LOUVAIN DRUG RESEARCH INSTITUTE

REPORT 2020

President:

Nathalie Delzenne

Vice-presidents:

Raphaël Frédérick (up to December 2020)
Bénédicte Jordan
Giulio Muccioli
Françoise Van Bambeke

Layout: Isabelle Alloo, Hoang Nguyen

Sources of information: UCL Health Sector Administration - official UCLouvain databases (ADRE/ADFI/SPER - DIAL)

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

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

For a copy of this report, please send a mail to secretaire-ldri@uclouvain.be

UCLouvain

FOREWORD

The general objective of the **Louvain Drug Research Institute (LDRI),** created in 2010 and located on the Health Sciences Campus of the *Université catholique de Louvain* (UCLouvain) in Brussels, is to develop fundamental and/or applied cutting-edge research, in the field of drugs, from target identification & validation to clinical practice. LDRI is a **multidisciplinary Institute**, covering original research topics related to Microbes, Inflammation, Cancer, Ageing and Chronic Diseases, via the implementation of New Technologies.

"Bridging sciences for better health" is LDRI's motto. The LDRI is proud of the diversity and wealth of its research. Over 170 motivated members share common objectives in terms of quality of science and well-being, thereby creating optimal conditions for networking. The convivial atmosphere of the LDRI was crucial this year to face the working conditions imposed by the Covid-19 pandemia. Despite the situation, all the programmed PhD thesis were successfully defended, and most weekly research seminars were organized online, attracting a lot of attendees. The increase in yearly number of publications was confirmed, reaching the highest number (151) and the highest mean impact factor (IF 6,19) since the creation of the institute. LDRI external visibility was also confirmed. According to the prestigious QS World University Ranking 2020, our research activities in "Pharmacy and Pharmacology" were confirmed in the Top 51-100 Universities over the world, UCLouvain being ranked as the first French-speaking University in Belgium. In 2020, five LDRI researchers, including four principal investigators, were among the highly cited researchers worldwide. LDRI researchers were particularly successful this year in getting highly competitive grants from Belgian funding agencies (FNRS-FRS, WELBIO…) and confirmed their place as leaders in international networks (European Research Council- Joined Program Initiative – Horizon 2020…).

Thanks to our internationally competitive research, we are creating and fostering new knowledge with a direct impact on healthcare. This is also supported by our ambition to develop efficient partnerships with industry and society. The Fondation Louvain supported our current partnership with Pharmacie Servais, and allowed a financial contribution for innovative technology acquirement upon Fonds Jacques Moulaert.

In this report, we present a brief overview of our objectives and mission statements as well as a detailed description of the scientific outcomes of the seven research groups and of the two technology platforms of our institute. The data on human resources, funding, and the scientific output of the LDRI are mostly based on data collected in 2020 using the University's official databases.

We take the opportunity to thank the persons who have highly contributed to LDRI management for years, including Raphaël Frederick, who was replaced as vice-president

by Bénédicte Jordan, and Patrice Cani, who was replaced as President of the Council by Raphaël Frédérick.

Even if uncertainty persists linked to the peculiar context linked to Covid-19, we will go on promoting research and management approaches for a “better health” for all, in line with our moto. Adhering to UCLouvain Horizon 600 plan, and taking into account the suggestions of our Scientific Advisory Board, will be helpful to meet our objectives.

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

Nathalie Delzenne,

President of the LDRI

Bénédicte Jordan (replaced Raphaël Frédérick in December 2020), Giulio Muccioli and Françoise Van Bambeke

Vice-Presidents of the LDRI

Abbreviations:

ADDB: Advanced Drug Delivery and Biomaterials

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

BPBL: Bioanalysis and Pharmacology of Bioactive Lipids

CLIP: Clinical Pharmacy

CMFA: Medicinal Chemistry

FACM: Pharmacologie cellulaire et moléculaire

FNRS: *Fonds National de la Recherche Scientifique*

FRIA: Fund for Research Training in Industry and Agriculture

FRS: Fonds de la Recherche Scientifique

FSR: *Fonds spécial de la recherche*

FTE: Full Time Equivalent

GNOS: Pharmacognosy

IF: Impact Factor

MNUT: Metabolism and Nutrition

PI: Principal Investigator

PMGK: Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics

REMA: Biomedical Magnetic Resonance

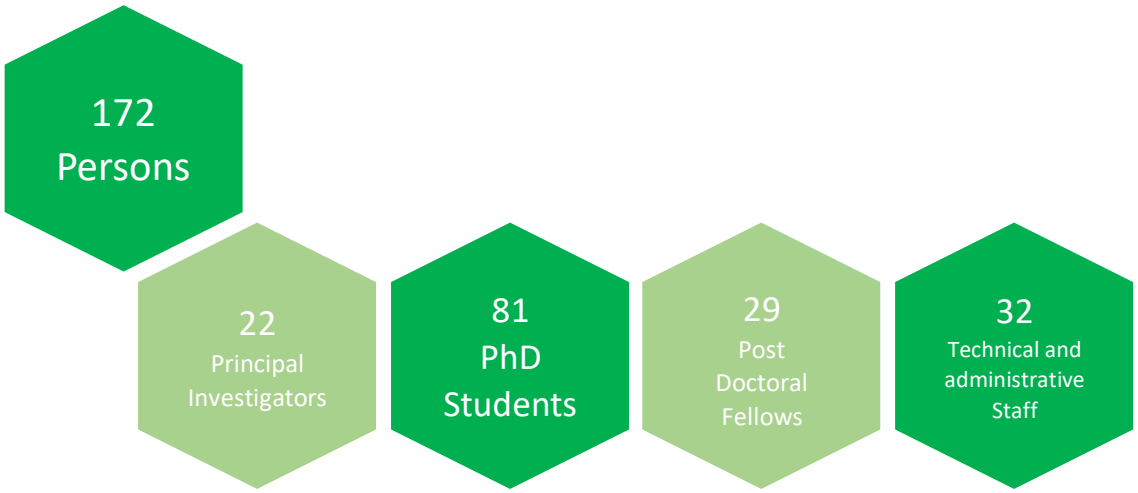
SME: Small and Medium Entreprise

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

Support the LDRI in its development and actions:

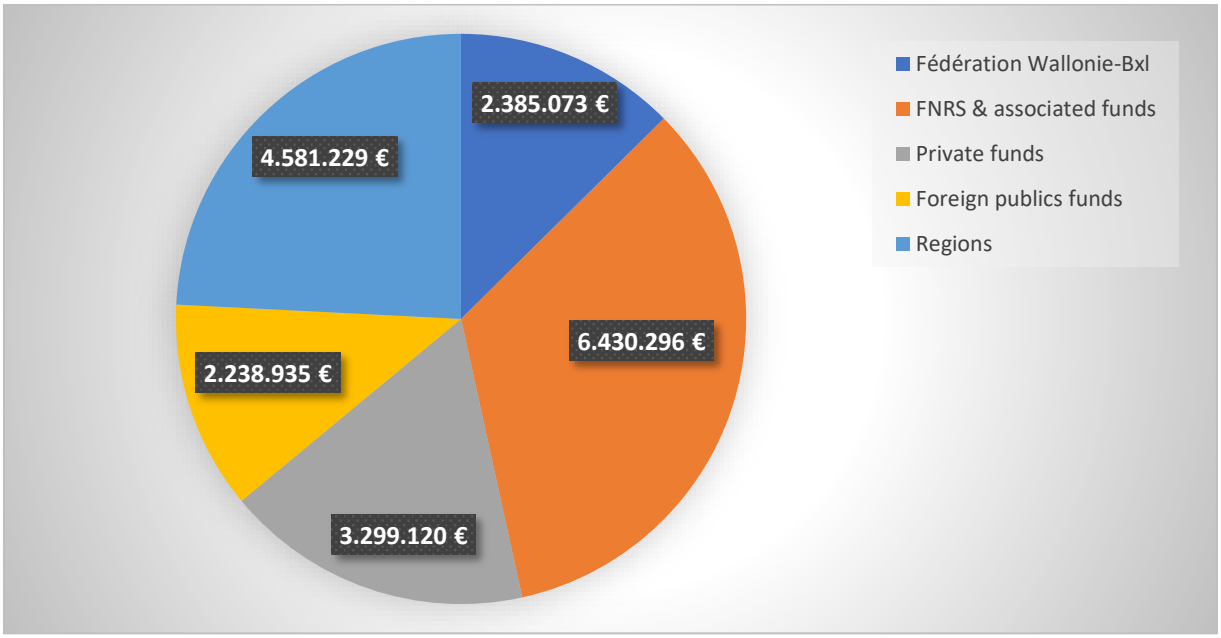
Account Number: BE29 2710 3664 0164 / BIC: GEBABEBB with the communication **"don LDRI 13.21100.001"**. You can add a specific team, person or research project.

LDRI – 2020 HIGHLIGHTS

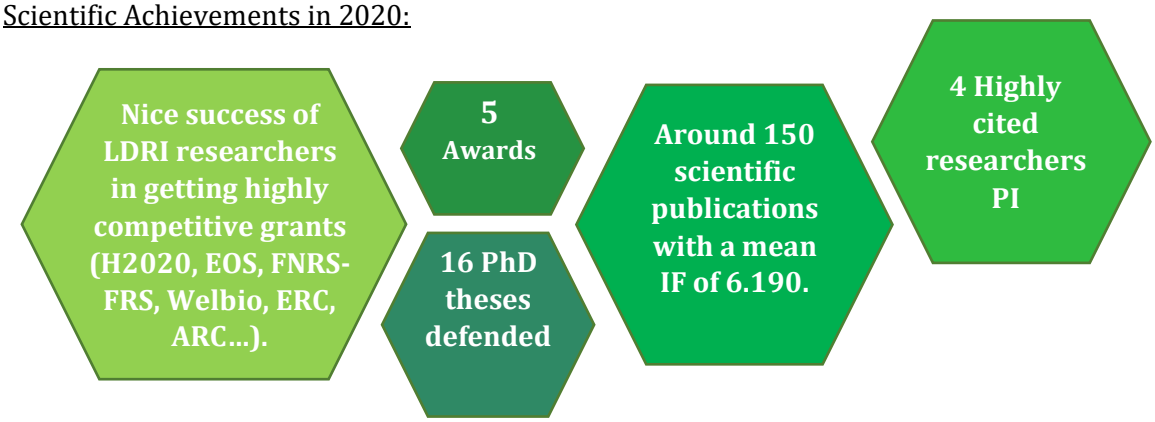


Funding:

Sources of funding of the LDRI (Global amount in EUR for projects running in 2020)

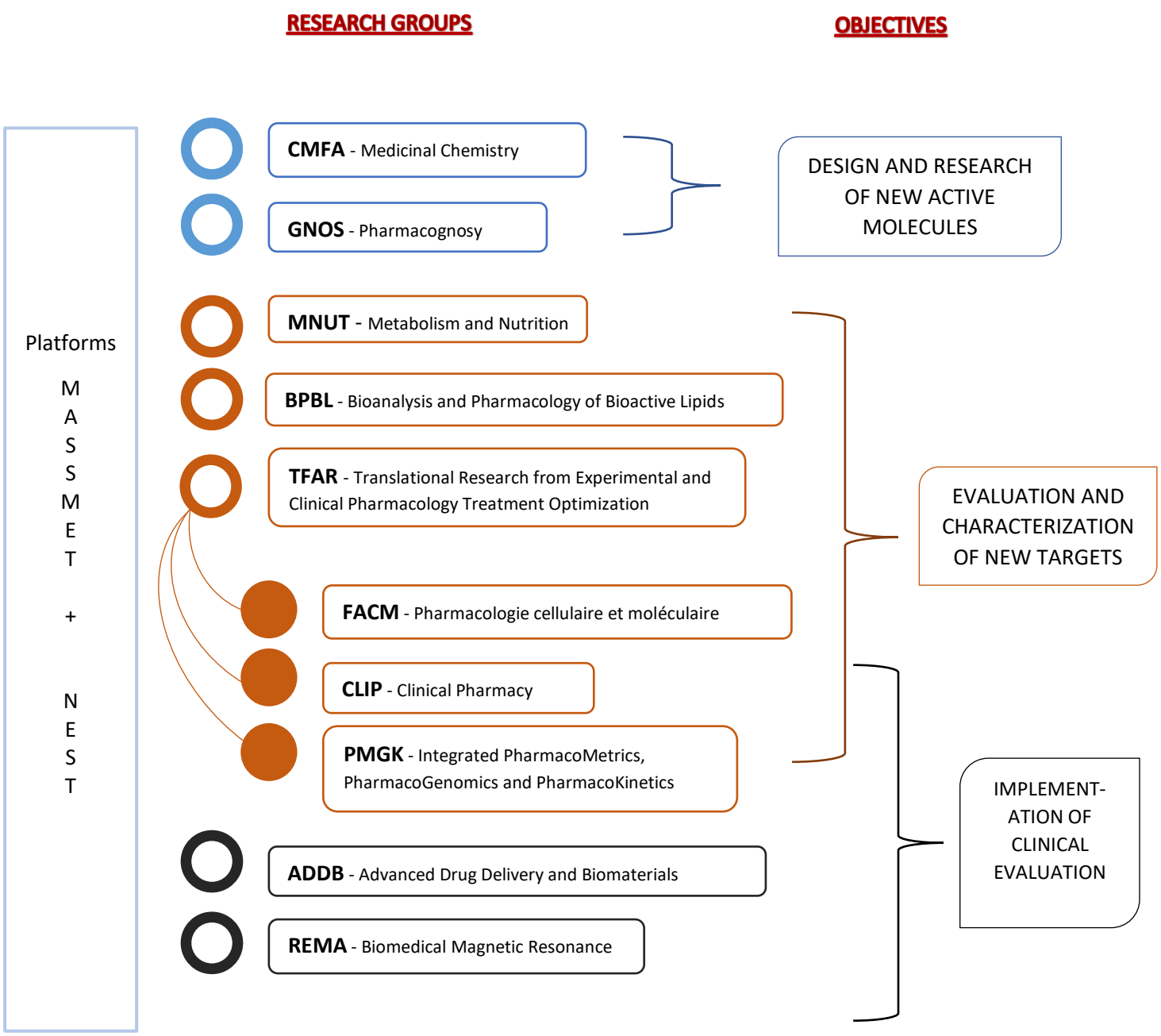


Scientific Achievements in 2020:



SECTION I – LDRI GENERAL PRESENTATION

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


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

I. Objectives and mission statements

The general objective of the Louvain Drug Research Institute (LDRI) is to develop a cutting-edge translational research in the field of drugs, within the Health Sciences Sector of the Université catholique de Louvain (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 standard analytical tools in the field of mass spectrometry (MASSMET) and pre-clinical magnetic resonance (NEST).

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

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

II. Research fields and groups

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

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

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

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

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

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

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

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

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

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

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

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

Therefore, all major aspects of the drug are covered from its design to its optimal use. 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 (Nathalie Delzenne) and three Vice-Presidents (Bénédicte Jordan- who recently replaced Raphaël Frederick-, Giulio Muccioli and Françoise Van Bambeke) who are elected by the LDRI Council.

N. Delzenne

F. Van Bambeke

B. Jordan

G. Muccioli

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 (2), scientific (2) and administrative and technical staffs (2).

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

An International Scientific Council is composed of five well-known researchers, who cover the research areas of the Institute, and provide advices on research and recruitment strategy, namely upon every three years on site meetings. C. Hughes (Primary care Pharmacy) (Queen's University, Belfast, Ireland); J.L. Veuthey (Analytical Chemistry) (Université de Genève, Switzerland), P. Ferré (metabolism, pathophysiology and molecular biology) (Inserm-Université Pierre et Marie Curie, Paris, France) and P. Herdewyn (Medicinal Chemistry) (KULeuven, Belgium).

Prof. C. Hughes (UK)

Prof. J.L. Veuthey (CH)

Prof. P. Ferré (FR)

Prof. P. Herdewyn (BE)

IV. Human Resources

The **total staff** of the LDRI in December 2020 was 162 full members (affected to LDRI as main entity) and 10 affiliated members. It represents 172 persons involved in LDRI's activities, with a proportion of 39% men and 61% women.

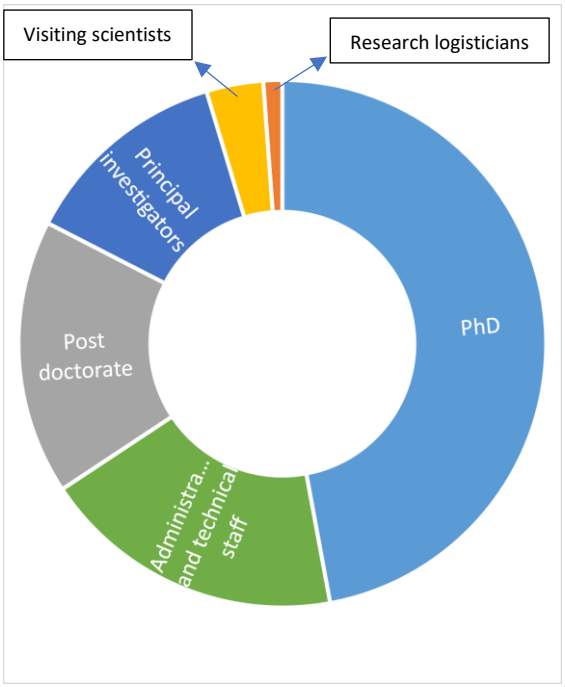
Academic staff:

Due to part-time contracts, the 22 Principal Investigators stand for 20.5 FTE (Full Time Equivalent). Among them, 7 FTE senior researchers are paid by the FRS-FNRS.

