ICI institute for chemical immunology

Interview

PROBING THE SECRETS OF PROTEASES

MATTHEW BOGYO
Professor of pathology, microbiology, immunology and (by courtesy) of chemical and system biology
Stanford University, CA, USA

When something goes wrong in our body, it often involves inflammation. And when there's inflammation, proteases take up a key role. Using an array of advanced chemical probes, Matt Bogyo, professor at Stanford University School of Medicine, is creating completely new ways to identify and understand this huge class of pivotal enzymes.

Even the short description of your research manages to comprise an impressive diversity of topics, ranging from studies on parasites to tumor imaging. What connects all these dots?

"There is a central element and that is our technology platform based on the use of chemical tools, or 'probes', to study specific enzymes through the formation of covalent bonds. That platform can be broadly applied in biology, including cancer-related imaging, identifying enzymes that pathogens use to infect humans host cells or studying bacterial enzymes in the microbiome."
"Within these biological applications, we mostly focus on proteolysis. I started out in chemistry but moved on to immunology to do my PhD in the group of Hidde Ploegh at MIT. It took me a while to get familiar with all the immunology lingo, it really felt like learning a new language. We worked on MHC I and II antigen presentation pathways which are designed to present peptide antigens that are created through proteolysis. That is how I got interested in proteins like cathepsins and the proteasome."

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Can you explain the essence of the chemical tools you use?

"We design and create molecular probes that contain what we call a 'warhead', which is an electrophile that covalently binds to a nucleophilic residue on a protein target. These warheads allow highly selective targeting of a protein of interest. But the molecules we use as carriers to deliver the electrophiles, are often short, linear peptides that have limited room for additional structures and functionalities to enhance binding and/or specificity. This simplicity of structure enables us to use chemical synthesis to create and modify our probes, but we are now exploring various strategies to diversify our tools."

Such as?

"We are currently working on the use of phage display to create new classes of highly selective probes with much more complex structures. Phages are viruses that infect bacteria and that can be engineered to express billions of diverse peptide sequences on their coat proteins. By using phage display, it is possible to screen all that peptide diversity to identify molecules that bind with high affinity to a target protein. The phage containing the 'winning' binders can be isolated and amplified to enrich for the best binders."
"In general, this process results in very specific binding molecules because your selection pool is so large. We figured out that it was possible to do chemistry on the coat proteins

"When there's inflammation, proteases take up a key role"

of the live phage viruses to introduce our favorite 'warheads' that drive covalent binding. We synthesize what we call 'linkers' containing a warhead and functional groups that react with two cysteine residues on the phage coat protein. This results in the formation of rigidified and stable cyclic peptide 'probes' on the phage coat that can be selected and amplified just like regular linear peptides. Because cyclic peptides are more constrained, they tend to bind proteins with higher degree of selectivity compared to the more flexible, linear molecules. One of the main chemical challenges we are facing now is to find the optimal reactivity of an electrophile to enable rapid covalent binding, while not being so reactive that it binds to more than you aim for."

Why go through all this trouble to make a very selective binder? What problem does such high specificity solve?

"When it comes to imaging of targets that are associated with a given disease, with cancer being the most obvious application, you need high specificity to get the necessary imaging contrast. In biology, you're dealing with complex systems. Therefore, you need super selective probes if you want to study just one of those proteins. We have a particular interest in covalently binding probes because they make it possible to directly determine what proteins are being targeted since the probes stick and don't come off."

"In the end, it is all about inflammation, that is where most health problems start"

How does this relate to your projects on pathogens?

"Most pathogens also use proteases to mediate various aspects of pathogenesis. We are now using our technology with pathogenic bacteria, like *Staphylococcus aureus*. We recently found a new family of serine hydrolases that are present in the bacterial biofilm and therefore are potentially valuable targets for imaging *S. aureus* infection sites. For example, in cardiac infections, it would be helpful to have a way to image the location and size of the infection and then monitor response to antibiotic treatment using a non-invasive imaging method like ultrasound. It may also be possible to use these probes to target antibiotics to the site of infection."

And in your microbiome projects, what is the leading question there?

"This is an area we are just starting to explore. We are starting to use our protease probes to take inventory of the proteases and hydrolases that these gut microbes produce. We found that commensal bacteria secrete proteases that target human protease-activated receptors, PARs that regulate diverse biological signals involved in gut barrier defense, inflammation and pain signaling. Therefore, it is likely that bacterial in the gut can control important processes such as inflammation to regulate their local environment and compete with other microbes."

Taking it to a higher level, what do all these insights teach us about immunology?

"In the end, it is all about inflammation, that is where the problems start for most human health conditions. And we know that when inflammation kicks-off, proteases play a vital role. That is what makes them so relevant to study."

So, proteases will remain a key topic in your work?

