Interview

ADDRESSING OLD PROBLEMS USING NEW TECHNOLOGIES

ALBERT HECK
Professor at Utrecht University and Scientific Director of the Netherlands Proteomics Centre

Albert Heck is internationally renowned for his technological breakthroughs that continuously take the proteomics field to new levels. But even though his heart is with technology, he always looks for a match with exciting biological questions.

Why did you join ICI? What does the combination of immunology and chemistry have to offer to a mass spectrometrist?

"In general, I want to know how proteins function, and since our immune system comes with a wealth of intriguing protein-related problems, it is an inspiring topic for a biomolecular mass spectrometrist. Through interactions with immunologists I soon realised that the immune system is extremely complex and dynamic, making it very challenging to study using current analytical techniques. You need to push the technology to its limits to understand immunology and, that is exactly where I like to contribute. I want to work on problems that increase our biological understanding and push mass spectrometry to the next level. My drive is to devise and enable truly novel experiments; to cover uncharted territory in immunology using mass spectrometry in completely new applications."

How does that drive translate to the ICI program?

"Our focus within ICI is mostly on two topics. One is complement activation and the other concerns the proteasome degradation machinery. In both these ICI projects, we see how technological breakthroughs transform our understanding of 'old' problems in immunology." ►

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Let's start with the project on complement activation. What is the role of mass spectrometry here?

"In collaboration with Piet Gros's group, our ICI colleagues here in Utrecht and a team led by Paul Parren at Genmab, a company specialised in antibody development, we succeeded in elucidating how the complement pathway is initiated, when the complement factor C1 binds to IgG. It was known that C1 binds to IgG, but only very weakly. So how can this binding be the priming step in activation of the whole complement cascade? The C1 structure forms a hexamer and thus our hypothesis was that IgG should also form a bigger complex. In earlier crystal structures IgG did form hexamers, but this was regarded by most as an artefact of the crystallisation.
"Then Genmab created mutations that potentially could stabilise IgG hexamers. We then used native mass spectrometry to establish their propensity to hexamerise in solution and next measured their strongly enhanced binding to C1. Now, by mass spectrometry we could follow the whole initial process of complement activation: who does what and when. Research into the complement system has a long history, but only now do we have the techniques that allow us to really study this system in molecular detail."

"You need to push the technology to its limits to understand immunology"

And did it generate new leads for further technology development, that mutual inspiration you mentioned before?
"Yes, this topic will bring us to another technology area: glycoproteomics. The complement cascade contains many glycosylated proteins and we want to know which glycans are present and what their functions are. So far, MS has not been well suited because glycosylation creates immense heterogeneity in protein mass. But we are now creating technical breakthroughs introducing a novel mass spectrometer to deal with this heterogeneity. It is really fun to work on something that will allow a whole new field of research to open up."

Are these new MS applications also involved in the proteasome project?

"No, here we employ novel peptide sequencing methods we recently developed. In cells, proteins are continuously broken down by the proteasome into short peptides that can subsequently be presented by MHC I molecules as antigens. The clear majority of these peptides are 'self' peptides. But when, for whatever reasons, mutations or modifications occur in a protein, some of the peptides will have an altered sequence. We developed technologies to efficiently eluate, sequence and analyse MHC I-bound peptides. Using the standard methods, we initially could match only 10% of the peptides to the genome databases. What was going on here? It turned out that the proteasome not only 'cuts' a protein into smaller pieces, it also can recombine these pieces, generating completely novel peptide sequences that never can be detected on the genome as they have no analogue DNA sequence.
"After a lot of work, implementing a novel peptide sequencing method called Electron Transfer Dissociation and Collision-induced Dissociation, EThcD, and building a new database of all possible recombinations the proteasome could generate, we were able to deduce that no less than 30% of the peptides are spliced and stitched by the proteasome. Such a finding turns immunology potentially upside down. To me, it also shows the importance of ongoing technology development even when you don't have a direct application in mind. When we started working on EThcD several years ago, there was a great deal of scepticism in the field. Many doubted the relevance for proteomics applications, but we have clearly proven the value in sequencing these proteasome spliced peptides. Nobody saw that coming, and that is how it often goes. You see an opportunity to improve and advance a technology and then, suddenly, an application pops."

But how do you ensure that your technology and such an unexpected application meet? You have to be in touch with the biological topics to enable a match.

"This is why I strongly like and support large-scale collaborations like ICI and our Roadmap Proteins@Work initiative. That is the best way to bring experts in technologies and research questions together. To me, collaboration with all kinds of fields is inherent to our work in MS. As I said before, I want to keep on developing MS in the context of relevant biological questions, and collaborating with other groups is the only way to do that."

