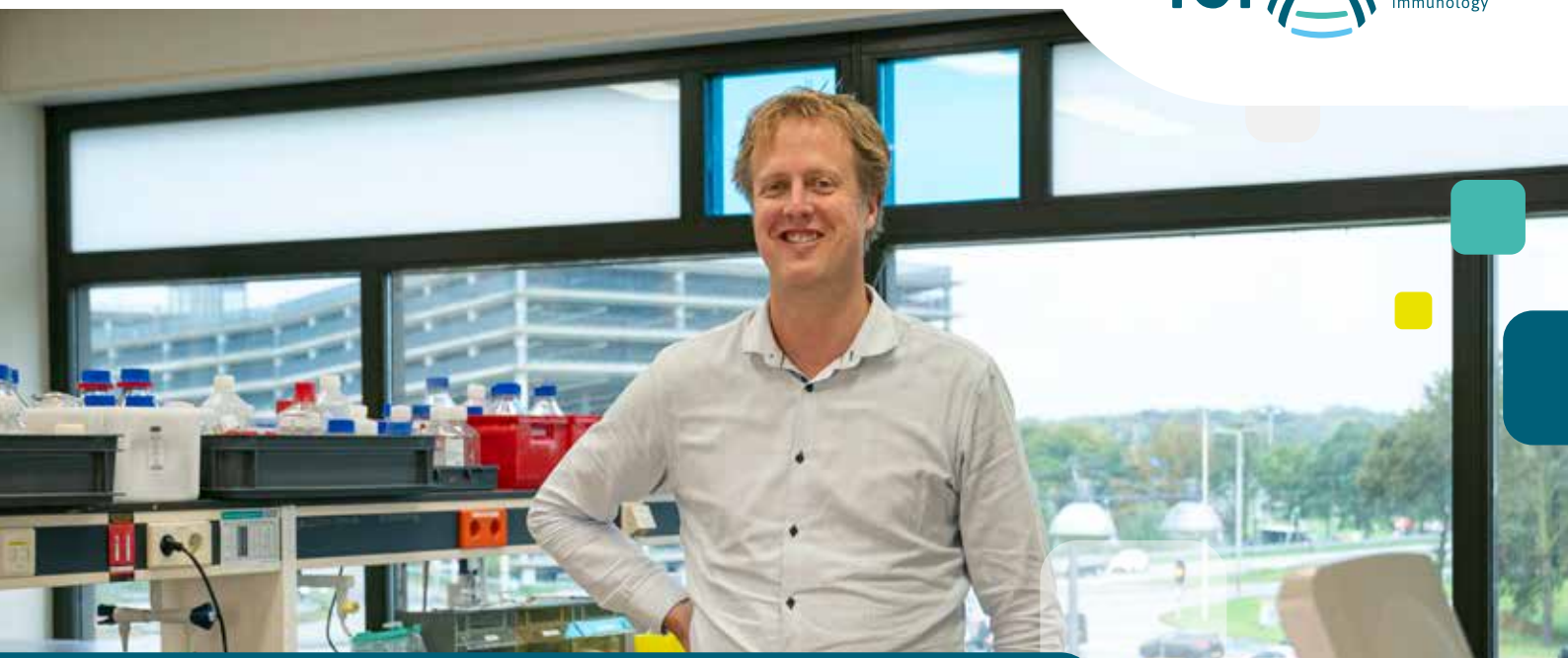
ICI institute for chemical immunology



Interview

OFF-SWITCH FOR CHRONIC PAIN MAY RESIDE IN THE IMMUNE SYSTEM

NIELS EIJKELKAMP
Associate professor Translational Immunology
UMC Utrecht

Niels Eijkelkamp, principal investigator at the Center for Translational Immunology of UMC Utrecht, has always been fascinated by the interactions between the nervous and the immune system. "It is real system biology as both cover the whole body. I find it highly intriguing for example that wound healing is influenced by stress." Eijkelkamp recent studies put forward another interaction: chronic pain may be a result of an error of the immune system.

Your research group is one of the few in the Netherlands studying the mechanisms underlying chronic pain, tells your website.

Unfortunately, that's true. Chronic pain affects more than one in five people and significantly reduces quality of life. Actually, more people suffer from chronic pain than from cancer, heart disease and diabetes together. However, chronic pain does not kill you directly. That's probably one of the reasons why it gets less attention. In the Netherlands, the main research funding agencies have not had a dedicated program on chronic pain in the past fifteen years. Internationally, there is more interest, in particular in finding non-opioid based treatments in the USA because of the current opioid crisis. Fundamental insights into the underlying mechanisms of pain may provide clues to better solutions than addictive opioids. ▶

"Studying the mechanisms underlying pain"

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How did you become interested in chronic pain?

During my PhD studies at UMC Utrecht the group's interest in inflammatory processes guided me to the topic. At that time, I was the first immunology PhD student working on pain and the subject really grabbed me. I've stayed in the field ever since and educated myself in pain mechanisms. For example, as a post-doc in the laboratory of John Wood at the University College London, specialized in the field of pain research.

Many will associate the immune system with fighting of dangerous pathogens not with pain. How does it work?

In response to viruses, bacteria or other intruders the immune system elicits an inflammatory response. Part of the process is sending signals to the nervous system to induce pain sensations. That happens, for example, in rheumatoid arthritis. Currently, the dogma in medical sciences is that upon cessation of the inflammation the pain dies away too,

"In rheumatoid arthritis, pain may persist even though there are no signs of active disease"

because this pain-inducing signaling stops. Often, however, the pain does not disappear whilst the inflammatory drivers have dissipated. In rheumatoid arthritis, for example, pain may persist even though there are no signs of active disease. We have reasons to believe that the immune system needs to actively switch off pain. When that does not happen properly, chronic pain independent of the original inflammation is the result.