Scientific staff:

29 post-doctoral fellows and 81 PhD students, including 24 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.

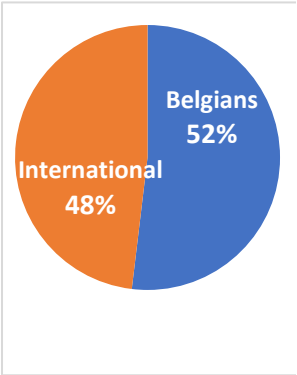
The technical and administrative staff affected to the LDRI represents 32 persons.



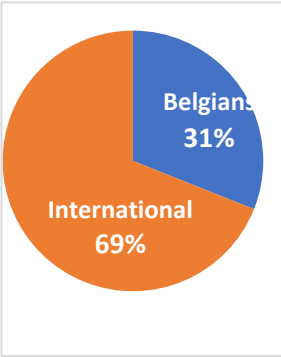
Proportion of the different categories of LDRI staff (includes affected and affiliated members).

The LDRI is continuously attracting numerous **international researchers**, as illustrated in the graphs below. 48% of the PhD students and 69 % of the post-doctoral fellows are coming from abroad.

PhD students



Post-doctoral fellows

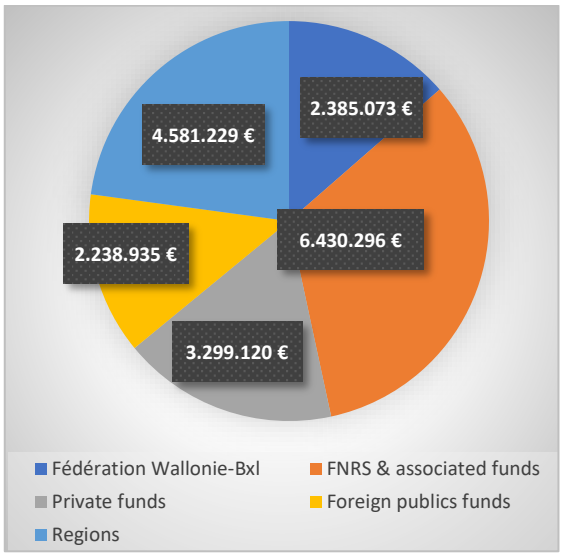


The background of the scientists is quite diverse reflecting the multidisciplinary research performed within the Institute. The PhD students within the LDRI's research groups have trained as pharmacists, bioengineers, engineers, chemists, biologists, physicists, MD or masters in biomedical sciences.

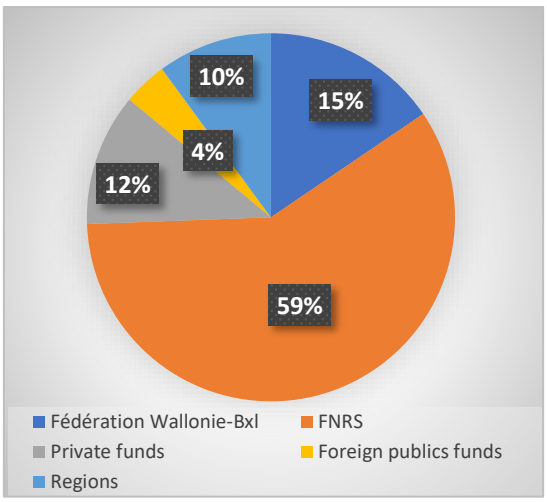
V. Fundings

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 and financial contribution for innovative technology acquirement upon Fonds Jacques Moulaert.

Sources of funding of the LDRI (Global amount in EUR for projects running in 2020)

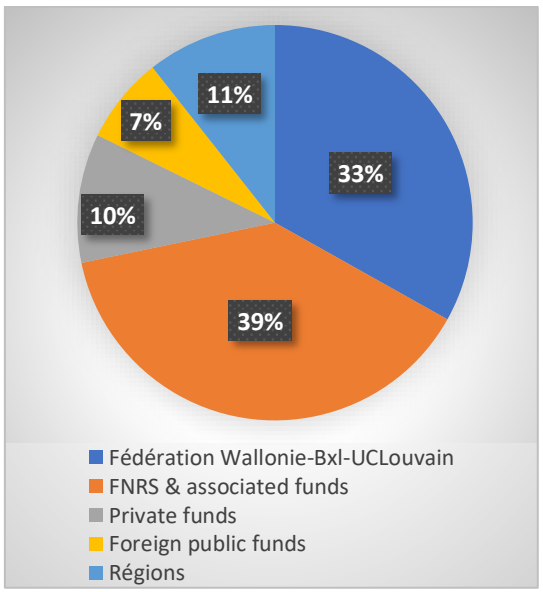


Sources of Funding of the LDRI (Percentage of the number of projects running in 2020)



The main sources of staff funding (see graphic below) are the FNRS (39%), UCLouvain/Fédération Wallonie Bruxelles (33%), the Belgian regions (11%), private funds (10%) and foreign public funds (7%). The FNRS pays the salary of 7 permanent principal investigators, 12 postdoctoral researchers, and 44 PhD students (including FRIA - Fund for Research Training in Industry and Agriculture - and Televie doctoral grants). 26 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 2020*



** This graph includes full and affiliated members.*

VI. Scientific Output

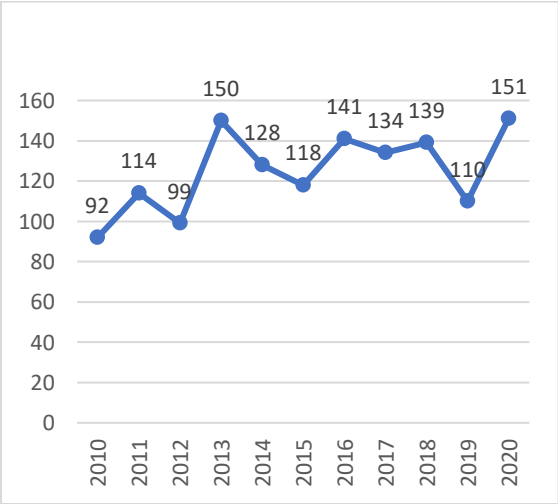
The scientific output of the LDRI can be appreciated by well-cited publications in recognized international journals and through the training of young researchers (illustrated by the high number of PhD and post-docs per PI). The members of LDRI are also involved in missions devoted to the dissemination 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

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

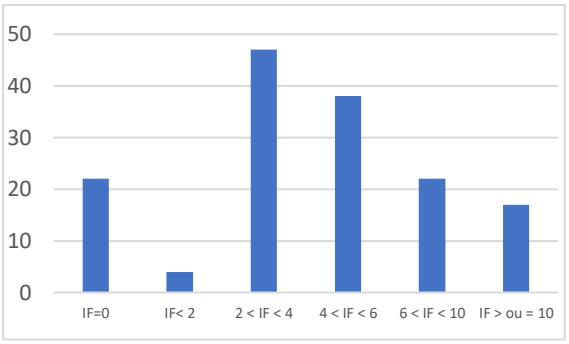
Altogether, the research groups of the LDRI published 400 scientific articles in international journals or book chapters during the last three years (2018-2020).

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



The mean Impact Factor (IF) of the publications during the period 2010-2019 was 4.78 (including review papers and educational papers). In 2020, the IF is **6.190**.

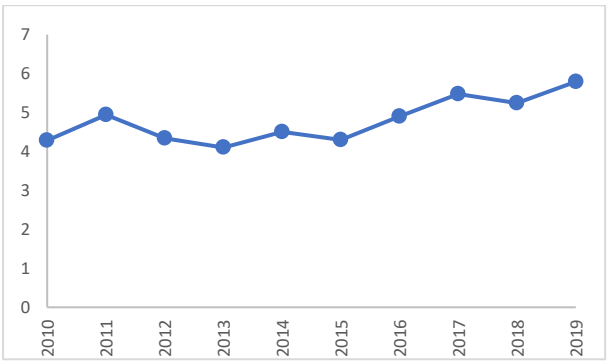
The distribution of publications per impact factor is shown hereafter.



(Papers with IF=0 are book chapters, educational papers or papers published in recent journals without IF, data 2020)

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

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



Training in research

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

Moreover, all the PIs of the LDRI are 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 (at least once a week). Their subjects alternate between presentations by junior scientists

from the Institute and by senior scientists (from the Institute, from other Institutes within the University, from other Universities in Belgium or abroad, from the Industry).

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

Expertise

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

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

- Royal Academy of Medicine (5 members)

- European Medicines Agency (EMA)

- Public Health Institute

- Federal Agency for Medicines and Health Products

- Federal Agency for Nuclear Control

- French National Research Agencies (ANR and HCERES)

- Fonds Wetenschappelijk Onderzoek (FWO)

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

Collaborative projects with the industry

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

KEY AWARDS 2018-2020

2020

Patrice Cani and Clara Depommier (MNUT): Scientific award from the Belgian Endocrine Society for the best paper published in 2018-2019 in the field of diabetes and metabolism, presented during the annual BES meeting 2020.

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

Alexandra Degraeve (TFAR PMGK/MNUT): Best Young Scientist Poster Presentation in Therapeutic Drug Monitoring, at the annual meeting of the International association of therapeutic drug monitoring and clinical toxicology.

Emilia Hoste (TFAR PMGK): Best Young Scientist Poster Presentation in Clinical Toxicology, at the annual meeting of the International association of therapeutic drug monitoring and clinical toxicology.

Albert Ruiz Sorribas (TFAR FACM): Prize for the best oral presentation at the virtual meeting of the Belgian Society for Microbiology. (oct 2020)

Patrice Cani, Nathalie Delzenne, Amandine Everard and Véronique Préat, highly cited researchers, Clarivate analytics Web of Sciences.

2019

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

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

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

Rodriguez Julie (MNUT): Prize of the Belgian Society of Clinical Nutrition.

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

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

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

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

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

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

2018

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

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

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

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

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

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

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

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

PLENARY LECTURES by EXTERNAL SPEAKERS 2020 (online from March 2020)

Prof. Pierre WEISS
Université de Nantes
« Biomatériaux innovants pour la régénération osseuse »

Dr. Alexis GAUDIN
Université de Nantes
« Stratégies de modulation de la réponse inflammatoire pulpaire, exemple d'un microgel biphasique en tant que système à libération de lipoxine »

Prof. Bruno SARMENTO
INEB Universidado do Porto
"Bioengineered-surface nanomedicines for the no-invasive delivery of anti-diabetic peptides"

Dr. Jérôme DUISIT
IREC, UCLouvain
"Vascularized composite tissues and organ decellularized scaffolds: the ideal biomaterials?"

Prof. David ALSTEENS
LIBST, Nanobiophysics lab, UCLouvain
"Probing virus binding to cell surfaces in physiologically relevant conditions using AFM"

Prof. Giulia LIBERATI
IoNS, Institute of Neuroscience, UCLouvain
"In search of a neural biomarker for pain: insights from intracerebral electroencephalography"

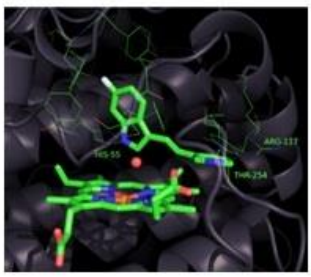
SECTION II – RESEARCH GROUP PRESENTATION

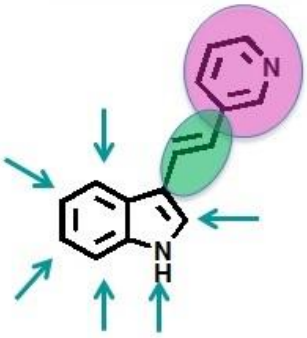
I. Medicinal Chemistry (CMFA)
II. Pharmacognosy (GNOS)
III. Metabolism and Nutrition (MNUT)
IV. Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)
V. Translational Research from Experimental and Clinical Pharmacology to Treatment Optimization (TFAR)
VI. Advanced Drug Delivery and Biomaterials (ADDB)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

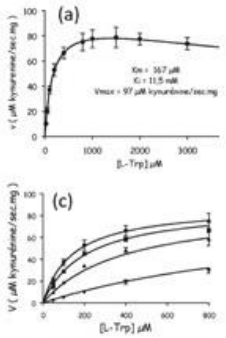
MEDICINAL CHEMISTRY (CMFA)

« Towards the discovery of innovative pharmacological tools and drugs »

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







In silico studies
Structure-based design

Chemical synthesis
Structural analysis

In vitro and *in vivo* assays

Screening

PhysChem properties

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Raphaël Frédérick
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raphael.frederick@uclouvain.be

MEDICINAL CHEMISTRY (CMFA)

Post-Doctoral fellows	PhD Students	Adm. & Techn. Staff
Liberelle M.	Ameryckx A. Ancia M. Brustenga C. Dechenne J. Deskeuvre M. Kozlova A. Leone G. Marteau R. Prevost J. Savoyen P. Tan Y. Thabault L.	Ribeiro F. 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

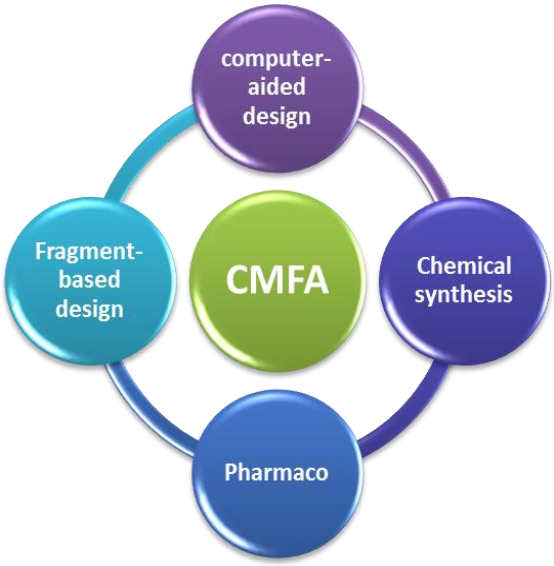
The discovery of new innovative medicines is a priority for human health. It is in this context that the Medicinal Chemistry Research Group (CMFA) is pursuing its research activities

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 pHLIP (pH-Low Insertion Peptides) that are peptides exploiting pH differences between healthy and diseased cells as a biomarker for targeting and delivering therapeutic to cells in acidic diseased tissues.



RESEARCH RESULTS

Anticancer immunotherapy

Tryptophan catabolism is an important mechanism of peripheral immune tolerance contributing to tumoral immune resistance, and indoleamine 2,3-dioxygenases (IDO and TDO) inhibition is a promising strategy for anticancer drug development. IDO and TDO are unrelated heme-containing enzymes catalyzing the oxidative cleavage of the indole ring of L-tryptophan (L-Trp), the first and rate-limiting step along the kynurenine pathway. The implication of IDO in the phenomenon of tumoral immune resistance is the focus of intense researches and the enzyme is now recognized as a validated target for anti-cancer therapy. Therefore, a number of groups, including us, are actively searching for novel original IDO inhibitors. In contrast, the effect of TDO expression on the immune response has only been recently investigated in detail. Indeed, we showed in collaboration with the group of Prof Van den Eynde that TDO was effectively overexpressed by a number of human tumors and that this expression prevented rejection of tumor cells. We designed a novel TDO inhibitor and proved, in a preclinical model, the concept that TDO inhibition promotes tumoral immune rejection. Interestingly, blocking both TDO and IDO to improve the efficacy of cancer immunotherapy would be complementary: in a series of 104 human tumor lines of various histological types, we showed that 20 tumors expressed only TDO, 17 only IDO and 16 expressed both enzymes. Therefore, targeting both IDO and TDO would allow reaching 51% of tumors instead of either 32% or 35% with a compound inhibiting IDO or TDO alone, respectively. The design of IDO, TDO or dual IDO/TDO inhibitors is thus of major importance. Interestingly, our recent works (PhD thesis of Arina Kozlova) led to the discovery of new TDO inhibitors. These data (still unpublished) are very encouraging to pursue the search for new anticancer agents targeting Trp catabolism.

Besides Trp catabolism, Arginine (L-Arg) catabolism by Arginase 1 (Arg1) is another mechanism contributing to tumoral immune resistance. In recent works (PhD thesis of Julien Prévost and Juhans Dechenne), we have identified novel boronic acids compounds as promising Arginase inhibitors.