Your collaborations are very diverse, in ICI with immunologist and synthetic chemists, but you are also active in many other areas.

"The best thing about collaborating is that I get a free crash course on a variety of topics, by leading experts. Ranging from plant biology to oncology and now through ICI, I get immersed in immunology. I step into each new field with my range of techniques and an almost 'naïve' perspective on established problems, and sometimes the techniques fit the problem very well and we can make an impact. That is why I think ICI is very innovative; we deploy new techniques to address 'old' problems in immunology and that leads us in unexpected, exciting directions." ■

"I want to keep on developing MS in the context of relevant biological questions"

INNOVATION ENGINE FOR NEXT GENERATION OF CANCER MEDICINES

Genmab is a leading international biotechnology company focused on developing innovative antibody therapeutics for cancer. "Our passion for understanding how antibodies function in our body leads to breakthrough insights into molecular design of effective antibody medication for cancer," says Jan van de Winkel.

"My dream is to transform cancer into a chronic illness and to offer patients a high quality of life," Genmab's CEO and co-founder reveals. And this dream is edging ever closer to reality. Genmab succeeded in creating a first-in-class immunotherapy for the treatment of multiple myeloma: daratumumab. "The results for this CD38 monoclonal antibody have been impressive," says van de Winkel. Daratumumab (trade name DARZALEX®) has been approved for certain indications in the United States and Europe by Genmab's collaborating partner, Janssen Biotech (a subsidiary of Johnson & Johnson). "Drug development and marketing require an almost military operation, which Genmab could not have done at the time we signed the deal for the drug. Genmab therefore entered a license and development agreement with Janssen for daratumumab."
DARZALEX is currently on the market for certain multiple myeloma indications. Multiple myeloma is a type of bone-marrow cancer and the third most common type of blood cancer. The mechanism of action of DARZALEX is based on attacking cancer cells in six different ways. "One of the mechanisms of action which may prove important is that it probably not only kills tumour cells but also stimulates the patient's immune system to clear up malignant cells. If this really is the case, then DARZALEX may work in many more types of tumours," van de Winkel explains.

Turning science into medicine

Genmab was founded in 1999 (Copenhagen, Denmark) and is publicly traded on Nasdaq Copenhagen and under an ADR in the US. The company employs just over 200 people divided among three offices: corporate functions and clinical development in Copenhagen; commercial, financial and administrative departments and clinical development in Princeton (US); and the R&D activities in the Netherlands at the Science Park in Utrecht. "Such an environment, where it's all about education, research, entrepreneurship and healthcare, provides an excellent innovation ecosystem for our R&D," van de Winkel says.
By participating in fundamental research projects, Genmab gains new knowledge which the company subsequently could turn into innovative antibody products. Van de Winkel is absolutely convinced that interaction, cooperation and sharing knowledge are the pillars of progress and innovation. "Our HexaBody® platform, for instance, was inspired by joint research with Professor Albert Heck and Professor Piet Gros of Utrecht University. While studying antibody working mechanisms at the molecular level, we discovered that antibodies operate in groups of six molecules after binding to their target on the cell surface. This 'hexamer finding' was then further developed by Genmab scientists into our HexaBody technology." In addition to the company's validated DuoBody®platform for producing bispecific antibodies this new platform proved to be a great asset to the company."

Next step

Today Genmab is the biggest biotech company in Europe and is one of the few profitable ones. Genmab has operated in the black since 2013, only 14 years after its foundation, whereas biotech companies usually need at least 20 years to come out of the red. "In order to continue the success we need to keep investing in early stage programs, establishing our pipeline of the future," concludes van de Winkel. "The next step is marketing products ourselves, which I very much hope will be realised within 6 or 7 years." ■

Genmab

W www.genmab.com
E info@genmab.com
T +31 30 2 123 123

How ISG15 fights off viruses

PhD project: Mechanisms of action of the ubiquitin-like modifier ISG15 in immune regulation

Without a protein called ISG15 we would be much more prone to viruses. Yet how ISG15 protects us is still largely a mystery. Jolien Luimstra and Victoria Iglesias are trying to resolve the question by synthesising the protein and studying its actions in the human cell.

"The protein looks like two connected ubiquitins," Jolien Luimstra says. She is the chemist of the ICI duo studying ISG15 or 'interferon-stimulated gene 15'. Luimstra is synthesising ISG15 *de novo* as part of her PhD studies at Leiden University Medical Centre. The chemical biology laboratory has a great deal of experience with ubiquitin synthesis, but in Luimstra's experience synthesis of ISG15 is a more tedious job. "The protein has about 150 amino acids, which is too long to synthesise in one run using an automated peptide synthesiser. Our first approach was to synthesise two halves and couple them, but synthesis of one of the halves is challenging. There is too much steric hindrance and the peptide has low solubility."

