ICI institute for chemical immunology

Interview

"CURRENT IMMUNOTHERAPIES ARE ONLY THE TIP OF THE ICEBERG"

KARIN DE VISSER
Netherlands Cancer Institute
Professor of experimental immunobiology, LUMC

When cancer spreads, the prospects for patients quickly deteriorate. Karin de Visser, group leader at the Netherlands Cancer Institute and Oncode Institute, and professor of experimental immunobiology of cancer at the Leiden University Medical Center is determined to find out why metastases are so skilled at keeping the immune system at bay. "Understanding how tumor cells communicate with neutrophils and other immune cells is key."

Your research group works on metastasized breast cancer. Why this focus on metastasis?

"Because it poses an urgent clinical challenge. Once a breast tumor has spread beyond the lymph nodes, treatment can still slow down disease progression, but that's it. Really curing the patient is virtually impossible by then. Of course, this is not unique to breast cancer. Metastasized cancer is always bad news."

Why is it so difficult to treat?

"For starters, we don't really understand what metastases are. Metastases are very different from the primary tumor that they originated from, for various reasons. Metastases are located in a completely different environment compared to the primary tumor, with different immune cells present. So, their tumor microenvironment has changed drastically. Moreover, it is very likely that only the toughest cells are able to leave the primary tumor, move somewhere else, settle there and survive in a new, foreign environment. Metastases ▶

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have gone through a selection process that yields the strongest, most aggressive cells. That makes them very hard to tackle."

What about immunotherapy? We have seen spectacular results in, for example, metastasized melanoma.

"So far, immune checkpoint inhibitors are much less effective in breast cancer, compared to melanoma or lung cancer, but there are differences between types of breast cancer. Some triple negative tumors show a response, whereas hormone sensitive tumors are less likely to respond. One of the reasons that breast tumors are, overall, less susceptible to immune checkpoint inhibitors is probably due to the presence of other types of immune cells that protect the tumor. Another important aspect is that external factors are involved in melanoma and lung cancer. Both UV radiation and smoking lead to DNA damage and as a result, these tumor types contain many mutations. And the more mutations, the easier it is for the immune system to recognize a tumor cell."

Are there other ways to deploy immune therapy to treat breast cancer?

"We are currently focusing on neutrophils as a target. In breast cancer, as well as in other cancer types, we see chronic inflammation that is hijacked by the tumor. Tumor-induced chronically activated neutrophils can inhibit T-cell activity

"Only the toughest cells are able to leave the primary tumor"

and thereby protect tumors from destruction by the immune system. Increased levels of neutrophil production and activity are seen in many different tumor types. At the same time, we see a high level of heterogeneity among patients. Some patients do have those elevated neutrophil levels, while others, with the same tumor type, don't. We also know from mouse models that when you remove the neutrophils, the immune system can be unleashed and inhibit tumor growth again. We now need to find out which molecules the tumor employs to activate neutrophils and to stimulate their production. We have identified that mutations in the p53 gene play a role in neutrophil activation. Understanding the communication between tumor cells with p53 mutations and the neutrophils and other immune cells in the vicinity is key. How does this tumor cell subvert the immune system?"

How could we put such insights to use in the clinic?

"Understanding the mechanisms that tumors use to make immune cells work for them, may allow us to determine beforehand if a patient is likely to respond to a certain therapy or not. It will still take time, but I hope that in ten years or so we can offer a more precise, more targeted way to select the best therapy for a particular patient. In addition, I'm confident that these insights will also reveal new leads for therapy development. When it comes to immune therapy, we have only scratched the surface. CTLA4 and PD/PD-1 are only the tip of iceberg; we will see the discovery of many more targets and checkpoint inhibitors."

"We are focusing on neutrophils as a target"

But do you think that new therapies, which take a long time to reach the clinic, are the best way forward or is it better to be pragmatic and focus on combinations of existing therapies, including drugs that target the immune system but were not originally developed to treat cancer?

"The latter is definitely important, as long as it is done rationally. A trial-and-error approach is not only unethical, it is also not very informative. Our first aim is to understand which characteristics of the tumor are associated with a certain therapy response. That requires very specific and targeted monitoring of the patients in clinical trials in parallel with mechanistic experiments in the lab. Together with medical oncologist Marleen Kok at the NKI, we analyze therapy-induced changes in the immune system and look for similarities between patients with the same response. This is a highly discovery-driven process; we collect large datasets and then try to find common denominators between responders and between non-responders. Our research is increasingly becoming intertwined with the clinic."

That wasn't always the case?

"No, this has really developed over the last five to six years that my group is working very closely with groups in the clinic. By joining efforts, we can gather as much information as possible from such studies and determine what we need to measure, which samples are needed, what kind of information can be derived etc. This takes a lot of effort, time and energy from everyone involved, but it is really worthwhile and essential to move forward. What I personally like about this way of working is that it enables me, as a basic researcher, to contribute to better care for patients even though I don't see them myself. That is very motivating." ■

"We don't really understand what metastases are"

BRINGING PROBLEM AND SOLUTION TOGETHER

Biotech company LAVA therapeutics develops bispecific antibodies that lure helpful T-cells towards tumor cells. A first product candidate has entered the clinic and the growing company is searching for scientists and analysts with strong team spirit.

"It's gone very fast," tells business manager Erik Ensing of Utrecht based LAVA Therapeutics. In 2018, he was the very first, official employee. Today, LAVA employs over sixty people in both the Netherlands and in the USA, is enrolling patients in a first clinical trial and is listed on the Nasdaq stock exchange. "Our fund raising has been very successful, investors believe in our technology."
Paul Parren, LAVA's head of R&D and endowed professor of Molecular Immunology at Leiden UMC: "The field of immuno-oncology has had enormous success in the past decade. For the first time in history, people with cancer have complete and lifelong remissions by stimulating their own immune system. However, the first generation of therapeutics proved not specific enough: not everyone benefits, side effects can be severe and the therapeutic window is often small. LAVA's bispecific antibody technology, the Gammabody platform, addresses these disadvantages."

