de Duve
Institute

UCLouvain

LUDWIG
CANCER
RESEARCH

Brussels

SCIENTIFIC REPORT 2022

Advancing knowledge, **transforming health care**

Scanning electron microscopy picture of red blood cells (imaging platform, Tyteca's group)

de Duve Institute

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42 nationalities

317 members

43%   57%

107 PhD students

46 postdoctoral students

105 publications

Most international lectures were postponed due to the Covid-19 pandemic

7 technology platforms

8000 m^2

From the Management

I t is with great pleasure that we introduce our scientific report for 2022. The year was marked by the end of the Covid restrictions, which had a big impact on the work at the Institute, as it made us realize how important our (international) collaborations are. We are proud that, despite these limitations, our teams made exciting discoveries in understanding diseases and improving their care. In this report we present some of these highlights (pages 6 to 14).

Donatienne Tyteca studies cell-membrane composition in healthy and pathological conditions, and highlighted its role in metastases of breast cancer. Wen-Hui Lien's group described the role of a key receptor in the continuous renewal of skin cells. Benoit Van den Eynde and his team made findings that may increase the efficacy of cancer immunotherapy. Noteworthy is also the important discovery by Guido Bommer's lab of a new mechanism explaining certain forms of Parkinson's disease.

Highlighting how fundamental research can lead to better health care, a clinical study based on Miikka Vikkula's research on arteriovenous malformations showed the efficacy of a new treatment, with great improvement in patients with severe forms of these malformations.

In 2022, we have started restructuring our research groups, with the aim of further optimizing collaboration within the Institute. As part of this process, we developed a new graphic identity, with our new logo as a backbone. The logo was designed to represent the main values of the Institute, such as excellence, creativity and collaboration. In the course of 2023, this new graphic identity will be displayed on a new website, which will also reflect the new structure of our groups currently under development.

The year 2022 was also the last of our 5-year collaboration agreement with the Ludwig Institute for Cancer Research, which supports several research groups within the Institute. We are very pleased to announce that this agreement has been renewed for another term starting in 2023. This continuous long-term funding allows the groups to embark on ambitious research projects. The Ludwig Brussels Laboratories, headed by Benoit Van den Eynde and Stefan Constantinescu, will now also comprise the group of Nathalie Vigneron, focusing on the processing of tumor antigens, and the group of Jingjing Zhu, studying immunosuppressive mechanisms in the tumor microenvironment.

The present report intends to give you an overview of the scientific focus and achievements of all our research groups. The Institute is a vibrant and warm community of people with a broad diversity in nationality, age and background. Together, we try to reveal the fundamental processes of life, in line with our motto:

<div align="center">"Advancing knowledge, transforming health care"</div>

Benoit Van den Eynde   Jean-François Collet   Miikka Vikkula   Sophie Lucas

About the Institute

Originally named International Institute of Cellular and Molecular Pathology (ICP), de Duve Institute was founded in 1974 by Professor Christian de Duve († 4th of May 2013) to develop basic biomedical research with potential medical applications.

Excellence and freedom of the researchers to choose their line of research are our core values as defined by de Duve. We attract excellent researchers from Belgium and from abroad, and give them the liberty to develop their original ideas in an inspiring environment. Discovery is the endpoint of their efforts and the only element taken into account for their evaluation.

We value collaborative work and interdisciplinary research. The Institute functions in symbiosis with the Faculty of Medicine of the University of Louvain (UCLouvain) and many of its senior members hold a Faculty position and have teaching appointments. The influx of doctoral students and postdoctoral fellows from the University is key to the success, as well as the close collaborations with clinicians of the University Hospital (Cliniques universitaires) Saint-Luc, located within walking distance. In addition, we have good contacts and many joint research projects with other research institutions in Belgium and all over the world.

In 1978 Ludwig Institute for Cancer Research decided to base its Belgian branch within the walls of de Duve Institute. A fruitful collaboration between the two institutions has been pursued ever since, and is today embodied in the full structural support of two research groups within de Duve Institute.

The ambition of de Duve Institute is to pursue research projects of high quality under conditions that allow original, long-term projects. Research is funded by public bodies, national and international, as well as by private donations. Most funds are awarded on a competitive basis. The Institute has an endowment, which is a source of key financing for priority issues, such as the creation of new laboratories for promising young researchers. The quality of our researchers, supported by sound organisational approaches, will enable de Duve Institute to remain at the forefront of European research. We are extremely grateful to all those who support the Institute.

Research Highlights

The multiple functions of the cell membrane

The membranes of our cells are mainly composed of lipids that are organized in domains. Donatienne Tyteca studies the structures and roles of these lipid domains in healthy and pathological conditions. They play an important role in breast cancer, her group found.

The membranes surrounding our cells are made up by lipids that are organized in bilayers. Different lipids are found in the membranes, cholesterol being one of them. The lipids are often considered as simple building blocks, forming a barrier that isolates the cellular content from the outside. But the lipids are much more than that, says Donatienne Tyteca. "They form well regulated domains with different lipid composition and biophysical properties. They have important functions, for example in cell migration, invasion and squeezing."

Donatienne Tyteca started to work on membrane biology during her postdoc in Groningen. When she came to de Duve Institute in 2003, she first studied endocytosis in the lab of Pierre Courtoy. But she soon returned to membrane biology and biophysics, which also became the focus of her own group in 2008. "There are so many interesting things to investigate. It's an overlooked topic, because there is a lack of relevant and validated tools to study it appropriately."

As there were no good tools available, Donatienne Tyteca decided to develop them herself. In collaboration with other groups, her team designed and optimized methods to visualize the lipids in cell membranes. They apply multiple imaging techniques, such as fluorescence, confocal, super-resolution and multiphoton microscopy. "It took a lot of time to find out how to label the lipids and to validate

the approaches so we can draw solid conclusions", she says. The most recent addition to their imaging arsenal is Atomic Force Microscopy, for which the group collaborates with the nanobiophysics lab of David Alsteens (UCLouvain). The technique does not require labeling and gives a very high spatial resolution, but lower time resolution. Every technique has its pro's and con's, she says: "Depending on what we want to investigate, we combine different microscopy techniques and biochemical assays." Her work thus requires a combination of technical skills, but that is what she likes about it. "Every day is different. There are new challenges, other problems to solve, new fundamental or applied questions to answer. It's not always easy, but it is very exciting and motivating!"

Her group first studied red blood cells, which have a biconcave shape – like a donut that is not fully pierced through. These cells have a very smooth surface with little protrusions and no endocytic pits. Therefore they are a model of choice to study lipid domains. "We found that the lipids are not homogeneously distributed at the red blood cell surface. Instead, they form domains in which specific lipids are enriched. We started to identify the functions of these domains. Some cluster together to support the high curvature areas needed for red blood cell squeezing. Other domains serve for calcium exchange by recruiting or activating specific proteins, but this remains to be clearly established."

> **"** *Upon cholesterol removal from the cell surface, the invasion capacity of the cancer cells strongly decreases.* **"**

When red blood cells lose their typical shape, the domain structure and distribution are disrupted. "This happens for example when red blood cells are stored for transfusion. The cells lose cholesterol-enriched domains during storage. In collaboration with the Red Cross, we are investigating how to minimize domain loss, to lengthen the storage time and improve the quality of the red blood cells before transfusion", says Donatienne Tyteca.

The lipid composition in the cell membrane is also deregulated in cancer. Cancerous cell lines were found to have different lipid content and distribution compared to their healthy counterparts. "In breast cancer, there is a higher abundance of cholesterol on the surface of malignant cells. We were wondering what role this plays in the progress of the disease."

By investigating different breast cancer cell lines, the group found that the cholesterol domains are indeed completely deregulated in breast cancer cells. "Importantly, this is specific for cancer cells and related to the aggressivity of the breast cancer cell lines. The most aggressive cancer cells have the highest abundance of cholesterol-enriched domains." With further research, they could uncover the mechanism that explains this. "The cholesterol-enriched domains on the malignant cell surface can contribute to cell invasion through invadopodia maturation. These are cell protrusions that are capable of degrading the extra-cellular matrix, thereby facilitating migration of tumor cells and metastasis. If we remove the cholesterol from the surface, the invasion capacity of the cancer cells strongly decreases." This knowledge can be used to develop a diagnostic or therapeutic tool.

Donatienne Tyteca's group continues the work on cancer. They plan to study if and how the membrane lipids are disorganized in glioblastoma, an aggressive brain tumor. "We want to further explore the potential role of the membrane lipids in other processes than cancer cell invasion, like resistance to apoptosis and escape from immune surveillance. It might then be possible to appropriately design molecules targeting membrane lipids to improve anticancer therapies."

Maja M, Mohammed D, Dumitru AC, Verstraeten S, Lingurski M, Mingeot-Leclercq MP, Alsteens D, Tyteca D. Surface cholesterol-enriched domains specifically promote invasion of breast cancer cell lines by controlling invadopodia and extracellular matrix degradation. Cell Mol Life Sci. 2022;79:417

Grasping the escaping cancer cells

Immunotherapy has become a standard treatment in various cancers, leading to unprecedented and durable remission in metastatic patients. However, many patients do not respond to the treatment. Benoit Van den Eynde tries to understand how cancer cells evade immune attacks, to make more patients benefit.

Cancer immunotherapy is an elegant way to treat cancer. Instead of directly attacking the tumor cells, as in surgery or chemotherapy, it stimulates the immune system – the natural defense force – to destroy the tumor. Though the concept was conceived long ago, few people believed it would work until the 1990's. "There was a lot of skepticism. Cancer cells are own cells, not foreign. People did not believe that the immune system could recognize them", says Benoit Van den Eynde. As a young biochemistry student with a fresh medical degree, he joined the lab of Thierry Boon in 1986. The lab, studying immunology in cancer, made an essential step towards cancer immunotherapy thanks to an original genetic approach. "We demonstrated that certain white blood cells can kill cancer cells. We identified the markers, called antigens, on the surface of cancer cells that these white blood cells recognize. This led to a new era. People accepted that immune cells can recognize cancer cells and started to believe in cancer immunotherapy."

Research on cancer immunology has boomed since then. Multiple treatments were developed and, in the last decade, approved for use in the clinic. Immunotherapy is today applied in more than 15 cancers. Benoit Van den Eynde is a leading scientist in the field. Next to heading a big research group at de Duve Institute, he leads a team at the University of Oxford. He founded a spin-off company, iTeos Therapeutics, which today has more than 100 employees and is notated on the Nasdaq. He published an impressive number of papers and filed multiple patents. On top of that he is director of de Duve Institute.

His research aims to improve the efficacy of immunotherapy treatments. "Despite the indisputable success, only 20 to 50% of the patients respond to the treatment and it works only in certain cancers. We try to understand what happens in non-responding patients." In fact, he explains, our immune system fights many battles against cancer. "Cancerous cells are formed regularly, but they are destroyed by immune cells. A cancer emerges when cancer cells hide from the immune response or resist to it, or when the immune response vanishes."

His lab has identified several mechanisms that cancer cells utilize to escape immune attacks. One of these findings was based on a paper he read in 1999. "It described that the enzyme indoleamine dioxygenase (IDO) is expressed at high levels in the placenta. The enzyme makes that the foetus, foreign to the mother's body, is not rejected." Benoit Van den Eynde decided to investigate whether the same immune suppression occurred in cancer. "Indeed, we found that IDO is expressed in many cancer cells. It degrades tryptophan, an amino acid that T cells need to kill cancer. When cancer cells express IDO, they cannot be rejected. Importantly, when we inhibited IDO, the rejection

> **" We now have a platform that is much better in stimulating CD8 T cells, which are the cells that kill cancer cells. "**

was restored." These findings were the basis of the foundation of iTeos Therapeutics in 2012.

A more recent discovery by the lab involves a type of immune cells called myeloid-derived suppressor cells (MDSC's). These cells were found to be present in high concentrations in tumors that are resistant to immunotherapy. "They induce the death of anti-tumor T cells. We found that a ligand on the surface of MDSC's, named FAS-ligand, is responsible for transmitting the signal to the T cells. When we block this ligand, the T cells survive and can attack the tumor cells. Blockers of the FAS-ligand could thus increase the efficacy of immunotherapy."

The Oxford group of Van den Eynde works on anti-cancer vaccines. Many researchers have worked on that, but with little success. Benoit Van de Eynde however believes that it can work. "In previous attempts, platforms were used that are not good to induce CD8 T cells, which are the cells that kill cancer cells. We now have a platform that is much better in stimulating these CD8 cells. This same platform was used in the Covid vaccine of AstraZeneca." The vaccines are currently tested in patients with lung cancer, in combination with an approved immunotherapy drug and chemotherapy.

In another important line of research, the group tries to understand how cancer cells produce the markers on their

surface. This is done in the proteasome, a complex in the cell that degrades damaged or unneeded proteins into small peptides. The group discovered that the proteasomes of cancer cells can differ from healthy cells and produce different peptides. They also did the remarkable finding that the proteasome does not only cuts, but also splices peptides, thus forming new fragments. Understanding these mechanisms will help to better target immunotherapy.

It's these unexpected findings that drive Benoit Van den Eynde, who, despite all his management activities, remains a real scientist. "The curiosity, the pleasure when doing a new discovery is what makes this work exciting. And the bonus is that it leads to better treatments of patients."

Untangling the skin's secrets

The cells in our skin are continuously renewed during life. The so-called Wnt signaling orchestrates these processes. It operates on a delicate balance, as Wen-Hui Lien recently proofed. The lack of a receptor of the pathway may lead to hair loss. Overexpression of this receptor is implicated in squamous skin cancer.

Wen-Hui Lien first set foot in a lab as a second-year biology student in Taiwan and was immediately impressed by biomedical research. "I didn't know much about the fundamental research that I was doing, because I didn't understand the scientific terms in English, but I loved to work on the bench. The techniques of molecular and cellular biology fascinated me. The first time you see DNA and living cells in a culture dish, that is amazing", says Wen-Hui Lien. The 1-year internship taught her two things: she wanted to be a researcher and she had to learn English, something that she had avoided up to that time.

Her passion for research would determine her career and life path. Still during her studies, she volunteered in a lab as part-time researcher to gain as many experiences as possible, after which she went to the US to spread her wings. She first worked as a research technician and did her PhD study at University of Washington in Seattle, then moved to New York for a postdoc at Rockefeller University. In 2013, de Duve Institute offered her the opportunity to establish her own research group and she came to Belgium. Meanwhile, she received multiple awards for her work, like the Research Creativity Award in Taiwan and the prestigious Harold M. Weintraub Award for her PhD work. An impressive and adventurous career, yet she only turns 45 this year.

Wen-Hui Lien's topic of research is a specific regulation mechanism in cells, called Wnt signaling. "Wnt signaling is important in tissue development, in stem cells and in cancer. There are several Wnt signaling pathways. Much is known about the canonical pathway. We study the non-canonical pathway, that we know little about", she says.

Her group tries to find out how this mechanism works in skin cells and what happens if it goes awry. The skin, the largest organ of our body, is constantly renewed throughout life. It's the skin stem cells that make this possible. "Stem cells are located in the inner layer of the skin epidermis. They divide and differentiate continuously, and the produced cells move slowly to the outside. They end as white dead cornified cells, forming the outermost skin barrier, which you might occasionally wash off during the shower." The hair follicles in the skin also contain stem cells, Wen-Hui Lien continues: "In contrast to the skin stem cells in epidermis, they are mostly in a quiescent state (not active). Only a part of them becomes active for a short period in the hair growth cycle. A stem cell then divides into one stem cell to maintain the stem cell pool, and one differentiated daughter cell that migrates downward to form the hair follicle."

Recently, the group identified a surprising role for a receptor of the Wnt pathway, called ROR2, in the regulation of hair follicle stem cells. Using genetic models and a cell

> **" The maintenance of the stem cell pool is very important to keep hair growth: without it, hair loss may occur. "**

culture system, they discovered that ROR2 is not only essential for the activation of these stems cells, but also for their self-renewal and maintenance. Without ROR2, the pool of stem cells is lost. "This is likely due to the fact that stem cells are unable to repair DNA damage when they are dividing, so that they are not able to divide into new stem cells (self-renewal) and consequently either fully differentiate into daughter cells only or die. The maintenance of the stem cell pool is very important to keep hair growth: without it, hair loss may occur." The findings suggest that ROR2 may be a therapeutic target to cure hair loss.