To Luimstra that means puzzling out how to find a suitable protocol using three or more fragments. At the same time she is studying possible mutant proteins. She would like to make a variant carrying a fluorescent label for tracing the protein within the cell. Another goal is an ISG15-mutant containing a chemical group that allows cross-linking to the proteins it binds. Luimstra: "It may take longer, but I hope to synthesise ISG15 in the coming months and start studying the mechanisms by which it boosts the immune system. After all, that is why I'm making them."

T cell activation

Luimstra's research partner Victoria Iglesias, who holds a master's degree in biochemistry and a PhD in neuroscience, works at the Netherlands Cancer Institute (NKI). "My research experience is foremost in molecular biology. But I've always had an interest in chemistry and I understand the language to a certain level. It's great to now be involved in a project that looks at such a complex molecule as ISG15 from both perspectives."

Iglesias is intrigued by the questions of *if* and *how* ISG15 stimulates the activation of T cells by dendritic cells. "That is an important step in our specific immune response *and* in immunotherapy. Knowing more about ISG15's working mechanism may improve this new therapy for the treatment of cancer." Iglesias is currently studying the impact of ISG15 on dendritic cells from an ISG15 knockout mouse. She compares the effect of a wild type ISG15 to a truncated version unable to bind other proteins. "By using this approach we can define whether ISG15 expression does indeed have an impact on T cell activation and if its conjugation to cellular proteins plays a role in this context."

> *"Synthesis of ISG15 is a more tedious job"*

As ISG15-conjugates may provide a clue to how the protein acts, Iglesias is also collaborating with proteomics scientists in analysing conjugates using advanced mass spectrometry. "We just settled the conditions for preparing the samples. The analyses will be carried out in the coming months."

Clear tasks

The two ISG15 researchers inform one another on their progress about once a month. Luimstra: "We don't need to discuss our work on a daily or weekly basis. We each have our own clear tasks and goals." For Luimstra the research project will hopefully result in a PhD thesis. For Iglesias it adds to a research portfolio that may help her to establish an own research group in Uruguay, her home country. "I know that's ambitious due to the limited national research budgets, but it's definitely my dream." ■

Jolien Luimstra, MsC
Chemical Biology
Leiden University Medical Centre

Victoria Iglesias, PhD
Molecular Biology
Netherlands Cancer Institute, Amsterdam

Designing a dendritic cell

PhD project: Polymer based synthetic dendritic cells

Dendritic cells play a crucial role in immunotherapy against cancer. They teach T cells which cells are the enemy. To simplify and speed-up immunotherapy, Jorieke Weiden and Dion Voerman work on an artificial off-the-shelf dendritic cell. "It might be an ambitious target, but it's very inspiring."

Biomedical scientist Jorieke Weiden is studying how T cells react to polyisocyanopeptide hydrogels. The gels were supposed to be a meeting point: T cells would enter the gel as it is functionalised with T cell recruiting proteins. Within the gel the cells would be programmed to attack tumour cells by MHC molecules that present tumour antigens, which are also attached to the polymer. That way the gel could take over the role of dendritic cells that so far have to be isolated from a patient's own blood.
Unfortunately the gel pores proved too small for T cells to enter. It was a disappointment, but also an inspiration. Weiden: "I'm now studying the possibility of using this gel as a local, slow release delivery system for immunotherapy. The idea is to lock in activated T cells and inject the gel close to the tumour. That's possible because the gel is fluid at low temperatures, but sets at body temperature." To achieve the original goal Weiden switched to alginate polymers.

Two worlds

The polymers Weiden studies are synthesised by chemist Dion Voerman. Both are PhD students in the Tumour Immunology group at Radboudumc. Weiden: "Thanks to our cooperation, I'm very well informed about the materials I use. We discuss what's possible and what not, and why. It inspired me to follow a course in organic chemistry as well."
Voerman prepares the polymers at the department of Organic Chemistry, which is close by but in another building. Sometimes the 'two worlds' give him a headache, admits Dion Voerman. "Try arranging two access cards or ordering chemicals on another department's account …" But he is full of enthusiasm about the science. "PhD students in organic chemistry usually go into great depth; I broadened my knowledge and discovered a whole new field."
Voerman has just figured out how to anchor one end of a polyisocyanopeptide to a solid surface. "None of the well-known coupling methods worked; they weren't compatible with the polymerisation conditions." So Voerman had to design a new synthetic route. "I've proved that it works. Now I'm starting experiments to attach the polymers to a nanoparticle."