Gamma delta

Monoclonal antibodies can 'betray' cancer cells: they make the deranged cells more visible to the patient's own immune system. Bispecific antibodies (BsAbs) can simultaneously lure helpful compounds or cells to the scene. Parren: "In our case, one arm of the antibody searches for a specific biomarker present on cancer cells, the other arm attracts T-cells."
BsAbs may thus increase the local immune response, thereby lowering the required doses and possible side effects. Today, about a quarter of all antibodies in development are BsAbs. LAVA's platform generates BsAbs which engage a particular subset of T-cells, called gamma delta T-cells. Parren: "It's known that when gamma delta T-cells are present in the tumor, a patient's survival chances increase." LAVA is not the only company focusing on gamma delta T-cells, but currently LAVA is the only one doing so by using BsAbs. Its very first clinical trial started this year. Parren: "Early in 2022 we expect to report initial results on safety, and in the course of 2022 we anticipate the first results on efficacy. An exciting year."
In LAVA's pipeline are four other publicly disclosed BsAb programmes, one of which is developed in cooperation with Janssen Biotech. Two of the BsAbs target solid tumors. "The holy grail in immuno-oncology," according to Parren. "We obtained proof-of-concept in preclinical studies on prostate cancer in cooperation with the VUmc. We have an ongoing partnership in innovation." That partnership results from close cooperation with Hans van der Vliet, professor in Medical Oncology at the Amsterdam UMC, but also founding father and Chief Scientific Officer of LAVA Therapeutics since its inception in 2016. Both Parren and Ensing joined the start-up in 2018 and succeeded in raising million in institutional funding to build, expand the business and launched a successful initial public offering.

Team players

LAVA will continue to expand its pipeline and is recruiting accordingly. Since recently, LAVA is also recruiting new talents fresh from university or higher professional education with expertise in immuno-oncology, antibodies, T-cells, bioinformatics, orbio-analysis. Parren: "Of course, we're searching for people who are very good at their job, but they also need to be real team players. For a young company like LAVA reaching milestones in time and within budget is sacred. And that's only possible when team result is top priority for all." What does LAVA offer? Parren: "A highly innovative environment, development of personal talents, team spirit, and a chance to grow along with the company." ■

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Looking at lipases in MS

PhD project: Visualizing lipid metabolism in multiple sclerosis

The autoimmune disease multiple sclerosis causes neurodegeneration by demyelination of axons. PhD students Kieran Higgins and Daan van der Vliet study the role of different lipases in demyelation, and also remyelination.

"Looking at living brain tissue in action under the microscope is really amazing," says neuroscientist Kieran Higgins. "Unfortunately, nerve cells are finicky," he adds. "It's not easy to keep brain cultures alive in the laboratory over weeks." Higgins does so to follow lipase activity in living cultures with special attention for microglia cells the resident immune cells in the brain that can't protect the crucial lipid-rich myelin layer in MS. Higgins: "These tiny cells are emerging more and more as vitally important in brain health, and there is still a lot to explore about them."
Higgins prepares living slices of animal brains, tissue from mice models of MS. He studies these with different imaging techniques, including two-photon spectroscopy, at the subcellular level, trying to measure and locate the activity of specific lipases, and follow changes in time. He also hopes to image acute slices, and when experiments are successful image lipase activity in living animals too: peering directly in a living brain through a cranial window.

Quite a mystery

Today, only nine months after starting his PhD studies, the goal is figuring out how lipase activity is best visualized using a probe made by his ICI PhD-partner, biochemist Daan van der Vliet. Van der Vliet: "The group I work in has a lot of expertise in synthesizing and using probes for visualizing lipase activity." He synthesized a fluorescent probe that covalently binds to and thereby inhibits the active site of the enzyme monoacylglycerol lipase, or MAGL. MAGL-inhibitors have shown efficacy in animal models of MS, but the exact role of the enzyme in MS is quite a mystery.
"If this probe works as we expect, I've finished the organic chemistry part of my PhD-studies," tells Van der Vliet. Where Higgins is focusing on imaging lipase activity in living animal microglia cells, Van der Vliet will study lipases in human brain tissue from the Netherlands Brain Bank. "I'm going to study parts of human brains that are clearly damaged by the disease and compare these with parts that are still untouched by MS or that are remyelinated." Where Higgins focuses on the subcellular level and on specific lipases, Van der Vliet investigates the broader total spectrum of lipases. He 'fishes' active lipases out of human tissue and uses mass spectrometry to answer the question which lipases are up- or downregulated in the various stages of the disease.

> *"Unfortunately, nerve cells are finicky"*

In real life

Like Higgins, also Van der Vliet is currently busy finding the best protocols for his experiments. Van der Vliet: "When we have both figured out the optimal conditions for our experiments, we will compare and discuss the outcomes and possibly give each other clues about further experiments." Higgins: "It has been very convenient for me that Daan started his PhD half a year before me. Not only, because the probe was ready when I needed it, but Daans knowledge of lipase pathways was extremely helpful for me in making a quick start. He tipped me what literature to read and we now regularly discuss the latest insights." Due to the pandemic, meetings in real life have been scarce, but that might change now. Higgins: "Leiden and Amsterdam are very close, especially in my Californian terms". ■

Kieran Higgins (left)
Netherlands Institute for Neuroscience, Amsterdam
Maarten Kole group

Daan van der Vliet
Leiden Institute of Chemistry, Leiden University
Mario van der Stelt group and Inge Huitinga group

Zooming in on lysosomal activity

PhD project: Elucidation of lysosomal biogenesis, repair and death during macrophage responses

Lysosomes clean up a cell's redundant biomolecules. Macrophages may easily contain over a hundred of these organelles. Do they specialize? And how do they react to changing conditions? PhD students Kristine Bertheussen and Max Louwerse want to find out.

