Interview

"LIGAND-BASED PHARMACODELIVERY IS A SIMPLE, POWERFUL CONCEPT"

PROFESSOR DARIO NERI
Lecturer at ETH, Zürich
CEO/CSO Philogen

One of Dario Neri's 'magic bullets' against cancer formed the basis of the Swiss-Italian pharmaceutical company Philogen. When its first product reached phase III clinical trials, Neri decided to join the company, run by his two brothers, full time. "It was a tough decision to shut down my own ETH-lab, but I wanted to bring my ideas to the market myself."

It takes Dario Neri just one slide to explain his ideas on how to beat cancer with crystal clarity. The slide shows PET-scans of four different patients with cancer, each treated with a different generation of radioactively labelled drugs.
The first and oldest scan is of a patient with lung cancer treated with a common cytostatic. The drug is visible in the liver, the spleen and urine, but at no moment in time there is a preferential accumulation around the tumor site. The second scan shows a patient with colorectal cancer treated with an IgG as the targeting agent. Neri: "We like to think of IgGs as magic bullets, but it took seven days to see some accumulation."
The third patient suffers from breast cancer and has liver metastases. She is treated with one of Philogen's therapeutic antibodies. After one day, the tumor-to-organ ratio of the drug was a good ten to one. Yet, the scan of the fourth patient shows what Neri is really aiming for: ultra-fast, preferential uptake of the anticancer drug at the tumor site. "Here we used a small organic ligand for targeting. It was selected from one of our DNA-encoded chemical libraries developed for targeting breast tumors. Within one hour we saw accumulation in the primary tumor and in bone and liver metastases. We probably set a world record." ▶

Contents

Your great-grandfather was a drug developer; he was the very first to treat a patient with anti-anthrax anti-sera. Did you want to follow in his footsteps when you were young?

Actually, I followed into my father's footsteps. He advised me to become a chemist like him. At that time, it was not something I was particularly enthusiastic about, but I never was a rebel. However, from the minute I got involved in synthesizing drugs during my studies in Pisa, I became very passionate. Organic chemists are often driven by the synthetic challenges, but I 'm in it for the medicines, for the cure. During my chemistry studies, I was always learning as much biology as I could. In retrospect, the advice of my father was pretty well chosen.

"Don't hold back, there are no stupid questions"

At some point, you became interested in immunology, too.

I did a PhD with Kurt Wüthrich on solving protein structures by NMR. I thought that learning about target proteins, would provide the knowledge to design better drugs. By the time I finished, I was convinced that proteins were highly interesting as drugs themselves. Therefore, I went to Sir Gregory Winter lab in Cambridge who was developing therapeutic antibodies. Later, I kind of went back to my roots. I discovered that small organic molecules can achieve many of the things that antibodies do, and I developed DNA-encoded chemical libraries to select them. Throughout my career I've focused on only one thing: ligand-based pharmacodelivery, delivering the drug at the site of the disease. It's a very powerful, simple concept which dates back to the vision of Paul Ehrlich of the 'Zauberkugel' or 'magic bullet'. It provides my challenges in science: finding the best target at the tumor site and the best ligand to deliver the drug.

Philogen was founded in 1996 by two of your brothers and yourself. Was it always your ambition to become an entrepreneur?

No, not at all, but in 1996 the opportunity came along when my colleagues and I discovered the first antibody that could selectively recognize tumor blood vessels. I realized the enormous potential for drug development. My brothers have a background in economics and biotechnology, and they have run operational activities for many years. I was their scientific adviser, working full-time as a university professor at ETH Zürich. When Philogen's products reached phase three clinical trials I had to make a decision. I could have had others running the growing activities, but I felt an urge to bring these findings to the market myself. Therefore, I decided to close my lab at ETH, not an easy decision as a full professor at 57.

Fortunately, ETH was kind enough to let me keep the title and I really enjoy still teaching there. Recently, I set up a new module on translational cancer research for PhD students from ETH and the University of Zürich, with teachers from both academia and industry. Roche and Novartis contribute too. We shouldn't have this distinction between academia and industry; they are two entities that go in the same direction.

You don't feel you've left fundamental science?

No, at Philogen we publish twenty to thirty articles every year, probably more than I used to publish in academia. We discover prototypes, we test them, we patent, we publish, and when prototypes are good enough, we move them to the clinic. I spend most of my time doing research with incredibly good scientists; there is little difference in my experience. The pressure, however, is bigger in industry. Investors constantly look at your performance, and your products need to be better than the standard of care. Yet, you can't do more than ensuring your science is solid. There is always a risk that you can cure a mouse, but not people. Hopefully, we will see as little as possible of that risk at Philogen, but one has always to be ready for failure.

"Academia and industry are two entities that go in the same direction"