"It would be a true milestone if the idea works"

The resulting particle is also an imitation of the human dendritic cell. It should attract T cells as it carries T cell binding peptides, and it contains tumour antigens. Voerman can't wait to see the reaction of T cells on his prototype. "It would be a true milestone if the idea works."
Both PhD students agree that building an artificial dendritic cell is an ambitious goal. Weiden: "I'm sure there are more straight-forward, less risky PhD projects." "But such a challenging goal is also inspiring," Voerman adds.

Reflection

Just as Dion introduces Jorieke to synthetic chemistry, Jorieke coaches Dion in immunology. Voerman: "What has surprised me is that such small amounts can induce a large biological effect. In chemistry I'm used to work with grams. Immunology experiments start by making seemingly endless dilutions."
Weiden enjoys the many contacts that an ICI project brings with it. "Almost instantly I had a national network in chemical immunology through the special PhD courses. That is very useful for resolving scientific problems, but we also reflect on many other troubles, and joys, that a PhD student encounters." ■

Jorieke Weiden, MsC
Biomedical Sciences
Radboudumc, Nijmegen

Dion Voerman, MsC
Organic Chemistry
Radboudumc, Nijmegen

Tracking the fate of metabolites

The unwanted proliferation of cancer cells and the desired expansion of immune cells both result from increased metabolic activity. Celia Berkers employs metabolomics to get a grip on the mechanisms underlying these metabolic adaptations.

Celia Berkers may be immersed in a research environment dominated by proteins, but that doesn't deter her from focusing on much smaller molecules. For Berkers it is all about metabolites: the small molecules that are continuously produced, transformed and re-used by the endless array of biochemical processes in a living cell. Still, being an assistant professor in the Biomolecular Mass Spectrometry Group at Utrecht University makes perfect sense, because Berkers applies the same analytical technique as her protein-minded colleagues do: mass spectrometry.
What can we learn from metabolites? And how is metabolomics linked to the ICI research program?
"Metabolites are the essence of metabolism. It is due to the continuous cascade of chemical transformations of the metabolites that the overall effect of 'metabolism' is generated," Berkers explains. "We know that the metabolism of cancer cells is strikingly different from that in healthy cells. To sustain their unbridled proliferation, cancer cells need to step up their metabolism."

Quick expansion

Changing one's metabolism is, however, not an exclusive ability of cancer cells. "We also see it in the immune system, for example in T cells. Once activated, T cells need to expand quickly, so they change their metabolism as well." But not all T cells adapt their metabolism in the same way, says Berkers. "Conventional T cells and regulatory T cells respond to the same signal that activates them to speed up their growth, but they adapt their metabolism in different ways." This difference may offer clues to influencing the balance between Tconv and Treg, which determines the strength of the response against cancer cells. The cytotoxic activity of Tconv is kept in check by Treg. But this balance is finely tuned. Too much Treg activity leads to weak, ineffective action against cancer cells. Too little Treg activity and the killer cells get out of control, resulting in damage to healthy tissues and autoimmunity.

"Different T cells adapt their metabolism in different ways"

Using metabolomics, Berkers aims to map the differences in the metabolic adaptation of Tconv and Treg, both in a qualitative and quantitative manner. Ultimately she wants to identify metabolic targets for pharmacological intervention to specifically inhibit Treg or Tconv. "Because metabolism is a highly dynamic process, static information on levels and concentrations are not very useful," Berkers points out. "You need to know how metabolite levels change over time. And you need to know the fate of a metabolite. It is not a straightforward cascade of reaction steps that turns A into B into C, but A can become many different compounds at different moments depending on different factors."

Stable isotopes

To this end, Berkers applies stable isotopes in conjunction with mass spectrometry. "We mostly use ^{13}C incorporated in sugars and amino acids. This allows us to track which source is used in which transformation step and to determine what happens to a metabolite. Where does it end up and in which form? All this provides us with detailed mechanistic insights and that is essential to really grasp what is going on in these complex networks," she explains enthusiastically. "It is all very complex. There is a lot we don't know, but using chemistry in a variety of techniques, there are many opportunities for discoveries. Metabolism really is a great puzzle to work on." ■

◀ *Celia Berkers supervises the ICI project on studying metabolic activity in conventional and regulatory T cells. The goal is to find metabolic targets for therapeutic intervention that can selectively modulate T cell activity.*

Inflammation shows the way

Detecting tumours can be tricky. Combining a variety of techniques, Hidde Ploegh is taking a seemingly indirect route. Using modified nanobodies for immunoPET, abnormal localisation of inflammatory activity becomes the tell-tale sign to look for. "It is like the canary in the coal mine."

