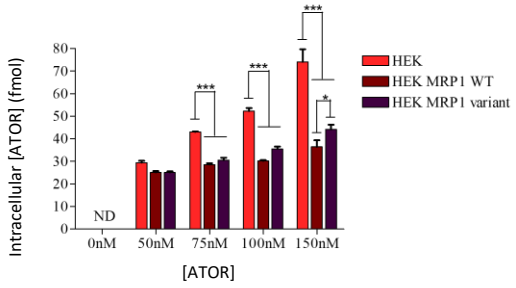

Figure 4: *Intracellular accumulation of atorvastatin in HEK293 (red) and in HEK293 stably transfected with a plasmid containing ABCC1 cDNA wild-type (bordeau) or variant for the rs... SNP (purple) after 2h of incubation with increasing concentrations of atorvastatin*

As both OATP and MRP transporters generate opposite drug transport, we will also assess the combined impact of these transporters when they are co-expressed. Finally, we plan to transpose and develop recombinant cultured primary differentiated human skeletal muscle myoblasts (HSMM) overexpressing drug transporters to weight the consequences on drug accumulation and myocyte toxicity with specific biomarkers.

Human studies

To pursue these investigations a step further, the group is now collaborating with Prof. Dr JL Balligand (FATH, IREC) to unravel the reasons for PK variability of atorvastatin in clinics. We also work with Prof Giulio Muccioli (BPBL) for the analytical part of the project and with Prof Vincent Haufroid (LTAP, IREC) for the pharmacogenetic aspect.

Our collaborative project will try to decipher the potential of popPK for optimization of statin therapy. Our study aims at recruiting 150 patients treated with atorvastatin for hypercholesterolemia. In those patients, drug and metabolites measurments are performed at 3 time-points and the patient is genotyped for some important biotransformation and transporter protein genes (e.g. CYP3A, MRPs,...). At present, we have recruited about 70 patients. Preliminary results show a high variability in both atorvastatin and its metabolites concentrations (figure 5).

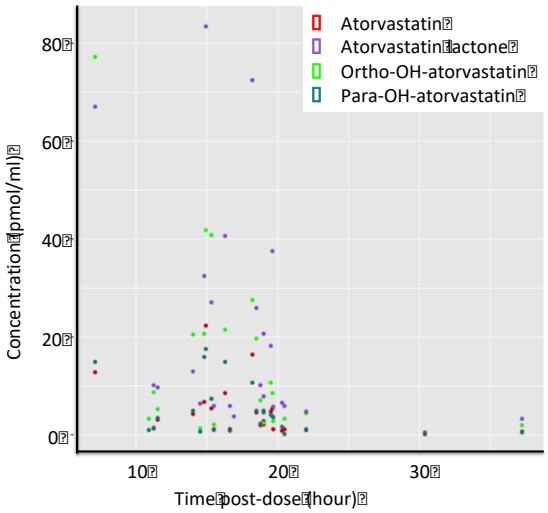

Figure 5: *plasma concentrations of Atorvastatin (red), atorvastatin lactone (purple), ortho- (light green) and para- (dark green) hydroxy-Atorvastatin in 30 patients.*

We expect that this innovative prospectively designed clinical study will ultimately allow predicting atorvastatin PK fluctuations and anticipating abnormal response (ADR or non-responsivness).

c) Anti-HIV drugs

Human studies

In close collaboration with the infectious disease unit of CUSL, in 2016, in a pilot study involving 135 patients treated with Darunavir, a potent protease inhibitor, we have demonstrated that significant PK drug-drug interaction exists between Darunavir and Etravirine, another coadministrated anti-HIV drug. We have also highlighted that this interaction is partly mediated by genetic polymorphisms in *CYP3A5*. Aside, in this study, we have shown that anti-HIV drugs accumulates differentially in circulating lymphocytes and that, for instance, Etravirine accumulates more efficiently in PBMCs compared to Darunavir. This is particularly important as lymphocytes represent the site where the drug exerts his therapeutic action. Our lab is at present trying to confirm and quantify these effects through popPK models. The recruitment phase is now completed (n=140). Ultimately, our Pop PK model will integrate different parameters to propose quantitative computations leading to a tailored drug dosage. The following step will be to evaluate the appropriateness of these new recommendations in a prospective randomized study.

d) New anticoagulants

In vitro investigations

In collaboration with CLIP, we have investigated the in vitro impact of *ABCB1* genetic polymorphisms on the transport activity towards rivaroxaban. We found that the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs had no significant effect on the efflux of rivaroxaban (Figure 6). However, the intracellular accumulation of rivaroxaban was influenced by the overexpression of ABCB1, confirming its involvement in the active transport of this oral anticoagulant. This information is crucial to manage potential drug-drug interaction through modulation of P-gp activity at the gut barrier.

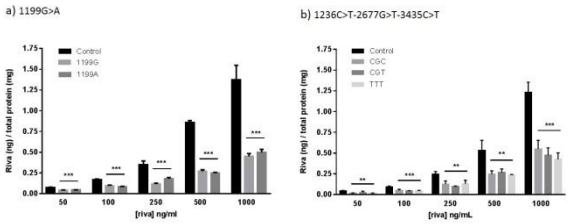

Figure 6: *Intracelllular accumulation of rivaroxaban after 120 min of incubation (n=3) at different concentrations in (a) $HEK_{control}$ (i.e. empty vector), HEK_{1199A} or (b) $HEK_{control}$, $HEK_{1236C>T-2677G>T-3435C>T}$. *p<0.05, **p<0.01, ***p<0.001 compared to $HEK_{control}$*

2) OUTLOOKS

The PK world is currently evolving from a descriptive explanatory tool towads a predictive modelling patient-centered method that allows proactive anticipations and individualized treatments through the identification of biomarkers. However, even if data are generated everyday, there is a lack of exhaustive unification of information. Our ambition is now to explore a more exhaustive and innovative track in pharmacotherapy that is the creation of a multi-omics integrative network for predicting drug PK (figure 7).

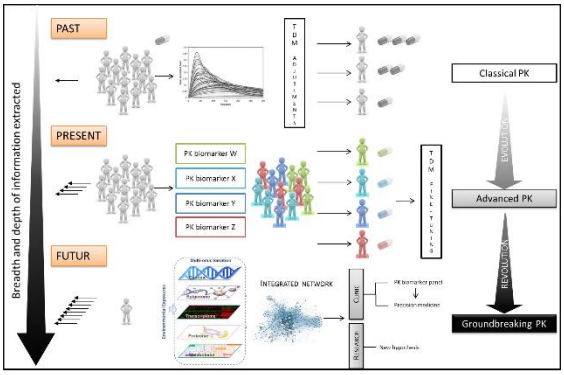

Figure 7: *Evolution of PK and future prospects.*

SELECTED PUBLICATIONS
Laure ELENS

Jean-Baptiste Woillard, Michel Mourad, Michael Neely, Arnaud Capron, Ron H. van Schaik, Teun van Gelder, Nuria Lloberas, Dennis A. Hesselink, Pierre Marquet, Vincent Haufroid and Laure Elens. "Tacrolimus Updated Guidelines through popPK Modeling: How to Benefit More from CYP3A Pre-emptive Genotyping Prior to Kidney Transplantation" Front. Pharmacol., 08 June 2017 https://doi.org/10.3389/fphar.2017.00358.

Anne-Laure Sennesael, Nadtha Panin, Catherine Vancraeynest, Lucie Pochet, Anne Spinewine, Vincent Haufroid and Laure Elens. "Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines". Sci Rep. 2018 Jul 12;8(1):10514.

Nuria Lloberas, Dennis A Hesselink, Ron HN van Schaik PhD, Josep M Grinyò LD, Helena Colom, Teun van Gelder and Laure Elens. "Detection of a rare CYP3A4 variant in a transplant patient characterized by a Tacrolimus poor metabolizer phenotype". Pharmacogenomics. 2018 Mar;19(4):305-310.

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Elens L., Vandercam B., Yombi J-C., Lison D., Wallemacq P., Haufroid V. Influence of host genetic factors on efavirenz plasma and intracellular pharmacokinetics in HIV-1-infected patients. Pharmacogenomics (2010), 11: 1223-34.

THESES IN PROGRESS

Degraeve Alexandra: "Tacrolimus pharmacokinetic pathway and microbiota: study of the complex bidirectional partnership for explaining metabolic variability and modulations".
Directors: Laure Elens, Laure Bindels (MNUT)

Stillemans Gabriel: "Optimization of Darunavir therapy through population pharmacokinetic modeling, simulations and dosage guidelines".
Directors: Laure Elens, Vincent Haufroid (IREC)

Ngougni Pokem Perrin: "Therapeutic monitoring of beta-lactams antibiotics to improve their efficacy".
Directors: Françoise Van Bambeke, Laure Elens

Clinical Pharmacy (TFAR - CLIP)

Our research focuses on the epidemiology and the quality of use of medicines in clinical practice, including the detection of inappropriate prescriptions and drug related admissions, and the individualisation of specific drug treatments.

In particular, our work is performed in different practice settings and focuses on high risk populations (older people, patients with chronic diseases, patients in intensive care), high risk medications (anticoagulants, antibiotics), and high risk situations (polymedication, multimorbidity, infections, and patients transiting across settings of care).

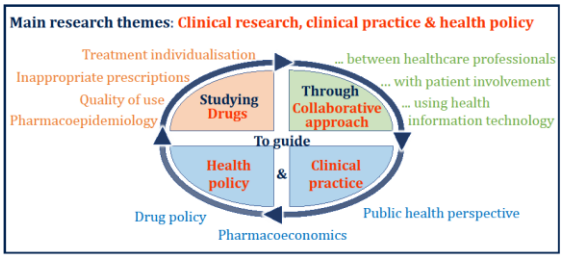
We use quantitative as well as qualitative research methods, and we:

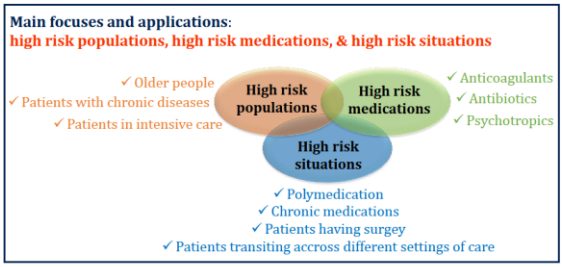
- *develop and/or validate instruments and tools to measure the quality of use of medicines;*
- *collect and use observational data for pharmacoepidemiological reasearch to assess the appropriateness of prescription and use of drugs, as well as their effects on patients in daily practice to optimise and individualize treatments;*
- *perform qualitative studies and/or surveys to identify the determinants of suboptimal practice and to evaluate patients' attitudes;*
- *design, implement and evaluate various approaches for optimisation, that address the causes of suboptimal practice. Evaluation usually involves using (quasi)-experimental designs, continuous quality improvement studies and observational studies;*
- *conduct systematic reviews on the effect of approaches for optimisation.*

Our three group leaders (Anne Spinewine, Olivia Dalleur and Séverine Henrard) have part-time activities in other settings (clinical appointment at UCL teaching hospitals, or other research institute). This feature brings a singular dynamic to our group and is a strength to elaborate a sound research group strategy, to reinforce leadership, facilitate development, collaboration and raise funding.