In another line of research, the group investigates the role of Wnt signaling in skin cancer. Dysregulation of the pathway has been implicated in the development of skin cancers. Aberrant signaling cascades not only give rise to tumor initiation, progression and invasion, but also maintain cancer stem cells which contribute to tumor recurrence. In collaboration with surgeon Benoît Lengelé of University Hospital Saint-Luc, the group focuses on a non-melanoma skin cancer, called squamous cell carcinoma. They found that in this type of tumor cells the ROR2 receptor is overexpressed. "We also showed in a mouse model that depleting ROR2 inhibits tumor growth and progression. Because normal skin cells express lower levels of this protein, it may be an interesting target for cancer treatment." This promising work is being continued to find out how the receptor functions in tumor cells.

The remarkable career of Wen-Hui Lien has not gone unnoticed in Taiwan. Being selected by the Taiwanese Ministry of Science and Technology as one of the Outstanding Taiwanese living abroad, she has become a role model for young Taiwanese students. She hopes that she can inspire young people who wish to choose scientific research for their future career, and she will definitely not hesitate to share her experiences if the opportunity arises. Wen-Hui Lien: "Sometimes you would encounter things that you prefer to avoid, but if you do, it will eventually come back to you. Facing it and giving it a try may bring you a gift that you have never expected."

Veltri A, Lang CMR, Cangiotti G, Chan CK, Lien W-H. ROR2 regulates self-renewal and maintenance of hair follicle stem cells. Nat Commun. 2022;13:4449.

IN A NUTSHELL

TREATMENT FOR SEVERE ARTERIOVENOUS MALFORMATIONS PROVES EFFECTIVE

Arteriovenous malformations (AVMs) are difficult-to-treat vascular anomalies in which abnormal connections are formed between high-pressure blood vessels (arteries) and low-pressure blood vessels (veins). Lesions are painful and may cause recurrent and sometimes life-threatening bleeding, ulceration and functional disability.

Research by the laboratory of Miikka Vikkula has identified several genetic causes of AVMs. These were shown to activate the signaling inside the cells of blood vessel walls, and to promote the abnormal formation of blood vessels (angiogenesis). This led them to wonder about the possibility of treating patients with thalidomide, a drug with strong inhibitory effects on angiogenesis.

After the team demonstrated that a vascular malformation could be corrected in a mouse model, Prof. Laurence Boon from the Centre for Vascular Anomalies at University Hospital Saint-Luc, started in 2010 a clinical study into the use of thalidomide in AVMs. She recruited 18 patients, aged between 19 and 70, with severe malformations that could not be treated by conventional approaches.

"All the patients experienced a rapid reduction of pain, together with cessation of bleeding and the healing of ulcers when these were present", says Prof. Vikkula. "Three patients with cardiac overload also saw this problem resolved, and one AVM appeared to be completely cured after 19 months of thalidomide and an eight-year follow-up". The researchers showed in five patients that combined treatment with embolization permitted thalidomide dose reduction with clinical improvement. Reducing the dose led to less side-effects during the treatment.

Reference
Boon LM, Dekeuleneer V, Coulie J, Marot L, Bataille A-C, Hammer F, Clapuyt P, Jeanjean A, Dompmartin A, Vikkula M. Case report study of thalidomide therapy in 18 patients with severe arteriovenous malformation. Nat Cardiovasc Res. 2022;1:562-7.

A LINK BETWEEN SUGAR METABOLISM AND PARKINSON'S DISEASE

Some patients with a rare, hereditary form of Parkinson's disease have inactivating mutations in an enzyme called PARK7. This enzyme, which is abundant in human, animal and plant cells, was described in literature to have many different functions. This wide range of functions made Guido Bommer wonder whether the real function of PARK7 might still be missing and whether the observed functions were just secondary effects.

Indeed, Guido Bommer's group discovered that the function of PARK7 was intimately linked with sugar metabolism. They found that an intermediate in sugar metabolism, called 1,3-bisphosphoglycerate, spontaneously forms a novel reactive intermediate that avidly reacts with amino groups. PARK7 acts by destroying this intermediate, thereby preventing the formation of damaged proteins and metabolites. Consequently, inactivation of PARK7 leads to the accumulation of these damaged compounds.

This is an extraordinary discovery. The process of glucose breakdown (glycolysis) is believed to hold no secrets anymore, yet the team found a fundamental new aspect. The reactive compound so far eluded discovery because it is very unstable and short-lived. The team had to deduce its existence, as it could not be detected. "This also explains the large amounts of the PARK7 enzyme. It must be abundant and efficient, so that it can react with the harmful compound before it attacks cellular structures", says Guido Bommer.

Because PARK7 can also be inactivated by oxidative stress, the accumulation of damaged compounds may also have a role in nonhereditary types of Parkinson's disease. The results suggest that reducing cellular levels of the intermediate compound should be explored as future therapeutic or preventive approaches.

Reference
Heremans IP, Caligiore F, Gerin I, Bury M, Lutz M, Graff J, Stroobant V, Vertommen D, Teleman AA, Van Schaftingen E, Bommer GT. Parkinson's disease protein PARK7 prevents metabolite and protein damage caused by a glycolytic metabolite. Proc Natl Acad Sci U S A. 2022;119:e2111338119.

DNA methylation alterations predict the aggressiveness of lung adenocarcinoma

Epigenetic mechanisms determine in part which genes are expressed by a cell. These epigenetic mechanisms often involve chemical modifications of DNA, in particular methylation, and are essential for the proper functioning of cells and the whole organism. Dysregulation of the epigenetic mechanisms can drive cancer development. Several studies have shown that DNA methylation marks are largely lost in most tumors. Charles De Smet discovered several years ago that this loss of methylation causes tumors to engage a gene expression program that is normally only present in testicular germ cells.

The group now showed that, in pulmonary adenocarcinoma (a common type of lung cancer), demethylation is also accompanied by the activation of other expression programs, namely programs specific for cells in the skin, throat and vagina. These three apparently unrelated tissues have one thing in common: they all contain a stratified epithelium, that is, a sheet formed by several cell layers stacked on top of each other. The group demonstrated that the identified genes are indeed part of an 'epithelial stratification' expression program.

In this research, the group performed computational analyses on large-scale genomic and epigenomic data from pulmonary adenocarcinoma and healthy lung cells, in combination with biochemical assays on cell lines. The researchers subsequently found that demethylation and activation of epithelial stratification genes were very closely correlated with poor survival prognosis in patients with lung adenocarcinoma. These results suggest that the methylation status of these genes can serve as a biomarker to estimate the aggressiveness of the tumor.

Reference
Diacofotaki A, Loriot A, De Smet C. Identification of tissue-specific gene clusters induced by DNA demethylation in lung adenocarcinoma: More than germline genes. Cancers. 2022;14:1007.

3D imaging of a developing pancreas

The formation of an organ like the pancreas is a complex process, finely tuned in time and place during embryogenesis. It involves the activation of specific genes in the pancreatic progenitor cells as well as the interaction of these pancreatic cells with other cells in the microenvironment. Full understanding of this process should guide bioengineering strategies to build a three-dimensional (3D) organ or research model.

The current knowledge of the spatial organization of cells in the embryonic pancreas is still largely based on two-dimensional analyses. The lab of Christophe Pierreux used a light-sheet fluorescence microscopy approach to image the pancreas in three dimensions and map cell positions and interactions at key developmental time points.

To this aim, they established protocols and optimized the technique to visualize the three main cell populations in the developing pancreas: epithelial, mesenchymal and endothelial cells. They also defined computational solutions for image analysis and quantification that enable to extract a multitude of relevant information for pancreas organogenesis.

They demonstrated the utility of their 3D approach by providing the topological organization and the relative abundance of epithelial, mesenchymal and endothelial cells within the developing pancreas, and by imaging the intimate interactions between nascent islets of Langerhans (the regions that will produce hormones) and blood vessels. They have made their data available to the scientific community in an open-source online repository ('the Pancreas Embryonic Cell Atlas') to enable further investigation of pancreas organogenesis and the development of bioengineering solutions for pancreatic diseases.

Reference
Glorieux L, Sapala A, Willnow D, Moulis M, Salowka A, Darrigrand JF, Edri S, Schonblum A, Sakhneny L, Schaumann L, Gómez HF, Lang C, Conrad L, Guillemot F, Levenberg S, Landsman L, Iber D, Pierreux CE, Spagnoli FM. Development of a 3D atlas of the embryonic pancreas for topological and quantitative analysis of heterologous cell interactions. Development. 2022;149:dev199655.

In a nutshell

Promising results with immunotherapy in a type of blood cancer

Primary myelofibrosis is a type of blood cancer, characterized by the build-up of scar tissue in the bone marrow (fibrosis) and failure to make enough normal blood cells, which leads to profound anemia, bleedings, immune deficiency, and sometimes acute leukemia. Patients with primary myelofibrosis show abnormal expansion of megakaryocytes (large bone marrow cells that produce platelets) and aberrant cytokine production. One of these cytokines, which are thought to contribute to the pathology, is TGF-β1.

Until recently, it was unknown which cells produce the deleterious TGF-β1 in primary myelofibrosis. The lab of Sophie Lucas hypothesized that it is produced by cells expressing a protein called GARP. In previous research, her lab had derived antibodies that block production of TGF-β1 by GARP-expressing cells. They decided to examine whether an anti-GARP antibody would exert therapeutic activity in a murine model of primary myelofibrosis.

They observed that the antibody reduced both the fibrosis and the expansion of cancerous cells. They could also show that the therapeutic effect implies targeting GARP on Tregs, which are white blood cells with immunosuppressive capacities. The therapeutic effects also required CD8 T cells, another type of white blood cells. The researchers concluded that the antibody blocks production of TGF-β1 by GARP-expressing Tregs, thereby increasing a CD8 T cell-mediated immune reaction that limits the expansion of cancerous cells. Interestingly, the anti-GARP antibody exerted immune-mediated effects on its own, without the need to combine it with another therapeutic agent. These results suggest a novel approach of cancer immunotherapy that could be tested to treat patients with primary myelofibrosis.

Reference
Lecomte SS, Devreux J, de Streel G, van Baren N, Schröder D, Vaherto N, Vanhaver C, Vanderaa C, Dupuis N, Pecquet C, Coulie PG, Constantinescu SN, Lucas S. Therapeutic activity of GARP:TGF-β1 blockade in murine primary myelofibrosis. Blood. 2023;141:490–502.

Different pathogens use the same trick to escape the immune response

For microbial infections to be successful, they must escape the immune responses of the host. The team of Thomas Michiels discovered that several viruses and bacteria use the same mechanism for this. Remarkably, these microbes are unrelated, which means they developed the mechanism independently.

The original observation was made by Frédéric Sorgeloos, postdoc in Thomas Michiels' team. He found in 2010 that a virus protein called L protein interacts with the host's RSK-kinases, which are important enzymes for the control of cell survival and growth. It was known that other pathogens also target these kinases, however that doesn't mean they do it in the same way.

When they studied the underlying mechanisms, they proved that the same mechanism is shared by four pathogens: Theiler's virus, a human herpes virus that may cause cancer, the varicella virus and the bacterium responsible for the plague, Yersinia pestis. These four pathogens produce proteins that contain a similar peptide sequence. This peptide sequence binds to the RSK enzyme, which keeps it continuously activated. Proteins produced by the microbes recruit and force the contact between the RSK enzyme and a cellular substrate, rendering the substrate phosphorylated. The substrate then acts to inhibit the immune response. It is remarkable that, even though the microbes activate the RSK enzyme identically, they disarm the immune system in a different way according to their own need. The results suggest that a drug blocking this shared mechanism could be used to fight multiple types of infections.

Reference
Sorgeloos F, Peeters M, Hayashi Y, Borghese F, Capelli N, Drappier M, Cesaro T, Colau D, Stroobant V, Vertommen D, de Bodt G, Messe S, Forné I, Mueller-Planitz F, Collet JF, Michiels T. A case of convergent evolution: Several viral and bacterial pathogens hijack RSK kinases through a common linear motif. Proc Natl Acad Sci U S A. 2022;119:e2114647119.

Research Groups

Cancer

A cancer starts when cells acquire the ability to grow fast and the body's defence system cannot control them. We study the signaling mechanisms that regulate cell growth and how genetic mutations lead to aberrations in these mechanisms, inducing for example blood cancers or child tumors. Our institute proudly was at the basis of today's cancer immunotherapy treatments. We now investigate how the efficacy of these treatments can be enhanced.

Stefan Constantinescu

We study how blood is formed under the influence of small proteins denoted as cytokines that act via specific receptors coupled with Janus Kinases (JAKs). We decipher how blood cancers appear and can be targeted.

Cell Signaling & Molecular Hematology

Formation of blood requires small proteins, denoted as cytokines, such as erythropoietin, thrombopoietin or interleukins. These proteins induce survival, growth and differentiation of blood precursors. They act by binding on the surface of target cells to 'receptors', which function like 'antennae' that transmit a signal to the cell interior. We study how these specific receptors assemble on the cell membrane and couple at the cell interior to JAKs, which are absolutely required to transmit a signal. We found that mutations in JAKs or in receptors themselves, such as the receptor for thrombopoietin (TpoR/MPL) confuse the cells and make them grow indefinitely, leading to blood cancers, specifically myeloproliferative neoplasms (MPNs). Our hypothesis is that a curative treatment n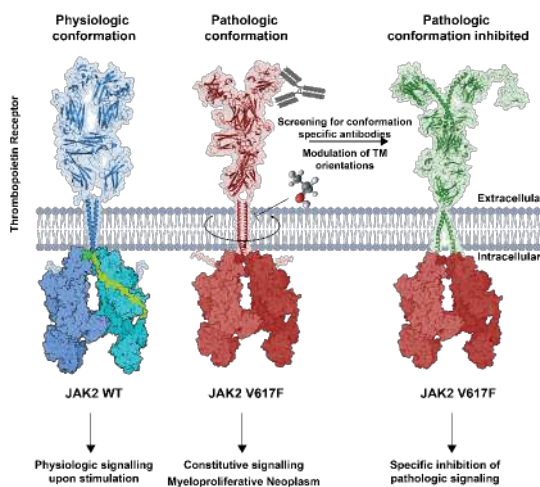eeds mutant-specific inhibitors. To this end, we delineated the circuit of JAK2 kinase activation by the V617F acquired mutation in the pseudokinase domain that could be a target for specific inhibition. We also found that the conformation of receptors bound to JAK2 V617F differs from that bound to wild type JAK2 and this allows us to try novel avenues for specific inhibition. In 2006 we discovered active mutants of the thrombopoietin receptor where one juxtamembrane residue W515 is mutated. Our structural studies on the juxtamembrane regions of cytokine receptors led to a new model of activation by mutations around the membrane domain and pointed to several extracellular exposed residues to be targeted by inhibitors or protein therapeutics.

The identification that the thrombopoietin receptor adopts different conformations (or 'shapes') when it is attached to wild-type JAK2 (left) or mutant JAK2 V617F (middle) may allow for the development of novel specific therapies (right).

We have also discovered that mutations can endow a chaperone protein, calreticulin, the oncogenic ability to persistently activate the TpoR. We showed that mutant CALRs are exposed at the cell-surface and can be targeted by antibodies. More recently we found that such mutant CALRs are secreted and can act *in trans* to stimulate cells that co-express TpoR and endogenous mutant CALR. Furthermore, current focuses are delineation of the pathway by which MPNs evolve to leukemia and the study of myeloproliferation and myeloid leukemia in children, especially those being resistant to treatment. This work is supported by 'Les avions de Sébastien'.

