

Faszination Forschung

TUM Research Highlights

Technical University of Munich

The Science Magazine

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Virology and Infection Control

Hepatitis B – A therapeutic vaccination can heal

Oncolytic Viruses – A weapon against cancer

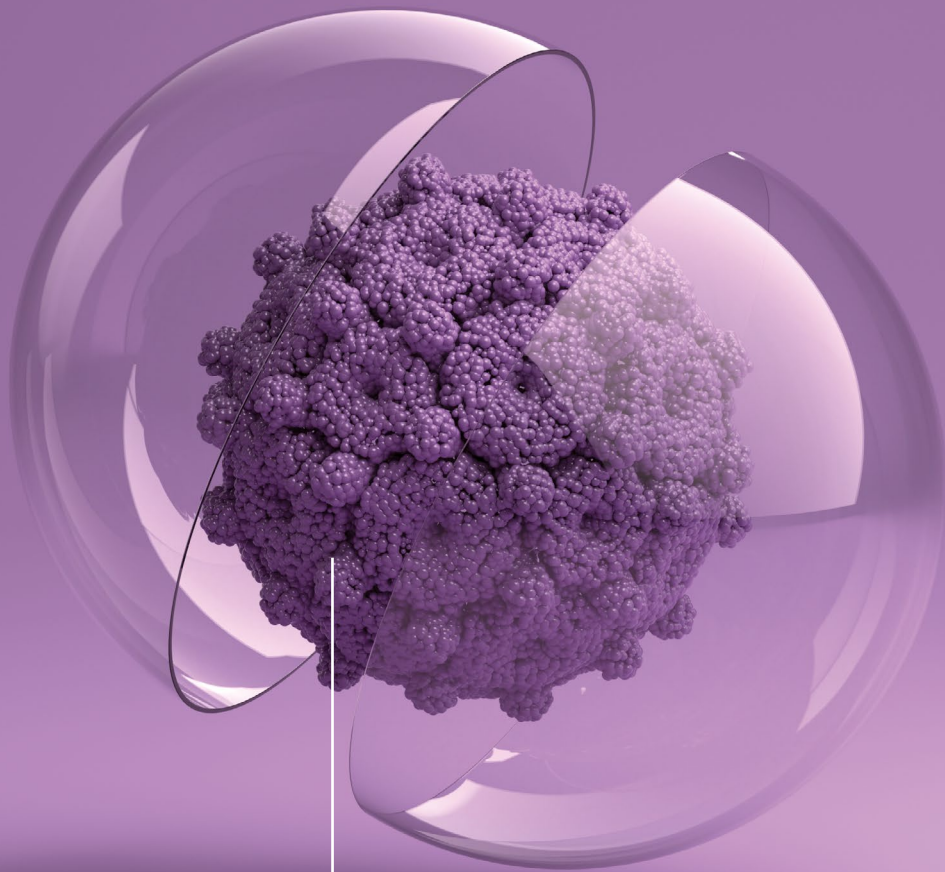
Global Health – Neglected tropical diseases

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Hepatitis B virus

**A therapeutic vaccination that
can cure hepatitis B infections**

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Dear TUM friends and associates,

We are exposed to viruses and bacteria every day, usually without even realizing it. The human immune system is sufficiently robust to repel most attacks before we notice any adverse effects.

Regional outbreaks of viruses such as COVID-19 are a common occurrence, yet no virus has ever dealt us such a severe blow. It is against this backdrop that we have produced this issue of *Faszination Forschung*, dedicated to virology and infection control. Although the field of medicine takes a prominent role, I am sure you will be surprised by the other angles and research approaches that TUM scientists have adopted in their efforts to investigate these topic areas.

TUM virologist Prof. Ulrike Protzer has been researching the hepatitis B virus for years. The virus can lead to a chronic, incurable infection. While newborn babies in Germany are vaccinated against hepatitis B, in many parts of the world, this is simply not possible. Consequently, Protzer is developing a therapeutic vaccination that aims to cure people already infected with the virus.

Prof. Andreas Pichlmair is investigating viruses at the molecular level. His interest lies in identifying which proteins are required for a virus to interact with human host cells. Pichlmair is also analyzing whether newly discovered biologically relevant signal pathways also come into play in other diseases for which effective medications already exist. If so, this could significantly accelerate the development of new therapeutic compounds.

If the immune system is unable to get a handle on infections or tumors, it switches into a lower functional state. Prof. Dietmar Zehn has identified the TOX protein as the molecular circuit breaker that causes the immune system to switch between active and reduced functional states. The ability to systematically reactivate exhausted immune cells would pave the way for new treatment approaches for both chronic infections and tumors.

Viruses require host cells in order to reproduce – something Dr. Jennifer Altomonte hopes to exploit in order to turn viruses into a weapon against cancer. Her team has produced a hybrid virus in the lab that multiplies in tumor cells and destroys them.

Unlike viruses, bacterial infectious diseases can be treated effectively with antibiotics. Bacteria, however, are increasingly developing resistances against such substances. Chemical



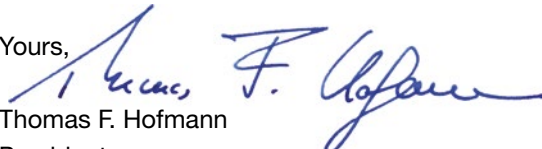
scientist Prof. Stephan A. Sieber has discovered a substance capable of combating dangerous multiresistant germs. A team of students has also taken up the fight against multi-resistant germs. They have developed a new method by which to produce bacteriophages – viruses that attack and kill bacteria. The concept won several awards at the international 2018 iGEM Competition. Today, the team hopes to bring their production process to market by founding a company.

Prof. Clarissa Prazeres da Costa conducts research into infectious diseases that are almost unheard of in affluent, industrial nations. Worm infections lead to digestive problems, anemia, and severe growth and development disorders in children. The parasites release molecules that actively inhibit the immune response in order to keep their host alive for as long as possible. Exactly how this mechanism works is the question Prof. da Costa is striving to answer.

Prof. Alena Buyx emphasizes the importance of listening to voices other than virologists and epidemiologists during a viral pandemic. In her work, the medical ethicist has observed that people are prepared to act in solidarity with others in times of crisis.

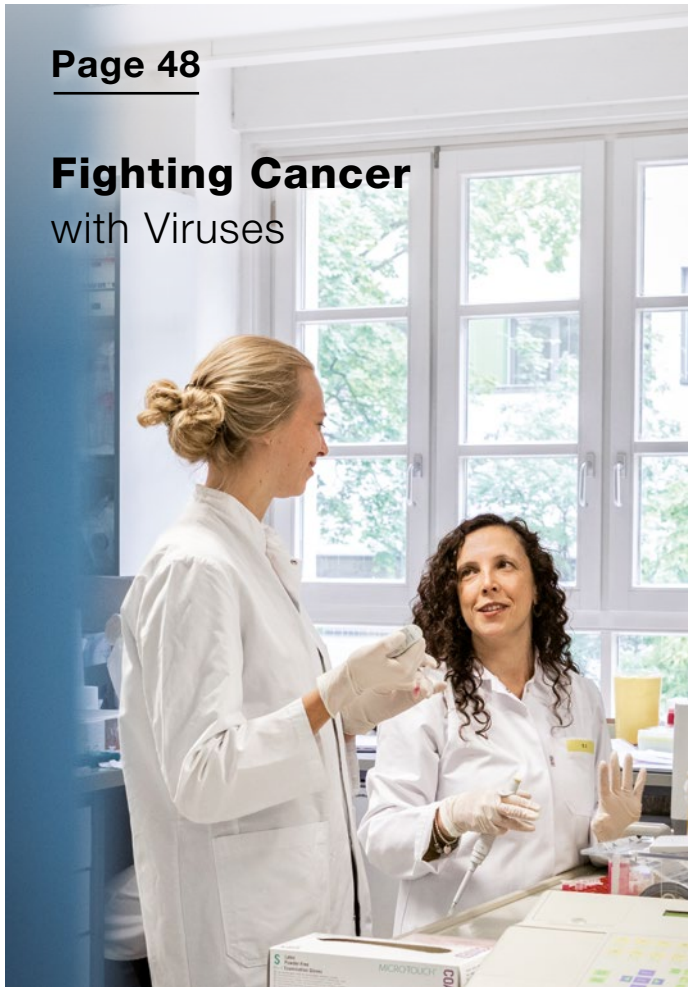
This new issue of *Faszination Forschung* is an emphatic reminder that, through their basic research, scientists and researchers at TUM are making outstanding contributions to the health and wellbeing of all humanity.

Yours,


Thomas F. Hofmann
President

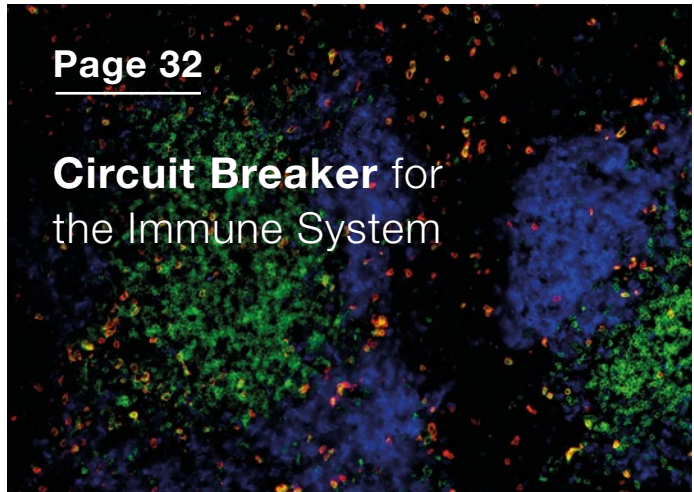
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Circuit Breaker for the Immune System



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“This Vaccination Should Benefit every Country”

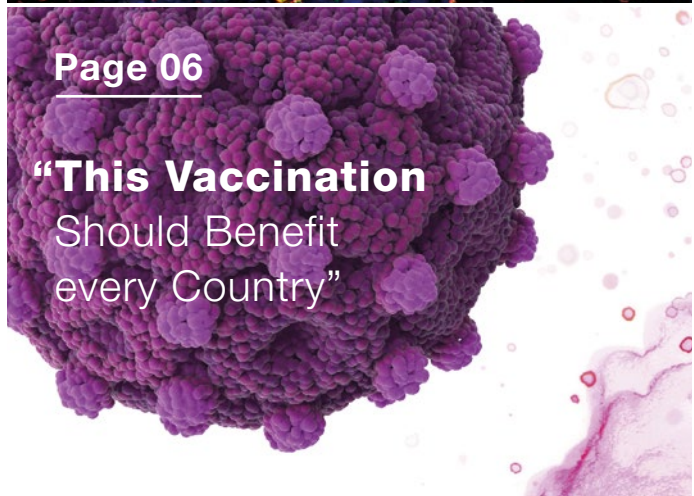


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Worm infections are widespread in low-income countries. Clarissa Prazeres da Costa investigates how these neglected tropical diseases affect the immune system.



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Fighting Superbugs
with Superdrugs

Picture credits: Jull Eberle, TUM; Graphics: edlundsepp (source: TUM, Markus Bernards, Stephan A. Sieber); 3D Models Virus and Worm: turbosquid

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“Ensuring healthy lives for all” is one of the United Nations’ Sustainable Development Goals. The Center for Global Health at the TUM School of Medicine is addressing these topics.

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Jennifer E. Altomonte harnesses the lethal power of viruses to combat malignant tumors. To optimize the therapeutic potential, they engineer these oncolytic viruses by means of genetic modification.

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Stephan Sieber has discovered a substance that very effectively fights dangerous, multi-resistant hospital germs.

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Highly specialized viruses offer an effective treatment for bacterial infections. TUM spin-off Invitris makes the production process for these bacteriophages faster and more cost effective.

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German edition available as a PDF here:

www.tum.de/faszination-forschung-25

“This Vaccination Should **Benefit every Country**”

Chronic hepatitis B infections often lead to cirrhosis and cancer of the liver. There is currently no known cure for the disease. However, a cure is precisely what Prof. Ulrike Protzer and her team of researchers are striving to develop with the help of a therapeutic vaccination to drive the virus from liver cells.

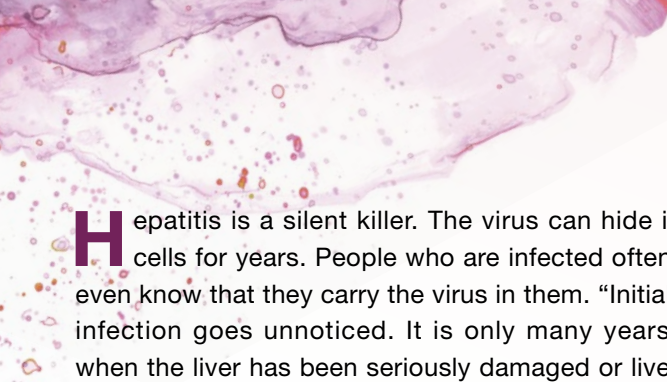
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„Diese Impfung soll allen Ländern  zugutekommen“

260 Millionen Menschen weltweit sind chronisch mit Hepatitis B (HBV) infiziert. Das Virus existiert in ihren Leberzellen. 890.000 Todesfälle pro Jahr sind die Folge der Infektion. Bisher gibt es nur Medikamente, die das Virus in Schach halten. Prof. Ulrike Protzer hat einen neuen therapeutischen Impfstoff entwickelt, der Hepatitis B tatsächlich heilen könnte. Er stimuliert ganzheitlich das körpereigene Immunsystem und versetzt es in die Lage, das Virus effektiv zu bekämpfen und zu eliminieren. Die Prime-Boost-Methode läuft in mehreren Phasen ab. Zunächst erhalten die Patienten zwei Mal einen Proteinimpfstoff. Das stimuliert die B-Zellen: Sie bilden Antikörper gegen HBV. Auch die T-Zellen werden bereits alarmiert. Dann folgt eine weitere Impfung mit einem eigens entwickelten, komplexen MVA-Impfstoff. Der aktiviert vor allem die T-Zellen, welche die Virus-DNA aus den Leberzellen entfernen und infizierte Zellen zerstören. Die klinische Prüfung soll Anfang 2021 starten. □

Link

web.med.tum.de/en/home/



Hepatitis is a silent killer. The virus can hide in liver cells for years. People who are infected often don't even know that they carry the virus in them. "Initially, the infection goes unnoticed. It is only many years later, when the liver has been seriously damaged or liver cancer develops, that the virus becomes evident," explains Prof. Ulrike Protzer, Director of the Institute of Virology at TUM and the Helmholtz Zentrum München.

If you come into contact with the hepatitis B virus (HBV) as an adolescent or an adult, your immune system will be able to keep it in check. Although the immune response will trigger a liver infection, in most cases your immune system will be able to fight and eliminate the virus, and only five to ten percent of all patients develop a chronic infection. The situation for babies and young children is much more dramatic. Their immune systems are not yet fully matured and lack the defense mechanisms required to fight off HBV. Consequently, the virus is able to settle in liver cells in 90 percent of cases in this age bracket – where it remains for the rest of the individual's life.

Around 260 million people suffer from a chronic infection with the virus. Most of them are unaware of this – and those who are aware often hide it due to the stigma attached to it. People with a hepatitis B infection often face accusations of drug abuse, despite the fact that there are many other origins of infection. Liver cirrhosis and liver cancer are common long-term consequences of a chronic HBV infection. The hepatitis B virus kills around 890,000 people every year as a result of such complications, putting it among the most deadliest diseases. And, while the number of deaths caused by malaria, HIV and tuberculosis show consistent declines, deaths from hepatitis B continue to rise. A prophylactic vaccination for hepatitis B

has been available for almost 40 years; in Germany, all children receive the vaccine within the first 12 months of life. In other parts of the world, however, the situation is quite different. Mothers infected with the virus can easily transmit it to their newborn child. In such cases, the vaccination must be administered within 24 hours of a child being born. In geographically remote areas of Africa and Asia, that is simply not feasible.

Consequently, it will not be possible to eradicate hepatitis B through prophylactic vaccination. Medications have so far only been able to control the infection. What efforts to fight hepatitis B actually need is a way to remove the virus from liver cells once infected.

A therapeutic vaccination heals

It is hoped that a new treatment developed by Ulrike Protzer called TherVacB will be able to cure hepatitis B infections. With the help of a therapeutic vaccination, the treatment aims to strengthen the immune system, enabling it to fight the virus and drive it from the body. In preclinical models, the novel concept behind TherVacB outcompetes other vaccine candidates currently in clinical trials. To understand how it works and why it may be superior, however, we first need to look at how the hepatitis B virus works.