« Optimising the use of medicines in daily practice is central to the quality of patient care »

The **Clinical Pharmacy Research Group** performs innovative multidisciplinary scientific research closely linked to clinical practice and pharmacy education





Main research settings:
✓ Hospitals ✓ Community pharmacies ✓ Nursing homes ✓ General practice

Main workforce:
Multidisciplinary team:
✓ Clinical and hospital pharmacists
✓ Medical doctors (geriatrician,...) & nurses
✓ Statisticians & epidemiologists
✓ Public health experts

Main research methods:
✓ **Quantitative methods** adapted to
✓ Clinical trials
✓ Observational studies
✓ Big data
✓ **Qualitative methods**

RESEARCH RESULTS AND PERSPECTIVES

a) Quality of use of medicines in older people

The use of medicines is a fundamental component of the care of older people, but inappropriate prescribing – in the form of over-, mis- or under-prescribing – is frequent. This causes substantial morbidity, impairs quality of life for patients and increases costs for the society. Measuring appropriateness of prescribing in older people is complex, and we develop methods for better measuring inappropriate prescribing and its adverse consequences. We then use these methods to describe the prevalence of inappropriate prescribing in various settings. By exploring the reasons underlying inappropriate use and the patient's perspectives, we aim to identify important factors that need to be taken into account when designing approaches for optimization. Finally, we implement and evaluate the effect of approaches for optimization.

- ***OPERAM***

The OPERAM project is a European project (H2020, 2015-2020) led by University of Bern. The core part of the OPERAM project is a large-scale **cluster RCT** to evaluate the effect of a complex intervention on drug-related admissions and other clinical and patient-reported outcomes. The intervention comprises clinical decision support using an electronic system called STRIPA, and medication review performed by a geriatrician and a clinical pharmacist. Patient recruitement was completed in Octobre 2018, and almost 400 patients were recruited from the Belgian site (Cliniques universitaires Saint-Luc). Patient follow-up was completed in October 2019. Final results will be available in 2020.

As leaders of the work package on "clinical outcomes and patient perspective", we have (a) developed a core outcome set (COS) for clinical trials of medication review in older patients with multi-morbidity and polypharmacy (b) developed a method to adjudicate drug-related admissions in older people (c) conducted a substudy about the patients' experience of medication review.

(a) A **COS** defines a minimum set of outcomes to be reported in all clinical trials in a particular research area. A systematic review, interviews with older people and an international Delphi survey and consensus meetings were performed. Consensus was reached on seven outcomes, which constitute the core outcome set. These outcomes are measured in the context of the OPERAM trial. In 2019 we have collaborated with other OPERAM partners to operationalize measurement of these outcomes from the OPERAM database. We have also finalised an international Delphi survey to validate a list of potentially clinically significant drug-drug interactions in older people.

(b) The **DRA adjudication** guide was developed with international and multidisciplinary input. This is the first standardized instrument to identify DRA in multimorbid older persons. The method has been used for the measurement of the primary outcome measure of the OPERAM trial. Inter-rater reliability was evaluated in 2019. Comparison of this DRA adjudication guide with another method of DRA adjudication is ongoing.

(c) In 2019 we completed a multi-centre mixed methods study embedded in the OPERAM trial to explore older multi-morbid **patients' experience** of hospital-initiated medication changes. Semi-structured interviews and the Beliefs about Medicines Questionnaire were conducted with a purposive sample of 48 patients from the four European countries enrolled in the OPERAM trial. Interviews were analysed using the Framework approach. Quantitative data on the level of patient participation

from clinicians' perspectives were also collected. Despite positive attitudes of most patients towards medication review, we found that an interplay of factors may affect effectiveness of medication review. Results will be made available in 2020.

- ***COME-ON (nursing home setting)***

The COME-ON (Collaborative approach to Optimise MEdication use for Older people in Nursing homes) study, led in collaboration with KULeuven (Prof V Foulon), was a **multicentre cluster-controlled trial** set up in Belgian nursing homes, with the aim to evaluate the effect of a complex, multifaceted intervention on the appropriateness of prescribing of medicines for older people in Belgian nursing homes. The results on the primary and secondary outcomes, as well as detailed data on the process evaluation have been published in 2019.

In 2019 we continued to perform additional posthoc analyses on the Come-On database. In-depth evaluations of data on the use of **antidepressants** and of **benzodiazepines** have been completed. As for benzodiazepines, we found that use and potentially inappropriate prescribing were highly prevalent. Deprescribing occurred in one fourth of NHRs. Being in the intervention group was associated with higher odds of deprescribing, as compared to the control group. We have also evaluated appropriateness of prescribing in a subgroup of frail NHRs, using the **STOPPFrail** criteria. Finally, an evaluation of the occurrence of potentially clinically relevant **drug-drug interactions** is ongoing.

- ***Deprescribing***

We participated in a study led by the University of Limoges (Prof ML Laroche), whose aim is to validate a French version of the 'revised **Patients's Attitudes** Towards Deprescribing (rPATD)' questionnaire. Results are expected in 2020

In 2019, we have launched new research projects focusing on **benzodiazepine deprescribing in older people**. Several aspects are addressed, including: current practices in benzodiazepine deprescribing in the inpatient, outpatient and nursing home settings; patient as well as healthcare professionals' attitudes towards benzodiazepine deprescribing; and effect of an approach that includes patient participation and interprofessional collaboration.

b) Pharmacoepidemiology in older people and people with chronic diseases

This new research dimension is being developed by Séverine Henrard, who joined our research group in 2016.

- ***Heterogeneity of type 2 diabetes in older patients: pathophysiology and therapeutic implications***

The aim of the project is to assess the heterogeneity in older patients with type 2 diabetes, with the aim of improving the therapeutic management of the different profiles of patients. The first published paper of this project focused on the description of the heterogeneity of metabolic and pathophysiologic profiles of older type 2 diabetes patients using latent profile analysis.

This project, which started in 2017, is designed as a multidisciplinary translational investigation, gathering metabolic, bio-clinical and (pharmaco-)epidemiological approaches and is conducted in collaborations with clinicians from Saint-Luc University Hospital.

c) Use of oral anticoagulants

In 2019 we continued some research word on the challenges associated with the appropriate use of Direct oral anticoagulants (DOACs) and vitamin-K antagonists (VKA).

We systematically reviewed the impact of **computerized clinical decision support systems** (CDSS) and described CDSS features associated with success or failure. We found that CDSS might positively impact the use of oral anticoagulants in AF patients at high risk of stroke. The scope of CDSS should however evolve to assist prescribers in selecting the most appropriate and tailored medication. Efforts should also be made to improve the relevance of notifications and to address implementation outcomes

d) Use of anti-infective drugs

In collaboration with FACM (see other section of this report), we perform pharmacokinetic studies in specific patients populations (haemodialysis patients) in order to propose optimize therapeutic doses, and pharmacoepidemiological studies to evaluate the off-label use of specific antibiotics.

- ***Rational peri-operative use of antibiotics in Benin***

In 2016, we started an international collaboration with Université d'Abomey-Calavi to assess the impact of a multidisciplinary approach, including a clinical pharmacy intervention and germ resistance characterization, to rationnalize the use of antibiotics in c-section practice in Benin. In these women, our research team observed that less than one woman out for five receives appropriate antibioprophylaxis. Since 2018, this project is extended to digestive surgery. In 2019, the characterization of germs involved in surgery site infections and the exploration of healthcare professionals' perception of antibioprophylaxis and hand hygiene continued.

e) Clinical decision support

Health information technologies are important tools to explore for the quality and safety of use of drugs. In collaboration with the Cliniques universitaires Saint-Luc, our research team evaluates decision support for medical prescription and medication validation by the pharmacist.

f) Use of neuroleptics

In 2019, a new project started in collaboration with PMGK on the appropriate use of neuroleptics in patients suffering from schizophrenia. The expertise of CLIP on medication appropriateness, DRA, and deprescribing and the expertise of PMGK on pharmacokinetics and pharmacogenomics will be combined to prevent inappropriate polypharmacy and reach precision pharmacotherapy in these patients.

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Olivia DALLEUR

Thevelin S, El Mounaouar L, Marien S, Boland B, Henrard S, Dalleur O. Potentially Inappropriate Prescribing and Related Hospital Admissions in Geriatric Patients: A Comparative Analysis between the STOPP and START Criteria Versions 1 and 2. Drugs & Aging — Vol. 36, no. 5, p. 453-459 (2019).

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Cillis M, Spinewine A, Krug B, Quennery S, Wouters D, Dalleur O. Development of a tool for benchmarking of clinical pharmacy activities. Int J Clin Pharm. 2018;40(6):1462-73.

Thevelin S, Spinewine A, Beuscart JB, Boland B, Marien S, Vaillant F, Wilting I, Vondeling A, Floriani C, Schneider C, Donzé J, Rodondi N, Cullinan S, O'Mahony D, Dalleur O. Development of a standardized chart review method to identify drug-related hospital admissions in older people. Br J Clin Pharmacol. 2018;84(11):2600-14.

Desmedt S, Spinewine A, Jadoul M, Henrard S, Wouters D, Dalleur O. Impact of a clinical decision support system for drug dosage in patients with renal failure. Int J Clin Pharm. 2018;40(5):1225-33.

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Séverine HENRARD

Christiaens A, Hermans M, Boland B, Henrard S. Distinction of cardiometabolic profiles among people ≥ 75 years with type 2 diabetes: A latent profile analysis. BMC Endocr Disord. 2019;19(1):85.

van den Akker M, Vaes B, Goderis G, Van Pottelbergh G, De Burghgraeve T, Henrard S. Trends in multimorbidity and polypharmacy in the Flemish-Belgian population between 2000 and 2015. PLoS One. 2019;14(2):e0212046.

Paul J, Vaillant F, Vanden Bossche O, Perpersack T, Henrard S, Boland B. Orthostatic blood pressure drop and cardiovascular and psychotropic medication dosages in acutely ill geriatric inpatients. Acta Clinica Belgica — , p. 1-8 (2019)

Smeets M, Vaes B, Mamouris P, Van Den Akker M, Van Pottelbergh G, Goderis G, Janssens S, Aertgeerts B, Henrard S. Burden of heart failure in Flemish general practices: a registry-based study in the Intego database**.** BMJ Open. 2019 ;9(1):e022972.

Henrard S, Vandenabeele C, Marien S, Boland B, Dalleur O. Underuse of anticoagulation in older patients with atrial fibrillation and $CHADS_2$ Score ≥ 2: Are we doing better since the marketing of direct oral anticoagulants? Drugs Aging. 2017;34(11):841-50.

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Anne SPINEWINE

Anrys P, Strauven G, Roussel S, Vande Ginste M, De Lepeleire J, Foulon V, Spinewine A. Process evaluation of a complex intervention to optimize quality of prescribing in nursing homes (COME-ON study). Implement Sci. 2019 Dec 11;14(1):104

Strauven G, Anrys P, Vandael E, Henrard S, De Lepeleire J, Spinewine A, Foulon V. Cluster-Controlled Trial of an Intervention to Improve Prescribing in Nursing Homes Study. Journal of the American Medical Directors Association 2019 ;20:1404-11.

Marien S, Legrand D, Ramdoyal R, Nsenga J, Ospina G, Ramon V, Spinewine A. A User-Centered design and usability testing of a web-based medication reconciliation application integrated in an eHealth network**.** International Journal of Medical Informatics 2019;126: 138-146.

Beuscart JB, Knol W, Cullinan S, Schneider C, Dalleur O, Boland B, Thevelin S, Jansen P, O'Mahony D, Rodondi N, Spinewine A. International core outcome set for clinical trials of medication review in multi-morbid older patients with polypharmacy. BMC Med. 2018;16(1):21.

Spinewine A, Schmader KE, Barber N, Hughes C, Lapane KL, Swine C, Hanlon JT. Appropriate prescribing in elderly people: how well can it be measured and optimised? Lancet. 2007;370(9582):173-84.

AWARDS 2018-2019

Séverine Henrard
Award 2018 of the Belgian Society for Gerontology and Geriatrics for the best poster presentation at the 28th autumn meeting (Liège, Belgium).

Antoine Christiaens
Award 2019 of the best oral presentation at the PhD day of the thematic doctoral school public health, health, and society.

