LOUVAIN DRUG RESEARCH INSTITUTE

REPORT 2022

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Giulio Muccioli

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

https://fr-fr.facebook.com/LouvainDrugResearchInstitute

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UCLouvain

Table des matières

FOREWORD 3
2022 HIGHLIGHTS 4

SECTION I – LDRI GENERAL PRESENTATION 5
I. Objectives and mission statements 6
II. Research fields and groups 6
III. Decision-making and management 7
IV. Human Resources 8
V. Fundings 9
VI. Scientific Output 10

SECTION II – RESEARCH GROUP PRESENTATION 15
Medicinal Chemistry (CMFA) 16
Pharmacognosy (GNOS) 25
Metabolism and Nutrition (MNUT) 32
Bioanalysis and Pharmacology of Bioactive Lipids (BPBL) 52
Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR) 60
Advanced Drug Delivery and Biomaterials (ADDB) 89
Biomedical Magnetic Resonance (REMA) 104

TECHNOLOGY PLATFORMS 113
SUPPORTING ORGANIZATIONS 119
APPENDIX 121
Complete list of 2022 LDRI Publications and patents 121

FOREWORD

The **Louvain Drug Research Institute (LDRI)**, created in 2010, is located on the Health Sciences Campus of the *Université catholique de Louvain* (UCLouvain) in Brussels. The LDRI develops cutting-edge fundamental and applied research in the field of drugs, from target and lead identification to implementation in clinical practice.

"**Bridging sciences for better health**" is the moto of our institute. As a multidisciplinary institute, we cover original research topics in four main directions (i.e. Microbes, Inflammation, Cancer, and Ageing), from the discovery of new targets to the design or identification of new drugs, as well as the optimization of their delivery and use, to move from bench to bedside. Two technical facilities offer innovative technologies related to imaging (NEST) and mass spectrometry analysis (MASSMET).

Around 180 motivated members share common objectives in terms of quality of science and well-being, thus creating optimal research conditions for all the members of the Insitute. In line with our motto, and adhering to our University "Horizon 600" plan, we aim at cultivating research excellence by extending the frontiers of knowledge, with the overall objective of a "better health for all".

In 2022, 16 young scientists of the LDRI obtained their PhD. The same year, over 120 papers were published in reputed peer-reviewed journals. And in 2022, as in the recent past, the QS World University Ranking confimed our research activities in "Pharmacy and Pharmacology" in the Top 51-100 Universities over the world. Moreover, in 2022 four LDRI researchers were among the "TOP 1% highly cited researchers worldwide" (Web of Science). As in the past, LDRI researchers have been particularly successful in obtaining highly competitive grants from Belgian funding agencies and as leaders in international networks.

Last year, two of our leading researchers, Prof. Marie-Paule Mingeot and Prof. Véronique Préat, were appointed Professor Emeritus. All the members of the Institute thank them for their great contribution to the research and life of the Institute.

In September 2022, after a thesis at the UCLouvain and postdoc positions at the University of Texas and Universiteit Utrecht, Joseph Lorent joined our Insitute as new academic PI in the Cellular and Molecular Pharmacology (FACM / TFAR) research group.

2022 was also the last year for Nathalie Delzenne as President of our Institute. All the members of the Institute are grateful for the time she has devoted – during her two terms – to the management of the LDRI, always keeping in mind the common good.

This report summarizes our achievements, objectives and mission statements, and details the scientific outcomes of the seven research groups and technology platforms.

Enjoy the reading!

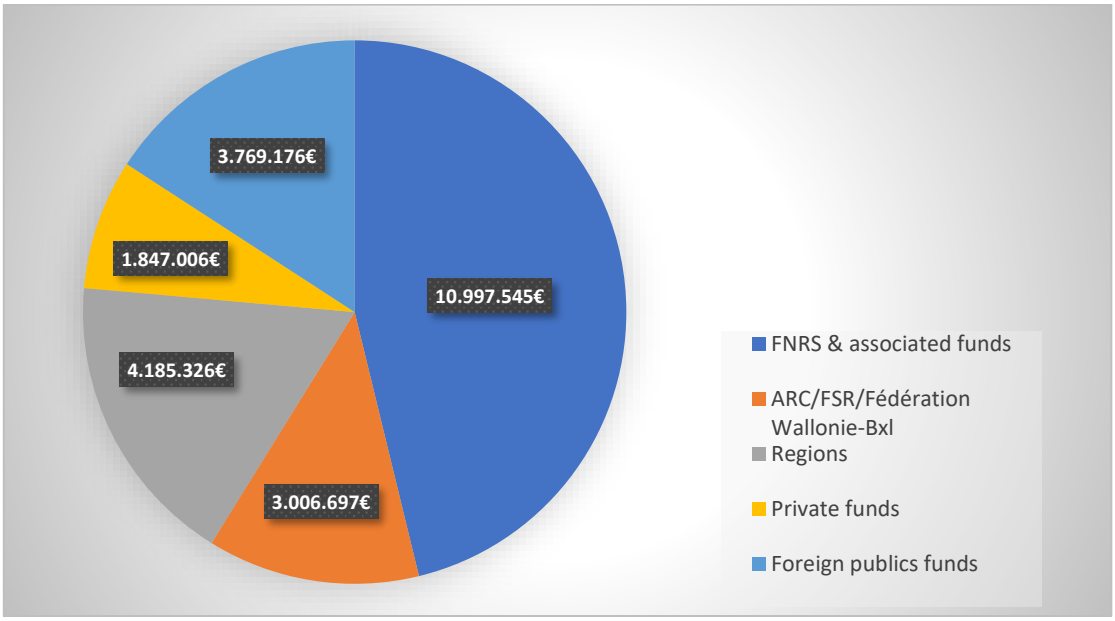
Giulio Muccioli (President)

Patrice Cani, Bénédicte Jordan, and Françoise Van Bambeke (vice-Presidents)

2022 HIGHLIGHTS

Human resources and scientific output

Sources of funding of the LDRI (Global amount in EUR for projects running in 2022)



The data on human resources, funding, and the scientific output of the LDRI are mostly based on data collected in 2022 using the University's official databases.

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

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: Cellular and Molecular Pharmacology
FNRS: Fonds National de la Recherche Scientifique
FRIA: Fonds pour la Formation à la Recherche dans l'Industrie et dans l'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

I. Objectives and mission statements

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

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 technology platforms with high-end technologies in the field of mass spectrometry (MASSMET) and pre-clinical magnetic resonance (NEST).

The research 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

The research activities are conducted 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 drug development are:

1) Design and research of new active molecules. This mainly involves 2 research groups:

(i) Medicinal Chemistry (CMFA) developing the expertise on rational-based synthesis of new compounds;

(ii) Pharmacognosy (GNOS) specialised in the extraction and identification of new bioactive molecules isolated from plants.

2) 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), focusing on lipid mediators in health and 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 means to improve therapeutic outcomes of drugs;

(iv) Biomedical Magnetic Resonance (REMA) that develops magnetic resonance-based innovative tools 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.

Thus, all the major aspects of a drug - from its design to its optimal use - are covered by our research activities. Importantly, active collaborations are established with UCLouvain-related University Hospitals (*Cliniques Universitaires St Luc* – located within walking distance of the LDRI - and *CHU UCL-Namur*).

III. Decision-making and management

The LDRI Management Committee is currently made up of a President (Giulio Muccioli) and three Vice-Presidents (Patrice Cani, Bénédicte Jordan, and Françoise Van Bambeke) who were elected by the LDRI Council.

G. Muccioli

P. Cani

B. Jordan

F. Van Bambeke

The LDRI Board (5 meetings/year) and Council (2 meetings/year) are respectively preparing and approving major decisions.

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

The Council is composed of the permanent scientific and academic LDRI members and representatives of the scientific, administrative and technical staff. The roles of the Council, Board and President, as well as the mode of elections, are described the "internal regulations" that were established in accordance with the general regulations of the University.

The International Scientific Council of the LDRI is composed of four reputed researchers, who cover the main research areas of the Institute, and provide advices on research and recruitment strategy. The current distinguished members of our International Scientific Council are P. Ferré (metabolism, pathophysiology and molecular biology) (Inserm & Université de Paris, Paris, France); P. Herdewyn (Medicinal Chemistry) (KULeuven, Belgium); C. Hughes (Primary care Pharmacy) (Queen's University, Belfast, Ireland); J.L. Veuthey (Analytical Chemistry) (Université de Genève, Switzerland).

Prof. C. Hughes (UK)

Prof. J.L. Veuthey (CH)

Prof. P. Ferré (FR)

Prof. P. Herdewyn (BE)

IV. Human Resources

The **total staff** of the LDRI in December 2022 was 162 full members (affected to LDRI as main entity) and 17 affiliated members. It represents 179 persons involved in LDRI's activities, with a proportion of 39% men and 61% women.

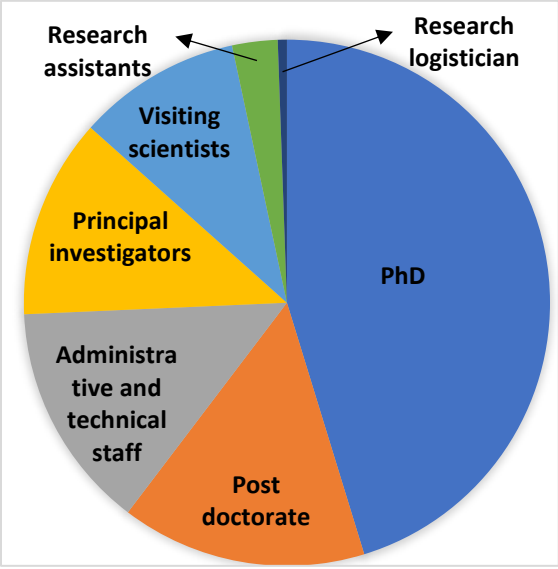
Academic staff:

Due to part-time contracts, the 22 Principal Investigators stand for 20.5 FTE (Full Time Equivalent). Among them, 8 FTE senior researchers are paid by the FRS-FNRS.

Scientific staff:

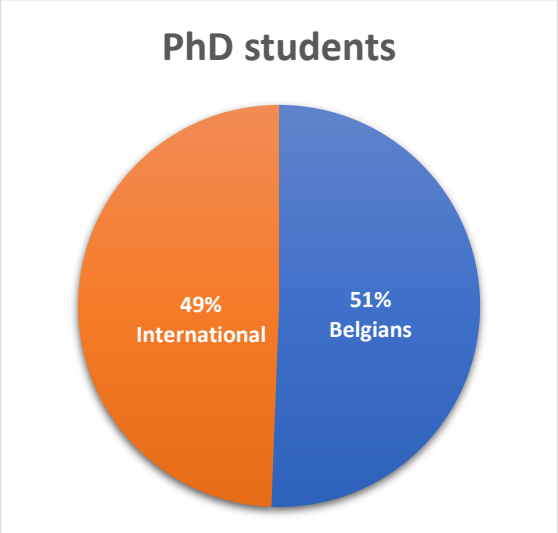
27 post-doctoral fellows and 81 PhD students, including 19 teaching assistants, are fully integrated in LDRI's research groups. In addition, several PhD students and post-doctoral fellows are affiliated to the LDRI, being co-supervised by LDRI members, but having their main affiliation in other Institutes or Universities.

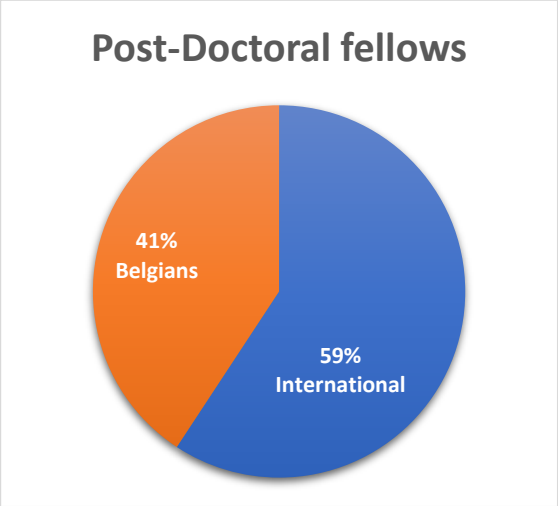
The technical and administrative staff affected to the LDRI represents 25 persons.



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

The LDRI is continuously attracting numerous **international researchers**, as illustrated in the graphs below. Half of the PhD students and around 60% of the post-doctoral fellows are coming from abroad.



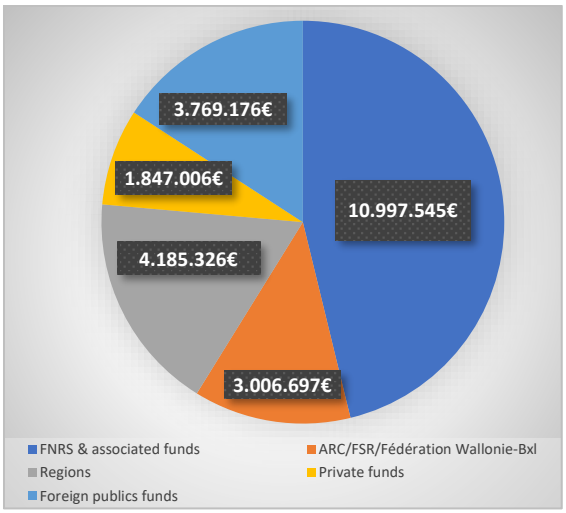


The background of the scientists is quite diverse reflecting the multidisciplinary research performed within the Institute. The PhD students within the LDRI's research groups trained as pharmacists, bioengineers, engineers, chemists, biologists, physicists, MD or masters in biomedical sciences.

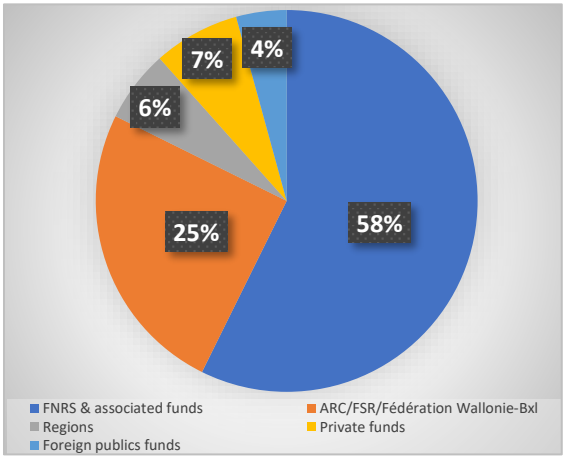
V. Fundings

The members of the LDRI are very active and successful in obtaining **financial incomes from third parties**, (as illustrated below), which are essential to cover the costs of wet-lab research activities, of innovative technologies implementation, and to guarantee the salary of part of the scientific and technical staff. The LDRI receives an annual fee from the Health Sector calculated upon criteria based on the number of academic and scientific staff involved in research activities. This year, we welcome funding provided by the *Fondation Louvain* related to partnership established with *Pharmacie Servais*.

Sources of funding of the LDRI (Global amount in EUR for projects running in 2022)

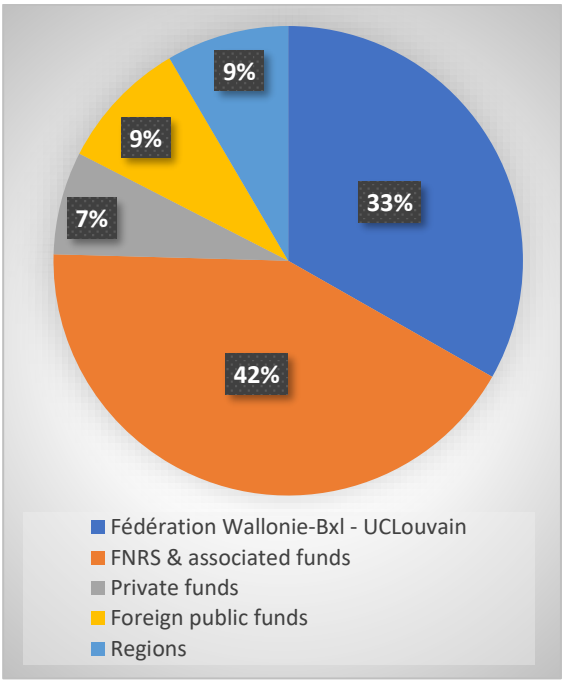


Sources of Funding of the LDRI (Percentage of the number of projects running in 2022)



The main sources of staff funding (see graphic below) are the FNRS (42%), UCLouvain/Fédération Wallonie Bruxelles (33%), the Belgian regions (9%), foreign public funds (9%) and private funds (7%). The FNRS pays the salary of 8 permanent principal investigators, 13 postdoctoral researchers, and 32 PhD students (including FRIA - Fund for Research Training in Industry and Agriculture - and Televie doctoral grants). 20 teaching assistants are paid by UCLouvain, and spend 50% of their time for research (50% in teaching activities).

Sources of funding of the LDRI staff in 2022*



** This graph includes full and affiliated members.*

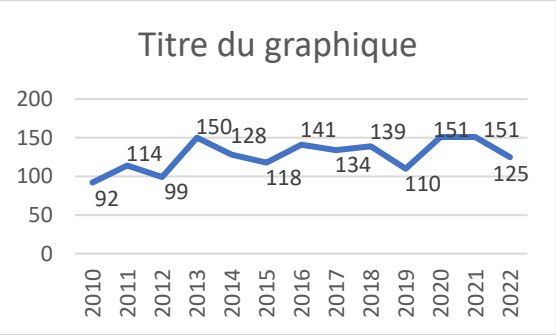
VI. Scientific Output

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

During the last three years, the researchers of the LDRI published more than 400 scientific articles in international journals or book chapters. A full list of publications can be found here https://uclouvain.be/en/research-institutes/ldri/publications.html

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



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 (DFAR) that organises semiars for and by the PhD students.

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

Seminars-Symposia

Researchers and other professionals having an interest for the scientific areas related to drugs attend the LDRI seminars (organised at least once a week). The topics of the seminar 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).

A "LDRI PhD day" is organised each year by the scientific staff. Talks and poster presentations by the PhD students allow 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 the partnership of regional and international industries and SME.

KEY AWARDS 2022

Prof. Ana Beloqui (ADDB) was awarded the Prix Galien for her work on the oral administration of peptides.

Prof Laure Bindels (MNUT) becomes Collen-Francqui Research Professor (2022-2025).

Prof Mireille Alhouayek (BPBL) becomes member of the *Collegium* of the Académie Royale de Belgique.

Laure Elens (TFAR-PMGK) received the Léopold et Marthe Delsaux-Champy Prize in cardiology (2022).

Four LDRI researchers are among the 2022 Clarivate's Highly cited researchers: Prof. Patrice Cani (MNUT), Prof. Nathalie Delzenne (MNUT), Prof. Amandine Everard (MNUT) and Prof. Véronique Préat (ADDB).

Prof. Nathalie Delzenne (MNUT) appears among the 10 Belgian women nominated, in the 1st edition of Research.com ranking of top female scientists.

Dr Antoine Christiaens (TFAR-CLIP) received the Alvarenga de Piauhy Prize from the Royal Academy of Medicine of Belgium.

Dr Antoine Christiaens (TFAR-CLIP) received the Lucie et Edouard Chaffoteaux Prize 2022, from the French Society of Geriatrics and Gerontology (SFGG), and the Fondation de France which hosts the Edouard and Lucie Chaffoteaux Prize Foundation.

Emilie Moens de Hase (MNUT) was awarded the "best poster presentation" award in the category: "Dietary intake and nutritional policy + gut microbiota" at the 3rd International Conference on Food Bioactives and Health in Parma.

Camille Lefevre (MNUT) was awarded a "Travel Award" for the Iscam 2022 Congress (Turin),

Pauline Bottemanne and Hafsa Ameraoui (BPBL) were awarded "best poster presentation" awards at the 8th EWLM (Stockholm).

Chloé Buyse (REMA) was awarded the "best poster" award at the 12th International Workshop on EPR in Biology and Medicine (Krakow)

Sarah Pötgens (MNUT) received the Prize of the Belgian Society of Clinical Nutrition 2023 (the best abstract selected at ESPEN 2022)

Gert-Jan Wijnant (TFAR- FACM) received an award for the best oral presentation from the International Society for Antinfective Pharmacology (ISAP) during its annual meeting in April 2022.

PLENARY LECTURES by EXTERNAL SPEAKERS 2022

Dr. Brice KORKMAZ
Université de Tours, France
"Therapeutic targeting of cathepsin C: from pathophysiology to treatment"

Chloé Martens
ULB, Unité de recherche en Structure et Fonction des Membranes biologiques
"H/D exchange coupled to Mass Spectrometry: a multifaceted tool to study membrane proteins"

Dr. Maria Rohm
Head of Division Tissue Cross Talk in Cancer Metabolism, Institute for Diabetes and Cancer, Helmholtz
"Lipid metabolism in cancer cachexia"

Prof. Donatienne Tyteca and Dr. Patrick Van Der Smissen
UCLouvain - DDUV/CELL and DDUV/PICT
"The Zeiss LSM980, a new generation confocal microscope equipped with a multiphoton laser installed at PICT in 2020: from equipment overview to applications"

Prof. Souleymane MBOUP
IRESSEF: Institute for Health Research, Epidemiological Surveillance and Training, Dakar, Senegal
"A unique Research Platform in West Africa". Presentation of the institution and the research conducted there

Dr. Vianney Delplace
Groupe RMeS de l'Université de Nantes
"Dynamic Hydrogels as Synthetic Extracellular Matrices: One Step Closer to Biology"
"Les hydrogels dynamiques comme nouveaux outils ajustables et polyvalents pour la culture 3D, la thérapie cellulaire et la bio-impression"

Prof. Yolanda Sanz
Professor of Research of the National Research Council (CSIC) at the Institute of Agrochemistry and Food Technology (IATA-CSIC) – Group "Microbial Ecology, Nutrition and Health", Valencia, Spain
Identifying microbiome signatures linked to obesity

Prof. Jean-Baptiste WOILLARD
Limoges University, France
"Estimation of immunosuppressant exposure using machine learning: towards a new way to perform therapeutic drug monitoring?"

Dr. Emily REEVE
Centre for Medicine Use and Safety, Monash University, Australia
"Informing implementation of deprescribing into clinical practice: Consumer attitudes towards deprescribing and the role of guidelines"

Prof. Séverin ANAGONOU
Université d'Abomey-Calavi, Bénin
"Management Tuberculosis and HIV co infection in Cotonou Benin"

Prof. Jeremy GRIMSHAW
MBChB, PhD, Senior Scientist and Full Professor - Centre for Implementation Research, Clinical Epidemiology Program, Ottawa Hospital Research Institute - Faculty of Medicine, University of Ottawa, Canada
"De-implementing Wisely – the science of de-implementing low value care"

Alain NINANE, PhD
UCLouvain - Expert/Analyste Cybersécurité
"Cybersécurité, la théorie, ses ratés. Comment s'en prémunir?"