In order to pursue our aims, we use molecular biology approaches biophysical approaches, like deuterium exchange mass spectrometry as well as in vivo transgenesis, microscopy and fractionation, mouse bone marrow transplantation, as well as investigation of primary patient cells. microscopy and fractionation, mouse bone marrow transplantation, as well as investigation of primary patient cells.
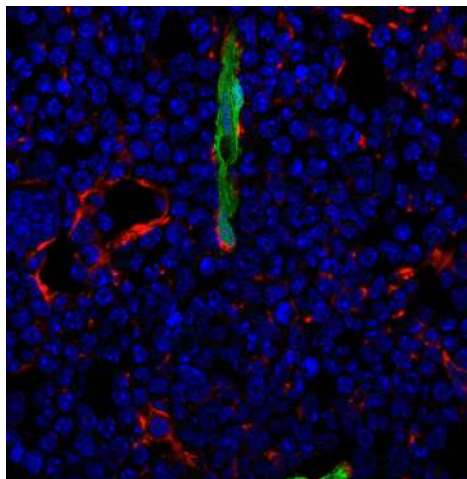
Nick van Gastel

Cellular Metabolism & Microenvironment

We aim to understand how metabolic interactions between cells in the bone marrow control blood cell production, how these pathways become dysregulated in leukemia, and how we can leverage this information for therapeutic purposes.

Converting nutrients from the environment into energy and biomass is a fundamental challenge of life. Unicellular organisms, like bacteria or yeasts, face this challenge in each cell individually. However, they can release certain metabolites as communication signals to indicate changes in nutrient availability, the presence of environmental stress or to coordinate growth. An important advantage of multi-cellularity is the ability of cells to metabolically diversify and work together, with metabolites produced in one cell type used as nutrients by another. In complex organisms such as mammals, metabolites are still used for cellular communication, but the extent and importance of these interactions is not well understood.

One example of a tissue in which many different cell types work together is the bone marrow, where daily 200 billion red blood cells, 10 billion white blood cells, and 400 billion platelets are made. The correct functioning of this process relies not only on blood stem cells, but also on their interactions with other cell types in the bone marrow microenvironment such as bone cells, blood vessel cells, immune cells and connective tissue cells, collectively referred to as the bone marrow niche. The bone marrow niche is also involved in the development and progression of blood malignancies such as leukemia, in which blood stem cells start to grow uncontrollably and no longer form normal blood cells.

Microscopy image showing blood vessel cells (green) and connective tissue cells (red) in the bone marrow of mice, both of which play an important role in blood cell formation and leukemia. The blue color shows all cells by staining the DNA in their nuclei; most of these are blood cells at different stages of maturation.

Our laboratory explores how metabolic communication between different cells in the bone marrow regulates blood stem cell function, blood cell production and leukemia growth. We use state-of-the-art metabolic analysis techniques in combination with cell and animal models to investigate how metabolites produced by one cell type influence the behavior of the other cells in its environment.

The goal of our research is to identify niche-derived metabolic signals important for the maintenance of blood stem cells and the production of red blood cells, white blood cells and platelets. This information can then be used to improve bone marrow transplantation or help in the treatment of blood diseases. By comparing normal and leukemic blood cell production, we further aim to identify changes in metabolic communication systems that can be targeted to reduce the growth of leukemia cells or improve the efficacy of current treatment methods such as chemotherapy.

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Jean-Baptiste Demoulin

Our team analyzes the signaling pathways that promote cancer cell proliferation. Recently, we made significant progress in understanding infantile myofibromatosis. We showed that these life-threatening tumors are caused by *PDGFRB* gene mutations and identified a treatment.

Cancer Signaling

We have a long-standing interest in platelet-derived growth factors (PDGF), which act via two receptor-tyrosine kinases, namely PDGFRA and PDGFRB. These proteins play important roles in the development of the embryo, as well as in cancer and other human diseases. We analyze signaling cascades activated by these receptors, with a particular interest for transcription factors, such as STAT, SRF, FOXO, HBP1 and SREBP.
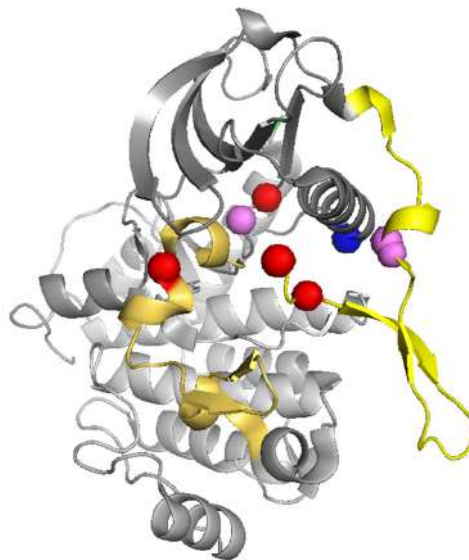
PDGF receptors can be aberrantly activated by gene mutation or gene fusion in cancer. Gene fusions involving PDGF receptors cause a rare type of leukemia characterized by proliferation of eosinophils (a blood cell type). Our team has studied the mechanism whereby these fusion products stimulate cell growth and differentiation into eosinophils by introducing mutated receptors in hematopoietic progenitors. In collaboration with the hematology unit of the University Hospital Saint-Luc, we also discovered new fusion genes.

Disease-causing mutations (indicated by colored spheres) in the PDGF receptor kinase domain.

Recently, using deep sequencing, we identified mutations in *PDGFRB* as a cause of childhood soft tissue tumors (infantile myofibromatosis). The disease is characterized by the presence of multiple tumor masses, which can be life-threatening, particularly in young children. The mutations aberrantly activate the kinase domain of the receptor (Figure). Similar mutations were also found in patients suffering from rare congenital disorders, such as Kosaki overgrowth syndrome, Penttinen premature aging syndrome, or hereditary progressive mucinous histiocytosis.

In a preclinical study, we showed that these mutants are sensitive to a drug named imatinib, which potently blocks PDGF receptors. Based on our results, this drug was tested successfully in children harboring *PDGFRB* mutations.

Finally, dominant *PDGFRB* mutations were also associated with primary familial brain calcification (formerly 'Fahr disease'). In this case, patients do not develop tumors, but suffer from severe neurological symptoms. We showed that these mutations cause a partial loss of receptor function (in sharp contrast to the mutations described above).

In conclusion, we have shown that alterations of PDGF receptors cause different human diseases. Our promising results suggest that some patients may benefit from PDGF receptor inhibitors. We now aim to understand these diseases in more detail and validate treatments.

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Pierre Coulie

Human Tumor Immunology

Cancer immunotherapy is a breakthrough but only for a minority of cancer patients. We explore early stage tumors for signs of anti-tumor T cell immune responses, such as antigenicity and inflammation.

Our immune system protects us by detecting and destroying foreign bodies like bacteria and viruses. Key effectors in this process are T cells, a type of white blood cells. T cells can also recognize tumor cells, as demonstrated by T. Boon and colleagues at de Duve Institute when they discovered the specific markers recognized on tumor cells. It paved the way for clinical applications and remarkable clinical results have been obtained since 2010 with immunostimulatory antibodies that enhance the activity of anti-tumor T cells. However, many patients do not respond to currently available immunotherapies. We try to understand the mechanisms of these limitations to eventually improve cancer immunotherapy.

> *" Analyzing human tumors at a very early stage, which is possible for example with in situ breast carcinomas, is key to understand why anti-tumor immunity is present or absent in a given patient. "*

One project focuses on T cells present within human bladder carcinomas, at an early phase of their development. Patients with these cancers benefit from intravesical instillations of live *Bacillus Calmette Guérin* (BCG), which is the vaccine used against tuberculosis. Thus it is a form of immunotherapy, even though its mechanisms of action remain poorly defined. We explored the hypothesis that CD8^{+} T cells stimulated by the BCG treatment recognize tumor-specific antigens. We examined also the CD4^{+} T cell response against BCG and observed that, surprisingly, most of the anti-BCG CD4^{+} T cells present in the bladder after the BCG instillations displayed cytolytic activity. These immune cells might participate in an antitumor reponse with a clinical benefit to the patient.

Another project deals with inflammation, which is normally a local response to microbes or various types of cellular stress. Inflammation depends on soluble factors, notably cytokines including IL-1β. In nascent cancer, inflammatory signals are faint and probably often absent. Increasing them locally could induce or increase anti-tumor T cell responses. In this context, we study the secretion of IL-1β by monocytes, another type of white blood cells and an important source of IL-1β. Monocytes can secrete IL-1β while they die, thus releasing their intracellular content. However we have observed that under certain conditions monocytes secrete IL-1β but do not die. We try to understand the mechanism of this secretion. Using CRISPR-Cas9 libraries we identified genes involved in this secretion. Specifically blocking or increasing IL-1β secretion could have important medical applications in chronic inflammatory diseases or cancer.

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Sophie Lucas

The immune system defends us against cancer and infections, but requires tight control to avoid allergy or auto-immunity. We study how Tregs and TGF-β control immune responses, hoping to design immunotherapeutic approaches for cancer or auto-immunity.
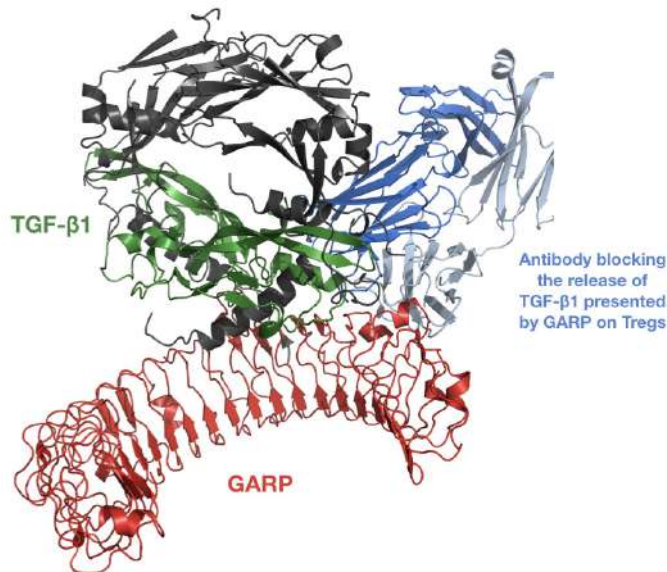
Immune cells can protect us against infections and cancer by killing microbial pathogens, infected cells, and tumor cells. Because they are very potent, immune cells need to be kept under tight control to avoid unwanted destruction of healthy tissues. Specialized cells called regulatory T cells or Tregs control immune cells, which they suppress to prevent auto-destructive reactions. Patients with insufficient Tregs suffer from autoimmune diseases. In contrast, excessive Treg activitycontributes to cancer progression and chronic infections.

We seek to identify the mechanisms by which Tregs suppress immune responses. We found that Tregs produce a protein called TGF-β1, which delivers inhibitory messages to immune cells. We also found that production of the immunosuppressive TGF-β1 requires another Treg protein called GARP. We developed tools (e.g. monoclonal antibodies) that bind GARP and block TGF-β1 production by Tregs. We solved the 3D structure of a protein assembly comprising GARP, TGF-β1 and a blocking antibody (Figure). This allowed us to understand how GARP presents TGF-β1 for activation on Tregs, and how our antibody can block this process. We showed that anti-GARP antibodies inhibit Treg immunosuppression and favor the elimination of tumor cells in several mouse models of cancer. In the case of 'liquid' cancers (i.e. when tumor cells are in the

3D structure of TGF-β1 presented by GARP on Tregs, bound and blocked by an anti-GARP antibody currently tested in the clinics for the immunotherapy of cancer.

blood or the bone marrow), anti-GARP antibodies alone were sufficient to exert therapeutic activity. In the case of 'solid' tumors, anti-GARP antibodies worked better when they were combined with another immunotherapy known as PD1/PD-L1 blockade. The latter is the best currently available immunotherapeutic approach for cancer, but it is still insufficiently efficient when used on its own in most patients. Our antibodies were licensed to a pharmaceutical company, who initiated an early phase clinical trial to test anti-GARP antibodies in combination with anti-PD1 for the treatment of patients with advanced, metastatic solid tumors. We are participating to the trial in collaboration with the University Hospital Saint-Luc. We analyze blood and tumor biopsies to study in depth the anti-tumor immune responses that may occur after administration of anti-GARP antibodies, hoping to define better which patients could benefit from this novel form of cancer immunotherapy.

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Benoit Van den Eynde

Improving Cancer Immunotherapy

Cancer immunotherapy is showing clinical benefit in a subset of cancer patients. Our work studies the basic mechanisms of immune recognition and rejection of cancers, aiming to increase the fraction of cancer patients who respond to immunotherapy.

Cancer immunotherapy works by helping the immune system to fight cancer. Its cornerstone is the notion, pioneered at de Duve Institute, that tumor cells express markers, called 'tumor antigens', which are absent on normal cells and allow the immune system to identify and destroy cancer cells. These tumor antigens are recognized by cytolytic T lymphocytes, which have the capacity to kill tumor cells. However, many tumors manage to resist immune rejection. This can be linked to two mechanisms: they can either lose expression of the tumor antigen, or they can produce immunosuppressive factors that paralyze the immune system. Our group studies these mechanisms, hoping to devise therapeutic strategies able to counteract resistance to immunotherapy.

The T cell-mediated delivery of immunotherapy drug anti-PD-L1 in the nanobody format (right) allows better tumor penetration and better coverage of the PDL1 target (red) than the injected classical antibody format (left). [from Petit et al., Cancer Immunol Res, 2022]

Tumor antigens are made of small protein fragments, named peptides, which are presented at the cell surface by class I molecules of the Major Histocompatibility Complex (MHC, also named HLA in human). These peptides generally come from the degradation of intracellular proteins by the proteasome, a proteolytic particle localized in the cytoplasm and the nucleus. We have characterized different types of proteasomes, which differ in their ability to produce peptides corresponding to tumor antigens. This means that the antigens presented at the surface of cancer cells partly depend on the proteasome composition of these cells, a notion that can explain the variability in tumor antigen expression. We further study how some cancers, while losing expression of classical tumor antigens, unmask other antigens that we are characterizing. We also discovered a new function of the proteasome, which enables the splicing of peptides, i.e. the production of peptides from noncontiguous fragments in the parental protein, following a 'cut and paste' process.

In addition, we are also researching the immunosuppressive mechanisms acting in the tumor microenvironment. We observed that tumors can selectively induce the death of T lymphocytes by apoptosis, and can produce immunosuppressive factors such as tumor-growth factor beta (TGFβ). Tumors also paralyze T lymphocytes by starving them of key amino acids, such as tryptophan. They do so by expressing enzymes, such as indoleamine dioxygenase (IDO) and tryptophan dioxygenase (TDO), which rapidly degrade tryptophan. We try to develop therapeutic strategies that can block these immunosuppressive mechanisms and thereby improve the clinical efficacy of cancer immunotherapy.
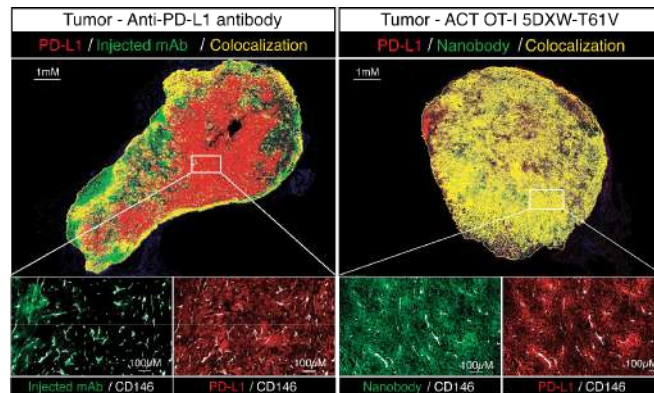
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Pierre van der Bruggen

We study the interactions between cancer cells and the human immune system. We ask why tumor-infiltrating lymphocytes (TILs) are often unable to eliminate tumors, and aim to find strategies to overcome this challenge.

T Lymphocyte Dysfunction

Most tumors are not ignored by the immune system of cancer patients. They contain immune cells, particularly T cells directed against tumor antigens.

In the '90s we identified the gene MAGE-1, which encodes the first known antigen that is expressed by many tumors but not by normal tissues, and that is recognized by cytolytic T lymphocytes. We subsequently identified genes with the same expression profiles (e.g. gene families MAGE, BAGE, and GAGE). Antigenic peptides encoded by these genes can be recognized by both CD4 and CD8 T cells. We designed approaches to identify the antigenic peptides and measure patients' T cell responses to vaccines.