THESIS DEFENDED IN 2018-2019

Bastos Miranda Ana: "Towards optimization of temocillin exposure in haemodialysis patients: a translational apprpoach from bedside-to-bench-to-bedside".
Directors: Françoise Van Bambeke, Anne Spinewine

THESES IN PROGRESS

Christiaens Antoine: "Elderly-onset type 2 diabetes: description, pathophysiology, and therapeutical implications".
Directors: Benoit Boland, Séverine Henrard, Michel Hermans

Dohou Angèle: "Nosocomial infections in surgery and development of bacterial resistance: impact of the Belgian approach of clinical pharmacy to rationalize the use of antibiotics in Benin".
Directors: Olivia Dalleur, Françoise Van Bambeke.

Evrard Perrine: "Deprescribing benzodiazepines in the nursing home setting".
Directors: Anne Spinewine, Séverine Henrard

Fiogbe Dessièdé Ariane: "Multidisciplinary strategy for the prevention and control of health-care associated infections in digestive surgery".
Directors: Olivia Dalleur, Françoise Van Bambeke

Lagreula Juliette: "Optimizing pharmacotherapy of antipsychotics in clinical daily practice: Moving towards individualized care".

Directors: Olivia Dalleur, Laure Elens

Pétein Catherine: "Assessing older adults' attitudes towards deprescribing benzodiazepines and z-drugs (in the ambulatory setting)'
Directors: Séverine Henrard, Anne Spinewine

Sibille François-Xavier: "Deprescribing benzodiazepine receptor agonists in hospitalized older patients: opportunities and challenges (DeBeHOP)'
Directors: Marie de Saint-Hubert, Anne Spinewine

Thevelin Stefanie: "Medication review to prevent avoidable hospital admissions in the multimorbid elderly".
Directors: Olivia Dalleur, Anne Spinewine

Thirot Hélène: "Study of risks associate with the off-label use of antibiotics".
Directors: Françoise Van Bambeke, Anne Spinewine

Yehouenou Carine: "Surgical Site Infections in Benin: description of hospital hygiene practices and molecular mechanisms of resistance of associated germs".
Directors: Olivia Dalleur, Anne Simon

Advanced Drug Delivery and Biomaterials (ADDB)

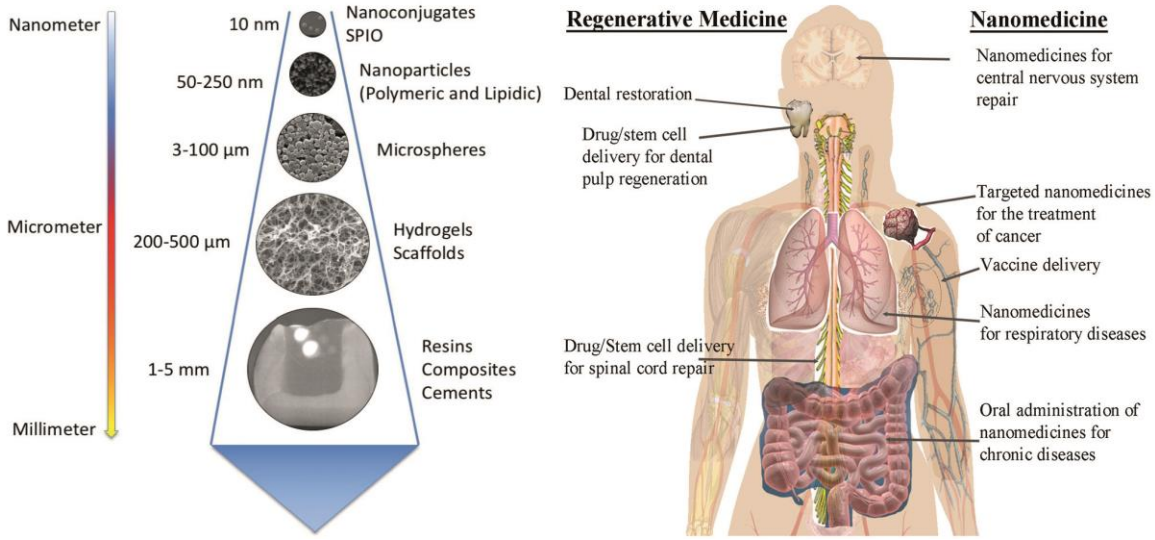

Figure 1: A. Drug delivery systems and biomaterials developed/used by the ADDB group; B. Biomedical applications targeted by the ADDB group.

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Advanced Drug Delivery and Biomaterials (ADDB)

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Bozzato E.
Buya A.
Fourniols Th.
Gilli M.
Gratpain V.
Hardy C.
Hollaert T.
Labrak Y.
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The objective of our research is to use drug delivery systems and biomaterials as a mean to improve therapeutic outcomes of drugs. We develop drug delivery systems going from nano-scale, through micro-scale up to macro-scale (Figure 1A).

Our different research applications can be gathered into two research themes (Figure 1B):

1. Nanomedicines:

a. Cancer: this theme focuses on targeted theranostic nanoparticles loaded with anticancer drugs or siRNA, adjuvanted recombinant antigens and gene vaccines.

b. Mucosal delivery routes: this theme includes the research on oral delivery using nanomedicines, cutaneous delivery and pulmonary delivery.

c. CNS diseases: the objective is to develop nanomedicines that would stimulate CNS repair by local, systemic, or mucosal delivery.

2. Regenerative medicine: *this theme focuses on tissue regeneration and restoration and gathers the research on spinal cord regeneration, dental restoration and skin wound healing.*

1) NANOMEDICINES FOR TARGETED OR LOCAL DRUG DELIVERY FOR CANCER TREATMENT (V. PRÉAT)

Our research mainly focuses on (i) intravenous delivery of drug-loaded of nanoparticles targeting the tumoral endothelium and cancer cells ii) local delivery of anticancer drugs.

Several main mechanisms of delivery of drug-loaded nanoparticles to tumors have been reported (*Figure 2*): (i) passive targeting through leaky vasculature surrounding the tumors, described as the enhanced permeability and retention effect (EPR) (ii) "active" targeting by grafting specific ligands of cancer cells or angiogenic endothelial cells to the surface of the nanocarrier (iii) magnetic targeting of SPIO (small paramagnetic iron oxides) loaded nanoparticles. We formulated various nanocarriers (micelles and untargeted or targeted nanoparticles) loaded with several anti-cancer drugs to specifically target tumors and improve the therapeutic index of anti-cancer drugs by nanomedicine. For example, PLGA-based nanoparticles formulated for the delivery of paclitaxel, a new cyclin dependent kinase inhibitor and doxorubicin induced a higher regrowth delay of tumors *in vivo* than free drugs. Exploiting the $\alpha_v\beta_3$ integrin overexpression by tumoral endothelium and tumor cells, we designed PLGA-based nanoparticles grafted with the RGD peptide and demonstrated the "active" targeting of these PLGA-based nanoparticles. We formulated multi-functional nanoparticles for the encapsulation of a therapeutic drug and a contrast agent (SPIO) that can be targeted by magnets and significantly enhanced drug biodistribution and tumors.

Anticancer drug-loaded nanomedicines are developed for the local treatment of glioblastoma. In particular lauroyl gemcitabine forming hydrogel significantly and photopolymerized hydrogel loaded with anti cancer drugs improved the survival of glioblastoma bearing mice when perisurgically injected in the resection cavity.

Our current projects are focussed on the mechanisms of action of nanomedicines, in particular their effect on the tumor microenvironment.

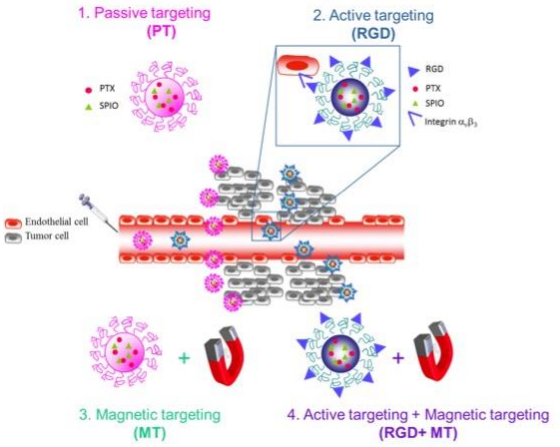

Figure 2: Passive, active and magnetic targeting of anticancer drug-loaded nanomedicines

We also aim to develop formulation (nanoparticles) and physical methods (electroporation) for the delivery of DNA and RNA with a particular interest in vaccination and cancer treatments (*Figure 3*). Electroporation of DNA was optimized to deliver plasmid vaccines into the skin or the muscle. This potent delivery method allows high level of expression. Optimised plasmids encoding tumor antigens elicited humoral and cellular immune response and induced tumor control or regression. Our current research focuses now on the combination of optimized anticancer DNA vaccines and immune checkpoint inhibitors.

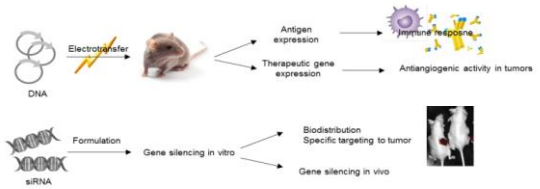

Figure 3: Plasmid DNA and siRNA delivery

2) NANOMEDICINES FOR ORAL DELIVERY (A. BELOQUI GARCÍA)

The oral route is the most preferred route of drug administration. It is easy to administer, pain free and cheaper compared to other routes of administration. However, this route is sometimes inefficient due to the partial/inadequate absorption of the drug, first-pass metabolism, the instability of the drug in harsh gastrointestinal conditions (such as intestinal pH or enzyme degradation). There is an unmet need for the administration of biologicals via the oral route of administration, especially in the treatment of chronic diseases where a daily painful administration is required.

The aim of our research is developing improved alternative drug delivery systems to fulfill the potential of the oral route of administration. For this purpose, we are exploiting the unique pathophysiology of the gut towards the development of novel drug delivery strategies, focusing on the treatment of two main chronic diseases: type 2 diabetes mellitus and inflammatory bowel diseases. Our recent results have shown the ability of lipid-based nanocapsules to induce endogenous GLP-1 and GLP-2 secretion (Figure 4).

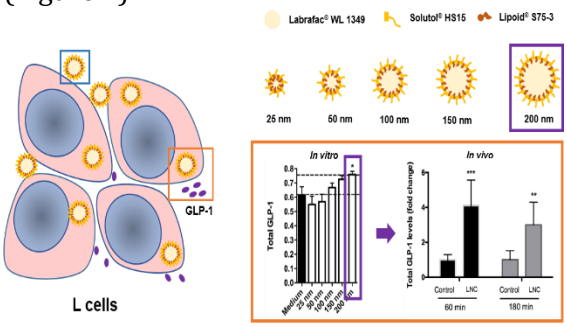

Figure 4: Size effect on lipid nanocapsule-mediated GLP-1 secretion from enteroendocrine L cells (from Xu et al, Mol. Pharm., 2018)

Unraveling the mechanisms of nanoparticle transport across the intestinal barrier is essential for designing more efficient nanoparticles for oral administration. For this purpose, we have development *in vitro* models of the intestinal epithelium and follicle-associated epithelium containing M cells to evaluate the mechanisms of transport of our drug delivery systems at the intestinal site. In concrete, we study the physicochemical parameters that dictate the fate of the drug delivery systems across the intestinal barrier (Figure 5).

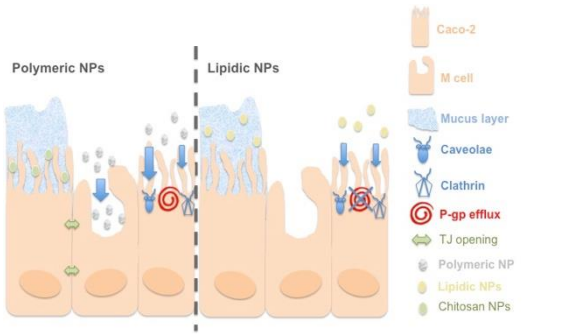

Figure 5: Polymeric versus lipidic NP mechanisms of transport across the intestinal barrier *(from Beloqui et al, Adv. Drug Deliv. Rev., 2016)*

3) NANOMEDICINES FOR PULMONARY DELIVERY (R. VANBEVER)

The research aims at improving the treatment or prophylaxis of severe respiratory diseases by designing nanomedicines to enhance the local efficacy of drugs. Our approaches include i) the preparation of polyethylene glycol (PEG)-drug conjugates to sustain drug release within the lung, and ii) the formulation of nanocarriers to target vaccines to lung dendritic cells.