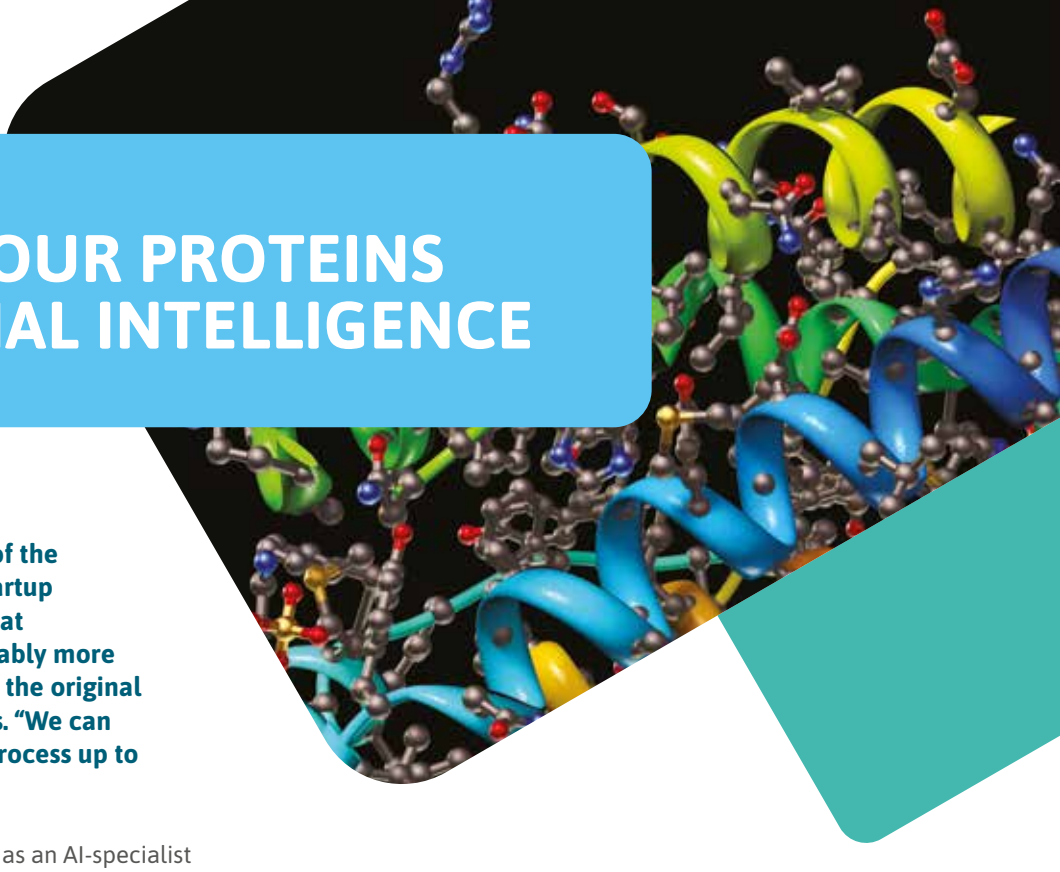
Phylogen has multiple clinical trials running. These are exciting times?

Yes, we have completed successfully, phase three clinical testing in stage three melanoma for one lead compound and also good data in phase two trials in non-melanoma skin cancer. Another product is being investigated in registrational trials in soft tissue sarcoma and in glioblastoma multiforme. These pivotal trials will read out in 2024 and 2025.

You've had two Nobel prize winners as teacher, and you are a known scientist and entrepreneur yourself. Can you share some tips on how to become a successful researcher?

Technologies will come and go in science. The fundamental challenge in research, certainly in pharmaceutical sciences, is to ask the important question: what do I want to do? The follow-up question is of course: how do I do it? If you don't know a technology, you need to learn it or develop it. We cannot be competent on everything, but you need to have the courage to ask. Don't hold back, there are no stupid questions. Last, but not least: go for clean experimental data. Only clear-cut answers are a good basis to build science on. I believe, you can't go wrong when your science is solid. ■

IMPROVING YOUR PROTEINS WITH ARTIFICIAL INTELLIGENCE



'Design better proteins' is the credo of the Swiss-Dutch company Cradle. The startup develops artificial intelligence (AI) that generates sequences which are probably more stable, active, or selective variants of the original enzyme, antibody, and other proteins. "We can accelerate the protein engineering process up to tenfold."

"One of my cofounders at Cradle worked as an AI-specialist at Google where optimizing advertisements is an important application. He wanted to make a more positive impact on the world with AI," says biochemical engineer Elise de Reus. She was one of five who established Cradle two and a half years ago. "I was working in Silicon Valley for biotech companies. They run more and more high throughput experiments, but still struggle hard to actually bring products to the market. Then I learned about AI and met people who were convinced that machine learning could come up with better designs. That led to the founding of Cradle."

Machine learning

When a company wants to improve the activity, stability, or specificity of a particular protein, they can upload its sequence at Cradle's platform. This generative AI model will then provide suggestions for improvements. De Reus: "Machine learning is excellent in optimizing multiple properties at the same time. So, you can ask for more stability and more activity, too."

Cradle, for example, supported the engineering of a P450 enzyme, typically a very challenging enzyme. The customer provided data from more traditional design methods to train cradle's models. The algorithm found still headroom within the mutations tested, and also provided completely novel mutations. De Reus: "The project progressed three times faster towards their goal."

Company researchers can download the suggestions and test them in their own lab in their specific assays. The results are fed back into the platform so that the models learn round over round and improve. Customers pay for the services based on annual subscription. The startup doesn't demand royalties or any IP of discoveries and information that enters the platform stays private.

Data is still the 'secret sauce' in protein design, emphasizes De Reus. "You can't find protein structures and functions on the internet as easily as labelled cat pictures. We have quite a large machine learning team doing really extensive research in making models that can learn from smaller data sets."

Biomedical scientist Richard Dela Rosa joined Cradle about nine months ago and works on Cradle's own wet lab. He runs high throughput experiments that are used to continuously improve the generative AI models. "Training sets needed to contain tens or hundreds of thousands of data points, but our current machine learning models only really requires about 96 points to be optimized."

Open to cooperation

Cradle's customers are drug developers or biotech companies. That was an important reason for Dela Rosa to apply for a job. "It's very important to me to work on creating sustainable products rather than creating more problems to society." For academics Cradle's services are free for non-commercial use. Dela Rosa: "We're open to cooperation with scientists who want to optimize a cytokine or antibody, but also to other immunotherapeutics and interesting proteins."