"It sounds a bit strange, but diseases are fascinating," says Max Louwerse, PhD student at Leiden University. "Why does somebody get ill? What went wrong? It's exciting to solve such a puzzle and perhaps add to a solution, too." The puzzle that Louwerse is working on for his PhD studies concerns lysosomal storage disorder in which a particular biomolecule is not or hardly broken down and accumulates in the cell. Louwerse: "There are identical twins of which one half suffers from such a disorder but the other not, while both carry the mutation known to be the cause. Very intriguing."
Lysosomes are known as the housecleaners of the cell. The spherical organelles contain a large variety of hydrolytic enzymes that break down redundant biomolecules. Macrophages easily contain over a hundred lysosomes. These are necessary to digest all the material – sometimes complete cells – those macrophages sweep up. Lysosomes are highly divers in size, move easily around in a cell and probably vary in composition and tasks. Still little is known about what is going on in the lysosomes at subcellular level. Kristine Bertheussen, PhD partner of Louwerse at ICI: "Only recently, it became clear that lysosomes in macrophages are more than garbage processors; by their hydrolytic activity they also influence the immune response."

"Still little is known about what is going on in the lysosomes at subcellular level"

Training

Louwerse wants to reveal lysosomal activity by using probes that tag specific hydrolytic enzymes. He studies macrophage cell lines, but also fresh cells. Louwerse: "Macrophages don't divide, therefore cell lines are by definition not representative of nature." He cultures fresh macrophages from monocytes isolated from donor blood. "I investigate which enzymes are present and how their concentrations change under various stress conditions. In practice that means pouring an awful lot of gels," he laughs. In a later stage, Louwerse also wants to study lipid composition of lysosomes, and protein and RNA-expression both in normal macrophages and that of patients with lysosomal disorders.
Bertheussen uses similar probes for imaging hydrolytic enzymes in lysosomes at *sub*macrophage level. She uses correlative light-electron microscopy (CLEM). "With CLEM you can measure the fluorescence of labelled enzymes and lay the results over an image of the intracellular structure so you can see activity differences between lysosomes in one cell. It's a complex technique, I use three different large and expensive machines." Bertheussen is currently training herself in the technique by finishing up a running project on fatty acids. "A different target, but the same technology and chemistry." Once she has finished and masters the apparatus completely, she will switch to imaging hydrolytic enzymes in macrophages.

Team spirit

Both PhD students are still in their first year of studies and haven't worked together much. "We both need to set up our experimental methods first," tells Bertheussen. "But we are in contact. We discuss science but also life as a PhD. I think science is never an individualistic effort, and I wouldn't choose to do PhD studies in just any laboratory, having nice colleagues and a good team spirit is as important to me as the science." Louwerse: "Once Kristine and I have our experiments running, we will start exchanging experiences on working with the probes and discuss results on lysosome composition. We don't really need to plan meetings, we work one floor apart, we can just take the stairs and knock on each other's door." ■

Max Louwerse
Leiden Institute of Chemistry, Leiden University
Hans Aerts group

Kristine Bertheussen
Leiden Institute of Chemistry, Leiden University
Hermen Overkleeft group

Willy van Heumen Prize for Ferenc Scheeren

Producing monoclonal antibodies requires substantial numbers of animals, but thanks to a new method developed by the Scheeren and Verdoes labs, a serious reduction is within reach. For that achievement, Ferenc Scheeren was awarded the Willy van Heumen Prize 2021.

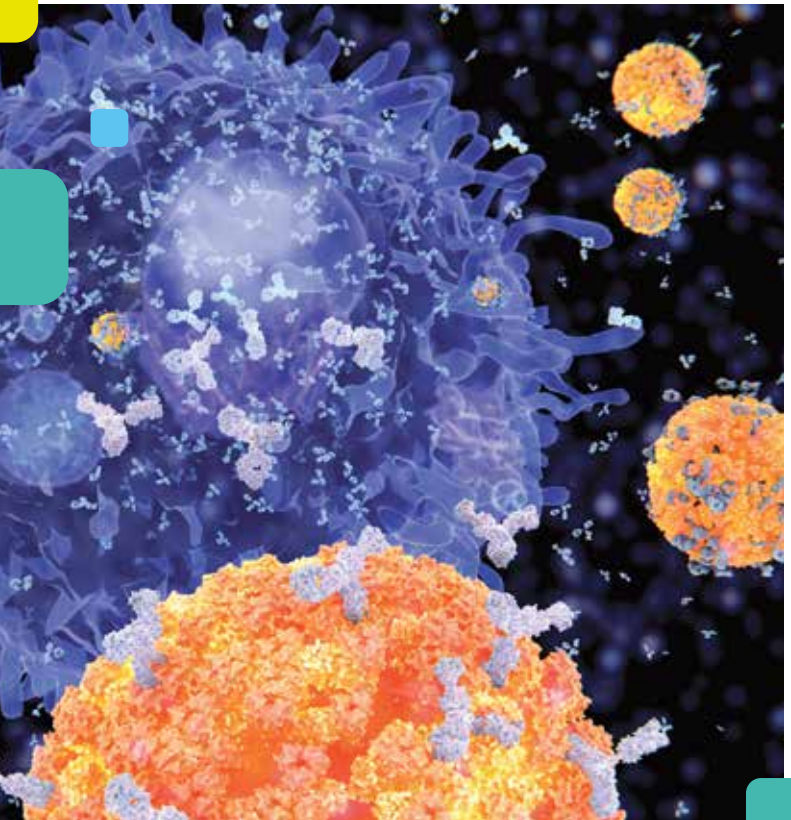
Sometimes, benefits of a new technology pop up in unexpected areas. In 2019, the groups of Ferenc Scheeren (LUMC) and Martijn Verdoes (Radboudumc) published a new, CRISPR-based method to engineer hybridomas; immortal cell lines used for the production of monoclonal antibodies. Their approach – fueled by frustration over existing expensive and time-consuming techniques – offers a fast, easy and cheap way to tune the characteristics of the desired antibody. But as it turns out, their approach also contributes to a reduction in animal use. For the latter, Ferenc Scheeren was awarded the Willy van Heumen Prize 2021, a Dutch prize that recognizes scientific contributions to the development of alternatives to animal experiments.