Tumor metabolism

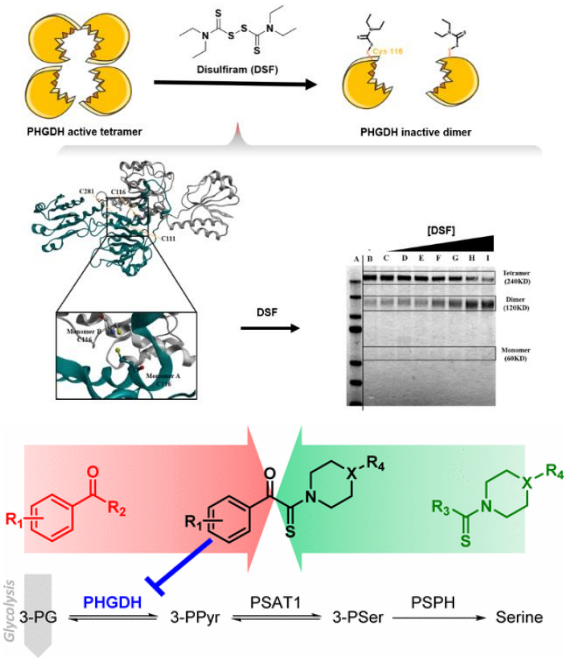
The last ten years have witnessed an increased regain of interest for tumor metabolism. Recent advances in this field have shed light on how tumors fuel rapid growth by preferentially engaging biosynthetic pathways. Although cellular metabolic pathways are rich pickings for drug targets, pinpointing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

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

In this project (PhD thesis of Quentin Spillier), our aim is to understand the role of the serine pathway in tumor progression and in particular to develop pharmacological tools to evaluate the extent of tumor addiction to this metabolic path and their therapeutic potential by exploring potential side effects on healthy tissues. To this end, novel innovative pharmacological inhibitors

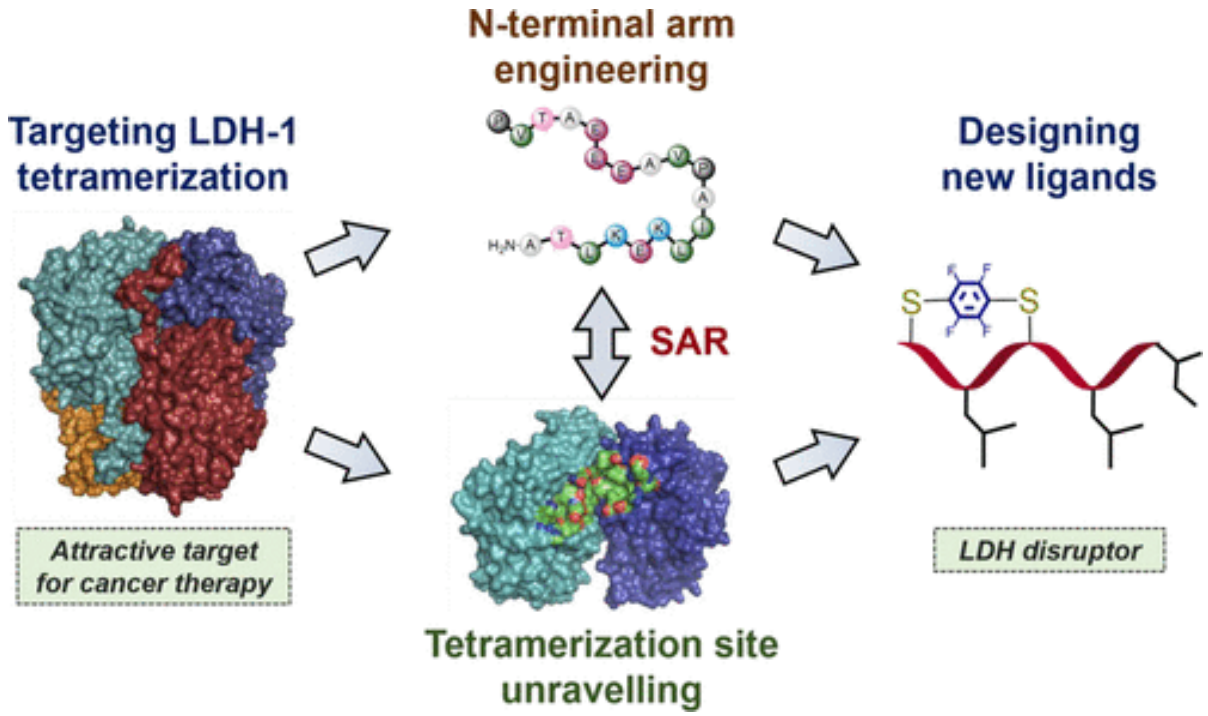
of PHGDH the first enzyme of the serine pathway, were designed and chemically synthesized.

A screening campaign of an *in-house* library of compounds was performed and led to the serendipitous discovery of two original series of very active PHGDH inhibitors: the first are derived from the FDA-approved drug Disulfiram, whereas the second is characterized by an original α-ketothioamide scaffold. Interestingly, the use of chemical biology methodologies such as cross-linking experiments and an original photoactivatable diazirine chemical probe (unpublished results) with mass spectrometry experiments led us to hypothesize that each series would act through a covalent allosteric mechanism, probably involving the ACT-regulatory domain, hence promoting disruption of the PHGDH tetramer. Although repurposing of Disulfiram is suggested and PHGDH inhibition could account for its anticancer activity, its direct optimization is not our intend here, but these preliminary results inspire the design and discovery of more specific covalent modifiers.



2. Tumor cells are also characterized by a remarkable metabolic plasticity allowing them to survive and proliferate in hypoxic and extracellular acidic environments. In tumor cells, this plasticity allows the coexistence and coordination of several metabolic phenotypes, leading to an optimal use or resources. Hypoxic cells 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 fueled the Krebs cycle and NADH uses the malate-aspartate shuttle to directly fuel the mitochondrial respiration chain. The oxidative use of lactate in the oxygenated tumor compartment therefore optimizes the availability of glucose for cells of the hypoxic compartment, thus constituting a unique metabolic cooperation.

If the use of lactate by oxidative cells is a proven fact, the advantage it gives them remains largely unknown. A first series of studies showed that lactate can act as a pro-angiogenic agent. This signaling activity also depends on the oxidation of lactate to pyruvate by LDHB, allowing pyruvate to inhibit enzymes of the prolylhydroxylase family and activate the hypoxia-inducible transcription factor factor-1 (HIF-1) independently of hypoxia. In addition, a recent collaborative led with the team of P. Sonveaux (IREC) has shown that the oxidative use of lactate promotes autophagy, ie, a process of degradation and recycling of proteins and organelles requiring formation of specialized structures, autophagosomes, and their fusion with lysosomes. To promote autophagy, LDHB physically interacts with V-ATPase, a proton pump located on the surface of lysosomes, which it feeds with the protons produced during the lactate + NAD^+ ⇌ pyruvate + NADH + H^+ reaction. This observation seems



important to us as autophagy participates in tumor progression by recycling damaged proteins and organelles when cancer cells are exposed to oxidative stress, and because it provides cells with energy substrates under metabolic stress conditions.

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

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

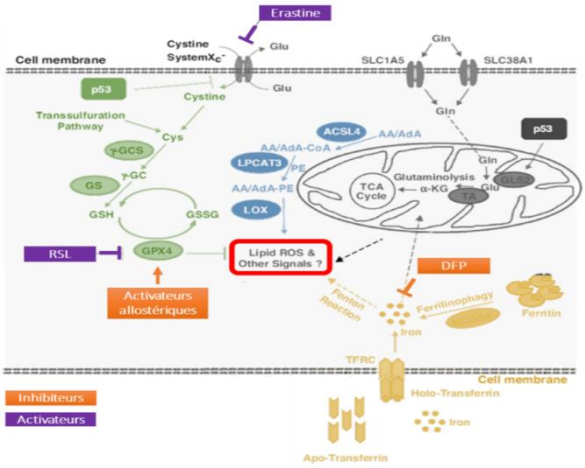
So far, our pivotal collaborative works led to (a) the delineation of hot spots at the LDH tetramerization site, (b) the design and synthesis of original (stapled) peptides capable of preventing LDH self-association and/or disrupting a preformed LDH tetramer, and (c) the development of some chemical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

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 grounds on important preliminary findings generated in our lab (PhD thesis of Romain Marteau). 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.



Antibiotics

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

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

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

Many diseases such as cancer (solid tumors), ischemia, stroke or infection lead to the development of local hypoxia and acidosis. Acidosis results from enhanced glycolytic flux which produces lactate and H+ ions (but also in tumors from hydration of CO_2 which

represents another source of H^+ ions). Hence, extracellular acidity might serve as a general marker for detecting and targeting ischemic tissues and tumors. However, since the bulk extracellular pH in these diseased tissues is only 0.5–0.8 pH units lower than the extracellular pH in healthy tissue, this strategy remains particularly challenging. Several pH-sensitive imaging and drug delivery systems have actually been envisioned as diagnostic or therapeutic modalities specifically triggered by the acidic tumor microenvironment. Among these are the pH Low Insertion Peptides (pHLIP) family derived from the bacteriorhodopsin C helix. This family represents a unique class of water-soluble membrane polypeptides which were found to insert across a membrane to form a stable transmembrane α-helix.

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

In our research project (Phd thesis of Marine Deskeuvre) we are studying, developing and applying the pHLIP technology in two promising fields of cancer therapy: tumor lipid metabolism and the response of T cells to tumor microenvironment acidification.

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, on the TSPYL5-USP7 complex. Biophysical and biochemical experiments will be undertaken to identify the hot spots of this 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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Ameryckx Alice: "Design and synthesis of DD-ligases inhibitors: Peptidoglycan intracellular biosynthesis as antibiotics target".
Director: Raphaël Frédérick
Co-director: Françoise Van Bambeke

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

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: "lnterrogating 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

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

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)

Prévost Julien: "Design and synthesis of Arg1 inhibitors for anticancer immunotherapy".
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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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.*

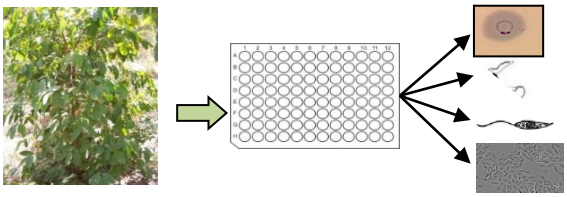
To allow these researches, we developed an expertise in extraction, purification, structure determination of compounds from complex matrices and development of quantification validated methods, while most of the pharmacological experiments are realised in collaboration with teams having expertise in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

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

1/ CRUDE EXTRACTS AND PURE COMPOUNDS EVALUATIONS

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

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

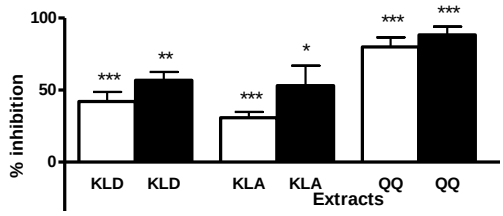


Keetia leucantha

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

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

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



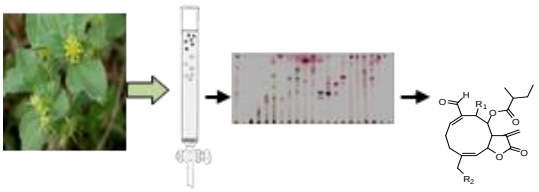
Efficient extracts and pure compounds on mice infected with *Trypanosoma brucei* were also identified and their highest tolerated dose determined.

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

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

2/ ISOLATION AND STRUCTURE IDENTIFICATION OF BIOACTIVE NATURAL COMPOUNDS

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



Acanthospermum hispidum

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

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

or essential oil components from Vietnamese plants.

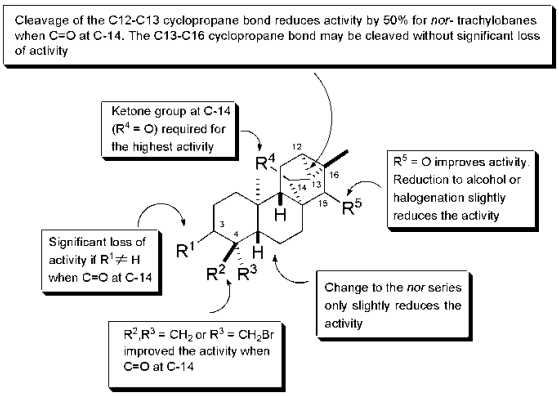
Identifications of antimalarial compounds is also guided by supervised metabolomics studies of crude extracts (collaboration with Prof. Choi, Leiden) while collaborations with LIST (Luxembourg Institute of Science and Technology, Dr André) allowed us to identify and produce potential bioactive compounds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

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

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

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

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



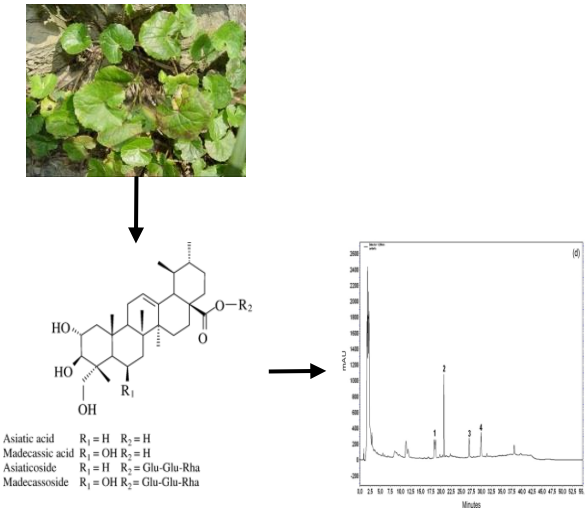
Structure-activity relationships for the vasorelaxant activity of trachylobanes

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

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

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.

SELECTED PUBLICATIONS
Joëlle Quetin-Leclercq

Catteau L, Schioppa L, Beaufay C, Girardi C, Hérent MF, Frédérich M, Quetin-Leclercq J. Antiprotozoal activities of triterpenic acids and ester derivatives isolated from the leaves of *Vitellaria paradoxa.* Planta Medica (2020) Dec 7. Doi: 10.1055/a-1286-1879

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

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

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

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

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

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

THESIS DEFENDED IN 2020

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

THESES IN PROGRESS

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

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

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

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

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

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Leclercq S.	Degraeve A.	Pötgens S.	Blave I.
Lefevre C.	Depommier C.	Rastelli M. L	Charlier J.
Leyrolle Q.	de Wouters d'Oplinter A.	Stevens P.	Danthinne H.
Mallaret G.	Gillard J.	Thibaut M.	Debluts P.
Neyrinck A.	Gourgue F.	Uwimana A.	Es Saadi B.
Pelicaen R.	Hiel S.	Yelek C.	Genten S.
Régnier M.	Huwart S.		Gesche L.
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Our research group proposes an approach based on integrative physiology, metabolism and nutrition, to investigate the role of the gut microbiota in the development of metabolic and behavioral disorders associated with obesity and cardiometabolic risk, alcohol dependence, cancer development and cachexia. In collaborative projects, we also evaluate the implication of the gut microbiota in xenobiotic metabolism and wound healing.

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

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

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

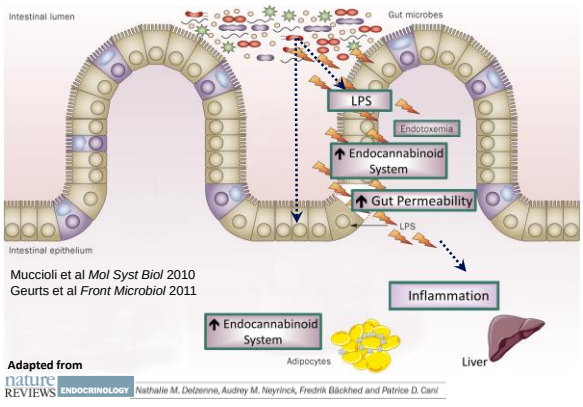
On one side, specific *in vitro* models, such as "Precision-Cut Liver Slices (PCLS)" and mouse adipose explants, have been implemented to study the contribution of tissue-fixed macrophages and other cell types in the metabolic response to nutrients, drugs and microbial compounds. We also developed intestinal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

A decade ago, one of our breakthroughs has been the identification of the role of the endocannabinoid system and its interaction with the gut microbiota in the development of adipose tissue and metabolic inflammation associated with obesity, insulin resistance and type 2 diabetes. To this aim, specific animal models of tissue specific (i.e., gut, liver, adipose), genetic deletions of genes involved in the host-bacteria interaction or in the synthesis of endocannabinoids have been or are currently developed and studied.

Both *in vivo* and *in vitro* models are proposed 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, surgical interventions, molecular, (meta)genomic and metabolomics approaches in biological fluids and tissues.

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



1) BACKGROUND

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

Our experimental data suggest their potential to improve metabolic disorders associated with obesity. In rodents, changing the gut microbiota composition using fructans reduces food intake, improves glucose homeostasis and steatosis, and decreases fat mass development, these events being clearly related to the modulation of endogenous gut peptides production. Indeed, changing the microbiota with dietary prebiotics administration leads to an increase in the differentiation of stem cells into endocrine L cells in the proximal colon of rats, and therefore promotes the production of glucagon-like peptide-1 and 2 (GLP-1 and GLP-2) in this organ. The relevance of the GLP-1 in the improvement of metabolic disorders is shown through experiments performed in mice lacking functional GLP-1 receptor: those mice are resistant to the beneficial effect of fructans on obesity and glucose metabolism. In addition, the GLP-2 is known to improve gut barrier function, here we found that the endogenous production of GLP-2 is a key event responsible for the reduced gut permeability observed upon severe obesity and type 2 diabetes.

Some of these metabolic alterations are modulated by the gut microbiota through specific bacteria-derived compounds such as pathogen-associated molecular patterns (PAMPs). Among them, we have identified the key role played by the lipopolysaccharides (LPS) in the onset of metabolic inflammation and glucose homeostasis disorders in the context of obesity and associated disorders, as well as in the inflammation linked to alcohol dependence in humans. The alteration of the gut barrier is one important cause of the translocation of bacterial elements (e.g., LPS, peptidoglycans) and metabolites which promote inflammation and metabolic disorders occurring in nutritional or behavioral disorders (diabetes and obesity, cancer cachexia, alcohol dependence…).