Inhalation aerosols offer a targeted therapy for respiratory diseases. However, the therapeutic efficacy of inhaled drugs is limited by their rapid clearance from the lung (*Figure 6*).

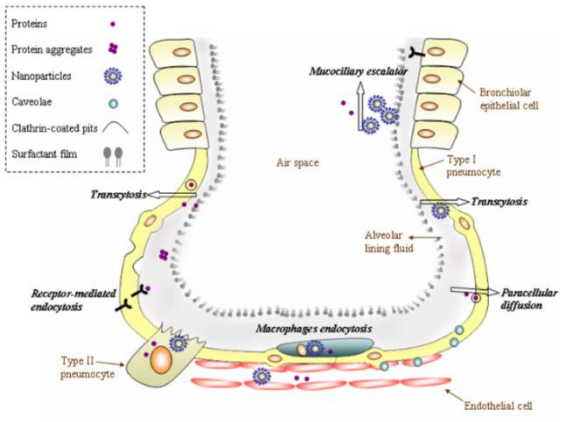

Figure 6: Schematic view of the fate of drugs in the lungs (from Todoroff & Vanbever, Curr. Opin. Coll. Interf. Sc., 2011)

Anti-IL17 and anti-IL13 antibody fragments were conjugated to a large PEG chain and conjugation was shown to greatly prolonge the presence of these fragments within the lung of mice, rats and rabbits. The prolonged pulmonary residency of the anti-IL-17 PEG-F(ab')$_2$ translated into an improved efficacy in reducing lung inflammation in a murine model of house dust mite-induced lung inflammation. The molecular weight of PEG, the nature of the antibody fragment and the site of delivery within the respiratory tract had an impact on the residence time of antibody fragments in the murine lung. PEGylated proteins were principally retained within the lung lumen rather than the nasal cavities or lung parenchyma. PEG increased the pulmonary retention of antibody fragments through mucoadhesion, reduced uptake by alveolar macrophages and epithelial cells rather than increased hydrodynamic size or improved enzymatic stability. A PEGylated Fab' antibody fragment and 40kDa PEG alone did not induce any lung toxicity following acute or chronic pulmonary administration of high doses in mice.

We have also applied this PEGylation strategy to recombinant human deoxyribonuclease I (rhDNase). rhDNase is the mucolytic agent most widely used for the treatment of respiratory disease in cystic fibrosis. However, rhDNase I is rapidly cleared from the lungs, which limits its therapeutic efficacy and implies frequent dosing. rhDNase was monoPEGylated on its N terminal residue and the conjugated enzyme preserved the full enzymatic activity of the native protein both on DNA solutions and cystic fibrosis sputa. PEGylated rhDNase was retained in the lungs for more than 15 days, compared to a few hours for unconjugated rhDNase. The sustained presence of PEGylated rhDNase in the lung presents interesting perspectives for the development of a long-acting rhDNase with a

reduced therapy burden for patients with cystic fibrosis.

We developped liposomes for targeting vaccines to lung dendritic cells. Nanoliposomes were prepared with cationic lipids presenting immunostimulatory capacities. These formulations were shown to successfully co-encapsulate both antigenic peptides and adjuvants with high loading efficiency. Nanoliposomes encapsulating calcein as a tracer were mainly taken up by alveolar macrophages following delivery to the lungs in mice. Few dendritic cells took up the liposomes, and interstitial macrophages did not take up liposomal calcein more than they took up soluble calcein. Stimulation of the innate immune system using liposomal CpG strongly enhanced uptake of calcein liposomes by all phagocytes in the lungs.

4) ADVANCED DRUG DELIVERY FOR CENTRAL NERVOUS SYSTEM (A. des RIEUX)

We develop nanomedicines for the central nervous system (CNS) repair. Our objective is to stimulate brain repair by either recruiting neural stem cells (NSC) at the site of injury, stimulating their differentiation and/or resolving inflammation.

Regarding NSC recruitment, we developed SDF-1 PLGA nanoparticles that, once implanted at the site of a traumatic brain injury, were able to recruit NSC at the damaged area (L. Zamproni, Universidade Federal de Sao Paulo, BR).

We also showed, in collaboration with P. Saulnier and J. Eyer (Université d'Angers, FR) that by modifying the surface of lipidic nanocapsules (LNC) with a peptide (NFL), we were able to specifically target NSC of the brain and to stimulate their differentiation toward the oligodendrocyte lineage (Thesis of D. Carradori) (*Figure 7*).

Our ongoing projects focus on new nanomedicines aiming at stimulating the differentiation of oligodendrocyte progenitor cells and resolving inflammation in the brain, more particularly in the scope of multiple sclerosis.

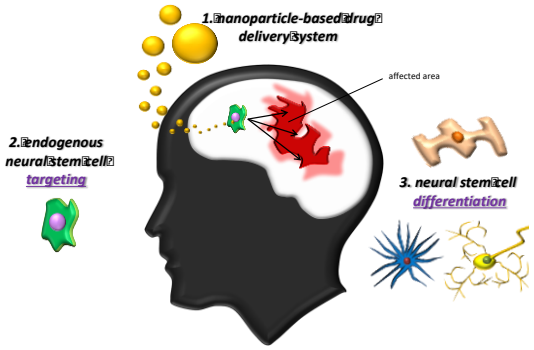

Figure 7: Targeted drug delivery for CNS repair.

5) DRUG AND CELL DELIVERY FOR TISSUE ENGINEERING (A. des RIEUX)

The research aims at developing implants (hydrogels, polymeric scaffolds, microcarriers) delivering growth factors, drugs and cells that provide sustained delivery of bioactive molecules, support survival, infiltration and proliferation of cells for tissue engineering, and in particular spinal cord injury.

Our group has gained expertise in drug delivery to the spinal cord that we combined with transplantation of adult mesenchymal stem cells; more particularly human dental stem cells. Indeed, human dental stem cells display superior neural stem cell properties than bone marrow-derived mesenchymal stem cells since they originate from the neural crest.

We have first evaluated the impact of growth factor delivery encapsulated in micro- and nanoparticles from injectable hydrogels. Then, we decided to explore the therapeutic potential of stem cells from the apical papilla (SCAP) for spinal cord injury. We tested different ways of administration in rat spinal cord injury models.

1. Sustained delivery of growth factors from injectable hydrogel

We evaluated the effect of VEGF and GDNF delivery, free or encapsulated, from an alginate:fibrinogen hydrogel injected in a rat spinal cord hemisection model. Local VEGF delivery from alginate:fibrinogen hydrogel gelifying *in situ* induced angiogenesis and neurite growth but no functional improvement. However, local GDNF delivery significantly improved functional recovery of rats. Indeed, the animals treated with free GDNF-loaded hydrogel experienced superior functional recovery compared to the animals treated with GDNF microsphere-loaded hydrogels and non-treated animals *(in collaboration with Prof. Blanco-Prieto, Navarra University, Spain, Drs Schakman and Deumens, UCL, IoNS).*

2. Stem cell delivery

As a source of human mesenchymal stem cells, we selected human dental stem cells of the apical papilla (SCAP) due to their neural crest origin but also because they are easily accessible (obtained from extracted wisdom tooth roots). They express numerous neuronal markers, display enhanced neural stem cell properties compared to bone marrow-derived mesenchymal stem cells and possess higher proliferation and differentiation rates compared to dental pulp stem cells. The studies performed on SCAP have been done in collaboration with Prof. Diogenes (San Antonio University, San Antonio, USA).

We used 3 strategies to deliver SCAP: in their original niche (apical papilla), incorporated in hydrogels or seeded on PLGA microcarriers (*Figure 8*).

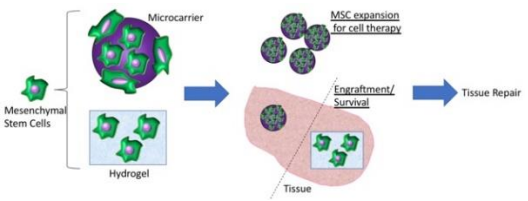

Figure 8: SCAP delivery strategies.

a. Implantation of a whole papilla in a spinal cord lesion

We hypothesized that isolating and expanding SCAP would change their properties and characteristics while keeping them in their niche would not. When rats were treated with a human apical papilla implanted as a whole, we observed a significant improvement of motor function compared to the control groups (lateral hemisection model) *(collaboration with Prof. Leprince, LDRI, UCL) (Figure 9)*. This might be explained by injury stabilization (papilla still in place after 6 weeks) and by the action of the cells present in the papilla (cells positive for human mitochondria in the papilla after 6 weeks).

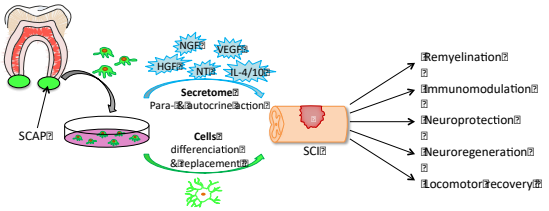

Figure 9: Therapeutic potential of SCAP for spinal cord repair.

b. Incorporation of SCAP in hydrogels

To deliver and maintain SCAP at the lesion site, we have selected injectable hydrogels (fibrin, alginates and Corgel®). No study previously compared the impact of hydrogel properties on SCAP *(collaboration with Prof. Dupont, IMCN, UCL)*. We observed that fibrinogen concentration in fibrin hydrogel impacted SCAP neurodifferentiation *in vitro,* but also proliferation and angiogenesis *in vivo*. When comparing different alginates and Corgel®, not a single property, but the appropriate combination of surface and mechanical characteristics dictates SCAP fate.

We also studied the influence of decellularized extracellular matrix-based hydrogels (dECMh) originating from different organs (bone, dentin and spinal cord) (*Erasmus Mundus NanoFar, collaboration with Prof. Shakesheff and Dr.*

White, University of Nottingham, UK). dECMh are thermosensitive (gelation at 37°C), contain preserved cell adhesion sites and active molecules specific of the organ of origin. We demonstrated that dECMh origin impacted hydrogel properties and SCAP viability and neuronal gene expression, spinal cord dECMh being the most favorable for neural differentiation.

c. Development of growth factor loaded microcarriers for SCAP delivery

Another strategy to deliver cells is to seed them on microcarriers designed to support cell adhesion and viability and to deliver growth factors. In the scope of an Erasmus Mundus project, we co-supervised a PhD thesis with Prof. Montero-Menei (*Angers University, FR*) that aimed to optimize the formulation of BDNF-loaded pharmacologically active microcarriers (PAM). We demonstrated that PAM supported the viability of mesenchymal stem cells and impacted their secretome and proteome. BDNF-PAM and SCAP were then combined and injected in a spinal cord contusion model. An improvement of rat locomotor function, a decrease of inflammation and neuroprotection were observed when SCAP where implanted associated with BDNF-PAM.