Recycling

Scheeren explains how their method helps to reduce the need for live animals in the production of antibodies. "Our method allows the recycling of existing hybridomas, which surely caught the jury's eye. All around the world there are massive amounts of hybridomas available and with our technique, these can be adjusted and used again. That already means a major reduction in animal use." And the method allows for more precision, adds Verdoes. "Our method in itself requires fewer hybridomas because you have precise control over the antibody that you generate." Finally, the jury applauded their open science approach. Scheeren: "Our technique is available to anyone and so far, we have received a lot of attention from researchers and many requests for collaborations.
Both Scheeren and Verdoes are encouraged by the fact that the jury saw the value of their technique even though it was not specifically developed as an animal-free alternative. "They clearly looked beyond the dedicated research into alternative and also considered other developments that can really make a difference," says Scheeren. "I think that refocusing your research is more important than just concentrating on finding animal-free alternatives." Verdoes agrees. "We even used animal experiments in the development of our technique, because we needed those to demonstrate that our method works and that is the way to convince the community." Scheeren: "I hope that our work shows that you can contribute to the discussion, also when your own research involves animal experiments."

> *"Our technique is available to anyone and so far, we have received a lot of attention and many requests for collaborations"*

▼ *An easy, cheap and fast technique for producing monoclonal antibodies. Besides, this approach proves to reduce laboratory animal use. The method was awarded the Willy van Heumen Prize 2021, a Dutch prize that recognizes scientific contributions to the development of alternatives to animal experiments.*



Immunotherapy

Instead of rigorously trying to avoid using animals, both agree that the starting point should be the question you want to answer. "If an animal experiment offers added value to answer your question, then I have no problem with performing such an experiment. But if the differences between a mouse and a human are too large, then you will learn nothing useful from a mouse study. In that case, don't do the animal experiment," says Scheeren. "However, it is important to acknowledge that studies in mice have taught us a wealth of important insights. Think for example about all the breakthroughs in immunotherapy. The fact that we now have checkpoint inhibitors is all thanks to basic research using mouse models. Animal experiments are certainly not useless when it comes to human biology, but benefits and alternatives should be carefully considered." ■

Reference

Van der Schoot JMS *et al.* Functional diversification of hybridoma-produced antibodies by CRISPR/HDR genomic engineering, *Science Advances*, 28 Aug 2019, Vol 5, Issue 8
DOI: 10.1126/sciadv. aaw1822

Understanding PD-1 blockade by perturbing tumor biopsies

What happens in a tumor when immunotherapy is applied? With their new ex vivo fragment platform, Daniela Thommen and her team offer a '3D human cancer model' that allows mechanistic studies on human tumors in a human setting. Using this platform, they revealed that PD-1 blockade leads to reactivation of T-cells and a broad intratumoral T-cell response.

It all started quite a while back, when Daniela Thommen was still working as a postdoc in her native Switzerland. "For developing new drugs, you often start with cell lines and then move to mouse models for mechanistic studies," says Thommen, who is now group leader at the Netherlands Cancer Institute. "But translating those mechanistic insights from mice to patients is very difficult, mostly because of the heterogeneity of human cancers and of patients.
That got me thinking about something in between. A method in which we use patient-derived material that can still be perturbed. That would enable us to perform mechanistic studies and reveal how a drug acts on a tumor and how the tumor responds." That 'something in between' developed into an innovative ex vivo tumor fragment platform, published in Nature Medicine, that turns out to be highly predictive of the clinical response to anti-PD-1 therapy.

"There was complete overlap between clinical responders and biopsy responders"

3D human cancer model

In Nature Cancer Community blogpost, first author Paula Voabil describes the platform as a '3D human cancer model'. A very apt description. "We use tumor biopsies as human models," says Thommen. "We collected biopsies from patients before their treatment with PD-1 inhibitors and in parallel with their treatment in the clinic, we treated the biopsies with the same drugs." The results showed a stunningly high correlation between the two 'groups': there was complete overlap between clinical responders and biopsy responders.
Impressive, but the starting point of the whole endeavor was to tackle the mechanism underlying PD-1 blockade. That became clear as well. "The first step in the immunological response is the reactivation of the tumor-resident T cells followed by the production of several chemoattractants, which then may lead to the recruitment of new, peripheral T cells," Thommen explains.
Despite its strong predictive power in these studies, Thommen does not immediately foresee the use of the platform as a prognostic tool. "I think that the main first application of our platform is to further study the immunological responses of tumor tissue to various immunotherapies and unravel the underlying mechanisms. This will hopefully also lead to the discovery of new biomarkers.
Another application would be to use the biopsies to screen the response to multiple therapies to identify responders and aid in treatment decisions."