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

For reviews:

Everard et al, Best Pract Res Clin Gastroenterol 2013, Rev Endocr Metab Disord. 2014

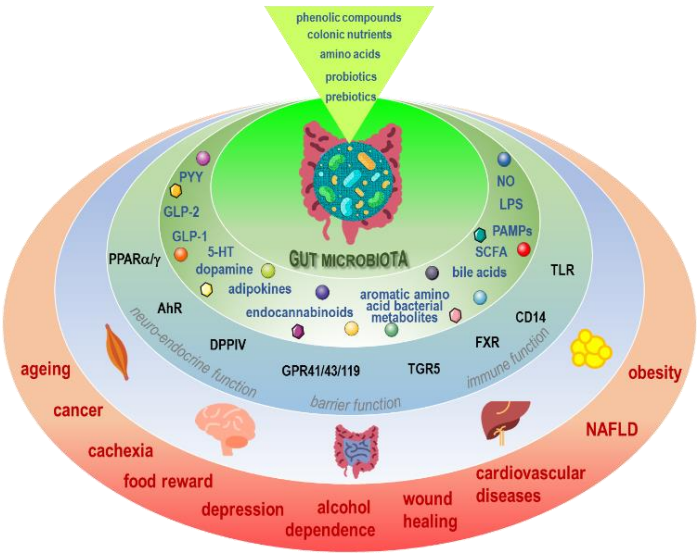
Bindels et al, Nat Rev Gastroenterol Hepatol. 2015, Clin Nutr Exp 2016

Cani Mol Nutr Food Res. 2016, Nat Rev Endocrinol. 2016, Int J Obes Suppl. 2016, Gut 2018, Nature Metabolism 2019, Nat Rev Endocrinol. 2019

Delzenne et al, Nature rev 2011, Diabetologia 2015, Proc. Nutr. Soc. 2019, Rev Endocr Metab Disord. 2020

2) RESEARCH ACTIVITIES

1. to develop experimental models mimicking metabolic and behavioral disturbances occurring during obesity, cancer development, addiction;
2. to evaluate the implication and therapeutic interest of the gut microbiota and associated microbial metabolites in the occurrence of metabolic and behavioral disorders and the progression of cancer;
3. to investigate the role of the gut microbiota in the control of food intake, food reward, alcohol dependence, and depression;
4. to investigate the role of the endocannabinoid system and of specific receptors responding to gut microbial components or metabolites;
5. to decipher the role of the innate immune system in the development of obesity, inflammation, insulin resistance, oxidative stress, type 2 diabetes, hepatic steatosis, or behavior in mice;
6. to evaluate the involvement of key gut functions (endocrine, immune, endothelial, barrier functions) alterations in the occurrence of behavioral and metabolic disorders associated to obesity, alcohol consumption and cancer progression.
7. to develop specific surgical interventions in mice and techniques of real-time imagery (i.e., mouse colonoscopy), in order to evaluate the role of the mucosal microbiota on wound healing.
8. to evaluate how drugs such as immunosuppressive agents can affect the gut microbiota and how conversely the gut microbiota can affect the pharmacokinetics and pharmacodynamics of such drugs.
9. to study the impact of nutrition and gut microbiota on immune system and the susceptibility to develop infectious diseases (e. g. malaria).
10. to study how nutrients targeting the gut microbiota may affect metabolic homeostasis, gut functions, immune system and organs (liver, muscle, brain, adipose tissue).
11. to evaluate – by using untargeted and targeted metabolomics- the relevance of microbial cometabolites in the changes in behavior (depression, social behavior and food related behavior) in the models of mice transferred with the gut microbiota from obese or alcohol-dependent human volunteers.



3) OVERVIEW OF THE RECENT RESULTS

a) *In the context of cardiometabolic disorders*

Akkermansia muciniphila

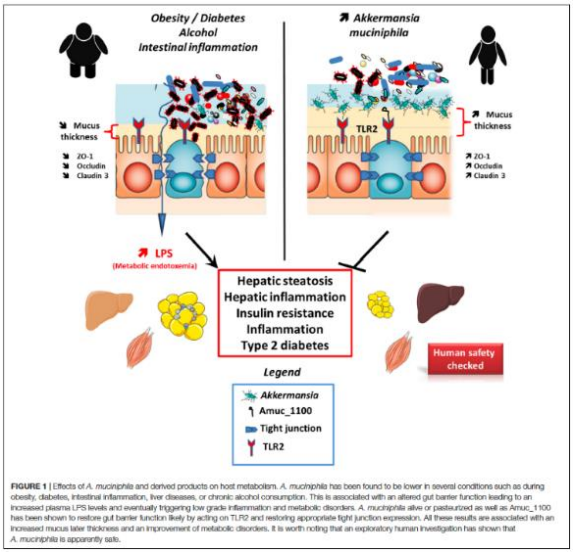
In 2013, we have identified *Akkermansia muciniphila* as a key bacterium involved in the control of the gut barrier function and host metabolism (Everard et al PNAS 2013 and patents). We demonstrated that *A. muciniphila*, a mucin-degrading bacterium that abundantly colonizes the mucus layer, negatively 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. Dietary fatty acids composition strongly contributes to the modulation of the abundance of *A. muciniphila*. Indeed, mice fed with a saturated fatty acid-rich diet exhibit a significant decrease in gut *A. muciniphila*, whereas omega 3 fatty acids -enriched diet dramatically increases *A. muciniphila*. This effect is associated with improvement of gut barrier function and adipose tissue inflammation, a phenomenon that can be transferred to germ-free recipient mice (Caesar et al. Cell Metabolism 2015).

Aging is also linked to a decrease in *A. muciniphila* (Schneeberger et al Sci Reports 2015). We found that HFD feeding influences adipose tissue profile and intestinal microbiota in a way that mimicks aging. In the same set of experiments, we found by using multifactorial analysis that these changes in *A. muciniphila* were robustly linked with lipid metabolism and inflammation markers in the blood and adipose tissue, as well as several blood markers (Schneeberger et al Sci Reports 2015).

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., 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). We demonstrated that *A. muciniphila* cultured on this media retains its efficacy (Plovier et al, Nature Medicine 2017 and patent pending). Unexpectedly, pasteurizing *A. muciniphila* even enhanced its capacity to reduce fat mass development, insulin resistance and dyslipidemia in mice, those effect being notably associated with a modulation of host urinary metabolomics profile and higher intestinal energy excretion. Then we wanted to understand why *A. muciniphila* behaved differently when live and pasteurized. By combining genomic and proteomic analyses of *A. muciniphila,* our collaborators identified proteins encoded by a specific Type IV pili gene cluster in fractions enriched for outer membrane proteins, Amuc_1100 being the most abundant protein (Plovier et al Nature

Medicine 2017). We showed that a His-tagged Amuc_1100 produced in *E. coli* recapitulated the beneficial effects of pasteurized *A. muciniphila* (Plovier et al Nature Medicine 2017 and patents pending). Interestingly, this protein also remains active after heating at 70°C (pasteurization). We demonstrated that Amuc_1100 interacts with TLR-2 and improves the gut barrier (see figure).



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

Finally, we also developed the production of *A. muciniphila* at a large scale in order to test the safety and efficacy of the bacterium on parameters associated with cardiometabolic risks factors. The major aim of the study Microbes4U© - published in Nature Medicine (Depommier et al. 2019)- was to evaluate the safety and tolerability of *Akkermansia muciniphila* in individuals with excess body weight by supplementing them with different doses of live *Akkermansia muciniphila* (Akk Synthetic - 10^{10}) or pasteurized *Akkermansia muciniphila* (Akk Pasteurized - 10^{10}). 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.

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

Besides *A. muciniphila* Prof. Cani and his team have isolated several novel bacteria including one novel genus/species/strain. The bacterium is called *Dysosmobacter welbionis* in reference to the project WELBIO which is supporting this innovative research since 2012 (Le Roy et al IJSEM 2019, and patent pending PCT/EP2019/068539). The metabolic effects of these novel bacteria are currently under investigation for their interest in the context of obesity, diabetes, inflammatory bowel diseases and cancer.

The innate immunity

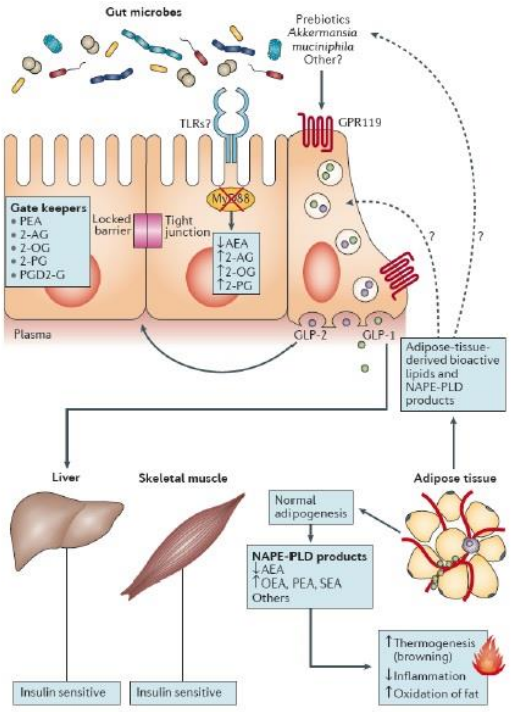
In 2014, we found that a link between the intestinal innate immune system (i.e., the protein MyD88) and energy homeostasis. We found that deactivating the protein MyD88 in intestinal cells of high fat diet fed mice delays type 2 diabetes development, reduces adiposity and deleterious inflammation and 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).

The endocannabinoid system

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

Actually, we discovered that mice harbouring inducible intestinal epithelial cell (IEC)-specific deletion of NAPE-PLD (Napepld$^{\Delta IEC}$) were hyperphagic upon first exposure to a high-fat diet and developed exacerbated diet-induced obesity and hepatic steatosis, involving as mechanisms a defect in hypothalamic Pomc neurons and alterations in intestinal and plasma NAE and 2-acylglycerols. After long-term HFD exposure, Napepld$^{\Delta IEC}$ mice presented a lower energy expenditure. A higher intestinal and hepatic lipid uptake contributed to exacerbated steatosis. Napepld$^{\Delta IEC}$ mice displayed altered gut microbiota composition. Strikingly, treatment with *A. muciniphila*, by modulating NAE, endocannabinoids and related mediators, partly counteracted the effects of the deletion. These results suggest that intestinal NAPE-PLD is a key sensor in nutritional adaptation to fat intake and energy homeostasis and thereby constitute a novel target to tackle obesity and related disorders (Everard*, Plovier*, Rastelli* et al Nature Communications 2019). To further understand the mechanisms responsible for aberrant gut-brain signaling leading to hyperphagia in the Napepld$^{\Delta IEC}$ mice, we exposed mice to different forms of lipid challenge (HFD or gavage). We discovered that Napepld$^{\Delta IEC}$ mice displayed reduced OEA in brain and intestine, suggesting an impairment of the gut-brain axis in this model; we speculated that decreased level of OEA likely contributes to reduce GLP-1R activation, explaining the observed hyperphagia in this model. Altogether, we elucidated novel physiological mechanism regarding the gut-brain axis, namely how intestinal NAPE-PLD regulates appetite rapidly after lipid exposure (Rastelli et al Am J Physiol Endocrinol Metab 2020). Taken together, these findings indicate that bioactive lipids produced by the NAPE-PLD contribute to changes in the gut microbiota even at distance of the organ targeted (e.g., the adipose tissue). These changes then participate in the altered metabolic disorders observed following NAPE-PLD deletion. These results provide strong support for the crosstalk between the gut microbiota and the endocannabinoid system as a potent mediator.



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

Nutritional strategies

Recently, several prebiotics have been tested in original mice models of endothelial dysfunction and gluten-induced obesity. Those data revealed that the improvement of the endothelial dysfunction by fructans and chitin-glucans is associated with specific changes in microbiota and increased intestinal production of nitric oxide release (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).
In the context of a European project (MyNewGut, http://www.mynewgut.eu/), we have also highlighted a potential interest of amino-acid microbial metabolites to counteract hepatic inflammation and of microbial conjugated polyunsaturated fatty acids to improve lipid metabolism (Beaumont et al FASEB J 2018, Pachikian et al Plos One 2018), thereby extending the concept of prebiotics and related bioactives.

Other classes of food products have been evaluated in term of microbiota modulation in preclinical models of nutritional disorders. Among them, green tea - rhubarb- curcuma- or pomegranate- extracts, as well as spirulina counteract inflammation associated with nutritional disorders (Neyrinck et al Plos One 2013, Mol Nutr Food Res 2016, J Nutr Biochem 2017, Nutrients 2017). 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 that modulating the gut microbiota with prebiotics (i.e., oligofructose) modifies the actions of enteric neurons, thereby controlling duodenal contraction and subsequently attenuating hyperglycaemia 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

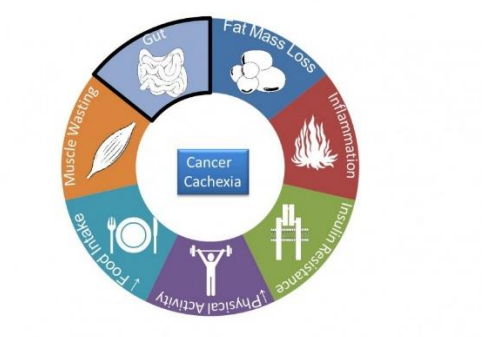
neurotransmitter synthesis were altered in the duodenum of both diabetic mice and humans (Abot et al GUT 2020).

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). A 3 months placebo controlled, multicentric intervention with inulin rich food with supplement in obese patient for 3 months, allowed to point out that inulin intervention better improved fasting insulinemia, BMI, diastolic blood pressure than placebo (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 decrease BMI and to improve mood depends both on the initial gut microbiota composition, whereas the effect on liver and muscle dysfunction depends on specific modulation of gut microbes upon inulin intervention (Rodriguez et al, Gut 2020; Leyrolle et al, Clin Nutr 2020).

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.



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). This microbial signature was not due to the anorexia observed in the last stage of the disease (Bindels et al, Plos ONE 2011; Bindels et al, The ISME J 2016. More recently, we have highlighted that

Klebsiella oxytoca was the Enterobacteriaceae species that was fostered in cancer cachexia. We evidenced a mechanism of emergence for this bacteria similar to the one described for the bloom of Enterobacteriaceae during antibiotics consumption. This framework includes a reduction in Treg cells in the intestine, together with a glycolytic switch and a host-derived production of nitrate (Pötgens et al, Sci Rep 2018).

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

Last but not least, we reported several times that nutritional interventions targeting the microbiota, such as prebiotics or probiotics, decreased cancer progression, reduced morbidity and fat mass loss, and/or increased survival of cachectic mice with leukemia (Bindels et al, the ISME J 2016; Bindels et al, Plos ONE 2015; Bindels et al, Br J Cancer 2013). Our data highlight propionate, a short-chain fatty acid produced through the fermentation of prebiotics, as a potential mediator of this anti-cancer effect observed in leukemic mice with cachexia. Indeed, administration of inulin-type fructans (a well-known prebiotic) increased portal levels of propionate which is able to control the proliferation of leukemic cells (Bindels et al, Br J Cancer 2013).

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 (recent implementation of H^1-NMR metabolomics) 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, manuscript under revision).

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. 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 (Thibaut et al, J Cach Sarc Muscle 2020).

c) In the context of food intake and food reward

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

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

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

In order to proof a causal link between gut microbiota and alterations of hedonic response to food intake associated with obesity, we use gut microbiota transplantation. Preliminary data suggest that transferring the gut microbiota from high-fat diet-induced obese mice into control diet fed mice is enough to alter the dopaminergic 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 suggest for the first time the implication of the gut microbiota into the alteration of hedonic regulation of food intake during obesity. These preliminary data need to be confirmed and we will investigate the mechanisms involved in these interactions between the gut microbiota and the hedonic regulation of food intake during obesity.

d) Clinical trials

Clinical trials are essential to evaluate the translational potential of our findings issued from preclinical models. For this reason, several clinical trials have been launched by the MNUT team in close collaboration with clinicians, whose names and affiliations are details on the related websites. Four of them are described below: the **MicroAML**, the **FOOD4GUT**, the **FIBERTAG**, the **Gut2Brain_ClinX** and the **Microbes4U©** studies. MNUT PI are also involved as collaborators for other international studies.

To evaluate the translational value of the experimental work linking the gut microbiota to cancer cachexia, we 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 (additional details available online at https://uclouvain.be/fr/node/43424). Recruitment was completed early 2020 and sample analyses are ongoing.

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

Our data obtained in alcohol dependent patients allowed to point out that the gut microbiota characteristics (and related gut barrier) are factors driving depression, craving and altered sociability in those patients (Leclercq et al PNAS 2014; Leclercq et al Cell Rep 2020). 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-hydrdoxybutyrate in brain dysfunction. An intervention study (**Gut2Brain_ClinX** 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.

The **FiberTAG** project aims at establishing a set of biomarkers linking dietary fiber (DF) intake and gut-microbiota related health effect, by using existing cohorts of healthy or overweight populations (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, Gut Microbes 2020) and in patients at risk for cardiometabolic health.

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).

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Patrice D. CANI

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Nathalie DELZENNE

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Amandine EVERARD

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AWARDS 2019-2020

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

Justine Gillard
Best poster communication Award at the 9th annual meeting of the Belgian Nutrition Society 2019

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, Clarivate analytics Web of Sciences

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

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

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

Julie Rodriguez
Prize of the Belgian Society of Clinical Nutrition 2020 (the best abstract selected at ESPEN 2019)

Clara Depommier & Patrice D. Cani
Prize of the Belgian Endocrine Society for the best publication in endocrinology between 2019-2020 for the paper Depommier et al Nature Medicine 2019).