Chronic pain is a result of a malfunctioning 'off-switch' in the immune system?

Yes, that is what we think. So, it's not just a question of disappearing pain stimuli or dampening pain-inducing signals. There seems to be an off-switch, a kind of reset button for pain. This is a different, new view on pain cessation. It's not a passive, but an active process. We've found evidence in animal models that macrophages are key in this off-switch. Please note, however, that we are not the only ones, to suggest that the immune system is involved in regulation of pain. Actually, several years ago evidence was shown that the cytokine interleukin 10, or lipids like resolvins reduce pain. However, the evidence we have suggests this off-switch is more than a cushioning effect. It is an active mechanism that resolves pain.

How can macrophages switch off pain?

The process involves macrophages of a specific phenotype, so-called M2-like macrophages. While M1-like macrophages are involved in maintaining pain in chronic pain models, M2-like macrophages are involved in resolving pain in transient pain models. We see these M2-like macrophages accumulating in dorsal root ganglia where the cell bodies of sensory neurons are located. When we remove these macrophages, pain does not resolve anymore. When we provide M2-like macrophages again, the pain disappears. Of course, we wanted to know what these M2 macrophages do to resolve pain. They turn out to donate mitochondria, our energy providing organelles, to sensory neurons. That macrophages donate mitochondria was quite a surprise. We checked and double checked this using genetics and pharmacological experiments, in vitro and in vivo, to be really sure. But all these experiments suggest that mitochondria from M2-like macrophages are transferred in eliminating chronic pain. When we silence the mitochondria in the vesicles in which they are transferred, the pain reducing effect is gone.

What do these additional mitochondria do?

That's what we want to know too and are currently investigating. Transfer of mitochondria between cells has been reported in the literature before. Astrocytes donate mitochondria to nerve cells after a stroke to limit damage, and stem cells are also known to donate mitochondria. At the moment, our findings raise more questions than they answer. How do these mitochondria enter sensory neurons and what changes do they induce? What causes the macrophages to donate mitochondria? Do M2-like macrophages is involved? Do these macrophages somehow sense which neurons are in need? Can we find other compounds in the vesicles essential for pain resolution? There are a lot of fascinating questions waiting to be answered.

"Resolving pain is not a passive, but an active process"

How may people suffering from chronic pain profit from the research?

The work om macrophages and pain resolution is still very fundamental in nature. We need to understand much more of the underlying mechanisms to identify potential pharmacological targets to resolve chronic pain. Studies on interleukins in the treatment of chronic pain are in a much further stage at UMC Utrecht. These provide hope for a therapy for chronic pain. Around 2010, we discovered that fusing two interleukins into one molecule can induce a much stronger pain reducing effect than the combination of 'loose' interleukins do. After preclinical studies, a spinoff company called Synerkine Pharma was established. They aim to bring this molecule to the clinic, which may contribute to a better quality of life for people suffering from chronic pain. ■

SCIENCE AND BUSINESS GO HAND-IN-HAND

Startup Laigo Bio develops a new platform technology to remove membrane-bound proteins. The spin-off company is built on an idea by professor Madelon Maurice, Principal Investigator of Oncode Institute and UMC Utrecht. "In principle, our idea could result in hundreds of different medicines for the treatment of cancer and other diseases."

Pitching business ideas for investors wasn't exactly on top of Madelon Maurice's bucket list. "I'm foremost an academic, interested in molecular processes that drive diseases. I'm not trained as an entrepreneur, but I'm learning fast and I'm keen to contribute my knowledge and expertise." Maurice also wouldn't want to miss this phase of Laigo Bio. "In principle, this technology could result in hundreds of different medicines. Currently, we are selecting the most promising drug candidates based on solid science."

The idea behind Laigo Bio slowly took shape when Maurice was studying the function of a subset of E3 ligases at her molecular biology lab in Utrecht. These ligases comprise membrane-bound proteins that can bind receptors and other proteins on the cell's surface. Binding is a 'kiss of death'. Once the ligase has attached a ubiquitin molecule to the target protein it is marked for destruction by the cell.

Maurice wondered: Would it be possible to 'convince' E3 ligases to bind to a particular receptor by just bringing the two closely together? A heterospecific antibody could do that job. And if so, would the receptor indeed be ubiquitinated and removed? That would be an entirely new way to target membrane proteins. Not by using small inhibiting molecules or blocking monoclonal antibodies like the famous checkpoint inhibitors, but by 'guiding' selected targets to self-destruction by the cells own machineries to give the same biological effect.

Patented screening technology

Maurice proposed her idea in a brainstorm session in 2018 for a new Dutch ubiquitin research consortium and received very enthusiastic reactions. A PhD in her lab started the first experiments and succeeded eventually in removing a selected receptor completely from the cell surface in cancer cell lines. Proof-of-principle for the technology (named SureTACs: Surface Removal Targeting Chimeras) was thus established. Maurice: "We found that some combinations of ligases and target proteins work well while others don't. Therefore, we developed a method which predicts if a target may be removed from cell surface membranes using specific E3 ligases."