Chemical immunology may appear to be a new research area for many, but for Hidde Ploegh it has been the focus of his research for more than three decades already. "In the 1980s, my group at the Netherlands Cancer Institute was one of the first in the Netherlands to apply chemistry to immunological problems," says Ploegh. Since then he has held faculty positions at MIT and Harvard Medical School; he is currently a senior investigator at Boston Children's Hospital.

Imaging inflammation

Currently one of the key topics in the Ploegh lab is using nanobodies as an imaging tool. Nanobodies, or VHHs, are single domain antibodies. Their size of approximately one-tenth of a conventional antibody allows for higher tissue penetration and faster clearance from circulation, improving the signal to noise ratio. Screening for nanobodies against surface proteins yielded an inventory of nanobodies that bind specific immune cells. "By including immune cells involved in the inflammatory response you can use them to track down a tumour. Instead of concentrating on direct binding of your probes to tumour cells, you look for an inflammatory response that guides you to the right spot."

To this end the nanobodies need to be equipped with additional groups that allow imaging. Enter *sortagging*, a technique developed by Ploegh in which enzymes called sortases are used to modify proteins, including nanobodies. Using sortagging, nanobodies can be uploaded with all kinds of functional groups, for example including PET (positron emission tomography) isotopes. Using labelled glucose as a PET probe is a well-established method for detecting sites of high metabolic activity, a characteristic of tumour cells. "But the increase in activity can also originate from an increase in T cell volume, which also shows increased metabolism," says Ploegh. "With our immunoPET approach, we look for abnormal localisation of inflammatory activity. That is the canary in the coal mine: when you see an abnormal location of inflammatory cells in a tumour-bearing animal that is probably where the tumour resides."

Dynamic behaviour

In addition to enabling quick, real-time detection of tumours, it turns out that immunoPET also has potential in (immuno) therapy monitoring and even prediction. CD8+ T cells can be detected using PEGylated nanobodies. "These can infiltrate the very dense stroma of the tumour, which is out of reach of most conventional techniques. When we applied these nanobodies to mouse models treated with checkpoint blockade inhibitors, we found a relationship between the distribution of the CD8+ T cells and therapy outcome. Tumours that had a homogenous localisation of T cells responded very well to immunotherapy, whereas those with heterogeneous distribution showed no response."

The behaviour of T cells in the tumour turns out to play a huge role, which underlines the need for dynamic imaging technologies. Ploegh: "We see the localisation of T cells changing over time, but we don't know yet what that entails. Are they moving to a different area, or are they dying in one spot and are new T cells recruited to a different spot? Either way, it is clear that the static snapshots generated by most imaging techniques are of limited use in understanding what is going on inside the tumour." ■

▼ *Hidde Ploegh, senior investigator at Boston Children's Hospital and member of the ICI Scientific Advisory Board, presented a lecture about 'Imaging Immunity' during the 3rd Chemical Immunology Conference (March 10th, 2017)*

"We found a relationship between T cell localisation and therapy outcome"

Partner in the fight against cancer

The Dutch Cancer Society is seeking collaborations to fight cancer faster and better

The Dutch Cancer Society (KWF Kankerbestrijding) has the ambition to fast track results from scientific research to practical applications. To facilitate this ambition, the Program Research & Implementation (PR&I) has been launched. The funding framework covers the entire field: from basic and translational research via a development track to implementation in clinical settings. The ultimate goal is to translate results of research to meet patients' needs as quickly as possible.

Less cancer, more cure and a better quality of life for cancer patients: that is the mission of the Dutch Cancer Society. The ideal world is one in which no one dies from cancer ever again. That is why the Dutch Cancer Society finances scientific research, influences policy, and shares knowledge about cancer and its treatment. "We're getting there together," according to Michel Rudolphie, Managing Director. "In order to ensure that every euro invested achieves the maximum impact for our mission, we have launched the Program Research & Implementation (PR&I): a new method of funding, reviewing and cooperating."

Flexibility is the core

"Each research phase needs tailored services, which is guaranteed by the flexibility in the PR&I," explains Yuri Souwer, program coordinator Immunotherapy. "The goal of the funding framework is to offer the best support in terms of funding, review methodology, monitoring and continuation options."

The PR&I introduces guideline amounts for project budgets rather than maximum budgets. Souwer: "This means there are no longer limits on the budget that can be requested for a project. Researchers can request what they need to take a new discovery/development through to the next phase."