THESIS DEFENDED IN 2020

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

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

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

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

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

THESES IN PROGRESS

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

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

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

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

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

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

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

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

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

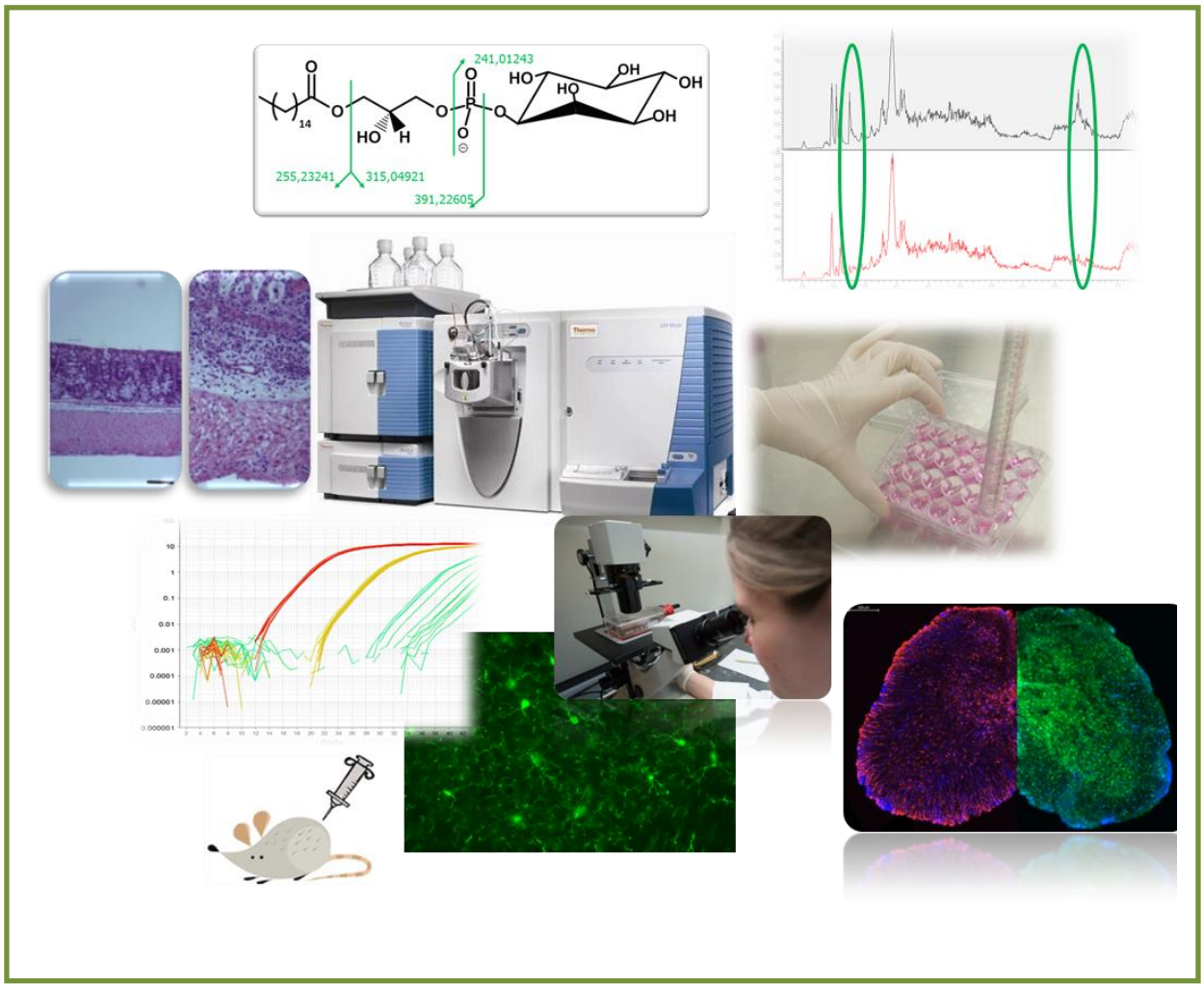
Emilie Moens de Hase: **"**Investigation of the effects of newly discovered bacteria Dysosmobacter welbionis on metabolism and inflammation**."**
Director: Patrice D. Cani

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

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

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

Bioanalysis and Pharmacology of Bioactive Lipids (BPBL)



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BPBL

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

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

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

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

1) OVERVIEW

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

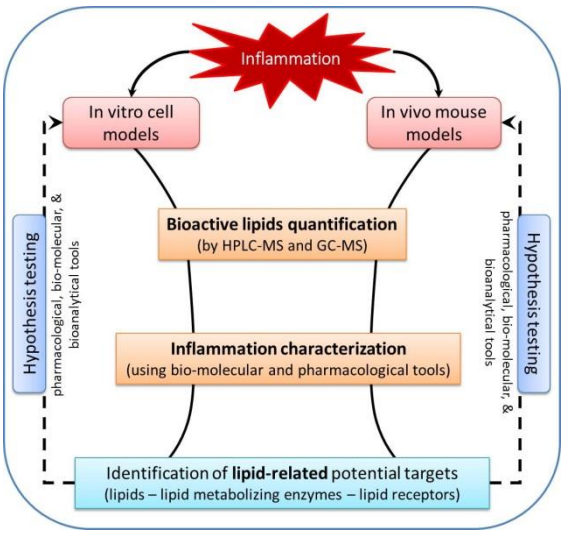


Figure 1. BPBL research group's strategy overview

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 2011; Alhouayek et al. FASEB J 2015; Guillemot-Legris et al. J. Crohns Colitis 2019). Other examples of models currently used in our research group are models of lung inflammation (LPS-induced inflammation, house dust mite-induced inflammation), of multiple sclerosis (EAE model in mice), and of inflammatory pain (carrageenan, LPS, capsaicin) (e.g. Bottemanne et al. FASEB J 2019; Buisseret et al. BBA Mol Cell Biol Lipids 2019; Mutemeberezi et al. J. Neuroinflammation 2018; Orefice et al. Elife 2020). 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 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).

We recently 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).

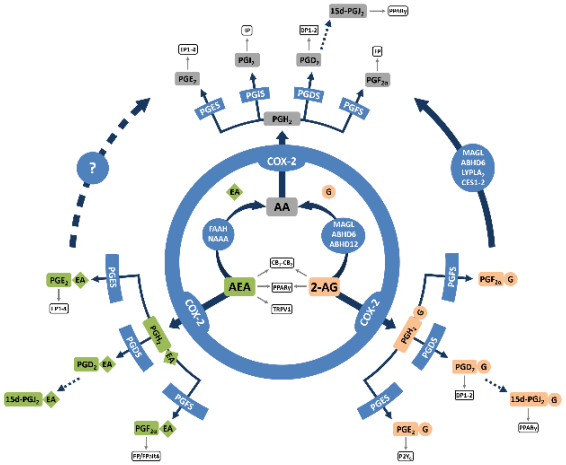


Figure 2: The two main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are part of a large network of bioactive lipids (Buisseret et al., Trends Mol. Med., 2019).

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).

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

b) Oxysterols

Oxysterols are now considered as important lipid mediators, beyond their role in controlling lipid metabolism (Guillemot-Legris et al., Trends Mol. Med., 2016; 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 are now investigating the consequences of these alterations on obesity in vivo and ex vivo (using mice and human adipose tissue explants).

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

Orefice NS, Guillemot-Legris O, Capasso R, Bottemanne P, Hantraye P, Caraglia M, Orefice G, Alhouayek M*, Muccioli GG*. miRNA profile is altered in a modified EAE mouse model of multiple sclerosis featuring cortical lesions. Elife (2020);9:e56916.

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

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

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

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

THESES DEFENDED IN 2020

Pauline Bottemanne : "Endocannabinoids and 25-hydroxycholesterol as modulators of inflammation".
Director: Giulio Muccioli
Co-director: Mireille Al Houayek

Baptiste Buisseret : "Effect of oxygenated derivatives of endocannabinoids on mechanical hyperalgesia induced by peripheral inflammation".
Director: Giulio Muccioli
Co-director: Mireille Al Houayek

THESES IN PROGRESS

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

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

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

Ben Kouidar Youssef: "Investigation of the interest of *N*-acylethanolamine acid amidase inhibition in diet-induced obesity, its inflammatory and neuroinflammatory consequences and the chronicization of post-operative pain".
Director: Giulio Muccioli

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

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

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

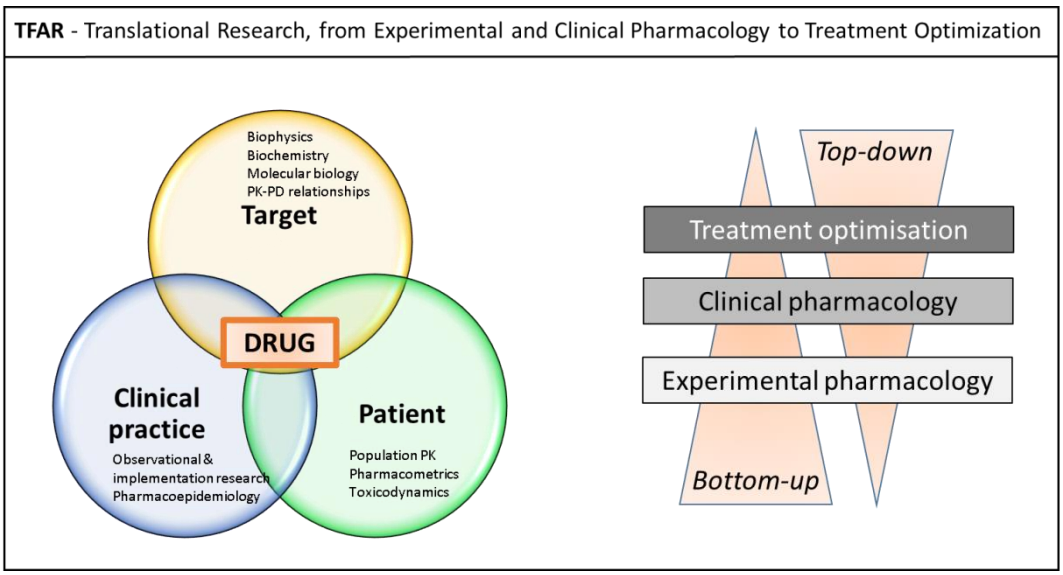
Mwema Ariane: "Nose-to-brain delivery of nanomedicines to stimulate remyelination in the scope of multiple sclerosis".
Director: Anne des Rieux
Co-director: Giulio Muccioli

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

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

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

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



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

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

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

*The main **disciplines** that are covered include: (1) in the field of experimental research: biophysics and molecular pharmacology, in vitro pharmacokinetics and 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 and Françoise Van Bambeke) is mainly oriented towards experimental research; **PMGK** (integrated pharmacometrics, pharmacogenomics and pharmacokinetic group; Laure Elens) towards clinical research; and **CLIP** (clinical pharmacy research group; Olivia Dalleur, Séverine Henrard and Anne Spinewine), towards clinical practice and implementation research.*

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

Examples of recent and ongoing translational research

The number of translational research projects within TFAR has increased over the last 5 years. In 2020, there were several ongoing projects implying eight PhD students that illustrate the type of integrative approaches existing between the groups:

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

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

Cellular and Molecular Pharmacology (TFAR - FACM)

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

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

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

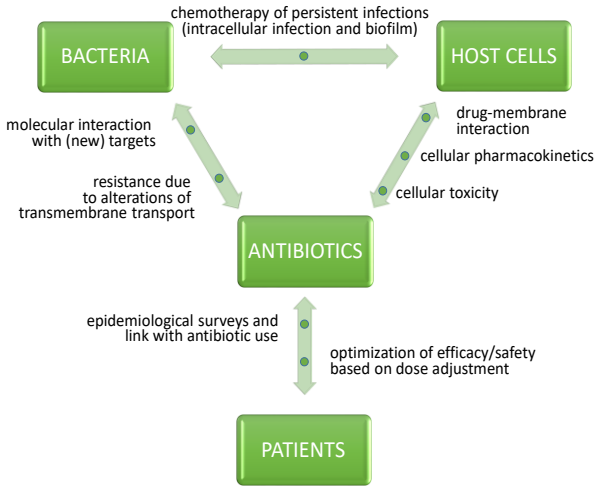
To this effect, we explore

- *at the cellular level, the cellular pharmacokinetics of antibiotics (accumulation, distribution, and efflux in eukaryotic cells), in relation with their activity against intracellular pathogens and with their capacity to cause cellular toxicity (lysosomal or mitochondrial alterations; apoptosis).*
- *at the molecular level, (i) the interaction between antibiotics and membrane lipids and consequences 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 6 main directions. These are, however, closely linked to one another.

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

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

Cardiolipin domains as a target for amphiphilic aminoglycoside derivatives?

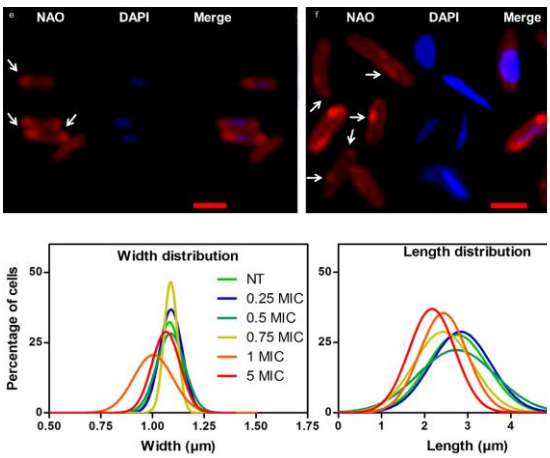
Combination of existing lipid diversity and functions with biophysics of bacterial membranes is a unique opportunity to discover new antibiotics. Bacteria (as mammalian cells) have capacity to maintain specialized zones in their membranes for fruitfully fill in their biological functions.

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

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

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

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



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

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

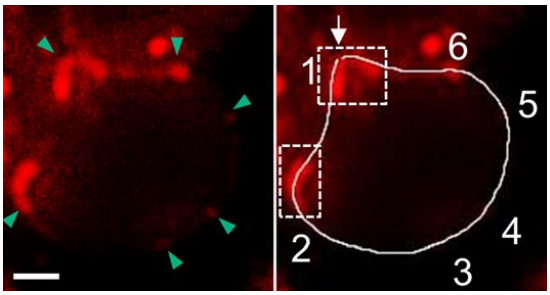
Lipid-proteins interactions could be also critical in cell physio- and pathology of erythrocytes. In this context, we investigated (i) whether enriched lipid domains in cholesterol and sphingomyelin could contribute to function-associated cell (re)shaping, (ii) whether the seminal concept of highly ordered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

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

In this aspect, we are exploring the role of *P. aeruginosa* outer membrane asymmetry and

of the Outer Membranes Vesicles (OMVs) formation in bacterial physiology as well as the potential impact for the activity of amphiphilic aminoglycoside antibiotics.

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



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

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

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

2) Cholesterol-enriched domains and cellular toxicity

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

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

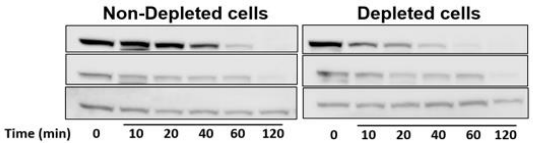
I) Cholesterol-enriched domains and cellular toxicity of anticancer drug (Ginsenoside Rh2)

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

one of the active principles of *Panax ginseng* root. This work is performed in close collaboration with J. Leclercq's team.

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

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



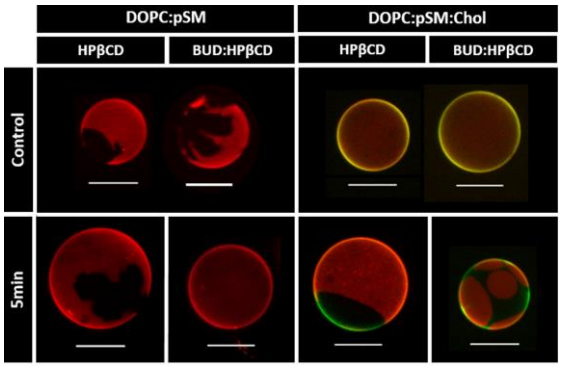
Rh2 decreases the phosphorylation of Akt faster upon cholesterol depletion. Cells depleted in cholesterol with 5 mM MβCD for 2 h, or not, were incubated for the indicated times with 60 µM Rh2 or with 0.1% DMSO (vehicle). Equal amounts of cell extracts were subjected to western-blot analysis for pAkt,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

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

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

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

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

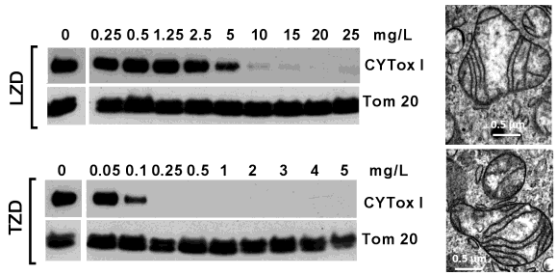


Confocal fluorescence microscopy imaging of membrane phase separation in GUVs upon incubation with BUD:HPβCD, and HPβCD. Imaging of membrane domains in GUVs composed of (left) DOPC:pSM (1:1) and (right) DOPC:pSM:Chol (1:1:3) before (top, control) and after (descending) 5, min with the BUD:HPβCD complex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

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

III) Mitochondrial toxicity of oxazolidinones

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



Left: Influence of increasing concentrations of linezolid (LZD) and tedizolid (TZD) on CYTox I expression in HL-60 promyelocytes incubated for 120 h in the presence of increasing concentrations of these drugs. Western blots of CYTox I (protein encoded by the mitochondrial genome) and of Tom 20 (encoded by the nuclear genome) of mitochondrial protein fractions. Right: electron microscopy images of mitochondria from HL-60 cells exposed to 15 mg/L linezolid or 3 mg/L of tedizolid. Milosevic et al, 2018, Antimicrobial Agents and Chemotherapy

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

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

I) Cellular pharmacokinetics

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

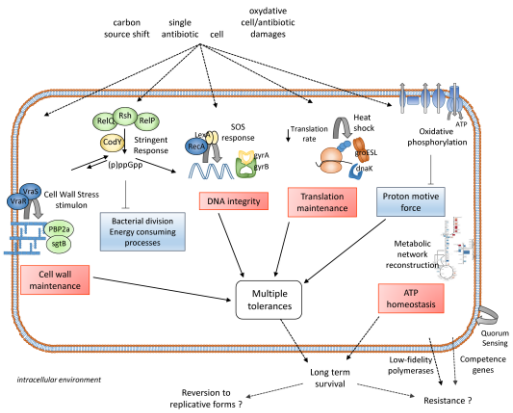
compartments. We try to decipher the mechanisms for their penetration and distribution within the cells. Over the last years, we have focused our interest on new antibiotic classes, like lipoglycopeptides, ketolides, new fluoroquinolones and new oxazolidinones now present on the market. We are now examining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

II) Cellular pharmacodynamics

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

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.