De Reus never envisioned herself as an entrepreneur, but she describes it as 'the best job'. "It's very special to work on a project that we care so deeply about. Moreover, Cradle is unique in combining different disciplines: artificial intelligence, protein sciences and software design. The only way to grow is to be extremely curious about what is happening in the other domains, to share ideas and give feedback. We all feel that no one holds the whole truth in his or her head." ■

E team@cradle.bio
W www.cradle.bio

How bacterial metabolites influence Parkinson's disease

PhD project: Targeting metabolism of the enteric glial cell in Parkinson's disease

The enteric nervous system possibly plays a key role in Parkinson's disease, making it a target for possible treatment. Anastasia Markidi at Utrecht University set studies the effects of bacterial metabolites on enteric glial cells.

Parkinson's disease (PD) is not limited to the brain, as we have known for a while. It is a widespread neuronal disorder involving not only the central nervous system. Increasing evidence also points to the gut-brain axis which connects the central and enteric nervous system (ENS). Inflammation in the ENS can trigger PD, scientists believe.

To study the enteric nervous system (ENS) a model system, which was developed recently, is most suitable. This 'ENS on a dish' uses embryonic stem cells (hESCs) which are induced to become enteric neurons and glial cells. The model system is highly suited to study the immunometabolism of the enteric glial cell. A team consisting of Paula Perez Pardo, Aletta Kraneveld and Celia Berkers applied for an ICI grant to set up this system and perform the study.

Anastasia Markidi was doing her master's in the same group. Coming from Greece where studying pharmacology usually means you will run your own pharmacy eventually, she was more interested in immunology and neuroscience. She jumped at the chance to study these fundamental aspects of Parkinson's disease in the ICI PhD project. "I found the proposal really interesting, and they were doing exciting experiments in the lab."

Anastasia Markidi
Utrecht University
Pharmacology, Aletta Kraneveld and Paula Perez-Pardo group
Veterinary medicine, Metabolomics, Celia Berkers group

ENS on a dish

Anastasia started with reproducing and testing the protocol to make the ENS on a dish. She then managed to isolate the enteric glial cells that she wanted to study because these play an important role in neuroinflammation and neuronal function. She was able to model the activation of these cells and cause inflammation by exposing them to proinflammatory cytokines.

Based on earlier results, the researchers hypothesized that particular acids such as butyrate and acetate that bacteria produce could prevent inflammation. Adding these compounds indeed stopped the induced inflammation. To know more about the fate of these acids, Anastasia labeled them with ^{13}C and traced them with metabolomics techniques. "It was really cool to see where they end up in the cells."

"It was really cool to see where they end up in the cells"

Next, Anastasia wanted to model a more realistic situation. Parkinson's disease is a chronic condition characterized by a chronic, low-grade inflammation. "We therefore exposed the cells to a lower dose of cytokines for longer," explains Anastasia. She expects the results of this study soon.

Alpha-synuclein aggregates

For the last year of her PhD, Anastasia has yet another project planned. Exposure of the ENS to bacterial curli proteins might induce the misfolding of alpha-synuclein, one of the protein aggregates that are associated with Parkinson's disease. These aggregates are then believed to spread to the brain much like prions. The project has just started, and Anastasia hopes to get results before her PhD ends. "If we see alpha-synuclein aggregation after long term exposure to curli, along with immunometabolic changes in the ENS cells, that would be of great interest in our field. But I'm already satisfied with everything that I have done so far."

Working in both a pharmacology and a metabolomics group has taught her the best of both worlds, Anastasia says. "My cells are here in the pharmacology department, but I move between the two labs and I've learned a lot from both." ■

Chemical probes to study triggers of Alzheimer's disease

PhD project: The role and effect of pathogenic amyloid proteins in the central nervous system

Enebie Ramos Cáceres is a jack of all trades: his PhD project comprises chemistry and immunology and he is doing all that on his own. He not only develops chemical probes to study key enzymes related to NETosis, relevant to Alzheimer's disease, but he also collects, prepares, and studies human blood samples to test his probes.

Enebie moved from his homeland on the Spanish island of Gran Canaria to The Netherlands to be with his Dutch partner in 2016 and was lucky to find that the first Molecular Life Sciences study in English had just been launched in Nijmegen. "I studied chemical engineering at home, which was 90 percent engineering. I wanted to study more chemistry and I've been fascinated by biology since I was a kid. So, the study in Nijmegen was perfect."
He went on to get a master's degree in molecular life sciences with (among others) Kim Bonger, who offered him a PhD position right after. "I knew little about immunology, but I took a leap in the dark. To make chemistry useful to understand life is the most fascinating thing to me."

Exploding cells

Enebie studies a relatively newly discovered way that neutrophils (white blood cells that play a crucial role in the innate immune system) attack pathogens. In a process called NETosis, the entire neutrophil explodes and spreads its contents to form extracellular fibers that trap and kill pathogens. But, as Enebie explains: "We don't know how NETosis affects the rest of the body. In large amounts it may do damage. And we don't have a lot of ways to study the process which involves a lot of enzymes. I was hired to make tools to study the process of NETosis in live cells and tissues."
Enebie developed and synthesized probes with a fluorophore that only lights up when it is bound to a specific enzyme. While such probes were known, these are the first ones that are applied to NETosis-related enzymes. The first paper is about to be published, in which the researchers show that their probes can be used to detect myeloperoxidase enzyme activity in living cells under wash-free conditions.
A key step is to test the probes on human blood samples as opposed to standardized cell lines. Enebie learned how to collect and prepare human donor blood for this. To convince potential healthy donors he promises them an image of their own white blood cells. "People love it! It really helps," claims Enebie. "I'm proud of the images, they look very cool. The probes help to see that there is something actually going on. It is regular confocal fluorescent microscopy but if you pay attention, you can take a very nice picture. And a picture is worth 1000 words."