" There is a need to understand how immuno-suppressive neutrophils, which are present in most cancer patients, participate in immune suppression. "

Today, we remain dedicated to studying T cells and the different immunosuppressive mechanisms that operate in human tumors. Tumor-infiltrating T cells (TILs) are often dysfunctional, and we focus on three factors that might limit TIL function: extracellular galectins, TIL exhaustion and immunosuppressive neutrophils. We discovered that extracellular galectin-3 secreted by tumor cells and macrophages binds glycoproteins at the T cell surface, which blocks human TIL functions.

TILs become less functional in tumors, a phenomenon often named exhaustion. We are characterizing in-depth CD8 T cells infiltrating human tumors functionally, phenotypically and molecularly. We abrogate or increase the expression of specific transcription factors to study how they contribute to or prevent TIL exhaustion. The results may help to improve adoptive transfer therapies with engineered CAR-T cells.

We also ask how immunosuppressive neutrophils impede T cell functions. While rare in healthy individuals, immuno-suppressive neutrophils are found in greater numbers in patients with some chronic diseases or cancer. We assess the suppressive functions of immunosuppressive neutrophils from blood and tumors in T cell co-cultures and by transcriptomic approaches.

Most of our clinical samples are obtained from ovarian or lung cancer patients. Ovarian cancer is often diagnosed at an advanced stage and patients receive chemotherapy before surgery. However, today it is still impossible to predict if and how a patient will respond to chemotherapy. To explain these heterogeneous responses, we collect samples before and after treatment, and we study the immune cells, the tumor microenvironment and the genetics of the tumors. For lung cancer, chemotherapy and immune checkpoint inhibitors are the standard of care. To achieve the best outcomes and to minimize side effects, it is important to predict how patients will respond to treatment. We examine if high level of immunosuppressive neutrophils in the blood before treatment correlates to poor clinical response rates. We dream of identifying factors that could predict therapeutic outcomes or that could be targeted to improve the clinical management of future patients.

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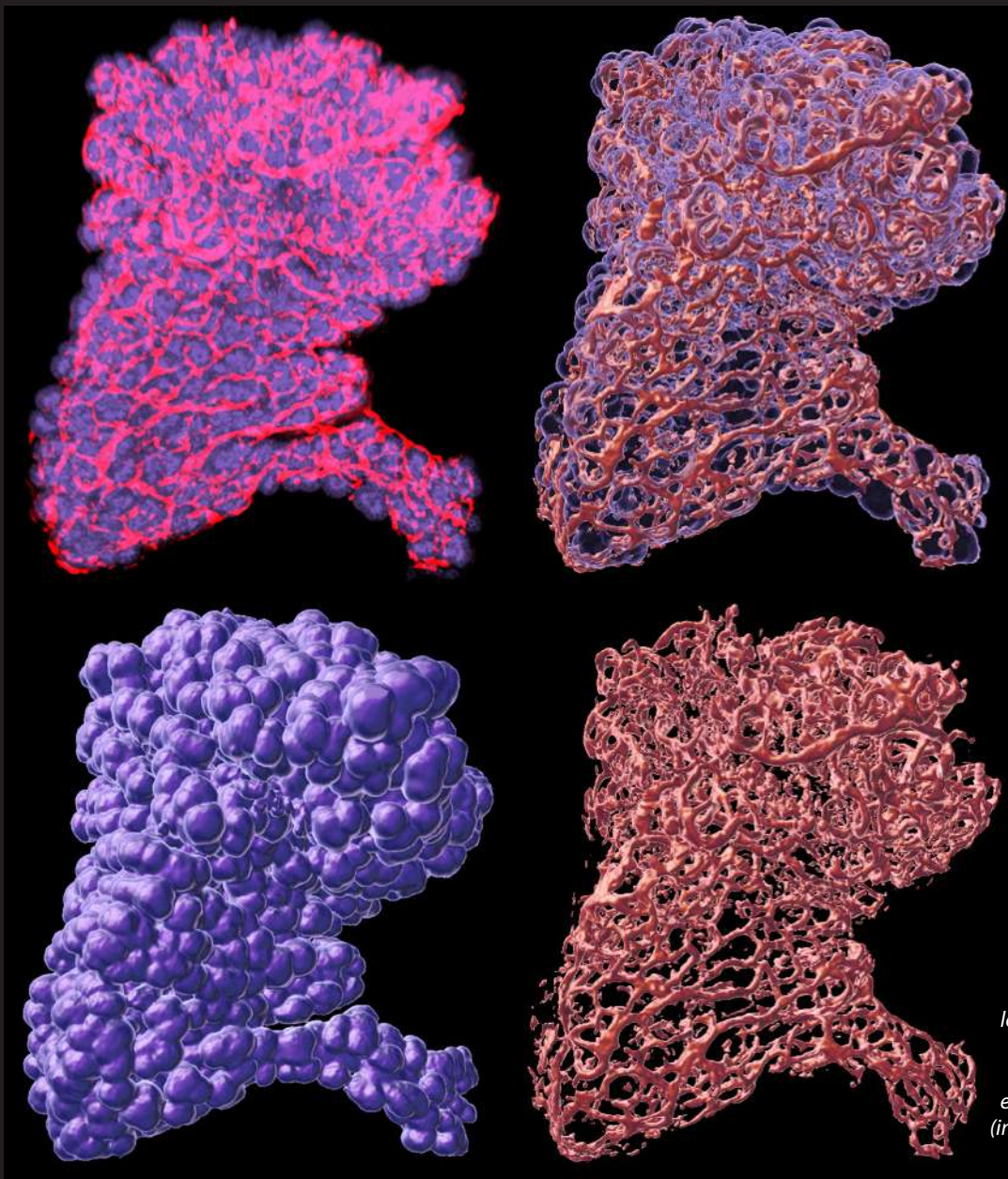
3D reconstruction of embryonic pancreas (stained with antibody against E-cad and Ve-cad, upper left) illustrating the complexity and the 'honey-comb' structure of the vascular network (red) around the epithelial budding structures (blue) (imaging platform, Pierreux's group)

Genetics and Development

All cells of a human body originate from one cell that, directed by the genetic information, divides, grows and differentiates into a fully functional organism. How do our cells develop in the embryo? Which genetic mutations lead to diseases? How do cells maintain themselves during a lifetime and how do they age? How is the expression of genes regulated? Our groups in genetics and development try to elucidate these secrets of life.

Donatienne Tyteca

Cell deformation is critical for numerous pathophysiological processes. Our group explores how plasma membrane biophysical properties contribute with the cytoskeleton and membrane bending proteins to cell deformation and how this interplay is deregulated in diseases.

Membrane Biology

In their environment, cells face a variety of stimuli and stresses inducing cell deformation. Typical examples are shear stress by squeezing of red blood cells (RBCs) in the narrow pores of spleen sinusoïds, stretching of muscle cells during contraction or pressure exerted by tumors on surrounding cells. We aim at elucidating how plasma membrane lipid composition and biophysical properties contribute to cell deformation, as a prerequisite towards understanding diseases.

Using high-resolution confocal imaging and atomic force microscopy (coll. D. Alsteens, UCLouvain), we discovered the existence of stable submicrometric lipid domains at the living human RBC plasma membrane. Three types of domains coexist, showing differential lipid enrichment (cholesterol vs GM1 ganglioside/cholesterol vs sphingomyelin/cholesterol), membrane curvature association, lipid order and role in the physiological RBC deformation process.

In contrast, during RBC storage in blood tubes and in RBC concentrates intended for blood transfusion (coll. Croix-Rouge de Belgique), cholesterol-enriched domains are specifically lost from the RBC membrane by vesiculation, opening the possibility of targeting them to limit vesicle release in RBC concentrates before transfusion.

Membrane lipid domains and biophysical properties are deregulated in RBC-related diseases. Those include RBC membrane fragility diseases due to cytoskeleton defects (i.e. hereditary spherocytosis and elliptocytosis; coll. B. Brichard & C. Lambert, University Hospital Saint-Luc),

" Elucidating the role of membrane lipids in disease paves the way towards use as diagnostic biomarker and manipulation for therapeutical benefit. "

lipid/lipoprotein metabolic disorders (i.e. sitosterolemia and hypobetalipoproteinemia; coll. R. Van Wijk, Utrecht University) and erythroleukemia, a rare type of acute myeloid leukemia with poor prognosis.

As RBCs, mouse myoblasts also exhibit lipid domains that are reorganized upon migration and contribute to this process by controlling the distribution of focal adhesions and myoblast polarization.

The relevance and deregulation of lipid domains has finally been proved in breast cancer. Thus, using the MCF-10A mammary cell line series which offer the same background but increasing invasivity and several other cell lines with different mutations and breast cancer subtypes, we have shown that the plasma membrane stiffness and the abundance of cholesterol-enriched domains are increased in malignant cells as compared to pre- and non-malignant cells. Those domains specifically promote malignant cell invasion by controlling invadopodia and extracellular matrix degradation. Our data open the possibility to target cholesterol-enriched domains by a pharmaceutical approach to limit breast cancer cell invasion and to use them as a biomarker for classification of early breast cancer subtypes.

Staff members
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Nisha Limaye

Genetics of Autoimmunity & Cancer

We study genetic factors underlying diseases potentiated by inappropriate immune responses: inadequate (cancers) or excessive (autoimmunity). As extremes of the same spectrum, the insights we gain into disease mechanisms of one have profound implications for the other.

Our immune system, responsible for defending us against harmful pathogens such as certain bacteria and viruses, can sometimes turn on us. It may mistake components of our own cells for foreign invaders, or react too zealously against perceived threats, causing significant collateral damage to our own tissues and organs. The resulting autoimmunity and systemic autoinflammation can be devastating. On the flip-side, inadequate surveillance or responsiveness of the immune system to abnormal 'self' cells can allow for the unchecked growth of cancers.

Our laboratory explores the contribution of genetics to immune dysfunction, in rheumatic (auto-immune, autoinflammatory) conditions such as systemic sclerosis and systemic lupus erythematosus, and in Hodgkin lymphoma, a hematological malignancy. In very rare cases, these diseases run in families. By sequencing the genomes of multiple members of such families, we identify genetic variants that are shared by the affected individuals, but not their healthy relatives. These genes may therefore contribute to disease.

This is no trivial task: we all carry tens-of-thousands of genetic variants, i.e., have slightly different 'versions' of each gene, relative to one another. The more closely related we are, the more of these variants we share with

each other. A tremendous amount of accumulated information, knowledge-based predictions, and data-processing is therefore required to distinguish the one-to-a-few genetic variants that actually impact disease, from the thousands of others incidentally shared by family members. Once we identify a genetic variant that we hypothesize may cause disease, we test for functional evidence of its impact: we induce cells to express the faulty version of the gene, and study how this changes their appearance, behavior and function.

Another important focus of our research is the organs that are damaged by systemic immune dysfunction. More specifically, we study the interaction between immune and non-immune cells in the kidney in lupus nephritis, a frequent and severe complication of systemic lupus erythematosus. Our goal is to understand why some patients go on to have chronic or even end-stage renal disease despite therapy, while others respond well. Ultimately, by understanding the genetic and molecular bases of these rare diseases, we seek to better predict, prevent, and treat them.
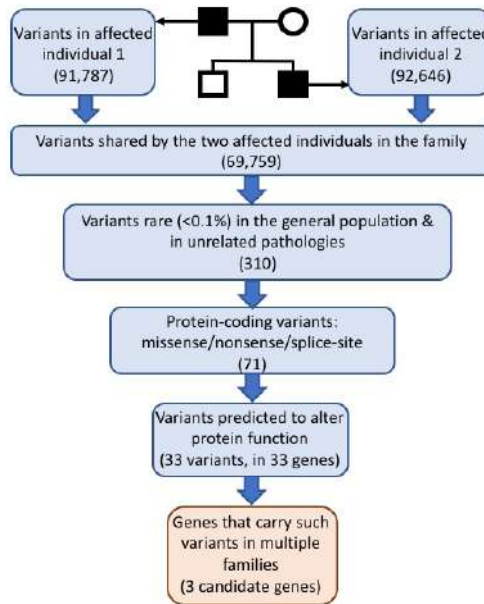
Strategy to identify candidate disease-causative genes, i.e. genes with rare, potentially pathogenic variants that co-segregate with disease in multiple families. Brackets: number of variants retained after each filtering step.

Staff members
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Miikka Vikkula

We study the mechanisms underlying cardiovascular disorders, cleft lip and/or palate, and some cancers. We evaluate the contribution of genetic variation to human disease by using bioinformatics and artificial intelligence. We also generate models to test novel molecular therapies.

Human Genetics

The bases of many disorders remain unknown, and treatments are often aimed at alleviating symptoms. We try to identify the causes of vascular tumors and malformations, lymphedema, and cleft lip and palate. This research is based on blood and tissue samples collected from patients in collaboration with clinical expert centers worldwide, and especially with University Hospital Saint-Luc. We generate large amounts of data of the patient's genome using high-throughput DNA and RNA sequencing, and analyze them with our own specialized bioinformatic tool Highlander and other academic programs (Figure). We manage the UCLouvain Genomics Platform (PGEN) with its important computational cluster.
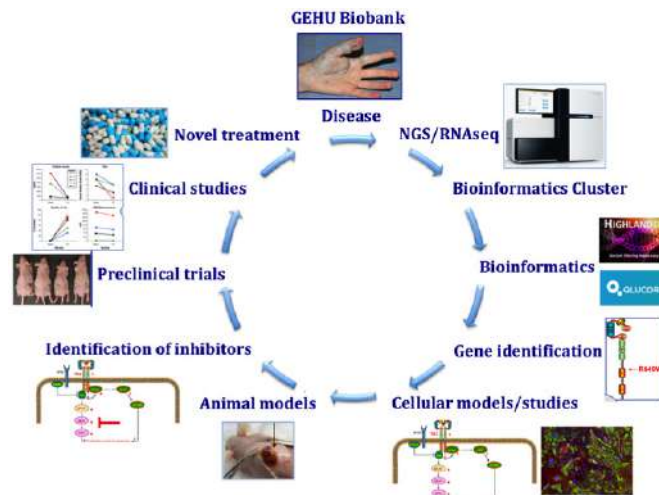
Workflow from bedside to bench back to patients

We have identified several genes that are mutated and cause inherited forms of vascular malformations. We have also discovered that the much more common non-hereditary forms are due to somatic mutations. Due to these mutations, the PI3K/AKT or the RAS/MAPK signaling pathway is abnormally activated, which we could show in cellular models (Figure). With our murine model for venous malformations, we demonstrated that the mTOR inhibitor rapamycin can control expansion of lesions. We also demonstrated its effectiveness in patients and a phase III European trial, called 'VASE', is ongoing, coordinated by Prof. L. Boon at University Hospital Saint-Luc (Figure). More recently, we have generated a model for arteriovenous malformation, and it is being tested for efficacy of medical treatments. We already demonstrated thalidomide to be effective on patients with arteriovenous malformations. We also provided insight to the pathogenesis of Gorham-Stout disease, a complex lymphatic anomaly, and discovered a novel treatment option for these patients.

A large part of our efforts is dedicated to understanding lymphedema, which causes chronic swelling of legs and arms and predisposition to infections. We have discovered several genes that can be mutated and predispose to lymphedema. In 2022, we added a completely new gene and signaling pathway to the ever-increasing picture of underlying causes. Altogether, 29 genes are now known, explaining about 30% of the cases. Our current work focuses on identification and explanation for the other 70%.

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Charles De Smet

Epigenetics in Cancer

Epigenetic mechanisms are essential to maintain proper gene expression programs in human tissues. Dysregulation of these mechanisms can lead to disease, including cancer. Studies in our group explore the causes and consequences of epigenetic alterations in tumors.

Maintenance of gene expression programs is essential to ensure proper functioning of the various cell types that make up the body. To this end, cells have evolved "epigenetic" regulatory mechanisms, based on the addition of chemical modifications on defined genes. Among such modifications, DNA methylation has an essential role in the long-term inactivation of tissue-specific genes. Importantly, the distribution of DNA methylation marks is profoundly altered in most tumors, and there is evidence that this contributes to cancer progression. The causes and consequences of this epigenetic disruption in tumor cells remain however unclear.

" Altered DNA methylation patterns in tumors often lead to aberrant activation of genes that normally display specific expression in germline cells."