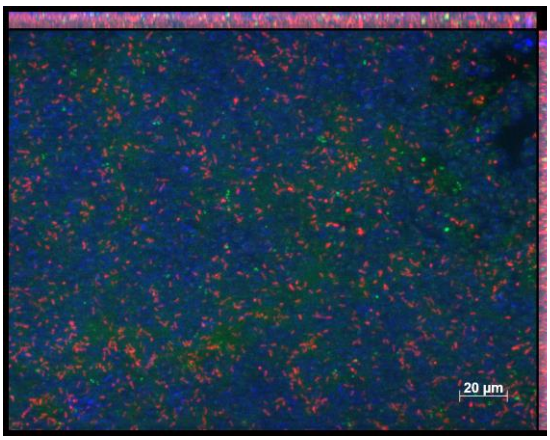


Overview of intracellular persistence regulation of S. aureus. In vacuolar nutrient-rich compartments, persisters are metabolically active cells shielding cell wall, DNA and translation products. Under environmental factors from the host cell, including a carbon source shift and antibiotic pressure, 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

4) Antibiotic activity against biofilms

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

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

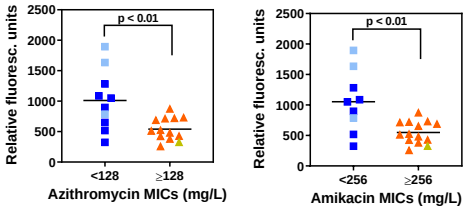


Fluorescence microscopy imaging of a multispecies biofilm made of *Candida albicans* (glycans stained in blue), *Staphylococcus aureus* and *Escherichia coli* (stained respectively in green and red by FISH probes)

5) Antibiotic efflux and permeability resistance mechanisms

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

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



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

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

6) Novel antibiotic targets and drug design

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

lactams in *Staphylococcus aureus or active against Leishmania Mexicana Mexicana and Trypanosoma brucei brucei.*

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

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

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

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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.

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Françoise VAN BAMBEKE

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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

Mustafa M-H., Khandekar S., Tunney M.M., Elborn J.S., Kahl B.C., Denis O., Plésiat P., Traore H., Tulkens P.M., Vanderbist F., Van Bambeke F. Acquired resistance to macrolides in *Pseudomonas aeruginosa* from cystic fibrosis patients. European Respiratory Journal (2017) 49:1601847

Chalhoub H., Pletzer D., Weingart H., Braun Y., Tunney M.M., Elborn J.S., Rodriguez-Villalobos H., Plésiat P., Kahl B.C., Denis O., Winterhalter M., Tulkens P.M., Van Bambeke F. Mechanisms of intrinsic resistance and acquired susceptibility of Pseudomonas aeruginosa isolated from cystic fibrosis patients to temocillin, a revived antibiotic. Scientific Reports (2017) 7:40208

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THESES DEFENDED IN 2019-2020

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

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

Diaz Iglesias Yvan: "Activity of antibiotics against in vitro mono-species biofilms of P. aeruginosa and S. aureus in the context of cystic fibrosis : the influence of the culture mediumx".
Director: Françoise Van Bambeke

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

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

Nguyen Tiep Khac: "Antibiotic resistance in Vietnam in relation with persistent forms of infection. "
Director: Françoise Van Bambeke

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

THESES IN PROGRESS

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)

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

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

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

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

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

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

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

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

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

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), and high risk situations (polymedication, multimorbidity, infections, and patients transiting across settings of care).

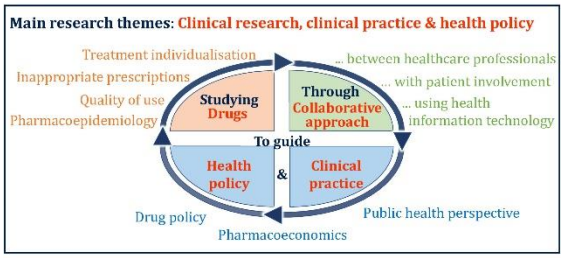
We use quantitative as well as qualitative research methods, and we:

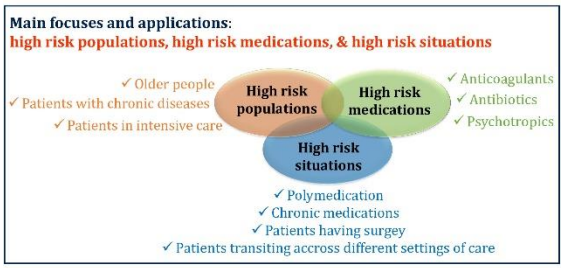
- *develop and/or validate instruments and tools to measure the quality of use of medicines;*
- *collect and use observational data for pharmacoepidemiological research to assess the appropriateness of prescription and use of drugs, as well as their effects on patients in daily practice to optimise and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

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

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

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





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

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

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

✓ **Qualitative methods**

RESEARCH RESULTS AND PERSPECTIVES

a) Quality of use of medicines in older people

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

- ***OPERAM***

The OPERAM project is a European project (H2020, 2015-2020) led by University of Bern. The core part of the OPERAM project is a large-scale **cluster RCT** to evaluate the effect of a complex intervention on drug-related admissions and other clinical and patient-reported outcomes. The intervention comprises clinical decision support using an electronic system called STRIPA, and medication review performed by a geriatrician and a clinical pharmacist. Patient recruitement was completed in October 2018, and almost 400 patients were recruited from the Belgian site (Cliniques universitaires Saint-Luc). Patient follow-up was completed in October 2019. The final results will be published in 2021, and numerous substudies are ongoing.

As leaders of the work package on "clinical outcomes and patient perspective", we have (a) developed a core outcome set (COS) for clinical trials of medication review in older patients with multi-morbidity and polypharmacy (b) developed a method to adjudicate drug-related admissions in older people (c) conducted a substudy about the patients' experience of medication review. The results of the latter will be published in 2021.

In addition, we have participated in the Health Economics work package to identify health economic characteristics of interventions to improve pharmacotherapy in older multimorbid people. We have assessed resource use, cost implications and cost-effectiveness of the OPERAM randomized clinical trial intervention in the 4 countries in which data were collected (Switzerland, Ireland, Belgium, the Netherlands). A cost-effectiveness analysis was performed in 2020, and its methodological challenges was assessed.

Recently, we evaluated the performance of the trigger tool developed to detect DRAs using data from the 1235 hospitalizations adjudicated for 832 OPERAM patients. A revised trigger tool was then developed based positive predictive values of triggers, correlations between triggers, and on the analysis on non-triggered events.

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

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

In 2020 we have continued to perform additional posthoc analyses on the Come-On database. In-depth evaluations of data on the use of **benzodiazepine receptor agonists (BZRA)** have been completed. We found that BZRA use and potentially inappropriate prescribing were highly prevalent. Deprescribing occurred in 28.1% of BZRA users at the end of the study. Being in the intervention group was associated with higher odds of deprescribing, as compared to the control group.

We have also evaluated appropriateness of prescribing in a subgroup of frail NHRs, using the **STOPPFrail** criteria. Among the 308 frail nursing home residents (NHRs), two third of them had ≥1 potentially inappropriate medication (PIM) at baseline. Although the prevalence of PIM remained high at 8 months follow-up (50%), a significant and encouraging decrease was observed over time after the implementation of the medication review process, except for some medication classes (i.e. calcium, multivitamins and antidiabetic agents).

Finally, an evaluation of the occurrence of potentially clinically relevant **drug-drug interactions** is ongoing.

- ***Deprescribing***

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

In 2019, we have 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 adluts' attitude towards BZRA deprescribing, the rPATD questionnaire was adapted into a BZRA specific instrument ith the implication of healthcare professionals and older adults taking BZRA. Ongoing current work includes the assessment of the psychometric properties of this adapted questionnaire in a sample of 240 older adults taking a BZRA in the ambulatory setting or in nursing homes. The recruitment of patients is still ongoing, and is highly impacted by the COVID19 crisis.

When designing approaches to BZRA deprescribing, it is important to account for barriers and enablers to deprescribing. To that end, we are currently conducting (a) a systematic review of the barriers and enablers of BZRA deprescribing, and (b) a qualitative study with healthcare professionals and residents in the nursing home setting. For both studies, we use the Theoretical Domains Framework to categorize barriers and enablers.

b) Pharmacoepidemiology in older people and people with chronic diseases

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

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

The aim of the project is to assess the heterogeneity in older patients with type 2 diabetes, with the aim of improving the therapeutic management of the different profiles of patients.

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.

In 2020, we have conducted a retrospective study on older geriatric inpatients ≥75 years with type 2 diabetes and taking a glucose lowering therapy (GLT), and assessed the inappropriateness of GLT prescribing defined by the 2019 Endocrine Society guideline on diabetes treatment in older adults, and the one-year mortality rate. GLT overtreatment was present in 57.2% of these geriatric patients, undertreatment in 17.9%, and appropriate treatment in 24.8%. Poor health status (*vs* intermediate), as well as overtreatment (*vs* appropriate) were both associated with a significantly higher 1-year mortality rate, but not undertreatment. A special attention should be paid to individualisation of the HbA1c target goals in geriatric patients with diabetes, and to GLT de-intensification in those being over-treated.

Finally, we have conducted and are finalizing a systematic review of Clinical Practice Guidelines recommendations on the individualization of glycaemic management in older people with type 2 diabetes.

c) Use of oral anticoagulants

In 2020 we have continued some research word on the challenges associated with the appropriate use of Direct oral anticoagulants (DOACs) and vitamin-K antagonists (VKA).

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

d) Use of anti-infective drugs

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

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

In 2016, we started an international collaboration with Université d'Abomey-Calavi to assess the impact of a multidisciplinary approach, including a clinical pharmacy intervention, 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.

In 2020, we identified 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 addition, we described the first detection of a plasmid-encoded New-Delhi metallo-beta-lactamase-1 (NDM-1) producing Acinetobacter baumannii isolated in Benin (to be published in 2021).

We observed healthcare workers from these 6 hospitals to describe hand hygiene actions in surgical care units. Overall, hand washing (72.1%) or alcohol rubbing (27.9%) was performed in 33.3% of hand hygiene opportunities. When hand hygiene was applied, technique and duration were not appropriate. Exploration of healthcare professionals' perception of antibioprophylaxis and hand hygiene will continue in 2021.

e) Clinical decision support

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

f) Use of neuroleptics

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. In 2020, a multicentric retrospective study started to describe the use of neuroleptics in this population in hospital setting.

SELECTED PUBLICATIONS
Olivia DALLEUR

Yehouenou CL, Kpangon AA, Affolabi D, Rodriguez-Villalobos H, Van Bambeke F, Dalleur O, Simon A. Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Benin. Ann Clin Microbiol Antimicrob. 2020 Nov 25;19(1):54.

Yehouenou CL, Dohou AM, Fiogbe AD, Esse M, Degbey C, Simon A, Dalleur O. Hand hygiene in surgery in Benin: opportunities and challenges. Antimicrob Resist Infect Control. 2020 Jun 15;9(1):85.

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Séverine HENRARD

Christiaens A, Boland B, Germanidis M, Dalleur O, Henrard S. Poor health status, inappropriate glucose-lowering therapy and high one-year mortality in geriatric patients with type 2 diabetes. BMC Geriatr. 2020;20(1):367.

Evrard P, Henrard S, Foulon V, Spinewine A. Benzodiazepine use and deprescribing in Belgian nursing homes: Results from the COME-ON study. J Am Geriatr Soc. 2020;68(12):2768-77.

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Anne SPINEWINE

Evrard P, Henrard S, Foulon V, Spinewine A. Benzodiazepine Use and Deprescribing in Belgian Nursing Homes: Results from the COME-ON Study. Journal of the American Geriatrics Society 2020; 68:2768-77.

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

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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 2019-2020

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

THESIS DEFENDED IN 2019-2020

Thevelin Stefanie: "Medication review to prevent avoidable hospital admissions in older people with multi-morbidity: Measuring outcomes that matter to patients".
Directors: Olivia Dalleur, Anne Spinewine

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

THESES IN PROGRESS

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

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

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

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

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

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

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

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

Yameogo Téné Marceline: "Potentially inappropriate prescription: Development of an analytical tool for French-speaking ECOWAS countries".
Directors: Séverine Henrard, Anne Spinewine

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

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*
- *Anti-HIV*
- *Anticoagulants*
- *Antibiotics*

1) RESEARCH RESULTS

a) Immunosuppressants used in renal transplantation

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

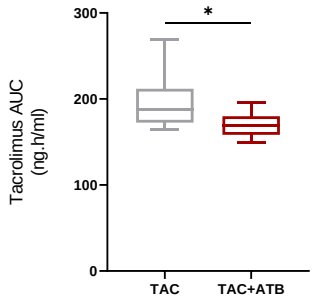

Figure 1: *Tacrolimus Area under the concentration curve (AUC) at day 7 post-exposure in mice treated qith Tac alone or with AB cocktail.*

Further experiments in mice are currently performed to investigate the etiology behind this important observation.

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 (Figure 2).

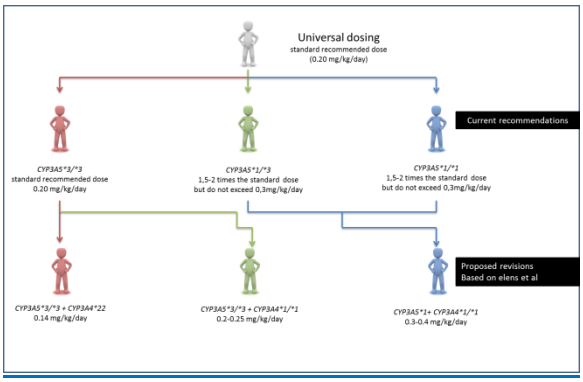

Figure 2: *New dosing guidelines for tacrolimus therapy in renal transplant recipients according to CYP3A genotype.*

In collaboration with the CUSL (Prof. Michel Mourad), We are currently conducting a 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 aim at recruiting 100 patients under Tac-based IS therapy (at present, n=50). We expect that this project will lead us to validate new biomarkers of Tac (and other IS drugs) 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).

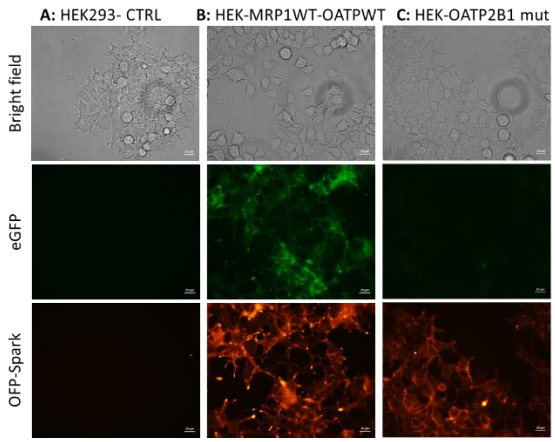

Figure 3: *(a)HEK293 non-transfected cells (b) stable double transfectants overexpressing OATP2B1 (OFP spark-red fluorescent tag) and MRP1 (Green Fluorescent protein [GFP] tag) and (c) stable single transfectant overexpressing an OATP2B1 variant.*

In collaboration with the group of 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) whereas overexpression of MRP1 (efflux) was associated with a significantly decreased accumulation (data not shown).

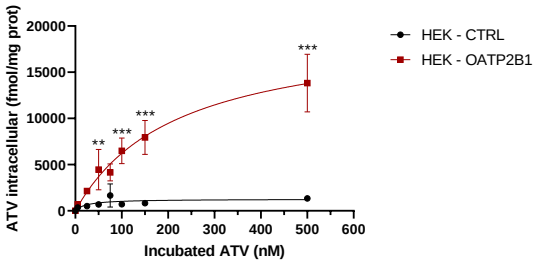

Figure 4: *Intracellular accumulation of atorvastatin in HEK293 (black) and in recombinant overexpressing OATP2B1 (red) after 2h of incubation with increasing concentrations of atorvastatin.*

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 5)

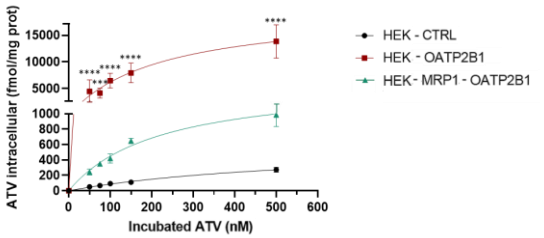

Figure 5: *Intracellular accumulation of atorvastatin in HEK293 (black), in HEK293 overexpressing OATP2B1 (red) and HEK overexpressing both OATP2B1 and MRP1 (green) after 2h of incubation with increasing concentrations of atorvastatin.*

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 6).