This method was patented and Laigo Bio was founded in Utrecht about a year ago with the help of Oncode Institute and the start-up incubator ArgoBio. Maurice: "Their role is beyond providing capital. ArgoBio and Oncode Institute also give advice on translational drug development and biotech business; we have lots of discussions about the science and business opportunities." Today, the team has grown to about ten scientists with support from six more biotech professionals at ArgoBio and Oncode. Maurice: "We are still working in my academic lab, moving to a location at a science park would be a logical next step. It is great to witness the technological development, but also to see the people grow. It has become an excellent team with my former PhD student Maureen Spit in a leading position.

Phase-I trial

What's ahead for Laigo Bio in the coming years? Maurice: "With the current pace of results, we'll be able to start our first phase-I trial within a couple of years. Currently, the focus is on degrading a number of immune checkpoint inhibitors. Our approach may result in less side-effects. In addition, emphasis is on a number of membrane-bound receptors that are of interest in oncology but are currently deemed 'undruggable'."

Does Laigo Bio focus only on cancer therapies? "We consider broadening our field of interest in the future, but for now, the focus is indeed on oncology," explains Maurice. ■

Laigo bio

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Unraveling interactions of herpes viruses hiding in nerve cells

PhD project: Cell adhesion molecules as receptors in virally induced neuroinflammation

Herpes viruses can invade the nervous system and stay there unnoticed for a long time, before causing disease. But not much is known about the interactions between the virus and the nerve cells. Sabrina Krepel finds out using advanced techniques such as mass spectrometry and electron microscopy.

Sabrina Krepel loves electron microscopy. "EM is the most beautiful technique I know. I always wanted to solve my own protein structure, and now I've accomplished that with EM." During her Bachelor's, Krepel was interested in the relationship between chemistry, molecules and biology. After a side step into astrobiology abroad, she became fascinated by electron microscopy. After a Master's in cryo-electron microscopy (Cryo-EM) at the University of Groningen she went on to find a PhD position which combined all these aspects. She found this position in two research groups at Utrecht University: the Structural Biology group of Bert Janssen and the Biomolecular Mass Spectrometry and Proteomics group of Joost Snijder. She explains: "My research is a combination of those two. If we have a scientific question, we can decide to solve it with mass spectrometry or with Cryo-EM." In addition to the techniques, Krepel is interested in the overall scientific issue that underlies her research: "We study how something small as a virus has a large influence on something big like a disease."

Sabrina Krepel
Biomolecular Sciences, Utrecht University
Bert Janssen and Joost Snijder groups

Elusive interactions

The herpes viruses that Krepel studies are neurotropic which means they can invade and survive in the nervous system, she says. "A herpes virus can bind to nerve cells which are not regenerated often. It uses these cells to enter a latent phase. They re-activate for example when your immunity is weakened. We don't really know how the viruses enter the nerve cells. A large part of my research is dedicated to finding out more about this."

"If we have a scientific question, we can decide to solve it with mass spectrometry or with Cryo-EM"

Glycoproteins play a key role in the attachment and invasion of the virus into the nerve cells. A glycoprotein on the surface of the virus interacts with Myelin Associated Glycoprotein (MAG) in the membrane of cells in the nervous system. Krepel now uses advanced MS and Cryo-EM techniques to investigate this protein-protein interaction in detail. "The first tests showed that the interactions are very weak. Mapping these turns out to be quite a challenge."
Additionally, glycans, a class of sugar molecules which are building blocks of glycoproteins, play a key role in the interaction between the virus and the nerve cells. Using mass spectrometry, Krepel found out which glycans are present and using electron microscopy she can determine which protein-protein interactions play a role.

Treatment against viral diseases

Krepel also cooperates with Robert-Jan Lebbink from the infection & immunity group at UMC. "Together, we develop tools to study herpes viruses. The ultimate goal is to develop a treatment against virus infections such as herpes but also corona viruses which operate in a similar way." In another collaboration, Sabrina Oliveira (cell biology) can isolate nanobodies which bind to the protein. Krepel can then use these to visualize the protein in the electron microscope. Krepel started her research in 2021. "I'm finally on a roll now and I am able to take a wider view to the research. As a new PhD, you need to learn so much, you make so many mistakes. To help me along, I really appreciated the courses that ICI offered, such as planning and other soft skills." ■

Developing a toolkit to study the brain's immune system

PhD project: The development of photochemical tools for in vivo microglial activation

Microglia play a crucial role in neuronal diseases such as MS or Alzheimer's. But there is a large gap in knowledge about the processes involving microglia and the development of these and other neurological disorders. Without understanding of the fundamentals, there is no chance of a cure. PhD student Viktor al-Naqib at NIN studies these fundamentals in cooperation with chemical immunologists in Leiden.

Microglia are the tissue macrophages of the brain, cleaning up cellular debris and constantly surveilling their environment. They form branches called ramifications with which they reach out to specific parts of the neurons, such as the axon initial segment. Viktor al-Naqib, neurobiologist in the Axonal Signaling Group of Maarten Kole at the Netherlands Institute for Neuroscience in Amsterdam, set out to study these interactions, because as he says: "We don't know why and under what circumstances the microglia are attracted to the axon initial segment."
But microglia are very difficult to study, found Viktor. "Normally, we study neurons in a slice of brain tissue. But microglia, being partially responsible for garbage disposal in the brain, become too activated and scavenge the damaged brain tissue." The solution is working with brain slice cultures, he explains. "The difference is the time scale. We now have days or even weeks to study the microglia. Right after slice preparation, they are still very activated, but eventually they chill out and go into their homeostatic state. Then we can observe what they do."