Prof. Greet KERCKHOFS
UCLouvain - Biomechanics Lab, Institute of Mechanics, Materials, and Civil Engineering (IMMC) and Institute of Experimental and Clinical Research (IREC)
"X-Ray based 3D histology of biological tissues"

Prof. Maria José ALONSO FERNANDEZ
University of Santiago de Compostela, Spain
"The alliance of pharmaceutical nanotechnology, biotechnology and functional materials"

Carina CARBIA SINDE, PhD
UCLouvain - Psychological Science Research Institute (IPSY) and Louvain for Experimental Psychopathology research group (LEP)
"Microbiome gut brain axis as a regulator of craving and social cognition in young binge drinkers"

Anna LECHANTEUR, Pharmacist, PhD, Professeure inviteé
Laboratory of Pharmaceutical Technology and Biopharmacy (LPTB) - CIRM, Department of Pharmacy, University of Liege
"3D printing of medicines"

Prof. Jean-Marie COLET
Human Biology and Toxicology Unit, Umons
"Rôles biologiques des "Suspects Usuels" en métabonomique : Exemples du fumarate et du succinate"

Dr. Frédéric GOORMAGHTIGH
Host-Pathogen interactions (Dirk Bumann), Biozentrum (Switzerland)
"Exploiting codon usage bias in bacterial pathogens to develop novel anti-virulence strategies"

Sophie LECLERCQ, PhD
UCLouvain - IoNS/NEUR - LDRI/MNUT
"Investigation of the gut-brain axis in alcohol-dependent patients"

LDRI Structure

RESEARCH GROUPS

OBJECTIVES

Platforms

MASSMET + NEST

CMFA - Medicinal Chemistry

GNOS - Pharmacognosy

DESIGN AND RESEARCH OF NEW ACTIVE MOLECULES

MNUT - Metabolism and

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

SECTION II – RESEARCH GROUP PRESENTATION

I. Medicinal Chemistry (CMFA)
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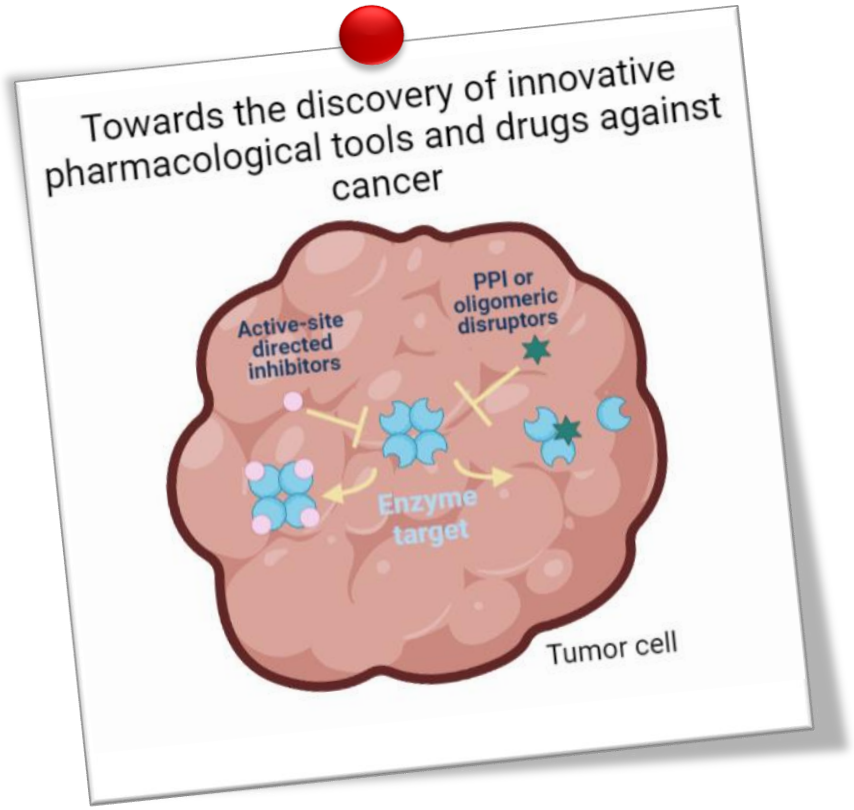
VII. Biomedical Magnetic Resonance (REMA)

MEDICINAL CHEMISTRY (CMFA)



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MEDICINAL CHEMISTRY (CMFA)

Post-Doctoral fellows

Nadal-Bufi F.
Porte K.
Spillier Q.

PhD Students

Ancia M.
Brustenga C.
Dechenne J.
Deskeuvre M.
Lacour S.
Leone G.
Marteau R.
Mathieu C.
Mazhari D.
Pierrard F.
Savoyen P.
Tan Y.

Adm. & Techn. Staff

El Aakchioui A.
Yildiz E.

Website CMFA: https://uclouvain.be/en/research-institutes/ldri/medicinal-chemistry-cmfa.html

Address: Van Helmont building – Tower 73, 4th floor, avenue E. Mounier 73, B1.73.10. B-1200 Brussels.

Phone: +32 2 764 73 41

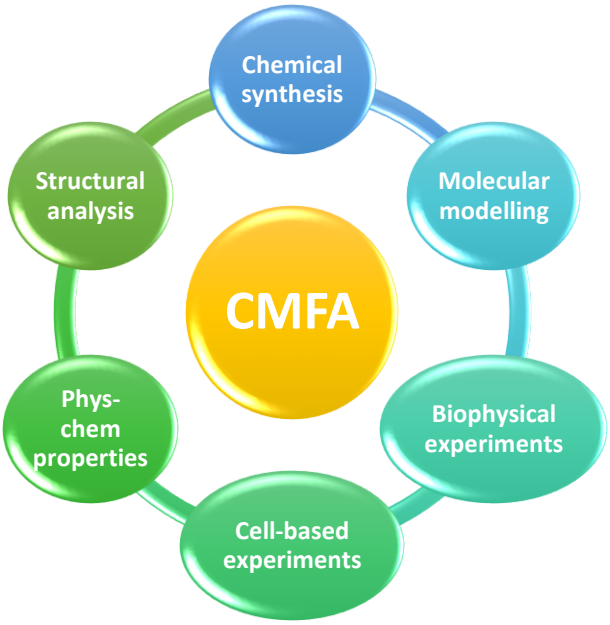
The discovery of new innovative medicines is a priority for human health. It is in this context that the Medicinal Chemistry Research Group (CMFA) is pursuing its research activities

Our team develops his expertise in the design and discovery of novel chemical tools and drugs to interrogate/target biological systems.

Over the past years, the group successfully designed and developed various series of inhibitors for anticancer immunotherapy and tumor metabolism using structure-based and fragment-based drug design approaches. The strategy was also applied to the discovery of new antibacterial agents.

More recently, our team has been interested in the development of molecules/peptides targeting protein-protein interactions using biophysical tools such as microscale thermophoresis (MST), NMR saturated transfer difference experiments or Differential Scanning Fluorimetry (DSF).

We are also studying drug conjugates such as pHLIP (pH-Low Insertion Peptides) and pegylated drug conjugates to develop new nanomedicines.

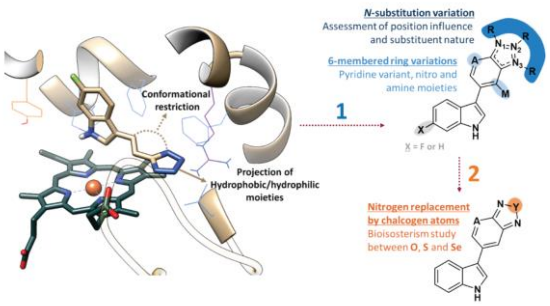


RESEARCH RESULTS

Anticancer immunotherapy

Tryptophan and arginine catabolism are important mechanism of peripheral immune tolerance contributing to tumoral immune resistance, and indoleamine 2,3-dioxygenases (IDO and TDO) and arginase (Arg1) inhibition are validated strategy for anticancer drug development.

The implication of IDO in the phenomenon of tumoral immune resistance was the focus of intense research and the enzyme is now recognized as a validated target for anti-cancer therapy. In contrast, the effect of TDO expression on the immune response has only been relatively recently investigated in detail. Indeed, we showed in collaboration with the group of Prof Van den Eynde (DDUV) 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. Our recent works (PhD thesis of **Séraphin Lacour**) led to the discovery of new TDO inhibitors acting at the TDO active site. Interrestingly, because TDO is only active in the tetrameric form, we have also recently started to study the TDO oligomeric interface with the goal to discover potential TDO oligomeric disuptors (PhD thesis of Mrs **Caroline Mathieu**).

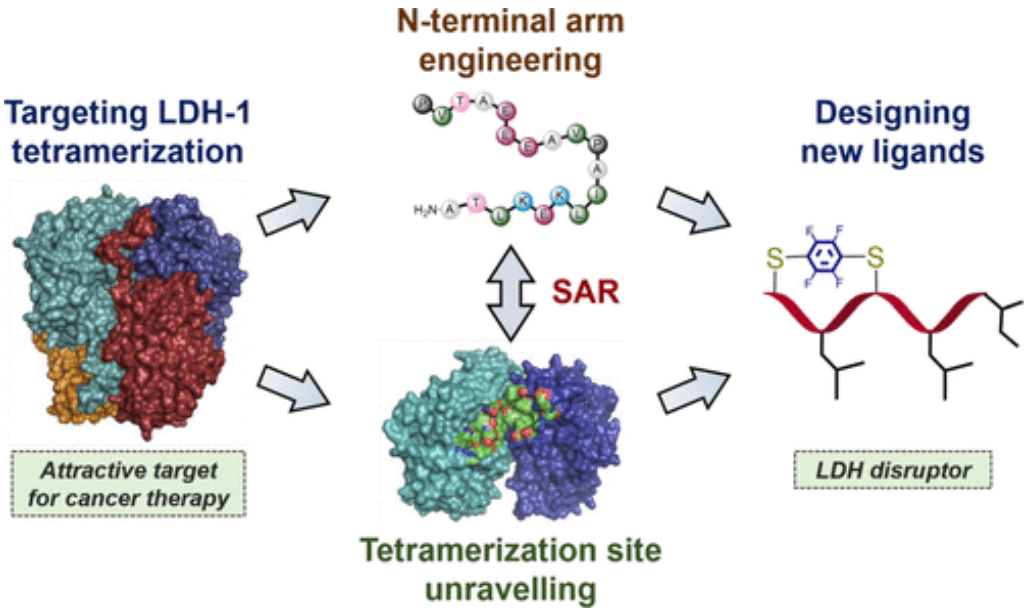


Arginine (L-Arg) catabolism by Arginase 1 (Arg1) is another mechanism contributing to tumoral immune resistance. In recent works, we have identified novel boronic acids compounds as promising Arginase inhibitors. But because boronic acids are not characterized with adequate PK properties we have recently undertook a new strategy targeting the oligomerization site of Arg1 that is a trimer (PhD thesis of **Juhans Dechenne**). So far, several mutants of Arg1 were produced as well as truncated and wild-type Arg1. The stability and activity of these Arg1 mutants as well as the effect of peptide disruptors are currently being investigated by biophysical and biochemical methodologies.

Tumor metabolism

Tumor cells are 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 use 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 fuel 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.

Several observations made in collaboration with the team of Pierre Sonveaux (IREC) suggest that LDHB may be a new target in cancer therapy. However, there is currently



no selective inhibitor of this enzyme, and the consequences of systemic inhibition of LDHB activity remain largely unknown.

In this project (Postdocs **Ferran Nadal-Bufi** & **Quentin Spillier**, and PhD thesis of **Chiara Brustenga** & **Perrine Savoyen**), our aim is 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.

So far, our pivotal collaborative works led to (a) the delineation of hot spots at the LDH tetramerization site, (b) the design and synthesis of original (stapled) peptides capable of preventing LDH self-association and/or disrupting a preformed LDH tetramer, and (c) the development of some chemical biology tools to interrogate LDH tetramerization using NMR spectroscopy (STD and WaterLogSy experiments), thermal shift, microscale thermophoresis, and fluorescence spectroscopy experiments.

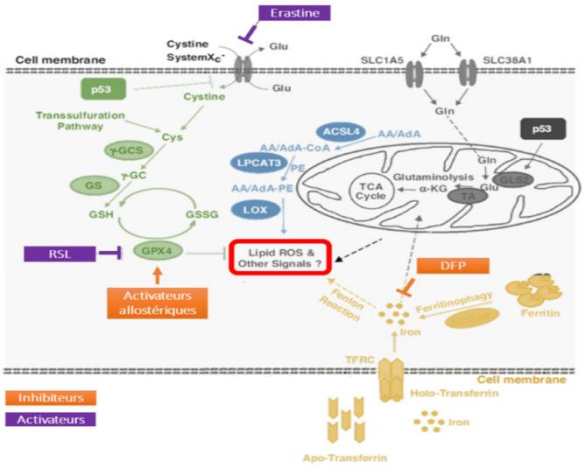
In collaboration with the team of Olivier Riant (IMCN), we are also developing novel chemical biology tools such as novel peptide stapling methodologies, the synthesis of fluorogenic probes including self-immolative linkers, etc. These innovative tools will be very helpful to interrogate LDH self-association (Phd thesis of **Yonghua Tan** & **François Pierrard**).

Ferroptosis

Ferroptosis, first coined in 2012, is a regulated cell death (RCD) characterized by iron-dependent accumulation of lipid hydroperoxides associated with an insufficient capacity to eliminate these oxidation products. A recent report uncovered acyl-CoA synthetase long-chain 4 (ACSL4) as a critical contributor to ferroptosis execution. Therefore, ACSL4 inhibitors are emerging as attractive anti-ferroptotic agents. The goal of our research program is to develop novel ACSL4 inhibitors to help establish the potential link between ACSL4, ferroptosis and NDDs. On a

longer-term perspective, it should constitute a strong basis for the development of first-in-class drugs for the treatment of NDDs.

The present project, led in collaboration of colleagues from the University of Lille, France (Prof. Séverine Ravez and Jamal El Bakali) grounds on important preliminary findings generated in our lab (Postdoc **Karine Porte** and PhD thesis of **Romain Marteau** & **Darius Mazhari**). A screening of the Selective Optimization of Side Activity (SOSA) library against ACSL4 was undertaken by TSA. Typically, this approach starts with the screening of a set of limited and structurally diverse drug-like compounds known to possess good bioavailability and safety in humans. So far, we identified three series of molecules that stabilize the folded state of ACSL4 and we validated these hits in our optimized enzymatic assay. The identified micromolar-range inhibitors of ACSL4 represent original starting points for our lead discovery program.



Drug conjugates - pH Low Insertion Peptides (pHLIP)

Targeting enzymes involved in tumor metabolism is a promising way to tackle cancer progression. The inhibition of carnitine palmitoyltransferase 1 (CPT1) by etomoxir (Eto) efficiently slows down the growth of various cancers. Unfortunately, the clinical use of this drug was abandoned because of hepatotoxic effects. In this project (Phd thesis of **Marine Deskeuvre**) we are developing pH-sensitive peptide (pHLIP)-drug conjugate to deliver Eto selectively to cancer cells exposed to acidic microenvironmental conditions. A newly designed sequence for the pHLIP peptide, named pHLIPd, was compared with a previously published reference pHLIP peptide, named pHLIPr. We showed that the conjugate between pHLIPd and Eto has a better pH-dependent insertion and structuration than the pHLIPr-based conjugate inside POPC vesicles. We observed antiproliferative effects when applied on acid-adapted cancer cells, reaching a larger inhibitory activity than Eto alone. Out works thus bring the first evidence that pHLIP-based conjugates with a CPT1 inhibitor have the potential to specifically target the tumor acidic compartment and exert anticancer effects while sparing healthy tissues.

Drug conjugates – PEGylated drugs as new nanomedicines

Inhalation is a convenient and effective route of administration of actives as it offers local treatment; nevertheless, small and lipophilic drugs are rapidly absorbed in the systemic circulation following delivery to the lungs. This translates in peaks in blood concentration of the active with consequent occurrence of systemic side effects.

In line with this, Treprostinil, a prostacyclin analogue used in the treatment of pulmonary arterial hypertension (PAH), is inhaled 4 times a day by patients. The quick absorption, with consequent emergence of systemic side effects, implies that the dose administered must be lowered in order to prevent systemic toxicity. Lower doses, more often administered through the day, partially answer the issue of systemic side effect, but the high frequency of administration represents a significant therapy burden for the patient.

The goal of this project, in collaboration with the team of Rita Vanbever (ADDB) (PhD thesis of **Giuditta Leone**) is to address these limitations by developing a nanomedicine with polyethylene glycol (PEG). The formation of a prodrug between the polymer and the small molecule will allow a slow release of the active, therefore sustaining Treprostinil residency, release and therapeutic activity within the lungs as well and improving its safety and water solubility.

Telomeres

Sarcomas, neuroblastomas and brain tumors frequently activate an alternative and telomerase-independent mechanism of telomere maintenance, dubbed ALT, based on homologous recombination events between telomeric sequences. Being absent from normal cells, the ALT mechanism offers new interesting perspectives for specific and targeted anticancer therapy. However, "druggable" ALT-specific targets are still awaiting identification.

TSPYL5 is suggested as a possible ALT target candidate. Recent discoveries indicated that TSPYL5 depletion induces ALT+ cell death without impacting normal or telomerase-expressing cells. Cell death results from strong DNA damage activation in response to telomere deprotection due to the proteasomal degradation of POT1 telomeric protein. Our current hypothesis is that, through its competitive binding to USP7 deubiquitinase, TSPYL5 inhibits the recruitment of USP7 to telomeres and the subsequent degradation of POT1.

To give a better understanding this ALT+ mechanism, we will focus, in collaboration with the team of Anabelle Decottignies (DDUV), on the TSPYL5-USP7 complex (PhD thesis of **Marine Ancia**). Biophysical and biochemical experiments will be undertaken to identify the hot spots of this protein-protein interaction and to discover small molecules capable of disrupting the TSPYL5-USP7 interaction. These pharmacological tools will help establishing the proof-of-concept that TSPYL5-USP7 disruption can exert anticancer activity in ALT+ tumour cells.

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Julien Prévost: "Towards the discovery and synthesis of new Arginase 1 inhibitors".
Director: Raphaël Frédérick

THESES IN PROGRESS

Ancia Marine: "Towards a better understanding of the Alternative Lengthening of Telomeres mechanism by the discovery of TSPYL5/USP7 protein-protein interaction inhibitors".
Director: Raphaël Frédérick
Co-director: Anabelle Decottignies

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

Dechenne Juhans: "Interrogating the self-assembly of arginase and tryptophan 2,3-dioxygenase, two proteins involved in cancer immunotherapy".
Director: Raphaël Frédérick

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

Lacour Séraphin: "Design, synthesis and evaluation of new pharmacological tools to study tryptophan-2,3-dioxygenase (TDO2) in cancer immunotherapy"
Director: Raphaël Frédérick

Leone Giuditta: "Inhaled vasodilator nanomedicine".
Director: Rita Vanbever
Co-director: Raphaël Frédérick

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

Mathieu Caroline: "Inhibition of tryptophan 2,3-dioxygenase (TDO2) by targeting its self-assembly: from the development of analytical methodologies to the identification of new potential disrupters."
Director: Raphaël Frédérick
Co-director: Marianne Fillet (ULiège)

Mazhari Darius: "Deciphering the exact role of the acyl-CoA synthetase long-chain family member 4 (ACSL4) in cancer"
Director: Raphaël Frédérick
Co-director: Jamal El Bakali (Univ. lille2, France)

Pierrard François: "Development of new chemical tools for interrogating the lactate dehydrogenase B (LDHB) tetramerization site with stapled peptides"
Director: Olivier Riant
Co-Director: Raphaël Frédérick

Savoyen Perrine: "Design and synthesis of peptide macrocycles as inhibitors of lactate dehydrogenase".
Director: Raphaël Frédérick
Co-director: Pierre Sonveaux

Tan Yonghua: "Biocompatible catalysts to release prodrugs".
Director: Olivier Riant
Co-director: Raphaël Frédérick

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Ortiz S.		Gelders F.
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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 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.*
5. *Analyse bioavailability of natural extracts/compounds*

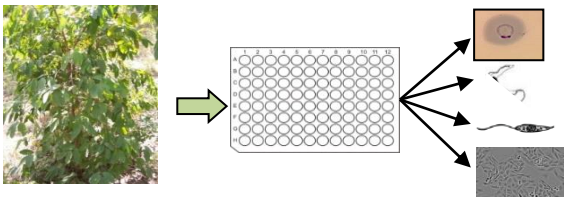
To allow these researches, we developed an expertise in extraction, purification, structure determination of compounds from complex matrices and development of quantification validated methods, while most of the pharmacological experiments are realised in collaboration with teams having expertise in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

1/ CRUDE EXTRACTS AND PURE COMPOUNDS EVALUATIONS

Plants used in traditional medicine in different countries are obtained through research collaborations (for example: Marocco, Benin, Congo Democratic Republic, Rwanda, Madagascar, Mauritius in Africa, Pakistant and 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 UCLouvain or Belgian partners): in the last years we mainly focused on antimicrobial and antiparasitic activities, but two new projects were developed dealing with antidiabetic and anti-inflammatory activities.

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



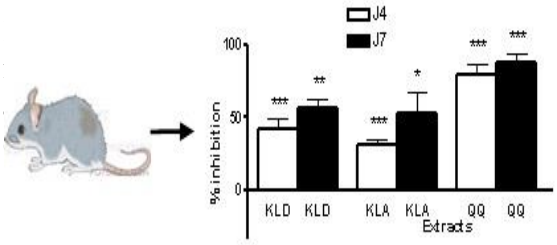
Keetia leucantha

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 or Prof. Kestemont UNamur). Extracts from i.e. *Keetia leucantha Carpolobia lutea, Vitellaria paradoxa* and *Acanthospermum hispidum* as well as isolated triterpenic esters proved to have antiparasitic properties on infected mice *in vivo.*



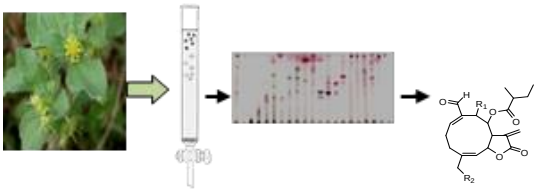
Efficient extracts and pure compounds on mice infected with *Trypanosoma brucei* were also identified and their highest tolerated dose determined.

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) and possess anti-diabetic properties (collaboration with Prof. A. Belocqui). We also collaborate with this team to and develop formulations to improve oral bioavailability of natural compounds.

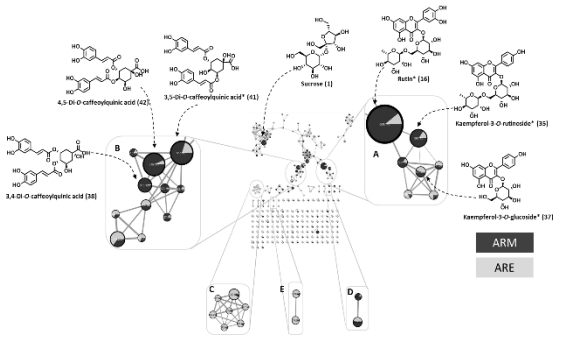
2/ ISOLATION AND STRUCTURE IDENTIFICATION OF BIOACTIVE NATURAL COMPOUNDS

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 also helped identifying known bioactive compounds and pointing out those that should be isolated. Structural identification is based on UV, IR, SM, 1D and 2DNMR spectra.



Molecular networking of C. arvensis bioactive fractions

In addition to known compounds, we identified several new molecules which are found for the first time in plants. Among them, we can cite several promising specific antiparasitic terpenic derivatives isolated form *Keetia leucantha*, *Curcuma longa*,

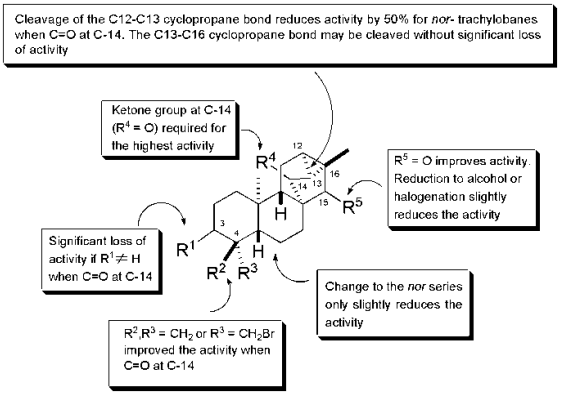
Vitellaria paradoxa or *Cymbopogon* species or essential oil components from Vietnamese plants.

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.

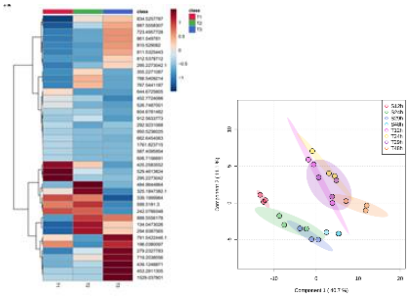
Researches on pure isolated compounds allowed us to determine some structure-activity relationships for the vasorelaxant effect of trachylobanes diterpenes (collaboration with N. Morel, IREC). Targets were identified as voltage dependent calcium channels.



Structure-activity relationships for the vasorelaxant activity of trachylobanes

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 antiplasmodial *in vitro* activity, in the same range as artemisinin.

Their effects on parasitic cells are now studied by metabolomics using LC-MS and NMR data (in collaboration with M. Frédérch and P. De Tullio from ULiège and B. Govaerts and SMCS from UClouvain) to determine the biochemical pathways modified by these natural pure compounds and identify their targets.



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…).

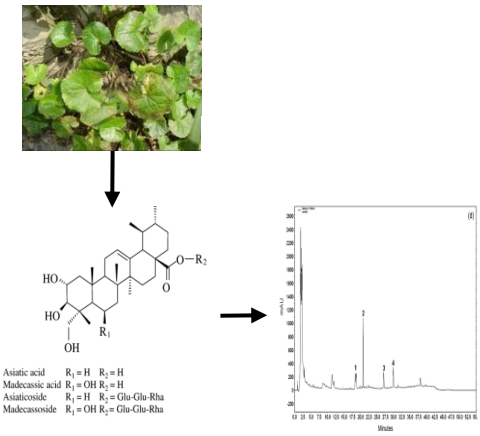
Analytical methods are useful:

- 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 and pharmacokinetic data 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 validated methods to analyse metabolic stability, identify metabolites and quantify natural or hemi-synthethic active compounds in blood or culture media.