Furthermore, the PR&I consists of various types of funding, which can be applied for during each research phase. Besides the traditional *Research projects* with a limited scope in terms of time and research groups, there are the *Young Investigator Grants* for young, talented researchers and the *Unique High Risk* projects, which offer the possibility of performing short-term preliminary work on the basis of a good, but not entirely crystallised idea. The funding type *Research consortium* has been introduced to fund research projects within more complex and/or bigger collaborations. The consortium proposal is intended for applications involving at least four organisations, including companies and/or sponsors. Finally, pilots for *Infrastructural initiatives* and *Implementation projects* were launched. The goal of the former is to stimulate existing or new infrastructure initiatives which support scientific cancer research and faster translation to concrete applications for patients and the public. The goal of the latter is to fund the implementation of concrete applications, which are positively evaluated in clinical settings and show demonstrable added value compared to the current practice.

Once a project is approved for funding, the Dutch Cancer Society remains expressly involved in order to offer optimum support to the funding. "We are interested in the progress and the results. Moreover, we work with the researchers, for example in seizing opportunities or tackling bottlenecks," Souwer explains.

New method of funding, reviewing and cooperating

From laboratory to patient

The new funding program was launched in 2016 including two calls for proposals (April and October). Last December the first call was completed, resulting in 88 approved projects. Almost € 50 M went to excellent oncological research covering the entire field of cancer: improved diagnostics; better treatment; new applications of immunotherapy; and better quality of life for patients. More than € 17 million (33 projects) were approved for basic research, which is essential for obtaining new insights into the origin and behaviour of cancer.

The remaining 55 approved projects focus on transforming basic insights into practical applications. Such research involves drug development or clinical trials, for example. The proceeds of the Alpe d'HuZes 2016 (over €11 M) were spent on 27 projects, including seven Young Investigator Grants (Bas Mulder Awards) for young, talented researchers, and nine Unique High Risk projects for breakthrough research ideas.

KWF KANKER BESTRIJDING

Submitting proposals

Last March Souwer visited the ICI Conference 2017 in De Rode Hoed (Amsterdam). He noticed the high-quality chemical immunology research and its high impact in the field of cancer. He encourages the researchers and project leaders involved to submit project proposals during the next PR&I call (deadline August 2; register via www.kwf.nl/poi, which will be open from May 29).
In conclusion, Souwer says three criteria are used for reviewing the projects: scientific quality, feasibility and relevance. "In fact, that means the research should contribute to our mission: less cancer, more cure and better quality of life." ■

For more information: www.kwf.nl/research

Using the immune system to fight cancer

The immune system's natural capacity to detect and destroy abnormal cells may prevent the development of cancers. "Improved knowledge of molecular and cellular interactions in our immune system gained momentum in the development of therapeutic cancer vaccines," says Ferry Ossendorp, professor of molecular defined vaccine biology at LUMC.

It was discovered some decades ago that T cells can recognise virus infected cells and tumours via the detection of foreign proteins presented as short peptides on the cell surface. This knowledge opened the way to immunotherapy against cancer. "We succeeded in identifying tumour-associated peptide antigens and developing vaccines based on synthetic analogues of the identified antigens," explains Ossendorp. In the early nineteen nineties these therapeutic vaccines proved to be efficacious in mice. Further unravelling molecular interactions in the immune system revealed prominent roles for peptide presentation by Human Leukocyte Antigen (HLA) molecules, the cell biology of dendritic cells (DC) and Toll-Like receptor (TLR) signalling. These findings led to rapid progress in developing cancer vaccines. "Vaccination seems a promising new treatment method for cancer patients," foresees tumour immunologist Ossendorp.

Next generation

Ossendorp's group works in close cooperation with chemists, pharmacists and bioinformaticians to design next generation cancer vaccines. He summarises some of the challenges: "A crucial requirement is the inclusion of appropriate adjuvants, e.g. TLR ligands, which stimulate the immune system to work even more effectively. Together with chemists we designed suitable small molecules which are covalently coupled to the peptide antigen. Such TLR ligand-peptide conjugates proved to show superior DC targeting and simultaneous DC activation."
Utilising pharmacist's knowledge, the Leiden researchers strive to find effective routes of administration. For instance, peptide antigens packaged in nano-particles together with an adjuvant turned out to induce stronger immune responses than their soluble forms. "Recently we found further improved immunogenicity of peptide epitope vaccines using novel nanocarriers based on self-assembling materials."
Another research project focuses on *in vivo* imaging of the therapeutic vaccine from the injection site to the draining lymph nodes, in order to offer maximum insight into the immunisation process. Organic chemists succeeded in connecting a near infrared fluorophore label to the therapeutic peptides enabling non-radioactive imaging deep in the tissue. "This approach substitutes traditional empirical research methods with step-by-step design, in which pharmaco kinetics and pharmaco dynamics are taken into account, including time, place, formulation and dosage aspects."