When a person becomes infected with the virus, it circulates in their blood until it reaches the liver cells. Once there, it is smuggled into cells by one of the cells' own transporters, which are actually tasked with transporting bile acid, and then imports its tiny genome into the cell's nucleus. This genome is just 3,200 base pairs long, circular and only double-standard in sections. By way of comparison, the human genome is a million times larger; even a herpes virus has 50 to 70 times more DNA. ▶

1,500,000

people died in 2017 of viral hepatitis and its consequences



Cardiovascular disease

is the top health cause of death globally



Cancers

claim the second most lives



Respiratory diseases

rank number 3 in death rates across the world with nearly 4 million deaths



Lower respiratory infections

are responsible for 2.6 million deaths per year globally, the fourth-largest number



Dementia

claims about 2.5 million lives each year



Digestive diseases

cause the death of 2.4 million people a year



Neonatal disorders

claim the lives of 1.8 million babies each year



Diarrheal diseases

rank eighth and lead to the death of 1.6 million people per year



Liver diseases and acute hepatitis

kill 1.5 million people each year

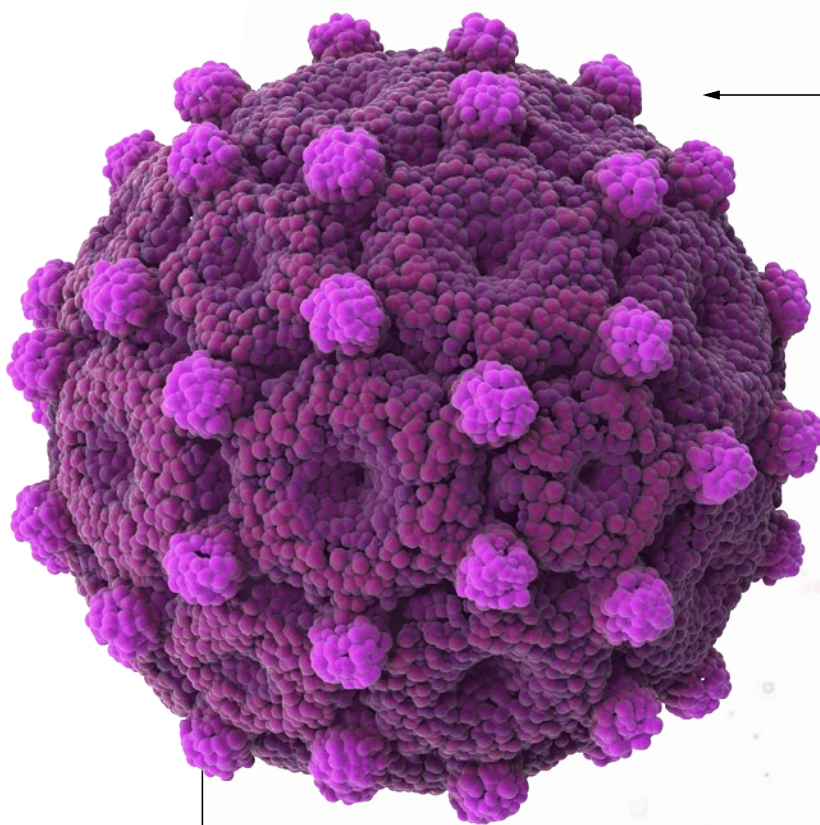


Diabetes

kills 1.4 million people a year around the world

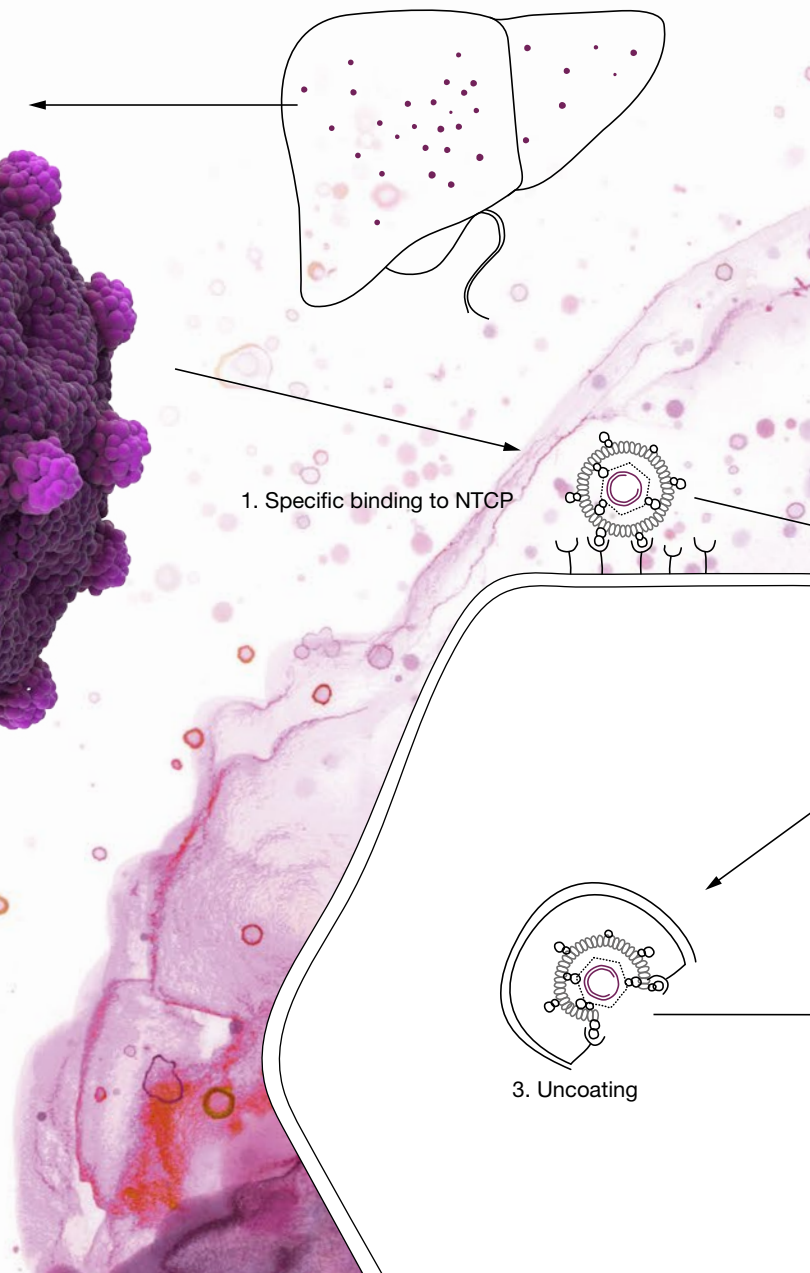
The liver cells' repair mechanisms incorrectly identify this viral DNA as belonging to the cell and thus begin to fill in the shorter DNA string with the corresponding base pairs. This creates what is known as cccDNA, which stays in the nucleus virtually forever. Only once this cccDNA has been created can viral genes be transcribed and proteins produced. One approach, therefore, would be to inhibit replication of the virus by interrupting protein synthesis or RNA transcription. "To date, however, efforts to do so have also damaged cellular functions," explains Ulrike Protzer. An alternative method is known as RNA interference. "By using small interfering RNAs (siRNAs), we can specifically inhibit the production of virus proteins without damaging

the host cell. Unfortunately, that alone is not enough to eliminate the virus." siRNAs are in the early stages of clinical trials and should be available in the near future. The treatment method most likely to cure hepatitis B infections is to teach the immune system to remove the cccDNA from liver cells or to destroy infected cells. The immune system is actually capable of doing so – but the immune response of people with a chronic infection is inadequate to fight off the invader. It is therefore hoped that a therapeutic vaccination will enable the body to expel the virus completely. "The failure of the immune response relates to all of its arms – so our vaccination also needs to stimulate all arms of the immune system," explains Protzer. ▶



Hepatitis B virus

The hepatitis B virus uses liver cells for reproduction.

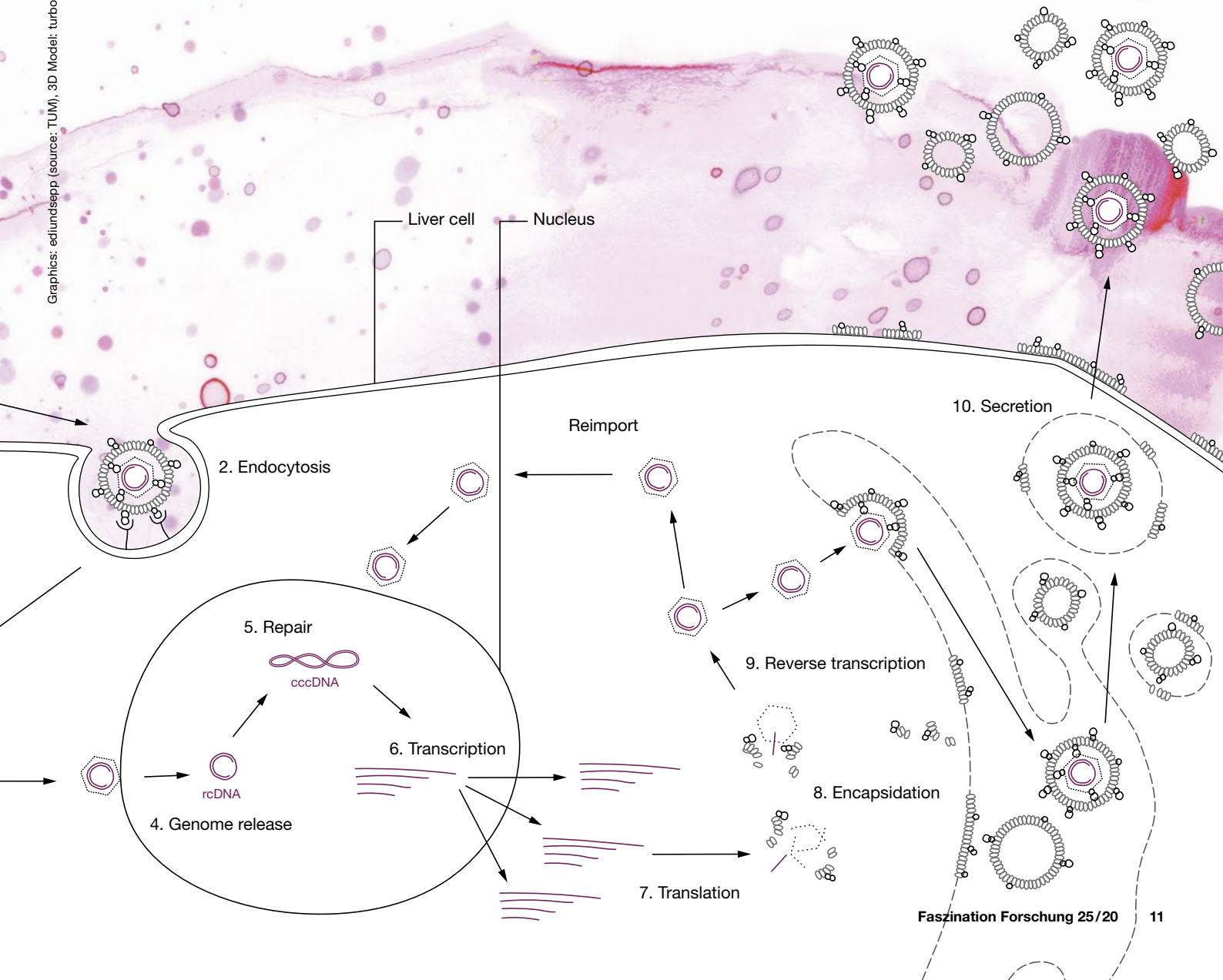




Prof. Ulrike Protzer

Ulrike Protzer studied medicine at universities in Erlangen, Basel and Durban (South Africa). She holds specialist qualifications and passed board exams in two areas, namely internal medicine plus microbiology, virology and infection epidemiology. From 2002 to 2007, she led a junior research group at the University of Cologne's Center for Molecular Medicine. She assumed the Chair of Virology at TUM in late 2007 and has since become Director of the Institute of Virology at TUM and the Helmholtz Zentrum München.

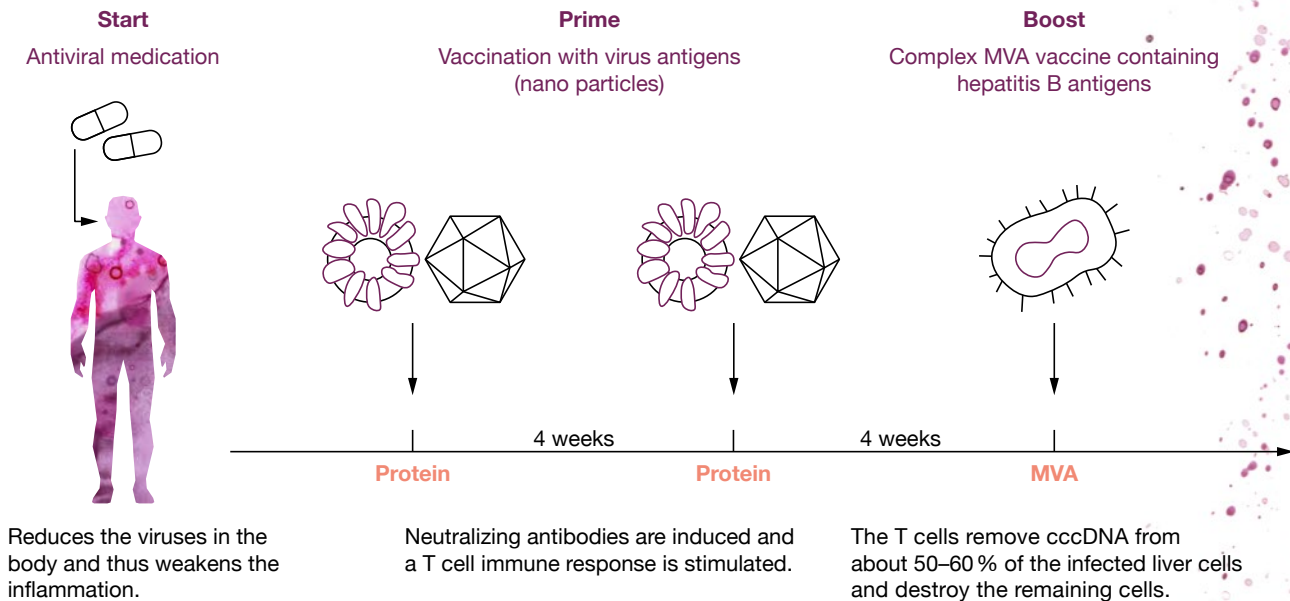
Graphics: edlundsepp (source: TUM), 3D Model: turbosquid; Picture credit: Kurt Bauer





The failure of the immune response relates to all of its arms – so our vaccination also needs to stimulate all arms of the immune system.

Ulrike Protzer



The phases of the therapeutic vaccination that can heal hepatitis B (TherVacB).

Several vaccination phases

The vaccination is administered in several stages in a process the virologist has dubbed “prime-boost”. The first step effectively lays the groundwork by giving patients antiviral medication that inhibits replication of the virus’ DNA in their liver cells. As soon as siRNAs are clinically available, they will replace this as the first step. Next, for the prime stage, the patients receive two vaccinations four weeks apart containing different virus antigens, thereby stimulating the T helper cells and warning the T cells that an intruder will soon arrive. In parallel, this activates B cells in the patients’ immune system. Neutralizing antibodies are then formed that prevent the virus from spreading further. After another four weeks, the treatment moves on to the next step – the boost. Patients are given a vaccine that shows the T cells the antigens of the hepatitis B virus, which the T cells would not otherwise be able to identify in the liver. At this stage, the T cells have two tasks. First, they release cytokines that instigate a complex signal cascade and degrade the cccDNA in liver cells. This removes virus DNA from around 50 to 60 percent of infected liver cells. The remaining infected cells are then destroyed by T killer cells.