"These compounds will be amazing tools to study microglial biology in a highly targeted manner"

Photocages

In this ICI-project, the aim is to develop a toolkit to study these complex cells, both by manipulating the immunological state of the microglia and studying the influence of certain molecules such as ATP that are known to be involved in their function.
In the Molecular Immunology group of prof. Sander van Kasteren at Leiden University, PhD student Yixuan Wang designed and synthesized so-called photocaged compounds. These compounds are inactive as long as they are bound to a protective group. This group is photolabile and can be removed by shining specific light on it.
For Viktor, this means he can prepare a brain cell culture with microglia he wants to study, using fluorescent reporter lines so they are visible under a microscope. He then adds the photocaged ATP (or other photocaged compounds) and specifically activates it near one microglia cell. He can then study what happens. In the case of photocaged ATP he expects the microglia to become attracted to the activated, uncaged area. But he can also study what happens if he adds an inhibitor of ATP receptors, or when all the neurons around the microglia stop communicating, for example.
"Although the experiments with the photocaged ATP are not fully working yet, I am happy that we now have a nicely working protocol to study the activity of microglia and the axon in detail. When the compounds will work, they will be amazing tools to study microglial biology in a highly targeted manner. Once we have a better fundamental understanding of how microglia are activated and how they talk to neurons, we can start to work towards an understanding of neurological diseases associated with them."

Close cooperation

Working in such close cooperation in a multidisciplinary team has been very exciting, says Viktor. "We have frequent meetings where we get ideas and suggestions. I get to observe how they do problem solving. We work at the interface of quite different disciplines, but we have a shared language which is science." ■

Viktor al-Naqib
Netherlands Institute for Neuroscience, Amsterdam
Axonal signaling
Maarten Kole group

Checkpoint inhibitors also mobilize 'the bad guys'

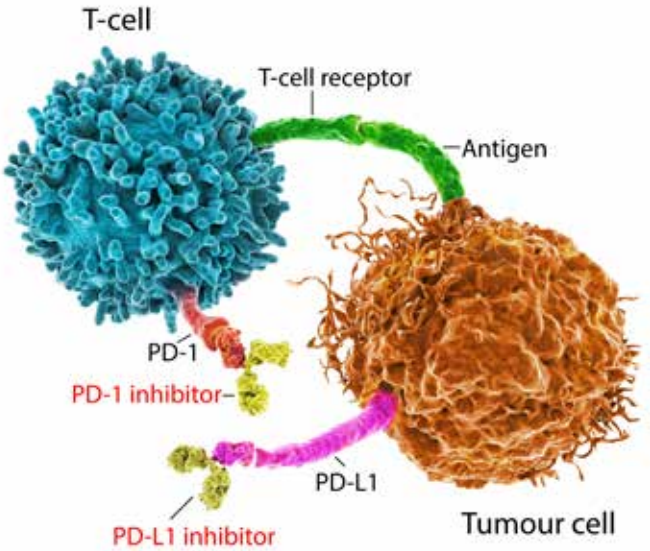
Unfortunately, many people with cancer don't experience durable benefits from immunotherapy. They develop therapy resistance, a still poorly understood phenomenon. Recently, Leiden/Rotterdam scientists revealed a fundamental factor behind the development of therapy resistance: checkpoint inhibitors also upregulate regulatory T-cells: 'the bad guys'.

"Within two months, the article was downloaded 11.000 times," tells Thorbald van Hall, professor of Experimental Tumorimmunology at Leiden UMC. "I'm still not quite familiar with this way of measuring impact, but when first author Mandy van Gulijk told me the number, she looked very proud, and rightly so."

Unbraked

Immunotherapy with checkpoint inhibitors has revolutionized cancer therapy in the past decennia. Antibodies increase T-cell levels by blocking the receptor PD-1/PD-L1 (programmed death-ligand 1). Van Hall: "You take away an important brake of the immune system, bringing it to full speed. However, our paper shows that checkpoint inhibitors also take away the brakes on regulatory T-cells, and that's not what you want. Where T-cells are the 'good guys' in the fight against cancer, regulatory T-cells are the 'bad guys'."
The paper in *Science immunology* shows that both processes take place at the same time, by the same route. Checkpoint inhibitors activate regulatory T-cells (T_{reg}) in exactly the same way as normal T-cells through blocking of PD-1/PD-L1. When immunotherapy is successful, checkpoint inhibitors rustle up more of the good guys (T-cells) than the bad guys (T_{reg} cells). Van Hall: "The role of T_{reg} is to balance the immune system, to prevent an 'overshoot'. The cells have been found in high numbers in the tumor micro-environment of therapy-resistant patients. However, it was unclear if the high numbers of T_{reg} are causing therapy resistance or if they are a consequence thereof." Using various knockout mouse models the Dutch scientists could provide proof-of-principle that upregulation of T_{reg} is a direct result of the immunotherapy and *causes* therapy resistance. Van Hall: "Depletion of T_{reg} from the tumor environment also eliminates the resistance in animal models."

▼ *Checkpoint proteins, such as PD-L1 on tumor cells and PD-1 on T-cells, help keep immune responses in check. The binding of PD-L1 to PD-1 keeps T-cells from killing tumor cells in the body. Blocking the binding of PD-L1 to PD-1 with an immune checkpoint inhibitor (anti-PD-L1 or anti-PD-1) allows the T-cells to kill tumor cells.*



> *"Upregulation of Treg is a direct result of the immunotherapy"*

Caution

Although the presence of T_{reg} cells in the tumor environment is discouraging sign for immunotherapy success, it does not necessarily predict therapy resistance. Van Hall: "When therapy starts and we notice that T_{reg} gets upregulated, therapy resistance will occur. However, how soon and how severe the resistance is, depends also on other tumor characteristics."
How may this new fundamental knowledge improve immunotherapy? Van Hall: "Removing T_{reg} cells eliminates therapy resistance in animal models. In the UK, first human trials have been started where checkpoint inhibitors are combined with depletion of T_{reg} cells. We're trying to start such trials too, at Erasmus MC. With high cautiousness. T_{reg} has a key role in balancing the immune system. You need to be aware of side effects."