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

5/ DEVELOPMENT OF IMPROVED FORMULATIONS FOR ORAL DELIVERY

After identifying promising natural molecules or derivatives, formations are developed in collaboration with Prof. A Belocqui to improve their oral bioavailability.

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Joëlle Quetin-Leclercq

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Abdul Khaliq H., Ortiz S., Alhouayek M., Muccioli G.G., Quetin-Leclercq J. Dereplication and Quantification of Major Compounds of *Convolvulus arvensis* L. Extracts and Assessment of Their Effect on LPS-Activated J774 Macrophages. Molecules. 2022;27(3): 963

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Schioppa L, Beaufay C, Bonneau N, Sanchez M, Girardi C, Leverrier A, Ortiz S, Palermo J, Poupaert JH, Quetin-Leclercq J. Design, Synthesis and Biological Activity of C3 Hemisynthetic Triterpenic Esters as Novel Antitrypanosomal Hits. Chemistry Open. 2021;10(9): 896-903

THESES IN PROGRESS

Tchetan Esaie *: 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

Kavungere Kambale Espoir**: Lipid nanocapsules containing green synthesized zinc oxide nanoparticles using Congolese plant extracts for the treatment of type 2 diabetes mellitus
Directors: Ana Belocqui, Patrice Memvemba (UNIKIN), Joëlle Quetin-Leclercq

*Co-promotion PhD UCLouvain and UAC (Université d'Abomey-Calavi)

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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 (including food behavior disorders and stress) associated with obesity and cardiometabolic risk, alcohol dependence, cancer development and cancer cachexia. In collaborative projects, we also evaluate the implication of the gut microbiota in xenobiotic metabolism and wound healing.

We mostly focus on bacterial supplementation or 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 microbial (co-)metabolites, gut endocrine and barrier functions and repair (wound healing), endocannabinoid

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 mechanisms 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 *ex vivo/in vitro* models, such as "Precision-Cut Liver Slices (PCLS)", primary mouse adipocytes, and mouse adipose explants, have been implemented to study the contribution of adipocytes, hepatocytes, 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 microbiota-related proteins.

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 deletion of genes involved in the host-bacteria interaction or in the synthesis of endocannabinoids have been or are currently developed and studied.

Both *in vivo* and *in vitro/ex vivo* models are exploited to analyze the modulation of metabolic, oxidative, and inflammatory stresses by nutrients, ingredients and/or pharmacological compounds.

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, behavioral analyses, 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 from the St Luc University Hospital, Gent University Hospital and Leuven University Hospital, as well as with colleagues from abroad.

1) BACKGROUND

A link exists between the composition of the gut microbiota – that is profoundly modified in genetic (*ob/ob, db/db*) 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, arabinoxylans 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.

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, which occur in nutritional or behavioral disorders (diabetes and obesity, cancer cachexia, alcohol dependence).

High-throughput molecular analysis of bacterial 16S rRNA gene allowed to point out interesting bacteria (*Bifidobacteria*, *Akkermansia muciniphila*, *Roseburia* spp., *Lactobacillus* spp., *Klebsiella oxytoca*, *Parabacteroides* spp., …) or yeast (*Saccharomyces boulardii*) in the control of host metabolic status, food intake behavior, adiposity, gut barrier function and immunity.

For recent reviews:

Thibaut & Bindels, Trends Mol Med 2022; Lefevre & Bindels, Curr Osteroporos Rep 2022.

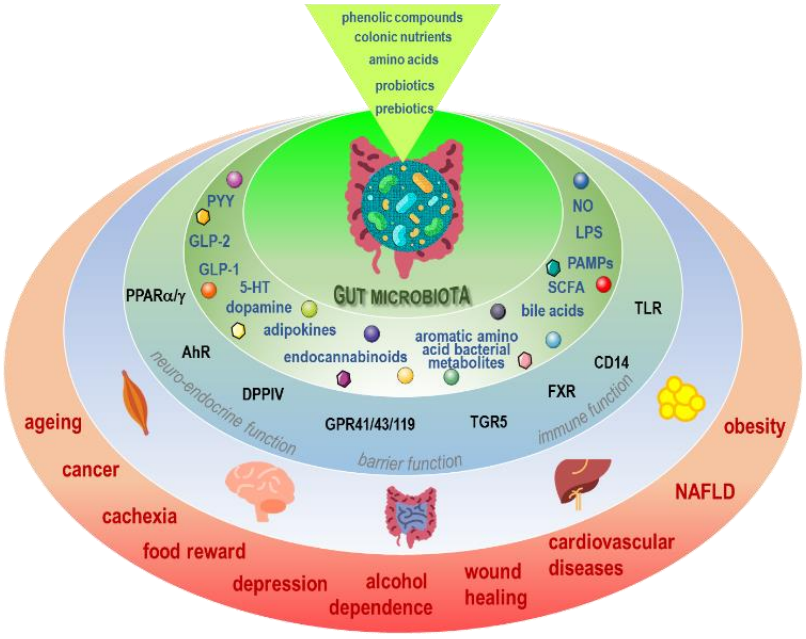
Cani, Depommier, Derrien, Everard, de Vos, Nat Rev Gastroenterol Hepatol. 2022 ; de Vos,Tilg, Van Hul & Cani Gut 2022.

Delzenne et al, Rev Endocr Metab Disord. 2020; Rodriguez, Olivares and Delzenne Mol Nutr Food Res 2021; Rodriguez and Delzenne, J Endocrinol 2021

de Wouters d'Oplinter, Huwart, Cani and Everard., Front. neurosci. 2022

2) RESEARCH ACTIVITIES

1. to develop experimental models mimicking metabolic and behavioral (including food behavior disorders and stress) disturbances occurring during obesity, cancer development, and addiction; as well as models of fecal material transfer (FMT) from human to mice for mechanistic studies.
2. to evaluate the implication and therapeutic interest of the gut microbiota and related microbial metabolites in the occurrence of metabolic and behavioral disorders, cancer progression and related cachexia, disturbed control of food intake and reward, alcohol dependence, anxiety and depression;
3. to investigate the role of the endocannabinoid system and of specific receptors responding to gut microbial components or metabolites;
4. to decipher the role of the immune system in the development of obesity, inflammation, insulin resistance, oxidative stress, type 2 diabetes, hepatic steatosis, infectious diseases (e.g. malaria) or behavior in mice;
5. to evaluate the involvement of key gut function alterations in the occurrence of behavioral and metabolic disorders associated to obesity, alcohol consumption and cancer progression.
6. 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.
7. to evaluate how drugs such as immunosuppressive agents can affect the gut microbiota and conversely how the gut microbiota can affect the pharmaco-kinetics and pharmacodynamics of drugs.
8. to study how nutrients targeting the gut microbiota may affect metabolic homeostasis, gut functions, behavior, immune system (including infectious di and organs (liver, muscle, brain, adipose tissue).
9. to use untargeted and targeted metabolomics in biological fluids and breath to analyze the relevance of microbial metabolites in the changes in behavior (depression, social behavior and food related behavior), and metabolic disorders related to cancer or obesity.
10. to isolate novel bacteria/metabolites to tackle obesity, diabetes, inflammation, cancer cachexia and some cancers.



3) OVERVIEW OF THE RECENT RESULTS

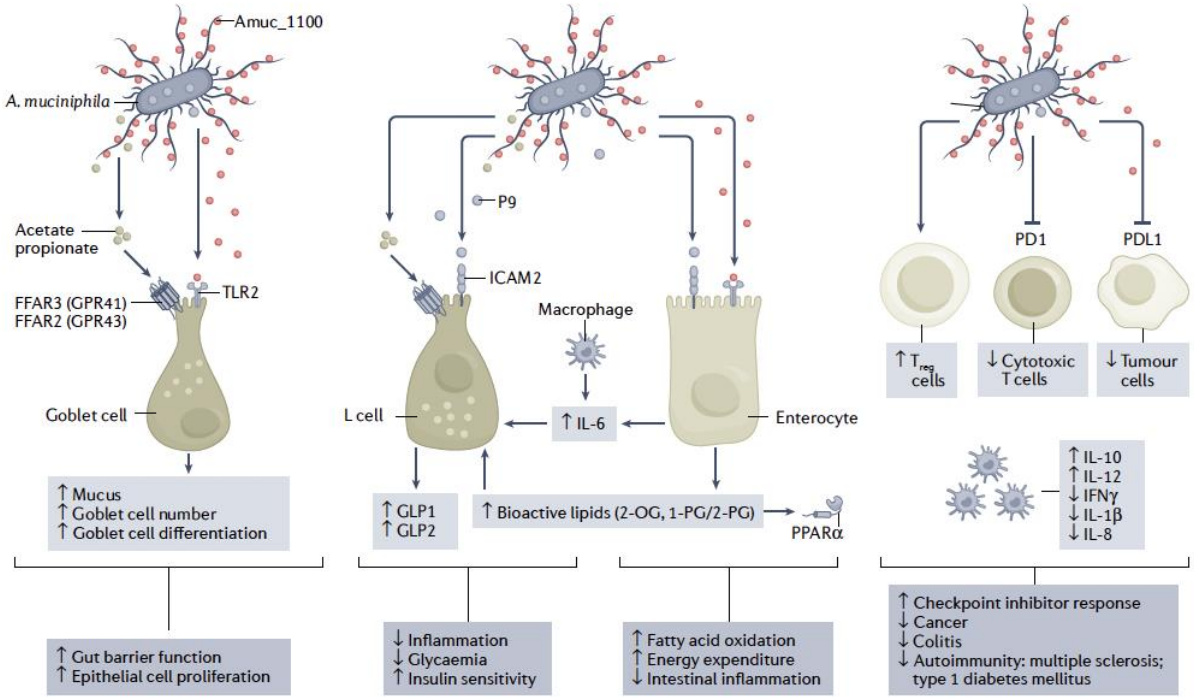
a) In the context of cardiometabolic disorders

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* negatively correlates with body weight and is decreased under high-fat diet (HFD). Moreover, daily administration of *A. muciniphila* for 4 weeks to high-fat-diet-induced obese mice – decreases weight gain, restores mucus layer thickness and antimicrobial peptides production and counteracts metabolic endotoxemia and insulin resistance (Fore Review: Cani et al *Nature Reviews Gastroenterology & Hepatology* 2022).

In accordance with the data obtained in rodents, we have shown in obese humans, that the abundance of *A. muciniphila* is inversely related to fasting plasma glucose levels, visceral fat accumulation, and adipocyte size in subcutaneous adipose tissue (Dao et al., GUT 2016). Subjects with higher *A. muciniphila* abundance have a lower fasting glucose, triglycerides and improved body composition. In addition, upon caloric restriction, obese individuals with higher baseline *A. muciniphila* displayed greater improved insulin sensitivity markers and other cardiometabolic risk factors (Dao et al., GUT 2016), whereas upon gastric bypass *A. muciniphila* is drastically increased (Dao et al. Am J Phyisol Endocrinol Metab. 2019). All these data suggest that *A. muciniphila* merits further investigation in humans. However, the culture conditions of *A. muciniphila* (growth requirement, oxygen sensitivity) compromise putative therapeutic opportunities in humans.

The team of Prof. Cani has contributed to solve these critical issues by developing synthetic medium compatible with human administration (collaboration with Prof. Willem de Vos, Wageningen).



Major mechanisms associated with the effects of Akkermansia or related molecules in diseases from.Cani et al, Nature Reviews Gastroenterology & Hepatology 2022

We demonstrated that *A. muciniphila* cultured on this media retains its efficacy (Plovier et al, Nature Medicine 2017 and patent granted) and that pasteurized *A. muciniphila* is even more efficient than the alive bacteria. We also confirmed these effects and extended the mechanisms using metabolic chambers (Depommier et al Gut Microbes 2020). With our collaborators, we identified a key protein called Amuc_1100 (Plovier et al Nature Medicine 2017). We showed that Amuc_1100 recapitulated the beneficial effects of pasteurized *A. muciniphila* (Plovier et al Nature Medicine 2017 and patents granted). We demonstrated that Amuc_1100 interacts with TLR-2 and improves the gut barrier (see figure).

Several preclinical data have been then confirmed in humans. The study Microbes4U© - published in Nature Medicine (Depommier et al. 2019) demonstrated that the administration of live or pasteurized bacteria grown on the synthetic medium is safe in humans and improves numerous cardiometabolic risks factors, including insulin sensitivity, insulinemia, inflammation, liver enzymes, cholesterol as well as markers of reinforced gut barrier. By using both lipidomic and metabolomic analysis in humans treated with either live or pasteurized *A. muciniphila*, we have discovered that *A. muciniphila* treatment induces specific modulation of different bioactive lipids that are identified as PPAR-alpha agonist (2-PG and 1-PG) (Depommier et al Cells 2021). Strikingly, using untargeted metabolomic analysis we have been able to reconstruct a metabolic pathway pointing towards the activation of fatty acid oxidation via the beta-oxidation and all the metabolites identified were converging towards a higher mitochondrial activity also under the control of PPAR-alpha (Depommier et al Gut Microbes 2021).

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 "*The Akkermansia company*, formerly named *A-Mansia Biotech SA*" in 2016 The use of pasteurized *Akkermansia* is validated by the EFSA as novel food since September 2021. In September 2022, a product has been launched on EU markets (Be, NE, Lux, IT, FR).

Dysosmobacter welbionis

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 2020, and patent pending PCT/EP2019/068539). This newly identified bacterium was detected in 70% of the healthy population. Strikingly, in obese humans with a metabolic syndrome, the abundance of *Dysosmobacter* genus correlates negatively with body mass index, fasting glucose and HbA1c. In mice, supplementation with live *D. welbionis*, but not with the pasteurized bacterium, partially counteracted diet-induced obesity development, fat mass gain, insulin resistance and white adipose tissue hypertrophy and inflammation. *D. welbionis* administration protected the mice from brown adipose tissue inflammation in association with increased mitochondria number and non-shivering thermogenesis. (Le Roy, Moens de Hase et al GUT 2022, cover of March issue). The metabolic effects of these novel bacteria are currently under investigation for their interest in the context of stress, anxiety, inflammatory bowel diseases and breast cancer (patent filed).

The innate immunity

In 2014, we found that a link between the intestinal innate immune system (i.e., the protein MyD88) and energy homeostasis. We found that deactivating the protein MyD88 in intestinal cells of high fat diet fed mice delays

type 2 diabetes development, reduces adiposity and deleterious inflammation and reinforces the gut barrier, thereby preventing the translocation of unsuitable bacterial compounds from the intestine. Deactivating MyD88- related immune thus has a therapeutic effect (inducement of weight loss), despite the fact that the animals were already obese and diabetic. Surprisingly, we found that it is possible to partially protect 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 on 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 bioactive 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).

Wound healing and microbiota

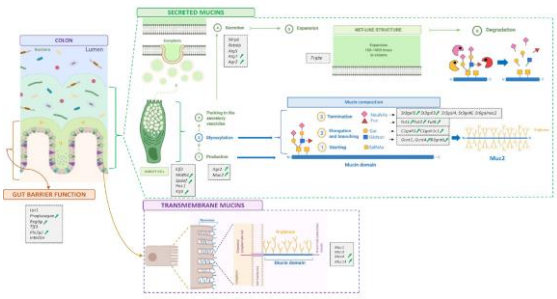
Anastomotic leakage is a major complication following colorectal surgery leading to peritonitis, complications, and mortality. Because we discovered that *Akkermansia muciniphila* has beneficial effects on the gut barrier function, we tested wether *A. muciniphila* could reduce peritonitis and mortality during colonic leakage. To do so we used the colonoscopic leakage mode that we developed in 2020, Bachmann, Van Hul et al Gut 2020). In addition, we investigated whether *A. muciniphila* can directly modulate the expression of genes in the colonic mucosa in humans. This has never been studied before. Hence, we investigated the effects of a pretreatment (14 days) with live *A. muciniphila* prior to surgical colonic perforation on peritonitis, mortality, and wound healing in mice. We used mice with an inducible intestinal-epithelial-cell-specific deletion of MyD88 (IEC-MyD88 K) to investigate the role of the innate immune system in this context. This model of cell specific deletion has been developed in our lab, see Everard et al Nature Communciations 2014).

Seven days after colonic perforation, *A.-muciniphila*-treated mice had significantly lower mortality and severity of peritonitis. This effect was associated with significant improvements of wound histological healing scores, higher production of IL22, but no changes in the mucus layer thickness or genes involved in cell renewal, proliferation, or differentiation. All these effects were abolished in IEC-MyD88 KO mice. In the proof of-concept pilot study, the healthy humans were exposed to *A. muciniphila* for 2 h and colonic biopsies taken before and after colonic instillation for transcriptomic analysis *(international collaboration with Sweden Finland, Belgium: Prof. Brummer, Prof. Repsilber, Prof. Rangel, Prof. de Vos)*. Finally, human subjects exposed to *A. muciniphila* exhibited an increased level of the bacterium at the mucus level 2 h after instillation and significant changes in the expression of different genes involved in the regulation of cell cycling, gene transcription, immunity, and inflammation in their colonic mucosa. In conclusion, *A. muciniphila* improves wound healing during transmural colonic wall defect through mechanisms possibly involving IL22 signaling and requiring MyD88 in the intestinal cells. In healthy humans, colonic administration of *A. muciniphila* is well tolerated and changes the expression of genes involved in the immune pathways.

Nutritional strategies

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 (Catry et al Gut 2018, Neyrinck et al Sci Report 2019). The change in bile acid profiling by inulin-type fructans support their potent contribution to the improvement of gut endocrine and vascular functions. We have also shown that arabinoxylo- and fructo-oligosaccharides are 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 able to modify the digestion of other nutrients in the upper part of the gut, as previously shown for dietary lipids and disaccharides (Suriano, Bindels et al Sci Report 2017; Neyrinck et al, Plos One 2016; Hiel et al Nutrient 2018). Although, we have previously demonstrated that prebiotic treatment with oligofructose (FOS) counteracted the effects of diet-induced obesity, together with changes in the gut microbiota composition, we had never studied if the intestinal mucus layer could be involved. In Paone et al Gut microbes 2022, we demonstrated that in addition to preventing high-fat diet induced obesity in mice, the treatment with FOS increased the expression of numerous genes involved in mucus production, glycosylation and secretion, the expression of both secreted and transmembrane mucins, and the differentiation and number of goblet cells. We are currently analyzing other prebiotics and their effects on the mucus composition and their degradation induced by specific microbes.



Paone et al Gut Microbes 2022

In the context of European projects (MyNewGut, http://www.mynewgut.eu/ and GUT2BEHAVE, https://www.gut2behave.eu/), we have also highlighted a potential interest of nutrients and environmental-derived microbial metabolites to modulate inflammation, behavior and metabolism (Beaumont et al FASEB J 2018, Pachikian et al Plos One 2018; Knudsen et al J Nutr 2021; Leyrolle et al Nutrients 2021), 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 -berberine-rhubarb- curcuma- or pomegranate-extracts, as well as spirulina counteract inflammation associated with nutritional disorders (Neyrinck et al Plos One 2013, Mol Nutr Food Res 2016, J Nutr Biochem 2017, Nutrients 2017, Nutrients 2021). Rhubarb extract seems highly potent to counteract diet-induced obesity and related metabolic disorders. Interestingly, these effects are strongly associated with the bloom of *A. muciniphila* in the gut of the treated mice (Régnier et al. Nutrients 2020). By comparing the metabolic effects of different dietary fibers in mice, we have shown that despite common endpoint – improved glucose tolerance – the mechanism being the effect and the changes in the gut microbiome were different (Van Hul et al Am J Physiol Endocrinol Metab 2020).

Recently, we discovered the concept of enterosynes (for review Knauf et al Neuroendocrinology 2020 and Fried et al Neuropharmacology 2021). We also found that modulating the gut microbiota with prebiotics (i.e., oligofructose) modifies the actions of enteric nervous system (ENS) and enterosynes production, thereby controlling duodenal contraction and subsequently attenuating hyperglycemia in diabetic mice; the signaling pathway depends on the synthesis of a bioactive lipid 12-

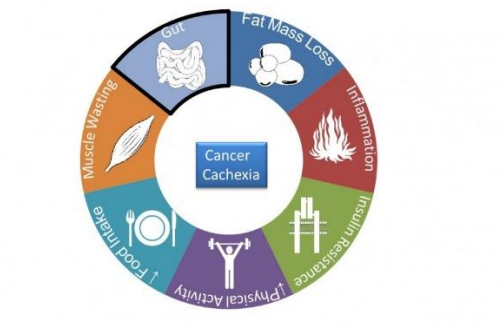
hydroxyeicosatetraenoic acid (12-HETE) and the presence of mu-opioid receptors (MOR) on enteric neurons. Interestingly, the expression of enzymes implicated in enteric neurotransmitter synthesis were altered in the duodenum of both diabetic mice and humans (Abot et al GUT 2021).

In 2019, we also published the first data showing the interest of food naturally rich in inulin in the context of the Food4gut project (www.food4gut.be). 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). Multicentric Intervention studies with inulin rich food and inulin in obese patients have been performed and are reported below in the section related to clinical studies.

b) In the context of cancer cachexia

Our work highlights the importance of the gut –including the microbial component- to target cancer cachexia, paving the way to new therapeutic opportunities (Genton et al, Front Cell Infect Microbiol 2019; Pötgens et al, Curr Opin Nutr Metab Care 2018).

Cancer cachexia is a complex multi-organ syndrome characterized by body weight loss, weakness, muscle atrophy and fat depletion. Clinically, cachexia results in increased morbidity and mortality rates as well as reduced tolerance to anti-cancer treatments. 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 (Pötgens et al, Cur Opin Metab Care 2018). In this context, targeting the gut microbiota represents an exciting opportunity for this public health issue.



Laure Bindels & Jean-Paul Thissen. Clin Nutr Exp 2016.

Links between gut microbiota and cancer have been studied for years. 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). 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 bacterium 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 (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).

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 team of Prof Bindels. To achieve such goal, her team is using targeted and untargeted metabolomics analyses using the NEST and MASSMET platforms. These data are integrated with targeted microbial metagenomics and transcriptomics to highlight new pathways involved in this crosstalk. Using such approach, we confirmed several hepatic metabolic alterations previously reported in the literature (such as a reduction in hepatic glycolysis) while revealing new pathways potentially involved in cachectic features. Specifically, we highlight (i) an activation of the hexosamine pathway in the liver, likely as a consequence of an endoplasmic reticulum stress and an unfold protein response, that may impact the hepatic signaling through O-GlcNAcylation; (ii) a reduction in the carnitine levels and its biosynthesis, and in the phosphatidylinositol pathway as potential contributors to the hepatic steatosis found in these mice; (iii) a reduction in the transformation of carbohydrates and proteins by the gut bacteria, that associates to specific host genic modulations (metabolic and gut barrier functions) (Pötgens et al, J Sarcopenia Cachexia Muscle 2021).

We further investigated the contribution of bile acids, one of the bacterial cometabolites identified through this metabolomic study. This led us to highlight a cholestasis in cancer cachexia (2 mouse models and one cohort of 94 patients) and to unequivocally demonstrate that systemic inflammation strongly contributes to the impairment of the hepatobiliary transport system in cancer cachexia. Targeting the enterohepatic circulation, we showed that bile acids contribute to hepatic inflammation and disorders (Thibaut et al, J Cach Sarc Muscle 2021). Along with alterations in the hepatobiliary transporters, bile flow was reduced in cachectic mice. Using a non-cachexia-inducing C26 cell line, we demonstrated that alterations in the bile acid pathways and profile were directly associated to cachexia and could not be attributed only to the tumoral presence. We revealed that ursodeoxycholic acid, a choleretic compound commonly used in the treatment of chronic cholestatic diseases, did not improve hepatic inflammation and worsened muscle atrophy in cachectic mice, along with a decreased TGR5 activity, suggesting that TGR5 agonists could potentially help counteracting cachectic features (Thibaut et al, Cancers 2021). Altogether, our work highlights a vicious circle between bile acids and inflammation and paves the way to new therapeutic strategies targeting bile acids to control hepatic inflammation and metabolic disturbances in cancer cachexia. In this context, we are now particularly interested to understand how the crosstalk between bile acid-activated receptors and the gut microbiota can impinge on these pathophysiological processes (Thibaut & Bindels, Trends Mol Med 2022).

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 but opioïds and endocannabinoids are also key mediators. This mesocorticolimbic system encodes for the three psychological components 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 scarce.