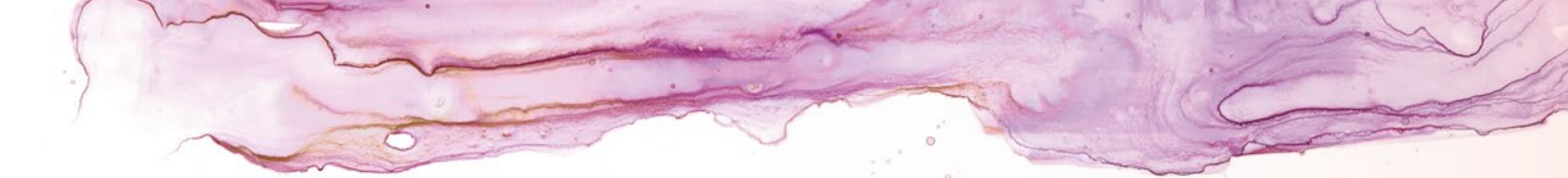
This vaccine is a complex MVA vaccine developed by Protzer herself. “MVA is the perfect vaccination vector as it boosts B and T cells in equal measure,” outlines Protzer.

A specifically developed vaccine

MVA is the abbreviation for Modified Vaccinia Virus Ankara, an attenuated virus from the poxvirus family. Although it can infect human cells, it is not able to reproduce in these cells and does not elicit any symptoms. The genome of the MVA virus serves as the backbone of the vaccine. Researchers in laboratories can then introduce new gene sequences that code for the antigens of other viruses and thus trigger an immune response against these antigens.

In principle, it should be possible to use this technique to produce all manner of vaccines. A clinical study of an MVA-based influenza vaccine is currently underway. Efforts to test the viability of a coronavirus vaccine using this system are also underway.

In most cases, only one new gene is built into the MVA backbone. Sometimes two. Yet, Ulrike Protzer wanted her hepatitis vaccine to be effective against almost all hepatitis B strains, all around the world. One or two genes were not enough to achieve this; Protzer needed to use five. “I’m not sure whether anyone has ever been crazy enough to pack five new genes in there,” she laughs. Many simply did not believe it would work – but it has. However, another hurdle lay in the researchers’ path. They needed to find a suitable adjuvant – an agent to intensify the initial immune response. “There are, in effect, no freely available adjuvants,” says Protzer. ▶



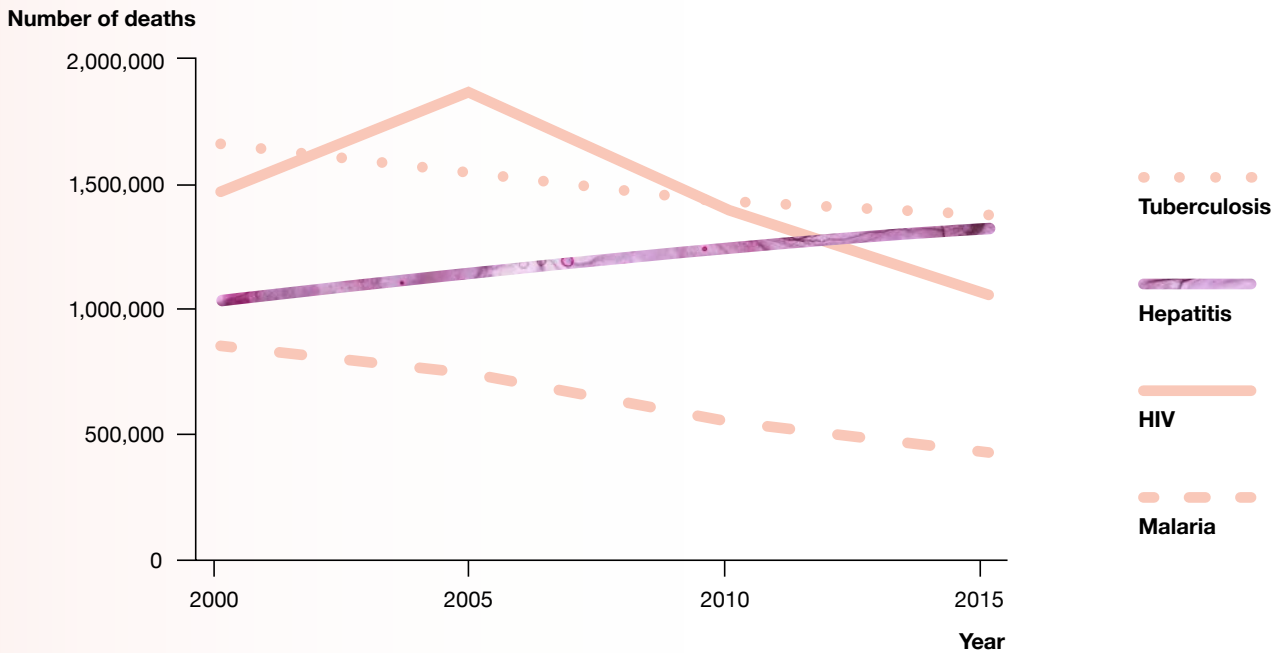
“The patents are almost all held by a single pharma company.” The company did make a product available – but wanted all patent rights to Protzer’s new TherVacB vaccine in return. She turned the deal down. Ultimately, the researchers still found what they needed and can now rely on CpG 1018, an adjuvant already employed in a prophylactic hepatitis B vaccine and therefore approved for use. In preclinical mouse models, Protzer has successfully demonstrated that the immune system reacts as she had hoped to the prime-boost vaccination. The hepatitis B antigens disappear from the blood and are replaced by numerous antibodies against the virus. The T cells do the rest – and cure the liver completely. “We are convinced that the principle we have developed would also be suitable for a vaccine against the novel SARS coronavirus,” she says.

The task now is to produce the vaccine in accordance with Good Manufacturing Practice (GMP) regulations. This will be followed by toxicity tests on mice and rats. A phase 1a clinical trial on human subjects is then scheduled to start

early next year (see box). The first step in this trial will be to vaccinate healthy subjects and determine the best combination of agents for the prime-boost technique. It is extremely rare for a clinical trial in the academic sphere to proceed without the involvement of the pharmaceutical industry. “We’re only able to do so on this project because we are working hand in hand with Klinikum rechts der Isar, LMU Munich and our partners throughout Europe,” explains Protzer. “Otherwise, it simply would not be possible.”

The researchers have already obtained close to €20 million funding for the project, all of which is from public funds – a fact of which Ulrike Protzer is particularly proud. “It means we’re independent of investors, who not only pursue humanitarian interests but also want to make money,” she says. “Ultimately, we hope our vaccine will one day benefit patients in all parts of the world.”

■ *Claudia Doyle*



Hepatitis B and C diseases are an increasing concern globally. While the number of deaths caused by malaria, HIV or tuberculosis is falling, the curve is continuously rising in the case of hepatitis.

Clinical studies – From the lab to the hospital

All medications have a long road to travel from the lab to being used in hospitals. First, a potential agent is usually tested on cells in a petri dish. If it takes effect, tests are then carried out on animal models that reconstruct the illness as accurately as possible. “Only then can the first clinical trials take place with humans, divided into four phases,” explains Christoph D. Spinner, senior physician and infectious disease specialist at TUM. Working together with Germany’s Federal Institute for Drugs and Medical Devices, the scientists involved in a trial have to determine how many patients need to be included for the results to be significant.

Phase 1 of a clinical trial examines the safety of a substance. “The aim is to exclude the risk of completely unexpected reactions occurring in the complex human organism,” explains Spinner. Consequently, these trials are usually conducted on healthy subjects. Phase 2 serves to identify the correct dosage and involves a small group of patients. In phase 3, the efficacy and safety of the agent are examined on the trial subjects. If the results are again positive, the substance can be approved for use. Phase 4 is therefore also referred to as a post-authorization study. The purpose is to highlight any side effects or long-term effects not identified to date.

TUM is very well positioned to facilitate studies of this type. “We can activate the infrastructure at our disposal overnight,” says Christoph Spinner. “This has enabled us to work intensively on the development of medications to combat COVID-19 since early this year.”



What a Virus really Needs

When a virus infects a cell, it interacts in many ways with its host. Prof. Andreas Pichlmair investigates these interactions at the protein level applying modern mass spectrometry technologies. He uses the collected data for network analyses. As a virologist, Pichlmair's goal is to understand which cellular components and signaling pathways are essential for viruses' propagation. His goal is to promote the development of new medications that target these pathways.

Was ein Virus wirklich braucht

D

Viren infizieren Wirtszellen und bedienen sich der molekularen Maschinerie der Zelle für ihre eigene Vervielfältigung. Dafür sind viele Interaktionen des Virus mit den Proteinen der Zelle notwendig. Prof. Andreas Pichlmair, Virologe der TUM, nutzt die Massenspektrometrie, um die beteiligten Proteine zu detektieren. Diese eignen sich potenziell als Ausgangspunkt für die Entwicklung von Therapien, auch gegen das neuartige Coronavirus SARS-CoV-2. Die Massenspektrometrie misst die Masse von Molekülen. Weil jedes Molekül eine einzigartige Masse besitzt, können die Forscher ihrem Messwert anhand einer Datenbank den jeweiligen Namen zuordnen. Zusätz-

lich lässt sich auch die Menge des jeweiligen Proteins erfassen. Pichlmair und sein Team beobachten, wie sich die Proteinzusammensetzung nach einem Virusinfekt in bestimmten Zeitintervallen verändert. Die Veränderungen, die sie dabei beobachten, verrechnen die Forscher auf Basis der Daten von Netzwerkanalysen. So lassen sich Signalwege der Zellen erkennen, die für Viren überlebensnotwendig sind. Parallel dazu versuchen sie herauszufinden, ob diese Signalwege auch bei anderen Erkrankungen eine Rolle spielen, für die es bereits Medikamente gibt. Denn dies könnte die Entwicklung neuer Wirkstoffe deutlich abkürzen. □

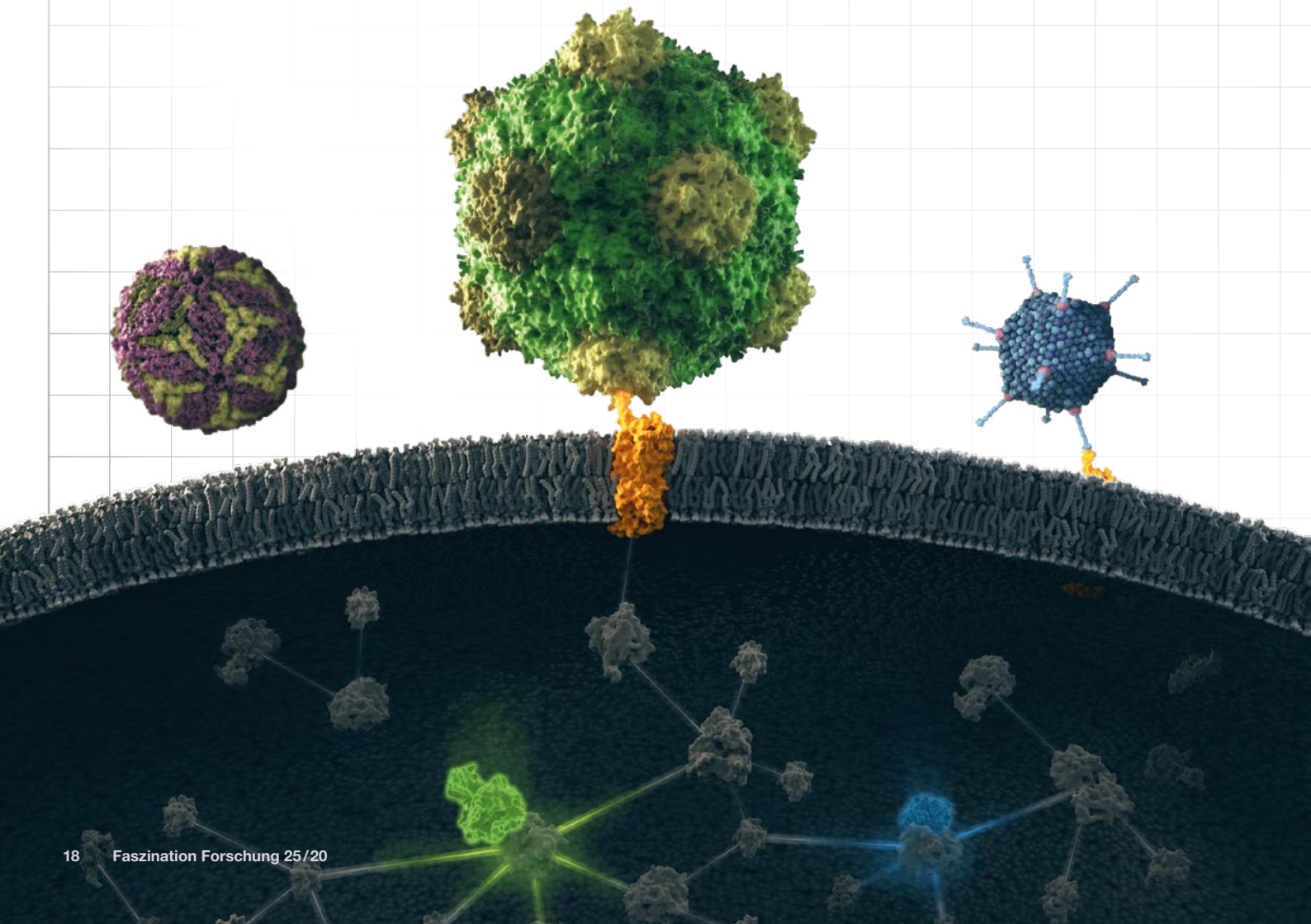
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www.innatelab.org

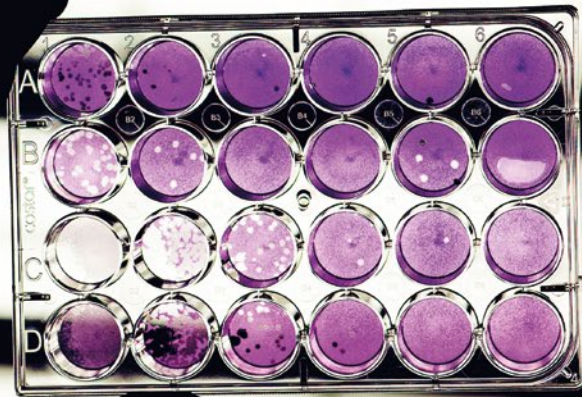
Viruses depend on foreign cells to multiply. Without a host, they cannot reproduce. After settling in a host cell, viruses take advantage of the cell's molecular machinery for their own reproductive purposes. First, however, they need to escape their host's immune system. They achieve this either by disguising themselves to avoid detection or by manipulating the signaling pathways that the host's immune system would otherwise use to raise the alarm. All of these steps involve interactions between the virus and certain cellular factors. Prof. Andreas Pichlmair is interested in the proteins involved in this process: The ones which the virus often specifically disables in the host cell to stay unrecognized and others which facilitate the virus's reproduction. As the TUM virologist explains, "these proteins could provide a starting point from which to develop therapies", including for the novel coronavirus (SARS-CoV-2).

Viruses control the cell's protein production

If a virus successfully delivers its genetic material into a host cell, it takes control of the cellular production mechanisms. This means, for instance, that the virus is able to restrict the production of proteins that the host cell's immune system requires to defend itself. At the same time, it can ramp up production of the proteins it requires for its own reproduction. Both cells and viruses are predominantly made up of proteins that form structures and, in the form of enzymes, facilitate chemical reactions. Each cell contains around 20,000 different genes carrying the code for specific proteins. While some proteins are not produced (expressed) at all or only with a few copies, others occur in millions of copies. Pichlmair and his team are using mass spectrometry to investigate which proteins are of greatest interest to viruses. This technique measures the mass of molecules such as proteins. Since



Viruses cause cytopathic effects in infected cells. Cells are fixed and stained with a violet dye. Wherever the virus replicates, it destroys the cell layer and thus reduces the violet color in the respective dish.



every molecule has a unique mass, scientists can identify the repertoire and quantity of individual proteins in a given sample. The researchers then compare the proteins in healthy cells with those in infected cells. This allows them to examine how the protein composition of cells changes at set intervals after a viral infection.

After conducting their measurements, the researchers ultimately obtain a list of several thousand proteins and their respective quantities.

The function of the proteins serve as an approach for possible medications

Certain proteins, for instance the ones required for energy metabolism, change in the case of almost all infections. Other proteins, however, only change when a cell is infected by a specific virus. All proteins that occur in greater or lesser quantities following an infection are presumably important for the virus. The precise function of many of these proteins remains unknown – and it is these proteins that interest Pichlmair. He wants to understand why a virus needs them. It would allow new medications to be developed quickly in response to specific viruses. The researchers are applying network analysis, a technique based on the analysis of complex processes within cells. Different stimuli within cells trigger a wide range of inter-

actions between molecules. Some of these molecules activate or deactivate other molecules. Others enable or disable the production of proteins. While some of these interactions within cells have already been identified, our understanding of others remains rudimentary. Protein production often plays a role. These interactions inside cells are also known as signaling cascades or pathways as one reaction acts like a signal and triggers and often amplifies numerous other reactions. The fact that these signaling cascades often interact with one another gives the cell a wide opportunity to respond to pathogens, but makes it more complicated for scientists to understand the processes involved.