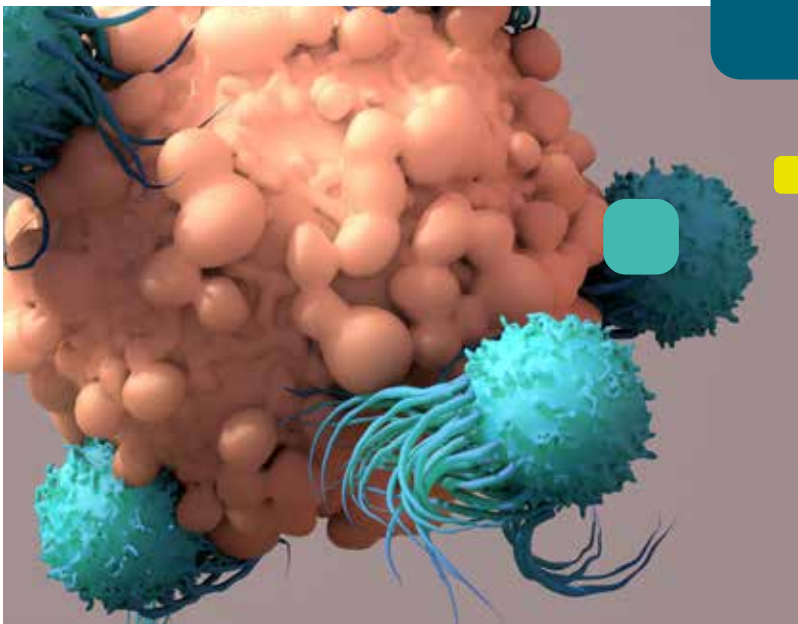
Lot of interest

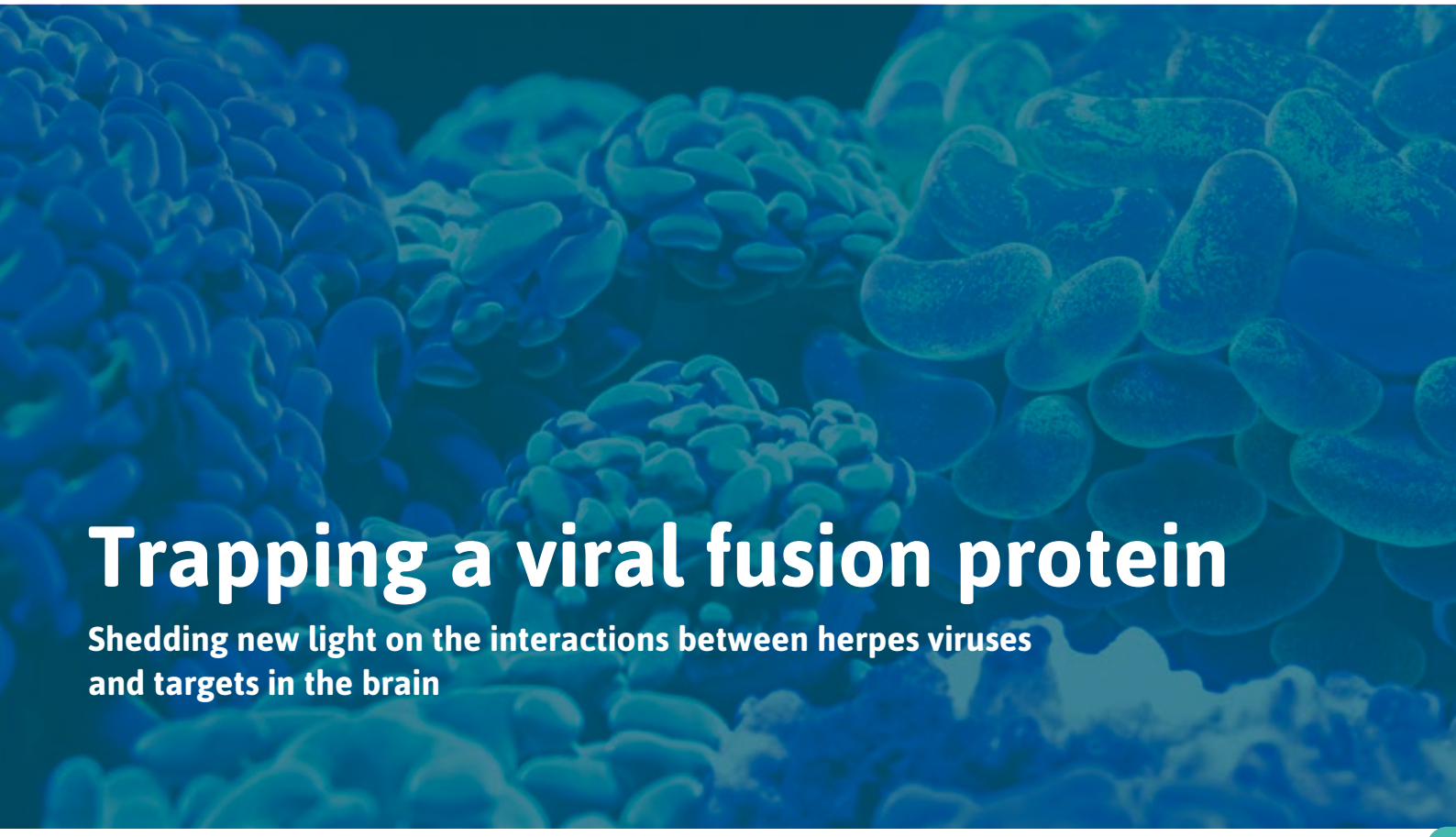
Since the publication, the team has attracted lots of interest in the platform, but Thommen warns that implementing their approach requires quite some work. "We are still facing all kinds of challenges, especially relating to logistics. We are lucky to be integrated with the Antoni van Leeuwenhoek hospital, so we are close by to get the samples. Which is essential because the speed of processing and the handling of the fragments are paramount. It may look simple, but there is more to it than just cutting up the tumor into small pieces."

Reference

Voabil P, de Bruijn M, Roelofsen LM *et al.* An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat Med* 27, 1250–1261 (2021). doi.org/10.1038/s41591-021-01398-3

▼ *Cancer immunotherapy is the artificial stimulation of the immune system to treat cancer, improving on the immune system's natural ability to fight the disease. Cancer immunology is a growing subspeciality of oncology. While some cancer patients respond spectacularly well to immunotherapy, many others do not benefit at all from such a treatment. By treating small pieces of tumor tissue from patients in the lab, Daniela Thommen wants to improve this: linking the right treatment to the right patient.*





Trapping a viral fusion protein

Shedding new light on the interactions between herpes viruses and targets in the brain

Herpes viruses are virtually impossible to avoid and responsible for variety of health problems, ranging from everyday nuisances to very serious medical conditions. Including brain inflammation-related diseases. A new ICI project aims to resolve the structure of an essential herpes virus fusion protein to shed new light on the interactions between the virus and its targets in the brain.