"Yeah, proteases are interesting because they are mechanistically well mapped and there are plenty of them, yet we barely know what any of them are actually doing in health and disease. I won't run out of proteases to study in my lifetime." ■

CLEANING UP CLOTTING PROTEINS

The Amsterdam-based start-up VectorY develops gene therapy for neurodegenerative disorders and is growing rapidly. "We have the knowledge, experience and capital to move fast," tells co-founder and CTO Sander van Deventer.

Many neurodegenerative diseases are caused by wrongly folded proteins that clump together and damage neurons. VectorY develops a gene therapy-based solution against this type of diseases. A viral vector introduces a gene producing a peptide with antibody-like structure that attaches to the aggregates marking them for clearance. Van Deventer: "Traditional gene therapy works by overexpression of a particular gene or by silencing it, neither is very suitable in diseases involving clotting proteins. We use vectorized antibodies to remove only the harmful form of proteins. We have got proof-of-principle that this strategy works well."
The vector, an adeno-associated virus, needs to deliver its package to the correct cells where it must be translated efficiently, and the vector may not evoke immune response itself. Van Deventer: "With our advanced technologies, we can guarantee a safe product without any unwanted immune effects and at relative low production costs. We can scale up to two thousand litre bioreactors which puts the technology at the same cost levels as monoclonal antibodies, with the crucial difference that this is gene therapy. Patients need treatment only once."
The first VectorY-therapy targets the neuromuscular disease ALS, later projects will concern Parkinson's and Huntington's disease. Alzheimer's may be a long-term target. Van Deventer: "Thus far, untangling plaques in Alzheimer's disease has not resulted in many benefits for the patients. We focus on diseases in which removing the protein aggregates is expected to result in a clear health effect."

In-house

"The secret behind VectorY's fast growth is a team with an impressive amount of experience in both biotechnology and business," says Van Deventer. "We have experienced all the major problems, challenges and pitfalls in this field, there isn't a missing puzzle piece that can surprise us in the final stages of development." VectorY has all necessary facilities in-house, from research to manufacture. Van Deventer: "That is crucial to move fast, too."
The CTO has ample experience, himself. He was one of the driving forces behind the first commercial monoclonal antibody (Remicade), the first commercial gene therapy (Glybera), is co-founder of uniQure, a Professor in Translational gastro-enterology at Leiden UMC, and has more than twenty years of experience at boards of private, Euronext- and Nasdaq-listed companies. At 67, he enjoyed initiating another start-up from scratch. "We screwed together the first desks ourselves, in a brand new, empty building. Today, it is a busy place with over fifty workers and a set of running bioreactors. That gives me energy."

State-of-the-art

VectorY is continuously searching for reinforcements. The team is a highly international mix of scientists and technicians with backgrounds in cell biology, protein synthesis, genetics, biotechnology, immunology, and computation. Van Deventer: "Employees immediately get a lot of responsibility; they are working with state-of-the-art equipment and technologies: high throughput tools, organoids, lab-on-a-chips, ... It is amazing that we can measure the contraction of muscle cells on a chip today."
The capital venture firm Forbion seeded the start-up; BGV and Eli Llily followed as important investors. First clinical studies are foreseen for the second half of 2023: vectorized antibodies against the TDP43-positive protein depositions characteristic for ALS. Van Deventer: "The trials will run abroad because of the restrictive Dutch legislation on gene therapy. A real pity. I have been advocating for changes for more than twenty years now; the Netherlands are missing out in a highly innovative industry." ■

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In search for the optimal conjugate in cancer vaccines

PhD project: Synthetic peptide conjugates for cancer therapy

By synthesising and analysing a series of conjugates of peptide antigen and TLR-ligand, the most effective combination as cancer vaccine must pop up. Two steadfast PhD students run the many experiments in Leiden.

"Science is always teamwork, therefore I was immediately enthusiastic about this project with Marjolein," tells medical biologist Giulia Castello, PhD student at Leiden UMC. "Marjolein synthesizes the conjugates and I test their immune activity. We strongly depend on each other, but have our own individual studies, too." Chemist Marjolein Isendoorn, Castello's research partner and PhD student at Leiden University LIC: "I really like it that I get feedback almost immediately. It gives me the feeling that I am really working towards a possible therapy."
Castello has always envisioned herself as a researcher, developing remedies for diseases in a laboratory. She studied in Italy, the US and the Netherlands, and focussed more and more on immunology. Isendoorn was trained as a chemical analyst and had already started a professional career, when she realized she missed depth and intellectual challenge. Therefore, she picked up her education again with a master's in chemistry. "After my master graduation, I was asked to think about a PhD position. Honestly, I never thought of myself working in academia, but I really liked the idea of running a research project by myself. Up till now, it is as nice as I imagined it could be."