New clues

Next steps for the Van Hall-research group are studying T_{reg} cells in their activated stage. The RNA-expression profiles are studied to figure out which pathways are triggered in T_{reg} by checkpoint inhibitors and how this results in therapy resistance. "Such knowledge may lead to new clues on how to neutralize T_{reg}-activation in immunotherapy." ■

Reference

Mandy van Gulijk, Anneloes van Krimpen, et al. **PD-L1 checkpoint blockade promotes regulatory T-cell activity that underlies therapy resistance.** Science Immunology 2023, 8 (83), eabn6173
DOI: 10.1126/sciimmunol.abn6173

Threonine analogue simplifies studying newly synthesized proteins

Every cell will react to the presence of a virus, medicine or signaling molecule. Studying newly synthesized proteins provides insights into what pathways are up- or downregulated. A new threonine analogue makes studying the proteins easier, reports a team of researchers from Nijmegen.

BONCAT, bioorthogonal non-canonical amino acid tagging, shows which proteins a cell produces in response to a particular compound, virus, bacterium or other stimulus. It works by providing the stimulus together with a modified amino acid. This analogue will be built into all newly synthesized proteins. Because it carries a reactive group the analogue enables visualization and identification of the newly synthesized proteins by conjunction to fluorescent dyes or affinity tags.
Today, the most commonly used analogues in BONCAT are azidohomoalanine (AHA) and homopropargylglycine (HPG). Both replace methionine. "A disadvantage is that you need to grow your cells on a methionine-free medium, otherwise too little of the analogue gets incorporated," tells Bob Ignacio, postdoc at Radboud University. "We felt that there was a need for an easier-to-use and more sensitive method." "The use of AHA or HPG is particularly challenging when you are studying cells that grow slowly such as primary cells or intracellular growing bacteria," adds associate professor Kim Bonger. "A simpler BONCAT method would fill a gap in the market in biological sciences."

New standard

Molecular life scientist Ignacio took up the challenge. "From literature, we learned that some threonine analogues are readily incorporated in proteins, but unfortunately none of these contained a bioorthogonal group. Digging further in the literature, we came across a threonine analogue called β-ethynylserine (βES) that does. It's a quite unknown analogue, only produced by an exotic bacteria called *S. cattleya*. I synthesized βES, and luckily it turned out to be very useful!"
The new method was named THRONCAT: threonine-derived non-canonical amino acid tagging. It doesn't require a special growth medium, you just add βES to your regular medium, tells Ignacio. At equimolar concentrations, one in every fortieth threonine in newly synthesized proteins will be replaced by βES without disturbance of metabolic processes. In an article in *Nature Communications*, the scientists show that βES also allows fast visualization, identification and quantification of newly synthesized proteins, both in cell cultures and *in vivo* experiments.
Does THRONCAT also has disadvantages? Ignacio: "You need to be aware that a tiny fraction of the proteome, about 2,5 percent, doesn't hold threonine residues and thus can't be detected. However, that is less than the fraction of methionine-free proteins." The only disadvantage of THRONCAT Ignacio can think of is that he is currently the only supplier of βES. Which means that everyone who would like to use THRONCAT should contact him. "We've had quite a number of requests for βES since we published the paper and are involved in about thirty new cooperations."
Most of these new projects are in neurosciences where THRONCAT is quickly becoming the new standard. There are, however, also cooperations in oncology, immunology, in proteomics and even in plant science. Bonger: "Everyone who thinks THRONCAT may be useful in their studies may contact us. We're happy to help." Iignaco: "It's quite fun to be involved in so many different projects, and we gather a lot of additional information about the method."

"A roughly halved cortisol plasma level is most striking"

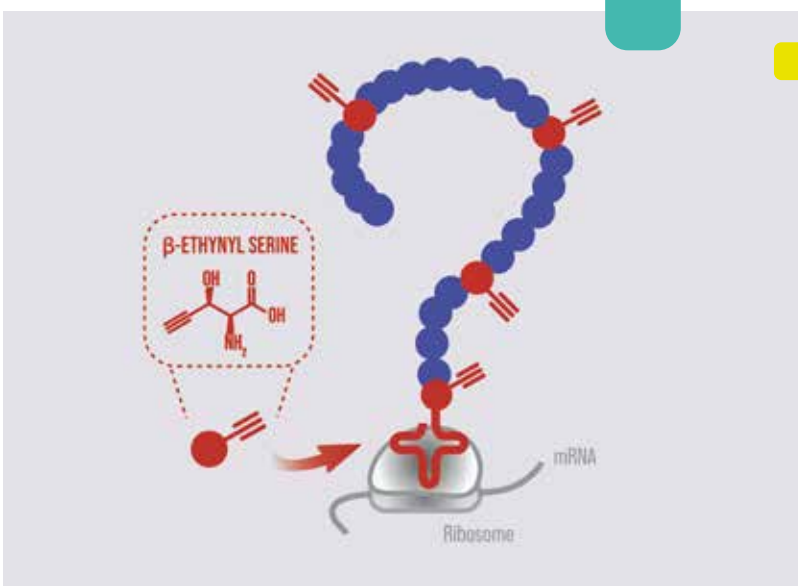
Spinoff

Because of the large interest in THRONCAT, Bonger considers creating a spinoff company which will produce βES commercially and provides advice and support to clients. Then Ignacio can also continue with his own research project: finding other amino acid analogues that may be helpful in studying cells responses. Ignacio: "We've plenty more ideas for analogues that may either improve sensitivity or may be useful under special, challenging conditions." ■