Studying brain tissue

Over the next months, using the probes he developed Enebie will study the interaction of neutrophils with amyloid proteins, which form aggregates and plaques in the brain and are associated with Alzheimer's disease. Some amyloids are shown to trigger NETosis, but the question is how much and via which pathways. "Ultimately my goal is to use the probes to study brain tissue from deceased Alzheimer's patients."
Even though Enebie does not have an ICI partner to cooperate with, which he regrets, he's grateful for the program ICI offers. "The workshops and courses and conferences are very good; I have attended most of them. I met a lot of people and have contacts that I would not have had without ICI." ■

"To make chemistry useful to understand life is the most fascinating thing to me"

Enebie Ramos Cáceres
Radboud University Nijmegen
Synthetic Organic Chemistry
Kim Bonger group

Ubiquitous glycolipid reveals its role in broad immunization against influenza

Early 2024, a remarkable publication appeared in Science in which Florian Winau at Harvard and his team describe how they discovered the function of a specific glycolipid called Gb3 found on B cells. From their results, they conclude that Gb3 has the potential to act as an adjuvant to improve vaccines against viral infections such as influenza.

Florian Winau, an associate professor of Pediatrics at Harvard Medical School (US) studies 'basic mechanisms that explain how the immune system functions'. As can be read on the groups' website, they have a strong interest in identifying targets for possible translation to future immunotherapies. But if therapy is not an option, vaccination is a strategy to control infectious disease.

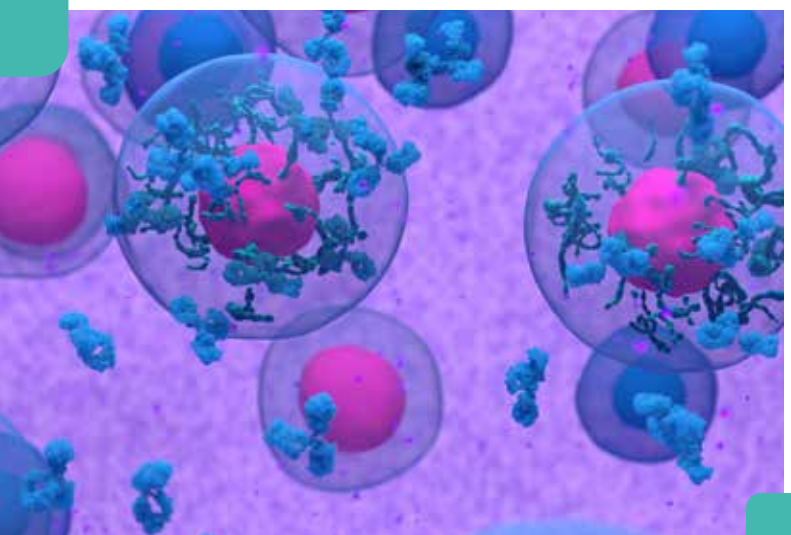
Vaccines induce the production of antibodies. These should not only have high affinity and broad reactivity but also be diverse to ensure their protective action when viral strains mutate. B lymphocytes that function in the humoral immunity component of the adaptive immune system, are recruited to the so-called germinal centers where they mutate their antibody sequences and, in this way, promote diversity and antibody affinity maturation. A type of glycosphingolipid called globotriaosylceramide (Gb3) is abundantly found on the surface of these cells, but the researchers noted that it was unknown what role they play in the function of the B cells.

Mouse models

The researchers used genetically modified mouse models to investigate several steps in the process of B cell maturation and mutation (somatic hypermutation, B cell receptor signaling, GC B cell cycling, and MHC-II presentation) to understand the impact of the lipid Gb3 on antibody affinity and diversity.

They found that in a first step, Gb3 binds to the plasma membrane glycoprotein CD19. Binding detaches it from its chaperone CD81 allowing it to help bring the B cell to its receptor. Additionally, the researchers found that without Gb3 several other steps in the cascade could not take place such as FOXO1 degradation. This ultimately leads the researchers to conclude that "without Gb3, B cells were not able to undergo affinity maturation in germinal centers and failed to produce high-affinity antibodies." Additionally, they found that Gb3 amplifies antibody diversity by exposing subdominant epitopes that are not normally recognized in the immune response.

▼ B cells that function in the humoral immunity component are recruited to the so-called germinal centers where they mutate their antibody sequences and, in this way, promote diversity and antibody affinity maturation. (Photo: B cells producing antibodies)



> *"They shed a fundamentally new light on the regulation of B cell response where ceramides play a role"*

René Toes, immunologist at LUMC and not involved in this research, noted another interesting result in the publication: "Gb3 also increases type I interferon signaling. Although it is not described how this works, interferon signaling improves B cell response and therefore antibody response. This is relevant because interferon signaling is important in many other (auto)immune diseases such as lupus."

Finally, the researchers hypothesized that Gb3 could enhance the efficacy of influenza vaccines. They administered Gb3 to knockout mice and investigated the uptake of Gb3. They found that Gb3 was incorporated in the membrane of B cells and observed that this "promoted broadly reactive antibody responses and cross-protection" concluding that Gb3 could be an adjuvant in influenza vaccines.