Giulia Castello (left)
Immunology, Leiden UMC
Ferry Ossendorp group

Marjolein Isendoorn
Bio-organic Synthesis, LIC, Leiden University
Dmitri Filippov group and Jeroen Codee group

Conjugates

Isendoorn synthesizes a series of cancer vaccines consisting of a range of different peptide epitopes coupled to a variety of different small molecules which are all ligands for TLR-receptors present on the dendritic cells of the immune system. "The idea is that the conjugates activate the immune system more than the two components given together but 'loose'. I also add a fluorescent label to track the conjugates. We want to know what is happening in detail." Isendoorn designs synthetic routes and has always some experiments 'cooking'. "Synthesizing variants parallelly, has the advantage that whenever there is a setback, there are always experiments running successfully, too."

> *"In the end, the most important thing to me is that it works"*

Castello analyses which of the conjugates elicits the strongest immune response. "I test their activity and possible toxicity *in vitro* in human and mouse cells. In the future, I will be using human skin and animal models, too. I just finished the training. In the end, I hope to bring the project to the point when clinical tests are the next logical step. You cannot know if that is feasible, but it would be wonderful."

Focus on application

The best moments in the laboratory for Castello are when she draws conclusions from the fresh data. "In the middle of an immunology experiment it is often not clear if it is going in the desired direction. When you finally get the overview, and it looks well ... that is great. In the end, that is the most important thing to me: that it works." Castello's ambitions focus on a research job with a pharmaceutical company. "I like the teamwork in companies and have a focus on applications." Isendoorn has not yet thought beyond her PhD-studies. "That feels still far away, I am not even halfway through my PhD studies. I am learning and developing myself every day. I would like to do some immunology experiments, for example, too. I always feel that you understand things better when you do them yourself. That lead me here, perhaps a bit to my own surprise." ■

Stimulating and steering immune response with sugars

Project: Amsterdam-Leiden-Nijmegen cancer vaccine consortium

Cancer vaccines can activate the immune system, but often the response is not strong enough to cure. The Amsterdam-Leiden-Nijmegen vaccine consortium searches for the necessary boost, for example by adding a 'sugar coat' to an antigen-adjuvant-construct.

Zach Wijfjes, PhD student at RadboudUMC Nijmegen makes 'triple A constructs'. He is a molecular scientist and couples an **A**ntigen, a cancer-specific peptide, with an **A**djuvant known to increase the immune response and with an **A**ntibody fragment recognizing dendritic cells. The construct must boost the uptake of the antigen by the dendritic cell (DC). An important first step because processing of antigens by this type of immune cell will activate cytotoxic T-cells that may kill cancer cells. Wijfjes: "Thus far, these constructs have given good results in cell cultures, and some mixed results in mice models."
In cooperation with Ward Doelman, a post-doc in bio-organic chemistry at Leiden University, Wijfjes will test a slightly different construct, too. The antibody fragment will be replaced by sugar groups. Doelman: "My PhD study handled about adding sugar coats to antigenic peptides to improve antigen uptake by dendritic cells. Our strategy was not to select rather simple sugars that are known to target DCs, but with low affinity. By adding multiple copies of these sugars, the binding affinity should increase. I have made constructs with up to six sugar groups, some indeed boost the uptake. We also added a fluorophore to measure the binding to dendritic cells."

"I like solving problems, the complex, the better"

Click chemistry

Doelman is the first to act in the new project. He will create multivalent constructs containing several different trisaccharides ("I still have a stock in the freezer") which Wijfjes will use to build several cathepsin probes. Doelman is optimistic: "Chemistry-wise It does not seem very different from what I have done before, and sugar chemistry is not as hard as many believe it to be. Using my favorite click reaction, the copper-catalyzed azide-alkyn cycloaddition, it should work." Doelman plans to prepare three or four sugar-constructs, each carrying a different type of sugar, and send them to Nijmegen, where Wijfjes will complete the total constructs and perform first tests to investigate their uptake by DCs.

"Depending on the results we will make further adjustments," says Wijfjes. "I am quite sure we will be able to synthesize the construct and measure its activity. When they indeed show good activity, our second goal is to discover if we can use our insights to steer the DCs response towards more B- or either T-cell activity." Endosomes within dendritic cells vary in types of enzymes which may lead to different processing of the antigen and as a result to different presentation on the DC's cell surface. Doelman: "There are indications that different types of sugars find their ways to different types of endosomes."

Really cool

Both scientists hope that their cooperation will be intense. Doelman: "We do perform immunology experiments in our laboratory, but not the complete repertoire. Therefore, we have a lot of collaborations. That may mean 'putting a stamp' on the compounds we synthesize, but sometimes you exchange results and try to optimize them together. I enjoy that." Wijfjes: "From the start of my studies, I have looked forward to being a PhD student. I like solving problems, the complex, the better. I have the feeling that I finally arrived at the level of complexity I enjoy. Perhaps, that is also why I have always been attracted to immunology, ever since I learned about it in secondary school. The immune system is so intricated and highly precise. Really cool." ■

Zach Wijfjes (right)
Chemical Immunology, RadboudUMC
Martijn Verdoes group

Ward Doelman
Bio-organic Synthesis, LIC, Leiden University
Sander van Kasteren group

New insights on what triggers MS

Two recent papers provide important clues to understand the development of Multiple Sclerosis (MS). One describes a large-scale epidemiological study that clearly demonstrates the involvement of Epstein-Barr virus (EBV). The other presents a new mechanism to explain how the virus contributes to disease onset. But neither study proves that EBV is the trigger, says Bert 't Hart.