The scientists get clues from network analyses

A number of these cascades have been identified and are stored in databases enabling Pichlmair to analyze the list of proteins generated through mass spectrometry. He uses the results of this analysis to help him interpret what happens in a cell once it is infected; his aim is to determine the reactions that take place within cells and pinpoint which signaling pathways are activated when a virus has infected a cell. “The more of this complex data we have, the more precise the picture becomes,” says Pichlmair. By the same token, the more we know about a specific >

signaling pathway, the easier it is to identify whether the virus needs it. Pichlmair, however, also issues a warning: “There are many measurement readings we simply cannot interpret because the network data is too sketchy or is missing entirely.”

Coronavirus: Searching for analogies with other diseases

The novel coronavirus SARS-CoV-2 is made up of around 27 proteins. While the functions of some of these proteins are known, others remain unclear. “What mass spectrometry shows us is that, as in other viruses, there are certain proteins both in the virus itself and in the host cell that are of particular importance to the virus,” says Pichlmair. The researchers’ goal is to identify signal cascades that are activated when a cell is infected with the coronavirus. In parallel with this, they are seeking to discover whether these cascades might also play a role in other diseases for which medications are already available. If so, it would significantly accelerate the identification of therapeutic agents that may be active against SARS-CoV-2. This approach, known as drug repurposing, looks at medications that have been approved for use with other diseases – or are at least at an advanced stage of testing. Medications that intervene in signal cascades are primarily known for their use in treating cancers. ▶

≈ 20,000

proteins are engaged by the virus

29

proteins are expressed by the virus

1,000

proteins are bound by the virus proteins

“What’s special about our work is that we are linking mass spectrometry, bioinformatic analyses, virology and cell biology.”

Andreas Pichlmair



The cells are stored in nitrogen.

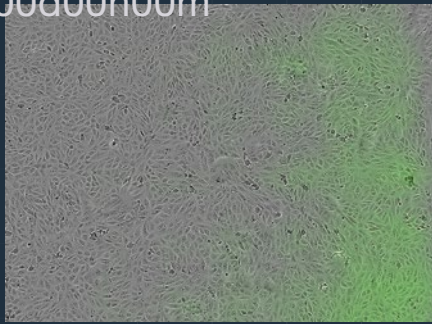


Picture credits: Magdalena Jooss

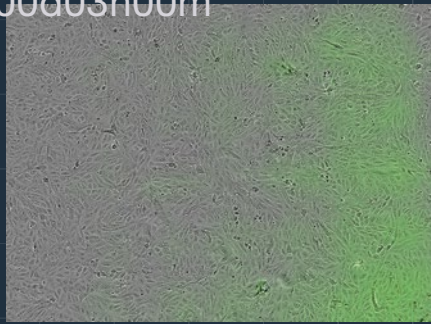
Prof. Andreas Pichlmair

Andreas Pichlmair studied at the University of Veterinary Medicine, Vienna, and performed his doctoral studies at the University of Freiburg/Breisgau. Subsequently, he obtained his doctorate from the London Research Institute of Cancer Research UK (now the Francis Crick Institute) and then spent three years as a postdoc in Vienna at the Austrian Academy of Sciences' Center for Molecular Medicine (CeMM). From 2011 to 2017, he established his own laboratory at the Max Planck Institute of Biochemistry in Munich as a Research Group Leader. Pichlmair was appointed Associate Professor at TUM in 2017. His research focuses on the interaction between viral proteins and their host organisms. He primarily uses mass spectrometry and combines this with other techniques, such as network analyses and other systems analyses. As a virologist, Pichlmair aims to understand which cellular components and signaling pathways are essential to viruses' survival – and thereby represent suitable targets for the development of drugs.

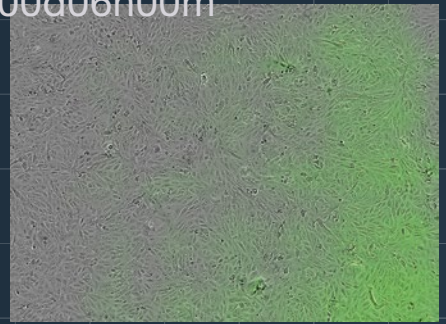
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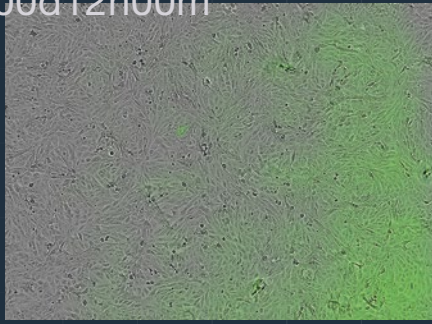
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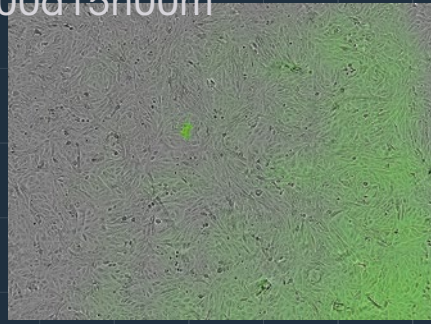
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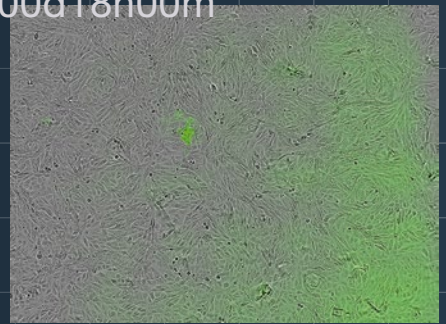
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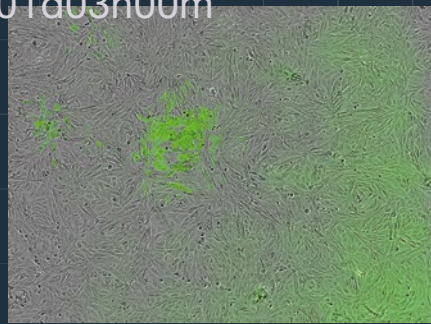
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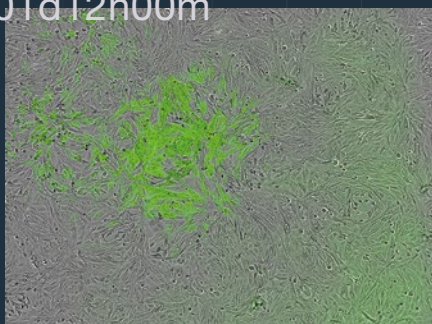
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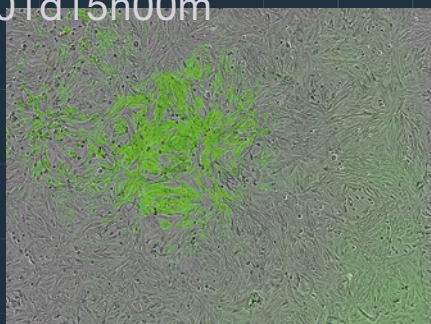
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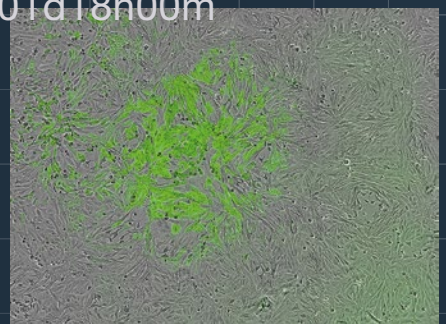
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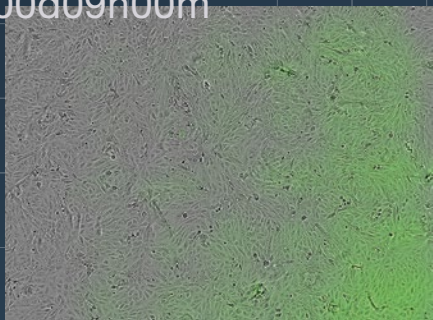


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A cell culture is exposed to a SARS-CoV-2 which is marked with a green fluorescent protein. Infected cells in which the virus is reproducing show up in green. The screening microscope (right) allows the same measurement to be performed every three hours to follow the replication of the virus. The development and spread of the virus can thus be seen over time.

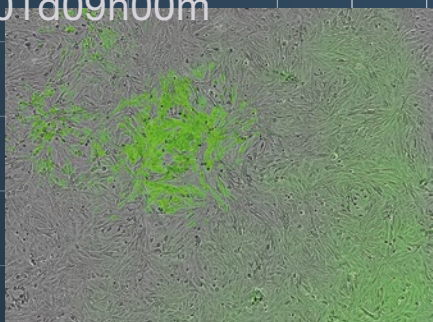
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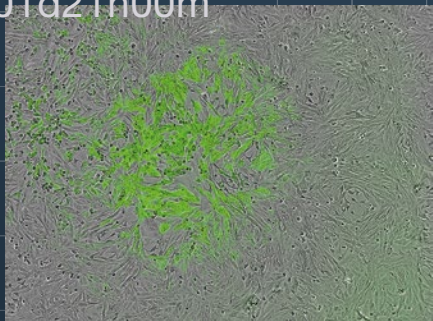
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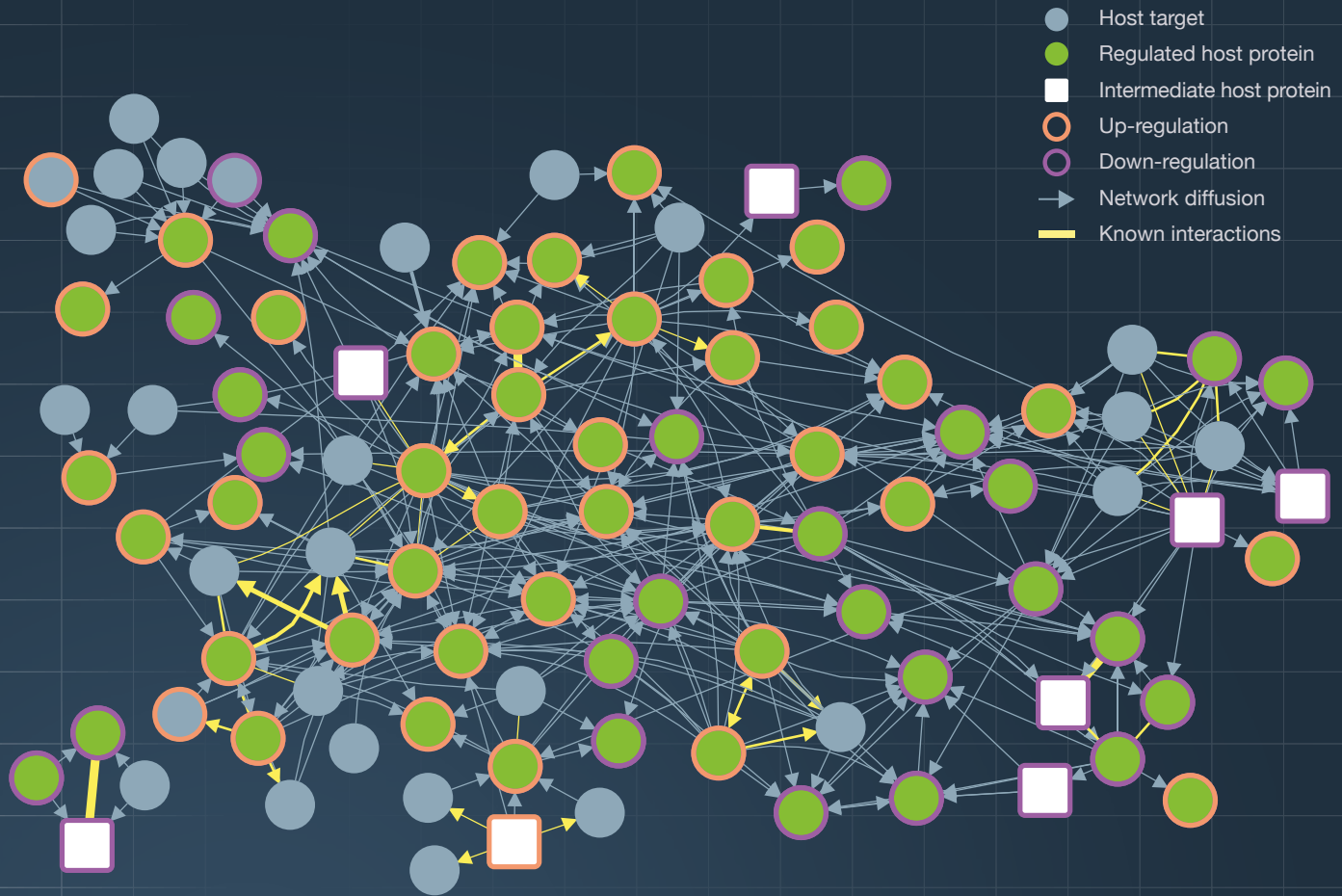


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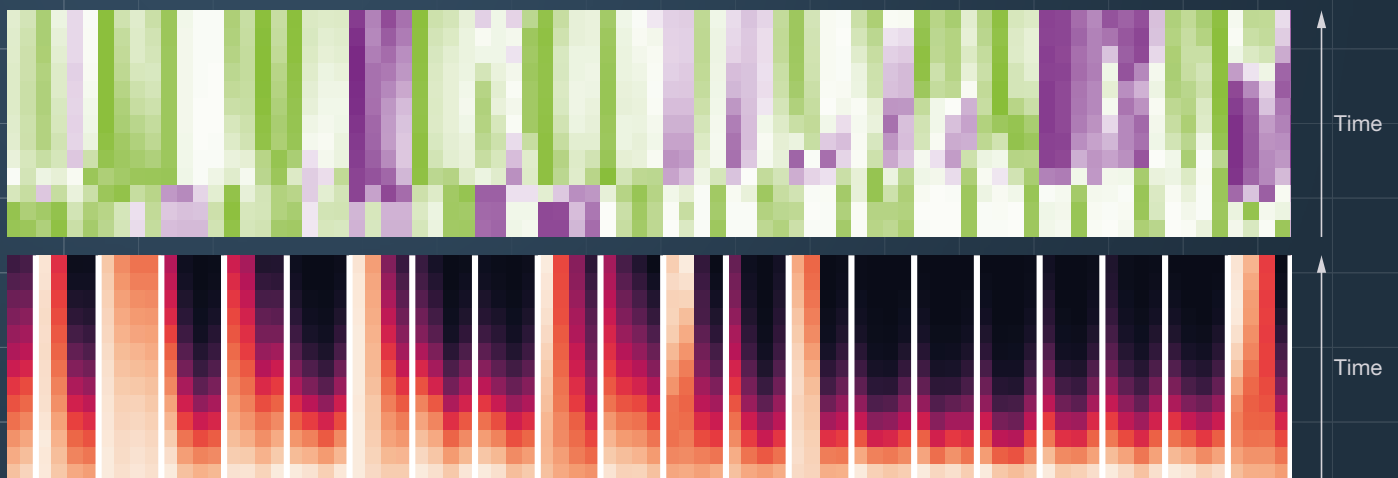


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The network in this picture shows what happens after the SARS-CoV-2 ORF7a protein is expressed in a cell. Each network node depicts a specific protein. Grey nodes indicate cellular proteins, to which the virus protein binds. Green nodes illustrate the downstream changes in host protein expression. An orange border stands for an abundance increase, purple for decrease. These proteins are targets for the viral protein, as it changes their abundance rate. The yellow lines denote the current knowledge of molecular interactions in the cell. Thus the network analysis helps to gain a deeper molecular understanding of the virus protein activity in the cell.