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 our 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. Our data demonstrate that transferring the gut microbiota from high-fat diet-induced obese mice into control diet fed mice is enough to alter the dopaminergic signaling 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 signaling 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 demonstrate for the first time the implication of the gut microbiota into the alteration of hedonic regulation of food intake during obesity (de Wouters d'Oplinter et al. Gut Micorbes 2021). We are now investigating the bacteria/metabolites as well as the mechanisms involved in these interactions between the gut microbiota and the hedonic regulation of food intake during obesity. Recently, we identified *Akkermansia muciniphila* as a novel actor able to improve the dysregulated reward behaviors associated with obesity, potentially through a decreased activation of inflammatory pathways and lipid-sensing ability in the striatum (Huwart et al. Cells 2022).

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. Four of them are described below: the **MicroAML**, the **FOOD4GUT**, the **FIBERTAG**, the **GUT2BRAIN** 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 launched in 2016 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. Recruitment was completed early 2020, sample analyses were carried out and two manuscripts are under preparation.

The FOOD4GUT project (https://sites.uclouvain.be/FOOD4GUT) is a multidisciplinary and inter-university project lead by N. Delzenne until 2019, that is further exploited in the context of current projects (METABIOTIX, PDR T.0068.19; GUT2BEHAVEPINT-MULTI R.8013.19 , NEURON, call 2019, https://www.gut2behave.eu/). It demonstrates that changing the microbiota via food rich in inulin may impact on health and behavior. A simple-blind placebo-controlled randomized multi-center trial has been conducted to highlight the interest for of inulin-type fructans in obese adults. A three months intervention versus placebo in obese volunteers, may modulate obesity (Hiel, Rodriguez et al Clin Nutr 2020). Clinical data analysis and experiments of fecal transplantation from obese patients to mice allowed to demonstrate that the efficacy of inulin intervention to improve BMI, inflammation, glycemia and mood, depend on the initial gut microbiota composition, on drug treatment and on physical exercise level (Rodriguez et al, Gut 2020; BMC Medicine 2022; Leyrolle et al, Clin Nutr 2021; Brain Behav Immun 2021; Neyrinck, Rodriguez et al Eur J Nutr 2021). New microbiota-derived metabolites that characterize the alterations of mood in obese subjects highlighted new targets to tackle mood disturbances in this context (Leyrolle et al. Nutrients 2021). Fatty liver disease severity estimated in this cohort by transient elastography revealed new extra-hepatic components of liver steatosis and fibrosis in obese patients (Lanthier et al Sci Rep 2021). Myosteatosis, but not sarcopenia, was strongly and independently associated with liver stiffness in obese patients with liver disorders (Nachit et al, JHEP Rep 2021).

Our data obtained in alcohol dependent patients allowed to point out that insufficient dietary fiber intake and the gut microbiota characteristics (and related gut barrier) are factors driving depression, craving and altered sociability in those patients (Leclercq et al PNAS 2014; Amadieu et al Clin Nutr 2021). Our recent data, performed in the model of fecal material transfer from alcohol dependent patients to mice, elaborated the causal role of the gut microbial dysbiosis in the alteration of sociability and depression, pointing out the role of ethanol-producing bacteria and hepatic alterations of beta-hydroxybutyrate in brain dysfunction (Leclercq et al Cell Rep 2020). An intervention study (**GUT2BRAIN** study-completed in 2020) with inulin-type fructans versus placebo was conducted 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 (Amadieu et al, Gut Microbes 2022).

The **FIBERTAG** project aimed 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 (Neyrinck et al, Nutr. Bull. 2020). In addition, intervention studies with dietary fibers (chitin-glutan) were conducted in healthy volunteers (Neyrinck et al, Nutrients 2020, , Rodriguez, Neyrinck et al Gut Microbes 2020) and in patients at risk for cardiometabolic health. This protocol allows to monitor noninvasively breath volatile metabolites that could be proposed as new biomarkers of dietary fibre fermentation, potentially linked to their biological properties (Neyrinck,

Rodriguez et al Gut Microbes 2021, eBio medicine 2022).

The **Microbes4U** study investigates the effects of an oral administration of *A. 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 ***A. muciniphila*** 10^9 cells/day or 10^{10} cells/day and the last group receiving pasteurized ***A. 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. Nat. Med. 2019, Depommier et al Cells 2021, Depommier et al Gut Microbes 2021).

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Patrice D. CANI

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Nathalie DELZENNE

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AWARDS 2020-2022

Alexandra Degraeve
Best poster in the category Therapeutic Drug Monitoring at the Annual Meeting of the International association of therapeutic drug monitoring and clinical toxicology 2020

Patrice D. Cani, Nathalie M. Delzenne, Amandine Everard
Highly cited researcher 2020, 2021 and 2022, Clarivate analytics Web of Sciences

Julie Rodriguez
Prize of the Belgian Society of Clinical Nutrition 2020 (the best abstract selected at ESPEN 2019); Prize of the best oral presentation of eposter, Belgian Week of gastroenterology, Antwerpen, Belgium 2020.

Patrice D. Cani
Prize AstraZeneca foundation
Medal of the Royal Academy of Medicine of Belgium

Laure Bindels
Collen-Francqui Research Professor Mandate (2022-2025)

Sarah Pötgens
Prize of the Belgian Society of Clinical Nutrition 2023 (the best abstract selected at ESPEN 2022)

THESIS DEFENDED IN 2022

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

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

Alice de Wouters d'Oplinter: « 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

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

THESES IN PROGRESS

Manon Autuori: "Breath volatolome as a new tool to study gut microbiome – nutrition – host interactions"
Director: Nathalie M. Delzenne

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

Savannah Eeckhout: "Bacterial Amino Acid metabolites against Muscle wasting (bAAm/M)".
Director: 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

Sabrina Huwart: "Roles of gut microbiota induced neuroinflammation on hedonic and food reward alterations during obesity."
Director: Amandine Everard

Axell-Natalie Kouakou : « Study of the links between obesity, gut microbiota and melanoma progression and response to therapy »
Directors: Bénédcite Jordan, Giulio Muccioli, Patrice D. Cani

Sophie Lecop: "Evaluation of the impact of new probiotic candidates on the gut barrier function in the context of cancer cachexia."
Director: Laure Bindels

Chi-Hsien Lee : "Isolating and characterizing new microbes from the human gut to improve health"
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

Jean d'Amour Mutoni : « Relationships between parasite infections and gut microbiota : experimental models and clinical study ».
Directors: Patrice D. Cani, Jean-Paul Coutelier, Nadine Rujeni

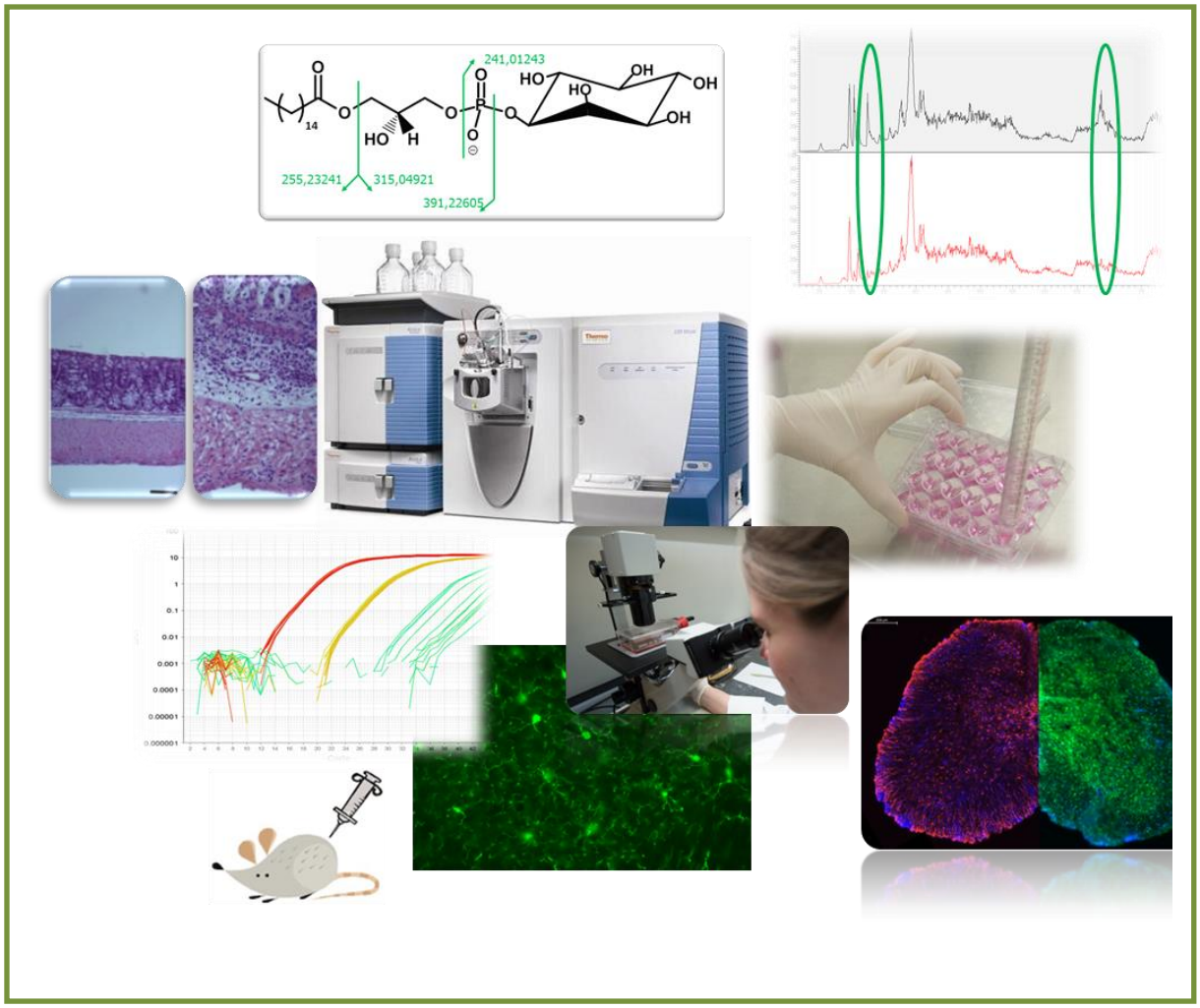
Khoi Nguyen Nguyen : "Dysosmobacter welbionis in health and diseases: novel insights from bionformatic approaches"
Director: Patrice D. Cani

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

Aline Uwimana: "Impact of nutrition on the outcome of malaria infection."
Directors: Léon Mutesa and Amandine Everard

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

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.

*Besides their role as essential constituents of biological membranes, numerous lipids possess biological activities and behave 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.

Dr Alhouayek was very recently appointed Research associate of the FRS-FNRS and will lead research in the field of inflammation resolution.

1) OVERVIEW

Bioactive lipids are important molecular mediators in inflammatory settings. As inflammation, and particularly 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.

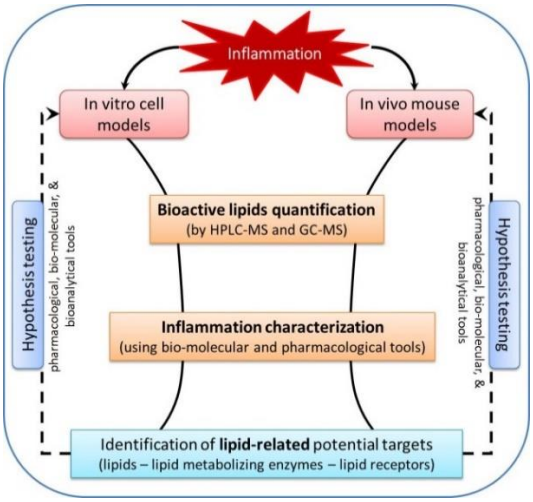


Figure 1. BPBL research group's strategy overview

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 LC-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, in two ongoing theses, we are developing quantification methods for challenging lipids due to their low abundance (e.g. PGD_2-G), as well as more general methods allowing the quantification of phospholipids and lysophospholipids (Pollet et al. Biomolecules, 2020). More recently, we developed a method allowing the quantification of linear and branched short chain fatty acids as well as TCA cycle metabolites. The method will be of large interest in the context of metabolic and microbiota studies (Paquot et al. in preparation).

b) Inflammation models

Over the years, we have implemented in our laboratory several in vivo and in vitro models to 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 2015; Alhouayek et al. FASEB J., 2018; Guillemot-Legris et al. J. Crohns Colitis 2019). Other examples of models currently used in our research group are 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; Mutemeberezi et al. J. Neuroinflammation 2018; Buisseret et al. BBA Mol. Cell Biol. Lipids, 2019; Orefice et al. Elife 2020, Buisseret et al. FASEB J. 2021; Bottemanne et al. Neurotherapeutics 2022). 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 (e.g. colon and adipose tissue). 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 showed that the ABHD6 inhibitor WWL70 strongly decreases 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 acute lung inflammation, 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).

We also demonstrated in vitro that ABHD6 inhibition in activated macrophages favors the production of PGD_2-G, a bioactive lipid

derived from 2-AG (*see Figure 2*), that we found to have potent anti-inflammatory effects (Alhouayek et al. PNAS, 2013).

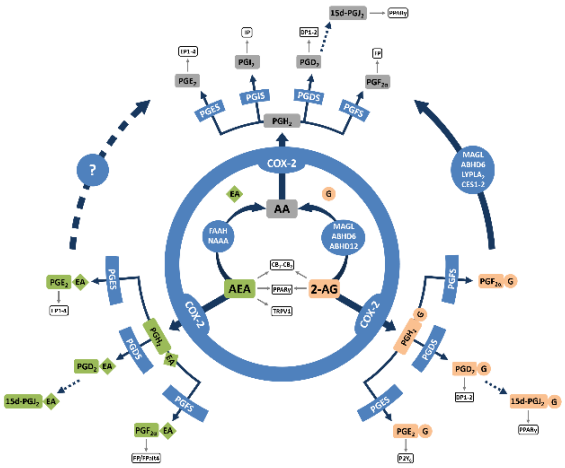


Figure 2: The two main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are part of a large network of bioactive lipids (Buisseret et al., Trends Mol. Med., 2019).

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 demonstrated 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).

Additionally, in the context of a PhD thesis (supervisors Prof. des Rieux (ADDB) & Prof. Muccioli), we have developped an innovative formulation for PGD_2-G, with the aim of increasing its delivery and efficacy in the CNS. Promising results obtained in a murine model of multiple sclerosis, the experimental autoimmune encephalomyelitis (EAE) model, support the feasibility and interest of this approach (Mwema A. et al. Nanomedicine. 2022).

Because inflammation can lead to painful sensations, we also asked whether PGD_2-G could reduce inflammation-induced pain. 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. Lipids, 2019). In a follow up study, we investigated the effects of the COX2-derived endocannabinoid metabolites (i.e. prostamides (PG-EAs) and prostaglandin glycerol esters (PG-Gs) on LPS-induced and carrageenan-induced hyperalgesia. Moreover, we compared in the same models the effect of the *S*- and *R*-enantiomers of flurbiprofen, the latter considered as a substrate selective COX inhibitor (Buisseret et al. FASEB J, 2021).

Besides these examples, 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 (Alhouayek et al. FASEB J, 2015; Alhouayek et al. BBA Mol. Cell Biol. lipids, 2017).

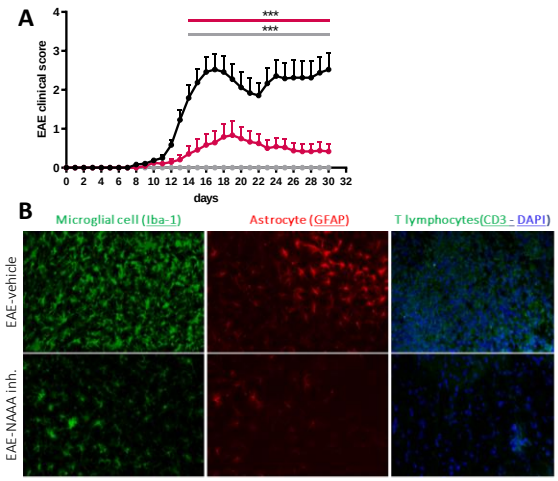


Figure 3: The NAAA inhibitor (AM11095) decreases (A) the clinical score of mice with EAE (EAE-vehicle in black, EAE-NAAA inhibitor in pink) and (B) microglia and astrocyte activation, as well as T lymphocyte infiltration in the spinal cord of EAE mice.

Case in point, we assessed the effect of NAAA inhibition in the EAE mouse model of multiple sclerosis. Our results show that NAAA inhibition decreases inflammation and demyelination in this model (*Figure 3*). Interestingly, this was not the case with FAAH inhibition in the same setting. (Bottemanne et al. Neurotherapeutics 2021).

b) Oxysterols

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

We 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 hardly 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 a mice model 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 then investigated the consequences of these alterations on obesity in vivo and ex vivo on mice and human adipose tissue explants (Guillemot-Legris, Leloup et al., in preparation).

We also reported 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 (reviewed in 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. 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 decipher further the underlying mechanisms, with several bioactive lipids as potential key mediators.

In conclusion, the 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, Guillemot-Legris O, Paquot A, Masquelier J, Malamas M, Makriyannis A, Alhouayek M, Muccioli GG. *N*-Acylethanolamine-Hydrolyzing Acid Amidase Inhibition, but Not Fatty Acid Amide Hydrolase Inhibition, Prevents the Development of Experimental Autoimmune Encephalomyelitis in Mice. Neurotherapeutics. (2021) ;18(3):1815-1833

Bottemanne P, Paquot A, Ameraoui H, Guillemot-Legris O, Alhouayek M, Muccioli GG. 25-Hydroxycholesterol metabolism is altered by lung inflammation, and its local administration modulates lung inflammation in mice. FASEB J. (2021);35(4):e21514.

Buisseret B, Guillemot-Legris O, Ben Kouidar Y, Paquot A, Muccioli GG*, Alhouayek M*. Effects of R-flurbiprofen and the oxygenated metabolites of endocannabinoids in inflammatory pain mice models. FASEB J. (2021);35(4):e21411

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

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

THESES IN PROGRESS

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

Auquière Marie: "Encapsulation of lipids and microRNAs in extracellular vesicles for the treatment of multiple sclerosis".
Director: Anne des Rieux
Co-director: Giulio Muccioli

Deltombe Matthieu: "The role of extracellular vesicle-associated miRNAs in the pathophysiology of multiple sclerosis".
Director: Vincent van Pesch
Co-director: Giulio Muccioli

Kouakou Axell-Natalie: "Study of the links between obesity, gut microbiota and melanoma progression and response to therapy"
Directors: Bénédcite Jordan, Patrice D. Cani, Giulio Muccioli

Laghouati Adam: "Study of the involvement of the endocannabinoid system in the resolution of inflammation".
Director: Mireille Al Houayek
Co-director: Giulio Muccioli

Leloup Romane: "GPR183 et ses ligands en tant que modulateurs de l'inflammation du tissus adipeux durant l'obésité".
Director: Giulio Muccioli

Ma Zhanjun: "Taking the best of two worlds to treat spinal cord injury: traditional chinese medicine Rosmarinic acid combined with stem cell derived-extracellular vesicles"
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

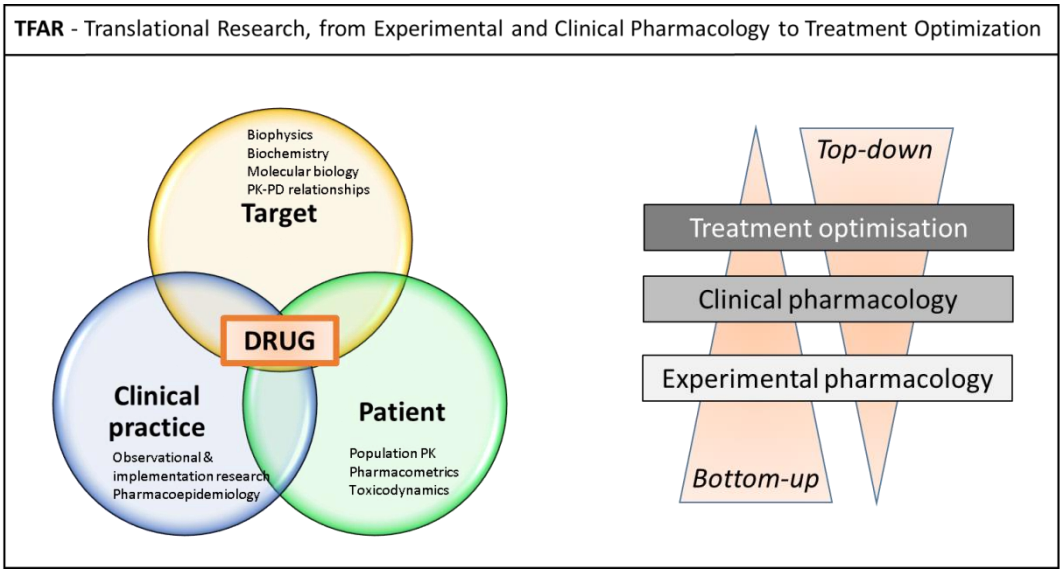
Paquot Adrien: "Development and validation of an HPLC-MS method to quantify the oxygenated derivatives of the endocannabinoids"
Director: Giulio Muccioli

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

Van Boxstael Elisabeth: "Study of the *N*-acylethanolamines and prostamides as modulators of glial cell functions in the context of multiple sclerosis".
Director: Giulio Muccioli
Co-director: Vincent van Pesch

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 pharmacodynamics (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, Françoise Van Bambeke and Joseph Lorent) 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 2022, there were several ongoing projects implying twenty-four 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)
- 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.*

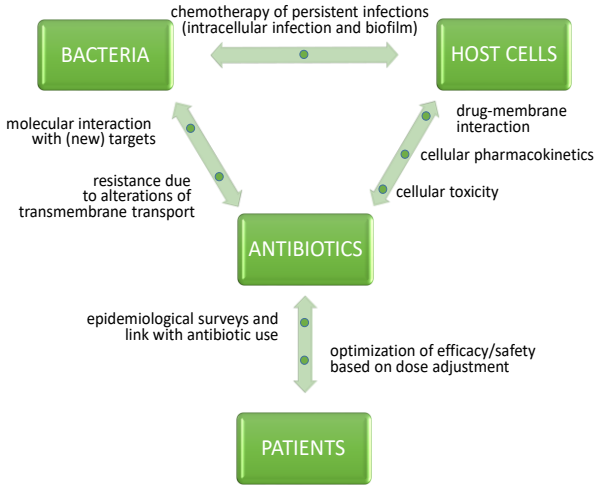
To this effect, we explore

- *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 thereof 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

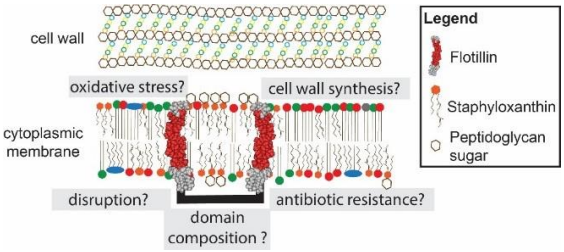
Over the last 5 years, we have published 80 papers, 83% of which directed related to our research dealing with anti-infective pharmacology and drug-membrane interactions (17% as reviews or book chapters and educational papers related to anti-infective pharmacology or pharmacotherapy, papers in the field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

Our experimental research is oriented in different topics closely linked to one another.

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.

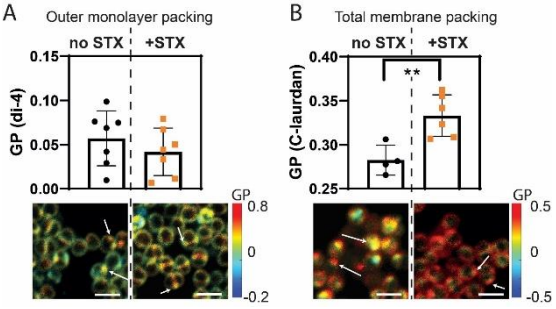
1) Bacterial microdomains as targets for antibiotic resistance in Staphyloicoccus aureus

Bacterial superbugs such as methicillin resistant staphylococcus aureus (MRSA) constitute major problems in common and hospital acquired infections by evading most antibiotic treatments. Recently it was shown that resistance towards β-lactams in MRSA depends on the presence of staphyloxanthin enriched membrane domains that can recruit the penicillin binding protein PBP2a. In this project, we want to study the lipid and protein structure of these domains, possible roles that emerge from the presence of staphyloxanthins, and ways to disrupt these domains to restore antibiotic susceptibility.



Membrane microdomains in MRSA are composed of staphyloxanthins, other unknown lipids and flotillins. They are implicated in numerous homeostatic functions such as antibiotic resistance

In first results, we were able to show that the presence of staphyloxanthins was not necessary to form microdomains but that it influenced inner leaflet membrane packing. This means that lipids besides staphyloxanthins can promote the formation of microdomains and that staphyloxanthins rather regulate domain properties.