It has long been suspected that the extremely common Epstein-Barr Virus (EBV) has something to do with MS. "Over the years many studies have pointed to a correlation between EBV and MS, but most of these were limited in scale," says retired neuroimmunologist Bert 't Hart, who studied MS for more than thirty years at the Biomedical Primate Research Centre, University of Groningen and VU University Amsterdam. In January of this year, Kjetil Bjornevik (Harvard) and co-workers published in Science the results of a study among 10 million US military recruits, which demonstrates that all cases of MS involved a prior infection with EBV. "This is a very important paper. It is now crystal clear that without an EBV infection, MS will not develop. The results were not surprising, but you need such a large-scale study to provide hard evidence."

Molecular mimicry

However, the study does not explain the huge gap between the high prevalence of EBV in the population and the very low prevalence of MS. According to 't Hart, this shows that the dominant concept in which EBV is the trigger for MS is incorrect. So, the question remains how EBV is involved in the development of MS. Also in January, but this time in Nature, Tobias Lanz (Stanford) and co-workers offered a new mechanism that may explain the role of EBV. In short, they discovered a molecular mimicry motif between the viral EBNA-1 transcription factor and GlialCAM, an endogenous protein, expressed on non-neuronal brain cells, such as oligodendrocytes and astrocytes. This explains cross-reactivity of anti-EBNA-1 antibodies with GlialCAM, leading to autoimmune brain pathology.

> *"It is now crystal clear that without an EBV infection, MS will not develop"*

▼ *Recently scientists demonstrated high-affinity molecular mimicry between the Epstein-Barr Virus transcription factor EBV nuclear antigen 1 (EBNA1) and the central nervous system protein glial cell adhesion molecule (GlialCAM). In their study, the research team provided structural and in vivo functional evidence for the relevance of this cross-reactivity in the development of Multiple Sclerosis.*

Major discussion

"An interesting mechanism," says 't Hart. "But we also know that many people who have anti-EBNA-1 antibodies in their blood do not develop MS. So again, this shows that EBV is not the trigger of the disease." That brings us to a major discussion within the MS research field, 't Hart explains. "There is the outside-in theory, in which an external trigger activates an autoimmune response in the brain. The Lanz paper is in line with the outside-in concept. And then there is the inside-in theory, in which an 'internal' problem precedes the autoimmune response." In this approach, the EBV infection is not so much the trigger of the disease, but the catalyst that drives the uncontrolled autoimmune response. The myelin sheath starts, for reasons still unknown, to disintegrate and the fragments induce an immune response. When the immune system is already hyperactive because of a common viral infection and cross-reactivity emerges due to molecular mimicry, the combination can lead to autoimmunity. But how does that explain the low prevalence of MS? "Even though EBV infections are quite common, only an exceedingly small fraction of B-cells has specificity for myelin and at the same time only a small portion of B-cells will contain EBV. So, the chances that EBV gets incorporated in exactly those B-cells that respond to myelin are still very small." ■

Reference

K. Bjornevik, et al., **Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis**, Science (2022), doi:10.1126/science.abj8222

T.V. Lanz, et al., **Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM**, Nature (2022), doi:10.1038/s41586-022-04432-7

Zooming in on targeted T-cells

With their SCARI method, published in Nature Chemical Biology, the groups of Ton Schumacher and Sander van Kasteren have created new approach to probe deeper into the cellular composition of a tumor. By combining spatial information with single cell sequencing, they aim to unravel what really makes a T-cell tick.

It started when Ton Schumacher (Netherlands Cancer Institute) called Sander van Kasteren (Leiden University) to provide a chemical perspective on a problem Schumacher was wrestling with. Van Kasteren: "Ton wanted to focus on specific groups of cells within a tumor. Tumors are very heterogeneous, they are composed of many different cell types, areas that each have their own characteristics. Ton's idea was to focus on the small part of the tumor, somehow mark the interesting cells in that area and then study those cells in detail using single cell sequencing. But a major drawback is that you lose all spatial information because you basically put everything in a meat grinder. As a result, you lose track of where that specific cell was located within the tumor."