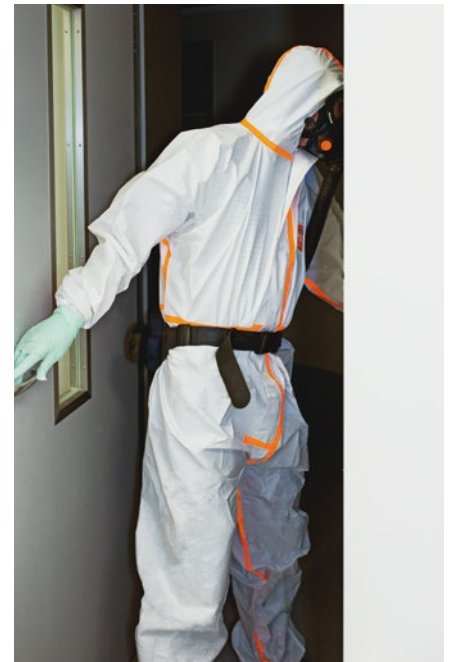
Results of a screening as shown on the previous page. Top: Signal of the green fluorescent protein; less green/more purple indicates less virus. Bottom: Cell growth; darker colors indicate higher cell densities. Each column represents the test with a different compound, each square within that column stands for one measurement. Dark red/purple colors indicate compounds which seem effective against the virus.

While some of Pichlmair's team continue to collect and analyze data, others are already testing the efficacy of identified substance classes. Do they still allow viruses to reproduce or do they inhibit this process? To do this, the researchers use cell cultures and coronaviruses marked with a fluorescent green dye. Infected cells in which the virus is reproducing show up in green. In cases where agents prove to be effective, the green coloring either disappears or does not appear at all. The researchers use a screening microscope, which allows them to process high volumes of samples. The device has been co-funded by the Max-von-Bauernfeind Association, a longstanding partner of TUM. Like all experiments which involve direct use of the virus, these tests are conducted in biosafety level 3 (BSL-3) laboratories. BSL-3 laboratories are sealed off and isolated from the outside world and are designed in such a way that pathogens cannot inadvertently escape. Thanks to their modern microscope, Pichlmair and his team can perform 574 independent measurements every three to four days.

A truly interdisciplinary team

“What’s special about our work is that we are linking mass spectrometry, bioinformatic analyses, virology and cell biology,” says Pichlmair. This approach demands extensive experience in entirely different fields. Pichlmair’s multidisciplinary team comprises virologists, cell biologists, engineers and bioinformaticians. His team of eighteen work hand in hand, each of them a specialist in their field and each contributing his or her part to complete the puzzle. At the same time, other team members are performing quality checks to avoid analyzing artifacts. Together, the team are working to understand exactly what viruses need in order to reproduce. “The challenge lies in not simply conducting blind tests where you don’t understand what’s happening. Instead, it lies in identifying substance classes of interest that, when combined with our knowledge, can help us to develop forward-thinking treatments,” says Pichlmair.

■ *Karoline Stürmer*



Many of the experiments are conducted in biosafety level 3 (BSL-3) laboratories. BSL-3 laboratories are sealed off and isolated from the outside world and are designed in such a way that pathogens cannot inadvertently escape. A special “dress code” and HEPA-filtered ventilation devices protect scientists from getting infected.



„Die Bereitschaft zu Datenspenden ist sehr hoch“



Pandemien sind Krisenzeiten. Aber sie bringen auch eine erhöhte Solidarität mit sich. Die Medizinethikerin Alena Buyx setzt hier an und plädiert dafür, persönliche medizinische Daten in Form von „Datenspenden“ für die medizinische Forschung nutzbar zu machen. □

“There is a Great Willingness to Donate Data”

Infectious diseases and pandemics entail a whole string of ethical conflicts that are difficult to resolve. During such times of crisis, however, positive social effects can also be identified, such as increased solidarity and greater willingness to donate. Medical ethicist Prof. Alena Buyx advocates viewing medical data not only from the perspective of individual autonomy, but also making this data available for medical research in the form of “data donations”.

Prof. Buyx, infectious diseases and pandemics have been a major topic in recent months. What ethical questions do you see arising in this context?

Pandemics involve a deep ethical conflict – something we have pointed out in the German Ethics Council. On the one hand, we need to ensure that our healthcare system remains able to function. It is therefore necessary to put measures in place to contain the spread of the pandemic. On the other hand, the knock-on effects of these lock-down measures include dramatic economic, social and psychological damage. There are, as it were, two sides to this coin. We have to find some way to balance the two aspects.

Link

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Can medical ethicists propose solutions or issue recommendations in the face of such conflicts?

It is not usually possible to resolve this conflict; however, we can attempt to attenuate its effects and preserve proportionality. We must therefore continuously examine whether the measures currently in place to contain the pandemic are still proportionate. This involves answering questions such as: has the evidence changed? Have we learned anything new – either about the virus or about whether and to what extent the measures in place are succeeding, etc.?





“We have a shared interest in protecting the health of everyone.”

Alena Buyx

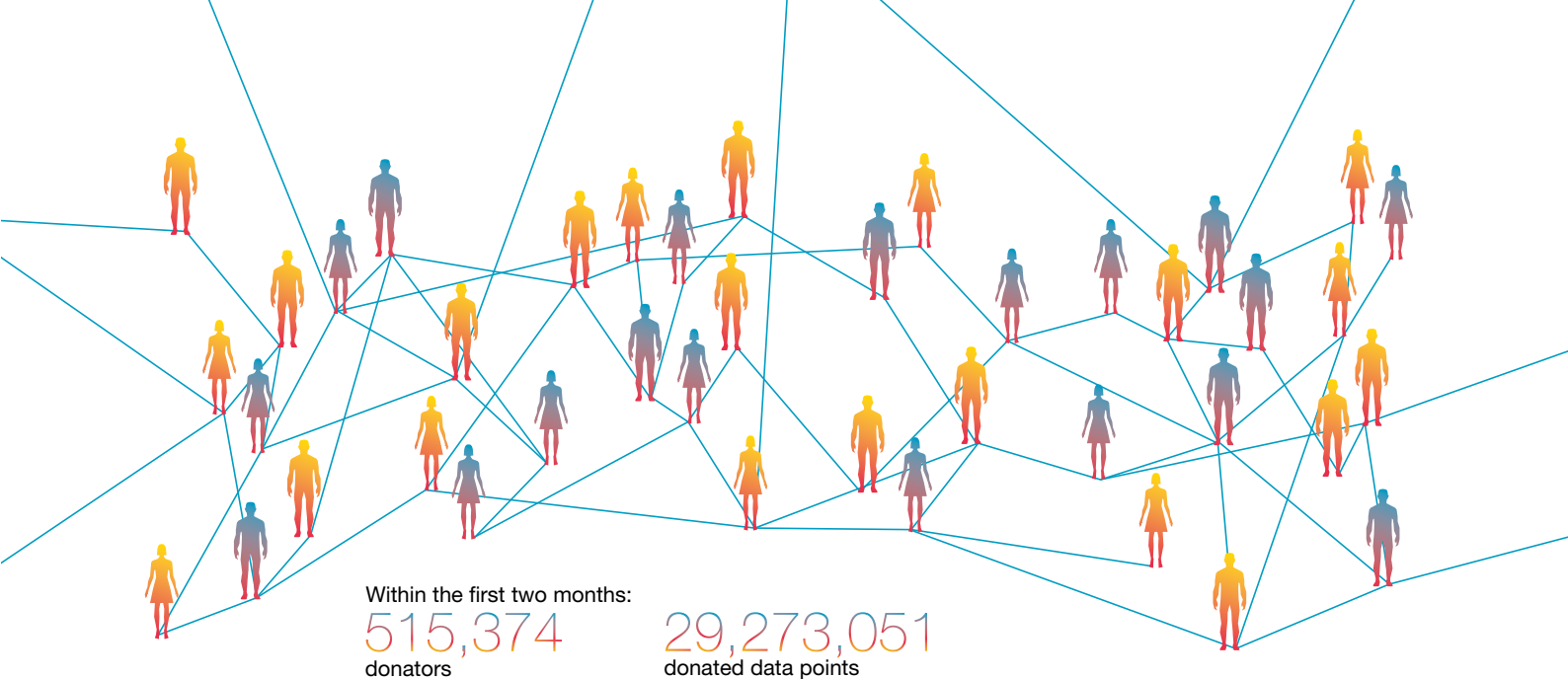
So, we should not simply leave these determinations in the hands of medical experts?

The Ethics Council has said that we should not adopt an approach in which we proceed exclusively or automatically on the basis of data presented by virologists and epidemiologists – and regard everything else as secondary. That is not acceptable. We need to make political decisions that cannot be dictated solely by data about the pandemic. Numerous other aspects and interests need to be taken into account, and balanced.

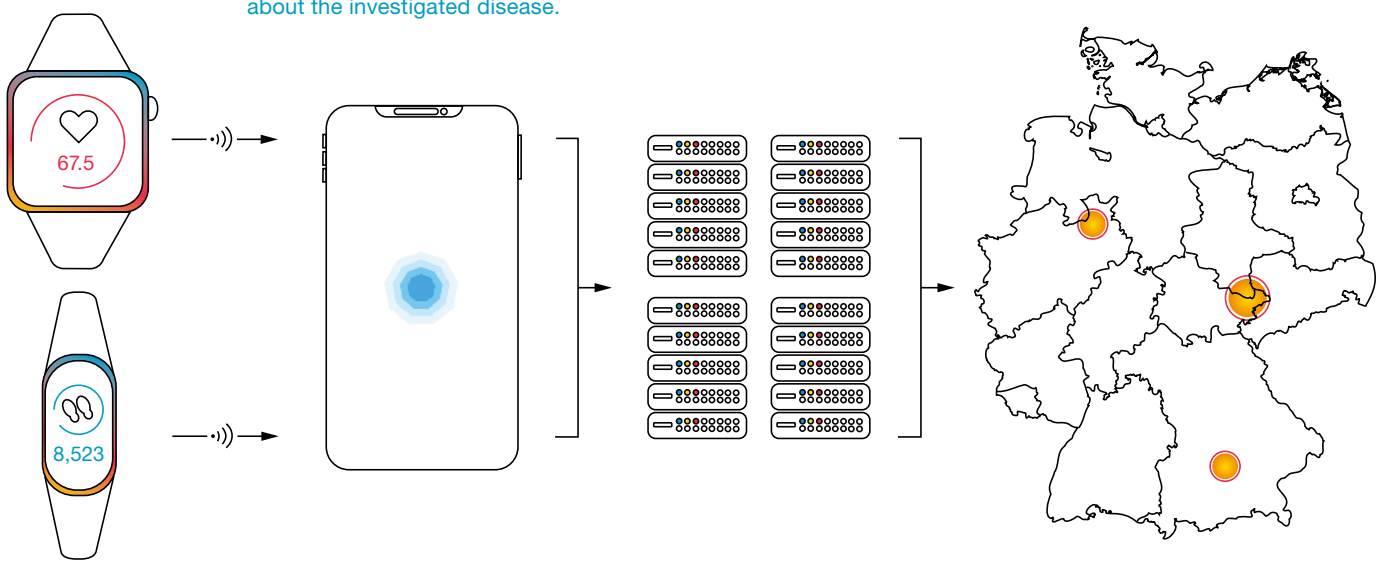
In the context of a crisis, people show more solidarity. Is this a topic that has played an important role for the Ethics Council and has been raised in discussions?

In the past, data protection legislation and regulations ensured that medical information was disclosed in a

manner that was very much geared towards minimizing the risks. The instrument of informed consent was intended to ensure this, and to enable data autonomy. In 2013, my colleague Barbara Prainsack and I published a paper in an international medical law journal that has been frequently cited in which we indicated that consent is about more than just autonomy and risk minimization. When people participate in research projects involving huge volumes of data, they also have a prosocial motivation for doing so. By this, we mean that people would like to do something voluntarily for others who might have a similar illness, even if doing so exposes them to minor risks, such as in terms of data protection. You might say that we have a shared interest in protecting the health of everyone. We should therefore also regard this form of research as solidarity in practice. ▶



Data donations like the Corona Data Donations app from the Robert Koch Institute are often about collecting as much data as possible from as many participants as possible. The more data, the better it can be searched for patterns that help answer questions about the investigated disease.



Fitness bands and smart-watches collect data like activity and heart rate.

Users have entered data like rough age, weight, height as well as their zip code into the app.

A central server processes the data.

Changes in the regional distribution of parameters like heart rate and change in body temperature are visible. Such data can help identify the onset of an infectious outbreak.

What is data donation?

For patients, donating data means making their medical data available for research purposes, usually in anonymized form. This data might relate to relatively neutral information such as their sex or weight, or data taken from fitness apps; however, it could also contain sensitive information such as X-ray images or the results of laboratory tests. Scientists can use the information gathered in this way to gain new insights and derive new therapies.

One current example is the German Corona Data Donations app developed by the Robert Koch Institute. The RKI has appealed for users to provide data from fitness trackers and smartwatches, as it is hoped that such data will provide indications of symptoms of a COVID-19 infection. The scientists hope to learn more about the virus' propagation as well as about the number of unreported infections. The information will help to derive better measures against the virus.

Graphics: edlundsepp (source: corona-datenspende.de/science); Picture credit: Juli Eberle

“Data donation can be structured so that it does not conflict with data protection provisions in any way.”

Alena Buyx

And voluntary data donation is one form of solidarity in practice?

The idea of donating data is now also being addressed as part of wider public discourse. To give some background on this: We performed several studies on the secondary use of data in clinical settings. Secondary use means that data that was primarily generated for a different purpose is used for other research questions. In our study, we identified that many people seek to act in a prosocial and solidary manner and are happy to donate their data – even if the precise research question this might help to address is not yet clear when the data is collected. That was the case for well over 80 percent of respondents, sometimes over 90 percent. We can therefore assume that a lot of people are highly socially motivated to donate their medical data. We also learned, however, that people only wish to do so when their donation serves the common good – in this case, publicly funded medical research. They would not be prepared to donate their data to companies such as Google or Facebook. Data donation must therefore be a truly solidary practice.

How exactly is donated data protected?

Obviously, donated data needs to be secured and protected. Donating data does not mean waiving all forms of data autonomy or data protection. Requirements regarding data protection still need to be observed. People expect there to be protective measures in place, such as anonymization, encryption, secure servers and access restrictions. This should not be a problem: Data donation can be structured so that it does not conflict with data protection provisions in any way. In such circumstances, people are very willing to donate their data and even their biomaterials to research for a purpose that serves the common good.

Do you think it would be sensible to make such forms of data donation mandatory, perhaps in the form of legislation?

I am always a fan of voluntary solutions where possible. In my opinion, mandatory data donation would be an absolute last resort – and in any case, I do not think we need to make it obligatory. If you explain what exactly you need the data for, there will be significant willingness to donate – simply because we are so willing to act in solidarity with others.

We should focus much more on this solidary motivation. Unfortunately, it has not yet been incorporated in our data protection regulations. I would advocate creating more opportunities for people to donate more of their data.

■

Klaus Manhart



Prof. Alena Buyx

Alena Buyx is Professor of Ethics of Medicine and Health Technologies and Director of the Institute of History and Ethics of Medicine at TUM. Professor Buyx is a doctor and holds degrees in philosophy and sociology. Her research covers the full breadth of ethics and biomedicine and public health, with a particular focus on the ethics

of medical innovations and health technologies, research ethics, issues of solidarity and new participatory approaches in biomedicine. She is chair of the German Ethics Council and member of the WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing in 2019.

Circuit Breaker for the Immune System

If the immune system is unable to get to grips with an infection or a tumor, it switches into a lower functional state. Prof. Dietmar Zehn has identified the molecular circuit breaker that causes the immune system to switch between active and reduced functional states. The ability to systematically and more effectively reactivate these exhausted immune cells could pave the way for new treatment approaches for both chronic infections and tumors.

Kurzfassung · Langfassung: www.tum.de/faszination-forschung-25

Schutzschalter für das Immunsystem

D

Wenn das Immunsystem Infekte oder Tumore nicht in den Griff bekommt, schalten die zytotoxischen T-Zellen nach einer Weile in einen reduzierten Funktionszustand. Das beeinträchtigt zwar ihre Fähigkeit, ein Virus zu eliminieren, hat aber für den Körper auch Vorteile, denn eine dauerhaft starke Immunantwort belastet Zellen und Gewebe schwer. Bei Krebspatienten lässt das Herunterschalten der T-Zellen allerdings Tumore massiv weiterwachsen.

Prof. Dietmar Zehn identifizierte den molekularen Schalter, der den Wechsel zwischen aktivem und reduziertem Zustand des Immunsystems auslöst - das TOX-Protein. Es bindet an die DNA der T-Zelle und sorgt dafür, dass bestimmte Gene abgelesen werden, die notwendig sind, um den Erschöpfungszustand auszulösen, der Oberflächenrezeptor PD-1 etwa.