6) DENTAL REGENERATIVE and INNOVATIVE MATERIALS (G. LELOUP, J. LEPRINCE)

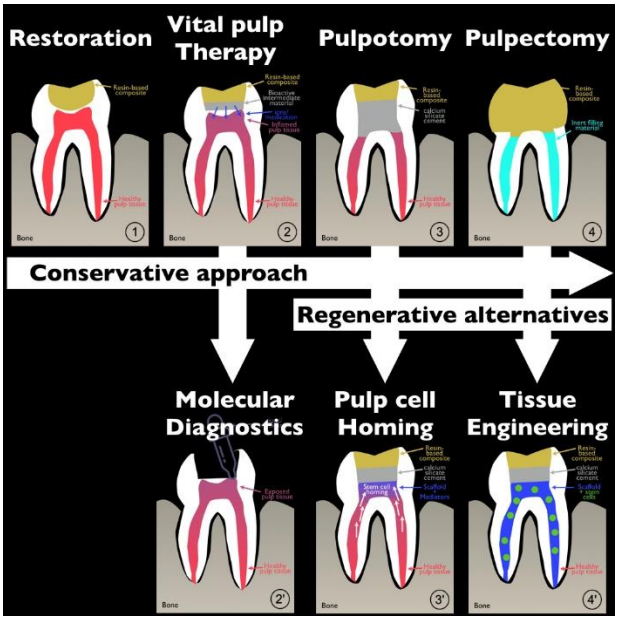

Figure 7: Current approaches for the treatment of tooth decay.

1) In case of tooth decay and a healthy pulp, a resin-based composite is used. 2) In case of tooth decay with an inflamed pulp, a calcium-silicate cement is applied on the pulp before the resin-based composite. 3) When the pulp is partially necrotic, this part is removed and replaced by a combination of calcium-silicate cement and resin-based composite. 4) When the complete pulp is necrotic, it is completely removed and replaced by inert filling material. Novel approaches for the treatment of tooth decay. 3') When the pulp will be partially removed, it will be replaced by a hydrogel loaded with growth factors in order to attract stem cells from the remaining pulp. 4') When the pulp will be completely removed, it will be replaced by a hydrogel loaded with stem cells in order to re-create a new dental pulp tissue.

In the treatment of tooth decay, restorative dental materials are required to exhibit excellent mechanical, biological properties and most uniquely, display good aesthetics. The research carried out focuses 1) on the characterization of currently available commercial materials, in relation with clinical requirements and 2) on developing new biomaterials for tooth restoration, from a conventional conservative but also from a more advanced regenerative standpoint.

7) STRATEGIES AND RESULTS

Conservative approach

The use of restorative materials allows for relatively fast treatments as they may be implemented directly in the oral cavity in a matter of minutes. They are also highly versatile. However several concerns exist with regards to the suitability of some materials in terms of mechanical or biological properties. Additionally the very mechanisms responsible for the setting of materials or interactions with the biological are little understood.

a) In vitro-methods

We are continuously invested in determining the most suited set of characterization methods to properly analyze both mechanical and biological properties of commercial materials, leading to innovative experimental research. Our previous results describe the setting kinetics and mechanical properties of ultra-fast polymerizing resin composites, based on a monoacylphosphate photoinitiator and bioactive calcium silicate cements. In collaboration with Pr. Möginger (University of Bonn-Rhein, Germany) and Pr. Will Palin (University of Birmingham, UK) an innovative combination of characterization techniques was set up, allowing for a precise analysis of polymerization kinetics in heavily filled composites. Moreover, the group has been recently awarded a grant to acquire a Raman spectrometer, to enable chemometric analyses, which nicely complements the previous developments.

b) In vitro-material development

The formulation of resin composites is fine-tuned (photoinitiator, resin composition, etc) to quicken kinetic, increase longevity and bring mechanical properties close to that of hard tissues. The use of micro hydroxyapatite particles and amorphous CaP nano particles is investigated for the release of Ca^{2+} and PO_4^{2-} with antibacterial and re-mineralizing potential. The impact of their introduction in model formulations on kinetics and mechanical properties is studied. Additionally, ceramics are investigated for their use as alternatives of resin composites following root canal treatment (Figure 6, item 4). Finally, we are currently working on the incorporation of anti-inflammatory drugs in tricalcium silicate cements (Figure 6, item 2) to modulate pulp inflammation and push the borders of vital pulp therapy.

c) In vitro-material/cell interactions

The interactions with pulp tissues and oral commensal bacteria are also researched. The potential of apatite-loaded resin composites is being evaluated, aiming both at *S.mutans/S.gordonii* biofilm reduction and increased dental pulp stem cells (DPSC) viability (induced osteo-differentiation is also analyzed). Further, as resin composites do not polymerize completely, the toxicity of monomers and un-reacted compounds on DPSC is investigated. Even in the absence of toxicity, some monomers may still induce oxidative stress and genotoxic effects. Methods are being developed to quantify ROS production and osteo-differentiation inhibition on a large number of samples. Again, the addition of the new Raman spectrometer will help characterize the resulting modifications in mineralized matrix produced by the DPSCs and/or the odontoblasts.

d) Clinical work

As a result of strong collaborations with the dental clinics, several studies are currently under way, focusing on the analysis of the suitability of resin composites for the treatment of large cavities, in a retrospective manner. Another study underway was designed to investigate prospectively the suitability of a pulpotomy strategy (more conservative approach) as permanent treatment in molars with irreversible pulpitis (Figure 6, item 3), which are currently treated by root canal therapy.

Regenerative approach

In modern dentistry, there is currently a paradigm shift from restorative procedures to strategies based on regenerative medicine. In this context, alternatives to current clinical restorative strategies where pulp tissue is partially or completely lost (irreversibly inflamed and necrotic dental pulps) must we designed by combining bioactive matrices and dental stem cells in a clinically relevant way.

a) Cells

Dental stem cells are mesenchymal stem cells that may be collected in large amounts from dental tissues. Such cells display a higher proliferation rate than bone marrow stem cells and have better neural and epithelial properties as they originate from the neural crests. Additionally dental stem cells can differentiate in multiple cells types, like osteo- odonto-, adipo-, neuro-, chondroblast-like cells… Among dental stem cells, we selected dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP) for their potential. While we have worked with SCAP (RP89 cell line), originating from one patient and obtained from Dr. Diogenes (University of Texas, USA), we recently created a pool of DPSC and SCAP from 10 different patients. These cell pools will be fully characterized by cell-surface markers analysis, by differentiation potential and by stem cell gene expression and used as an internal standard for all of our work. Such efforts will allow us to have a much genetically diverse and relevant cell source.

b) Scaffold

For the regenerative approach, cells must be properly delivered. The design of an "ideal" bioactive matrix is thus necessary. This one would be biocompatible, injectable and would ideally resemble the native pulp tissues in terms of mechanical properties and allow cell invasion, survival and proliferation. Therefore, we will test *in vitro* different hydrogels, which will be provided through different collaborations (Prof. Anne des Rieux, UCL; Prof. Berit Strand, NTNU, Norway; Prof. Patrick Henriet, UCL; Prof. Christine Dupont, UCL).

Fibrine/Alginate hydrogels are currently being investigated, testing for DPSC attachment and viability on the medium-term. Once an « ideal » bioactive matrix is designed, it will be implemented in two different regenerative strategies and tested *in vitro*/*in vivo*:

-Dental pulp stem cell homing from residual dental pulp tissue in case of *partial* pulp tissue removal, through the injection of a bioactive scaffold loaded with factors like SDF1, bFGF and TGF-B (Figure 6, item 3'),

-Exogenous dental pulp stem cell delivery in case of *complete* pulp tissue loss, to regenerate the lost tissue volume into a vascularized, innervated and functional de-novo dentin-pulp complex (Figure 6, item 4').

Molecular diagnostics

The tools currently available to the dentists for diagnostics purposes are limited. The extent of pulp and periapical inflammation are currently evaluated using mechanical and thermal stimuli, which are not enough reliable and have low level of evidence. A promising approach to better diagnose the inflammatory conditions of the pulp and periapical tissues in vital pulp therapy and endodontic treatments is to quantify the level of expression of pro-inflammatory and pro-resolution molecules. We are developing an in vitro and in vivo model to achieve these goals, in collaboration with Pr. Yusuke Takahashi (University of Osaka, Japan). Future strategies could be planned based on in-situ readings of such levels, leading to improved diagnostics and better patient care.

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AWARDS 2019

Highly cited researcher: Véronique Préat

Bastiancich Chiara: "Lauroyl-gemcitabine lipid nanocapule hydrogel for the local treatment of glioblastoma", Prix de thèse « formulation galénique » de l'académie nationale de pharmacie (France)

Beloqui Ana : Prix Paul Van de Velde. Amount: 2.500 €

Vandermeulen Gaëlle : Prix Cornélis-Lebègue (2016-2018), Académie royale de Médecine de Belgique (Belgium),

THESES DEFENDED IN 2019

Ganipineni Pallavi: "Multifunctional poly(lactic-co-glycolic acid) based nanoparticles for targeting and treating glioblastoma**".**
Director: Véronique Préat (ADDB/LDRI)

Germain Loïc: "Macroporous scaffolds for tissue engineering: 3D printing & modification of the surface mechanical properties".
Directors: Anne des Rieux (ADDB/LDRI); Christine Dupont-Gillain (IMCN)

Lopes Alessandra: "From molecular optimization to therapy combination: novel strategies to improve cancer DNA vaccines".
Director: Véronique Préat (ADDB/LDRI); Co-director: Gaëlle Vandermeulen

Zhao Mengnan: "Anticancer drug-loaded photopolymerizable hydrogel for the treatment of glioblastoma".
Director: Véronique Préat (ADDB/LDRI)

THESES IN PROGRESS

Beauquis Julien: "Understanding and management of the mechanisms of pulp inflammation".
Director: Gaëtane Leloup (ADDB/LDRI); Co-Director: Julian Leprince (ADDB/LDRI)

Bausart Mathilde: "Combined local chemotherapy and immunotherapy with systemic vaccination for the treatment of glioblastoma".
Director: Véronique Préat (ADDB/LDRI); Co-Director: Gaëlle Vandermeulen

Bozzato Elia: "Prevention of glioblastoma recurrence by injection in the resection cavity of a hydrogel formed by targeted lipid nanocapsules loaded with anticancer drugs".
Director: Véronique Préat (ADDB/LDRI); Co-Director: Chiara Bastiancich

Buya Aristote: "Self-nanoemulsifying systems for simultaneous oral delivery of antisickling agents".
Director: Véronique Préat (ADDB/LDRI); Co-Director: Patrick Menvanga (Universté de Kinshasa)

Conq Jérôme: "Strategies for a transient opening of the blood brain barrier to increase the delivery of nanomedicines to glioblastoma".
Director: Bernard Gallez (REMA/LDRI); Co-Director: Véronique Préat (ADDB/LDRI)

Fourniols Thibaut: "Targeting tumor microenvironment by a translational nanomedicine, toward an improve of colorectal cancer therapy".
Director: Véronique Préat (ADDB/LDRI)

Gilli Mathieu: "Paving the way for tomorrow's dentistry with hydrogel-based strategies for pulp tissue regeneration".
Director: Gaëtane Leloup (ADDB/LDRI); Co-Director: Julian Leprince (ADDB/LDRI)

Gratpain Viridiane: "Non-invasive delivery of activin A-loaded extracellular vesicles to stimulate remyelination in the central nervous system".
Director: Anne des Rieux (ADDB/LDRI)

Hardy Chloé: "Optimisation of the bonded indirect dental restorations".
Director: Gaëtane Leloup (ADDB/LDRI); Co-Director: Julian Leprince (ADDB/LDRI)

Hollaert Thibaut: "Optimization of dentin-substitute materials".
Director: Gaëtane Leloup (ADDB/LDRI); Co-Director: Julian Leprince (ADDB/LDRI)

Labrak Yasmine: "Targeted nanomedicines to stimulate the differentiation of oligodendrocyte progenitor cells in the scope of multiple sclerosis".
Director: Anne des Rieux (ADDB/LDRI); Co-Director: Giulio Muccioli (BPBL/LDRI)

Lasserre Jérôme: "Evaluation of new strategies to control dental biofilms and related diseases".
Director: Michel Brecx (MEDE); Co-Director: Gaëtane Leloup (ADDB/LDRI)

Liu Xiao: "Development of a novel form of alpha1-antitrypsin for inhalation".
Director: R. Vanbever (ADDB/LDRI)