Fundamental and applied

Toes is impressed by the complexity of the research combining chemistry and immunology, and the unexpected result. "They shed a fundamentally new light on the regulation of B cell response where ceramides play a role that was unexpected to me. The combination with the first application in a vaccine which showed the incorporation of Gb3 into the B cell membrane is remarkable. There is a lot of work ahead to apply this to human vaccines, but it is a good first step." ■

Reference

Pankaj Sharma, Xiaolong Zhang *et al.* **The lipid globotriaosylceramide promotes germinal center B cell responses and antiviral immunity**, Science 383, 720 (2024). DOI: 10.1126/science.adg0564

How the common cold virus binds to its host cell

Remarkably, we don't know everything about the common cold virus HKU1, part of the coronavirus family. A research team at Utrecht University recently not only proved where and how HKU1 binds to the host cell, but also showed how this binding triggers a remarkable conformational change of the spike protein that is fundamental to how this virus enters the cell. The results were published in Nature last October.

The common cold virus is part of the coronavirus (CoV) family with their characteristic spikes. All CoV spike proteins, that play an important role when a virus enters a host cell, are homotrimeric class I fusion proteins. These have three identical units of polypeptide in the so-called S1 domain that mediates receptor binding. The three units can be buried between their neighbors on the protein, called the closed state. Or they rotate and are sticking out, called the open state.
In this state they can bind to a receptor but are also vulnerable to antibody attack. For SARS and MERS CoV for example, we know that the spikes can spontaneously switch between a closed or open configuration, balancing host cell attachment and immune evasion. However, most CoVs, notably the common cold CoV HKU1, appear to be tightly closed. How then, does it invade a host cell?

Modeling the spike protein

ICI researcher Joost Snijder, co-author of the Nature publication, specializes in protein-glycan interactions. Recently, it was found that the HKU1 spike targets specific sialic acid receptors: α2,8-linked 9-O-acetylated disialosides which are glycan motifs typical of oligosialogangliosides such as GD3. Snijder explains: "We knew from Geert-Jan Boons' work that HKU1-A binds to sialic acid, but we were not sure if this would be the receptor and where it would bind. That would give us crucial information on how this virus infects the cell."
Snijder and his team set out to investigate receptor binding of HKU1. "We cooperated with Daniel Hurdiss (Veterinary virology at UU) who is an expert on cryogenic electron microscopy of viral proteins. The challenge was producing sharp high-quality EM-reconstructions to build atomic models, which has been the work of ICI postdoc Matti Pronker, and aligning those with the results of molecular dynamics simulations." This resulted in the most detailed structure of the unbound HKU1-A spike protein so far.

Binding triggers opening

But the real surprise for Snijder came after incubating the HKU1 spike protein with the disialoside receptor analogue. They observed a remarkable conformational change in the spike protein after binding. They identified two more distinct conformations in addition to the closed state: a partially opened state with a single S1^{B} domain rotated upwards and a fully opened state with three S1^{B} domains up. These domains are located at the other end of the protein at a distance of 40 Å from the disialoside receptor. Snijder: "We did not anticipate this large conformational change so far away from the binding site. That is remarkable. This mechanism is fundamental to how this virus enters the cell."

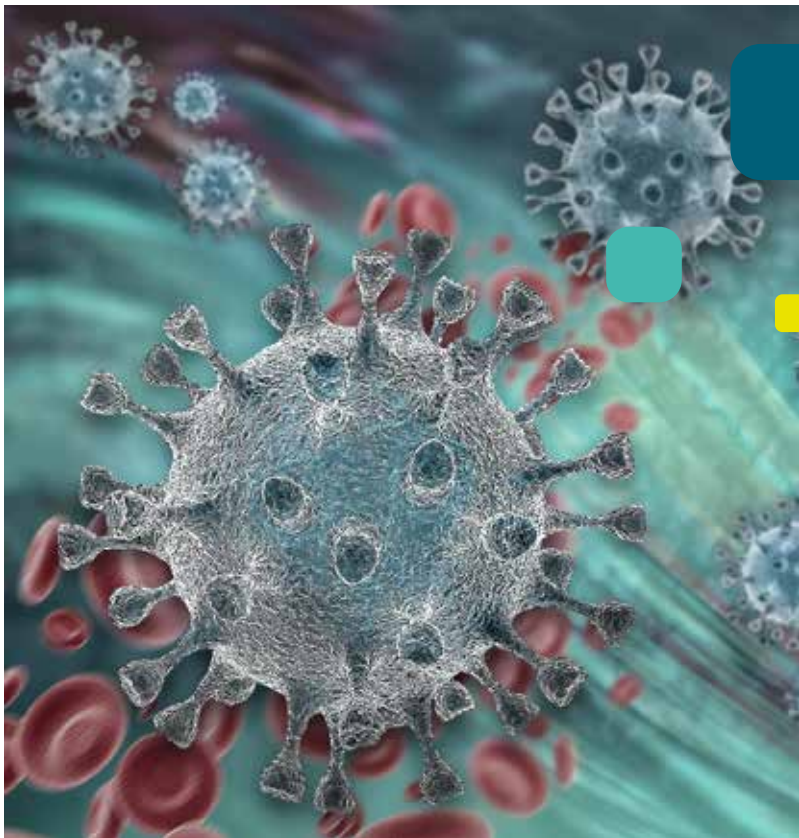
"This mechanism is fundamental to how this virus enters the cell"