Die Forscher verwenden das Mausmodell und einen Transfer gentechnisch veränderter T-Zellen. Dabei konnten sie zeigen, dass T-Zellen ohne TOX-Gen ihre Aktivität nicht drosselten. Allerdings machten die Forscher eine andere wichtige Entdeckung: TOX schützt nicht nur das betroffene Gewebe, sondern auch die T-Zellen vor einem frühen Tod. Diese Erkenntnisse eröffnen neue, molekular zentrierte Ansätze um die Abschwächung von Immunantworten zu verhindern. Gleichzeitig liefern sie neue Einblicke, wie das Überleben von T-Zellen in chronischen Infektionen reguliert wird. Beides ist wegweisend hin zu effektiveren Immuntherapien gegen chronische Krankheiten und Krebs. □

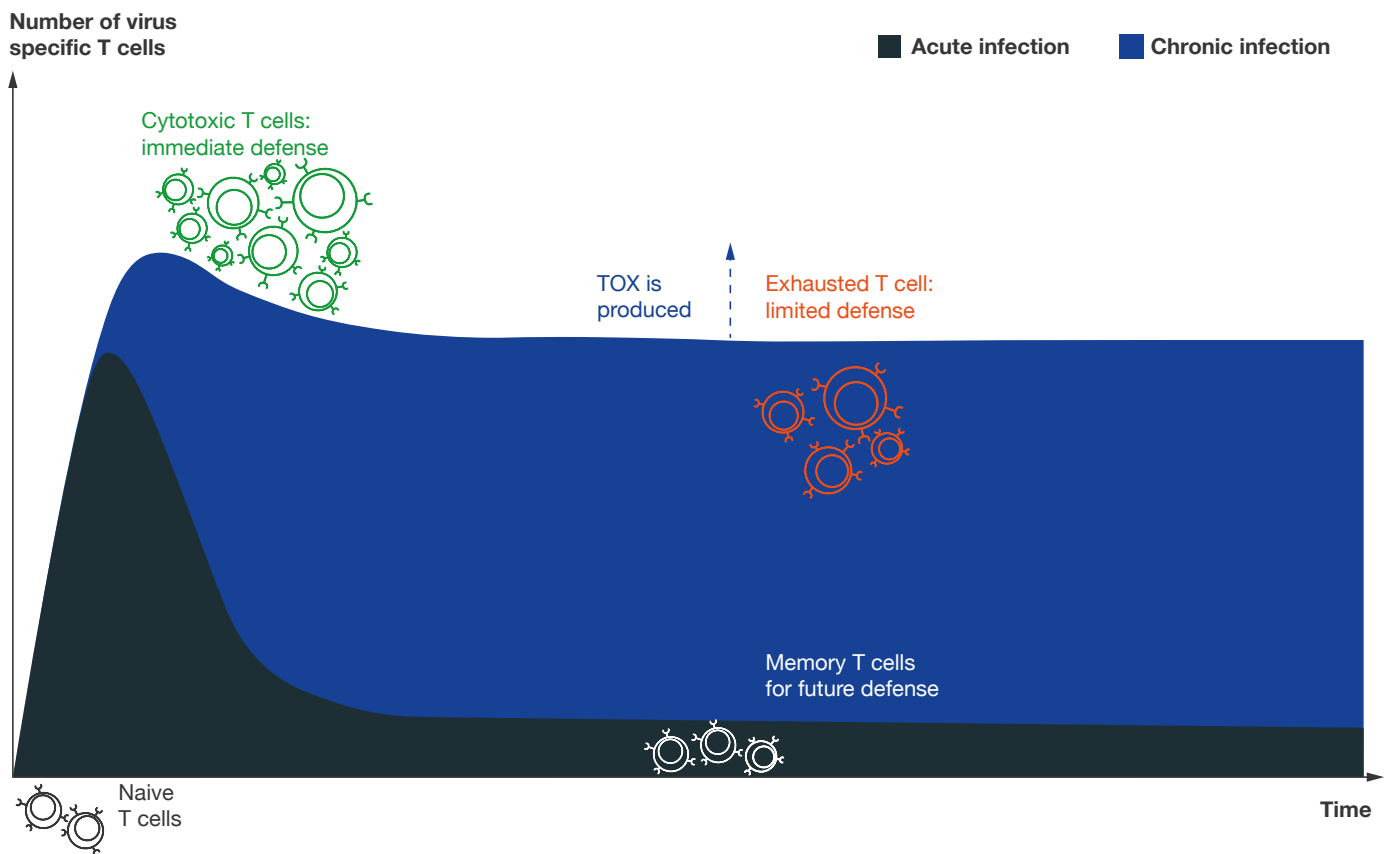


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Whether it involves a virus, bacteria or single-cell parasites known as protozoa, when the body becomes infected with a pathogen it puts the immune system on high alert. While the intruders infect the somatic cells, immune cells such as T cells and B cells activate, expand, and aggressively fight to eradicate the pathogen. Cytotoxic T cells – specialized cells in the immune system – play a particularly important role, as they are responsible for eliminating infected cells or tumor cells. They are able to do so because affected cells normally have different proteins on their surface that act as a red flag, identifying them to cytotoxic T cells. If T cells are unable to overcome the disease after several days or weeks, they often switch to a state in which their functions are significantly reduced – known as exhaustion.

Although the cells can keep the pathogen or the tumor in check to some degree in this state, they will not be able to defeat it completely. An acute infection, such as hepatitis C or HIV in humans, can then transition from an acute form into a chronic state. Nevertheless, the immune cells' state of exhaustion also has its benefits. For one, the relentless onslaught of an immune system on high alert causes significant damage to the affected tissue. By reducing T cell activity, the body finds a compromise between the disease and the collateral damage that a prolonged aggressive immune response would enact. This is not the case for cancer patients. For them, the state of exhaustion is unequivocally negative, as the T cells switching to reduced functioning allows tumors to grow a great deal faster.

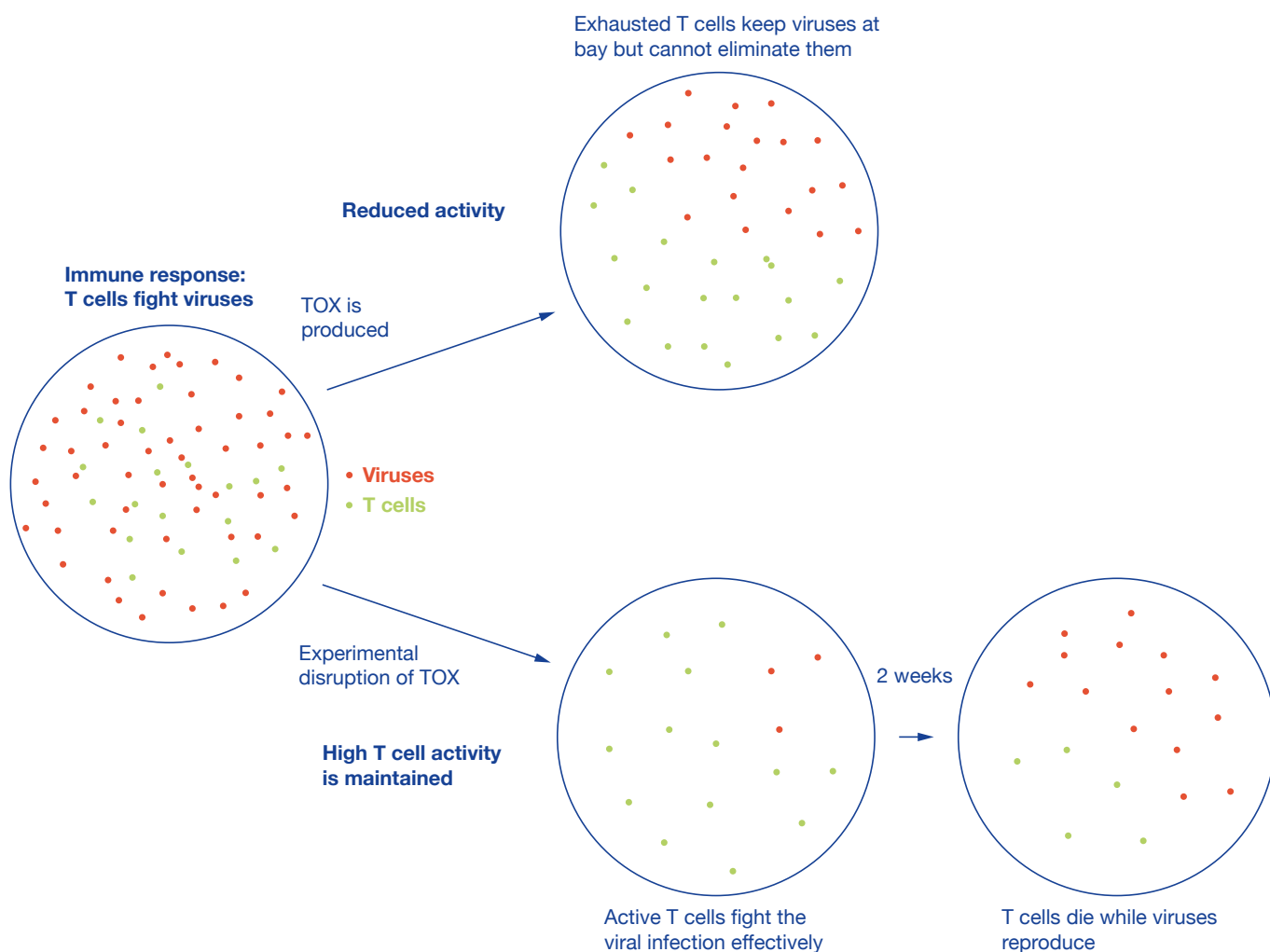


Progress of the immune response to viral infections. The immune system identifies cells infected by viruses as foreign structures. At the start of an infection, there are only few immune cells specific for cells afflicted by the virus. These virus specific, cytotoxic cells rapidly reproduce and destroy the cells which replicate the viruses. In the case of an acute infection (black area), this is successful. Subsequently, a few virus specific cells remain as memory T cells, forming a defense for future infections. For chronic infections (blue area), the cytotoxic T cells cannot completely eliminate the viruses. At some point they fall into an exhausted state. This state goes along with the production of the protein TOX. As a result, the body maintains a limited immune defense which holds the virus infection at bay but does not eliminate it.

Switching the cell's state on purpose

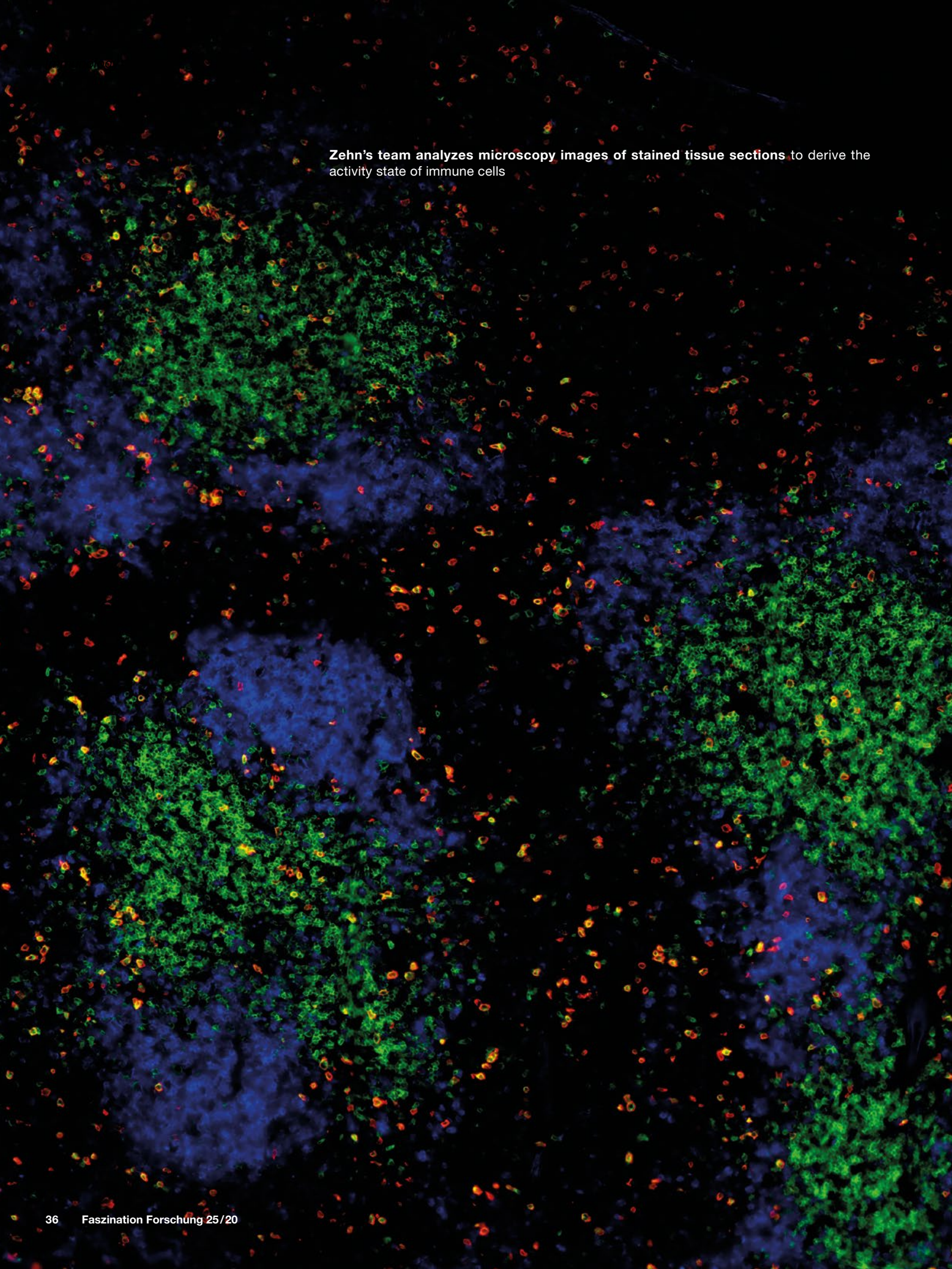
Prof. Dietmar Zehn's research team, supported by colleagues from the University of Freiburg, Germany, the USA and Israel, recently identified the molecular switch that causes cells to transition from active to exhausted state. The Professor of Physiology and Immunology at TUM's Weihenstephan School of Life Sciences published the results of this study in renowned academic journal "Nature" – at the same time that two other working groups from the USA independently arrived at the same result. The projects backed each other up on key points and showed that the protein TOX functions as a molecular regulator of exhaustion.

Researchers investigating tumors and infections had long been searching for a molecule that triggered cells to change between active and exhausted states. Following this breakthrough, researchers hope it might be possible to deliberately alter the functional state of these cells in the near future. A more effective ability to systematically reactivate exhausted immune cells would pave the way for better treatment approaches for both chronic infections and tumors. Before that can happen, however, researchers need to understand the underlying mechanisms in greater detail. ▶



Dietmar Zehn's team was able to show in experiments that T cells which can produce TOX reduce their activity in the course of the infection. The number of viruses drops but is not reduced to zero. A kind of balance develops. T cells without a TOX gene maintain their active state and are initially better able to fight viral infections. However, after some time, the number of T cells begins to fall and the virus increases again.

Zehn's team analyzes microscopy images of stained tissue sections to derive the activity state of immune cells



“The state of exhaustion actually appears to be useful because it protects the body in chronic infections from excessive and damaging immune responses.”

Dietmar Zehn

The protein TOX normally bonds in the nucleus with genetic material (DNA) and regulates the transcription of certain genes that are needed to trigger a state of exhaustion in T cells. When researchers inactivated TOX by removing its DNA binding domain, the infection no longer triggered a state of exhaustion. However, this only applied in an early stage of infection. “TOX is only needed to switch the T cells to a state of exhaustion but not to maintain this state,” says Zehn. Epigenetic changes in the T cells’ DNA appear to play a role in this. Bonds to chemical compounds permanently activate these genes, after which time the bond with the regulator itself is no longer needed. The state of exhaustion remains highly stable.

Precisely which gene TOX directly activates is not currently known. “However, we have identified a strong correlation with the PD-1 receptor,” says Zehn. Receptors are molecules on the cell surface, which allow other molecules to dock on and thus trigger signal processes in the cell. This receptor, named Programmed Death 1, was one of the discoveries for which the 2018 Nobel Prize in Medicine was awarded, after scientists in the USA and Japan showed that inhibiting receptors such as PD-1 enhances

the efficacy of tumor-fighting treatments. Zehn points out that this underlines the relevance of his field of research. The mechanism of action of TOX remains unexplained; however, interpretation of the results suggests that TOX activates a genetic program in the nucleus of T cells, which in turn causes PD-1 receptors to emerge on the cells’ surface. “That definitely isn’t the entire mechanism yet, but it is an important aspect,” says Zehn.