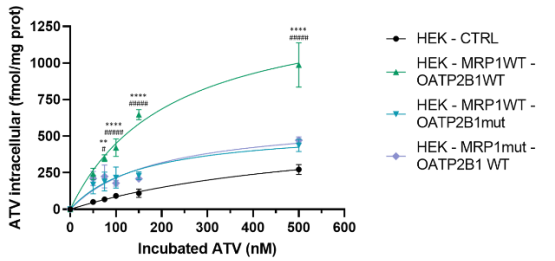

Figure 6: *Intracellular accumulation of atorvastatin in HEK293 (black), in HEK293 overexpressing WT OATP2B1+MRP1 (green), HEK overexpressing WT OATP2B1 but MRP1 variant (rs45511401) (purple) and HEK overexpressing WT MRP1 but OATP2B1 variant (rs12422149) after 2h of incubation with increasing concentrations of atorvastatin.*

Finally, we plan to transpose and develop recombinant cultured primary differentiated human skeletal muscle myoblasts (HSMM) characterized by physiological expression of drug transporters in order to weight the consequences of transporter modulation (DDI, SNPs…) on drug accumulation and myocyte toxicity with specific biomarkers.

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 now includes 83 patients treated with atorvastatin for hypercholesterolemia. In those patients, drug and metabolites measurements were performed at up ty 3 visits and patients were genotyped for some important biotransformation and transporter protein genes (e.g. *CYP3A, ABCs, SLCOs*…). PK results show a high variability in both atorvastatin and its metabolites concentrations (figure 7).

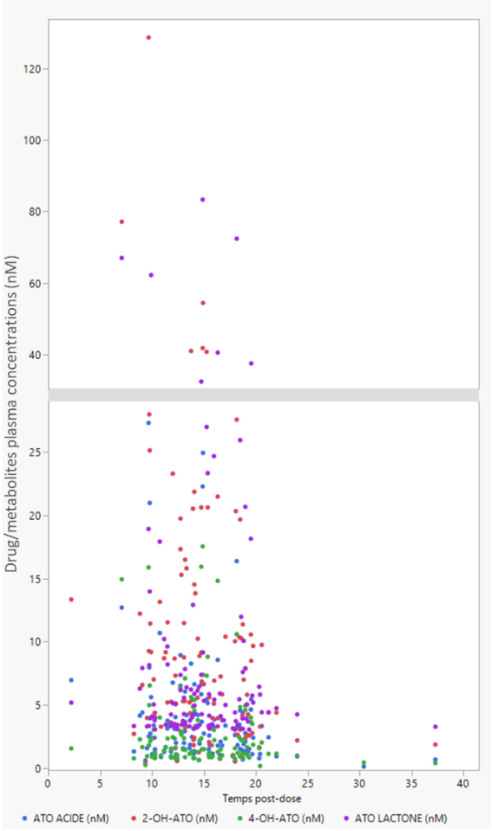

Figure 7: *plasma concentrations of Atorvastatin (red), atorvastatin lactone (purple), 4- (light green) and 2-(blue) hydroxy-Atorvastatin in 83 patients.*

By combining those data with a more rich-sampled cohort (collaboration, Anders Asberg, Oslo University Hospital), we have developed a two-compartmental PopPK model and tested the influence of covariates on PK parameters such as apparent clearance (CL/F). Our primary analysis indicates that CL/F was reduced in carriers of the *SLCO1B1* 521C allele compared to carriers of the T allele. The next step will be to model the PK-PD relationship and test whether genetic polymorphisms influence the clinical response to the drug.

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 recent study, we have characterized darunavir PK by developing a population model based on data collected in a large cohort of 140 Darunavir treated HIV-infected patients. Alpha-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.

d) New anticoagulants

In vitro investigations

In collaboration with CLIP, we have investigated the in vitro impact of *ABCB1* genetic polymorphisms on the transport activity towards rivaroxaban. We found that the *ABCB1* 1236C>T-2677G>T-3435C>T and 1199G>A SNPs had no significant effect on the efflux of rivaroxaban (Figure 8). However, the intracellular accumulation of rivaroxaban was influenced by the overexpression of ABCB1, confirming its involvement in the active transport of this oral anticoagulant. This information is crucial to manage potential drug-drug interaction through modulation of P-gp activity at the gut barrier.

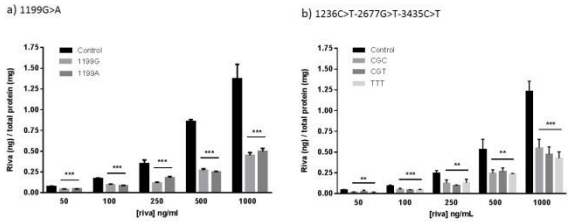

Figure 8: *Intracelllular accumulation of rivaroxaban after 120 min of incubation (n=3) at different concentrations in (a) $HEK_{control}$ (i.e. empty vector), HEK_{1199A} or (b) $HEK_{control}$, $HEK_{1236C>T-2677G>T-3435C>T}$. *p<0.05, **p<0.01, ***p<0.001 compared to $HEK_{control}$*

2) OUTLOOKS

The PK world is currently evolving from a descriptive explanatory tool 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 (figure 9).

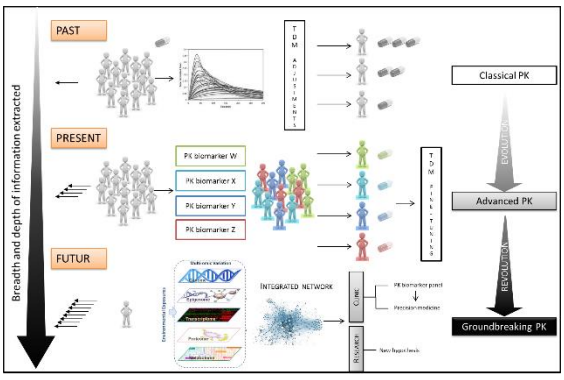

Figure 9: *Evolution of PK and future prospects.*

SELECTED PUBLICATIONS
Laure ELENS

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.

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

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

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

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Elens L, 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, Tyteca D, Panin N, Courtoy P, Lison D, Demoulin J-B, Haufroid V. Functional defect caused by the 4544G>A SNP in ABCC2: potential impact for drug cellular disposition. Pharmacogenet Genomics (2011), 21: 884-93.

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

THESES DEFENDED IN 2020

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

THESES IN PROGRESS

Degraeve Alexandra: "Tacrolimus pharmacokinetic pathway and microbiota: study of the complex bidirectional partnership for explaining metabolic variability and modulations".
Directors: Laure Elens, Laure Bindels (MNUT)
Hoste Emilia: "Atorvastatin toxicokinetics"
Directors: Laure Elens, Vincent Haufroid (IREC)
Ngougni Pokem Perrin: "Therapeutic monitoring of beta-lactams antibiotics to improve their efficacy".
Directors: Françoise Van Bambeke, Laure Elens

Advanced Drug Delivery and Biomaterials (ADDB)

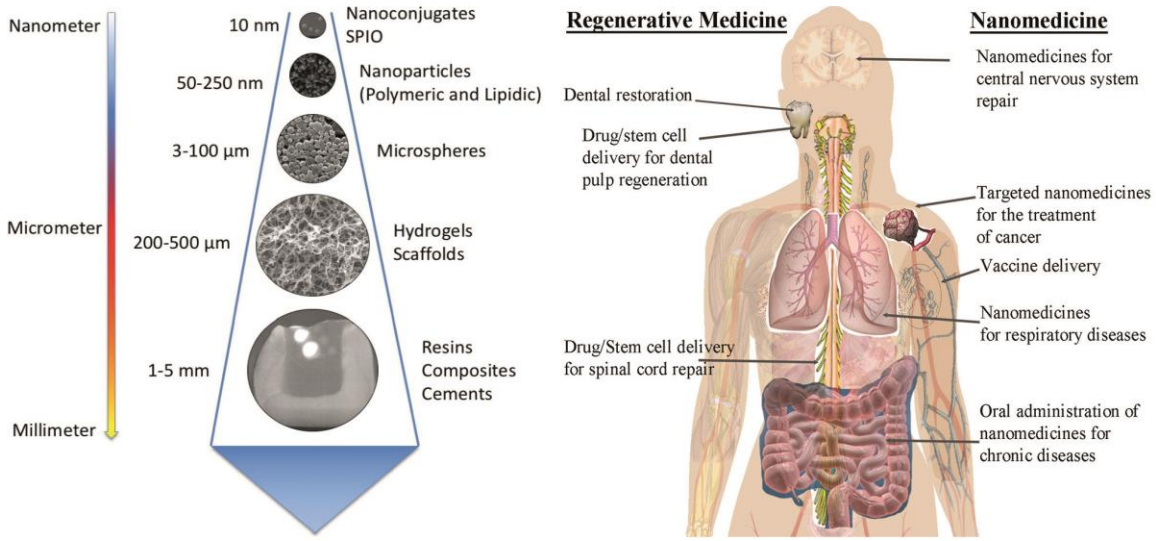

Figure 1: A. Drug delivery systems and biomaterials developed/used by the ADDB group; B. Biomedical applications targeted by the ADDB group.

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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 theranostic nanoparticles loaded with anticancer drugs or siRNA, adjuvanted recombinant antigens and gene vaccines.

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

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

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

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

Polymeric and lipidic nanomedicines are developed for the administration of poorly water soluble drugs, peptides, vaccines and nucleic acids. Our research mainly focuses on (i) intravenous delivery of drug-loaded of nanoparticles targeting the tumoral endothelium and cancer cells ii) local delivery of anticancer drugs.

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

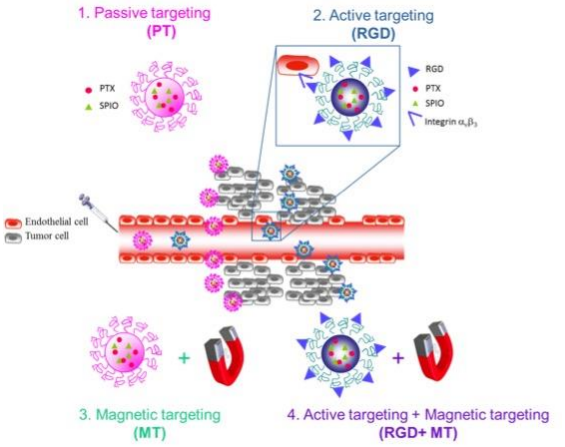

Figure 2: Passive, active and magnetic targeting of anticancer drug-loaded nanomedicines

We aim to develop formulation (nanoparticles) and physical methods (electroporation) for the delivery of DNA and RNA with a particular interest in vaccination and cancer treatments (*Figure 3*).

Electroporation of DNA was optimized to deliver plasmid vaccines into the skin or the muscle. This potent delivery method allows high level of expression. Optimised plasmids encoding tumor antigens elicited humoral and cellular immune response and induced tumor control or regression. Electroporation of plasmid coding for host defense peptides promoted wound healing in healthy and diabetic mice models.

Our current research focuses now on the combination of optimized anticancer DNA vaccines and immune checkpoint inhibitors.

RGD targeted Nanoparticles loaded with siRNA showed good biodistribution and antitumoral efficacy *in vivo*.

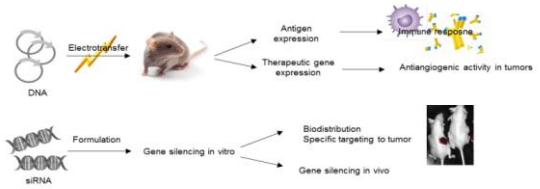

Figure 3: Plasmid DNA and siRNA delivery

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

The oral route is the most preferred route of drug administration. It is easy to administer, pain free and cheaper compared to other routes of administration. However, this route is sometimes inefficient due to the partial/inadequate absorption of the drug, first-pass metabolism, the instability of the drug in harsh gastrointestinal conditions (such as intestinal pH or enzyme degradation). There is an unmet need for the administration of 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 developing improved alternative drug delivery systems to fulfill the potential of the oral route of administration. For this purpose, we are exploiting the unique pathophysiology of the gut towards the development of novel drug delivery strategies, focusing on the treatment of two main chronic diseases: type 2 diabetes mellitus and inflammatory bowel diseases. Our recent results 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.

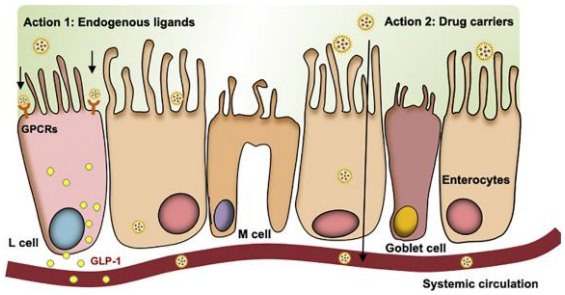

Figure 4: A schematic representation of the dual effect attained with our novel nanoformulation (from Xu et al., J Control Release, 2020).

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).

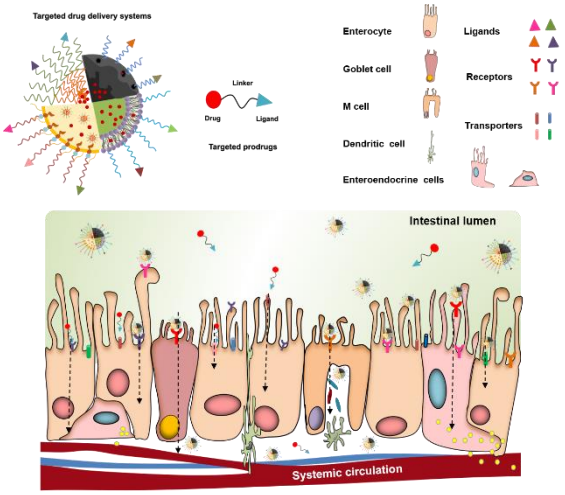

Figure 5: Targeting strategies towards the intestinal barrier *(from Xu et al., J Control Release, 2020).*

3) NANOMEDICINES FOR PULMONARY DELIVERY (R. VANBEVER)

The research aims at improving the treatment or prophylaxis of severe respiratory diseases by designing nanomedicines 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. PEGylated rhDNase was stable to jet nebulization. Multiple high-dose administrations of PEGylated rhDNase for up to three months did not cause any significant pulmonary or systemic toxicity, nor accumulation of the rhDNase or PEG moieties in biological fluids.

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. On the other hand, mucociliary clearance appeared to play a less prominent role in the clearance of those proteins after pulmonary delivery.

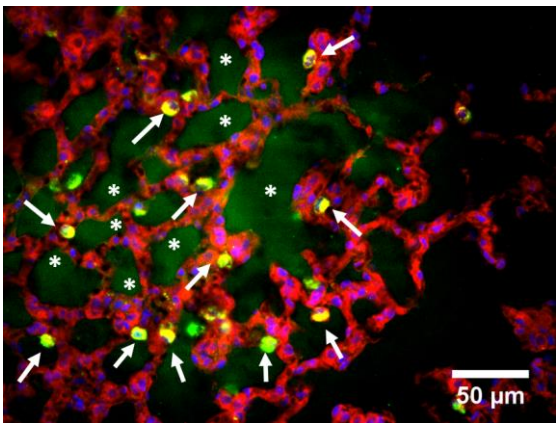

Figure 6. Localization of PEGylated rhDNase in mouse lungs by confocal imaging 4 days after intratracheal instillation. 1 nmol of Alexa488-PEG-rhDNase (green) was administered to NMRI mice by intratracheal instillation. Four days later mice were sacrificed and lung slices were imaged with Cell Observer 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, in press.

Next we investigated the mechanisms promoting the extended lung retention of PEG-rhDNase conjugates using cell culture models and lung biological media. Uptake by alveolar macrophages was also assessed in vivo. PEGylation reduced the uptake and transport of rhDNase across monolayers of Calu-3 cells cultured at an air-liquid interface. PEGylation also decreased the uptake of rhDNase by macrophages in vitro whatever the PEG size as well as in vivo 4 h following intratracheal instillation in mice. The uptake of rhDNase by macrophages was dependent on energy, time, and concentration and occurred at rates indicative of adsorptive endocytosis. Decreased transport across lung epithelial cells and uptake by macrophages appear to contribute to the longer retention of PEGylated rhDNase in the lungs.

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

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

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

Regarding NSC recruitment, we developed SDF-1 PLGA nanoparticles that, once

implanted at the site of a traumatic brain injury, were able to recruit NSC at the damaged area (L. Zamproni, Universidade Federal de Sao Paulo, BR).

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

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

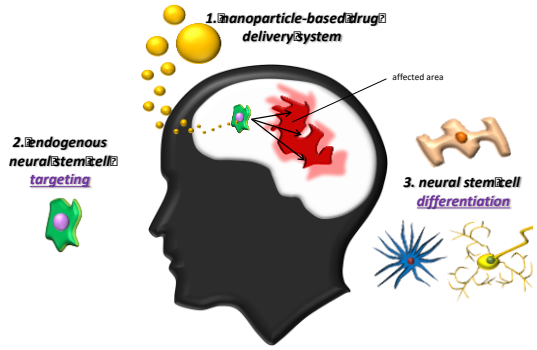

Figure 7: Targeted drug delivery for CNS repair.