"We had to combine spatial information with single cell sequencing"

Spatial information

The underlying question is what drives T-cell activation, says Van Kasteren. "Let's say you have a little corner in the tumor with many dendritic cells, then it is interesting to see how the T-cells in that same area are activated and whether that differs from T-cells in other parts of the tumor with fewer or perhaps no dendritic cells. Which genes are expressed in a T-cell in a specific part of the tumor? That may tell us more about how T-cell activation works and which factors play a role. But to do that, we needed to combine spatial information with single cell sequencing."
Translating that idea into a chemical question was quite a challenge. "It boiled down to the question of how you can mark an area in a primary human tumor. You cannot use the conventional manipulation techniques because you're not dealing with a lab-generated tumor from an immortal cell line. Also, you want to keep the cells alive. For a chemist, this makes it a lot more fun to think about, it's a completely new playing field." The solution was developed step by step, leading ultimately to a new approach called SCARI - Single Cell Analysis of Regions of Interest.

Two colors

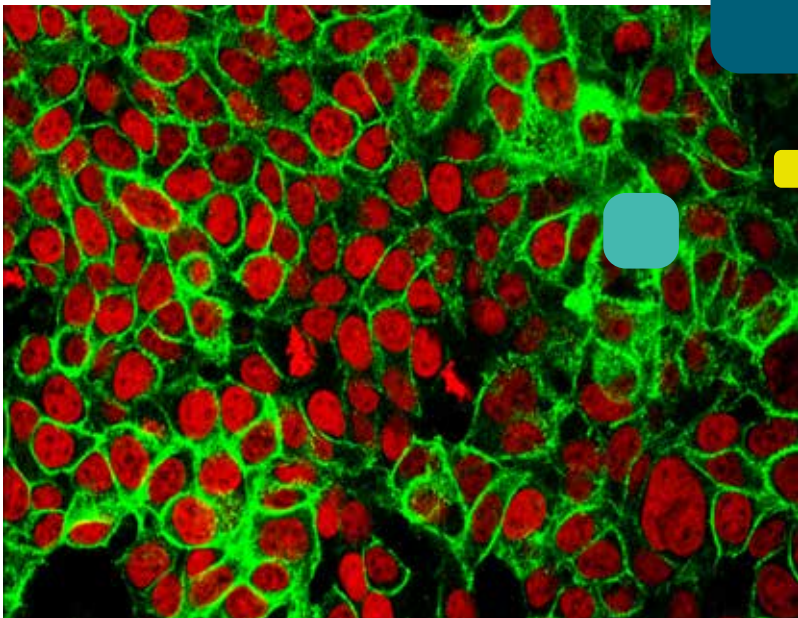
The key here is the use of a photosensitive cage that protects the fluorescently labeled nanobody, which targets the T-cells. By applying light to only a small part of the tumor, the part you want to study, the cage gets degraded and the fluorescent label is removed. As a result, the area of interest gets dark. However, to enhance resolution, a labeled antibody, with a different color, is added that can only bind to the uncaged tag on the targeted T-cells. "That was the breakthrough idea, to use two colors and thus two channels to enhance separation between targeted and non-targeted T-cells. Using FACS, you can then count and sort the T-cells, create two populations, and use single cell sequencing to map the differences."
Next on the list: make it broadly applicable. "Right now, it only works for nanobodies that have a sortase tag, but we plan to create a version that can be coupled to any antibody. What remains essential is the compatibility with primary human samples, because after all, that is the 'real' deal, that is what we need to understand." ■

"For a chemist, a major challenge in a completely new playing field"

Reference

A.M. van der Leun, et al., **Single-cell analysis of regions of interest (SCARI) using a photosensitive tag,** Nature Chemical Biology (2021), doi:10.1038/s41589-021-00839

▼ *The functional activity and differentiation potential of cells are determined by their interactions with surrounding cells. Approaches that allow unbiased characterization of cell states while at the same time providing spatial information are of major value to assess this environmental influence.*
To achieve that goal a photocage-based technology had been developed that allows isolation and in-depth analysis of live cells from regions of interest.



Working together pays off

For anyone who might think that professional matchmaking or speed dating events should be avoided, think again. It may open up a world of ideas and possibilities.

Consider the case of chemist Kim Bonger and immunologist René Toes. When they met during a match-making session at the very start of ICI, there was not an immediate connection between their respective lines of research. But that first meeting turned out to be the start of a very inspiring and fruitful collaboration that already spans eight years.

"The overall topic of our research is to specifically target autoreactive B-cells," says Bonger, associate professor of chemical biology at Radboud University. Autoreactive B-cells are key players in a variety of autoimmune diseases, but the project's primary interest is rheumatoid arthritis (RA). Current RA therapies are non-specific. They target all B-cells and although that results in the desired inhibition of the autoreactive cells, it also means that the protective B-cells are blocked. As a result, these therapies come with a variety of unwanted and dose-limiting side-effects. Bonger: "The medical field is therefore very interested in ways to enhance the specificity of these drugs." Next to the ICI funding, Bonger and Toes were also awarded a grant by 'Reumafonds', the Dutch Arthritis Foundation. That support underlines the need for new, better therapies.