*Staphyloxanthins (STX) have no effect on outer monolayer packing (A, di4-ANEPPDHQ) but increase membrane order in the whole membrane and hence the inner membrane (B, C-laurdan) in S. carnosus. Formation of domains (white arrows) does not rely on STX. Statistical analysis: One-way ANOVA, **p<0.01, white bar=2µm*

These results enlighten the complexity of bacterial microdomains and their possibly asymmetric composition. Understanding the physicochemical parameters which drive domain formation will be necessary to fully understand the resistance mechanism in MRSA and the development of potential solution to counteract domain mediated resistance.

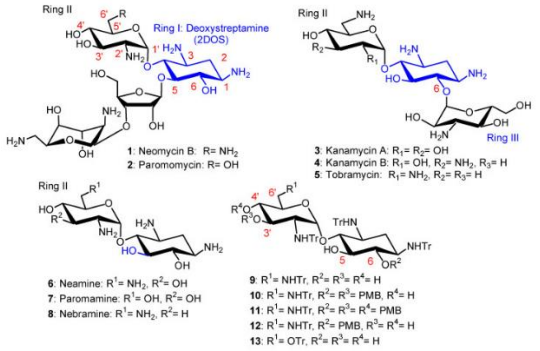
2) Cardiolipin domains as a target for new amphiphilic aminoglycoside derivatives?

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, the nature of the hydrophobic tail as well as the position and the number of substitutions 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 derivatives at sub-inhibitory concentrations.

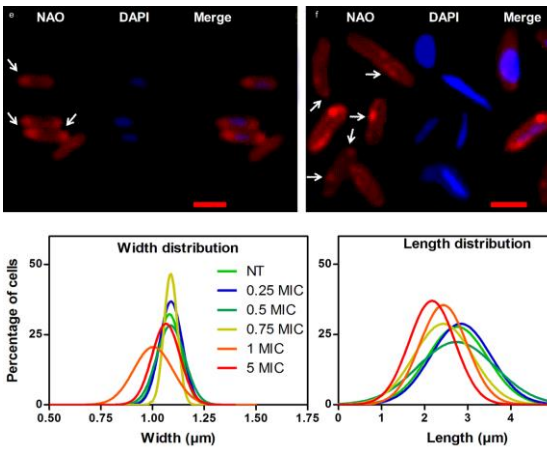


Structures of natural antibiotic aminoglycosides 1–5, of some corresponding constitutive derivatives 6–8 and of synthetic intermediates used to prepare amphiphilic aminoglycosides (AAGs) 9–13 (Tr = trityl group = triphenylmethyl, PMB = para-methoxyphenyl group). (Dezanet et al, 2020, Int.J.Mol.Sci)

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.

3) Cholesterol-enriched domains: involvement at cellular level and for nanomechanics

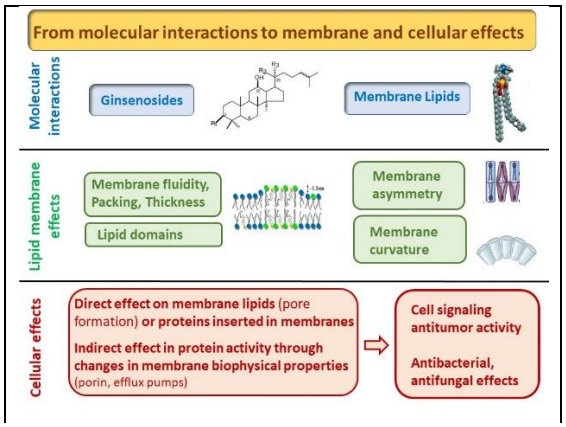
The existence of clusters of proteins and lipids and especially, the transient nanometric cholesterol- and sphingolipid-enriched domains, called rafts, are described as signaling platforms for a wide range of cellular responses to stimuli including reactive oxygen species (ROS) generation, inflammatory cytokines expression and cell death. They also play a role in nanomechanistics.

We first explored the role of cholesterol and cholesterol-enriched domains, in the mechanism of action of a potential anticancer drug, the steroid saponin (protopanaxatriol) known as one of the active principles of *Panax ginseng* root, the ginsenoside Rh2. Second, the role of cholesterol-enriched domains was investigated in the nanomechanics of breast cancer cells.

First, regarding the potential mechanism of action of the ginsenoside Rh2, 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.

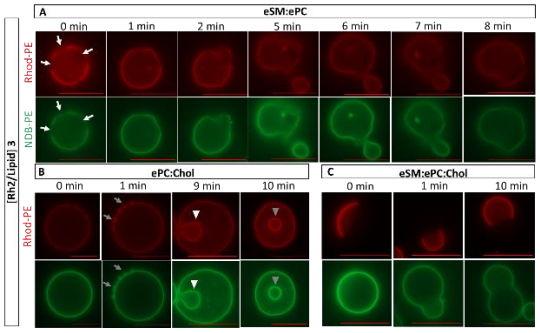
The cytotoxic activity of Rh2 is accelerated in human leukemic U937 cell lines upon cholesterol depletion via the pretreatment with methyl-β-cyclodextrin, a cholesterol-sequestering agent. Mechanistically, Rh2 alters plasma membrane fluidity by compacting the hydrophobic core of lipid bilayer (DPH anisotropy) 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).



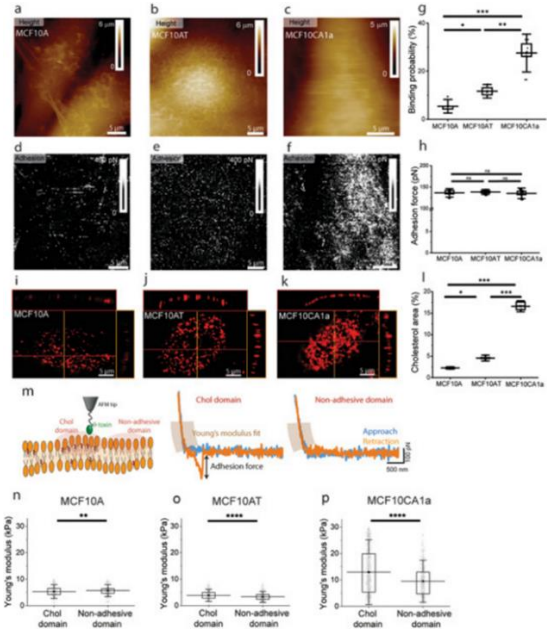
From molecular to cellular and biological effects induced by the interactions of ginsenosides with lipids (Verstraeten et al, 2020, Front. Microbiol.)

At the molecular level, Rh2 induces positive membrane curvature in the presence of SM and small buds followed by intraluminal vesicles in its absence.



GUVs were labeled with 18:1 Lissamine Rhodamine-PE (red) and NBD-PE (green) to reveal the liquid-disordered (l_d) and liquid-ordered (l_o) phases, respectively. GUVs were incubated with 1% DMSO (control, 0 min) or Rh2/lipid ratio of 3. Arrows point to s_o phases, grey arrows to small buds, arrowheads to inward membrane budding and grey arrowheads to intra-luminal vesicles. Scale bars, 20 µm.(Verstraeten et al, 2019, Sci Reports)

Second, we explored the biomechanical properties of plasma membrane (PM) of a series of MCF10 cell lines, used as a model of breast cancer progression. Notably, a strong correlation between the cell PM elasticity and oncogenesis is observed. The altered membrane composition under cancer progression, as emphasized by the PM-associated cholesterol levels, leads to a stiffening of the PM that is uncoupled from the elastic cytoskeletal properties. Conversely, cholesterol depletion of metastatic cells leads to a softening of their PM, restoring biomechanical properties similar to benign cells. As novel therapies based on targeting membrane lipids in cancer cells represent a promising approach in the field of anticancer drug development, this method contributes to deciphering the functional link between PM lipid content and disease.



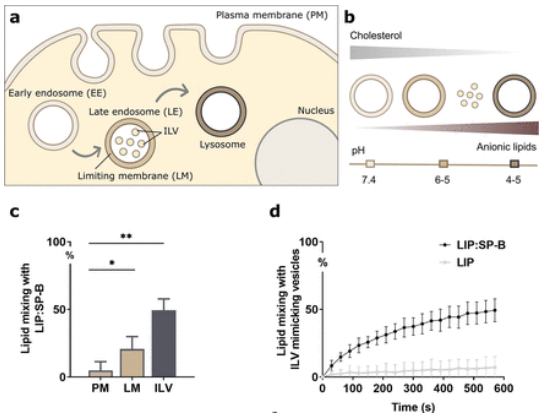
Plasma membrane cholesterol increases on malignant MCF10CA1a cells. a–c) FD-AFM height images; d–f) corresponding adhesion maps for MCF10A (healthy), MCF10AT (premalignant), and MCF10CA1a (malignant) cells; g) Binding probability; h) adhesion forces as extracted from adhesion maps; i–k) Confocal z-stack images of cholesterol staining with mCherry-θ-toxin on the surface of MCF10 cells m) A θ-toxin derivatized AFM tip probes the surface of the cellular PM; n-p) Elasticity of cholesterol and non-adhesive domains for MCF10A, MCF10AT, and MCF10CA1a. (Dumitru et al, 2020, Adv Sci).

4) Membrane biophysical properties and cellular delivery

Membrane properties like the ability to lipidic fusion is important, as for cellular traffic. Here we wanted to obtain mechanistic insights into the SP-B(Surfactant Protein B)-mediated cellular delivery of siRNA.

Using an *in vitro* non-small-cell lung carcinoma model with lipid mixing assays on vesicles that mimic the composition of (intra)cellular membranes, we demonstrated a strong correlation between SP-B-mediated fusion with anionic endosomal membranes and cytosolic siRNA delivery, a mode of action resembling that of certain viruses and virus-derived cell-penetrating peptides. This provides a

mechanistic understanding of SP-B-induced perturbation of intracellular membranes, offering opportunities to fuel the rational design of SP-B-inspired RNA nanoformulations for inhalation therapy



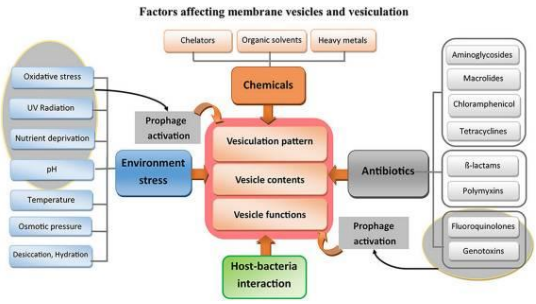
Lipid mixing of SP-B-containing liposomes and lipid-coated nanogels with liposome models of cellular membranes. (Guargliardo et al, 2021, ACS Nano).

5) Membrane remodeling and bacterial microvesicles

Membrane vesicles are the nano-sized vesicles originating from membranes. The production of membrane vesicles is a common feature among bacteria. Various physiological and ecological roles have been attributed to membrane vesicles under both homeostatic and stressful conditions. Nutrient deficiency, the presence of antibiotics as well as elements of the host's immune system are examples of stressors threatening pathogens inside their host. To combat stressors and survive, pathogens have established various defensive mechanisms, one of them is production of membrane vesicles. Pathogens produce membrane vesicles to alleviate the destructive effects of antibiotics or other types of antibacterial treatments.

Additionally, membrane vesicles can also provide benefits for the wider bacterial community during infections, through the transfer of resistance or virulence factors.

Besides, regarding that membrane vesicles play vital roles in bacteria, disrupting their production may suggest an alternative strategy for battling against pathogens.



Inducers of vesiculation in bacteria. The stresses that change the vesiculation in bacteria categorize into five major groups; (I) Environmental abiotic stresses, (II) Groups of antibiotics, (III) Chemical treatments, (IV) Prophage effects, and (V) effects of interactions between bacteria and their host. (Mozaheb and Mingeot-Leclercq, Frontiers Microb., 2020)

We now examine the difference in membrane fluidity between planktonic and biofilm modes of growth in P. aeruginosa and whether the capability to alter membrane rigidity in *P. aeruginosa* could be transferred via MVs.

6) 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

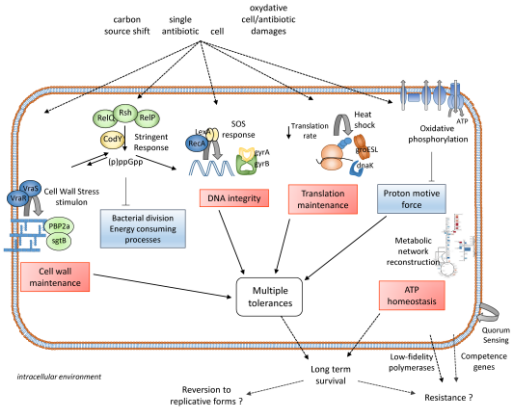
We study the cellular accumulation (including the mechanisms of entry) and the subcellular localization of novel molecules in preclinical and clinical development, as a basis for further studies examining their intracellular activities in specific compartments. We try to decipher the mechanisms for their 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 also examine 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. In collaboration with PMGK, we are now also examining the impact of genetic polymorphism of efflux transporters on antibiotic transport in host cells.

II) Cellular pharmacodynamics

In parallel, we study the activity of antibiotics against intracellular bacteria sojourning in different subcellular compartments, mainly *Listeria mono-cytogenes* (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.

We are now trying to elucidate the mechanisms by which intracellular bacteria become tolerant to antibiotics. We specially focus on trying to identify genes involved in intracellular persistence. To this effect, we ran a transcriptomic analysis of intracellular *S. aureus* surviving antibiotic exposure within permissive eukaryotic cells. We found that these survivors were persisters, i.e. phenotypic variants exhibiting transient non-growing state and antibiotic tolerance. This phenotype was stable but reversible upon antibiotic removal, unveiling a reservoir for relapsing infection. These persisters harbored a major transcriptomic reprogramming but remain metabolically active. Regulation mechanisms were not solely dependent on stringent response but included a network of responses displaying multiples entries, comprising the activation of cell wall stress stimulon, SOS and heat shock responses. These changes led to multidrug tolerance after exposure to a single antibiotic.

We have also characterized the capacity to survive inside these cells of clinical isolates collected from persistent infections and have shown that isolates showing a higher persister character are also those that acquire more rapidly antibiotic resistance and survive better to antibiotics intracellularly.



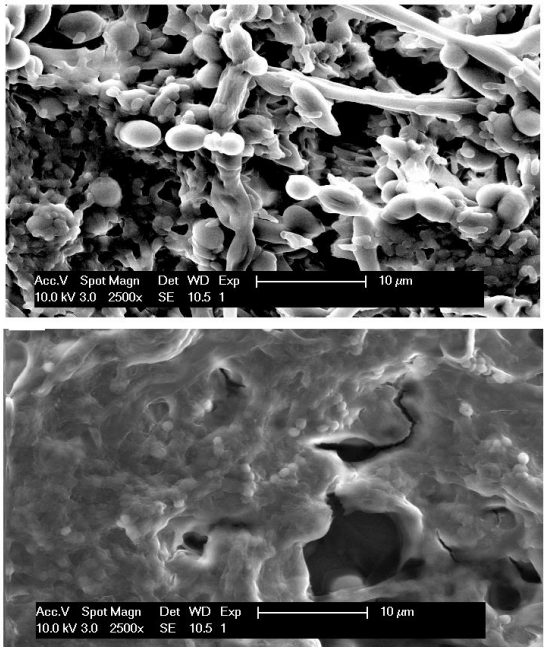
Overview of intracellular persistence regulation of S. aureus. In vacuolar nutrient-rich compartments, persisters are

metabolically active cells shielding cell wall, DNA and translation products. Under environmental factors from the host cell, including a carbon source shift and antibiotic pressure, persisters promote a network of stress or adaptive responses displaying multiple entries. Stringent response does not show signs of activity for prolonged periods but rather contributes partly to initiate the switch to a persister phenotype through (i) post-translational modifications, contributing to an almost immediate blockade of bacterial division, and (ii) transcriptional regulation, silencing energy-consuming processes. Regulation circuits also include the cell wall stress stimulon, the SOS response, and the heat shock response. These active responses, together with a decrease in oxidative phosphorylation and in translation levels, lead to multidrug tolerance upon exposure to a single antibiotic. This stable phenotype allows bacteria to maximize the chances of long-term survival. Finally, depending on the level of stress, this state could either revert to replicative forms, or promote the evolution to resistant forms, through increased probability of mutations and horizontal gene transfer. Peyrusson et al. 2020, Nature Communications

7) Antibiotic activity against biofilms

We developed in vitro pharmacodynamic models to evaluate the activity of antibiotics against biofilms made of *S. aureus* 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, among which the combination of antibiotics with enzymatic cocktails or with phages.

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.



Scanning electron microscopy of an interkingdom biofilm (C. albicans: S. aureus: E. coli) gown on Titanium, after 24 h incubation in control conditions (top) or after incubation successively with a protease during 1 h and the with the combination meropenem-caspofungin during 24h. Ruiz Sorribas et al, 2022, Microbiology Spectrum

8) Antibiotic efflux and permeability resistance mechanisms

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

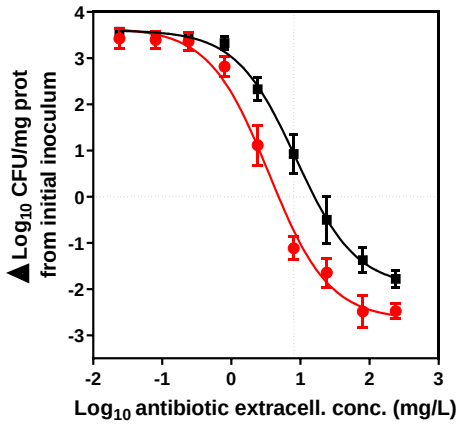
We have now evaluated the impact of this mechanism of resistance in *Achromobacter xylosoxidans,* a bacterial species which follows *P. aeruginosa* in the colonization of the lung of patients with cystic fibrosis (CF).

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.

9) 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 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, biofilms and activity against strains that show resistance to other antibiotic classes or mutations in their efflux systems.



Activity of doxycycline alone (black) over a broad range of extracellular concentrations (vertical dotted line, MIC) or combined with the polyaminoisoprenyl compound NV716 (red) against intracellular P. aeruginosa PAO1 after 24 h incubation. The graph shows that NV716 increases both the relative potency (static effect [horizontal dotted line] reached at a lower extracellular concentration of doxycycline) and maximal efficacy (more negative Emax value) of doxycycline.

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 patients with renal insufficiency, 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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Marie-Paule MINGEOT-LECLERCQ

Mozaheb N, Mingeot-Leclercq M-P. Membrane Vesicle Production as a Bacterial Defense Against Stress. Front Microbiol. (2020) Dec 9; 11: 600221.

El Khoury M., G. Sautrey, L. Zimmermann, P. Van Der Smissen, J.-L. Décout, and Mingeot-Leclercq M-P. Targeting Bacterial Cardiolipin Enriched Microdomains: A New Promising Antimicrobial Strategy? Sci.Reports (2017), 7:10697.

Sautrey G., M. El Khoury, A. Dos Giros L. Zimmermann, M. Deleu, L. Lins, J.-L Decout and Mingeot-Leclercq M.-P. Negatively-Charged Lipids as Potential Target for New Amphiphilic Aminoglycoside Antibiotics: a biophysical study. J. Biol. Chem. (2016), 291: 13864-13874.

Mingeot-Leclercq M-P., Deleu M, Brasseur R, Dufrêne YF. Atomic force microscopy of supported lipid bilayers. Nat Protoc. (2008), 3: 1654-1659.

Mingeot-Leclercq M-P., Tulkens PM. Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother. (1999), 43:1003-1012.

AWARDS 2022

Gert-Jan Wijnant received an award for the best oral presentation from the International Society for Antinfective Pharmacology (ISAP) during its annual meeting in April 2022.

SELECTED PUBLICATIONS
Françoise VAN BAMBEKE

Peyrusson F., Varet H., Nguyen T.K., Legendre R., Sismeiro O., Coppée J.Y., Wolz C., Tenson T., Van Bambeke F. Intracellular *Staphylococcus aureus* persisters upon antibiotic exposure. Nature Communications (2020) 11:2200

Peyrusson F, Nguyen TK, Najdovski T, Van Bambeke F. Host cell oxidative stress induces dormant Staphylococcus aureus persisters. Microbiology Spectrum 2022; 10(1):e0231321.

Miranda Bastos A.C., Vandecasteele S.J., Spinewine A., Tulkens P.M., Van Bambeke F. Temocillin dosing in haemodialysis patients based on population pharmacokinetics of total and unbound concentrations and Monte Carlo simulations. Journal of Antimicrobial Chemotherapy (2018) 73:1630-1638

Wang G, Brunel JM, Preusse M, Mozaheb N, Willger SD, Larrouy-Maumus G, Baatsen P, Häussler S, Bolla JM, Van Bambeke F. The membrane-active polyaminoisoprenyl compound NV716 re-sensitizes Pseudomonas aeruginosa to antibiotics and reduces bacterial virulence. Communications Biology 2022; 5(1):871.

Siala W., Kucharíková S., Braem A., Vleugels J., Tulkens P.M., Mingeot-Leclercq M-P., Van Dijck P., Van Bambeke F. The antifungal caspofungin increases fluoroquinolone activity against *Staphylococcus aureus* biofilms by inhibiting N-acetylglucosamine transferase. Nature Communications (2016) 7:13286

THESES DEFENDED IN 2021-2022

Dohou Angèle: "Infections prevention and control in caesarean section: multifacet analysis of determinants of rational use of antibiotics in Benin".
Directors: Olivia Dalleur, Françoise Van Bambeke

Ngougni Pokem Perrin: "Clinical pharmacokinetcis of temocillin: A way to optimize personalized dosing"
Directors: Françoise Van Bambeke, Laure Elens

Peyrusson Frédéric: "Intracellular persistence of Staphylococcus aureus: From observations to mechanisms".
Director: Françoise Van Bambeke

Poilvache Hervé: "In vitro and in vivo study of biofilm disruption strategies for the treatment of prosthetic joint infections".
Directors: Olivier Cornu (IREC), Françoise Van Bambeke

Ruiz Sorribas Albert: "Targeting inter-kingdom biofilms with antimicrobials and hydrolytic enzymes".
Director: Françoise Van Bambeke

Wang Gang: "Intracellular persistence of Staphylococcus aureus: from observations to mechanisms"
Director: Françoise Van Bambeke

THESES IN PROGRESS

Atrissi Jad: "Intracellular persistence of *Escherichia coli*"
Directors: Françoise Van Bambeke, Jan Michiels (KULeuven)
Bouzisa Mbuku Randy: "Improving the treatment of biofilm-related orthopaedic infections"
Directors: Olivier Cornu (IREC), Françoise Van Bambeke
Comein Audrey: "Study of the intracellular fate of *Pseudomonas aeruginosa:* balance between cytotoxicity and intracellular persistence"
Director: Françoise Van Bambeke
De Soir Steven: "Phage-Antibiotic Synergy for the treatment of biofilm related infections on orthopedic implants"
Directors: Françoise Van Bambeke, Jean-Paul Pirnay (Military hospital)
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
Mahieu Gwenaëlle: "Parameters that influence the cellular pharmacokinetics and pharmacodynamics of fluoroquinolones against intracellular infections"
Directors: Françoise Van Bambeke, Laure Elens
Rasouli Paria : "Pharmacological studies of combinations between antibiotics and potentiators active on the membrane of Gram-negative bacteria as an innovative strategy against persistent multidrug-resistant infections"
Directors: Françoise Van Bambeke, Joseph Lorent
Sleiman Ahmad : "Biophysical and biochemical principles of bacterial membrane microdomain formation."
Directors: Joseph Lorent, Françoise Van Bambeke
Thirot Hélène "Study of risks associate with the off-label use of antibiotics".
Directors: Françoise Van Bambeke, Anne Spinewine
Wang Zhifen: "Evaluation of the activity of antibiotics and adjuvant strategies against dual species biofilms in the context of cystic fibrosis."
Director: Françoise Van Bambeke

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, antipsychotics, glucose lowering drugs), and high risk situations (polymedication, multimorbidity, infections, low value care).