Say 'herpes' and plenty of nasty afflictions pop up in people's minds, but brain inflammation is probably not one of them. So why are Bert Janssen and Joost Snijder (both at Utrecht University), focusing on herpes viruses in their collaborative project within ICI's neuro-immunochemistry program? "Because there is a very clear connection between neuronal inflammation and herpes infections," says Snijder, "Herpes viruses are extremely widespread. Most people are infected, without knowing."

Cold sores

The family of herpes viruses is large, diverse and very common. There is herpes simplex 1 that gives you cold sores, and varicella zoster that causes chicken pox in children and shingles in senior adults. Other examples are Epstein-Barr virus, which causes glandular fever and is implicated in certain types of lymphoma, and cytomegalovirus, which can lead to serious birth defects. Snijder: "Herpes viruses cause longtime, latent infections and can suddenly emerge when the opportunity arises. For example, when your immune system is weakened by another infection, immune suppressive drugs or tissue damage. Or simply because of advanced age. Suddenly, elderly people can be struck by shingles. It may seem like they have contracted a new infection, but in most cases, it was there all along." This persistence is already nasty enough, but what really complicates matters is that herpes viruses can spread throughout the nervous system and even end up in the brain. "What starts as just a cold sore can over time develop into a full-blown neuro-inflammation problem." But Snijder warns against the easy assumption that there is a causal relationship between a herpes infection and diseases of the brain. "It is very difficult to unravel these links, it quickly becomes a chicken-or-egg problem. Take multiple sclerosis, which is clearly linked to myelin damage. But does that damage create an opportunity for a latent herpes virus become active or does that infection over time leads to myelin damage and the development of MS? At this point, we don't know what is causing what. But we do know that herpes viruses are very often implicated in brain inflammation."

> ***"We are dealing with some really basic questions here"***

Glycoprotein

What is still largely unknown is the mode of action of herpes viruses when they take up residence in the brain. To understand those mechanisms, detailed knowledge of both the virus and the cells it targets is essential, says Bert Janssen. "My research focuses on the interactions between cells in the central nervous system. For example, glia are important, such as the oligodendrocytes that stimulate myelin formation. How do these cells communicate, which surface proteins are involved and how does adhesion work?

> *"There is a very clear connection between neuronal inflammation and herpes infections"*

It is important to know and understand all these interactions because viruses use them for their own purpose." Fortunately, Janssen and Snijder are not completely left in the dark. It has been established that all herpes viruses have a fusion protein called gB, which interacts with myelin associated glycoprotein (MAG). This MAG is a transmembrane glycoprotein present on oligodendrocytes and is involved in the myelination process. Also, the structure of the ectodomain (extracellular part of the protein) of MAG has been resolved. So, there is something to start from, but it is not much. Janssen: "We know that gB and MAG interact, but we have no clue how. MAG is a glycoprotein, which could mean that the interactions are sugar-mediated. We have created MAG mutants that are no longer able to bind sugars. The mutants are no longer able to bind the GT1b ganglioside present on neurons. Disruption of MAG's sugar binding capacity has an immediate effect on the way it operates. But we don't know whether this is because of specific sugar binding interactions that are now blocked or that simply the presence of the sugar coating is necessary, but the effect is aspecific. And there is still the possibility that specific protein-protein interactions are also playing a role. We are dealing with some really basic questions here."

Fired up

A major obstacle in studying the gB-MAG interactions is the lack of structural information on gB in its pre-fusion state. The post-fusion structure has been resolved. Snijder: "But we need insight into the pre-fusion conformation to study the interaction with MAG in details, but also to look for leads relating to an application, such as an inhibitor that can block the fusion." The instability of the pre-fusion state really complicates matters. "This instability of the pre-fusion state is not because gB is an intrinsically disordered protein, but the post-fusion state is so extremely stable that the protein will switch to that state as soon as it can. It is as if gB is completely fired up for fusion, it only needs the slightest trigger to change its conformation." To make that conformational change a little less easy, gB is being engineered. "We study homology models of other gB's to see which interfaces we need to stabilize in the pre-fusion state," Janssen explains. "By introducing more hydrophobic interactions in the core of the protein, it will become more difficult for gB to switch its conformation. At the same time, the active sites at the surface of the protein remain undisturbed and that is crucial to study the interactions with MAG." This approach should result in more stable variants of the ectodomain of pre-fusion gB, which will then be studied using cryoEM to determine the protein's structure. First steps have been realized, according to Snijder. "PhD student Sabrina Krepel has succeeded in making gB constructs that allow us to express the ectodomain and we are currently exploring which mutations might enable more stability."

Detailed mapping

Once that stable pre-fusion state has been achieved, the team has a whole range of topics to address. Starting with an inventory of the interactions between gB and MAG, but also studying the structure of the gB-MAG complex and, even further ahead, performing immunology studies to produce antibodies and nanobodies against MAG and pre-fusion gB. Janssen: "The brand-new protein expertise center in Utrecht offers a whole suite of advanced equipment that will benefit the project. Using this, we will develop a toolkit that allows detailed mapping of cellular interactions." Next to broadly applicable tools, Snijder feels that more insight into this gB structure will show its relevance one day. "When in January 2020 the sequence of SARS-CoV-2 was published, the structure of the spike protein was resolved in no time at all. That was possible because researchers had been working on stabilizing the protein's pre-fusion state for a long time. So, when the need was there, they could act immediately. If we succeed in stabilizing the pre-fusion state of gB, it will be a huge boost to revolving the structure all gB variants within the family of herpes viruses." ■

New ICI theme

The immune system of the brain remains largely unknown. A serious a problem, because the interactions of the brain-resident immune cells play a major role in the development of both the healthy brain and brain-related diseases. The recently initiated ICI neuro-immunochemistry program encompasses the interplay between the immune system and neurological processes. ICI's chemical biology approach and its team-up approach between chemists, immunologists and brain researchers will be useful. Projects, like the here described collaboration between Bert Janssen and Joost Snijder will result in new insights to address major questions.