Inspiring to hear

"When you want to tackle autoreactive B-cells, the B-cell receptor (BCR) immediately becomes your priority," says Bonger. "The BCR is at the heart of the problem. We need to selectively bind that receptor if we aim to target a specific B-cell population." When such selective binding is possible, Bonger sees different ways to manipulate the behavior of the targeted B-cells. "Binding the BCR can simply inhibit B-cell activity, but we are thinking about deploying the BCR to incorporate *Antigen Drug Conjugates* into the B-cell that can block crucial signaling pathways and change the cell's behavior from within. Or maybe we can use such a route to stimulate the cell's normal function."

> ***"A world of opportunities emerged"***

These targeting approaches have become an integral part of oncological drug development, but for autoimmunity applications, this is all very new. Bonger: "When it comes to chemical biology methods and targeted drug delivery, I would say that the autoimmunity field is lagging behind compared to the oncology field." So, when during that ICI match-making event Bonger started explaining her research to René Toes, professor of experimental rheumatology at Leiden University Medical Centre, a world of opportunities

emerged. "I knew nothing of the type of work Kim was doing, so it was very inspiring to hear. Her expertise creates so many new possibilities to explore, which makes this a great project to work on."
Toes agrees that there is much to learn from the oncology field. "We know that it is possible to target T-cells and manipulate their behavior. If we can achieve that for B-cells as well, that we can specifically target those cell populations that are causing the problems, it would help tremendously. Those autoreactive B-cells really are the bad guys here."

"Getting rid of the bad B-cells"

A lot to discover

As it takes two to tango: why was Bonger interested in working on better therapies for RA? "Well, to be honest, my main interest is not necessarily RA. For me, the challenge is in targeted drug delivery: how can we achieve that? That is a very interesting problem for a chemist. The chemical question fuels my research, more than the specific disease that such a solution may be used for. Having said that, autoimmunity is a very intriguing area to work on, because the type of chemistry we apply here is still very new to this area. There is a lot to discover."
In a relatively new field, with still plenty of uncharted territory, there are many possibilities and approaches to explore. You may easily get lost, but luckily for Bonger and Toes there are some practical clues to start from. "We know that CCP's, cyclic citrullinated peptides, can target autoreactive B-cells and these are already used in diagnostics to identify ACPA's, the anti-citrullinated peptide antibodies, which are a hallmark of RA," says Bonger. "CCP is the antigen and when we connect our toxins and inhibitors to CCP, we can use that as a delivery vehicle to get our compounds to the B-cell receptor. By using the amino acid citrulline, we make our constructs selective against citrullinated epitopes."
But there is a catch to targeting the BCR. First, the drug must be able to reach the BCR without being scavenged by autoantibodies in the circulation. Next, the receptors must cluster together on the surface of the B-cell to enable delivery of a drug. "We need therefore to ensure multivalency in our compounds, to bring multiple BCRs together on the surface."
Despite all these challenges, the in vitro results of the combination of CCP and Btk inhibitors, which target a kinase that is crucial to B-cell development, are promising enough to start thinking about preclinical studies.
Toes: "The step towards studies in mice models is in sight. Our first objective there is to demonstrate specific silencing of B-cells. If we can establish that, it is fair to assume that the disease severity will diminish as well. Ideally, we can recognize and target multiple types of autoreactive B-cells with one CCP construct. My hope is that specific targeting allows us to increase the dosage and that is what we need to completely wipe out all the unwanted B-cells."

Limited room

But there are still some chemical obstacles on the road toward the much-anticipated preclinical phase. "We can make the various compounds, but it is a time-consuming process," says Bonger. "There is more to it than just the synthesis, we also need scale up the volumes that we need for the next phase and make sure that our compounds have the right physical-chemical properties. Such as solubility. That is essential, but as it turns out, it is not a given. We have tried many different variants, but a large number of them turned out to be insoluble, which makes them of no use for our intended applications."
Another crucial requirement is that the compounds are not toxic. Toes: "RA is not lethal, which really limits the room for acceptance of toxic side-effects. That is a big difference compared to anti-cancer drugs. But the Btk inhibitor is already in clinical phase III studies as an isolated compound, which makes it very likely that toxicity will not be an issue. As long as we can show in mice models that the right B-cells are cleared and that the auto-immune response disappears in a safe manner, then it should be enough to start clinical studies." But first things first, says Toes. "What we need to do now is scale up the production, so we have enough material towards the preclinical studies." ■

Reumafonds
Dutch Arthritis Foundation

Joining forces

Reumafonds, Dutch Arthritis Foundation, protects the interests of people with a rheumatic disease. The foundation provides information about the diseases and advises on how to live with arthritis. In addition, she funds scientific research for better treatment and cure. Since the start of ICI, there has been a collaboration with the Reumafonds (see ICI Bulletin issue 1, p 6-7, October 2015). The work described in this article shows that joining forces is paying off.