From molecule to man

The goal is to establish a chemistry-based precision immunotherapy platform, with the aim of developing novel, highly defined molecular structures to attack cancer from multiple immunological angles simultaneously. Thanks to multidisciplinary cooperation, market introductions of therapeutic vaccines are in sight. "Sharing knowledge and expertise plays a vital role in bringing 'molecule' to 'man', bringing scientific knowledge to the clinic," Ossendorp concludes. It perfectly links up with the goal of the new Programme Research & Implementation (PR&I) of the Dutch Cancer Society, as described in the accompanying article. ■

Hacking the immune system

Gaining a fresh perspective on your work is always worthwhile. With that in mind, researchers at the Nijmegen Tumour Immunology Lab challenged their colleagues to leave their comfort zone and be inspired by scientists from computer science and engineering.

What do you get when you mix theoretical biologists, cancer vaccine developers, computer engineers, tumour immunologists, bio-inspired computation experts, cell biologists and bioinformaticians? A symposium! On Friday February 17, approximately 120 participants, mostly PhD students and postdocs, gathered in Nijmegen for a day filled with exciting examples of how immunology, computer science and artificial intelligence can inspire and benefit each other. And not just in an instrumental sense in which bioinformaticians and computer scientists help the immunologists get a grip on the huge and complex datasets they generate: immunologists can also learn from the way computing experts, programmers and engineers approach a problem. That goes the other way around as well: the immune system offers a rich source of inspiration for computer scientists who are interested in systems capable of adaptation, learning and evolution. Nature-inspired computing so to speak.
The symposium, initiated by Johannes Textor and Carl Figdor, put together a diverse program featuring no fewer than nine invited speakers. In the following we strive to give you a flavour of what was presented and discussed.

▼ *Exploiting computational immunology to understand the immune system*

Mouse? Model!

Computer scientist Jon Timmis, professor of Intelligent and Adaptive Systems at the University of York, kicked off by sharing his experience as a computer scientist who entered the world of immunology. Whatever the biological topic or questions, the key element of his work is mathematical modelling. And building evidence to gain the trust of biologists in your mathematical model is perhaps the most difficult part of any interdisciplinary collaboration, he says. “When explaining your model to the users, you have to keep in mind that they do not necessarily think like an engineer or a computer scientist. On the other hand, biologists easily rely on the results they gain from animal experiments. But a mouse is model too. Building trust and a common language takes time.”
Most presentations started out from immunological problems and discussed how bioinformatics, theoretical biology approaches or computational methods were not only incorporated, but offered new insights for tackling the biological issues at hand. A highly original take on how immunology can inspire computing was presented by Emma Hart, professor of Natural Computation at Edinburgh Napier University. In her own words, she works on ‘everyday problems’ associated with optimisation of scheduling and packing.

Immune-based optimisation

“Most algorithms work very well for one particular problem. If the problem changes or a new problem comes along, you need a new algorithm,” Hart explained. “My interest is in adaptive algorithms that can cope with dynamic environments and develop themselves to improve their performance and use prior knowledge to address new tasks.” The immune system is a great example of an adaptive, life-long learning system. Hart: “It deals with a continuous stream of information and problems; it can re-use and adapt existing responses to tackle new problems; it generates new responses; and because there is competition between responses, it gets better and better at performing its tasks.” Whoever would have thought that the intricate operations of the immune system could be useful to the macroscopic world of transport and logistics? ■

▼ *Co-organizing PhD students: (from left) Till Mathan, Jorieke Weiden, Edyta Swider and Maxim Baranov*

News

Celia Berkers elected member KNAW Young Academy

Every year the KNAW Young Academy selects ten talented new researchers to add to its ranks. In addition to their proven research excellence, Young Academy members take a broad interest in science and in science communication. This year, Celia Berkers from the ICI and Utrecht University, has been selected as a new KNAW Young Academy member for a five-year period. She will be inducted on 8 June 2017 in Amsterdam.

Albert Heck named Honorary Member of the Spanish Proteomics Society

Professor Albert Heck, Utrecht University and member of the ICI executive board, has received an honory membership of the Spanish Proteomics Society. Heck was honored during the Opening Ceremony of the 6th Congress of the Spanish Proteomics Society in Cadiz on November 15th 2016.

Eveline Li and Tim Hoogervorst winners of ICI Poster Prize 2017

On 10 March 2017, the 3rd Chemical Immunology Conference took place at the Rode Hoed in Amsterdam. Over 150 people from inside and outside the ICI participated. During lunch several ICI PhD students presented their work. Poster prizes were awarded to Eveline Li (VUmc) and Tim Hoogervorst (LU) for their joint research project. An impression of the day can now be viewed online.

Recent publications

Bruins JJ, Westphal AH, Albada B, Wagner K1, Bartels L, Spits H, van Berkel WJ, van Delft FL.