Translating complex matter into clear visuals

Ensuring that your audiences understand the essence of complex scientific and medical concepts is key to the communication of science. "Clear visuals combined with well-focused text are essential for optimal communication," explains Joost Bakker, specialized in science visualization.

Can you make something spectacular out of this? Couldn't you brighten up my story with a cartoon or something? Usually, accompanied by a package of text pages or a PowerPoint with dozens of slides. Joost Bakker often receives such requests. "But visualization includes much more than that," he starts teaching the principles. "Visuals are powerful tools to successfully communicate complex scientific concepts and products in a clear and convincing way. Since graphics may represent information which is too complex to explain in words. This makes it easier for the audience to quickly understand the scientific concept and to consider the essence of a process or product." It is exactly Bakker's job to guide such scientific visualization.

Becoming expert

Originally trained as an immunologist, he further improved his skills in science writing at Genmab, where he worked at the department of Scientific Communication for 12 years. Here he gained expertise in developing infographics and corporate art work. Furthermore, he adopted principles of marketing communication strategies. In 2013, he decided to combine his scientific background, graphic skills and marketing knowledge by starting Scicomvisuals. His business idea has since grown into a successful company. "Ultimately, I think the biggest driving factor behind the success is that I simply love what I do. It is great to be able to combine my scientific background and expertise with my passion for visualization and design. To me, developing an illustration, infographic or animation that shows the essence of a technology or scientific concept is a true joy!"

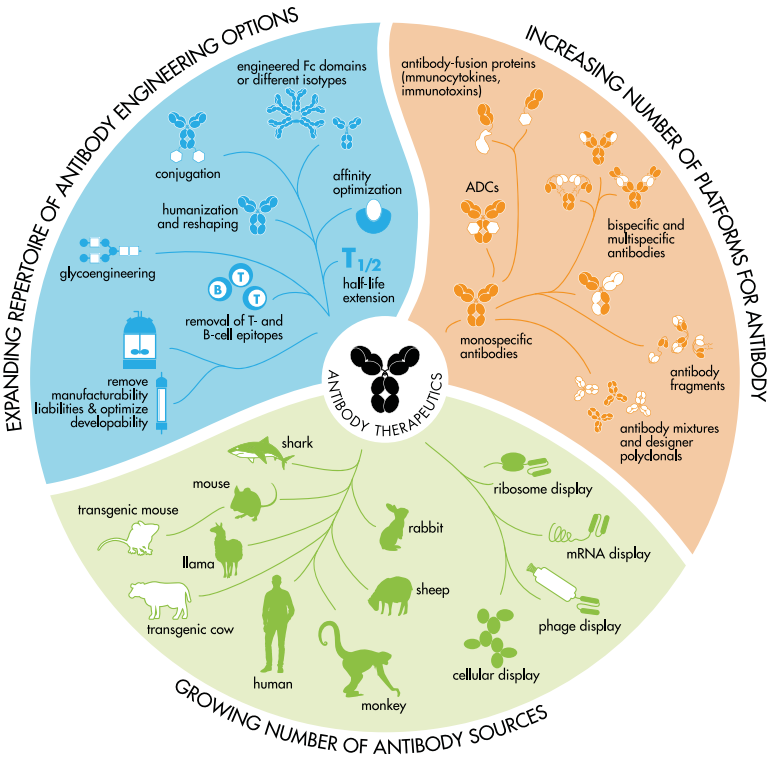
Scicomvisuals succeeded to attract national and international clients ranging from university research groups to companies in biomedical and agriculture sector. The website (www.scicomvisuals.com) shows a nice overview, including clear explanations, of completed assignments varying from detailed infographics to attractive cover illustrations and compelling animations. An example is shown in the illustration below.

An appealing poster

Bakker also provides training and courses for students and scientists to improve their skills in science communication and visualization. Next year, February 18th, together with science writer Esther Thole a workshop is organized for ICI PhD students called *How to make the perfect scientific poster*. A great challenge! "Too often, we see hanging long rows of posters all looking the same and following the rigid and uniform structure: title, abstract, introduction, materials and methods, results, discussion, references."

Both trainers are going to teach the students how to make an appealing poster by learning them to establish the vital message to be conveyed by the poster. "An appealing poster offers the possibility to drive traffic to your research. It is more than a summary of your research. You should envision it as the marketing message, the advertisement of your work," Bakker explains.

Visualization tools, when used properly, provide powerful support to make science communication effective and attractive, not only posters, but also oral presentations, reports, publications or grant applications. Which can be followed by unexpected collaborations, more funding, more citations and recognition. "In short, all this generates momentum and even more opportunities," he concludes. ■

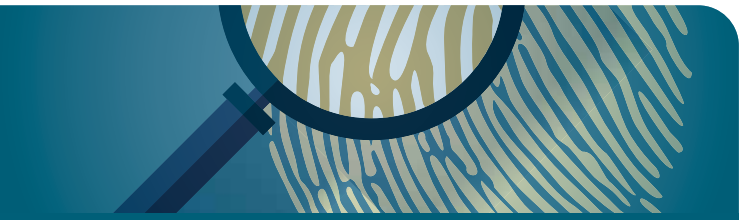


◄ *Infographics offer the opportunity to replace an endless list of bullet points. The idea of this visual was to give an overview of the vast increase in antibody innovations in sources, engineering options and platforms over the past decades. The figure shows an expanding toolbox for the generation of therapeutic antibodies that meet modern biopharmaceutical requirements. Therapeutic antibodies can be generated in many ways and capturing an antibody's source in a single syllable is therefore no longer possible.*

The infographic was published in a perspective by Paul Parren et al: Parren P.W.H.I. et al (2017) Changes to International Nonproprietary Names for antibody therapeutics 2017 and beyond: of mice, men and more, mAbs, 9:6, 898-906.
DOI: 10.1080/19420862.2017.1341029.