From student to professional

During their research period, ICI PhD students receive trainings both professionally and personally. Where the professional training comprises a variety of chemical and immunological courses, the personal training focuses on soft skills. Together with Louise Mennen from Mennen Training &Consultancy ICI launched a tailored training course.

Embarking on a PhD is a major milestone in any academic career and inevitably a daunting one as well. Taking on a huge scientific goal is exciting but it usually comes with great challenges. Besides struggles of a scientific nature, PhDs are confronted with socially oriented questions like 'how can you get the most out of your busy supervisor' and 'how do you plan four years in advance' or 'where do you want your career to go next.'

"In our coaching work we often see brilliant scientists who fail to achieve their full potential because of their lack of social skills," Mennen explains. To avoid this pitfall, all PhD students enrolled in ICI receive a 4-year program covering topics ranging from personal effectiveness to career development. The program consists of four two-day modules: (1) *time and project management*; (2) *communication in science*; (3) *personal leadership*; (4) *a PhD, what next?*

Completed two modules

The first batch of ICI PhDs has now graduated and most of them are full of praise for the skills they learned in this course. The second batch of PhDs has completed two modules. "Unfortunately, due to corona, the training sessions largely took place online," Mennen says. "We were faced with considerable challenges, but thanks to successful improvisation techniques such as working with break out groups, sending students out for a walk to reflect and inserting more pauses, the course has not lost any effect or value."

May last year, the PhDs were trained in time and project management. You do not earn the title of 'doctor' simply by arriving at the lab on time. "Therefore, students learn during this module how to manage and oversee the huge PhD research project and how to stay motivated, even after a lot of lab work without the desired results," Mennen explains. Participants have appreciated this module as evidenced by their comments: "This course was much more useful and relevant than I expected. Not only did it give me tools and ideas to better manage my PhD study in the short and long term, but by talking with other students about their work methods I discovered how to better control my time and attention."

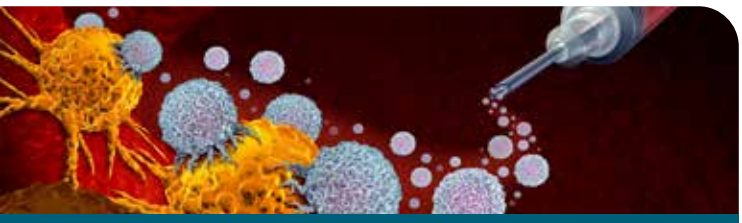
During the second module, December last year, the PhDs learned to deal with successful networking, giving a convincing presentation, designing a powerful poster and how to communicate effectively. There was also a stream of positive reactions to this training such as: "The course provided clear insight into my own communication styles and awareness of others' style. Confronting and eye-opening, but helpful to understand and apply to the real work situations."

Two of the three groups were lucky enough to experience this module live: "It was great having the training in person; not only to get meeting colleagues in real life, but also because of the interactive parts of the course. I very much appreciated all the tips and tricks we were getting."

Next sessions

Over the next two years, the PhDs will receive the other two modules, including personal leadership and career planning. Expectations are high, judging by statements like: "I think this will give a clear insight into my own strength and weaknesses," or "The training will help me to exploit specific skills that I have and help me become aware of the things that I need for a successful completion of my PhD and suitable follow-up career." ■

◄ *Participants experience the course as an eye opener in a lot of aspects: "It is really helpful to get insights in things, which are normally often not discussed during your 'daily' science work. You for example learn how to improve communication by knowing people's communication styles, how to give helpful feedback, how to network and how to make an attractive presentation and poster. This all gave useful insights where you normally never would think of, but that from of now I will implement in my daily work as a scientist."*



Oncode accelerator project

Cancer immunotherapy is a game-changing treatment that stimulates the T-cells of our immune system to kill cancer cells. However, the resistance of tumors to this T-cell-mediated therapy appears to be a major obstacle in practice. A group of Oncode investigators from several institutes are joining forces to face this problem. They will focus on mobilizing innate leukocytes to attack tumors. Dutch Cancer Society (KWF) funds the project with 3.4 million euros. Amongst others Sjaak Neefjes', Carl Figdor and Karin de Visser groups participate the project.

ERC Proof of Concept Grant

Martijn Verdoes (Tumor Immunology, Radboud UMC) has been awarded an ERC Proof of Concept (PoC) Grant. The honored project is based on a novel molecular vaccine strategy that effectively delivers target antigens to DCs to concomitantly induce robust and synergistic CD4+ and CD8+ T-cell responses. The PoC grant aims to support ERC grantees with the commercial or societal application of the results of their funded research.