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

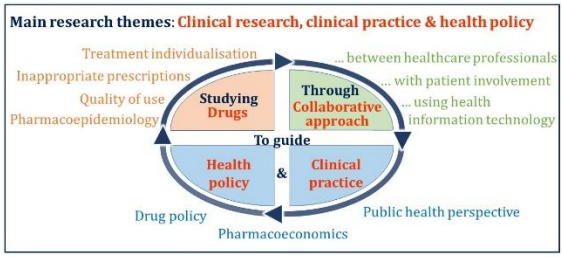
- *develop instruments and tools to measure the quality of use of medicines;*
- *collect and use observational data for pharmacoepidemiological research to assess the appropriateness of prescription and use of drugs, as well as their effects on patients in daily practice to optimise and individualize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

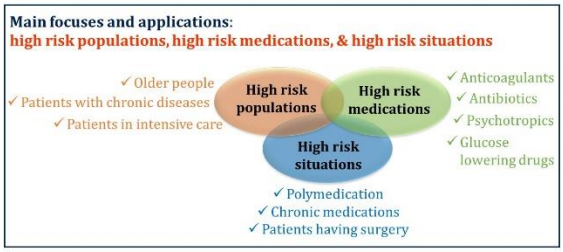
Our three group leaders (Anne Spinewine, Olivia Dalleur and Séverine Henrard) have part-time activities in other settings (clinical appointment at UCLouvain teaching hospitals, or other research institute). This feature brings a singular dynamic to our group and is a strength to operate a sound research group strategy.

In 2021-2022, Anne Spinewine spent a sabbatical year at the Ottawa Hospital Research Institute (OHRI) in the team led by J Grimshaw, to expand her skills in implementation science.

« 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 was a European project (H2020, 2015-2020) led by University of Bern. The core part of the OPERAM project was 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 results were published in 2021 in the BMJ in 2021. In a nutshell, the results are the following: (a) inappropriate prescribing was common in older adults with multimorbidity and polypharmacy admitted to hospital; (b) although inappropriate prescribing was reduced through the intervention to optimise pharmacotherapy, it did not significantly reduce drug related hospital admissions, the primary outcome measure. These data, together with additional data from substudies, are essential to design future interventions to optimize pharmacotherapy in this group. They will need to enforce prescribing advice implementation with greater involvement of the outpatient setting and to address more effectively doctors' and patients' perceived barriers to pharmacotherapy optimization.

In 2022 our group finalised and published several OPERAM substudies. First, we developed a revised trigger tool to detect DRAs (Zerah et al.). Second, we explored the patients' experience of medication review (Thevelin et al.). Third, we evaluated the association between diabetes overtreatment and clinical outcomes (Christiaens et al.). Fourth, we evaluated the prevalence of clinically significant drug-drug interactions using an explicit list that was previously validated by an international panel of experts (Zerah et al, Anrys et al.).

Additional substudies led by our group are currently being reviewed for publication – among which a substudy about drug related admissions related to antipsychotics (Bienfait et al.), and another substudy on the use and cessation of benzodiazepines during and after hospitalisation (Sibille et al.). Others are still ongoing, including a study on the comparison of drug-related admission detection methods.

- ***Deprescribing***

In 2019, we launched new research projects focusing on **BZRA 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.

In this context, as no validated tool exists specifically to evaluate older adults' attitude towards BZRA deprescribing, the rPATD questionnaire was adapted into a BZRA

specific instrument with the implication of healthcare professionals and older adults taking BZRA, and its psychometric properties were evaluated in a sample of 240 older adults taking a BZRA in the ambulatory setting or in nursing homes.

In 2022, our work focused on designing approaches to BZRA deprescribing. An important step is to assess barriers and enablers to deprescribing. To that end, we conducted (a) a systematic review of the barriers and enablers of BZRA deprescribing (Evrard et al.), and (b) a qualitative study with healthcare professionals and residents in the nursing home setting. For both studies, we used the Theoretical Domains Framework to categorize barriers and enablers. We are now designing two theory-derived interventions, one in the nursing home setting, and another in the outpatient setting. In 2023 we will evaluate their feasibility.

Two new and large research projects around deprescribing started in fall 2022 and will last for 5 years each:

(a) **BE-SAFE** is an EU project funded through Horizon Europe and coordinated by UCLouvain (A Spinewine). The goal of BE-SAFE is to improve patient safety by addressing knowledge and practice gaps related to the reduction of benzodiazepine and sedative hypnotics (BSH) used for sleep difficulties in Europe. BE-SAFE proposes an interdisciplinary and inter-sectorial approach with experts in guidelines, implementation, dissemination, case studies, geriatrics and sleep. BE-SAFE will emphasize patient involvement by establishing a Patient Partnership Advisory Council with patients, informal carers and patient organisations, which will advise on all aspects of the project. BE-SAFE will conduct a survey among patients, informal carers and healthcare professionals (HCPs) to identify barriers and enablers to BSH reduction. The results will inform the development of guidelines, implementation recommendations and patientcentred materials to support self-management. The approach will be tested in a multinational, cluster randomised controlled trial. Case studies conducted in the six countries will inform the development of country-specific and general logic models to facilitate the
scale up and spread of the BE-SAFE intervention and adapt inter-sectoral clinical pathways.

(b) **DI-PRESCRIBE** is funded through ARC (Actions de Recherche Concertée, UCLouvain)? In DI-PRESCRIBE we will explore how to enhance the implementation of deprescribing by better understanding the determinants of patients' willingness to engage in deprescribing, by better preparing future health care professionals, and by leveraging policy initiatives. In parallel, we will use the case of benzodiazepine deprescribing to perform an in-depth evaluation of implementation processes within and across three settings of care.

-

b) Individualisation of glycaemic management in older people with type 2 diabetes (T2D)

The aim of the project is to assess the heterogeneity in older patients with T2D, with the aim of improving the therapeutic management of the different profiles of patients. 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, and with researchers from King's College London (UK), Université de Bordeaux (France), and Sorbonne Université (France).

In 2022, different studies were conducted in this field. First, we published a systematic review assessing recommendations for individualised glycaemic management in older people with T2D from clinical practice guidelines (CPGs) from major scientific

societies. The 3 CPGs made 27 recommendations addressing individualised glycaemic management, a minority of which had a high level of evidence. Although there is a consensus on avoiding hypoglycaemia in older patients with T2D, significant discrepancies regarding individualised HbA1c targets exist between CPGs. Second, we have applied the definitions of glycaemic control appropriateness from these 3 major CPGs on data from clinical practice in geriatric older patients with T2D. Large discrepancy was detected across these CPGs and concerned mainly overtreatment, which may be an obstacle to the prevention of hypoglycaemia. We also conducted a third study on the overtreatment of T2D (by glucose-lowering therapy) in older patients. This sub-study of the OPERAM study, using multi-centre (European) data of 490 older (70+) inpatients with multimorbidity and polypharmacy, found that overtreatment was a frequent condition (one-third of the patients). Furthermore, we confirmed that overtreatment was independently associated with one-year mortality. Finally, on the topic of diabetes overtreatment, we conducted a literature review demonstrating that there is no standard definition of diabetes overtreatment for older patients with T2D, which leads to confusion for researchers and clinicians in this field. We therefore strongly suggested the establishment of a standardised "evidence-based" definition.

c) Use of oral anticoagulants

In 2022 we continued some research work on the appropriate use of Direct oral anticoagulants (DOACs) and vitamin-K antagonists (VKA) (led by AL Sennesael). Retrospective studies on DOAC levels in patients with low body weight and in patients receiving CYP450 inducers were performed. An ongoing project aims to describe DOAC PK in lung transplant patients and to investigate the impact of tacrolimus on DOAC disposition. Recruitment is ongoing.

In a retrospective study, we found a significant risk of reduced DOAC levels in patients taking strong P-gp and CYP3A4 inducers, especially those without risk factors for drug accumulation. These data call for DOAC measurement to help manage this relevant drug-drug interaction.

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 optimized therapeutic doses, and pharmacoepidemiological studies to evaluate the off-label use of specific antibiotics.

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

Since 2016, we collaborate with Université d'Abomey-Calavi to assess the impact of a multidisciplinary approach, including a clinical pharmacy intervention, to rationalize the use of antibiotics and promote hand hygiene in c-section practice and digestive surgery in Benin. This is the Multidisciplinary STrategy for Prevention and Infection Control (MUSTPIC) project.

Since 2020, we work at the identification and characterisation of the causative agents involved in surgical site infections (mainly *S. aureus*, 28.5%, in obstetrics, and *E.coli*, 38.4%, in gastrointestinal surgery) and the alarming rate of multidrug-resistant bacteria (90.8% of aerobic bacteria) in six public hospitals in Benin. In 2022, we described Carbapenem-Resistant Organisms Isolated in Surgical Site Infections.

Key factor for transmission of resistant organisms, healthcare professionals' behaviour towards hand hygiene was qualitatively explored through interviews. Which showed that environmental context

and resources (such as lack of water) was unsurprisingly a major barrier to HH practice. The presence of role models had a significant impact on the good practices of HH.

Another key factor is the irrational use of antibiotics. The use of antibiotics in 141 patients having undergone caesarean section was (1) frequent before admission (56.7% of patients), inappropriate in surgical antibioprophylaxis (31.2% of women received optimal antibiotic prophylaxis), and always prolonged for 5 to 10 days after the surgery. To deepen the knowledge on the use of antibiotics in surgery, our team interviewed 19 healthcare professionals. Determinants such as suboptimal patient status health, low confidence in antibiotics, some disagreement with the policy, and inappropriate infrastructures can explain poor practices.

In 2022, we also published quality analysis of antibiotics samples frequently used in surgery in Benin. Results showed that 97% (n = 32) of the samples (i.e. powders for injection: amoxicillin + clavulanic acid, ampicillin, ceftriaxone; solutions for injection: ciprofloxacin, gentamicin, metronidazole) passed visual inspection; 100% (n = 33) of the samples passed the pharmacotechnical tests, identification of active ingredients, and sterility test; 88% (n = 29) passed the test for percentage of active pharmaceutical ingredients.

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, pharmaceutical validation and clinical pharmacy practice.

f) Use of neuroleptics

Since 2019, expertise of CLIP on medication appropriateness, DRA, and deprescribing and the expertise of PMGK on pharmacokinetics and pharmacogenomics are combined in a project aiming to prevent inappropriate polypharmacy and reach precision pharmacotherapy in patients suffering from schizophrenia. A multicentric retrospective study in this population highlighted the large use of antipsychotics polypharmacy in Belgium, with 59,1% of patients discharged with inappropriate polypharmacy. Some factors were associated with antipsychotic polypharmacy, such as the use of first generation antipsychotics ($OR_{discharge}$ = 25.2, CI = 12.2-52.04), increased antipsychotic exposure ($OR_{discharge}$ = 19.89, CI = 10-39.54), or a greater number of hypno-sedatives ($OR_{discharge}$ = 4.18, CI = 2.53-6.91). In addition, we observed underuse of clozapine. Indeed, although 28.1% of patients were eligible for clozapine treatment, only 11% of patients were discharged with a clozapine prescription. In 2022, a multicentric prospective study started to describe the use of neuroleptics and the relationship between plasma levels, safety efficacy and genomics, in this population in hospital setting. Additionnaly, a survey was conducted to assess the professional social capital of psychiatrists and its influence on antipsychotics polypharmacy.

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Olivia DALLEUR

Lagreula J, de Timary P, Elens L, Dalleur O. Antipsychotic polypharmacy and clozapine prescribing patterns: evolution and correlates before and after a psychiatric hospitalisation. Ther Adv Psychopharmacol. 2022 Aug 23;12:20451253221112587. eCollection 2022.

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Thevelin S, Pétein C, Metry B, Adam L, van Herksen A, Murphy K, Knol W, O'Mahony D, Rodondi N, Spinewine A, Dalleur O. Experience of hospital-initiated medication changes in older people with multimorbidity: a multicentre mixed-methods study embedded in the OPtimising thERapy to prevent Avoidable hospital admissions in Multimorbid older people (OPERAM) trial. BMJ Qual Saf. 2022 Dec;31(12):888-898.

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Séverine HENRARD

Christiaens A, Henrard S, Zerah L, Dalleur O, Bourdel-Marchasson I, Boland B. Individualisation of glycaemic management in older people with type 2 diabetes: a systematic review of clinical practice guidelines recommandations. Age Ageing. 2021;50(6):1935-42.

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Anne SPINEWINE

Evrard P, Pétein C, Beuscart JB, Spinewine A. Barriers and enablers for deprescribing benzodiazepine receptor agonists in older adults: a systematic review of quantitative and qualitative studies using the Theoretical Domains Framework. Impl Sci 2022; 17:41.

Zerah L, Henrard S, Thevelin S, Feller M, Meyer-Massetti C, Knol W, Wilting I, O'Mahony D, Crowley E, Dalleur O, Spinewine A. Performance of a trigger tool for detecting drug-related hospital admissions in older people: anlaysis from the OPERAM trial. Age Ageing 2022; 51.

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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.

AWARDS 2021-2022

Anne Spinewine
Utrecht University Award for Excellence in Pharmacy (2021)

THESES DEFENDED IN 2021-2022

Christiaens Antoine: "Implications of health and metabolic heterogeneities for glycaemic management in older patients with type 2 diabetes".
Directors: Séverine Henrard, Benoit Boland, Michel Hermans

Dohou Angèle: "Infections prevention and control in caesarean section: multifacet analysis of determinants of rational use of antibiotics in Benin".
Directors: Olivia Dalleur, Françoise Van Bambeke

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

THESES IN PROGRESS

de Montigny Manon: "Management of the treatment of patients with polymedication in primary care: How pills connect people?"
Directors: Olivia Dalleur, Thérèse Van Durme

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

Fuchs Victoria: Evaluation of the impact of adherence, drug-drug interactions, polypharmacy, and inappropriate prescribing on efficacy and safety criteria for Direct Oral Anticoagulants in people with Atrial Fibrillation in the routine clinical practice setting.
Directors: Séverine Henrard, Anne Spinewine

Lagreula Juliette: "Optimizing pharmacotherapy of antipsychotics in clinical daily practice: Moving towards individualized care".
Directors: Olivia Dalleur, Laure Elens

Lopez Toribio Maria: "Contextual analysis and patient-centred care pathways description to enhance deprescribing of benzodiazepines and sedative-hypnotics in older adults"
Directors: Olivia Dalleur, Jean Macq

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

Shapoval Vladislav: "Deprescribing benzodiazepines and sedative-hypnotic use in older adults across Europe: from identification of barriers and enablers to process evaluation embedded in a randomised controlled trial"
Directors: Anne Spinewine, Séverine Henrard

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

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

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 mainly 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*
- *Antivirals*
- *Antipsychotics*
- *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) and Mycophenolate mofetil (MMF) 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 serious 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.

One of our current projects in collaboration with Laure Bindels (MNUT), combines in vitro, in vivo and clinical investigations aiming at characterizing how Tac PK and host microbiota are interrelated. The aim is to shed light on the mechanisms linking the gut microbiota to the Tac inter but also intra-individual PK variability. In a first animal experiment, mice (n=10 per group) were treated with Tac (3mg/kg body weight) +/- an oral non-resorbable antibiotic (ATB) cocktail that ensures gut microbiota depletion (2,6 log10 reduction). Our results demonstrate that effective ATB-mediated microbiota depletion decreases Tac systemic exposure (Figure 1) whereas no difference was observed in Tac oxidative metabolism. This observation suggests that ATB-mediated microbiota depletion is associated with a decreased Tac absorption without impacting on drug biotransformation

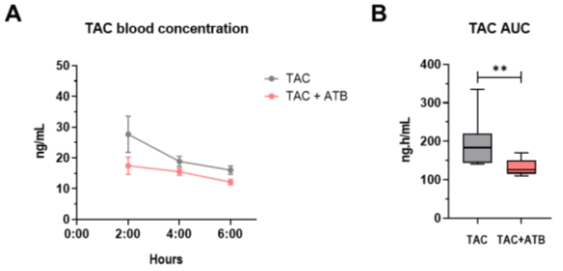

Figure 1: *A) TAC blood concentration in control (TAC) or ATB-treated mice (TAC+ATB). (B) TAC area under the curve (AUC) computed with the PK model for both mouse groups (n = 10/group). **p < 0.01.*

As Tac absorption is known to be limited by the efflux transporter abcb1a, we thus hypothesized that microbiota depletion promotes abcb1 overexpression and/or activity.

To test our hypothesis, we performed an additional experiment. Mice were divided into 4 groups of treatment (8 mice/group); vehicle, Tac, ATB, Tac+ATB. Intestinal and hepatic tissues were collected at necropsy for expression analysis by qPCR. As hypothesized, we observed a significant increased *abcb1* mRNA expression in the proximal intestine of microbiota depleted mice (Figure 2a), corroborating our original observation of a decreased Tac absorption in ATB-treated mice and correlates with TAC AUC (Figure 2b. This observation was further confirmed in vitro by treating Caco-2 intestinal epithelial cells with fecal aqueous solution prepared with feces collected in mice before or after ATB-mediated microbiota depletion. RNAseq analysis have demonstrated that this upregulation is mediated through the overexpression of CAR, a transcription factor implicated in the regulation of *ABCB1* expression.

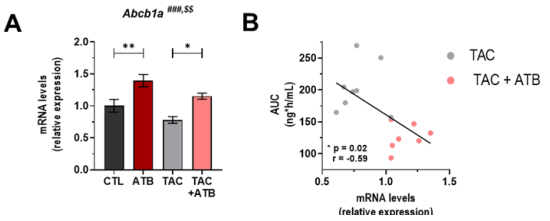

Figure 2: *(A) Comparison of the mRNA expression of Abcb1a in the proximal intestine of control and ATB-treated mice, with or without TAC treatment (n = 7-8/group). #significant ATB effect; $significant TAC effect; ***p < 0.001, **p < 0.01 and *p < 0.05. (B) Pearson's correlation between TAC area under the curve (AUC) and Abcb1a mRNA expression level in the different tissues.*

We next evaluated the impact of Tac on fecal microbiota composition by 16S rRNA sequencing and showed that Tac administration to mice had also a modulating effect on the gut microbiota composition. After 5 days of TAC treatment, the gut microbiota was significantly affected at the genus level when compared to vehicle-treated mice but also that TAC treatment reduces the α-diversity with a reduced richness and eveness in TAC treated mice. .

Human studies

During the last decade, we demonstrated 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.

In collaboration with the CUSL (Prof. Michel Mourad), we have conducted a cross sectional clinical study investigating the potential influence of a patient's microbiome on the tac dose requirement and the response to IS treatment to try to unravel the reason for the PK variability unexplained by host factors. We have recruited 100 patients under Tac-based IS therapy in combination with MMF. we have built a comprehensive biobank with fecal samples, serum, blood, urines & PBMCs. Bacterial DNA has been extracted from fecal material and rRNA16S gene sequencing analyses have been performed. In parallel, for PK profiling, we have quantified Tac and MPA(G) in blood at different times post-dose, popPK analysis have been performed and we were able to derive total AUC through machine learning.

New perspectives have emerged as we are now designing a prospective clinical study in renal transplant patients to monitor the changes in microbiota over time after transplantation. In parallel, a novel strategy with second-generation microsampling devices through volumetric absorptive microsampling (VAMS) will be applied. Basically, VAMS is an innovative strategy that can substitute venipuncture for monitoring drug levels in capillary blood with a minimally invasive finger prick procedure. Microsamples can easily be collected by the patient himself, at home, and, due to the low level of invasiveness, more samples can be collected, thereby providing a more intense PK profiling that allow to quantify precisely intra and inter-individual PK variabilities.

We expect that this project will lead us to validate new biomarkers of Tac (and other IS drugs such as MMF) PK variabilities and help refining our dosage recommendations. Moreover, important microbial biomarker identification might also become 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 fibers. We have developed recombinant HEK293 cellular models overexpressing either OATP2B1 or MRP1 or both transporters (see examples in Figure 3).

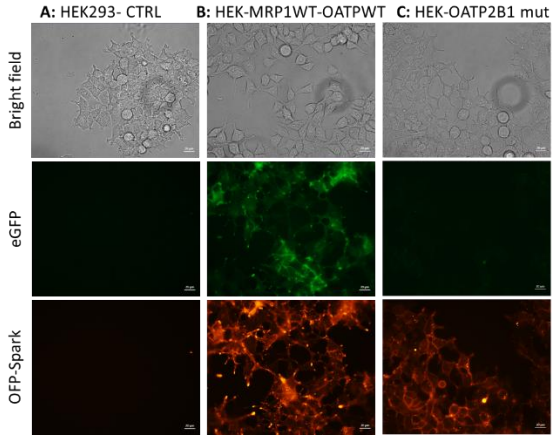

Figure 3: *(a)HEK293 non-transfected cells (b) stable double transfectants overexpressing OATP2B1 (OFP spark-red fluorescent tag) and MRP1 (Green Fluorescent protein [GFP] tag) and (c) stable single transfectant overexpressing an OATP2B1 variant.*

In collaboration with the group of Prof. Giulio Muccioli (BPBL), we have demonstrated that Atorvastatin is a good substrate of these 2 efflux pumps as we observed a significant increase in Atorvastatin intracellular concentrations in OATP2B1 (influx) overexpressing cells (Figure 4).

As both OATP and MRP transporters generate opposite drug transport, we have also assessed the combined impact of these transporters when they are co-expressed. In those double transfectants, we were able to show that ATV MRP1-efflux counteract OATP2B1 influx (Figure 4)

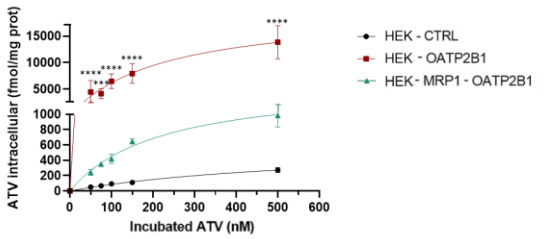

Figure 4: *Intracellular accumulation of atorvastatin in HEK293 (black), in HEK293 overexpressing OATP2B1 (red) and HEK overexpressing both OATP2B1 and MRP1 (green) after 2h of incubation with increasing concentrations of atorvastatin.*

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. With those mutagenetic experiments, we have pinpointed two natural genetic variations significantly affecting either MRP1 or OATP2B1 activity towards Atorvastatin (Figure 5).

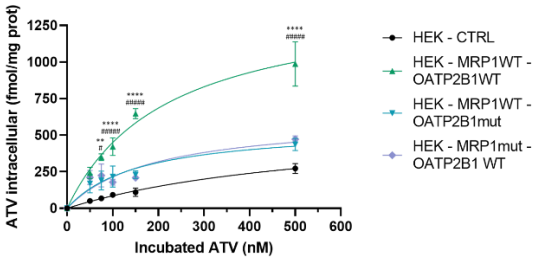

Figure 5: *Intracellular accumulation of atorvastatin in HEK293 (black), in HEK293 overexpressing WT OATP2B1+MRP1 (green), HEK overexpressing WT OATP2B1 but MRP1 variant (rs45511401) (purple) and HEK overexpressing WT MRP1 but OATP2B1 variant (rs12422149) after 2h of incubation with increasing concentrations of atorvastatin.*

In collaboration with Prof. Louise Deldicque (IONS, UCLouvain) We have now developed primary skeletal muscle cell culture from human biopsies to (i) check the physiological expression of our transporters in myotubes and (ii) assess the importance of those transporters in myotoxic response towards Atorvastatin. Recent qPCR results on differenciated myotubes indicate that the expression of transporters is highly variable among individuals. We are now investigating how it correlates with intracellular atorvastatin accumulation and possibly with myotoxic response.

Human studies

To pursue these investigations a step further, the group has collaborated with Prof. Dr JL Balligand (FATH, IREC) to unravel the reasons for PK variability of atorvastatin in clinics. In this project, we also worked 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 aims at deciphering the potential of popPK for

optimization of statin therapy. Our prospective study has included 70 patients treated with atorvastatin for hypercholesterolemia. A total of 132 PK samples at a maximum of three visits were collected in the 70 included patients. Patients were genotyped for some important biotransformation and transporter protein genes (e.g. *CYP3A, ABCs, SLCOs*…). With the collected data, we developed a two-compartment PopPK model that allowed estimating atorvastatin apparent clearance (CL/F) relatively precisely and considering the genotype of the patient for *SLCO1B1* c.521T>C SNP. Our results indicate that the estimation of the CL/F of atorvastatin through our PopPK model might help in identifying patients at risk of myalgia. We also observed that the CL/F was correlated with the efficacy outcomes. Notifying the clinician with this information can help in identifying patients at risk of myalgia and gives indication about the potential responsiveness to atorvastatin therapy. This work has been granted by the Léopold et Marthe Delsaux-Champy award

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. In a more recent study, we have characterized darunavir PK by developing a popPK model based on data collected in a large cohort of 140 Darunavir treated HIV-infected patients. α-1 acid glycoprotein level, sex, and genetic polymorphisms in the *CYP3A5* and *SLCO3A1* genes were found to be significant predictors of darunavir PK. The model was thoroughly evaluated using internal and external validation techniques.