We discovered that DNA methylation alterations often affect a particular group of genes, which normally display specific expression in germline cells. These genes lose methylation in many tumors, and become therefore aberrantly activated. Due to their particular expression profile, such genes were termed 'cancer-germline' (CG). Bioinformatics analyses are being conducted to better define the group of CG genes, and to explore possible mechanisms underlying their epigenetic de-repression in tumor cells (in collaboration with the team of Laurent Gatto).

Several CG genes were found to encode proteins that display oncogenic properties, and are therefore considered as potential targets for anti-cancer therapies. It is expected indeed that therapies directed against proteins expressed almost exclusively in tumors and germline cells will have only little side effects in cancer patients. Our group also isolated a CG gene (*CT-GABRA3*) that is not translated into a protein, but carries a clustered pair of miRNAs (miR-105 and miR-767). These miRNAs were found to promote tumor development, notably by favoring the formation of distant metastases.

More recently, we made the surprising observation that several CG genes produce long non-coding transcripts that overlap downstream promoters and thereby trigger their hypermethylation. Another consequence of CG gene activation in tumors is therefore the epigenetic repression of neighboring genes, which include tumor suppressor genes.

In order to determine the full spectrum of gene activations induced by genome demethylation in tumors, we performed a computational analysis of transcriptomic and methylomic data from lung cancer. This led to the identification of new transcripts activated by DNA demethylation in tumors, the majority of which are germline specific. Interestingly, we also identified two groups of transcripts that display specific expression in somatic tissues: one in the lower digestive tract, and the other in stratified epithelia. Genes belonging to the stratified epithelia cluster were most strongly associated with tumor grade and poor survival of lung cancer patients, suggesting that these genes might exert tumor-promoting functions. Studies are now being conducted to determine if DNA methylation sites within these genes can be exploited as prognostic biomarkers of lung cancer.

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Anabelle Decottignies

Telomeres are specialized protective structures present at chromosome ends. How to protect telomeres to delay cellular ageing or, conversely, how to damage telomeres to stop cancer cell proliferation, are two aspects of our research.

TELOMERES & EPIGENETICS

Telomeres are specialized protective protein-RNA-DNA structures at chromosome ends and shorten with successive cell divisions until they get too short, leading to a permanent exit from cell cycle and cellular senescence.

Although we found that some melanoma cells do not activate any telomere maintenance mechanism and yet form aggressive tumors, suggesting that indefinite replicative potential is not a general cancer cell hallmark, most cancer cells avoid telomere shortening. In 80% of tumors, telomerase expression is reactivated. In embryonic stem cells, telomerase counteracts telomere shortening, but its expression is lost upon cell differentiation. Sarcomas or central nervous system tumors (including pediatric tumors), however, frequently activate a telomerase-independent mechanism, called ALT (Alternative Lengthening of Telomeres), based on homologous recombination events. As ALT is not active in normal cells, this offers interesting perspectives for targeted cancer therapy. Thanks to a powerful genetic system, we identified TSPYL5 as a possible specific anti-ALT target. In collaboration with J. Messens (VIB-VUB) and R. Frédérick (LDRI, UCLouvain), we are currently working on the identification of anti-TSPYL5 drugs, notably through a nanobody-based approach. We also developed new assays for ALT diagnosis on tumor sections and established the first ALT+ mouse xenograft model. We are currently investigating various other aspects of ALT+ cell biology, including the connection between ALT and the cGAS-STING pathway.
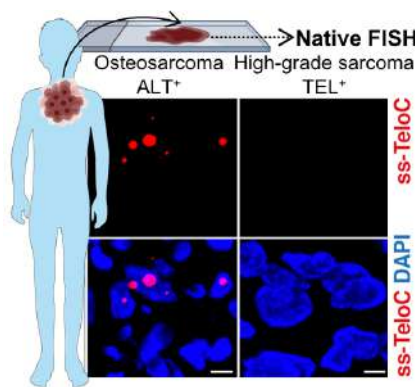
The native FISH assay to detect the ALT mechanism in solid paediatric and adult tumors: a red FISH probe containing the G-rich telomeric sequence is used to detect single-stranded C-rich telomeric DNA, a hallmark of ALT+ cells.

Another part of our research focuses on understanding how cells manage to replicate very long telomeres that experience replication stress.

Finally, we study cellular ageing, notably in the context of premature ageing diseases linked to defective telomere maintenance (telomeropathies). We studied the cellular defects induced by telomeropathy-linked *PARN* loss-of-function and evaluated telomeres and senescence in lungs from IPF patients. Telomeropathy diagnosis is achieved through telomere length measurement in blood cells using a technique called Flow-FISH, which was not available in Belgium. We set up Flow-FISH in collaboration with University Hospital Saint-Luc and enrolled about 500 healthy volunteers to establish the standard curves (to be published soon). The technique is now used by Belgian clinicians. These curves allowed us to show that most severely affected COVID-19 patients are characterized by short telomeres, further supporting the link between telomere length and resistance against viral infections. Over the last two years, we also investigated the link between mitochondrial genome sequence and telomere length using a cellular cybrid approach.

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Frédéric Lemaigre &
*Patrick Jacquemin*

Cell Differentiation

Our group identifies mechanisms that allow cells in liver and pancreas to acquire their mature functions during embryonic development. We also determine how these functions are perturbed in adults, which is essential to understand how cancer is initiated.

To develop into a complex organism cells in the embryo need to proliferate, differentiate and organize in three-dimensional tissues. While focusing on liver and pancreas, our group aims at identifying the mechanisms that promote cell differentiation and tissue morphogenesis in the embryo, and those that perturb differentiation in adults and induce liver or pancreatic cancer. We share our findings on normal differentiation with collaborators who transpose the information in cell culture protocols to produce hepatic or pancreatic cells for cell therapy. Our observations on disease mechanisms aim at promoting early cancer diagnosis and identifying targets for therapy.

Cholangiocytes are the cells which delineate the bile ducts in the liver and which form the epithelial lining of the gallbladder. We investigate the gene networks that drive cholangiocyte and bile duct development in the embryo and identified several regulators of normal biliary development, e.g. HNF6 – discovered in our laboratory –, and TGFβ signaling. We currently focus on interactions between developing bile ducts and adjacent liver vasculature. Parallel to our research on liver development, we investigate how cells transit from normal to precancerous and eventually invasive cancer states in gallbladder cancer and intrahepatic cholangiocarcinoma. This work is performed using spatial transcriptomics, organoid culture and mouse models. In that context, we identified genes that promote cholangiocarcinoma progression.

In pancreas, the most abundant cells are the exocrine cells. These comprise acinar cells that produce digestive enzymes, and ductal cells that delineate pancreatic ducts through which acinar enzymes flow to the gut. Implementing our expertise in cell differentiation, we identified ductal cells as a cell type of origin of pancreatic ductal adenocarcinoma (PDAC), as well as signaling cascades promoting formation of precancerous lesions and their evolution to cancer. We found how primary cilia, EGF signaling and peroxiredoxin control inflammation, a main driver of tumorigenesis. Importantly, we also uncovered a novel post-translational mechanism regulating the activity of KRAS – the most frequently mutated oncogene in PDAC.
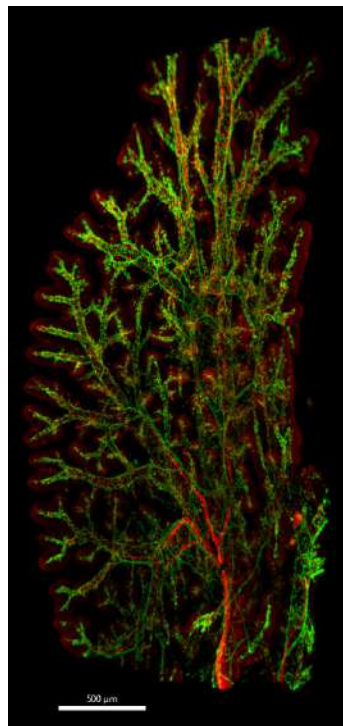
Three-dimensional visualization of the developing bile ducts (green) and adjacent hepatic artery (red).

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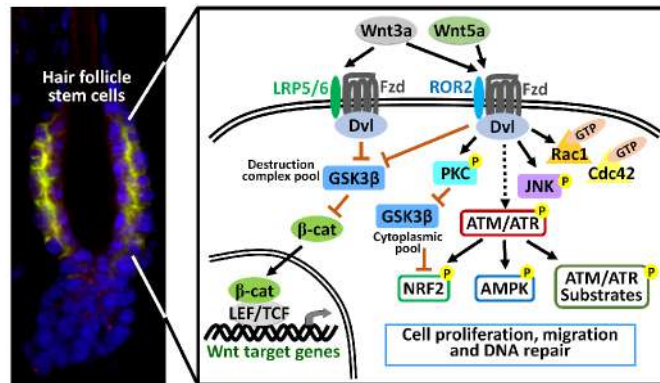
Wen-Hui Lien

The goal of our research is to understand how Wnt signaling pathways regulate skin stem cell maintenance and skin tumor development. Our studies provide an integrative view of signaling regulation and extend our knowledge for regenerative medicine and treatment of cancers.

Signaling Crosstalk

Throughout life, skin epidermis is constantly renewed and its appendage, hair follicles, undergoes cycles of regeneration. Skin epidermal stem cells that can self-renew and differentiate provide the unlimited source of cells required for tissue homeostasis and injury repair. The regeneration of tissues is fine-tuned by signaling cues from their microenvironment. Deregulation of this signaling may contribute to the development of tumors.

In mammals, Wnt signaling pathways, including canonical and non-canonical Wnt signaling, regulate diverse processes, such as cell proliferation, differentiation, migration and polarity. Canonical Wnt signaling, referred as Wnt/β-catenin signaling, is known as an important pathway that regulates developmental processes, tissue regeneration and cancers. While Wnt/β-catenin signaling has been extensively studied, the functions of non-canonical Wnt pathways are still underappreciated. Our group uses skin as a model system to investigate the roles of receptor tyrosine kinase-like orphan receptor 2 (ROR2), a Wnt receptor, in the regulation of skin development, stem cell maintenance and tumorigenesis.

Wnt signaling is shown to regulate adult stem cells, but exactly how it functions and for what purpose has been a matter of much debate. We conducted loss-of-function approaches by generating mutant mouse models to determine how ROR2 regulates skin development and

A model summarizing the roles of ROR2 in regulating hair follicle stem cell self-renewal and maintenance.

hair follicle regeneration. Using the cell culture system, we dissected the mechanism of ROR2 underlying stem cell self-renewal and maintenance. By generating double-mutant mouse models, we further investigated the cross-interaction between canonical and non-canonical Wnt signaling pathways in stem cell fate determination.

How non-canonical Wnt signaling regulates tumor development remains elusive. To address this important question, our group collaborates with a surgeon, Dr Benoît Lengele, at University Hospital Saint-Luc, to collect and analyze human non-melanoma skin tumors. Using these human specimens in combination with our mouse models, we identified the essential role of ROR2 in skin tumor development. We are currently investigating the underlying mechanism of ROR2 responsible for the tumor progression, including cell proliferation, migration, epithelial-to-mesenchymal transition and invasion. The ultimate goal of our research is to identify the clinical relevance of the main regulators involved in Wnt signaling and to use them as therapeutic targets to treat cancer and other diseases.

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Christophe Pierreux

Epithelial Differentiation

Our group studies paracrine communications, between epithelial cells and their environment, that govern epithelial (de)differentiation during thyroid and pancreas organogenesis and diseases.

Our body is composed of various cell types, among which epithelial cells fulfill different functions: gas exchange in lung alveoli, nutrient absorption in the intestine, digestive enzyme secretion from the pancreas, hormone production by the thyroid, ... To achieve these diverse and essential functions, epithelial cells form particular tridimensional structures, like closed spheres in the thyroid. They also gradually specialize by acquiring specific function(s), e.g. production of digestive enzymes in the pancreas. These happen during embryonic development through timely and tightly controlled epithelial differentiation programs. Loss or impairment of the tridimensional organization and specialization of these cells are frequently observed in pathological conditions.

" Deciphering tissular and cellular organization and identification of molecular mechanisms during embryonic development and disease pave the way towards organ bioprinting. "

Our group aims at understanding how thyroid and pancreatic epithelial cells organize and differentiate in response to signals from their environment. We have shown that thyroid and pancreatic progenitors first form a tridimensional mass of proliferating, non-polarized epithelial cells. Then, epithelial cells polarize and form monolayers that adopt a structure tailored to the organ's function: multiple independent closed spheres, the thyroid follicles, or a single, highly branched network of ducts and acini in the exocrine pancreas. We demonstrated the importance of VEGF signaling during thyroid and pancreas formation and uncovered a perfusion-independent function of blood vessels, mediated by paracrine signals from endothelial cells. Recently, we have identified the different cell types present in the embryonic pancreas, quantified their relative proportions and deciphered their precise 3D topological relationships. We also used tran-scriptomic and interactomic to identify ligands involved in intercellular communications. These data are important to design adequate bio-ink and instruct bioprinters for tissue bioprinting.

We are also investigating epithelial homeostasis in adult organs. On the one hand, we studied intercellular communications in thyroid cancers and identified a subpopulation of immunosuppressive macrophages and a sophisticated communication system that uses small (~100 nanometers) vesicles to transfer miRNA species between cells. Interestingly, these vesicles and two miRNAs were identified in blood of patients with thyroid cancer. On the other hand, we study blood vessels remodeling and macrophages during pancreatitis, a condition mainly affecting western countries, and investigate the molecular mechanisms causing these tissular changes.

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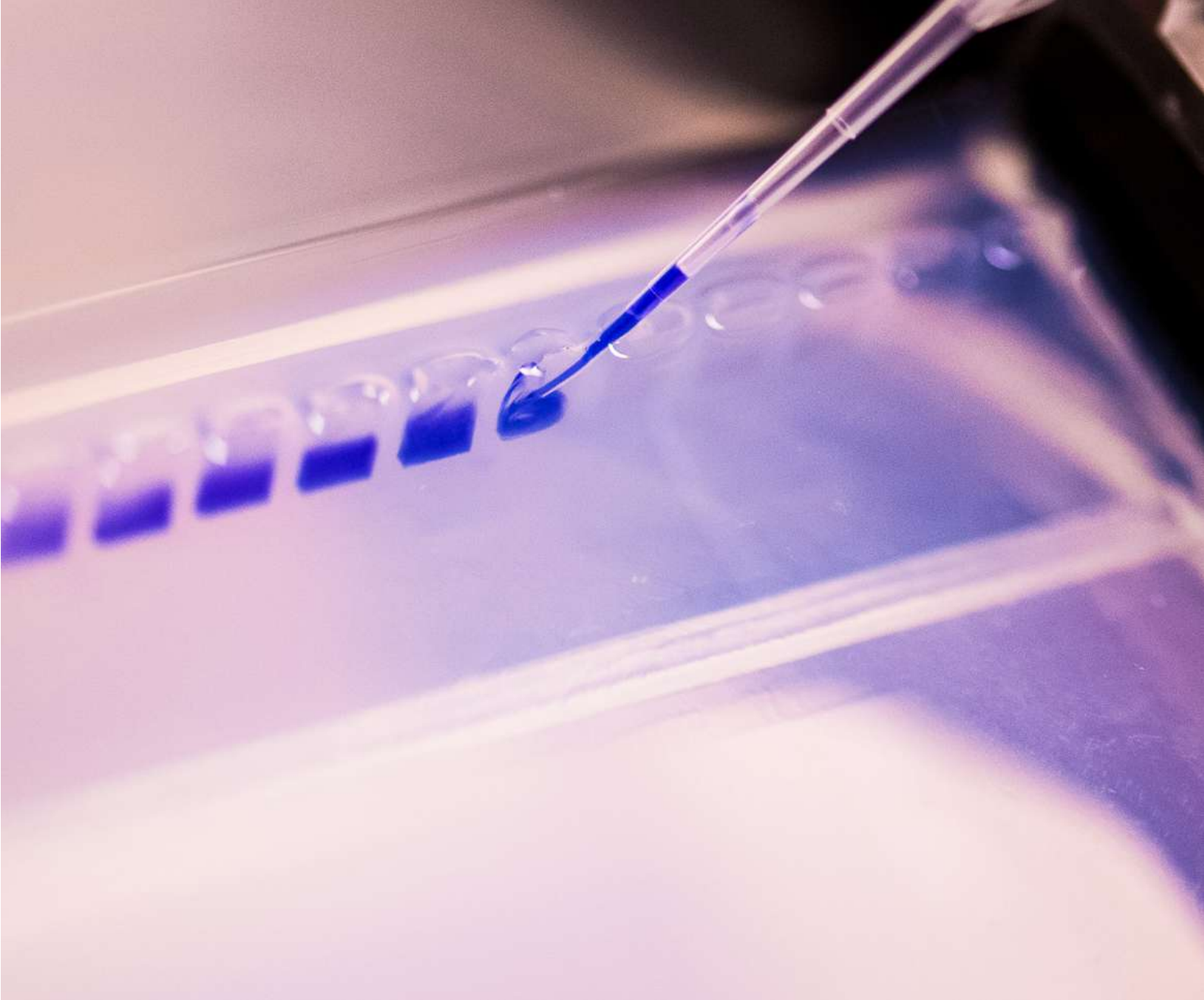
Our body deals with viral or bacterial infections by inflammatory responses of the immune system. Our groups investigate how viruses modulate the body's immune reactions, or escape from them. We also address the worrying emergence of bacteria resistant to all available antibacterial agents. When inflammation gets ill-controlled, it can induce inflammatory diseases, like Crohn's disease, asthma or psoriasis, of which we study key mechanisms.