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

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

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

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

1. Sustained delivery of growth factors from injectable hydrogel

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

2. Stem cell delivery

As a source of human mesenchymal stem cells, we selected human dental stem cells of the apical papilla (SCAP) due to their neural crest origin but also because they are easily accessible (obtained from extracted wisdom tooth roots). They express numerous neuronal markers, display enhanced neural stem cell properties compared to bone marrow-derived mesenchymal stem cells and possess higher proliferation and differentiation rates compared to dental pulp

stem cells. The studies performed on SCAP have been done in collaboration with Prof. Diogenes (San Antonio University, San Antonio, USA).

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

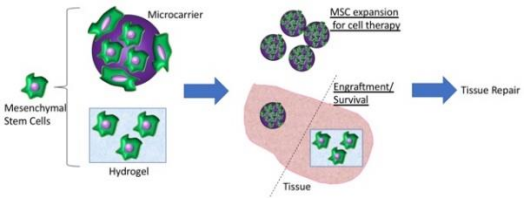

Figure 8: SCAP delivery strategies.

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

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

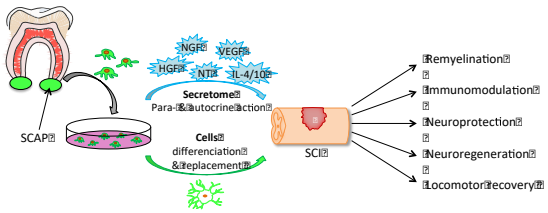

Figure 9: Therapeutic potential of SCAP for spinal cord repair.

b. Incorporation of SCAP in hydrogels

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

We also studied the influence of decellularized extracellular matrix-based hydrogels (dECMh) originating from different organs (bone, dentin and spinal cord) (*Erasmus Mundus NanoFar, collaboration with Prof. Shakesheff and Dr. White, University of Nottingham, UK*). dECMh are thermosensitive (gelation at 37°C), contain preserved cell adhesion sites and active molecules specific of the organ of origin. We demonstrated that dECMh origin impacted hydrogel properties and SCAP viability and neuronal gene expression, spinal cord dECMh being the most favorable for neural differentiation.

c. Development of growth factor loaded microcarriers for SCAP delivery

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

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

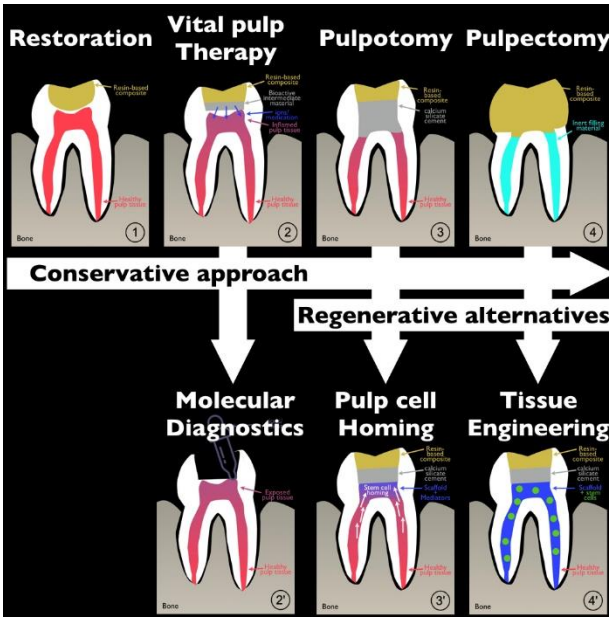

Figure 7: Current approaches for the treatment of tooth decay.

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

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

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) <u>In vitro-methods</u>

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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Véronique PREAT

Zhao M, Bozzato E, Joudiou N, Ghiassinejad S, Danhier F, Gallez B, Préat V. Codelivery of paclitaxel and temozolomide through a photopolymerizable hydrogel prevents glioblastoma recurrence after surgical resection. J Control Release (2019) 309: 72-81.

Bastiancich C, Bianco J, Vanvarenberg K, Ucakar B, Joudiou N, Gallez B, Bastiat G, Lagarce F, Préat V, Danhier F. Injectable nanomedicine hydrogel for local chemotherapy of glioblastoma after surgical resection. J Control Release (2017) 264:45-54

Schleich N, Po C, Jacobs D, Ucakar B, Gallez B, Danhier F, Préat V. Comparison of active, passive and magnetic targeting to tumors of multifunctional paclitaxel/SPIO-loaded nanoparticles for tumor imaging and therapy. J Control Release (2014) 194:82-91.

Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release (2012) 161:505-22.

Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release (2010) 148: 135-146.

SELECTED PUBLICATIONS

Rita VANBEVER

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)

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), in press

Vanbever R, Loira-Pastoriza C, Dauguet N, Hérin C, Ibouraadaten S, Vanvarenberg K, Ucakar B, Tyteca D, Huaux F. Cationic nanoliposomes are efficiently taken up by alveolar macrophages but have little access to dendritic cells and interstitial macrophages in the normal and CpG-stimulated lungs. Mol Pharm. (2019) 16: 2048-2059.

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.

Loira-Pastoriza C, Todoroff J, Vanbever R. Delivery strategies for sustained drug release in the lungs (invited review). Adv Drug Deliv Rev. (2014) 75: 81-91.

AWARDS

Highly cited researcher:

Véronique Préat since 2015

THESES DEFENDED IN 2020

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

Mahri Sohaib: "Mechanisms of retention of PEGylated recombinant human deoxyribonuclease (rhDNase) in the lungs".
Director: R. Vanbever (ADDB/LDRI)
Co-director: C Bosquillon (Univ. Nottingham)

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

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

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

THESES In progress

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

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

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

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

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

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

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

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

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

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)

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

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

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)

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

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

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

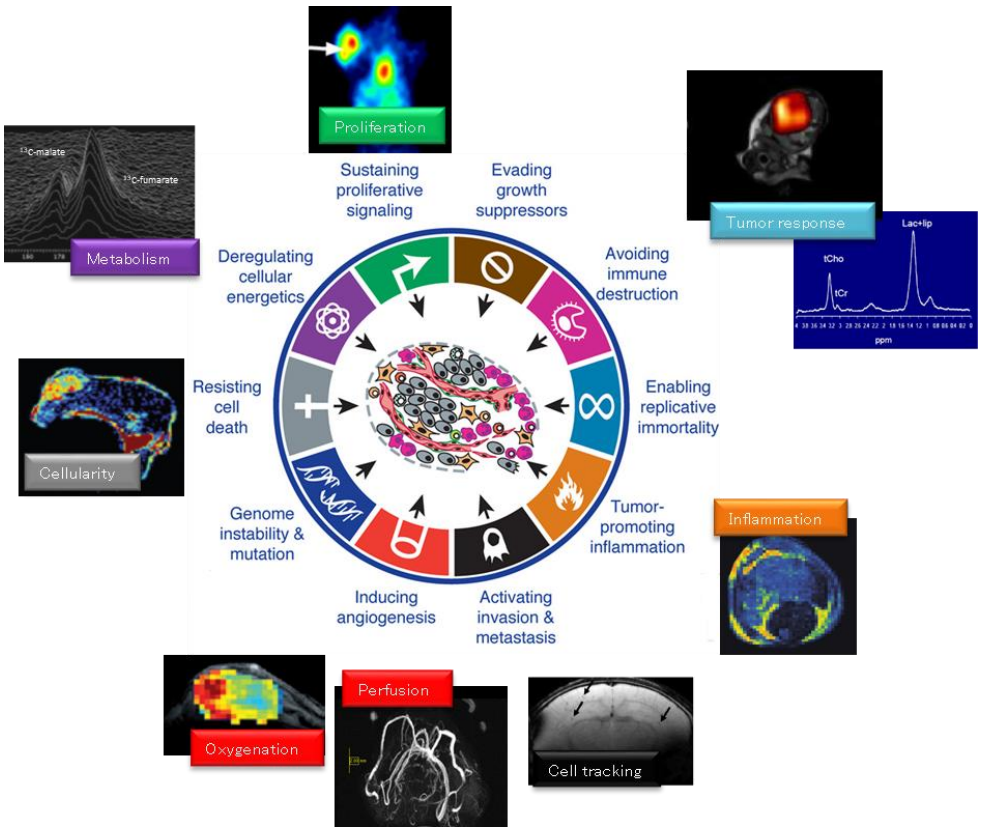
The major theme of the REMA Group is to understand how the tumor microenvironment influences the response to anti-cancer treatments, to identify early non-invasive markers of tumor response to treatment, and to identify metabolic shifts driving resistance to anti-cancer therapy. For that purpose, three 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 oxygen consumption; as well as the tumor proliferation and metabolic features.

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

Imaging hallmarks of cancer



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

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

We 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

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 imaging to assist in therapeutic guidance of treatments targeting proton extruders overexpressed by glycolytic cancer cells.

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

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

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

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

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

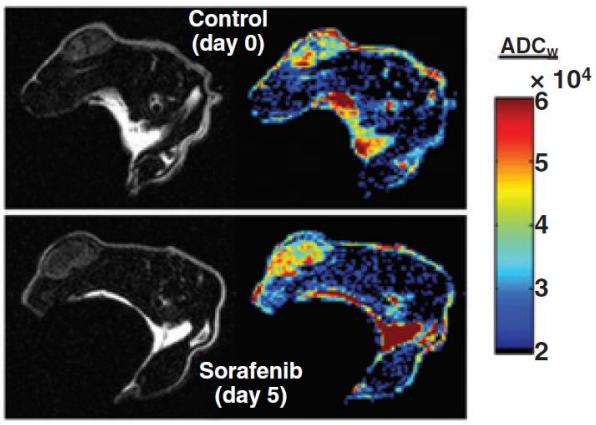


Figure 2. Typical ADCw (Apparent Diffusion Coefficient of water) maps obtained on mice xenografts in response to the multi-kinase inhibitor sorafenib. Note the increase in global ADCw in the tumor region at day 5 post therapy.

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

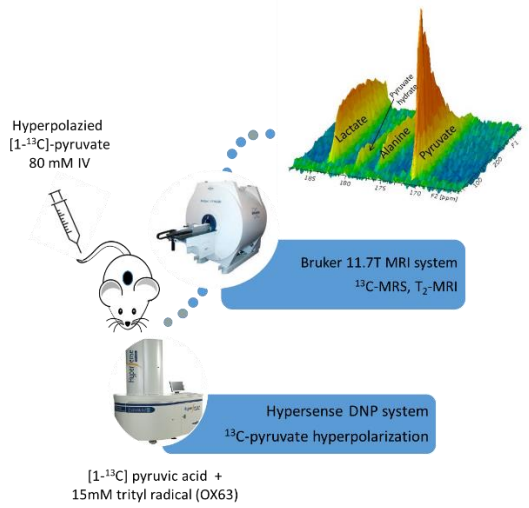


Fig.3 Schematic representation of an imaging session including ^{13}C-MRS of hyperpolarized ^{13}C-pyruvate. The ^{13}C-pyruvate substrate is first hyperpolarized and then directly injected intravenously to the tumor-bearing mouse that is concomitantly imaged in a small-animal MRI scanner for detection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

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

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

SELECTED PUBLICATIONS
Bernard GALLEZ

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.

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

Neveu MA, De Preter G, Marchand V, Bol A, Brender JR, Saito K, Kishimoto S, Porporato PE, Sonveaux P, Grégoire V, Feron O, Jordan BF, Krishna MC, Gallez B. Multimodality Imaging Identifies Distinct Metabolic Profiles In Vitro and In Vivo. Neoplasia (2016), 18, 742-752.

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

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

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Bénédicte JORDAN

Yelec C, Mignion L, Joudiou N, Terrasi R, Gourgue F, Van Hul M, Delzenne N, Gallez B, Corbet C, Muccioli G, Feron O, Cani PD, Jordan BF. Acetate, Friend or foe against breast tumour growth in the context of obesity? J Cell Mol Med. (2020).

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.

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

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

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

THESES DEFENDED IN 2020

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

Gourgue Florian: "Investigation of the apelin adipokine as a potential therapeutic target for breast cancer patients in the context of obesity".
Director: B.F. Jordan
Co-director: P.D. Cani

Schoonjans Céline: "Tumor microenvironment involvement in the metabolic reprogramming induced by the PDK inhibitor dichloroacetate".
Director: B. Gallez
Co-director: O. Feron

THESES IN PROGRESS

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

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

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

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

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

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

TECHNOLOGY PLATFORMS

I) MASSMET PLATFORM

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

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

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

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

ThermoScientific LTQ – ORBITRAP –XL

Waters xevo TQS

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

Contact person: Prof Giulio G Muccioli (Giulio.muccioli@uclouvain.be)

II) NUCLEAR & ELECTRONIC SPIN TECHNOLOGIES (NEST) PLATFORM

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

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

Nuclear Magnetic Resonance

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

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

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

Here are few example of application:

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

Magnetic Resonance Imaging

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

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

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

Dynamic Nuclear Polarization

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

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

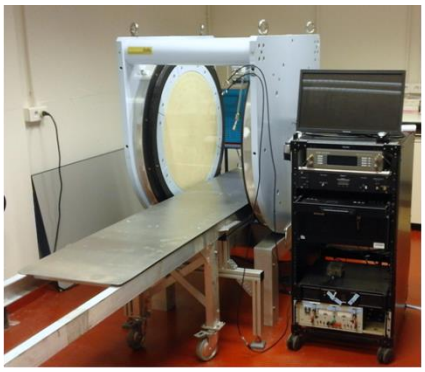
Electron paramagnetic resonance

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

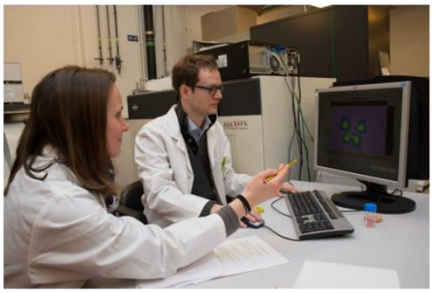
- X-band EPR spectrometer (Bruker EMX+, 9 GHz) for *in vitro* applications
- Benchtop X-band EPR spectrometer (Magnettech Miniscope, 9 GHz for *in vitro* applications
- L-band (1 GHz) and X-band (9 GHz) imaging EPR system (Bruker Elexys) for *in vitro* and *in vivo* applications
- L-band EPR spectrometer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

Benchtop X-band EPR

EMX+ X-band EPR



Clinical L-band EPR



Elexys EPR imaging

In vivo L-band EPR

EPR applications include (non-exhaustive list):

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

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.

Example of those recent development are:

- pH imaging using the chemical exchange saturation transfer (MRI) in addition to the previous methodology of pH measurement,
- Development of a bimodal imaging technique for ^{19}F MRI imaging and fluorescence imaging,
- Development of DNP method on spheroid in addition to the previous method on cells.

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

Illustrative examples involving LDRI or Health Sector research groups include:

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

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SUPPORTING ORGANIZATIONS

APPENDIX

2020 PUBLICATIONS

Research or review papers – first or last author

1. Acciardo, Stefania ; Mignion, Lionel ; Lacomblez, Estelle ; Schoonjans, Céline ; Joudiou, Nicolas ; Gourgue, Florian ; Bouzin, Caroline ; Baurain, Jean-François ; **Gallez, Bernard** ; **Jordan, Bénédicte**. *Metabolic imaging using hyperpolarized 13 C-pyruvate to assess sensitivity to the B-Raf inhibitor vemurafenib in melanoma cells and xenografts*. In: *Journal of Cellular and Molecular Medicine*, Vol. 24, p. 1924-1934 (2020). doi:10.1111/jcmm.14890. IF: 4.486

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3. Bachmann, Radu ; Van Hul, Matthias ; Léonard, Daniel ; **Delzenne, Nathalie M**. ; Kartheuser, Alex ; **Cani, Patrice D**.. *The colonoscopic leakage model: a new model to study the intestinal wound healing at molecular level*. In: *Gut*, Vol. 69, no. 12, p. 2071-2073 (2020). doi:10.1136/gutjnl-2020-321234. IF: 19.819

4. Beauquis J, Petit AE, Michaux V, Sagué V, **Henrard S, Leprince JG**. *Dental emergencies management in COVID-19 pandemic peak: a cohort study*. In: *Journal of Dental Research* 2020 (Accepted). IF: 4.914

5. Bayiha, Jules ; Evrard, Brigitte ; Cataldo, Didier ; De Tullio, Pascal De ; **Mingeot-Leclercq, Marie-Paule**. *The Budesonide-Hydroxypropyl-β-Cyclodextrin Complex Attenuates ROS Generation, IL-8 Release and Cell Death Induced by Oxidant and Inflammatory Stress. Study on A549 and A-THP-1 Cells*. In: *Molecules*, Vol. 25, no.21, p. 4882 (2020). doi:10.3390/molecules25214882. IF: 3.267

6. Buya Aristote; **Beloqui Garcia, Ana** ; Memvanga Patrick ; **Préat, Véronique**. *Self-Nano-Emulsifying Drug-Delivery Systems: From the Development to the Current Applications and Challenges in Oral Drug Delivery*. In: *Pharmaceutics*, Vol. 12 (2020) 1194. doi.org/10.3390/pharmaceutics12121194. IF : 4.421

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10. **Cani, Patrice D**. ; Van Hul, Matthias. *Microbial signatures in metabolic tissues: a novel paradigm for obesity and diabetes?* In: *Nature Metabolism*, Vol. 2, no.3, p. 211-212 (2020). doi:10.1038/s42255-020-0182-0. IF: /

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Patent

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