The model also allowed us to simulate the effect of alternative dose regimens in populations representative of clinical practice. A reduction of the standard 800 mg once-daily dosage to 600 or 400 mg once-daily was found to be safe in a large proportion of patients. On the other hand, intermittent therapy (five out of seven days) constituted an unsafe option in most subjects. Whether individual patients could benefit from these alternative regimens could be predicted by our model. Additionally, optimal sampling strategies for darunavir were derived, showing how to best design future studies or how to optimize therapeutic monitoring for this drug. The following step would be to evaluate the appropriateness of these new recommendations in a prospective randomized study. A collaboration with the group of Prof. Haufroid, investigates now the factors affecting the response to new anti-HIV drugs such as integrase strand transfer inhibitors among which the microbiota composition of the patient (Dr Julien Degreef PhD thesis).

2) OUTLOOKS

The PK world is currently evolving from a descriptive explanatory tool towards a predictive modelling patient-centred method that allows proactive anticipations and individualized treatments through the identification of biomarkers. However, even if data are generated every day, 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. As such, our team is involved in a consortium focused on

developing system biology network approaches to solve complex medical problems and advance alternative therapeutic strategies through big data integration with the recent foundation of the HYGEIA (**HY**pothesizing the **G**enesis of **I**nfectious diseases and **E**pidemics through an **I**ntegrated systems biology **A**pproach) initiative. The HYGIEIA study has been designed to respond to the enormous challenges of the COVID-19 pandemic through a multi-omic approach supported by network medicine. It is hoped that in addition to investigating COVID-19, the logistics deployed within this project will be applicable to other infectious agents, pandemic-type situations, and also other complex, non-infectious diseases.

SELECTED PUBLICATIONS
Laure ELENS

Hoste E, Paquot A, Panin N, Horion S, El Hamdaoui H, Muccioli GG, Haufroid V, Elens L. "Genetic polymorphisms in SLCO2B1 and ABCC1 conjointly modulate atorvastatin intracellular accumulation in HEK293 recombinant cell lines". Ther Drug Monit. 2022 Oct 11. Doi: 10.1097/FTD.0000000000001043. Online ahead of print

Stillemans G, Paquot A, Muccioli GG, Hoste E, Panin N, Åsberg A, Balligand JL, Haufroid V, Elens L. "Atorvastatin population pharmacokinetics in a real-life setting: Influence of genetic polymorphisms and association with clinical response". Clin Transl Sci. 2021 Nov 10. doi: 10.1111/cts.13185. Online ahead of print.

Stillemans G, Djokoto HP, Delongie KA, El-Hamdaoui H, Panin N, Haufroid V, Elens L. "Effect of four ABCB1 genetic polymorphisms on the accumulation of darunavir in HEK293 recombinant cell lines". Sci Rep. 2021 Apr 26;11(1):9000.

Stillemans G, Belkhir L, Vandercam B, Vincent A, Haufroid V, Elens L. "Optimal sampling strategies for darunavir and external validation of the underlying population pharmacokinetic model". Eur J Clin Pharmacol 2020 Online ahead of Print.

Stillemans G, Belkhir L, Vandercam B, Vincent A, Haufroid V, Elens L. "Exploration of Reduced Doses and Short-Cycle Therapy for Darunavir/Cobicistat in Patients with HIV Using Population Pharmacokinetic Modeling and Simulations". Clin Pharmacokinet. 2020 Online ahead of Print.

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Sennesael AL, Panin N, Vancraeynest C, Pochet L, Spinewine A, Haufroid V, Elens L. "Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines". Sci Rep. 2018 Jul 12;8(1):10514.

Lloberas N, Hesselink DA, van Schaik RHN, Grinyò JM, Colom H, van Gelder T, Elens L. "Detection of a rare CYP3A4 variant in a transplant patient characterized by a Tacrolimus poor metabolizer phenotype". Pharmacogenomics. 2018 Mar;19(4):305-310.

de Graan A J, Elens L, Sprowl J A, Sparreboom A, Friberg L E, Van der Holt B, de Raaf P J, de Bruijn P, Engels F K, Eskens F A, Wiemer E A, Verweij J, Mathijssen R H, Van Schaik R H. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. Clin Cancer Res. (2013), 19: 3316-24.

Elens L, Bouamar R, Hesselink D A, Haufroid V, Van der Heiden I P, Van Gelder T, Van Schaik R H. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. Clin Chem. (2011), 57: 1574-83.

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.

AWARDS 2021-2022

Laure Elens: Prix - Léopold et Marthe Delsaux-Champy in cardiology (2022)

Biocodex Microbiota Fondation Grant (2021)

THESES DEFENDED IN 2022

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

THESES IN PROGRESS

Boland Lidvine: "Metabolomics and immunosuppressive therapy in Renal transplant patients."
Directors : Vincent Haufroid; Laure Elens

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)

Hoste Emilia: "Atorvastatin toxicokinetics"
Directors: Laure Elens, Vincent Haufroid (IREC, LTAP)

Lagreula Juliette: "Optimizing pharmacotherapy of antipsychotics in clinical daily practice: Moving towards individualized care"
Directors: Olivia Dalleur, Laure Elens

Mahieu Gwenaëlle: "Study of the parameters modulating the cellular pharmacokinetics and pharmacodynamics of fluoroquinolones in an in-vitro pharmacodynamic model"
Directors: Françoise Van Bambeke, Laure Elens

Ward Bradley: "OMICS data exploratory analysis of COVID-19 phenotypes through the use of a network-based integrative approach".
Directors: Laure Elens, Leïla Belkhir (IREC, LTAP)

Advanced Drug Delivery and Biomaterials (ADDB)

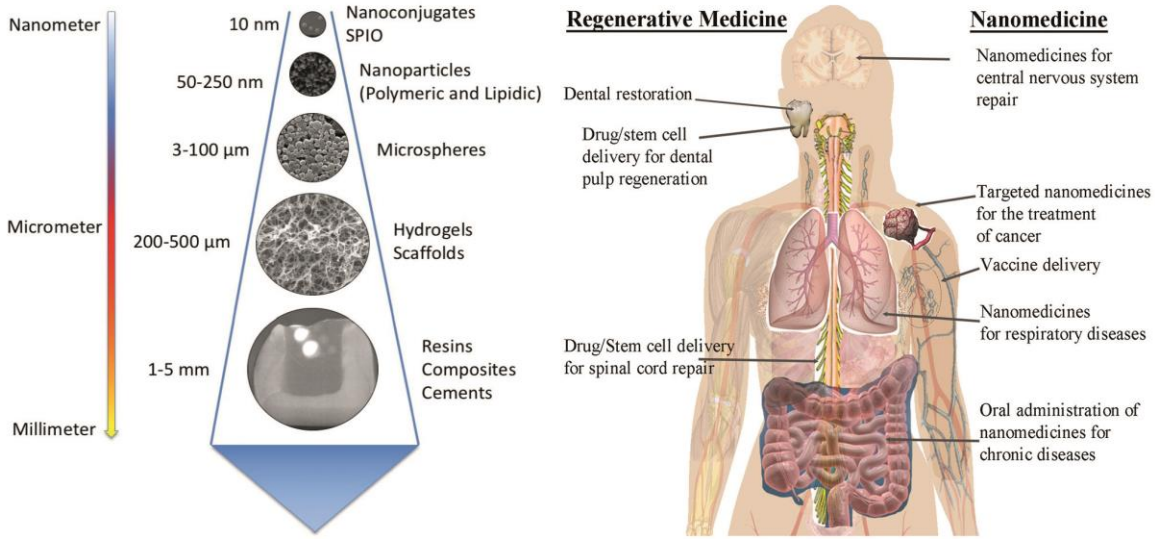

Figure 1: A. Drug delivery systems and biomaterials developed/used by the ADDB group; B. Biomedical applications targeted by the ADDB group.

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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 or local delivery of nanoparticles loaded with anticancer drugs and immunotherapy.

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 mechanisms of delivery of drug-loaded nanoparticles to tumors have been investigated (*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 nanomedicines. 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 in colorectal cancer and glioblastoma (GBM).

GBM is a particularly aggressive brain cancer associated with high recurrence and poor prognosis. The local delivery of active agents within the tumor resection cavity has emerged as an attractive means to initiate oncological treatment immediately post-surgery. Anticancer drug-loaded nanomedicines are developed for the local treatment of glioblastoma. (Figure 3) In particular lauroyl gemcitabine lipid nanocapsules forming hydrogel or PEGDMA photomerizable hydrogel loaded with anticancer nanomedicine significantly 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.

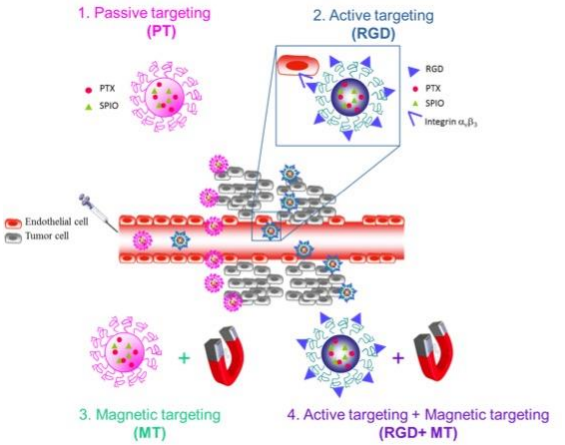

Figure 2: Passive, active and magnetic targeting of anticancer drug-loaded nanomedicines

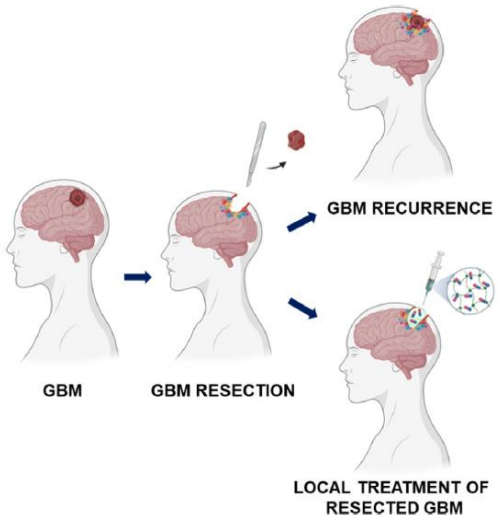

Figure 3. Schematic representation of the local treatment of GBM (From Bastiancich et al., Adv Drug Del Rev, 2021)

We aim to develop formulation (nanoparticles, polymer conjugates) and physical methods (electroporation) for the delivery of DNA and RNA with a particular interest in vaccination and cancer treatments. Optimised patented plasmids encoding tumor antigens elicited humoral and cellular immune response and induced tumor control or regression. Our current research focuses on the combination of optimized anticancer DNA vaccines (DNA vaccine and antigen-polymer conjugates) and immunomodulators.

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 biologics via the oral route of administration, especially in the treatment of chronic diseases where a daily painful administration is often required.

The aim of our research is to develop 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 three main chronic diseases: type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and inflammatory bowel diseases (IBD). Our recent results describe a novel nanosystem compatible with human use that synergizes its own biological effect with the effects of increasing the bioavailability of a GLP-1 analogue. The effects of the formulation were comparable to the results observed for the marketed subcutaneous formulation. This nanocarrier-based strategy represents a novel promising approach for oral peptide delivery in incretin-based diabetes treatment. A schematic representation of our strategy is depicted in Figure 4.

In the context of IBD, we are also developing innovative drug delivery systems for the local treatment of IBD via oral delivery of biologics (e.g. siRNA, mAb).

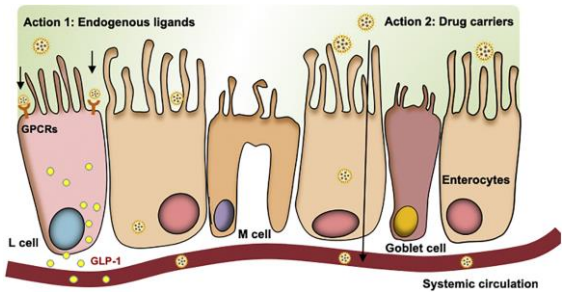

Figure 4: A schematic representation of the dual effect attained with our novel nanoformulation (from Xu et al., J Control Release, 2020).

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. This includes evaluating targeting strategies that could potentially ameliorate the transport of our drug delivery systems across the intestinal epithelium (Figure 5).

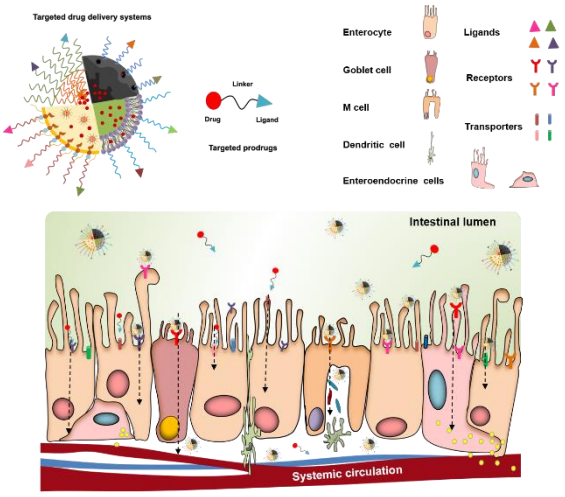

Figure 5: Targeting strategies towards the intestinal barrier (from Xu et al., J Control Release, 2020).

3) NANOMEDICINES FOR PULMONARY DELIVERY (R. VANBEVER)

The research aims at improving the treatment or prophylaxis of severe respiratory diseases by designing nanomedicines able 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 of recombinant human deoxyribonuclease I (rhDNase) is a gold-standard therapy in the management of cystic fibrosis. Yet, the rapid elimination of the mucolytic from the lungs requires its daily administration and rhDNase contributes to the high therapy burden of patients with cystic fibrosis. We have prepared a long-acting PEGylated version of rhDNase that could be delivered once weekly instead of once daily. Conjugation of rhDNase to a PEG chain sustained its presence and mucolytic activity within the murine lungs for more than 15 days. One single dose of PEGylated rhDNase was as effective as 1 daily dose of unconjugated rhDNase during 5 days to decrease the DNA content in the lungs of β-ENaC mice, a model of the CF lung disease.

We elucidated the biodistribution and elimination pathways of native and PEGylated rhDNase after intratracheal instillation in mice. In vivo fluorescence imaging revealed that PEGylated rhDNase was retained in mouse lungs for a significantly longer period of time than native rhDNase. Confocal microscopy confirmed the presence of PEGylated rhDNase in lung airspaces for at least 7 days (Figure 6). In contrast, the unconjugated rhDNase was cleared from the lung lumina within 24 hours and was only found in the lung parenchyma and alveolar macrophages thereafter. Systemic absorption of intact

rhDNase and PEG-rhDNase was observed. However, this was significantly lower for the latter. Catabolism, primarily in the lungs and secondarily systemically followed by renal excretion of byproducts were the predominant elimination pathways for both native and PEGylated rhDNase.

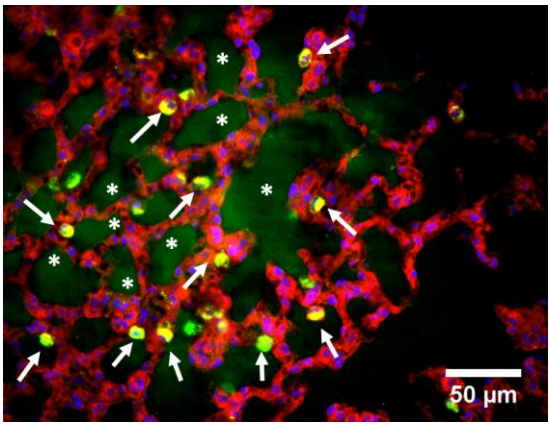

Figure 6. Localization of PEGylated rhDNase in mouse lungs by confocal imaging 4 days after intratracheal instillation. 1 nmol of Alexa488-PEG-rhDNase (green) was administered to NMRI mice by intratracheal instillation. Four days later mice were sacrificed and lung slices were imaged with Cell Observer Spinning Disk. Images were recorded in green (Alexa488-PEG30-rhDNase), red (tissue, MitoTracker Red CMXRos), and blue (nuclei, Draq5). Signal from Alexa488-PEG-rhDNase is indicated by arrows in alveolar macrophages and stars in alveolar spaces. Scale bars are 50 µm. From Guichard et al, Advanced Therapeutics, 2021.

A long-acting PEGylated version of alpha1 antitrypsine (AAT) for augmentation therapy was prepared and characterized. AAT is an endogenous inhibitor of serine proteases which, in physiological conditions, neutralizes the excess of neutrophil elastase and other serine proteases in tissues and especially the lungs. PEG-AAT conjugates preserved the ability to form a protease-inhibitor complex with neutrophil elastase and proteinase 3 as well as the full inhibitory capacity to neutralize neutrophil elastase activity. In addition, PEGylated ATT was less sensitive to proteolysis than unconjugated AAT while PEGylation had no effect on the structure and thermodynamic stability of the protein.

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 encapsulate oligodeoxynucleotides containing CpG motifs (CpG) and polyinosinic-polycytidylic acid (poly I:C) double-stranded RNA. However, the liposomes permanently entrapped CpG but could not efficiently withhold poly I: C. Both poly I:C and CpG delayed tumor growth in the murine B16F10 model of metastatic lung cancer. However, only CpG increased IFN-γ levels in the lungs. Pulmonary administration of CpG was superior to its intraperitoneal injection to slow the growth of lung metastases and to induce the production of granzyme B, a pro-apoptotic protein, and T helper type 1 cytokines and chemokines in the lungs. These antitumor activities of CpG were strongly enhanced by CpG encapsulation in liposomes.

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

The **general scientific objective** of **Anne des Rieux's research** is the development of new drug and stem cell delivery systems to address neurological unmet medical needs. Her interest focuses mainly on new delivery systems that potentiate the therapeutic action of bioactive molecules and cells. Over the years, Anne des Rieux has developed a strong expertise in nanomedicines for central nervous system (CNS) and tissue engineering.

Nanomedicines for Multiple Sclerosis

We develop nanomedicines for the central nervous system (CNS) repair. Our objective is to stimulate CNS repair by different strategies. So far, we were able to stimulate the repopulation of a brain white matter lesion by new oligodendrocytes with one ventricular injection of retinoic acid loaded lipid nanocapsules (LNC) (Thesis of D. Carradori) (*Figure 7*).

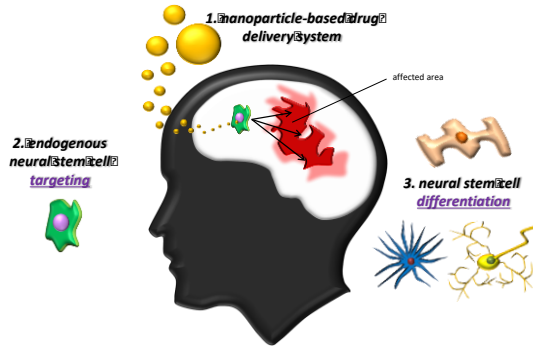

Figure 7: Targeted drug delivery for CNS repair.

We also designed SDF-1-loaded 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).

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 (MS) *(Figure 8)*. This work is performed in collaboration with Prof. Muccioli (BPBL). In that scope, we were able to graft an antibody (anti-PDGFRa) aiming to target oligodendrocyte progenitor cells at LNC surface (PhD project of Y. Labrak). We also optimized LNC by modification of their surface with a cell penetrating peptide (TAT) to enhance their Nose-to-Brain transport and to deliver to the CNS a potent anti-inflammatory lipid (PhD project of A. Mwema).

We recently started to develop nanomedicines based on extra cellular vesicles (EV), still in the scope of MS. We set a new protocol in the lab for their isolation and characterization and we are currently working on drug encapsulation in EV (PhD projects of V. Gratpain and M. Auquière). Together with Prof. Muccioli and Prof. van Pesch we obtained an ARC grant focused on EV and MS.

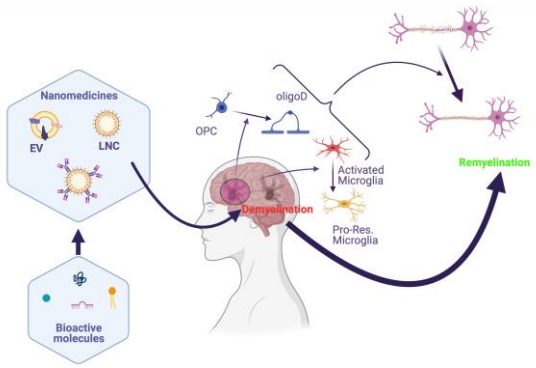

Figure 8: New drug delivery strategies for the treatment of multiple sclerosis.

Therapeutic potential of stem cells of the apical papilla for SCI repair.

Human dental stem cells of the apical papilla (SCAP) have been increasingly studied as an alternative source of mesenchymal stem cells due to their accessibility, their neural crest origin and their high proliferation rate. We have then selected them for our cell therapy projects as they have a high translational potential. This part of our research focuses mostly on spinal cord injury *(Figure 9)*.

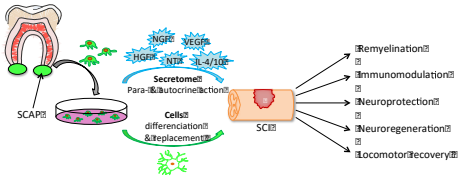

Figure 9: Therapeutic potential of SCAP for spinal cord repair.

We aim to develop new strategies for the delivery of stem cells that would ensure and support their viability and therapeutic efficiency *(Figure 10)*.

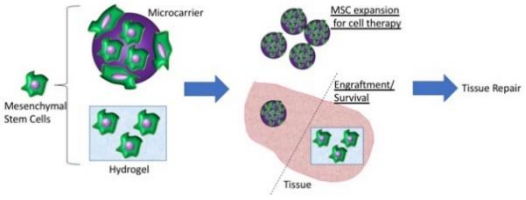

Figure 10: SCAP delivery strategies.

SCAP reduce inflammation and stimulate remyelination: when co-cultured with activated microglia cells (BV2 cells) and rat spinal cord organotypic cultures, SCAP decreased TNF secretion, increased oligodendrocyte survival and oligodendrocyte OPC differentiation, partially through activin A secretion (Thesis of P. De Berdt).

Impact of SCAP encapsulation in hydrogels: incorporation in hydrogels maintained SCAP viability, proliferation and neurodifferentiation in vitro but also proliferation, collagen secretion and angiogenesis in vivo (collaboration with Prof. Dupont, UCLouvain). Encapsulation in extracellular matrix-derived hydrogels also maintained their viability and proliferation while stimulating the expression of neural markers (collaboration with Dr. White, Nottingham University, UK). It did not affect SCAP ability to secrete immunomodulatory molecules like indoleamine-pyrrole 2,3-dioxygenase (IDO) upon pro-inflammatory stimulation nor to induce the decrease of the iNOS/Arginase ratio (TNF/IFN stimulation) (Thesis of N. Tatic).

To further enhance SCAP viability after transplantation, we used an alternative cell delivery system termed pharmacologically active microcarriers (PAMs) loaded with brain derived neurotrophic factor (BDNF)[18] (*collaboration with Prof. Montero-Menei, Angers University, FR*). We observed a significant increase of TSG-6, VEGF, activin A and IL10 gene expression when SCAP were grown on PAMs, as well as an improved locomotor function of rats subjected to a spinal cord contusion treated with BDNF-loaded PAMs, compared to non-treated animals (Thesis of S. Kandalam).

Apical papilla stimulates spinal cord repair: when implanted in a rat spinal cord hemisection model, the human apical papilla allowed the restoration of several gait parameters (Catwalk™) and the reduction of microglia activation (6 weeks post-lesion). One week after implantation, the apical papilla also decreased the concentration of pro-inflammatory cytokines in the spinal cord (collaboration with Prof. Diogenes, San Antonio University, USA). We observed a significant decrease of CD68, Iba-1 and GFAP (markers of activated glia) and increase of 5HT (marker for motoneurons (serotonin)) (Thesis of P. De Berdt).

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

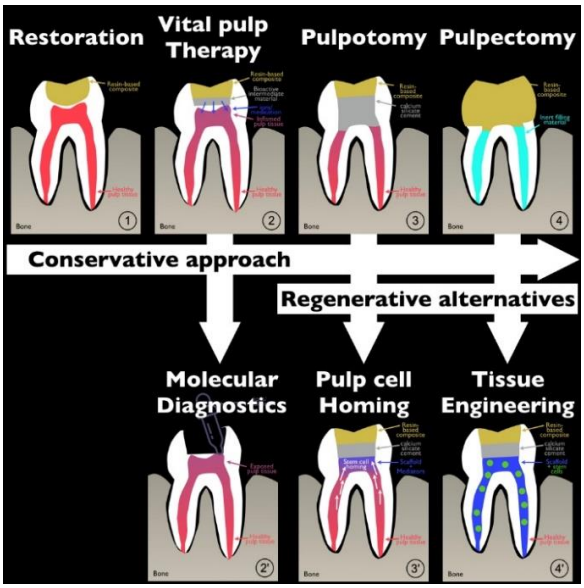

Figure 7: Current approaches for the treatment of tooth decay.