Infections and Inflammation

Jean-François Collet

Bacterial Stress Responses

It is urgent to develop new antibiotics against resistant bacteria. Our laboratory wants to contribute to the global effort aiming to prevent the return of untreatable epidemics by better understanding how bacteria respond to different types of environmental stress.

The overuse of antibiotics to treat bacterial infections in human and veterinary medicine has created a global resistance crisis that could lead to a surge in infection-related mortality. A recent report predicted that multidrug resistant bacteria will kill more people than cancer by 2050. A particularly serious threat is the emergence of a new wave of multidrug-resistant Gram-negative bacteria, including *Pseudomonas aeruginosa* and enterobacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. It is therefore urgent to develop new antibiotics against resistant bacteria, which requires a deep understanding of the biology of these microorganisms. Our laboratory wants to contribute to the global effort aiming to prevent the return of untreatable epidemics by better understanding how bacteria respond to the different types of stress to which they are exposed. In particular, we want to understand how bacteria defend themselves against oxidative stress and how they maintain the integrity of their cell envelope despite always changing environmental conditions.

The cell envelope is the morphological hallmark of Gram-negative bacteria. It is composed of two concentric membranes: the inner membrane (IM), which is in contact with the cytoplasm, and the outer membrane (OM), which constitutes the interface with the environment. The IM and the OM are separated by the periplasm, a viscous compartment that contains the peptidoglycan. The cell envelope is essential for bacterial viability. Proteins involved in envelope biogenesis and maintenance are therefore attractive targets for the design of new antibiotics.

> **" Proteins involved in envelope biogenesis and maintenance are attractive targets for the design of new antibiotics. "**

The long-term objective of our laboratory is to delineate and ultimately harness the mechanisms underlying the assembly and maintenance of the envelope. Our research will contribute to the global effort to find new antibacterials by identifying proteins that play important roles in envelope assembly and protection, and therefore are attractive targets for new antibiotics.

Since the lab started in 2005, a number of major discoveries were made. In particular, we identified two antioxidant systems that are active in the bacterial envelope. The first system protects single cysteines from oxidation by reactive oxygen species, while the second rescues methionines from oxidative damage. We also discovered that the lipoprotein RcsF is targeted to the cell surface, in contrast to the general view that OM lipoproteins remain inside the periplasm. We determined that RcsF export is mediated by Bam, the machinery that inserts β-barrel proteins (porins) in the OM. We now want to investigate if additional lipoproteins decorate the cell surface of *E. coli*, which would radically change the model of the cell envelope as it is currently presented in many textbooks.

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Géraldine Laloux

Predatory bacteria kill other bacteria while being inoffensive for eukaryotic cells, hence representing a promising strategy to fight antibiotics-resistant pathogens. We study the biology of these microbes to understand how they proliferate inside their prey.

Bacterial Cell Biology

Acquiring fundamental knowledge on the cell biology of bacteria is a prerequisite for many clinical applications, including the fight against pathogenic strains, the development of solutions to the problem of antibiotic resistance, and the appropriate use of bacteria with beneficial roles in the human body. In our group, we study how bacteria organize their cellular content in space and time to achieve complex lifestyles, using a combination of bacterial genetics, molecular biology, live fluorescence microscopy and quantitative image analysis at the single-cell level.

We focus on the predatory bacterium *Bdellovibrio bacteriovorus* for two main reasons: (i) *Bdellovibrio* is a promising complement to classical antibiotics, since it kills other Gram-negative bacteria (including antibiotic-resistant and biofilm-forming pathogens), while being harmless for eukaryotic (e.g. human) cells; (ii) *Bdellovibrio* has an astounding cell cycle (Figure), which challenges the paradigm of binary cell division in bacteria: while most model species produce two cells per generation, *Bdellovibrio* releases larger and variable numbers of descendants. How cellular processes are orchestrated to govern the sophisticated biology of *Bdellovibrio* is largely unknown. Yet, discovering the molecular determinants underlying the cell cycle of this micro-predator is critical to understand how it kills and thrives inside its prey.

Recently, we discovered that the single chromosome of *Bdellovibrio* (its genetic material) is compacted to an unprecedented level when the bacterium is outside its prey, and partially decondenses during the growth phase inside the prey. We are now investigating the molecular cues and physiological role of this unique cell-cycle-dependent organization. We also revealed the complex dynamics of chromosome replication and segregation, which led us to propose a model that explains how *Bdellovibrio* produces variable, odd or even numbers of daughter cells that do not follow a canonical exponential pattern. Moreover, we developed methodologies to quantitatively assess predation efficiency. In parallel, we examine the function of proteins that keep the content of the bacterial cell in order. This year, we revealed the impact of the prey morphology and physiology on the predation cycle, and we have initiated a project to characterize the molecular machine required for the prey-predator interaction. For all projects, we constantly develop new analytic tools to extract quantitative data from live microscopy images, at the single-cell and population levels.
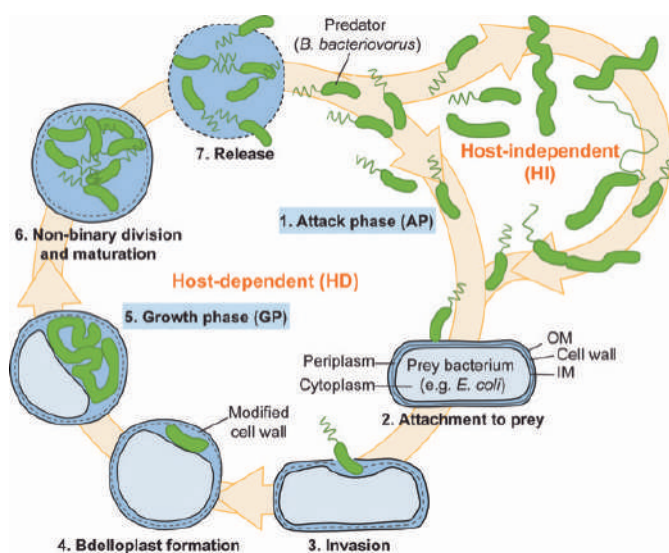
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Jean-Paul Coutelier

Infections & Immunity

Our project is to analyze the relationships between infectious agents and the immune microenvironment, and their consequences on unrelated diseases that develop concomitantly in the infected host, with a special focus on developing countries.

The possibility for evolved organisms to survive infections depends on the ability of their immune system to eliminate the pathogenic agent without induction of immunopathology. Therefore, both quantitative and qualitative parameters of the immune responses will determine the outcome of infections. For instance, infection with *Plasmodium* parasites may result in asymptomatic carriage, mild or severe malaria. Our main project is to determine in patients from Rwanda some of the causative environmental events that modulate anti-parasite responses and thus lead to severe forms of malaria or to asymptomatic persistence of the parasite. A dysbalance between proinflammatory and regulatory immune responses has been found in such distinct clinical forms of infectious diseases.

> **Modulation of the host immune microenvironment by infections enhances susceptibility to some diseases (blood autoimmune diseases, septic shock), but prevents the development of others (autoimmune encephalitis, some cancers such as myeloma).**

Using lactate dehydrogenase-elevating virus (LDV), and other common mouse viruses, we were first to show that viruses triggered a specific type of response, now called Type 1, characterized by increased proportion of IgG2a antibodies that were more efficient to protect mice against a fatal polioencephalomyelitis. Some of these characteristics of the immune responses are found also after infection with intracellular parasites such as *Plasmodium*, whereas helminths, including *Schistosoma*, induce a completely different response. Infections result therefore in a bias in the immune microenvironment of the host, which often leads to alterations of responses elicited against non-infectious antigens and of concomitant diseases with an immune component.

In mice, LDV- and *Plasmodium*-modulated immune microenvironment resulted in an enhanced susceptibility to diseases concomitant to the infection, but of unrelated cause, such as septic shock, through macrophage activation leading to enhanced TNF production. These infectious agents triggered an increased production of soluble receptors for bacterial lipopolysaccharide, which might serve as early indicators of this enhanced susceptibility to develop shock. Similarly, autoantibody-mediated hemolytic anemia and thrombocytopenia were aggravated by viral infection because of enhanced phagocytosis of opsonized erythrocytes and platelets by activated macrophages. This could explain how Immune Thrombocytopenic Purpura develops in children after infection with diverse common viruses.

However, modulation of the host immune microenvironment by infections could also protect against immune-mediated diseases such as graft-versus-host response and experimental autoimmune encephalitis. Similarly, mouse NK cell activation and IFN-γ production triggered by LDV infection or by ligands of immune receptors that mimick infections resulted in the inhibition of the development of some tumors such as plasmacytoma and mesothelioma. In contrast, *Schistosoma* antigen decreased both IFN-γ production and plasmacytoma prevention. Similarly, a study of Egyptian myeloma patients suggested an enhanced risk to develop cancer after *Schistosoma* infection.

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Thomas Michiels

Viruses developed fascinating strategies to hijack cellular signaling pathways and to counteract defenses of their host. Studying how viral proteins act provides insight into infection mechanisms as well as into important and physiological cellular processes.

Owing to their rapid multiplication, viruses constantly evolve to adapt to their host. They developed subtle strategies to counteract immune defenses. Our research focuses on topics related to the interplay between viral infections and the immune response of the host and in particular the IFN response.

Theiler's virus is a mouse picornavirus that has a striking ability to persist in the central nervous system despite a strong and specific immune response. We study how the leader (L) protein encoded by this virus and by related cardioviruses helps the virus to escape host defenses. L is a very short protein (76 amino acids) endowed with multiple activities. Our data show that L can hijack host kinases of the RSK family to interfere with the activation of PKR, a key effector of the IFN response as well as to deregulate the nucleo-cytoplasmic traffic of proteins in infected cells. Our data suggest the 'model of the clamp' whereby L recruits and activates cellular kinases of the RSK family through a short linear motif, and recruits specific substrates through another domain. In this way, L retargets RSK kinases toward these substrates, which, once phosphorylated, will act to facilitate the viral cycle or to escape host defenses (Figure).
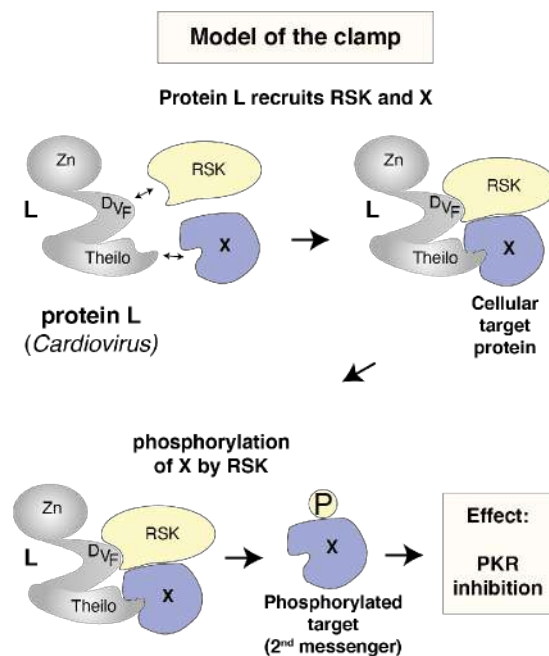

'Model of the clamp': the L protein binds RSK and recruits targets, which, once phosphorylated by RSK, act as second messengers to trigger the effects of L.

Ongoing research:
• We currently analyze whether additional pathogens use the same mechanism to hijack RSKs.
• We analyze how the recruitment of RSKs by cardiovirus L proteins triggers PKR inhibition as well as perturbation of nucleo-cytoplasmic trafficking in the infected cell.
• We further analyze the involvement of phosphorylation in the fine-tuning of PKR activity. It is known that a deficient PKR activity can lead to increased sensitivity to viral infections whereas a too strong PKR activity is associated with crippling autoimmune diseases such as Aicardi-Goutières syndrome or systemic lupus erythematosus.
• In view of the COVID-19 emergency, we also embarked in basic researches related to the SARS-CoV-2 coronavirus, the COVID-19 agent. We analyze the spike proteins determinants that define how the virus enters into cells. We also analyze polymerase properties that regulate viral replication with the hope that polymerase may be targeted by future antiviral agents.

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*Jean-Christophe Renauld &
Laure Dumoutier*

Inflammatory Disorders & Cytokines

Inflammation is a response to a variety of aggressions, like infections. It normally heals, but when excessive or ill-controlled, it can induce so-called inflammatory diseases such as Crohn's disease, asthma or psoriasis. We study the key mechanisms in these diseases.

In our laboratory, we work towards improving our understanding of the role of cytokines (small signaling proteins) in inflammation. More specifically, our research is focused on two cytokines, IL-9 and IL-22, crucial players in the inflammatory process, both of which were discovered by our lab.

IL-9 is a double-edged sword depending on disease. For instance, it is involved in the protection against worm infection whereas it plays a detrimental role in asthma. Asthma is a common chronic inflammatory disease of the airways, characterized by reversible airflow obstruction and bronchospasm. We showed that overexpression of IL-9 can cause bronchial hyperresponsiveness upon exposure to various allergens. In addition, we found that asthmatic patients produce increased amounts of IL-9. The potential aggravating role of IL-9 in asthma was confirmed by genetic analyses performed by others, pointing to both IL-9 and the IL-9 receptor genes as major candidates for human asthma. We collaborate with pharmaceutical companies to produce molecules that can block IL-9 activity, in order to improve the quality of life of asthmatic patients.

Recently, we investigated the role of IL-22 and IL-22-related cytokines in skin inflammatory disorders including psoriasis, allergic contact dermatitis and urticaria. In collaboration with the dermatology department of University Hospital Saint-Luc, we have shown that IL-22-related cytokines are highly expressed in the skin of patients with these three inflammatory diseases. These results strongly suggest that these cytokines are involved in skin inflammatory processes. Indeed, we have been able to show that in animal models of psoriasis, administration of an antibody blocking IL-22 activity is able to decrease some features such as scaly lesions and redness, demonstrating the deleterious role of this cytokine in the disease.

" IL-22 and its receptor are good therapeutic targets in skin inflammatory diseases. "

In contrast to the skin, we have shown that IL-22 plays a beneficial role in inflammatory bowel disease by protecting the gut mucosa. Crohn's disease and ulcerative colitis are the most common types of inflammatory bowel disease. They can affect any part of the digestive tract (Crohn's), or only the colon and rectum (colitis). Crohn's disease is caused by chronic inflammation, in which the immune system of the body attacks the gastrointestinal tract. Currently, there is no cure for this disease and treatments are restricted to controlling symptoms.

In the future, we aim to develop a therapy that blocks only the deleterious arm of IL-22 activity, leaving intact its beneficial functions in Crohn's disease.