ICI Conference 18 March 2022

We are pleased to invite you to the next ICI conference to be held March 18th 2022 at the Naturalis Biodiversity Center in Leiden. We are proud to announce the following confirmed speakers: Pamela Chang, Cornell University; Matthew Bogyo, Stanford University; Santiago Zelenay, Cancer Research UK Manchester Institute; Dmitri Filippov, Leiden University; Leila Akkari, Netherlands Cancer Institute; Bart Eggen, University of Groningen. Mark your calendar for 18 March 2022! More information on the program and registration will follow.



Our immune system as unique as a fingerprint

Every person appears to have a completely unique immune system. Researchers from Utrecht University discovered this immune diversity after mapping antibodies from healthy and sick individuals. The discovery could help explain why, for example, corona vaccines appear to be less effective for some people. Extreme diversity in immune responses could also create new possibilities for personalized treatments and vaccinations. The research was published in Cell Systems last September.

High contagious card game

Infecting each other with viruses and bacteria while protecting yourself with medicines and vaccinations. Sounds like a fun evening, right? Master students Life Science & Technology Rafael Jezior and Dennis de Beeld certainly think so. Together, they developed ImmunoWars: an exciting card game based on real science. Many are already infected with the game! (see also Column Page 12)

Recent publications

Ho NI, Camps MG, Garcia-Vallejo JJ, Bos E, Koster AJ, Verdoes M, van Kooyk Y, Ossendorp F.

Distinct antigen uptake receptors route to the same storage compartments for cross-presentation in dendritic cells.

Immunology. 2021 Nov;164(3):494-506. doi: 10.1111/imm.13382. Epub 2021 Jun 30. PMID: 34110622.

Bondt A, Hoek M, Tamara S, de Graaf B, Peng W, Schulte D, van Rijswijck DMH, den Boer MA, Greisch JF, Varkila MRJ, Snijder J, Cremer OL, Bonten MJM, Heck AJR.

Human plasma IgG1 repertoires are simple, unique, and dynamic.

Cell Syst. 2021 Sep 13: S2405-4712(21)00331-8. doi: 10.1016/j.cels.2021.08.008. Epub ahead of print. PMID: 34613904.

van der Leun AM, Hoekstra ME, Reinalda L, Scheele CLGJ, Toebes M, van de Graaff MJ, Chen LYY, Li H, Bercovich A, Lubling Y, David E, Thommen DS, Tanay A, van Rheenen J, Amit I, van Kasteren SI, Schumacher TN.

Single-cell analysis of regions of interest (SCARI) using a photosensitive tag.

Nat Chem Biol. 2021 Sep 9. doi: 10.1038/s41589-021-00839-x. Epub ahead of print. PMID: 34504322.

Zaal EA, de Grooth H-J, Oudaert I, Langerhorst P, Levantovsky S, van Slobbe GJJ, Jansen JWA, Menu E, Wu W, Berkers CR

Targeting coenzyme Q10 synthesis overcomes bortezomib resistance in multiple myeloma.

Molecular Omics 2021 Aug. doi: 10.1039/D1MO00106J

Peng W, Pronker MF, Snijder J.

Mass Spectrometry-Based De Novo Sequencing of Monoclonal Antibodies Using Multiple Proteases and a Dual Fragmentation Scheme.

J Proteome Res. 2021 Jul 2;20(7):3559-3566. doi: 10.1021/acs.jproteome.1c00169. Epub 2021 Jun 14. PMID: 34121409; PMCID: PMC8256418.

Jäger, E., Humajová, J., Dölen, Y., Kučka, J., Jäger, A., Konefał, R., Pankrác, J., Pavlova, E., Heizer, T., Šefc, L., Hrubý, M., Figdor, C. G., Verdoes, M.,

Enhanced Antitumor Efficacy through an "AND gate" Reactive Oxygen-Species-Dependent pH-Responsive Nanomedicine Approach.

Adv. Healthcare Mater. 2021, 10, 2100304. doi.org/10.1002/adhm.202100304

Column

THE AMAZING GAME OF OUR IMMUNE SYSTEM

KIM BONGER
ICI EXECUTIVE ADVISORY BOARD

Kim Bonger is assistant professor biomolecular chemistry at Radboud University

I immediately recognized the characters on the TV screen when I entered the room. My young daughters found the 'Once upon a time... life' series on Netflix. As a child I loved this show! The animation series, which originated from the 80s when broadcasted on television tubes with push buttons, depicts an educational view on the human body where leading figures represent the different cells and defend mechanisms. The "Immunology" episode that my daughters happened to be watching, beautifully depicted 'lymphocytes type B' as surveillance space shuttles, 'lymphocytes type T' as white little soldiers, antibodies as friendly fly-like figures and macrophages as multi-armed vacuum cleaners. I also immediately recalled why I always visualize bacteria as mean-looking blue monsters with purple dots.

For me as chemist, it is a big challenge to fully comprehend the field of immunology. The complexity of cell organization, cellular interactions and signaling molecules is just mind-blowing and any source of accessible information is very welcome in order to learn. At the start of the ICI, I followed several online courses from leading universities and greatly benefitted from educational YouTube channels to understand the different mechanisms of our protective system in more detail. I was especially amazed by the huge number of wonderful animations and inspiring lectures that were often freely available online.

This ease of access to scientific education is important. Besides the learning aspect, it changes views and creates interest and engagement. It goes beyond the web and textbooks. I recently learned about the new Kickstarter card game ImmunoWars that was developed by two Dutch Life Science & Technology students, who discussed the contours of the game over a drink during the pandemic. In the game you battle with friends and foes with viruses and bacteria and protect yourself using the different elements of the immune system as well as other resources, such as medicine and protective gear. The website and trailer video look amazing and the launch time is perfect due to the pandemic. As ICI community we may even contribute a little to the game as suggestions for new play cards are still welcomed on their website. I am much looking forward to the official release and put my pre-order in place! ■

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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