References

Bob J Ignacio, Jelmer Dijkstra, et al. **THRONCAT: metabolic labeling of newly synthesized proteins using a bioorthogonal threonine analog,** Nature Communications 2023, 14 (1), 3367. DOI: 10.1038/s41467-023-39063-7

▼ **THRONCAT**
THR*e***O***nine-derived* **N***on-***C***anonical* **A***mino acid* **T***agging. The β-ethynylserine labels are exclusively incorporated into newly synthesized proteins enabling visualization and identification of the protein by linking to fluorescent dyes or affinity tags.*



TACT: Targeted Anti-Cancer Therapies

Scientific excellence and innovative training network in one

The EU-project TACT combines state-of-the-art research in antibody-drug conjugates with extensive training of new researchers.

Immunotherapy is booming, and antibody-drug conjugates (ADC) are a fast-growing class of therapeutics. These consist of a cytotoxic drug connected to an antibody that targets a tumor marker. Although much research is aimed at elements of ADCs such as the drug payload or constructing nanobodies, the international research program TACT is bringing all aspects of this research together, from fundamental chemistry to the initial stages of biological assessment. ICI-partner WUR is involved.

"It's one of the best collaborative programs I have been involved in," says Christopher Scott, professor of Pharmaceutical Biosciences at the Queen's University Belfast (QUB, Northern Ireland, UK). Scott, like ICI-researcher Bauke Albada at WUR, leads one of the research groups collaborating in the EU-project Targeted Anti-Cancer Therapies, which is part of the European program Horizon 2020. The scientific goal of TACT is to "push the boundaries of antibody-drug conjugates" as Scott puts it. "This is a rapidly expanding area of cancer therapeutics. We do cutting edge research that companies would find too risky. We demonstrate that these technologies are worth investing in. Ultimately it will bring new cancer therapeutics."

Fruitful collaborations

TACT, involving nine research institutes and three pharmaceutical companies, relies heavily on the collaboration between the twelve organizations. Each academic institute hosts one or two PhD students, eleven in total. All projects have one or more assigned secondment partners where the PhD student will spend several months doing research outside their own lab. Scott's research group focuses on targeting systems; the development and bioassessment of nanobodies (nanoparticles functionalized with antibodies) to target pancreatic cancer cells, specializing in immunology. Bauke Albada, head of the Nanochemical Biology Group at WUR, is specialized in the fundamental chemical principles that can be used to couple small synthetic molecules to biomolecules. In a close collaboration exemplary for TACT, Irene Shajan working as a PhD in Albada's group spent a few months in Scott's laboratory in Belfast. She prepared bispecific antibodies (see box) and brought them to QUB for testing in bioassays. During the exchange, Shajan had the chance to learn abouT-cell biology and toxicity and the QUB students in turn learned about what is possible with the molecules she makes.

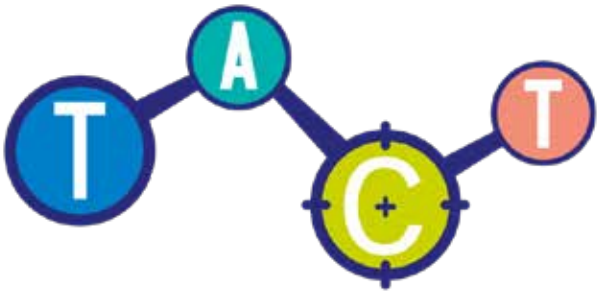
"Targeted anti-cancer therapies are the future of cancer treatment"

"The results were better than we expected," says Scott. "The constructs that Bauke makes are exceptionally potent. This could cause toxicity issues, but we didn't see any of that. Also, with this kind of chemical biology you can relatively easily modulate the activity. It really surprised me how good they are."
Albada adds: "It has been great to be able to test the constructs we make ourselves. We don't have access to these methods nor the biological samples that are needed. By collaborating with Scott we gain an insight in the way the molecules we make work. That helps us for example to design even better constructs and propose new research."

Extensive training

As important as the scientific part of TACT are the various training elements for students. The program is funded within the Marie Skłodowska-Curie Innovative Training Networks. It is a 'collaborative European Training Network' (ETN) and therefore provides funding for extensive training for the early-stage researchers, the PhDs. This training program resembles the ICI PhD training program and includes seven large themed meetings on entrepreneurship, careers and legal affairs as well as scientific topics. In addition, the institutions organize trainings on topics such as development or IP.