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Our metabolism assures that our cells always dispose of the energy they need, despite a fluctuating demand. We study the amazing networks of metabolic pathways, as well as the enzymes that are involved in it and the genetic mutations that cause a pathway to fail. We also investigate a remarkable example of a dynamic tissue: the uterine mucosa that is substantially destructed and again regenerated during every menstruation.

Metabolism and Hormones

*Emile Van Schaftingen &
Maria Veiga-da-Cunha*

Metabolite Repair

Our work focuses on the discovery of metabolite repair enzymes. Unlike what is usually assumed, enzymes of intermediary metabolism are not absolutely specific, they make significant amounts of side-products. Metabolite repair enzymes are indispensable to eliminate these side-products.

Work performed by our group in collaboration with Guido Bommer leads us to revise our ideas about the way that intermediary metabolism is structured. Intermediary metabolism is the sum of all enzyme-catalyzed reactions that allow cells to produce their own indispensable constituents. Biochemistry textbooks state that these enzymes are extremely specific and that this is enough to avoid the production of useless side-products

Yet the study of L-2-hydroxyglutaric aciduria, a neurological disease, unveiled the idea that enzymes of intermediary metabolism make significant amounts of side-products and that our cells have multiple 'metabolite repair enzymes', which serve to eliminate these toxic side-products. Thus, L-2-hydroxyglutaric aciduria is a genetic deficiency in the metabolite repair enzyme L-2-hydroxyglutarate dehydrogenase, which destroys L-2-hydroxyglutarate, a toxic side product made by side reactions of L-malate and L-lactate dehydrogenases on the classic metabolite α-ketoglutarate. Nit1 is another example of a highly conserved repair enzyme. It degrades a deaminated glutathione, a damaged form of glutathione resulting from side activities of various transaminases on this tripeptide.

The metabolite repair concept led us to understand the cause of the congenital neutropenia found in patients deficient in G6PC3, a phosphatase present in the endo-

plasmic reticulum, or in glycogen storage disease type 1b patients, deficient in G6PT, the glucose-6-phosphate transporter of the endoplasmic reticulum. These two proteins collaborate to destroy 1,5-anhydroglucitol-6-P, an abnormal metabolite made in vivo by side activities of glucose-phosphorylating enzymes. Lack of dephosphorylation of 1,5-anhydroglucitol-6-P leads to its intracellular accumulation and, as a result, to strong inhibition of glucose phosphorylation. This is toxic to neutrophils and explains the patients' neutropenia. An inhibitor of the sodium-dependent glucose transporter SGLT2, which causes a depletion of 1,5-anhydroglucitol in serum, has been successfully used to treat the neutropenia in G6PT and G6PC3 deficiencies. This effect is indirect, being due to inhibition of the kidney 1,5-anhydroglucitol transporter SGLT5 by the high concentration of glucose present in kidney tubules when SGLT5 is inhibited.

In collaboration with clinicians, we have recently identified a new neurological disease due to deficiency in an enzyme that serves to make high concentrations of glucose-1,6-bisP in the brain. Our data indicate that this deficiency does not lead to a glycosylation defect and that glucose-1,6-bisP plays an important role in the brain.
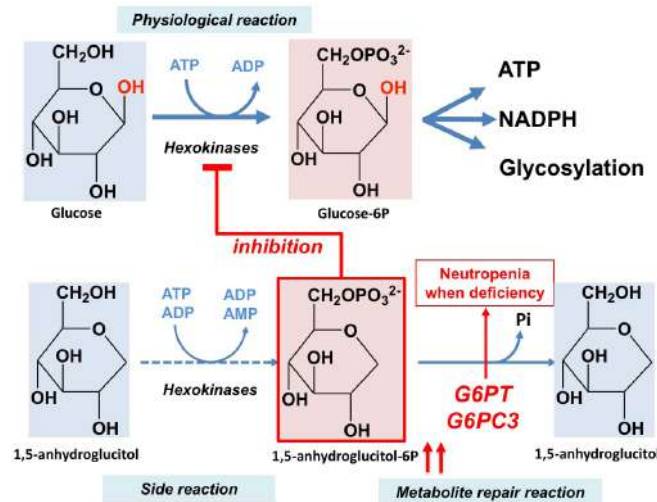
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Guido Bommer

Cells need to adjust their metabolism to fulfill changing needs for building blocks, energy and protection from stress. We search for vulnerabilities in known or newly discovered metabolic pathways that might be targeted in future therapies

Metabolism in Cancer & Other Diseases

The local 'success' of a cancer cell is measured by its ability to proliferate and survive better with the available nutrients than its neighboring cells. Like any other cell, a cancer cell needs to maintain cellular integrity and fulfill baseline housekeeping functions. All cell types need to synthesize ATP by breaking down nutrients in pathways such as glycolysis, citric acid cycle and mitochondrial oxidative phosphorylation. In addition, proliferating cells in general and cancer cells in particular need to generate bio-mass, composed of amino acids, nucleotides and lipids. Synthesis of these components starts with precursors that are intermediary products in the same pathways that are used to synthesize cellular ATP. Several adjustments of the flux through these pathways are needed to reconcile cellular demand for biosynthetic building blocks and for ATP synthesis.

Degradation of glucose in glycolysis leads to a newly discovered reactive metabolite that causes cellular damage, unless it is eliminated by the Parkinson's disease protein PARK7.

We are investigating the role of a series of enzymes, for which we have reason to believe that they might be involved in the synthesis of regulatory molecules. In these studies, we use a combination of state-of-the-art metabolomics (GC-MS and LC-MS) and genetic manipulation of cell lines to understand the cellular effects of novel regulatory molecules. Classical enzymological studies (in collaboration with the laboratory of Emile Van Schaftingen and Maria Veiga-da-Cunha) on purified proteins are then used to understand the molecular basis of the observed effects. Eventually, we hope that our work will reveal novel therapeutic targets in cancer. Currently, we are particularly interested in several phosphatases that might serve to eliminate metabolic side-products or metabolic regulators.

While we strive to understand processes involved in cancer biology, we remain very much open to surprising discoveries. As such, we have recently discovered a novel post-translational modification of α-dystroglycan by ribitolphosphorylation. Furthermore, we are following-up on observations that suggest that so far unknown biochemical changes may contribute to the development of Parkinson's disease.

In this context, we have recently discovered the function of the protein PARK7, which is inactivated by mutations in some hereditary cases of Parkinson's disease. We discovered that this enzyme serves as a guardian to prevent damage of metabolites and proteins caused by glucose metabolism. In fact, we found that a metabolite in glycolysis spontaneously converts into the reactive compound cyclic 1,3-phosphoglycerate that can attack amino groups in both proteins and metabolites. PARK7 eliminates this compound and thereby prevents damage. In contrast, deficiency of PARK7 leads to Parkinson's disease and the accumulation of damaged proteins and metabolites (which carry glycerate or phosphoglycerate modifications).
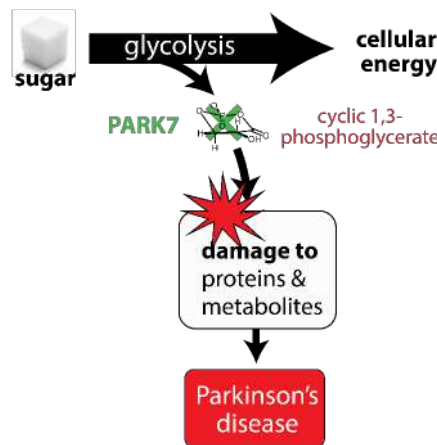
Staff members
Clinical Investigator: Joseph Dewulf • **Senior Investigator:** Isabelle Gerin • **Postdoctoral Scientist:** Marina Bury • **PhD Students:** Francesco Caligiore, Isaac Heremans, Jean Jacobs, Stéphanie Paquay • **Master Student:** Emilie de Kerchove d'Exaerde • **Research Assistant:** Julie Graff

Mark Rider

Protein Phosphorylation

We study control by protein phosphorylation in relation to diseases such as type 2 diabetes. AMP-activated protein kinase and insulin signaling are our main interests. We also run the mass spectrometry protein analysis facility.

Metformin is the most prescribed drug used for the treatment of type 2 diabetes (T2D) and its effects can partly be explained by activation of AMP-activated protein kinase (AMPK), which is the main focus of our research. AMPK acts as a sensor of cellular energy status, activated by an increase in the AMP:ATP ratio, as occurs during hypoxia or muscle contraction/exercise. The role of AMPK in the cell is to maintain ATP by stimulating ATP-producing pathways and at the same time inhibiting energy-consuming biosynthetic pathways. AMPK is now a well-recognized drug target for treating metabolic disorders such as T2D

> **" Drug targeting AMPK with novel compounds will be beneficial for the treatment of type 2 diabetes. "**

In white adipose tissue (WAT), we showed that pharmacological AMPK activation inhibits insulin-stimulated lipogenesis and glucose uptake to prevent lipid accumulation, a major cause of insulin resistance, but without affecting insulin-stimulated fatty acid esterification. In addition, AMPK activation in WAT inhibited noradrenaline-induced lipolysis, which could also be beneficial for treating T2D. In hepatocytes, a panel of small-molecule AMPK activators was found to inhibit glucagon-stimulated glucose production in an AMPK-dependent manner, although one compound (991) had off targets effects on basal gluconeogenesis by inhibiting pyruvate uptake and *sn*-glycerol-3-phosphate dehydrogenase-2. Our studies support the notion that direct pharmacological activation of hepatic AMPK as well as inhibition of pyruvate uptake could be an option for the treatment of T2D-linked hyperglycemia.

In addition to our work on AMPK, we run the mass spectrometry protein analysis facility (MASSPROT) on the Brussels campus of UCLouvain. The acquisition by the de Duve Institute of the High Resolution Orbitrap Fusion Lumos mass spectrometer has enabled us to perform quantitative proteomics and increase our capabilities to study other protein modifications. Indeed, we are currently establishing stable isotope-based absolute quantification of the AMPK subunits across rat, mouse and human tissues by exploiting the Orbitrap. We are also developing a similar approach for absolute quantification of the protein Tau in human post-mortem brain extracts from patients with neurodegenerative disorders. In close collaboration with the clinicians, we are part of a large integrated systems biology approach to better understand the biology of long-term COVID using human plasma differential proteomics.

Staff members
Emeritus: Louis Hue • **Senior Investigator:** Didier Vertommen (Platform Manager) • **Postdoctoral Scientists:** Clémence Balty, Manuel Johanns • **PhD Student:** Nathalie Kyalu Ngoie Zola • **Master Students:** Pauline Chateau, Clément De Comite, Axelle Vanparys • **Research Assistants:** Gaëtan Herinckx, Nusrat Hussain, Roxane Jacobs

*Etienne Marbaix &
Patrick Henriet*

Our group identifies mechanisms controlling physiological degradation and regeneration of the human endometrium at menstruation, with the aim to understand how their dysregulation results in dysfunctional uterine bleeding and endometriosis.

ENDOMETRIUM PHYSIOPATHOLOGY

Throughout the reproductive life, the human endometrium – the uterine mucosa – undergoes cyclic remodeling. Changes in endometrial structure condition fertility and must be perfectly orchestrated by sex hormones, namely estrogens and progesterone. Menstruation occurs at the end of every unchallenged menstrual cycle and results from an intense but locally restrained degradation of the endometrium when the circulating concentration of the sex hormones drops. Treatment of two endometrial pathologies, dysfunctional uterine bleeding (DUB) and endometriosis, should benefit from a better understanding of the molecular events surrounding menstruation. On the one hand, DUB results from local menstrual-like breakdown of the endometrium, suggesting inadequate response to sex hormones. On the other hand, endometriosis, a pathology characterized by the presence of endometrial tissue outside the uterus, is believed to often originate from retrograde menstruation, i.e. migration of menstrual endometrial fragments through the fallopian tubes and invasion of the peritoneal cavity, peritoneum and ovaries.

> **Treatment of two endometrial pathologies, dysfunctional uterine bleeding (DUB) and endometriosis, should benefit from a better understanding of the molecular events surrounding menstruation.**

Twenty-five years ago, our laboratory was the first to show that endometrial tissue breakdown at menstruation is performed by a group of proteolytic enzymes, the matrix metalloproteinases (MMPs). Our subsequent work aimed at characterizing the various molecular mechanisms that ensure the focal nature of progressive tissue lysis by locally tuning the global hormonal control. Our research is focused on three levels of control of MMP activity. In a first axis, we investigate how the different potential sex hormone receptors combine their specific effects to induce or repress MMP expression. In a second axis, we dissect the complex network of local regulators acting between hormone receptors and MMP genes. Our work has highlighted the role of cytokines and growth factors, such as interleukin-1α, TGF-βs and Lefty2, in the control of MMP expression. We recently turned to PGRMC1, a potential modulator of progesterone response involved in control of cell survival and proliferation. In a third axis, we explore mechanisms able to discard obsolete MMP activity. We have shown that members of the low density lipoprotein receptor family, LRP-1 and LRP-2, act as endocytic receptors able to bind MMPs complexed with their TIMP inhibitors, in order to induce their lysosomal degradation.

Following up on puzzling data from our previous whole genome transcriptomic analysis of the menstrual endometrium, we also investigate the molecular mechanisms coupling tissue lysis and subsequent scarless regeneration. Indeed, our results highlighted that genes required for early endometrial repair, in particular extracellular matrix components, are expressed concomitantly with MMPs during menstruation.

Staff members
PhD Student: Marie Van Wynendaele • **Master Student:** Laurie-Anne de Selliers

Computational Biology

Modern high throughput biology produces huge amounts of data that can be analyzed, and the challenges of modern biology are statistical interpretation and integration of these data. Research and developments in computational biology and bioinformatics aim to provide the methods and tools to comprehend these high dimensional data and understand their underlying biological processes.

Laurent Gatto

Our group uses statistical learning, computational techniques and visualization to analyze and understand high throughput and multivariate biological data and comprehend complex biological processes.

Computational Biology & Bioinformatics

For the last decades, biology and biomedical sciences have seen an impressive increase in the size of the data that are collected as part of routine research projects. The increase in amount and complexity of these data lead some to call it a data deluge. Indeed, we have reached a situation where the sheer volume of data that is produced is overwhelming the capacity of individual researchers and research groups to manage, analyze and extract meaningful information from them. This revolution is shifting biomedical research towards a quantitative, data-driven discipline. This evolution has been driven by technological breakthroughs that, today, allow us to sequence whole genomes, quantify the near complete set of transcripts or proteins, measure epigenetic modifications across whole genomes, assay proteins for post-translational modifications, interactions and localization. But the question remains: what to do with all that data?

Our group works on diverse projects and benefits from computational and biological expertise. We work on transcriptomics and proteomics gene expression projects in collaboration with other research groups at de Duve Institute, to identify differentially expressed genes and processes related to cancer development, cell signaling, or metabolomic disorders. We are also involved in single cell-level assays, at the RNA and protein level, to contribute to the identification of cell types and cell states in organ development or the immune response.

The lab is also heavily invested in the development of novel, open source research software, with a long-standing interest in mass spectrometry-based proteomics data. These include quantitative data processing and analysis,

> **" This revolution is shifting biomedical research towards a quantitative, data-driven discipline. "**

sub-cellular spatial proteomics methods, or the identification of protein-protein interactions.

Finally, the lab is also involved in integrative omics, i.e. the development of methods to integrate different types of omics data or experimental and publicly available resources. Indeed, it becomes essential to integrate different biological modalities or complementary resources to gain further insights into the complexity of biological processes and their regulation.

Clarity and traceability of the data and the analysis methodology enable us to better understand what we do, how and why we do it and consequently exploit complex data and comprehend the underlying biology. The collaborative and interdisciplinary nature of high throughput biology calls for open approaches, from communication between stakeholders, open research and development and open dissemination of all research outputs, which our lab fully adheres to.