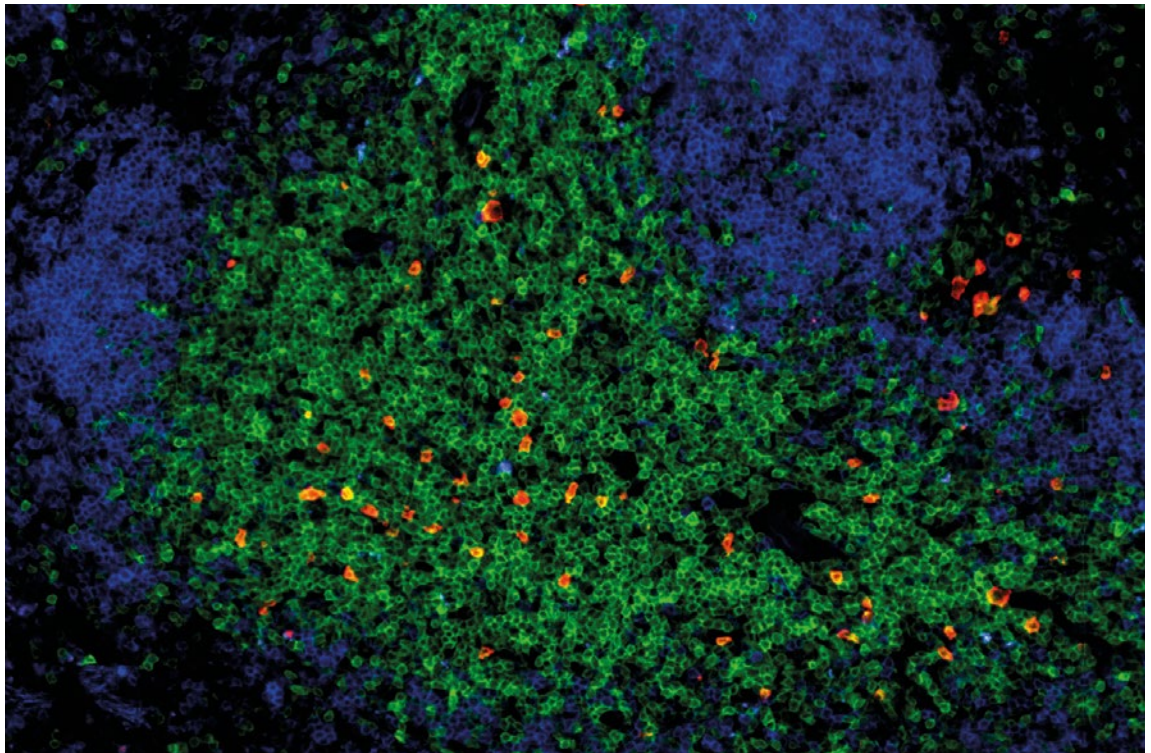
The protein TOX is the key

The researchers have been using laboratory mice infected with a virus and administering genetically modified T cells without a TOX gene. Using this method, they have been able to show that T cells without a TOX gene do not throttle back their activity. However, this change was associated with two major drawbacks. First, although mice with T cells without a TOX gene were initially better able to fight viral infections, their overall health was worse than that of their relations with unaltered T cells. This comes down to the fact that an unregulated immune system can actually cause greater damage than the infection itself.



“We will only be able to influence the immune system in a targeted manner in emergency situations once we understand its mechanisms and how it works.”

Dietmar Zehn



The position and accumulation density of cells helps researchers to learn about their state of activity. The picture shows a microscopy image of stained tissue sections of the spleen. Blue: B cell zone; green: T cell zone; red: pathogen specific model T cells that were activated during an infection.

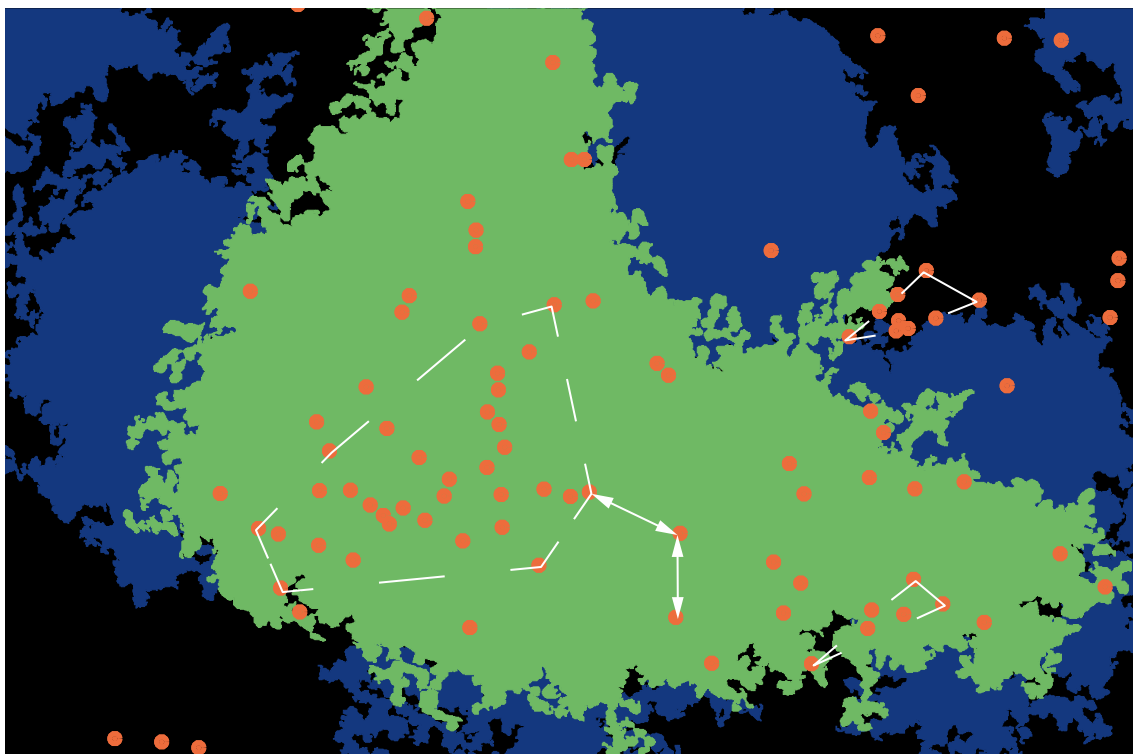
Without TOX, cells maintain their active state

Second, even without TOX, the T cells only remained active for two weeks. After that point, the virus continued to reproduce while the number of T cells without the TOX protein began to fall. “The cells probably die when they are strongly activated for long periods and cannot enter a state of exhaustion,” continues Zehn. TOX therefore not only protects the affected tissue from excess damage, but also saves the T cells from an early death.

Activated T cells are short-lived. “However, in the case of chronic diseases and tumors, a small subfraction emerges that constitutes extremely long-living T cells and a sort of stem cell population,” says Zehn. These stem cells maintain the immune response and continue to produce new virus specific cells. The more of these stem cells are present, the more likely it is that a patient will respond well to different tumor therapies. “We are currently work-

ing with colleagues on a way to apply this to develop a treatment for tumor patients,” says Zehn.

His next objective is to find molecules that make it possible to manipulate cells’ functional state. Switching in both directions could play an important role in developing new therapeutic approaches. Patients with chronic infections such as hepatitis C, HIV or tumors would benefit from an increase in cell activity. However, it is possible that reducing T cell activity could benefit patients with other illnesses, such as autoimmune disorders and even some infections. “For instance, in the context of COVID-19, caused by the coronavirus, it is conceivable that patients with severe symptoms might have immune systems that are overshooting the mark, with TOX playing a role in this,” says Zehn. The question of why most people handle the virus well while others struggle is one that interests Zehn.



The microscopy image is computerized into a vector graphic. The dotted lines indicate local accumulations, while arrows denote distance measurements. The researchers use these to calculate accumulations in specific areas and measure cell-cell distances.



Is there some kind of dysregulation – and, if so, why? Have patients who present with more serious symptoms perhaps previously experienced an infection with a similar or different pathogen? The immune system protects us against re-infections with the same pathogen. In some cases, however, a second infection with a similar but not identical virus takes a more severe course than the initial infection. It is still not clear whether this is the case for COVID-19. “What is certain is that we will only be able to influence the immune system in a targeted manner in emergency situations once we understand its mechanisms and how it works,” says Zehn.

■ *Karoline Stürmer*

Prof. Dietmar Zehn

After studying medicine at Charité in Berlin, Dietmar Zehn spent five years conducting postdoctoral research at a renowned immunological laboratory in the USA at the University of Washington. He devoted his time there to basic research into infectious and autoimmune diseases. Subsequently, Zehn managed a laboratory in Switzerland for six years, during which time he was awarded a sponsored professorship by the Swiss National Science Foundation (SNF). In 2015, he accepted a position at TUM and assumed the Chair of Physiology and Immunology at the Weihenstephan School of Life Sciences. His field of expertise comprises molecular and cellular mechanisms in T-cell-induced immune responses to acute and chronic infections and tumors, as well as immune tolerance and autoimmune diseases. Dietmar Zehn has received numerous awards and fellowships in the course of his career. In 2015, he received the European ACTERIA Prize for Immunology. In 2018, he successfully applied for an ERC Consolidator Grant, which directly followed the ERC Starting Grant he had obtained in 2013. Both grants run for five-year terms and are aimed at elite researchers distinguished by their excellent research. In 2020, he acquired a new Center for Integrated Infection Prevention for TUM, co-financed by the federal state and the federal government, in a competitive procedure.

EUR 40 Million for New TUM Institute

TUM is going to bundle its competencies for the development of innovative strategies for preventing, combatting and avoiding the spread of resistant pathogens in humans and animals.

The marked increase in resistant bacteria and the associated massive rise in the danger of infections in both humans and animals which cannot be treated with antibiotics is, in the long term, one of the biggest scientific, medical and social challenges of our time.

“Without innovations, we are at risk of regressing to the pre-antibiotic era in which simple injuries could develop into deadly threats,” state TUM professors Dietmar Zehn, Percy Knolle and Bernhard Küster. They represent the team of researchers who supported the application to found the institute. “The number of deaths caused by infections, which is just under one million per year, could rise to about ten million by 2050.”

TUM therefore combines its competencies for the development of innovative strategies for preventing, combatting and avoiding the spread of resistant pathogens within a new research facility, the Center for Integrated Infection Prevention (ZIP).

The federal government and the Free State of Bavaria will support the new construction project at the Weihenstephan campus equally to the tune of roughly EUR 40 million in total.

Fast transfer of research into practice

The new center’s research work is divided into three program pillars: modulation and dynamics of the microbiome, strengthening of the local immunity on microbially populated boundary surfaces, and innovative technologies.

With cross-species observation of resistant bacteria in humans and farm animals, the ZIP lays emphasis on the intersection of medicine, life sciences, microbiology, bioanalytics and information sciences, an emphasis that is unique in the world.

One of the most important goals pursued by the institute is to put new prevention strategies into practice as fast as possible. The idea is to largely avoid the use of antibiotics in livestock farming, to better control existing infections and to suppress transmission paths between animals and humans.

Positioned in a unique research environment

Bernd Sibler, Minister of Science, said about the funding of the ZIP: “Through their multidisciplinary research, TUM offers a nationally and internationally outstanding and stimulating research environment for the pressing future topic of bacterial resistance. It is therefore the right place for the Center for Integrated Infection Prevention, a state-of-the-art research facility in which their research competencies are combined to facilitate interdisciplinary approaches at the intersection of health research and big data, from the agricultural and nutritional sciences to biomedicine to computer sciences, and strengthened at an international level.”

“Infection prevention as a central objective of the ZIP is more relevant than ever and requires completely new research approaches,” says TUM president Prof. Thomas F. Hofmann. “In order to activate the enormous potential of the ZIP, we have already created the critical, interdisciplinary environment through strategic appointments for top-level positions, pooling of financial resources and successful efforts in the promotion of young researchers, equal opportunities, diversity and technology transfer.”



Andreas Battenberg (TUM)

“Infection prevention as a central objective of the ZIP is more relevant than ever and requires completely new research approaches.”

Prof. Thomas F. Hofmann

Gesundheit für alle

D

Wurminfektionen sind in Ländern mit niedrigem bis mittlerem Einkommen weit verbreitet. Prof. Clarissa Prazeres da Costa erforscht, wie diese vernachlässigten Tropenkrankheiten das Immunsystem beeinflussen. □

Link

www.mikrobio.med.tum.de/node/52

Worm infections are widespread in low to middle income countries. Prof. Clarissa Prazeres da Costa conducts research into how these neglected tropical diseases affect the immune system. As Co-Director of the Center for Global Health at the TUM School of Medicine, she furthermore promotes interdisciplinary and international research collaborations.

Healthy Lives for All



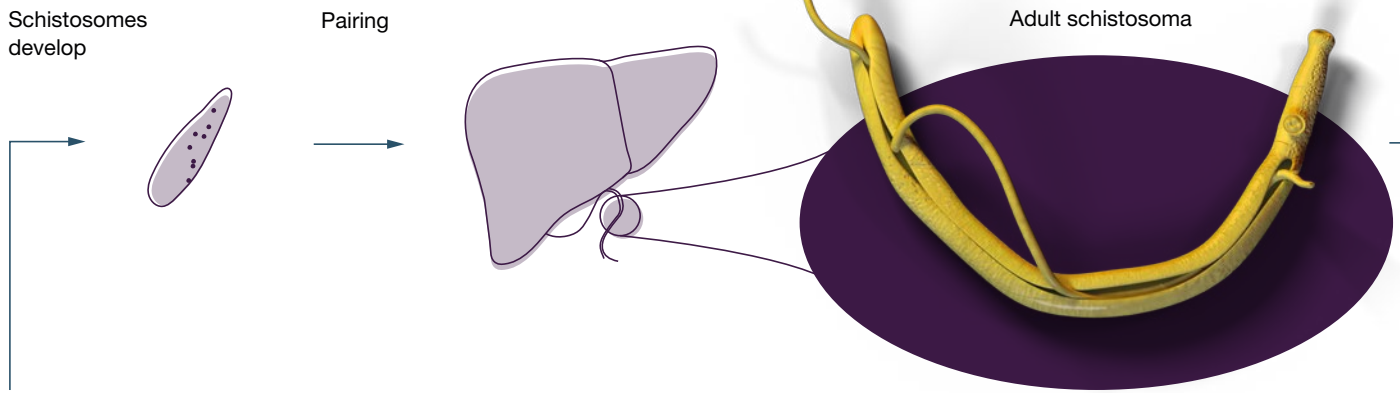
1.5 billion

people around the world are infected with parasitic worms



Approximately 1.5 billion people around the world are infected with parasitic worms. These trematodes, cestodes and nematodes are known in technical language as helminths. They can enter the human body in food or drinking water contaminated with urine and feces, through mosquito bites or by burrowing directly through the skin. They have complex life cycles and circulate between the environment and their hosts – humans. Until about a 100 years ago, these worms were as widespread in Germany and other economically developed countries as is now only the case in countries without adequate public sewage systems and sanitation facilities. Today, however, such infections are almost unheard of in Germany. Consequently, they are now classified as a group of so-called neglected tropical diseases (NTDs) strongly associated with poverty and are the subject of comparatively little research.