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.

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 cells and tissues are of importance with regards to the prediction of their performance. Since 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. Kerstin Galler, Regensburg, Germany).

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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SELECTED PUBLICATIONS
Gaëtane LELOUP/Julian LEPRINCE

Beauquis J, Petit A-E, Michaux V, Sagué V, Henrard S, Leprince JG. Dental Emergencies Management in COVID-19 Pandemic Peak: A Cohort Study. **J Dent Res, (2021) Vol. 100, no.4, p. 352-360.**

Leprince JG, Van Nieuwenhuysen J.-P. The missed root canal story: aren't we missing the point? **Int Endod J (2020) 53, 1162–1166.**

Leroy R, Bourgeois J, Verleye L, Declerck D, Depuydt P, Eloot A, Carvalho JC, Teughels W, Cauwels R, Leprince JG, Toma S, Michiels K, Aryanpour S, Vanden Abbeele A, De Bruyne M. Guideline on the prudent prescription of antibiotics in the dental office – Supplement. Good Clinical Practice (GCP) Brussels: Belgian Health Care Knowledge Centre (KCE) **(2020) KCE Reports 332S. D/2020/10.273/24.**

Hardy CMF, Bebelman S, Leloup G, Hadis MA, Palin WM, Leprince JG. Investigating the limits of resin-based luting composite photopolymerization through various thicknesses of indirect restorative materials. **Dent Mater (2018) 34: 1278-1288.**

Randolph LD, Palin WM, Bebelman S, Devaux J, Gallez B, Leloup G, Leprince JG. Photopolymerization of highly filled dimethacrylate-based composites using Type I or Type II photoinitiators and varying co-monomer ratios. **Dent Mater. (2016) 32: 136-48.**

Randolph LD, Palin WM, Leloup G, Leprince JG. Filler characteristics of modern dental resin composites and their influence on physico-mechanical properties. **Dent Mater (2016) 32:1586-1599.**

SELECTED PUBLICATIONS
Véronique PREAT

Bausart M, Vanvarenberg K, Ucakar B, Lopes A, Vandermeulen G, Malfanti A, Préat V. Combination of DNA Vaccine and Immune Checkpoint Blockades Improves the Immune Response in an Orthotopic Unresectable Glioblastoma Model, **Pharmaceutics (2022) 14 1025.**

Malfanti A, Catania G, Degros Q, Wang M, Bausart M, Préat V. Design of Bio-Responsive Hyaluronic Acid–Doxorubicin Conjugates for the Local Treatment of Glioblastoma, **Pharmaceutics (2022) 14 124.**

Bausart M, Préat V, Malfanti A, Immunotherapy for glioblastoma: the promise of combination strategies, **J Exp Clin Cancer Res (2022) 41 35.**

Lopes A, Bastiancich C, Bausart M, Ligot S, Lambricht L, Vanvarenberg K, Ucakar B, Gallez B, Préat V, Vandermeulen G. New generation of DNA-based immunotherapy induces a potent immune response and increases the survival in different tumor models, **J Immunother (2021) 9 e001243**

Bastiancich C, Malfanti A, Préat V, Rahman R. Rationally designed drug delivery systems for the local treatment of resected glioblastoma, **Adv Drug Deliv Rev (2021) 113951.**

SELECTED PUBLICATIONS
Rita VANBEVER

Rondon A, Mahri S, Morales-Yanez F, Dumoulin M, Vanbever R. Protein engineering strategies for improved pharmacokinetics. **Adv Funct Mater (2021) 2101633**.

Loira-Pastoriza C, Vanvarenberg K, Ucakar B, Machado Franco M, Staub A, Lemaire M, Renauld J C, Vanbever R. Encapsulation of a CpG oligonucleotide in cationic liposomes enhances its local antitumor activity following pulmonary delivery in a murine model of metastatic lung cancer. **Int J Pharm (2021) 600: 120504**.

Mahri S, Rondon A, Wilms T, Bosquillon C, Vanbever R. Biodistribution and elimination pathways of PEGylated recombinant human deoxyribonuclease I after pulmonary delivery in mice. **J Control Release (2021), 329: 1054-1065**.

Guichard M-J, Wilms T, Mahri S, Patil H P, Hoton D, Ucakar B, Vanvarenberg K, Cheou P, Beka M, Marbaix E Leal T, Vanbever R. PEGylation of recombinant human deoxyribonuclease I provides a long-acting version of the mucolytic for patients with cystic fribrosis. **Adv Ther (2021) 2000146 (1-17).**

Patil H P, Freches D, Karmani L, Duncan G, Ucakar B, Suk J S, Hanes J, Gallez B, Vanbever R. Fate of PEGylated antibody fragments following delivery to the lungs: Influence of delivery site, PEG size and lung inflammation. **J Control Release (2018) 272: 62-71**.

AWARDS

Highly cited researcher:
Véronique Préat since 2015

Ana Beloqui received the Prix Galien 2021 – awarded in May 2022 (5,000 euros)

Yining Xu received the APV Award for the most outstanding thesis in pharmaceutical sciences defended in 2021 – awarded at the PBP World Meeting in 2022 (2,500 euros)

THESES DEFENDED IN 2022

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

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

Liu Xiao: "Development of a long-acting version of alpha1 antitrypsin for augmentation therapy in alpha1 antitrypsin deficiency"
Director: Rita Vanbever (ADDB/LDRI)

THESES In progress

Auquière Marie: "Encapsulation of lipids and microARNs in extracellular vesicles for the treatment of multiple sclerosis"
Directors: Anne des Rieux (ADDB/LDRI), Giulio Muccioli (BPBL/LDRI)

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

Cassiers Céline: "Formulation approaches to overcome clearance mechanisms in the lungs"
Director: Rita Vanbever (ADDB/LDRI)
Co-Director: Markus Friden (Astra-Zeneca)

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)

Cheng Chen: "Nanoparticle-based oral drug delivery systems for inflammatory bowel disease and colorectal cancer treatment towards colon-specific release"
Director: Ana Beloqui García (ADDB/LDRI)
Co-Director: Yining Xu (ADDB/LDRI)

Debuisson Floriane: "Mesenchymal stem cells delivery in hybrid spheroids for treatment of spinal cord injury: innovative formulation and pre-clinical perspectives"
Director: Anne des Rieux (ADDB/LDRI)

Fongaro Benedetta: "Development of nanomedicines to tackle cystic fibrosis lung disease"
Director: Mireille Dumoulin (ULiège)
Co-Director: Rita Vanbever (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)

Godard Vanessa: "Design of long-acting nanomedicines for inhalation"
Director: Rita Vanbever (ADDB/LDRI)
Co-Director: Mireille Dumoulin (ULiège)

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)

Hendrickx Pauline: "Exploring the use of DNA nanotechnology for the cytoplasmic delivery of therapeutic miRNA for spinal cord injury repair".
Director: Anne des Rieux (ADDB/LDRI)
Director: Maartje Bastings (EPFL)-joined PhD

Kambale Kavungere Espoir: "Nanocapsules lipidiques à base de nanoparticules d'oxyde de zinc biosynthetisées à partir d'extraits de plantes congolaises pour le traitement du diabète mellitus de type 2"
Director: Ana Beloqui (ADDB/LDRI)
Co-directors: Joëlle Leclercq (GNOS/LDRI), Memvanga Bondo Patrick (UNIKIN)

Ladeiro Domingues Inês: "Exploiting the biological effect exerted by nanocarriers in non-alcoholic fatty liver disease treatment**"**
Director: Ana Beloqui (ADDB/LDRI)
Co-Director: Isabelle Leclercq (GAEN/IREC)

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

Leone Giuditta: "Design of a long-acting vasodilator nanomedicine for inhalation in pulmonary arterial hypertension"
Director: Rita Vanbever (ADDB/LDRI)
Co-Director: Raphaël Frédérick (CMFA/LDRI)

Ma Zhanjun: "Taking the best of two worlds to treat spinal cord injury: traditional Chinese medicine Rosmarinic acid combined with stem cell derived-extracellular vesicles"
Directors : Anne des Rieux (ADDB/LDRI) ; Giulio Muccioli (BPBL/LDRI)

Marotti Valentina: "Gene silencing of TNFα in inflammatory bowel disease treatment through a dual-shielding strategy via bioadhesive beads**"**
Director: Ana Beloqui (ADDB/LDRI)
Co-Director: Alessio Malfanti (ADDB/LDRI)

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

Redeghieri Paola: "Nanobodies to target proteases involved in inflammatory diseases of the lungs"
Director: Mireille Dumoulin (Center for protein engineering, ULiège)
Co-Director: Rita Vanbever (ADDB/LDRI)

Salim Mahmoud: "Development of nanomedicines to tackle cystic fibrosis lung disease"
Director: Rita Vanbever (ADDB/LDRI)
Co-Director: Mireille Dumoulin (ULiège)

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

Yang Jingjing: "Development of rationally-designed stimuli-responsive nanoparticles for neuroprotective purposes in spinal cord injury repair"
Director: Anne des Rieux (ADDB/LDRI)°

Zhang Wunan: "Local delivery of an anti-TNF-α monoclonal antibody in the treatment of inflammatory bowel diseases using foam-based drug delivery system"
Director: Ana Beloqui (ADDB/LDRI)

Biomedical Magnetic Resonance (REMA)

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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.

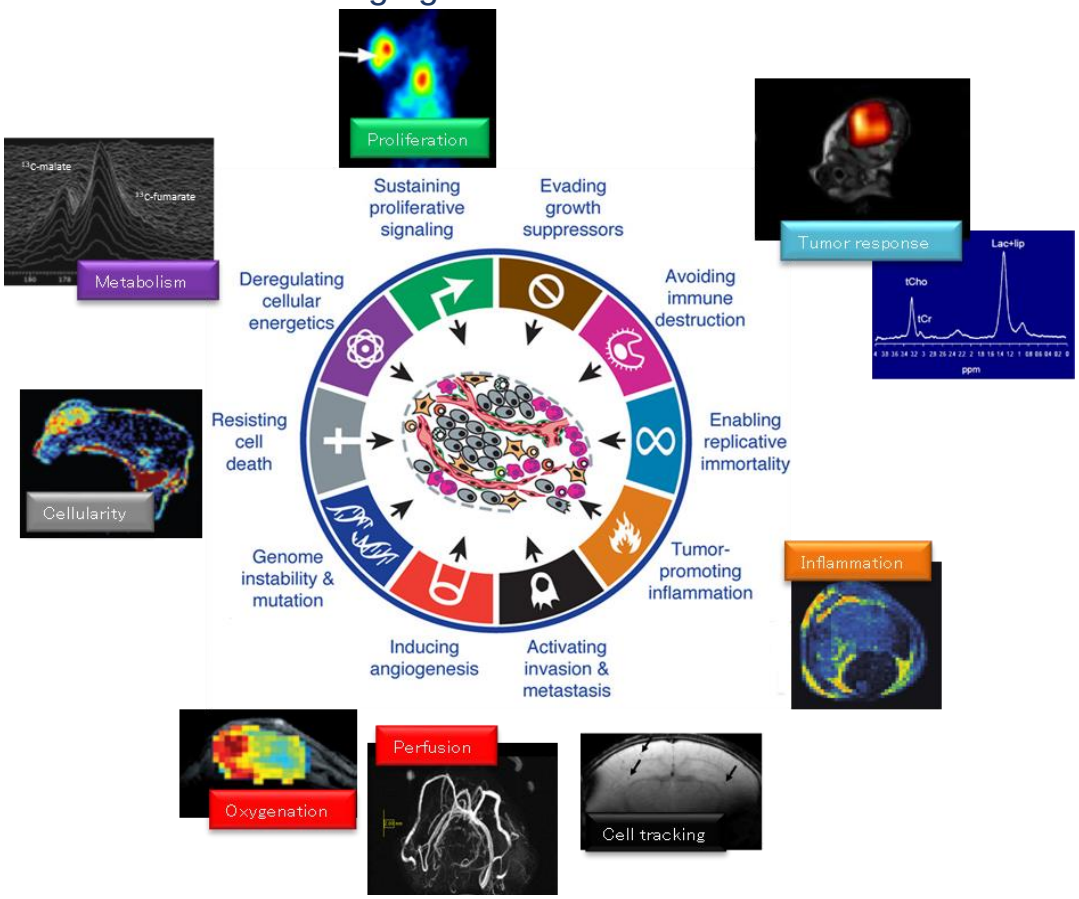
1) Development of tools for monitoring hallmarks of cancer by MR techniques:

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 consumption, redox status and superoxide production, as well as 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 allows carrying out clinical EPR studies in oncology and diabetology. In the purpose, a clinical study has been conducted to assess melanin in melanoma with the ultimate goal of stratifying malignant versus benign naevi. We have demonstrated for the first time the ability of EPR to detect noninvasively an endogenous free radical in human skin melanoma. The spectrometer has been upgraded with a unique capability to detect the EPR signal in multiharmonic mode in order to increase the sensitivity of the method. We have also been interested in developing ways to measure oxygen using MRI, namely by using ^{19}F relaxometry in order to map tumor oxygenation, or using endogenous contrast based on R_1 and R_2*.

Regarding hemodynamics, we are characterizing the tumor perfusion and permeability with Dynamic Contrast-Enhanced (DCE) – or Dynamic Susceptibility Contrast (DSC) MRI. We are also continuously developing new methodologies to measure tumor oxygen consumption *in vivo*, using ^{17}O-NMR and EPR oximetry.

Imaging hallmarks of cancer



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-MRS, steady-state ^{13}C-MRS, and dynamic hyperpolarized ^{13}C-MRS via ^{13}C-enriched substrates.

We validated mitochondrial redox nitroxide EPR probes to assess tumor redox status *in vitro* and *in vivo*, in response to the modulation of gluthatione and thioredoxin status. We are currently assessing innovative probes and combining detection with EPR and MRI for a better characterization of the redox status.

We also recently developed a mitochondrial 'toolbox' (mito-ToolBox) for measuring mitochondrial superoxide simultaneously to oxygen consumption rate (OCR) measurement. This unique versatile toolbox is presently used to assess the effect of treatments tackling the mitochondrial function of cancer cells as well as the effect of intoxicants on normal cells. *In vivo* translation of this tool is currently assessed using both EPR and MRI.

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. Another field of interest is the application of pH assessment using both CEST-MRI and EPR to assist in therapeutic guidance of treatments targeting proton extruders overexpressed by glycolytic cancer cells. Recent studies were focused on inhibitors of monocarboxylate transporters (MCTs) and mitochondrial pyruvate carrier (MPC).

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, EGFR, and CDK4/6 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.

In collaboration with the Metabolism and nutrition research group of the LDRI, we recently assessed the metabolic status of breast tumors growing on obese mice in comparison with lean mice and identified a unique metabolic feature linked to obesity, using hyperpolarized ^{13}C-pyruvate combined with ^{13}C-glucose tracing experiments involving both ^{13}C-MRS and mass spectrometry (collaborative work involving the NEST and MASMET platform of the LDRI).

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).

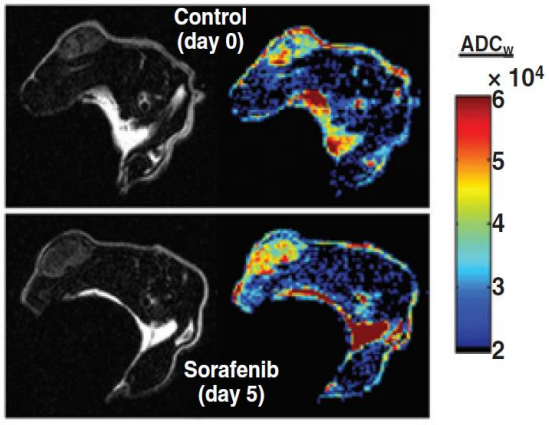


Figure 2. Typical ADCw (Apparent Diffusion Coefficient of water) maps obtained on mice xenografts in response to the multi-kinase inhibitor sorafenib. Note the increase in global ADCw in the tumor region at day 5 post therapy.

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.

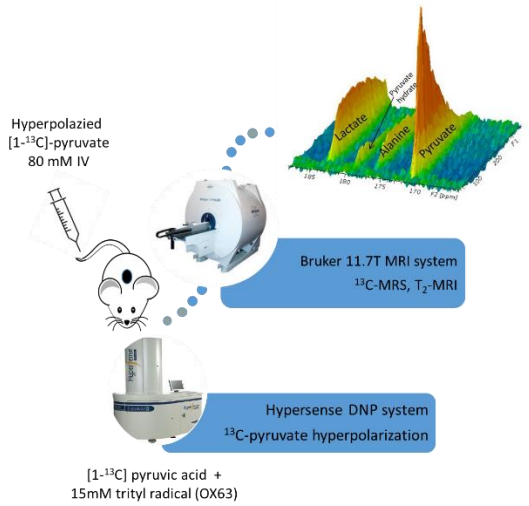


Fig.3 Schematic representation of an imaging session including ^{13}C-MRS of hyperpolarized ^{13}C-pyruvate. The ^{13}C-pyruvate substrate is first hyperpolarized and then directly injected intravenously to the tumor-bearing mouse that is concomitantly imaged in a small-animal MRI scanner for detection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

The steady-state assessment of ^{13}C-enriched substrates is also used in combination with hyperpolarized studies for identification of resistance mechanisms to currently available therapies, as well as 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, F. Duhoux and C. van Marcke). 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.

In the last five years, 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 and melanoma progression and metastatization.

SELECTED PUBLICATIONS

Bernard GALLEZ

Mignion L, Desmet CM, Harkemanne E, Tromme I, Joudiou N, Wehbi M, Baurain JF, Gallez B. Noninvasive detection of the endogenous free radical melanin in human skin melanomas using electron paramagnetic resonance (EPR). Free Radic Biol Med. (2022) 190, 226-233.

B. Gallez. The Role of Imaging Biomarkers to Guide Pharmacological Interventions Targeting Tumor Hypoxia. Front Pharmacol. (2022) 13, 853568.

D. d'Hose, P. Danhier, H. Northshield, P. Isenborghs, B.F. Jordan, and B. Gallez. A versatile EPR toolbox for the simultaneous measurement of oxygen consumption and superoxide production. Redox. Biol. (2021) 40, 101852

Schoonjans CA, Joudiou N, Brusa D, Corbet C, Feron O and Gallez B. Acidosis-induced metabolic reprogramming in tumor cells enhances the anti-proliferative activity of the PDK inhibitor dichloroacetate. Cancer Lett. (2020) 470, 18-28.

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.

SELECTED PUBLICATIONS

Bénédicte JORDAN

Yelek C, Mignion L, Paquot A, Bouzin C, Corbet C, Muccioli G, Cani PD, Jordan BF. Tumor metabolism is affected by obesity in preclinical models of triple negative breast cancer. Cancers (2022); 14(3):562.

Farah C, Neveu MA, Yelek C, Bouzin C, Gallez B, Baurain JF, Mignion L, Jordan BF. Combined HP ^{13}C Pyruvate and ^{13}C-Glucose Fluxomic as a Potential Marker of Response to Targeted Therapies in YUMM1.7 Melanoma Xenografts. Biomedicines. (2022);10(3):717.

Mignion L, Acciardo S, Gourgue F, Joudiou N, Caignet X, Goebbels RM, Corbet C, BouzinC, 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 (2020); 26(8):1932-1943.

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 IN PROGRESS

Buyse Chloé: "pH imaging by MRI as a guidance tool for therapies targeting tumor extracellular acidification".
Director: B. Gallez

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

Dehaen Natacha: "Study of the apelin adipokine as a driving factor of triple negative breast cancer metastates".
Director: B. F. Jordan
Co-director: P. D. Cani

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

Kouakou Axell-Natalie: "Study of the links between obesity, gut microbiota and melanoma progression and response to therapy".
Director: B. F. Jordan
Co-directors: P.D. Cani, G.M. Muccioli

Mathieu Barbara: "Towards in vivo mapping of mitochondrial ROS production in tumor models"
Director: B. Gallez
Co-director: P. Sonveaux

Rodella Giulia: "Radiotherapy-activated immunizing niches as nanomedicines intended for local implantation at the resection cavity in glioblastomas"
Director: B. Gallez
Co-director: A. Malfanti

Salman Yasmine: "Defining an MRI signature of tau pathology: a pathological and anatomical study of Alzheimer's disease and related disorders using post-mortem and in-vivo imaging data"
Director: B. Hanseeuw
Co-director: B. Gallez

Wehbi Mohammad: "Characterization of skin melanomas using multiharmonics EPR"
Director: B. Gallez

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. C

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 research groups within the LDRI and the "Health Sector", as well as for research groups of the "Sciences and Technology Sector".
To this aim, we share the use of analytical equipment located on the Brussels' campus (mainly at the LDRI) and on the Louvain-la-Neuve's campus (mainly at LIBST). The available equipment includes, among others:

- ThermoScientific LTQ – ORBITRAP –XL high resolution mass spectrometer
- Waters xevo TQS UPLC-MS/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.

Next year a new high-end Q-TOF mass spectrometer will be added to the available equipment thanks to a grant secured from the FRS-FNRS.

ThermoScientific LTQ – ORBITRAP –XL

Waters xevo TQS

The relevance of the expertise of the MASSMET platform is 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). A 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 been implemented in the platform. These technologies may provide biomarkers for monitoring (patho) physiological parameters and the response to pharmacological treatments.

The NEST platform, managed by 2 PhDs in Engineering and Biomedical Sciences, 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 +60°C)

Here are few examples 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 measurements
- pH measurements and pH mapping
- 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. This is suggested as a marker of response to anticancer therapy.

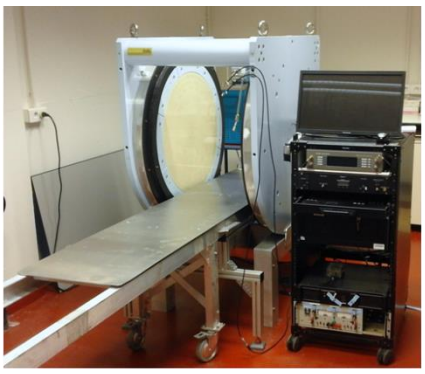
Electron paramagnetic resonance

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:

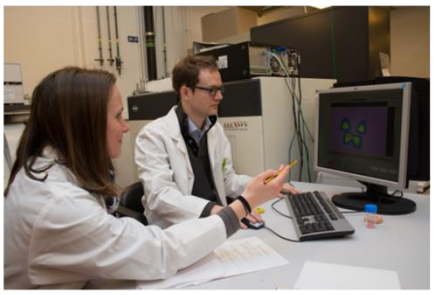
- X-band EPR spectrometer (Bruker EMX+, 9 GHz) for *in vitro* applications
- Benchtop X-band EPR spectrometer (Magnettech Miniscope, 9 GHz for *in vitro* applications
- L-band (1 GHz) and X-band (9 GHz) imaging EPR system (Bruker Elexys) for *in vitro* and *in vivo* applications
- L-band EPR spectrometer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

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):

- Characterization of free radicals by spin trapping
- Quantification of melanin / melanoma cells in tissues
- Spin labeling: 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

In order to improve the area of expertise of the platform recent implementations have been made or are under development. Those development are made in response of the need of our collaborators. Last months, we have improved sensitivity of the acquired signal by the clinical L-band EPR spectrometer with the acquisition and implementation of a multiharmonic system.

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,KULeuven);
- 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);
- oxygenation in ovarian grafts (GYNE/IREC);
- liver oxygenation (GAEN/IREC);
- oxygenation in 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)
- DNP & EPR: Dr Lionel Mignion (lionel.mignion@uclouvain.be)

Principal investigators responsible of the NEST platform:

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- Prof. Bernard Gallez (bernard.gallez@uclouvain.be)

SUPPORTING ORGANIZATIONS

APPENDIX

Complete list of 2022 LDRI Publications and patents

Research or review papers – first or last author

1. Abdul Khaliq, Hafiz; **Al Houayek, Mireille; Quetin-Leclercq, Joëlle; Muccioli, Giulio**. 5'AMP-activated protein kinase: an emerging target of phytochemicals to treat chronic inflammatory diseases. In: Crit Rev Food Sci Nutr, . 2022 Nov 30;1-26. doi: 10.1080/10408398.2022.2145264

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