Scott feels this helps the students a lot: "The students within TACT have opportunities that others have not. There is funding to send them abroad on courses and meetings everywhere. They have daily contact outside their group and form close bonds. They help each other out and learn to collaborate. As a result, their scientific work is faster and better."
With still a year to go, the program has been a success in all respects, Scott says: "The collaborations within TACT brought me to places I'd never been to and introduced me to people I would never have met because they were outside my academic circle. We are working towards a common goal and this creates a level of trust as well. We are working as a team." ■



The Albada Lab

TACT's scientific focus is on developing antibody-drug conjugates (ADCs) for new therapeutical principles specifically targeting cancer. The Albada Lab takes this concept various steps further. Bauke Albada explains: "An ADC is a drug bound to an antibody which delivers the drug locally to the tumor cell by recognizing and binding to it. A bispecific antibody is a similar construct, but it also contains a protein which binds to a T-cell from the immune system. In this way, it brings together the tumor cell and the T-cell and helps the immune system to clean up the cancer cells."
Albada's group is specialized in so-called click-chemistry. They used this method to synthesize the bispecific antibodies. "The magic word here is bio-orthogonal," says Albada. "That means we designed a chemical reaction that does not attack the rest of the biological molecule. For example, a reaction between a small molecule like a drug and the antibody." This way they can covalently bind different functional parts to the antibody.
Many different click-reactions are known, but Albada and his colleagues designed and patented a biogenic click reaction that utilizes a conserved tyrosine residue on the antibody. "The reaction between the small molecule and the tyrosine residue is much quicker than any other reaction on other parts of the antibody. Therefore, it occurs almost exclusively. There is no time for other reactions to take place." The company Synaffix founded by ICI researcher Floris van Delft holds the license to the tyrosine click-reaction patent and offers the method for custom-made protein conjugates.
To prove the concept of this bispecific antibody, Irene Shajan, Albada's PhD student working on this project, created a bispecific antibody with trastuzumab as the part that binds to a tumor cell. Trastuzumab is used to treat breast cancer, among others. She succeeded in binding two antigen-binding regions that bind to T-cells to trastuzumab. In activity tests done at Queen's University Belfast, they proved that the antibodies bind to the targeted cells and that the T-cells are indeed activated at very low picomolar levels. Just adding the separate components did not result in any binding to cells, which shows that the click chemistry that is used to link them is required. Next, they made a construct with one T-cell binding and two tumor-binding antibodies, instead of two to two. "The activity of the T-cells is a bit more subtle in the 2:1 construct. If the activity is too high, healthy cells are destroyed as well, potentially causing unwanted side-effects," Albada explains.
Albada is very happy with the results: "The best part of this research is when we got the positive results of the biological activity studies. After a few years of learning how the chemistry works and producing enough material for tests, that is beautiful to see."

The science of asking questions

We all know the feeling: a question pops into mind during a lecture, but when it comes to it, you're not raising your hand to ask it. 'It's not a good question', 'I haven't paid attention' or 'my English is bad' are some of the common excuses. Within ICI we believe it's important to coach and train PhD students not only in conducting scientific research but also in soft skills. At this year's PhD Day the theme 'Communication' encompassed the workshop 'Asking scientific questions'.

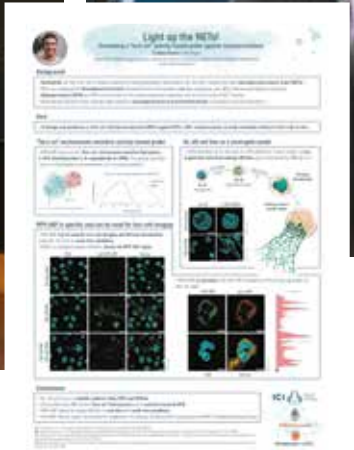
During last year's ICI conference in 2022 we noticed that very few PhD students asked questions. We had the impression they could benefit from discussing this topic, to investigate what keeps them from asking questions, how to overcome those issues, how to ask a question and to learn from the experience of others.
The workshop included a presentation by Agnes van Rossum about the blockages you may experience when it comes to asking questions after a lecture. Two ICI researchers shared their experiences on the matter and shared tips and tricks and do's and don'ts for asking questions. Finally, the PhD students had the opportunity to test their freshly gained knowledge by asking questions after Sabina van der Zanden's lecture about her research. Separately at the end of the day, Sara Cigna of Luris explained all about intellectual property, patents and valorization.

PhD day 2023

Everyone learned at least a little bit from the workshop, judging from the answers to the questionnaire we sent out afterwards. "It was an illustrative workshop that allows identifying key points to ask questions, avoiding common mistakes and use helpful tools to overcome wrong ideas about asking questions," said one of the participants. Participants learned to 'identify the advantages and disadvantages of asking questions', and to 'visualize how a presenter would face questions'. Not surprising for scientists, most respondents would have liked to hear a more in-depth analysis about the psychological science behind being scared to ask questions. Overall, the participants enjoyed an 'informative, well organized' PhD Day.
The traditional poster prize of the PhD Day 2023 was won by Enebie Ramos Cáceres from Radboud University. His poster received the most votes from the conference participants. Enebie said he felt nervous when he found out he won such a prize for the first time: "To be honest, I did not expect it. There were many other really good posters at the conference. However, I always try to do my best with this type of scientific communication. I'm a bit of an artist so I make hand-made illustrations and use eye-catching microscopy images to convey the purpose and goal of my research. One image is worth a thousand words, right?" ■

▼ *Winner Enebie Ramos Cáceres (r) receives the poster prize from Martijn Verdoes.*

▼ *Workshop on learning to ask questions.*



◄ *The winning poster 2023.*

Inaugural lecture Sander van Kasteren

After his appointment as professor of Molecular Immunology last year, Sander van Kasteren will accept the professorship on December 15 by delivering his inaugural lecture entitled “Chemical Adventures in Immunology”. His research focuses on studying and manipulating the uptake and routing of antigen in dendritic cells.