Mahri Sohaib: "Understanding the mechanisms involved in the lung retention of PEGylated proteins".
Director: R. Vanbever (ADDB/LDRI); Co-director: C. Bosquillon (U Nottingham)

Mwema Ariane: "Nose-to-Brain Delivery of Nanomedicines to stimulate remyelination in the scope of multiple sclerosis".
Director: Anne des Rieux (ADDB/LDRI); Co-Director: Giulio Muccioli (ADDB/LDRI)

Setbon Hugo : "Matériaux bioactifs en endodontie: aspects fondamentaux et applications cliniques".
Director: Gaëtane Leloup (ADDB/LDRI); Co-Director: Julian Leprince (ADDB/LDRI)

Tatic Natalja: "Modulation of macrophage phenotype for spinal cord repair".
Director: Anne des Rieux (ADDB/LDRI); Co-director: Lisa White (Univ. Nottingham)

Tsakiris Nikolaos: "Nanoplatform based drug delivery system: a combinational therapy against breast and colorectal cancer".
Director**:** Véronique Préat (ADDB/LDRI); Co-Director**:** Arnaud Vigneron (Centre du cancer, Lyon)

Wang Mingchao: "Nanomedicine for the treatment of glioblastoma
Director: Véronique Préat (ADDB/LDRI); Co-Director: Chiara Bastiancich (ADDB/LDRI)

Xu Yning: "Peptide-loaded nanoparticle oral delivery strategies towards diabetes treatment".
Director: Ana Beloqui (ADDB/LDRI); Co-Director: Véronique Préat (ADDB/LDRI)

Biomedical Magnetic Resonance (REMA)

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Biomedical Magnetic Resonance (REMA)

Post-Doctoral fellows

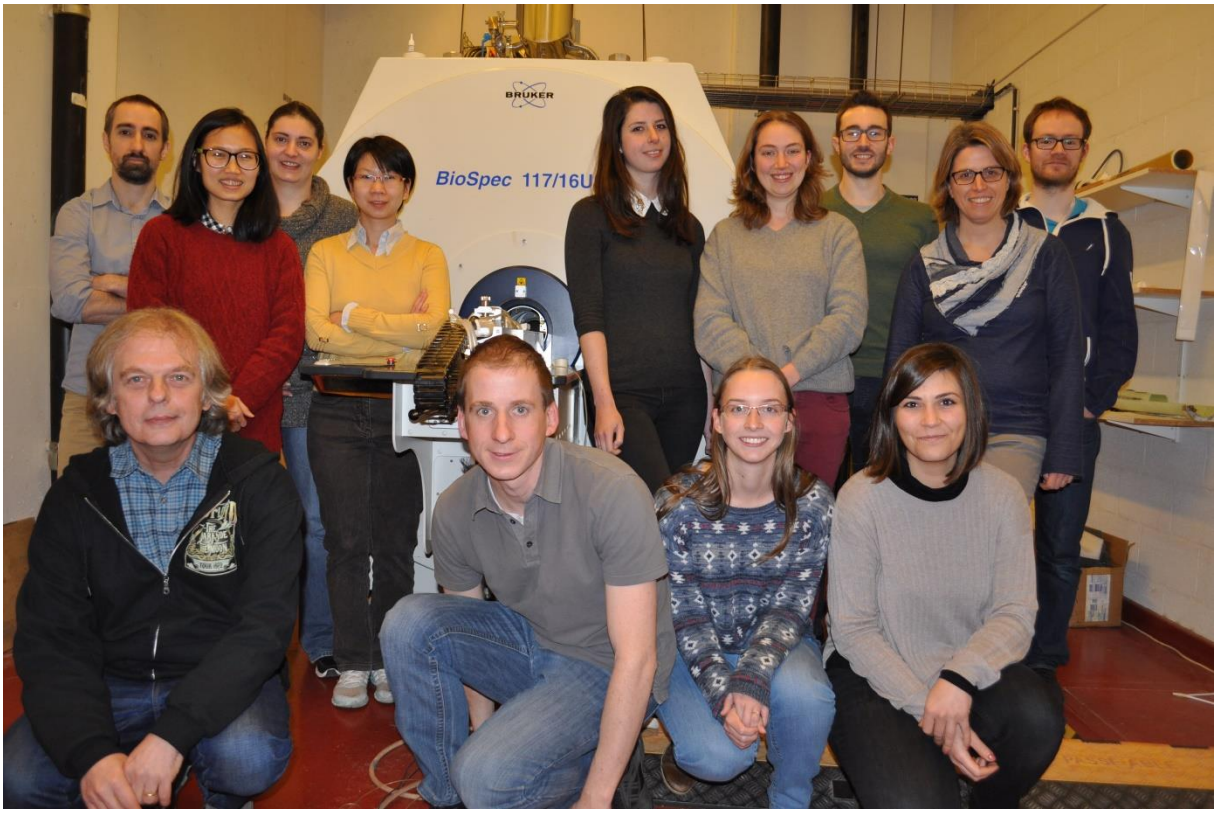
Desmet C.
Mignion L.

PhD Students

Acciardo S.
Cao Pham TT.
Conq J.
d'Hose D.
Farah C.
Gourgue F.
Nel J.
Schoonjans C.
Scheinok S.
Yelek C.

Adm. & Techn. Staff

Thibou D.
Picard V.



Website REMA: http://uclouvain.be/en/research-institutes/ldri/biomedical-magnetic-resonance-rema.html

Address: Van Helmont building – Tower 73, 4th floor, avenue E. Mounier 73, B1.73.08. B-1200 Brussels
Fax: +32 2 764 73 90

The goal of this research team is to carry out fundamental and pre-clinical research in biomedical magnetic resonance (NMR or Nuclear Magnetic Resonance, EPR or Electron Paramagnetic Resonance, and DNP or Dynamic Nuclear Polarization).

The research involves the development of innovative tools using advanced technologies, and the application of these tools to understand physiology and physiopathology, with a special interest in oncology.

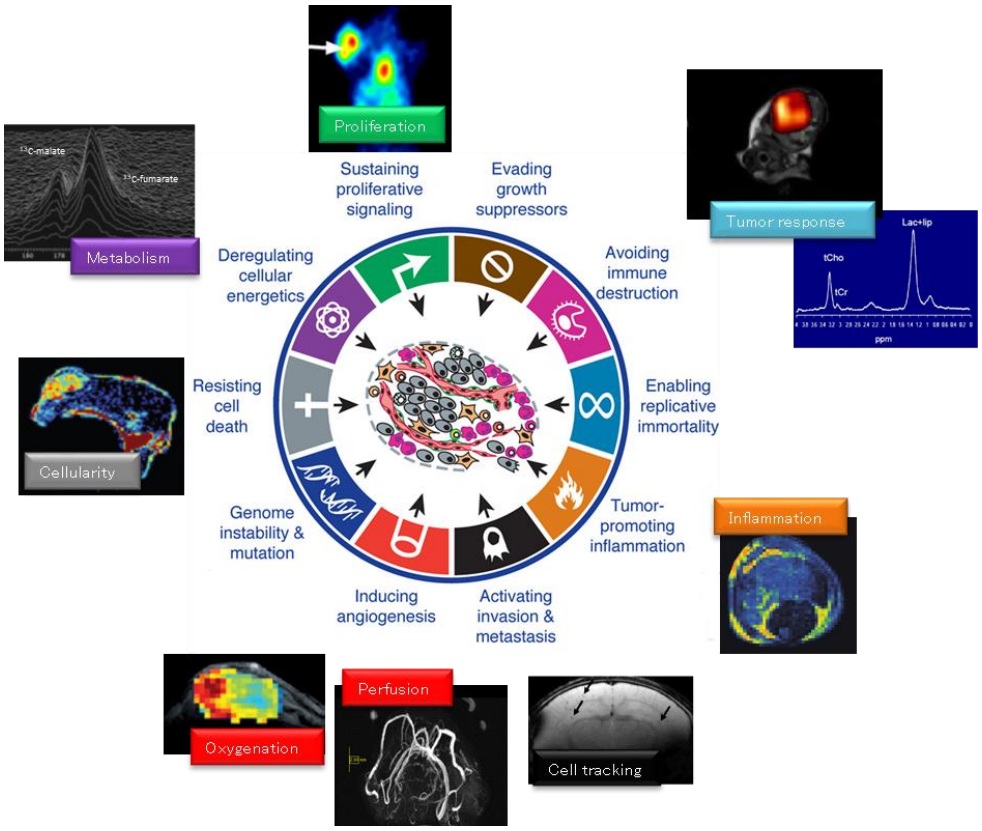
The major theme of the REMA Group is to understand how the tumor microenvironment influences the response to anti-cancer treatments, to identify early non-invasive markers of tumor response to treatment, and to identify metabolic shifts driving resistance to anti-cancer therapy. For that purpose, three main areas of research involve: (a) the development of tools for monitoring the tumor microenvironment by MR techniques, (b) the application of MR techniques to characterize the tumor microenvironment, and (c) the validation of early non-invasive surrogate markers of tumor response to treatment.

1) Development of tools for monitoring hallmarks of cancer by MR techniques:

Since several years, we are developing innovative MR technologies to characterize several hallmarks of cancer, including the tumor hemodynamics and their different components: tissue oxygenation, perfusion, oxygen delivery, and oxygen consumption; as well as the tumor proliferation and metabolic features.

We pioneered developments in EPR oximetry with the characterization of paramagnetic materials possessing favorable features for oximetry. Thanks to these developments, EPR oximetry is routinely used in the laboratory for studying the temporal evolution of tumor pO_2. The technique is unique in a sense that it monitors oxygenation inside a tissue non-invasively and repeatidly from the same site over time. In a translational approach, we also developed biocompatible forms of these systems. One clinical EPR system (second in the world) allows carrying out clinical EPR studies in oncology and diabetology. In the purpose, a clinical study is currently ongoing to assess melanin in melanoma with the ultimate goal of stratifying malignant versus bening naevi. We have also been interested in developing new ways to measure oxygen using MRI, namely by using ^{19}F relaxometry in order to map tumor oxygenation. More recently, we developed a technology based on endogenous contrast (i.e. no need for probe injection), called *MOBILE* (Mapping of Oxygen By Imaging Lipid relaxation Enhancement). This technique is based on the change in relaxation of the proton lipids induced by the oxygen, which is paramagnetic and acts as an endogenous oxygen sensor. We bechmarked this technique with other non-invasive oxygen-sensitive MR methods, based on R_1 and R_2* endogenous contrast.

Imaging hallmarks of cancer



Regarding hemodynamics, we are characterizing the tumor perfusion and permeability with Dynamic Contrast-Enhanced (DCE) – MRI. We are also continuously developing new methodologies to measure tumor oxygen consumption in vivo, using ^{17}O-NMR and EPR oximetry. We focused more recently on the tumor metabolism, which is a target of new therapeutic strategies. More specifically, studies are assessing *in vivo*: the extracellular pH, the glycolytic/oxidative tumor phenotypes and their potential role in tumor resistance to treatment, and the link between tumor cell metabolism and cell proliferation, using ^{1}H, ^{13}C-MRS, and hyperpolarized ^{13}C-enriched substrates.

We recently validated mitochondrial redox nitroxide EPR probes to assess tumour redox status in vitro and vivo, in response to the modulation of gluthatione and thioredoxin status.

We are also developing a mitochondrial 'toolbox' (mito-ToolBox) involving spin traps for measuring mitochondrial superoxide simultaneously to oxygen consumtion rate (OCR) measurement.

2) Applications of MR (EPR and NMR) to characterize the tumor micro-environment:

Our goal is to characterize how the tumor microenvironment influences the response to therapy. We are testing novel approaches using the modulation of the vascular network and/or the inhibition of the oxygen consumption by tumor cells to increase the response to radiation therapy and/or

chemotherapy. In this way, we are trying to define optimal schedule for an optimal therapy.