A question mark in the publication remained the identity of the second receptor to which the opened S1^{B} domains would bind. Demonstrating the importance of this research, within weeks other researchers had published the answer to that question: the receptor is TMPRSS2, a usual suspect in CoV research. "We would have investigated that ourselves, but this saves time," smiles Snijder. "The important thing is that our work has contributed to the knowledge on how to study this virus. Others can take advantage, which helps the field to progress." ■

References

Pronker, M.F., Creutznacher, R., Drulyte, I. et al. **Sialoglycan binding triggers spike opening in a human coronavirus**. Nature 624, 201–206 (2023). doi.org/10.1038/s41586-023-06599-z

▼ *Coronaviruses exhibit spike protein, also known as the S protein, on their surfaces; S is a class I fusion protein and is responsible for mediating viral entry as the first step in viral infection. (Photo: Coronavirus in human blood)*



Studying similar pathways with different focus

The enemy may be your friend in autoinflammatory disease

Supported by the Kennedy Trust, LUMC scientist Annemarthe van der Veen and Pierre Maillard of the London Blizard Institute are studying virus-derived peptides as a potential therapy for autoinflammatory diseases. "Viruses try to repress the immune system. We want to hijack their tricks to inhibit interferon production selectively."

Dutch biomedical scientist Annemarthe van der Veen and Swiss virologist Pierre Maillard met each other about ten years ago when they were postdocs at the London Research Institute (now part of The Francis Crick Institute). Van der Veen: "We were working on related projects and often had useful discussions and helped each other out." They have stayed in contact ever since and followed a similar path in academia that led them both to the position of group leader today.
Maillard's research group at the Blizard Institute tries to understand how a virus is detected, which defense mechanisms are triggered and how cells destroy the intruder. Maillard: "Viruses constantly evolve to escape or to block these defense mechanisms in order to survive and replicate." Therefore, the evading and thwarting tactics of viruses are another important topic in the Maillard group.
Van der Veen's group at the LUMC focuses on autoinflammatory diseases caused by overactivation of the innate immune system, including a group of genetic disorders called type I interferonopathies. In autoinflammatory diseases, body's own proteins or nucleic acids are mistakenly seen as foreign and dangerous, resulting in unnecessary and harmful inflammation.
"We have a different focus, but study similar pathways," says Van der Veen. "Pierre is looking at inflammatory pathways from the perspective of a virus; my approach is from the perspective of autoinflammation, or sterile inflammation, as we call it."

"We initially focus on providing a proof of principle"

Pilot study

Talking about their work at a conference, the scientists came up with the idea to put a viral 'trick' to beneficial use. Could a viral protein be used to subdue the overresponse of the innate immune system in autoinflammatory disease? The two scientists wrote a proposal that received a Research Ignition Award of the Kennedy Trust for Rheumatology Research. Currently, a PhD student (Leiden) and a postdoc (London) are jointly working on this project.
The scientists chose the rare, inherited autoinflammatory disease Aicardi-Goutières syndrome (AGS) for their pilot study. During viral infections, the detection of viral RNA by innate immune sensors triggers the production of type I interferons (IFNs), which are cytokines with antiviral function. This results in inflammation. Due to a genetic mutation, people with AGS continuously produce type I IFNs although no virus

is present. The mutation causes a failure to discriminate between self and non-self nucleic acids and consequently autoinflammation.
Van der Veen and Maillard want to copycat the ability of some viral proteins to reduce IFN production, by synthesizing a virus-derived peptide that interferes with certain receptors. Together, the scientists designed a small viral peptide which is still large enough to be functional. They considered questions such as: what is the optimal length, where should the peptide stop and start exactly, and should it be linear or cyclic? The actual synthesis is performed in Leiden where ample support and experience in peptide synthesis is available. Chemistry comes in to add click handles to the peptide for attaching a track-and-trace fluorophore and to add a peptide to ease transport across the plasma membrane of cells in an *in vitro* model of AGS.

Receptor specificity

Maillard: "Annemartha's lab has the tools and the model system to accomplish all that. In London, we focus on studying how this peptide works exactly. Which receptor does it bind to block IFN production and how? We suspect that it counteracts particular receptors, but we will need to verify that hypothesis."

The Maillard Lab

Research in the Maillard Lab aims to understand the various and complex mechanisms by which cells defend themselves against viruses. The lab is headed by Swiss virologist Pierre Maillard and is part of the Centre for Immunobiology at the Blizard Institute. The Blizard is part of the Queen Mary University of London which ranks 14th in the world for research quality. The Maillard lab studies how viruses trigger different defense mechanisms and how the various pathways are regulated. During replication, some viruses generate long double-stranded RNA (dsRNA). In vertebrates, including humans, dsRNA is sensed by receptors which initiate a signaling cascade resulting in the secretion of type I interferons (IFNs). These IFNs stimulate the expression of hundreds of genes involved in inhibiting virus replication. Recent evidence suggests that mammals also use another defense system triggered by viral dsRNA called antiviral RNA interference. This defense mechanism was previously thought to be used by plants and invertebrates exclusively. The Maillard lab is currently characterizing its role in mammals.

The target receptors (LGP2 and MDA5) are part of the innate immune system. They are normally activated by viral RNA, but in AGS they are triggered by endogenous (self) RNA, leading to unwanted IFN production and auto-inflammation. A protein of Paramyxovirus, a virus family that includes measles virus and mumps virus, is known to bind to LGP2 and MDA5 during a viral infection. This V protein shields the receptors to obstruct their alarm function as RNA sensors.