Inducible, Site-Specific Protein Labeling by Tyrosine Oxidation-Strain-Promoted (4 + 2) Cycloaddition.

Bioconjug Chem. 2017 Mar 16. doi: 10.1021/acs.bioconjchem.7b00046. [Epub ahead of print]

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Retinoic Acid and Immune Homeostasis: A Balancing Act.

Trends Immunol. 2017 Mar;38(3):168-180. doi: 10.1016/j.it.2016.12.006. Epub 2017 Jan 13.

Baggelaar MP, van Esbroeck AC, van Rooden EJ, Florea BI, Overkleeft HS, Marsicano G, Chaouloff F, van der Stelt M.

Chemical Proteomics Maps Brain Region Specific Activity of Endocannabinoid Hydrolases.

ACS Chem Biol. 2017 Mar 17;12(3):852-861. doi: 10.1021/acschembio.6b01052. Epub 2017 Feb 13.

Gagarinov A, Li T, Toraño JS, Caval T, Srivastava AD, Kruijtzer JA, Heck AJ, Boons GJ.

Chemoenzymatic Approach for the Preparation of Asymmetric Bi-, Tri-, and Tetra-Antennary N-Glycans from a Common Precursor.

J Am Chem Soc. 2017 Jan 18;139(2):1011-1018. doi: 10.1021/jacs.6b12080. Epub 2017 Jan 6.

Textor A, Schmidt K, Kloetzel PM, Weißbrich B, Perez C, Charo J, Anders K, Sidney J, Sette A, Schumacher TN, Keller C, Busch DH, Seifert U, Blankenstein T.

Preventing tumor escape by targeting a post-proteasomal trimming independent epitope.

J Exp Med. 2017 Feb;214(2):567. doi: 10.1084/jem.2016063601122017c. Epub 2017 Jan 17.

De Nardis C, Lössl P, van den Biggelaar M, Madoori PK, Leloup N, Mertens K, Heck AJ, Gros P.

Recombinant Expression of the Full-length Ectodomain of LDL Receptor-related Protein 1 (LRP1) Unravels pH-dependent Conformational Changes and the Stoichiometry of Binding with Receptor-associated Protein (RAP).

J Biol Chem. 2017 Jan 20;292(3):912-924. doi: 10.1074/jbc.M116.758862. Epub 2016 Dec 12.

Baranov MV, Revelo NH, Dingjan I, Maraspini R, Ter Beest M, Honigmann A, van den Bogaart G.

SWAP70 Organizes the Actin Cytoskeleton and Is Essential for Phagocytosis.

Cell Rep. 2016 Nov 1;17(6):1518-1531. doi: 10.1016/j.celrep.2016.10.021.

Column

TURNING A COLLECTIVE INTO A CONSORTIUM

ICI is an unusual collection of serious chemists and immunologists, and with their combined PhD projects I think they have made a terrific start. But now that we are further down the line, we have to ask ourselves whether ICI is becoming a true consortium or if it will just remain a funded collective.

The challenge at this point is to take the tools and insights developed so far and apply them in such a way that multiple projects get integrated and can be taken to the next level. That is necessary in order to create added value and enable results that wouldn't be achieved if these projects and groups worked solely on their own or in bilateral collaborations.

How to stimulate this much-needed integration? My first recommendation is to reserve part of the budget for what I call 'opportunity funding'. Once projects start running and the first results emerge, it is time to consider the possibilities for integrating various projects. That often demands additional funding, which is why it is wise not to spend the complete budget immediately. You don't know where the opportunities will arise, but you should at least expect them and be prepared to develop them further as soon as possible.

We all know that the best science happens by tripping over things, so you should create room for 'tripping'. That is my second piece of advice. How? Organise meetings at which small, multidisciplinary groups of scientists work on actual, cross-over problems. Then have the entire group review the solutions suggested by each working team and synthesise a plan for attacking the problem. The plan can be elaborated into a proposal for the 'opportunity funding'. That way you can benefit from the input of a diverse group of scientists and identify new approaches to important problems, the success of which can have a big impact within and outside the consortium.

It is important that ICI starts focusing now on these integrative activities in order to demonstrate the added value of a large-scale consortium and justify the substantial investments. In that way ICI certainly will turn into a true consortium. ■

RONALD N. GERMAIN, M.D., PH.D.
SCIENTIFIC ADVISORY BOARD ICI

Ronald Germain is chief of the Laboratory of Systems Biology at the National Institutes of Health, US

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 Organisation 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 mail to info@chemicalimmunology.nl.

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Contact

Padualaan 8
3584 CH Utrecht
T: 030-2536804
info@chemicalimmunology.nl

ICI institute for chemical immunology