We are also characterizing the evolution of the tumor microenvironment after therapies that are targeting the tumor metabolism. Thanks to the unique tools that have been developed in our laboratory, we propose new strategies to optimize radiation therapy, chemotherapy, and targeted therapies. As an illustrative example, we are studying the effect of statins on the tumor hemodynamics and response to therapies. In collaboration with the Luxembourg Institute of Health, we have also a research program on the modulation of tumor hypoxia to optimize immunotherapy in cancer.

A more recent research activity of the laboratory is focused on the anti-cancer strategies targeting the tumor metabolism. Using ^{13}C-NMR spectroscopy, we are assessing the effect of PDK, BRAF and EGFR inhibitors on glycolytic flux and tumor metabolism. The identification of alternative metabolic pathways used by tumor cells to sustain their proliferation can be considered as a major mechanism of resistance to this type of treatment. This research will provide a rationale for innovative combination of therapies targeting tumor metabolism.

Finally, activities in tumor cell labelling by imaging reporters (EPR, MRI, bioluminescence) allowed us to monitor the migration of the tumor cells and their homing in distant organs (metastatic process) and to evaluate determinant factors that influence the metastatic progression (including the role of HIF in the metetastatic progression of breast cancer).

3) Development of biomarkers predictive of treatment sensitivity or resistance to targeted therapies:

In the field of radiation therapy, ongoing studies assess the use of imaging biomarkers (^{18}F-FAZA, EPR oximetry, ^{19}F-MRI) to evaluate the efficacy of anti-cancer strategies such as dose painting and dose escalation.

In the field of chemotherapy, we are currently implementing methods that might be predictive of tumor response early in the treatment regimen and comparing their respective value: diffusion MRI (cellularity), ^{1}H-spectroscopy of choline (membrane turnover), ^{13}C-MRS (metabolism), ^{18}F-FDG (glucose uptake), ^{18}F-FLT PET (cell proliferation).

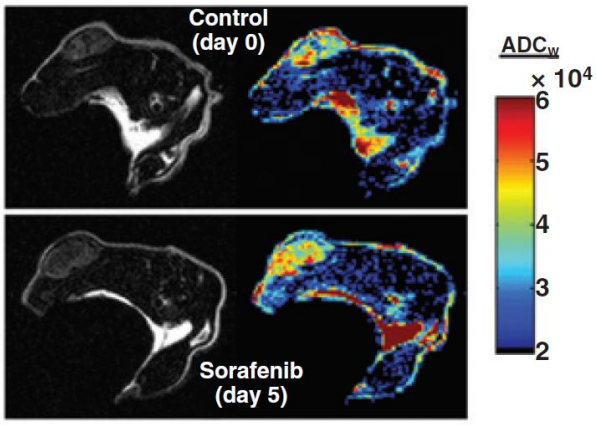


Figure 2. Typical ADCw (Apparent Diffusion Coefficient of water) maps obtained on mice xenografts in response to the multi-kinase inhibitor sorafenib. Note the increase in global ADCw in the tumor region at day 5 post therapy.

A Dynamic Nuclear Polarization (DNP, "Hypersense") system allows the study of metabolic fluxes using ^{13}C-MRS. We are looking to the value of ^{13}C enriched substrates (i.e. pyruvate-lactate exchange) as biomarkers of response to anti-cancer treatment, including EGFR (epidermal growth factor) inhibitors, MAPKinase inhibitors, as well as CDK4/6 inhibitors.

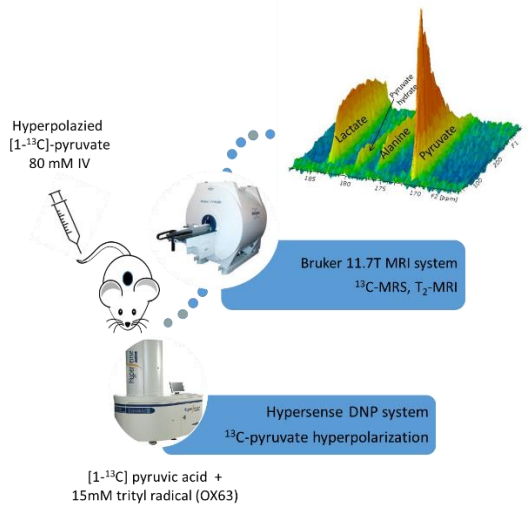


Fig.3 Schematic representation of an imaging session including ^{13}C-MRS of hyperpolarized ^{13}C-pyruvate. The ^{13}C-pyruvate substrate is first hyperpolarized and then directly injected intravenously to the tumor-bearing mouse that is concomitantly imaged in a small-animal MRI scanner for detection of exchange with lactate and alanine using ^{13}C-MRS, to assess metabolic fluxes in vivo in real time.

Hyperpolarized substrates are also used for the stratification of tumors that may benefit from innovative therapies that modulate the metabolism of cancer cells. This multi-modal strategy significantly contributes to the identification of early non-invasive imaging markers of tumor response to combined targeted therapies in the transition towards individualized cancer therapy, with a special focus on the resistance to first line therapy in advanced breast cancer, in advanced melanoma, and in Head & Neck tumors, in collaborations with medical oncologists of the Experimental and Clinical Research Institute (UCL, Profs. J-P. Machiels, S. Schmidt, J.F. Baurain, and F. Duhoux). The ultimate goal of this type of studies is to spare patient's cycles of futile therapy, and possibly allow them to move to other, possibly experimental therapies. These metabolic studies, by identifying resistance mechanisms to targeted therapies (such as glutaminolysis, fatty acid oxidation, or glycolysis inhibition), thanks to the developed imaging metabolic tracers, will provide rationale for new therapeutic combinations involving metabolic targeted therapies.

More recently, the influence of obesity on breast cancer progression and tumor response to treatment is also being studied in collaboration with Prof. P.D. Cani of the Metabolism and Nutrition group of the LDRI institute. This project involves the study of the role of adipokines and gut microbiota in breast cancer progression and metastatization.

SELECTED PUBLICATIONS
Bernard GALLEZ

B. Gallez, P. Danhier, M.A. Neveu, B.F. Jordan. Manipulation of tumor oxygenation and radiosensitivity through modification of cell respiration. A critical review of approaches and imaging biomarkers for therapeutic guidance**.** Biochim. Biophys. Acta (Bioenergetics) (2017) 1858, 700-711

Neveu MA, De Preter G, Marchand V, Bol A, Brender JR, Saito K, Kishimoto S, Porporato PE, Sonveaux P, Grégoire V, Feron O, Jordan BF, Krishna MC, Gallez B.

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Neoplasia (2016), 18, 742-752

De Preter G., Neveu M.A., Danhier P., Brisson L., Payen V.L., Porporato P.E., Jordan B.F., Sonveaux P. and Gallez B. Inhibition of the pentose phosphate pathway by dichloroacetate unravels a missing link between aerobic glycolysis and cancer cell proliferation. Oncotarget (2015), 7, 2910-2920.

Tran LBA, Bol A, Labar D, Karroum O, Bol V, Jordan B, Grégoire V, and Gallez B. Potential role of hypoxia imaging using ^{18}F-FAZA PET to guide hypoxia-driven interventions (carbogen breathing or dose escalation) in radiation therapy. Radiother. Oncol. (2014), 113: 204-209.

Diepart C., Karroum O., Magat J., Feron O., Verrax J., Buc-Calderon P., Grégoire V., Jordan B., Gallez B. Arsenic trioxide treatment decreases the oxygen consumption rate of tumor cells and radiosensitizes solid tumors. Cancer Res. (2012), 72: 482-490.

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Bénédicte JORDAN

Mignion L, Acciardo S, Gourgue F, Joudiou N, Caignet X, Goebbels RM, Corbet C, Bouzin C, Cani PD, Machiels JP, Schmitz S, Jordan BF. Metabolic imaging using hyperpolarized pyruvate-lactate exchange assesses response or resistance to the EGFR inhibitor cetuximab in patient-derived HNSCC xenografts. Clin. Cancer Res (2019), in press.

Acciardo S, Mignion L, Joudiou N, Bouzin C, Baurain JF, Gallez B, Jordan BF. Imaging markers of response to combined BRAF and MEK inhibition in BRAF mutated vemurafenib-sensitive and resistant melanomas. Oncotarget. (2018) 30; 9(24):16832-16846.

Mignion L, Danhier P, Magat J, Porporato PE, Masquelier J, Gregoire V, Muccioli GG, Sonveaux P, Gallez B, Jordan BF. Non-invasive in vivo imaging of early metabolic tumor response to therapies targeting choline metabolism. Int J Cancer. (2016) 15;138(8):2043-9.

Colliez F., Neveu M.A., Magat J., Cao Pham T.T., Gallez B., Jordan B.F. Qualification of a Noninvasive Magnetic Resonance Imaging Biomarker to Assess Tumor Oxygenation. Clin. Cancer Res. (2014), 20: 5403-5411.

Mignion L., Dutta P., Martinez G.V., Foroutan P., Gillies R.J., Jordan B.F. Monitoring chemotherapeutic response by hyperpolarized 13C-fumarate MRS and diffusion MRI. Cancer Res. (2013), 74: 686-694.

THESES DEFENDED IN 2019

Janske Nel: "Lipid nanocapsules as theranostic tools".
Director: L. Lemaire; Co-Director: B. Gallez

Scheinok Samantha: "Development of a new EPR tool to detect mitochondrial superoxide".
Director: B. Gallez; Co-Director: P. Sonveaux

THESES IN PROGRESS

Acciardo Stefania: "Identification of early non-invasive imaging markers of tumor response to BRAF inhibitors in combination with concomitant additional targeted therapy or immunotherapy in melanoma".
Director: B.F. Jordan; Co-Director: JF Baurain

Gourgue Florian: "Study of the role of the apelin adipokine in breast cancer progression and response to treatment".
Director: B.F. Jordan, Co-Director: P.D. Cani

Schoonjans Céline: "PDK inhibitors to block tumor cell proliferation: Rationale for combined treatment strategies counteracting compensatory mechanisms that sustain tumor growth."
Director: B. Gallez, Co-director: O. Feron

D'hose Donatienne: "Statins and oxygen consumption in tumors".
Director: B. Gallez, Co-director: B.F. Jordan

Yelek Caner: "Impact of bioactive lipids on tumor cell metabolism and cancer progression: novel insight from the gut microbiota."
Director: B.F. Jordan; Co-Director: P. D.Cani

Conq Jérôme: "Boosting nanomedicines delivery in glioblastoma"
Director: B. Gallez, Co-director: V.Préat

Farah Chantale: "Imaging metabolic plasticity in melanoma: relevance of combining metabolic modulators with BRAF or immune checkpoint inhibitors"**.**
Director: B.F. Jordan; Co-Director: JF Baurain

TECHNOLOGY PLATFORMS

I) MASSMET PLATFORM

The MASSMET platform is an analytical platform applying mass spectrometry analysis to small metabolites and to compounds of biological or pharmaceutical interest.

The platform provides a support in analytical chemistry mainly through the development of chromatographic methods coupled to mass spectrometry detection, with a particular focus on the detection, identification and quantification of "small molecules" in complex matrices. As such, the expertise provided by the platform is important for numerous labs within the LDRI and the "Health Sector", as well as for labs of the "Sciences and Technology Sector".

To this aim, we share the use of several analytical equipments located both in Brussels (mainly at the LDRI) and at Louvain-la-Neuve (mainly at the ISV). These equipments include (but are not limited to):

- ThermoScientific LTQ – ORBITRAP –XL high resolution mass spectrometer
- Waters xevo TQS UPLC-MS/MS
- ThermoScientific Trace GC-MS
- Thermoscientific LCQ Advantage mass spectrometer
- ThermoScientific DSQ GC mass spectrometer
- Several chromatographic systems (HPLC, UPLC, GC) using UV, DAD, or FID detectors are also available.