"We want to know everything about antiviral defense"

Receptor specificity is an important aim in the pilot study. A recent study showed that patients with AGS who are treated with inhibitors that block IFN pathways completely, are doing better in terms of inflammation, but suffer from infections because they become immunocompromised. Van der Veen: "With our strategy, we hope we can inhibit the receptors involved in IFN production more selectively, leaving some immune defense pathways against pathogens intact."

Next step

The grant of the Kennedy Trust is for two years. "A short period in which we want to provide proof of principle," says Van der Veen. That means synthesizing a small 'viral' peptide that indeed blocks IFN production in AGS models. "Thereafter, we could take the next step: writing a larger grant proposal to study these peptides in *in vivo* models." Ultimately, the peptides may hold clinical value for the treatment of AGS and possibly other autoinflammatory and autoimmune diseases in which IFNs are produced unwantedly such as type I diabetes or rheumatoid arthritis. Van der Veen: "In the bigger picture, this is a new treatment strategy for immune related disorders, one of the central goals of ICI." ■

Research Ignition Award

In 2023, the Van der Veen lab at the LUMC and the Maillard group received a Research Ignition Award (£100.000) from the Kennedy Trust for Rheumatology Research. The funds will be equally split between the collaborating labs. The Research Ignition Award is aimed to stimulate new and ambitious avenues in the field of inflammatory disorders. Van der Veen and Maillard use the support for piloting a new strategy: using viral peptides to subdue IFN production in autoinflammatory disease.

Data analysis is not as hard as it looks

Omics data sets are an increasingly important part of the research of wet-lab cell biologists and immunologists. But they are usually not equipped with enough bioinformatics or coding skills to be able to analyze these data sets. A first for PhD students in the ICI program, this spring they had the opportunity to participate in the online training 'Omic data analysis and visualization using R' which focuses precisely on these skills.

The course is developed for wet-lab scientists with no prior experience in coding. It is part of a wider bioinformatics training program, developed and delivered by bioinformatics specialist John J. Cole, manager of the Glasgow Bioinformatic Core at the University of Glasgow. He explains: "Anyone can do omics experiments nowadays. You just buy a kit and hire a company to produce the data for you. But the analysis of these data can take months. Labs can't afford to pay anyone to do that. Also, the wet-lab scientist knows best what to look for in their data. But without the skills to do the data analysis themselves, these labs get stuck."
The training focuses on R, a computer language and software package for statistical computing and graphics such as omics data analysis. The course starts with an introduction into R.

"The aim of the course is first of all to take away the fear of data and programming"

▼ Last March a dozen ICI PHDs and ICI researchers participated the on-line course 'Omic data analysis and visualization using R'. The course is given online several times a year by bioinformatics specialist John Cole from the Glasgow Bioinformatic Core (University of Glasgow).
https://www.immunology.org/events/omic-data-analysis-and-visualisation-using-r-0

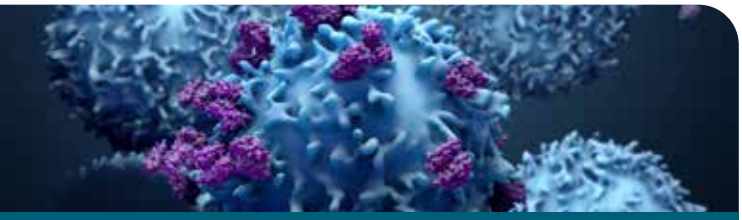
Using a provided data set, the students learn step by step to write a script to open and analyze the data set. The next step is to produce various kinds of analyses and plots such as a PCA analysis and an MA plot. In the end, the students have their own scripts and the skills to analyze any data set.

No fear

The aim of the course is first of all to take away the fear of data and programming, explains Cole. "We offer a structured program where we don't just throw bits at people but really explain how the different analyses and plots work."
This certainly worked for ICI principal investigator Sander van Kasteren who made the course available to ICI researchers: "I have no talent for programming, but this training was exactly what I hoped it would be. The basics are not as scary as I thought. I can now judge the quality of analyses in my own work and others and avoid pitfalls." One of the pitfalls is to keep looking until you find something, says Van Kasteren. "John explained that your data might not offer what you want. It is important to stick with the research question and analysis that you identified as the best up front and then stop."
Eva George Matlalcuatzi was one of the ICI PhD students who participated. She said: "I wanted to do more in-depth statistical analysis of my proteomics data using more common software like R. Also, it will help me to increase my capabilities on coding. I liked that it considered everything from simple coding to organize data until large and more complicated data management and different plots."
Anyone can sign up for this training, which gets extremely high reviews from participants worldwide, over 1200 so far. Van Kasteren hopes that in addition to PhD students more principal investigators will invest the time in this training. "The invested time was definitely not wasted. I use the knowledge on datasets for my own papers and when I'm reviewing others. And John is a great teacher who knew exactly what we did not know." ■

Millions in subsidies for 'brain atlas'

The Institute for Chemical Neuroscience (iCNS) will receive 23.23 million euros from the government as part of the Gravitation program. The money will be used for the development of a 'brain atlas' of psychiatric symptoms. Neurobiologists, chemists, psychiatrists, and data scientists work closely together on human brain tissue. Young scientists are trained in combining chemistry, artificial intelligence, and neurobiology.



'T-cell receptor challenge' awarded a grant

The MATCHMAKERS team, who submitted the 'T cell receptor challenge', is one of the five new global teams that are going to receive up to $25M in Cancer Grand Challenges funding over five years. The Netherlands Cancer Institute with Ton Schumacher's group is one of the MATCHMAKERS team members. This consortium aims to decipher the T-cell receptor cancer-recognition code. By harnessing advances in high-throughput approaches and computational prediction, the team will take an integrated approach to understand and predict how T cells recognize tumors, paving the way for personalized immunotherapies.