NWO Venture Challenge

For twenty years, Hermen Overkleeft's group has have been doing research on the proteasome, a large protein complex whose main function is to break down other proteins when they are superfluous or damaged. A recent breakthrough from the PhD research of Elmer Maurits paves the way for promising drugs. But bringing a discovery from the lab to the clinic is not so easily done. Thanks to NWO's Venture Challenge, Maurits and his team will receive ten weeks of guidance to start up a company.

Recent publications

Le Gall C, Cammarata A, de Haas L, Ramos-Tomillero I et al..

Efficient targeting of NY-ESO-1 tumor antigen to human cDC1s by lymphotactin results in cross-presentation and antigen-specific T cell expansion

J Immunother Cancer. 2022 Apr;10(4):e004309. doi: 10.1136/jitc-2021-004309. PMID: 35428705.

Wörner TP, Aizikov K, Snijder J, Fort KL, Makarov AA, Heck AJR.

Frequency chasing of individual megadalton ions in an Orbitrap analyser improves precision of analysis in single-molecule mass spectrometry

Nat Chem. 2022 Mar 10. doi: 10.1038/s41557-022-00897-1. Epub ahead of print. PMID: 35273389.

Hagemans IM, Wierstra PJ, Steuten K et al.

Multiscale imaging of therapeutic anti-PD-L1 antibody localization using molecularly defined imaging agents

J Nanobiotechnology. 2022 Feb 2;20(1):64. doi: 10.1186/s12951-022-01272-5. PMID: 35109860; PMCID: PMC8811974.

Ignacio BJ, Dijkstra J, Garcia NM, Slot EFJ, van Weijsten MJ, Storkebaum E, Vermeulen M, Bonger KM.

THRONCAT: Efficient metabolic labeling of newly synthesized proteins using a bioorthogonal threonine analog

bioRxiv 2022.03.29.486210; doi: https://doi.org/10.1101/2022.03.29.486210.

Stok JE, Oosenbrug T, Ter Haar LR, Gravekamp D et al.

RNA sensing via the RIG-I-like receptor LGP2 is essential for the induction of a type I IFN response in ADAR1 deficiency

EMBO J. 2022 Mar 15;41(6):e109760. doi: 10.15252/embj.2021109760. Epub 2022 Feb 14. PMID: 35156720; PMCID: PMC8922249.

Schluck M, Eggermont LJ, Weiden J et al.

Dictating Phenotype, Function, and Fate of Human T Cells with Co-Stimulatory Antibodies Presented by Filamentous Immune Cell Mimics

Adv. Therap. 2022; 5: 2200019. https://doi.org/10.1002/adtp.202200019

Weiden J, Schluck M, Ioannidis M et al.

Robust Antigen-Specific T Cell Activation within Injectable 3D Synthetic Nanovaccine Depots

ACS Biomater Sci Eng. 2021 Dec 13;7(12):5622-5632. doi: 10.1021/acsbiomaterials.0c01648. Epub 2021 Nov 4. PMID: 34734689; PMCID: PMC8672349.

Column

KILLING MANY BIRDS WITH ONE STONE

JOOST SNIJDER
ICI EXECUTIVE ADVISORY BOARD

Joost Snijder is assistant professor Biomolecular Sciences at Utrecht University

When I first read the headlines, it felt like a big moment for public health. Researchers from Harvard claimed they found a very strong association between a viral infection and MS (multiple sclerosis). In MS, chronic inflammation results in loss of the insulating material of nerve cells in the brain and spinal cord. It's a debilitating illness without treatment. The root causes of MS have been a big mystery and now the team from Harvard claims that in many patients it's the result of a simple virus infection. On the surface this might suggest that vaccines and antivirals can prevent and cure MS. Almost too good to be true.

Until I could finally sit down and read the study, I was going back-and-forth between excitement and skepticism about the discovery myself. After all, we have been here before. People have been quick to point to viruses as possible causes for chronic, complex and mysterious neurological diseases. But all the usual suspects from the family of herpesviruses are very widespread, both in neurological patients *and* healthy people. It's a chicken-and-egg problem: does the viral infection trigger inflammation, or is a latent infection reactivated in the inflamed tissue?

But the Harvard team's study had an impressive scope. They followed millions of US army recruits over a 20-year period and showed that the few people who seemingly never became infected with Epstein Barr Virus are more than 30 times less likely to develop MS later in life. I am convinced: this infection must play a crucial role for many who suffer from MS.

So, does that mean we should pull out the vaccines and antivirals, and that we can soon call MS a disease of the past? It seems that antibodies against a viral antigen cross react with host proteins to cause MS, so we better be careful that vaccination not also mimics the detriments of our immune reactions. But maybe a childhood vaccine to prevent infection in the first place, one that omits the risky antigens that cause the autoimmune response, will have the biggest impact? Besides MS, Epstein Barr Virus also causes mononucleosis (kissing disease) and many types of cancers. Such a vaccine could kill not just two, but many birds with one stone. ■

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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