Research Ignition Award 2023

Annemarthe van der Veen (department of Immunology of LUMC) and her collaborating partner Pierre Maillard (Blizard Institute, UK) received a Research Ignition Award from the Kennedy Trust for Rheumatology Research. The award is aimed to stimulate new and ambitious avenues in the field of disease pathways in rheumatological, musculoskeletal and related inflammatory disorders. The honored researchers will receive financial support for piloting a new strategy of therapeutic intervention in autoinflammatory diseases.

Sjaak Neefjes speaker at the Avond van de Chemie

His lecture was about the (re)discovery of anthracyclines as anti-cancer drug. Although these compounds are widely used for decennia, their toxicity is a barrier. What do you do if you have developed a less toxic variant, but do not have a strong patent position? How do you convince the authorities of its potency and how do you make the promising variant available to patients? The roads to achieve that goal prove to be sticky. In his lecture Neefjes made a case for developing medicines at the interface between academia and industry.

Recent publications

Wijfjes Z et al.
Controlling Antigen Fate in Therapeutic Cancer Vaccines by Targeting Dendritic Cell Receptors
Mol Pharm. 2023 Sep 18. doi: 10.1021/acs.molpharmaceut.3c00330. Online ahead of print. PMID: 37721387 Review.

Wu Y et al.
Cathepsin B abundance, activity and microglial localisation in Alzheimer’s disease-Down syndrome and early onset Alzheimer’s disease; the role of elevated cystatin B
Acta Neuropathol Commun. 2023 Aug 14;11(1):132. doi: 10.1186/s40478-023-01632-8.
PMID: 37580797 Free PMC article.

Weiss L et al.
Direct In Vivo Activation of T-cells with Nanosized Immunofilaments Inhibits Tumor Growth and Metastasis
ACS Nano. 2023 Jul 11;17(13):12101-12117. doi: 10.1021/acsnano.2c11884. Epub 2023 Jun 20.
PMID: 37338806 Free PMC article.

Schluck M et al.
Insights in the host response towards biomaterial-based scaffolds for cancer therapy
Front Bioeng Biotechnol. 2023 Jun 5;11:1149943. doi: 10.3389/fbioe.2023.1149943. eCollection 2023. PMID: 37342507 Free PMC article. Review.

Ligthart NAM et al.
A Lysosome-Targeted Tetrazine for Organelle-Specific Click-to-Release Chemistry in Antigen Presenting Cells
J Am Chem Soc. 2023 Jun 14;145(23):12630-12640. doi: 10.1021/jacs.3c02139. Epub 2023 Jun 3.
PMID: 37269296 Free PMC article.

Gall CLM et al.
CRISPR/Cas9-based Engineering of Immunoglobulin Loci in Hybridoma Cells.
Bio Protoc. 2023 Feb 20;13(4):e4613. doi: 10.21769/BioProtoc.4613. eCollection 2023 Feb 20.
PMID: 36845533 Free PMC article.

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Effect of Antigen Valency on Autoreactive B-Cell Targeting
Mol Pharm. 2023 Oct 20. doi: 10.1021/acs.molpharmaceut.3c00527. Online ahead of print.
PMID: 37862070

van Gelder MA et al.
Re-Exploring the Anthracycline Chemical Space for Better Anti-Cancer Compounds.
Journal of Medicinal Chemistry, 66(16), 11390-11398 (August 10, 2023)

Column

THE RENAISSANCE OF RNA VACCINES AND THERAPEUTICS

ANNEMARTHE VAN DER VEEN
ICI EXECUTIVE ADVISORY BOARD

Annemarthe van der Veen is an associate professor of Molecular Immunology at LUMC

The rapid distribution of two safe and effective mRNA-based vaccines against SARS-CoV2 made a crucial difference in halting the pandemic. They were developed at unprecedented speed, yet they are founded on decades of basic research by many scientists. Katalin Karikó and Drew Weissman were amongst these scientists. They firmly believed that RNA had great potential to be used in vaccinations or as therapeutics, if only its intrinsic potential to activate the innate immune system would be diminished. Diminished, but not eliminated, as some degree of innate immune activation favors an effective adaptive immune response.

The innate immune system recognizes RNA through a number of different receptors. Some of these are membrane-bound, such as Toll-like receptor 3, 7, and 8. Other RNA sensors are cytosolic, including the RIG-I-like receptors. These receptors have in common that they recognize foreign RNA, for example viral RNA, and discriminate it from our own RNA. This is a topic that is very close to my heart, as it is the main topic of the research in my group. Recognition of foreign RNA by innate immune sensors leads to a strong inflammatory response and translational shutdown. Therefore, RNA was deemed useless as a therapeutic.

Katalin Karikó and Drew Weissman were not discouraged by this notion. They formed a perfect team and started the quest to reduce the immunostimulatory capacity of RNA. In a landmark paper in Immunity in 2005, they elegantly demonstrated that modification of RNA, for example by pseudouridylation, reduces the activation of RNA sensing receptors on dendritic cells. This discovery, together with the formulation of mRNA into lipid-based nanoparticles to enhance delivery, opened the doors to the development of effective mRNA vaccines against SARS-CoV2. For this, Katalin Karikó and Drew Weissman were rewarded with the highest distinction in science, the Nobel Prize.

The enormous potential of mRNA vaccines has only just begun to be explored. Other mRNA-based vaccines that are in clinical trials involve vaccines against cytomegalovirus, respiratory syncytial virus, HIV, and malaria. But the future of mRNA vaccines is not limited to infectious disease. The next frontier is cancer vaccines. The success story of Karikó and Weissman is a beautiful example of how basic research eventually saved the lives of millions. Moreover, it marks the start of an era in which RNA vaccines and therapeutics will be dazzling! ■

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.

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