Staff members
Senior Investigator: Axelle Loriot • **PhD Students:** Julie Devis, Samuel Grégoire, Philippe Hauchamps, Christophe Vanderaa • **Research Assistant:** Andrea Vicini

Selected Publications

Stefan Constantinescu

1. Pecquet C, Papadopoulos N, Balligand T, Chachoua I, Tisserand A, Vertenoeil G, Nedelec A, Vertommen D, Roy A, Marty C, Nivarthi H, Defour JP, El-Khoury M, Hug E, Xu E, Zagrijtschuk O, Fertig E, Marta D, Gisslinger H, Gisslinger B, Schalling M, Casetti I, Rumi E, Pietra D, Cavalloni C, Arcaini L, Cazzola M, Komatsu N, Kihara Y, Sunami Y, Edahiro Y, Araki M, Lesyk R, Buxhofer-Ausch V, Heibl S, Pasquier F, Havelange V, Plo I, Vainchenker W, Kralovics R, Constantinescu SN. Secreted mutant calreticulins as rogue cytokines in myeloproliferative neoplasms. Blood. 2023;141:917-29.

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Nick van Gastel

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Jean-Baptiste Demoulin

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Benoit Van den Eynde

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Technology Platforms

Bioinformatics (BIOINFO)

The platform provides the scientific community with technical and methodological support in bioinformatics and high-throughput data analysis. It also organizes dedicated training and workshops. The platform is managed by Prof. Laurent Gatto and is run by Dr. Axelle Loriot.
[W] https://uclouvain.be/en/technology-platforms/bioinfo.html

Flow Cytometry and Cell Sorting (CYTF)

Flow cytometry technology allows simultaneous multiparametric analysis of thousands of cells per second, enabling trained users to rapidly analyze complex cell populations based on phenotypic and functional features. High-speed assisted cell sorting services provide researchers with physical separation of identified cell populations, for any downstream characterizations. The platform is managed by Prof. Laure Dumoutier and is run by Dr. Nicolas Dauguet.
[W] http://www.deduveinstitute.be/flow-cytometry-and-cell-sorting

Genomics (PGEN)

The genomics platform provides the scientific community with access to the latest technologies related to Next Generation Sequencing (Massive Parallel Sequencing), including bioinformatics. Theses techniques facilitate and speed up data analysis, which is beneficial for many different fields, such as biology, medicine, agronomy, ... Their use in clinical diagnosis also broadens the spectrum of molecular diagnosis and opens new ways for personalized medicine. The platform is managed by Prof. Miikka Vikkula and is run by Drs. Pascal Brouillard and Raphaël Helaers.
[W] http://www.deduveinstitute.be/genomics-platform

Imaging (PICT)

The imaging platform trains and provides the scientific community with fluorescence, confocal, multiphoton and super-resolution microscopy, as well as a wide range of sophisticated methods of vital confocal microscopy, immuno-labeling and dynamics. It is also a source of collaborations and advices, providing users with the necessary expertise at all stages of the experiment, from sample preparation to analysis and interpretation of data. The platform is managed by Prof. Donatienne Tyteca and is run by Dr. Patrick Van Der Smissen.
[W] http://www.deduveinstitute.be/pict-platform-imaging-cells-and-tissues

Laboratory Animals (LAF)

The platform produces mice under 'SPF' health status for academic research use, with no commercial purpose. It hosts 80 different mouse strains, both non-genetically and genetically modified, available under a very high sanitary status monitored via a sentinel program, for research teams of the University of Louvain and collaborators. The platform is managed by Prof. Sophie Lucas and is run by Dr. Pedro Gomez, with technical help from Pascale Bougard, Axel Capron, Laurent Hermanns and Quentin Lechien.
[W] http://www.deduveinstitute.be/laboratory-animals

Mass Spectrometry (MASSPROT)

The platform provides proteomics services principally through gel-free approaches coupled to mass spectrometry. It specializes in the identification and quantification of proteins from complex samples, and can also provide data on the

location of post-translational modifications, even in complex samples. The platform is managed by Profs. Jean-François Collet and Mark Rider and is run by Dr. Didier Vertommen.
[W] https://www.deduveinstitute.be/massprot-platform-mass-spectrometry

Transgenesis (TRSG)

The transgenesis platform offers transgene technology tools to research teams of the University of Louvain and other Belgian universities at the lowest possible cost. It also enables the sharing of expertise in designing and creating transgenic mouse lines and offers training opportunities to PhD students and post-doctoral researchers. The platform is managed by Profs. Patrick Jacquemin and Frédéric Lemaigre, and is run by Dr. Younes Achouri.
[W] http://www.deduveinstitute.be/transgenesis

Prizes, Awards and Honors

Mohamad ASSI • Dr Maurice Godin - Maria Savelkoul Prize 2019-2021
Awarded every three years by the Académie royale de Médecine to three Belgian researchers for the best work on cancer, Parkinson's disease or multiple sclerosis.

Guido BOMMER • Vicomtesse Valine de Spoelberch Prize 2021-2022
Awarded every other year to a Belgian researcher by the Queen Elisabeth Medical Foundation for a work in neurosciences.

Guido BOMMER • Bauchau Chair 2022 - University of Namur
Attributed every other year by the Adrien Bauchau Fund (University of Namur) to a professor from another Belgian university to give four lessons.

Jean-François COLLET • Associate Member of the Académie royale de Médecine de Belgique

Stefan CONSTANTINESCU • President of the Académie royale de Médecine de Belgique

Ophélie DELCORTE • Publication award of the Belgian Thyroid Club 2022
Awarded every year to a clinical/translational/basic researcher aged 50 or less active in Belgium, who has published an original study in the field of thyroidology or parathyroidology during the current or previous year.

Isaac HEREMANS • Dr Maurice Godin - Maria Savelkoul Prize 2019-2021
Awarded every three years by the Académie royale de Médecine to three Belgian researchers for the best work on cancer, Parkinson's disease or multiple sclerosis.

Nassim HOMAYUN-SEPEHR • d'Alvarenga, de Piauhy Medal 2021
Awarded every year to a Belgian researcher by the Académie royale de Médecine for the quality of his/her thesis work on a medical topic.

Géraldine LALOUX • Vice-Chair of the Board of Directors of the Belgian Society for Microbiology

Nick VAN GASTEL • Lambertine Lacroix Prize 2022-2023
Awarded every other year by the FNRS to a Belgian researcher from the Fédération Wallonie-Bruxelles aged 50 or less, for a translational research on cardiovascular diseases or cancerology (in rotation).

PhD Theses

Pierre-Florent PETIT • *New insights in cancer immunotherapy targeting the immune checkpoint PD-L1* • Promoter: B. Van den Eynde, co-promoter: J. Zhu

Estelle BALAN • *Influence of lifelong endurance training on hallmarks of aging in skeletal muscle* • Promoter: L. Deldicque, co-promoter: A. Decottignies

Christophe VANHAVER • *On the diversity of neutrophils in blood and tumor of cancer patients: CD45 expression discriminates low-density neutrophils with different suppressive capacities* • Promoter: P. van der Bruggen, co-promoter: A. Bruger

Lila GANNOUN • *Identification of mechanisms driving development of the bile ducts and hepatic arteries* • Promoter: F. Lemaigre

Kilian DEKONINCK • *It's a trap: new findings into the role of outer membrane proteins in the Rcs stress response system of Escherichia coli* • Promoter: J.-F. Collet

Elsa KHOURY • *The genetic basis of familial Hodgkin lymphoma* • Promoter: N. Limaye, co-promoter: M. Vikkula

Anna DIACOFOTAKI • *Epigenetic alterations and ectopic activation of tissue-specific gene clusters in lung adenocarcinoma* • Promoter: C. De Smet

Anne-Sophie CLOOS • *How plasma membrane propreties contribute to red blood cell vesiculation* • Promoter: D. Tyteca

Charlotte THIEFFRY • *Progesterone Receptor Membrane Component (PGRMC) 1: study of its expression, regulation and potential functions in the human endometrium and in endometriosis* • Promoter: C. Pierreux, co-promoter: E. Marbaix

Marie SOLVAY • *New insights into the role of the AHR in IDO1-mediated immonusupression* • Promoter: B. Van den Eynde

Julien DEVREUX • *Targeting GARP:TGF-β1 complexes on Tregs for the immunotherapy of myeloproliferative neoplasms* • Promoter: S. Lucas

Ariane SABLON • *Molecular characterization of FOXO1 and SRF oncogenic alterations* • Promoter: J.-B. Demoulin, co-promoter: V. Havelange

Eloïse CLAUDE • *Indentification of an ALT therapeutic target and re-evaluation of ALT markers in tumors and cell lines with long telomeres* • Promoter: A. Decottignies

Thibault HIRSCH • *The transcription factor IRF4 attenuates human CD8 T lymphocytes functions and promotes their proliferation and PD-1 expression* • Promoter: P. van der Bruggen

Clément TRIAILLE • *Deciphering transcriptomic heterogeneity in Rheumatoid Arthritis Synovium* • Promoter: N. Limaye, co-promoter: B. Lauwerys

Ophélie DELCORTE • *The miRNA content of extracellular vesicles in papillary thyroid cancer: from identification in mouse thyroid tumor to detection in the plasma of patients* • Promoter: C. Pierreux

Lectures & Scientific Events

23rd Heremans Lecture
Lluis QUINTANA-MURCI • *Collège de France & Institut Pasteur, Paris, France*
From Neanderthals to COVID-19: genetic and evolutionary sources of human immune response variation

Heremans lectures and de Duve lectures are given every other year by a prominent international scientist (for podcasts and a complete list of speakers: http://www.deduveinstitute.be/seminars).

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N.B. Due to the Covid-19 pandemic, some scientific events and lectures had to be postponed or cancelled.

Chantal HOUSSET • *Sorbonne University & Saint-Antoine Hospital, Paris, France*
Portal myofibroblasts, effectors of liver fibrosis distinct from hepatic stellate cells

Camille GOEMANS • *European Molecular Biology Laboratory, Heidelberg, Germany*
The impact of drugs on the human gut microbiota

Pedro MOURA ALVES • *Instituto de Investigação e Inovação em Saúde, Porto Unversity, Portugal*
Connections in the AHR: a ticket to multiple destinations in infection, immunity and drug therapy

Julie STOCKIS • *Cancer Research UK Cambridge Institute, University of Cambridge, UK*
Tissue-resident Tregs: novel insights into their function in the exocrine pancreas an beyond

Eric CASCALES • *Institut de Microbiologie, Bioénergies et Biotechnologie, Aix-Marseille University, France*
Assembly and mechanism of action of an antibacterial speargun: the type VI secretion system

Grégory VERDEIL • *University of Lausanne & Ludwig Institute for Cancer Research, Epalinges, Switzerland*
NFAT5 induction by the tumor microenvironment enforces CD8 T cell exhaustion

Philipp WEBER • *University of Vienna & Vienna Doctoral School of Ecology and Evolution, Austria*
Morphogenesis and chromosome organization in animal-attached bacteria

Maud MARTIN • *ULB Neuroscience Institute, Université Libre de Bruxelles, Gosselies, Belgium*
Breaking the symmetry during blood vessel formation: not too little, not too much, just right

Shai IZRAELI • *Schneider Children's Medical Center & Sackler Faculty of Medicine, Tel Aviv University, Israel*
JAK-STAT Acute Lymphoblastic Leukemias: challenging drivers

Yejing GE • *University of Texas MD Anderson Cancer Center, Houston, TX, USA*
Functional dissection of stem cell lineage plasticity in the skin epithelium

Christine & Jonathan SEIDMAN • *Harvard Medical School, Boston, MA, USA*
Cardiomyopathies and cardiovascular diseases

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Annual meeting of the 'Oxidative Processes and Antioxidants' F.R.S-FNRS contact group: The paradOX meeting, the good and bad sides of oxidation. Organized by J.-F. Collet

Speakers: Ursula JAKOB *(University of Michigan, Ann Arbor, MI, USA)*, Michel TOLEDANO *(Paris-Saclay University, CEA, France)*, Marianne ILBERT *(Aix-Marseille University, France)* & Gerd P. BIENERT *(Technical University of Munich, Germany)*.

> *One-day meeting focusing on redox biology, across all kingdoms of life. The 'Oxidative Processes and Antioxidants' F.R.S-FNRS contact group connects researchers in biochemistry and molecular biology at the national and international level, to promote scientific research on oxidative mechanisms and stresses.*

Third MACS® Immuno-Oncology Day, Benelux: 'MDSCs: cause or symptom of tumor immune suppression?'. Co-organized by P. van der Bruggen and A. Bruger

Speakers: Vincenzo BRONTE *(University of Verona, Italy)*, Bastian HÖCHST *(Technical University of Munich, Germany)*, Jo VAN GINDERACHTER *(Vrije Universiteit Brussel, Belgium)*, Mikaël PITTET *(University of Geneva, Switzerland)*, Marco CASSATELLA *(University of Verona, Italy)*, Sven BRANDAU *(University Hospital Essen, Germany)*, Zvi FRIDLENDER *(Hadassah-Hebrew University Medical Center, Jerusalem, Israel)*, María CASANOVA ACEBES *(Centro Nacional de Investigaciones Oncológicas, Madrid, Spain)*, Luca CASSETTA *(University of Edinburgh, UK)* & Diletta DI MITRI *(Humanitas Clinical and Research Center, Milan, Italy)*.

> *One-day annual meeting focusing on research in immuno-oncology using MACS® technology.*

Autumn meeting of the Royal Society for Cell and Developmental Biology: 'Endoderm and Endoderm Derivatives in Development and Disease'. Co-organized by F. Lemaigre and P. Jacquemin

Speakers: Luis ARNES *(University of Copenhagen, Denmark)*, Eduard BATTLE *(Institute for Research in Biomedicine, Barcelona Spain)*, Benjamin BECK *(Université Libre de Bruxelles, Belgium)*, Alain CHARIOT *(Université de Liège, Belgium)*, Jorge FERRER *(Center for Genomic Regulation, CIBERDEM, Barcelona, Spain)*, Martin GUILLIAMS *(University of Ghent, Belgium)*, Darrel KOTTON *(Boston University, MA, USA)*, Raquel MAJEAS LUQUE *(Technical University of Munich, Germany)*, Elke OBER *(University of Copenhagen, Denmark)*, Meritxell Rovira *(University of Barcelona, Spain)*, Francesca SPAGNOLI *(King's College London, UK)* & Sumeet Pal SINGH *(Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire, Brussels, Belgium)*.

> *Two-day semi-annual meeting focusing on novel mechanisms involved in the development of the endoderm and derived organs, and how they can be translated to understand and treat diseases. The RBSCDB connects Belgian and foreign cell and developmental biologists through high quality scientific meetings.*

EuroBioC Conference 2022. Co-organized by L. Gatto

Keynote speakers: Shila GHAZANFAR *(University of Cambridge, UK)*, Julio SAEZ-RODRIGUEZ *(Heidelberg University, Germany)*, Judith ZAUGG *(European Molecular Biology Laboratory, Heidelberg, Germany)*, Michael DORRITY *(University of Washington, Seattle, WA, USA)* & Björn GRÜNING *(University of Freiburg, Germany)*.

> *Three-day annual meeting aimed at biologists, bioinformaticians, statisticians, and users of the Bioconductor software, a widely used project for the analysis and comprehension of high-throughput genomic data.*

PhD Day

> *All PhD students of de Duve Institute present their work either as a talk or as a poster.*

de Duve Institute

De Duve Institute is constituted of two structural entities: the international non-profit association de Duve Institute aisbl (DDI) and de Duve Institute – UCLouvain (DDUV). The two entities are managed in close collaboration by a Coordination Committee comprising the three-member DDI directorate and the DDUV president.

Coordination Committee

Directorate of de Duve Institute aisbl (DDI)
Benoit Van den Eynde, Director
Jean-François Collet, Co-Director
Miikka Vikkula, Co-Director

President of de Duve Institute – UCLouvain (DDUV)
Sophie Lucas

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Ludwig Cancer Research

Ludwig-supported research groups are hosted at de Duve Institute.

Director

Benoit Van den Eynde

Research Groups

Stefan Constantinescu
Benoit Van den Eynde

Support

Pierre-Olivier Seroen (Administrator)

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ARES	Académie de Recherche et d'Enseignement Supérieur
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erc	European Research Council
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NIH	National Institutes of Health, USA
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Wallonie	Région Wallonne
télévie	Télévie
UCLouvain	Université catholique de Louvain

WELBIO Laboratories

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Confocal imaging of lipid domains at the surface of living red blood cells (imaging platform, Tyteca's group)