ThermoScientific LTQ – ORBITRAP –XL

Waters xevo TQS

The interest and importance of the expertise of the MASSMET platform are shown by the numerous publications that benefited from the data obtained using the equipment and/or expertise of the platform. Examples of such studies involving LDRI research groups include the quantification of antibiotics from cell cultures (TFAR-FACM), the quantification of transcellular transport (ADDB –TFAR - PMGK), the quantification of endogenous metabolites from microorganisms, cells and tissues (BPBL – MNUT – TFAR-FACM), the identification of metabolites from plants (GNOS), the quantification of endogenous and exogenous metabolites in plasma (BPBL – ADDB – GNOS – TFAR - PMGK) and the determination of the nature and purity of compounds of synthetic origin (CMFA). An exhaustive list of collaborations (within and outside the LDRI) and publications is available on the platform website (https://uclouvain.be/en/research-institutes/ldri/massmet.html).

Contact person:

- Prof Giulio G Muccioli (Giulio.muccioli@uclouvain.be)

II) NUCLEAR & ELECTRONIC SPIN TECHNOLOGIES (NEST) PLATFORM

The (pre)clinical magnetic resonance platform accommodates cutting-edge MR technologies: magnetic resonance imaging (MRI), electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), and Dynamic Nuclear Polarization (DNP); dedicated to studies on biological samples, and small animals. Human EPR measurements have also recently been implemented in the platform. These technologies may provide convenient biomarkers for monitoring (patho) physiological parameters and the response to pharmacological treatments.

The NEST platform, managed by 3 post-docs, provides expertise and services in magnetic resonance-related technologies such as DNP, EPR, MRI and NMR. The support from the experts of the platform take place from the design of experiment to publication of scientific communication.

Nuclear Magnetic Resonance

The Bruker Ascend 600MHz NMR system equipped with a broadband cryoprobe gives the possibility to access high resolution and high signal to noise ratio.

With this system, it is possible to work on any nuclei and to perform most of the liquid state experiment (1D or 2D experiment)

The system is equipped with a sample handling system that can be thermo-regulated (from +4°C to +40°C)

Here are few example of application:

- metabolomics study on biological samples (plasma, urea, etc…)
- saturation transfer difference
- 2D homonuclear and heteronuclear correlation (such as J-RES, COSY, TOCSY, etc…)
- HRMAS experiment on biopsies using the HRMAS probe

Magnetic Resonance Imaging

Equipped with a Bruker Biospec 11.7T MRI the platform can proceed to a wide range of *in vivo* studies. Along with this MRI comes a large set of coils that allow us to work on rats and mice on any anatomical area and different nuclei (^{1}H, ^{13}C, ^{17}O, ^{19}F and ^{31}P).

During the past years the platform has shown skills in this following fields:

- In vivo anatomical structures with high spatial resolution
- Metabolism (spectroscopy and spectroscopic imaging)
- Vessels architecture (micro-angiography)
- Tissue perfusion (by Dynamic Contrast Enhanced MRI, DCE-MRI)
- Oxygen and pH measurements
- Heart physiology (ventricle function)
- Cell death (Microscopic water diffusion)
- Cell tracking

Dynamic Nuclear Polarization

Hyperpolarization allows to considerably increase the sensitivity (>10.000) of MR spectroscopy. Our Hypersense (Oxford Instruments) system, used in combination with the Bruker Biospec 11.7T MRI system (for in-vivo application) or the with Bruker Ascend 600MHz NMR system (for in-vitro applications), is able to hyperpolarize ^{13}C-enriched substrates for the monitoring of metabolic fluxes in real time. The detection of the metabolites is performed using ^{13}C-MRS coils.

The follow-up of different metabolic fluxes, such as the ^{13}C-pyruvate to ^{13}C-lactate (or ^{13}C-alanine) exchange allows the monitoring of tumor metabolism and glycolysis.

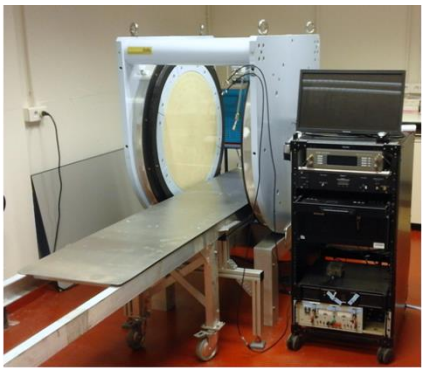
Electron paramagnetic resonance

Electron paramagnetic resonance spectroscopy/imaging is the gold standard method for detecting and quantifying free radicals and superparamagnetic species in living organisms. The NEST platform offers access to the following equipment:

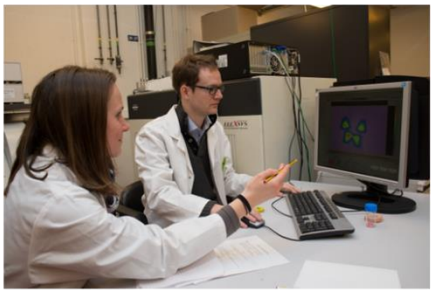
- X-band EPR spectrometer (Bruker EMX+, 9 GHz) for *in vitro* applications
- Benchtop X-band EPR spectrometer (Magnettech Miniscope, 9 GHz for *in vitro* applications
- L-band (1 GHz) and X-band (9 GHz) imaging EPR system (Bruker Elexys) for *in vitro* and *in vivo* applications
- L-band EPR spectrometer (Magnettech, 1 GHz) for *in vivo* applications (small animals)
- Clinical L-band EPR spectrometer (whole body, 1 GHz) for human studies

Benchtop X-band EPR

EMX+ X-band EPR



Clinical L-band EPR



Elexys EPR imaging

In vivo L-band EPR

EPR applications include (non-exhaustive list):

- Free radicals measurements and characterization by spin trapping
- Quantification of melanin / melanoma cells in tissues
- Molecular dynamics, microviscosity, micropolarity in tissues and drug delivery systems
- Dosimetry (retrospective dosimetry in bones and teeth)
- Dosimetry in phantoms (external beams, brachytherapy)
- Tissue oxygenation, Oxygen consumption
- Redox status, pH
- Superparamagnetic iron oxides nanoparticles (SPIO) quantification

The utility and importance of the expertise of the Pre-clinical MR platform is testified by the numerous publications that benefited from the data obtained using the equipment and expertise of the platform.

Illustrative examples involving LDRI or Health Sector research groups include:

- characterization of new drug delivery systems (ADDB/LDRI);
- characterization of spinal cord regeneration (ADDB/LDRI);
- identification of free radicals involved in toxicological processes (MNUT,MORF/LDRI,IREC, ADDB/LDRI);
- characterization of the tumor microenvironment (REMA/LDRI, FATH/IREC);
- tumor metabolism (REMA/LDRI, FATH/IREC);
- resistance to treatments (REMA/LDRI, MIRO/IREC, FATH/IREC);
- characterization of dental resins (ADDB/LDRI),
- characterization of angiogenic process (FATH/IREC);
- oxygenation of pancreas islets grafts (CHEX/IREC);
- ovarian grafts (GYNE/IREC);
- liver oxygenation (GAEN/IREC);
- endometrium grafts (CELL/DDUV);
- cardiac function (FATH, CARD/IREC);
- validation of PET tracers (MIRO/IREC);
- ligand-receptor interaction (CMFA/LDRI);
- metabolomics (MNUT/LDRI);

Research logisticians in charge of the NEST platform:

- NMR, MRI: Dr Nicolas Joudiou (nicolas.joudiou@uclouvain.be)
- EPR: Dr Pierre Danhier (pierre.danhier@uclouvain.be)
- DNP: Dr Lionel Mignion (lionel.mignion@uclouvain.be)

Principal investigators responsible of the NEST platform:

- Prof. Bénédicte Jordan (benedicte.jordan@uclouvain.be)
- Prof. Bernard Gallez (bernard.gallez@uclouvain.be)

SUPPORTING ORGANIZATIONS

APPENDIX

2019 PUBLICATIONS

Research or review paper – first or last author

1. Acciardo, Stefania ; Mignion, Lionel ; Lacomblez, Estelle ; Schoonjans, Céline ; Joudiou, Nicolas ; **Gallez, Bernard** ; **Jordan, Bénédicte**. *Metabolic imaging using hyperpolarized 13 C-pyruvate to assess sensitivity to the B-Raf inhibitor vemurafenib in melanoma cells and xenografts*. In: *Journal of Cellular and Molecular Medicine*, Vol. 2, no. ZZ, p. 5 (2019). IF: 4,658

2. Anrys P, Strauven G, Roussel S, Vande Ginste M, De Lepeleire J, Foulon V, **Spinewine A.** *Process evaluation of a complex intervention to optimize quality of prescribing in nursing homes (COME-ON study).* In: Implementation Science. 2019 Dec 11;14(1):104. IF: 4,525

3. Bastiancich, Chiara ; Bozzato, Elia ; Luyten, Urszula ; Danhier, Fabienne ; Bastiat, Guillaume ; **Préat, Véronique**. *Drug combination using an injectable nanomedicine hydrogel for glioblastoma treatment.* In: *Drug combination using an injectable nanomedicine hydrogel for glioblastoma treatment*, Vol. 559, no.3, p. 220-227 (2019). IF : /

4. Beaufay, Claire ; Henry, Guillaume ; Streel, Camille ; Bony, Emilie ; Herent, Marie-France ; Bero, Joanne ; **Quetin-Leclercq, Joëlle**. *Optimization and validation of extraction and quantification methods of antimalarial triterpenic esters in Keetia leucantha plant and plasma.* In: *Journal of Chromatography* B, Vol. 1104, p. 109-118 (2019). IF : 3,858

5. Benites J, Valderrama JA, Ramos M, Valenzuela M, Guerrero-Castilla A, **Muccioli GG**, **Buc Calderon P**. *Half-Wave Potentials and In Vitro Cytotoxic Evaluation of 3-Acylated 2,5-Bis(phenylamino)-1,4-benzoquinones on Cancer Cells.* In: Molecules. 2019 May 8;24(9). pii: E1780. IF : 3,060

6. Bottemanne P, Paquot A, Ameraoui H, Alhouayek M, **Muccioli GG**. *The α/β-hydrolase domain 6 inhibitor WWL70 decreases endotoxin-induced lung inflammation in mice, potential contribution of 2-arachidonoylglycerol, and lysoglycerophospholipids.* In : *Faseb Journal*. 2019 Jun;33(6):7635-7646. IF : 5,391

7. Buisseret B, Alhouayek M, Guillemot-Legris O, **Muccioli GG**. *Endocannabinoid and Prostanoid Crosstalk in Pain.* In: *Trends in Molecular Medicine* 2019 Oct;25(10):882-896. IF : 11,028

8. Buisseret, Baptiste ; Guillemot-Legris, Owein ; **Muccioli, Giulio** ; Al Houayek, Mireille. *Prostaglandin D2-glycerol ester decreases carrageenan-induced inflammation and hyperalgesia in mice*. In: Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids, (2019). IF : 4.402

9. **Cani PD**, Depommier C. *Reply to 'Simpson's paradox in proof-of-concept studies'.* Nat Med. 2019 Nov;25(11):1640-1641. IF : 30,641

10. **Cani PD**. *Is colonic propionate delivery a novel solution to improve metabolism and inflammation in overweight or obese subjects?* Gut. 2019 Aug;68(8):1352-1353. IF : 17,943

11. Cani PD. *Severe obesity and gut microbiota: does bariatric surgery really reset the system?* In: *Gut*. 2019 Jan;68(1):5-6. doi: 10.1136/gutjnl-2018-316815. Epub 2018 Jul 10. IF: 17,943

12. **Cani, Patrice D.** ; Van Hul, Matthias ; Lefort, Charlotte ; Depommier, Clara ; Rastelli, Marialetizia ; **Everard, Amandine**. *Microbial regulation of organismal energy homeostasis.* In: *Nature Metabolism*, Vol. 1, no. 1, p. 34-46 (2019). IF:/

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