Two-day anniversary symposium Hecklab

Past April 18th and 19th a symposium had been organized around the theme "Celebration of Biomolecular Mass Spectrometry and Proteomics". This two-day symposium combined Albert Heck's 25 years of employee ship at the UU and his 60th birthday this year. Besides alumni, fellow researchers, and students at the Hecklab the festive symposium was visited by an internationally group of scientists from the field of mass spectrometry and proteomics.

Recent publications

Jäger E et al

pH and ROS Responsiveness of Polymersome Nanovaccines for Antigen and Adjuvant Codelivery: An In Vitro and In Vivo Comparison

Biomacromolecules. 2024 Mar 11;25(3):1749-1758. doi: 10.1021/acs.biomac.3c01235. Epub 2024 Jan 18. PMID: 38236997

Becker AMD et al

Inhibition of CSF-1R and IL-6R prevents conversion of cDC2s into immune incompetent tumor-induced DC3s boosting DC-driven therapy potential

Cell Rep Med. 2024 Feb 20;5(2):101386. doi: 10.1016/j.xcrm.2023.101386. Epub 2024 Jan 18. PMID: 38242119

van Weijsten MJ et al

Effect of Antigen Valency on Autoreactive B-Cell Targeting

Mol Pharm. 2024 Feb 5;21(2):481-490. doi: 10.1021/acs.molpharmaceut.3c00527. Epub 2023 Oct 20. PMID: 37862070

Spruit CM et al

Contemporary human H3N2 influenza A viruses require a low threshold of suitable glycan receptors for efficient infection

Glycobiology. 2023 Oct 30;33(10):784-800. doi: 10.1093/glycob/cwad060. PMID: 37471650

Peng W et al

Reverse-engineering the anti-MUC1 antibody 139H2 by mass spectrometry–based de novo sequencing

Life Science Alliance Mar 2024, 7 (6) e202302366; DOI: 10.26508/lsa.202302366

Gerrits L et al

Tailoring of Physical Properties in Macroporous Poly(isocyanopeptide) Cryogels

Biomacromolecules. 2024 May 14. doi: 10.1021/acs.biomac.4c00086. Online ahead of print.
PMID: 38743442

Kaiser FK et al

Filamentous fungus-produced human monoclonal antibody provides protection against SARS-CoV-2 in hamster and non-human primate models

Nat Commun 15, 2319 (2024). doi.org/10.1038/s41467-024-46443-0

Column

THE BIRTH AND DEVELOPMENT OF THE INSTITUTE OF CHEMICAL IMMUNOLOGY

SJAAK NEEFJES
ICI EXECUTIVE BOARD

Professor of Chemical Immunology at Leiden University

Hermen Overkleeft and I were drinking a beer some 12 years ago and discussed the option of starting a new Gravity program. Chemical Biology was a field under development but the applications of chemistry within the Immunology domain was hardly considered. We invited a strong founder team consisting of Piet Gros, Carl Figdor, Ton Schumacher, and Albert Heck with the aim to establish a Chemical Immunology Society. The first attempt failed, but the second succeeded and was the start of the ICI Institute 10 years ago!

A lot has happened the last 10 years! Many research projects performed by 'scientific twins' were started integrating chemistry and immunology. In almost all cases it resulted in original research simply by using inspiration, tools, and applications from both fields. It turned out to work. ICI can be proud for the development of a *'new school of thought'* resulting in many great and important scientific breakthroughs and publications. And various new research groups have been founded based on ICI support. An all time low was the passing away of our friend and colleague Huib Ovaa. Huib was unique and a beloved member of the ICI society. His work was superb and his contributions to research involving chemistry, biology and immunology will be everlasting.

And now? ICI will be completed after the 10 years investment. Hermen and I passed on the baton to the next generation of chemical immunologists. Mission accomplished: chemical immunology is now established with even two professor chairs in Chemical Immunology in Leiden! The gap between immunology and chemistry is closed and the ICI trained scientists understand the different languages and apply their options in the two fields. Many results of fundamental as well as applied nature have been the result of the ICI program. Chemically modified vaccines are one spin-off of the ICI program and are moving to testing in patients. This also applies to anthracycline cancer drug variants that are also developed for testing in human patients and that never would have been developed without the ICI program.

The ICI gravity program is now completed, but that is not the end! ICI scientists have contributed to a new recently accepted Gravity program that is also interdisciplinary in nature: chemical neurobiology!
Chemical Immunology and its spin-offs move on!

About ICI

The Institute for Chemical Immunology (ICI) is a Gravitation project, made possible by the Ministry of Education, Culture and Science, in collaboration with the Netherlands Organization for Scientific Research (NWO). ICI will define and exploit a new field termed chemical immunology and train a novel generation of interdisciplinary scientists. It aims to promote academic excellence. The ICI publishes twice-yearly the 'ICI Bulletin' featuring ICI research, education and other relevant developments. To (un)subscribe please send an email to info@chemicalimmunology.nl.

Editorial Board	Martijn Verdoes, Sander van Kasteren, Sabina van der Zanden
Text and interviews	Marian van Opstal, Marga van Zundert, Bastienne Wentzel
Coordination and editing	Bèta Communicaties
Lay-out	SPRANQ, Alexander Kraaij
Photography	Thijs Rooimans, iStock image bank
Printing	Bestenzet BV, Zoetermeer

Contact

LUMC
Einthovenweg 20
2333 ZC Leiden
Tel: +31 71 5268727
ici@lumc.nl

ICI institute for chemical immunology