These parasitic worms have developed or co-evolved with the human immune system for a long time. They occupy almost every organ in the human body, with each parasite gradually carving out its own niche, from the skin and the liver to the brain. Symptoms of infection can range from mild digestive problems or anemia to severe growth and development disorders in children. Nevertheless, as Clarissa Prazeres da Costa explains, “it is relatively rare for people to die from these infections, though they often unknowingly carry the infection their entire lives.” Prof. da Costa is Head of the “Infection and Immunity in Global Health” Working Group at the Institute for Medical Microbiology, Immunology and Hygiene (MIH) at TUM. In order to survive in their host for as long as possible, worms need to outsmart the human immune system – and precisely how they achieve this is a fascinating process that da Costa wants to understand better. ▶

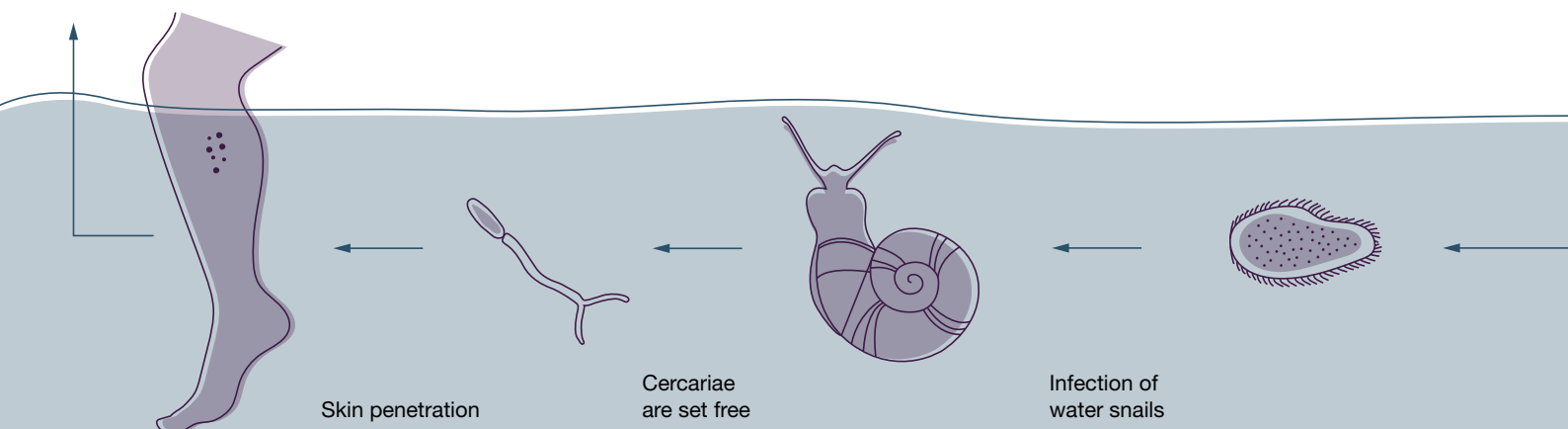


She and her team have discovered, for instance, that helminths release molecules that actively inhibit the body's immune response. In addition, the worms induce increased release of suppressor cells, which further suppresses active responses from the immune system. This "immunomodulation" also influences how people infected with helminths react to other conditions such as allergies, other infections like hepatitis B and C, and even vaccinations, namely by weakening the body's response in many cases. "As immunologists, we can learn a lot from our 'old friends' by better understanding how they manage to trick our immune system," says da Costa.

Interestingly, experiments in animals have shown that parasitic infections during pregnancy can have an impact on the development of the immune system even in the next generation. Studies are currently underway in the sub-Saharan country of Gabon to examine whether this also occurs in humans and the extent to which parasitic infections affect women's health in general. In fact, Prof. da Costa is driving this research forward at the TUM Center for Global Health (CGH), which she co-founded and leads with her fellow Co-Director, Andrea Winkler

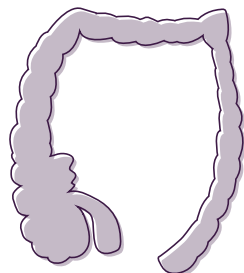
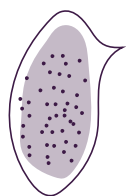
(Department of Neurology). "We feel obligated to pursue the UN Sustainable Development Goals, particularly the third goal of 'ensuring healthy lives for all'." Achieving this will require – among other measures – the elimination of neglected tropical diseases. This calls for multidisciplinary research approaches to facilitate sustainable innovation, something the CGH at TUM is seeking to promote. "We want to use the CGH to promote an exchange that spans disciplinary boundaries, especially during the COVID-19 crisis, that is impacting scientific research and scientists alike," says da Costa.

Pandemics like the novel coronavirus present particular challenges for countries in the Global South. In total, there are only around 2,000 ventilators across 42 African countries. By way of comparison, Germany alone has a regular working stock of 23,000 and is capable of ramping this up to 30,000. African countries also lack sufficient personal protective equipment for medical staff. Da Costa hopes to counter this lack of resources with innovation. Together with Fabian Jodeit and Petra Mela from the Chair of Medical Materials and Implants and Andreas Pichlmair from the Institute of Virology, as well as a



Egg production

Eggs pass through bowels and bladder



Prof. Clarissa Prazeres da Costa

Physician and infectious disease consultant Clarissa Prazeres da Costa specializes in tropical medicine, parasitology and immunology. She is the Head of the Department of Diagnostic Parasitology and lecturer at the Institute for Medical Microbiology, Immunology and Hygiene. Da Costa heads the “Infection and Immunity in Global Health” research group since 2005 and is Co-Director of the Center for Global Health (CGH), which she co-founded at the TUM School of Medicine in 2016.

collaboration with a firm called Plasmatreat, da Costa is seeking to explore potential methods of sterilizing disposable items with plasma-activated water vapor to thereby inactivate the SARS-CoV-2. This would enable the reuse of ventilators, protective masks and protective suits. “This method is cost-effective and mobile, providing the ideal preconditions for deployment in regions where infrastructure is lacking,” says da Costa, outlining the benefits.

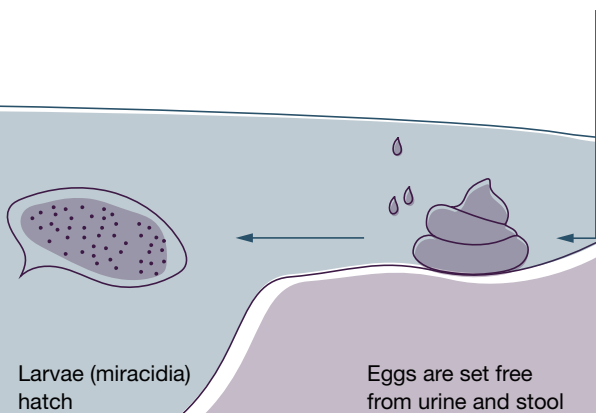
In collaboration with Eugénia da Conceição-Heldt and Janina Steinert from the TUM School of Governance and Martin Schlegel from the Clinic for Anesthesiology, she is also working on a project to investigate the role of the WHO as a crisis manager during the COVID-19 pandemic. An example of this is the WHO’s international SOLIDARITY Trial, a drug trial in which the Klinikum rechts der Isar is also registered.

In her work, da Costa feels bound by the motto of “ensuring healthy lives for all” – both in her basic research into helminths and her commitment to promoting interdisciplinary research at the CGH.

Claudia Doyle



Graphics: edlundsepp (source: hegasy.de, turbosquid); Picture credit: Stefan Rumpf



A complex life cycle: Parasitic worms like schistosomes enter the human body as larvae called cercariae, which are released by the intermediate host, the fresh water snails. They penetrate the intact skin and undergo several development stages in the body. Eventually, the female worms release eggs, which enter the bowel or bladder. This inflammatory process can cause typical disease symptoms. Miracidia larvae hatch from the excreted eggs. They infect the intermediate host and multiply again. The cycle is thus complete.

Global Health Needs a Multidisciplinary Approach

Since the onset of the COVID-19 pandemic, the topic of global health has once again come into sharp focus. “Ensuring healthy lives for all”, one of the United Nations’ Sustainable Development Goals, is also a key focus for Prof. Clarissa Prazeres da Costa, a specialist in the fields of medical microbiology and infectious disease epidemiology. Together with Prof. Andrea Winkler she founded the Center for Global Health at the TUM School of Medicine.

Prof. da Costa, what exactly is “Global Health”?

It is both a concept and a global commitment on the part of UN Member States to ensure healthy lives and promote wellbeing for all. However, the topic of global health is not limited to the field of conventional biomedicine. Instead, the focus is on the prospects for healthcare systems. It is therefore vital that we adopt a multidisciplinary approach to the issue.

As someone who conducts basic research, how did you become involved in the topic?

My father is of Indian descent; he worked for an American company and we traveled a lot. I have worked as a doctor in Nepal and the Philippines, where I saw the extent to which people suffer from avoidable acute and chronic infectious diseases. After completing my medical training, I immersed myself in the field of infectious diseases. Since then, I have expanded my focus, which now ranges from the lab to hospital beds. I just want to provide tangible benefits through my research and raise students’ awareness of global health through my teaching.

What diseases do poorer countries typically face?

Unlike in high-income countries, infectious diseases are among the most common causes of death in low-income countries. Even putting their generally weak healthcare systems to one side, these countries also lag behind in the development of new medications. To give you an example, 230 million people currently suffer from schistosomiasis, a worm infection that can result in chronic inflammation of the bladder and liver if left untreated. For context, the same number of people have malaria. Only one medication is currently capable of fighting the infection – but it is often not affordable and unavailable. This is why it is donated by pharma companies and administered in mass-medication

1 NO POVERTY



2 ZERO HUNGER



5 GENDER EQUALITY



6 CLEAN WATER AND SANITATION



9 INDUSTRY, INNOVATION AND INFRASTRUCTURE



10 REDUCED INEQUALITIES



13 CLIMATE ACTION



14 LIFE BELOW WATER



Weltgesundheit braucht einen multidisziplinären Ansatz

D

„Gesundheit für alle“ (Health for all) gehört zu den Nachhaltigkeitszielen der Vereinten Nationen (UN) und beschäftigt auch Prof. Clarissa Prazeres da Costa. Die Fachärztin für Medizinische Mikrobiologie und Infektions-epidemiologie zielt auf „One Health“ ab. Damit meint sie einen Forschungsansatz, der die Gesundheit von Mensch, Tier und Umwelt umfasst. □



programs supported by the World Health Organization, primarily to school children. This approach, however, raises the risk that the parasites will develop resistance to the medication. Although drug development is urgently needed, this work is rarely supported because the enormous costs involved make it less lucrative for pharma companies. At the same time, however, we also urgently need innovative diagnostic techniques that are robust and inexpensive in order to measure the success of such large therapy programs.

How can research become established in countries where there is little structure?

By initiating research networks. We are part of specific programs set up by the German Research Foundation and the Federal Ministry of Education and Research (BMBF) aiming to do exactly that. Another example would be the German Center for Infection Research (DZIF) – a research association bringing together more than 500 doctors and scientists working on new methods to prevent, diagnose and treat infectious diseases. It also has partnerships with four institutions in sub-Saharan Africa.

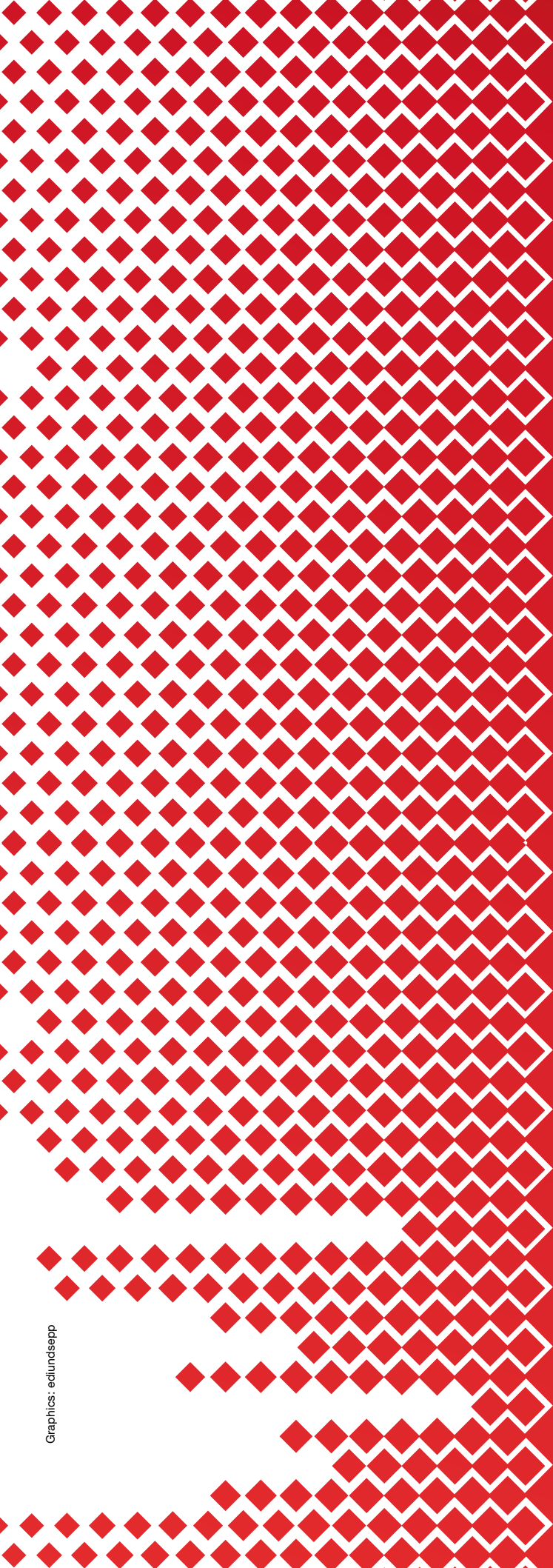
Could you briefly explain the objectives of the projects you are involved in?

In Cystinet Africa, a sub-Saharan research network supported by the BMBF, my colleague Andrea Winkler and I are working on an interdisciplinary approach to containing a parasitic pork tapeworm infection. This involves taking a “one health” approach to humans, animals and the environment. In another DFG-supported project in the Central African nation of Gabon, our working group is researching the immunological effects of worm infections, more specifically schistosomiasis, during pregnancy. In previous investigations, we have been able to show that an infection during pregnancy can protect the progeny against allergies. It is important to understand the mechanisms involved in order to understand their effects; initial indications are that maternal infection could even have an impact on the efficacy of vaccinations in children. German and African doctoral students are working hand in hand on this project. At present, we are establishing a network on the topic of global women’s health together with academics at TUM and internationally at the Center for Global Health. The research field of immunity and pregnancy will become embedded in this network.

Interview by Eve Tsakiridou

Fighting Cancer with Viruses

By nature, viruses exist at the cost of other living organisms. They often target specific organs. The novel coronavirus, for instance, targets the lungs, rabies targets the brain and HIV targets the immune system. At the Klinikum rechts der Isar, researchers hope to harness the lethal power of viruses to combat malignant tumors. Dr. Jennifer E. Altomonte and her team are engineering viruses to optimize their therapeutic potential in cancer cells. In so doing, immune cells are also called onto the scene, which then contribute to controlling the cancer.



Graphics: edlundsepp

Link

www.med2.mri.tum.de/en/research/ag-altomonte.php

Viren gegen Krebs

D

Viren als Waffe gegen Krebs? Die Idee klingt bestechend – und ihre Realisierung rückt näher. Einen entscheidenden Beitrag leistet Privatdozentin Dr. Jennifer E. Altomonte am Klinikum rechts der Isar der TUM. Ihr Team hat zwei onkolytische Viren (onko = Geschwulst, lyse = Zerfall) gentechnisch verändert und zu einem hybriden Virus kombiniert, der sich in Tumorzellen – und nur dort! – rasant vermehrt und sie dabei zerstört. Die sterbenden Zellen entlassen nicht nur tausende Kopien der Viren, die weiteres Krebsgewebe zerstören. Überdies rufen sie körpereigene Immunzellen auf den Plan, die nun ebenfalls gegen die Tumorzellen vorgehen. Die hybriden Viren haben sich bereits in Zellkulturen und Tierversuchen bewährt und erzeugen keine toxischen Nebenwirkungen. Damit sind die wichtigsten Hürden genommen, um die nächsten Schritte zu gehen: Geplant ist eine noch stärkere Aktivierung der Immunantwort, die kommerzielle Produktion der hybriden Viren, und schließlich die Erprobung der neuen Virustherapie am Menschen. □

PD Dr. Jennifer E. Altomonte

Born in 1976, private lecturer Dr. Jennifer E. Altomonte studied microbiology, biochemistry and molecular biology at Pennsylvania State University. In 1999, she moved back to New York, her birthplace. After returning, she first worked at the Institute for Gene and Cell Medicine at the Mount Sinai School of Medicine, researching the development of viral vectors in gene therapy for metabolic disorders. Four years later, she moved to another laboratory within the same institution, where she began her research on oncolytic viruses. In parallel to this, she conducted research at John Jay College of Criminal Justice into molecular methods to clarify the causes of Sudden Infant Death Syndrome and received her Master of Science in Forensic Science in 2016. In 2006, she moved on to work at Dr. Oliver Ebert's laboratory at the Klinikum rechts der Isar and later obtained her doctorate at TUM before assuming management of the laboratory in 2016.



Picture credit: Juli Eberle

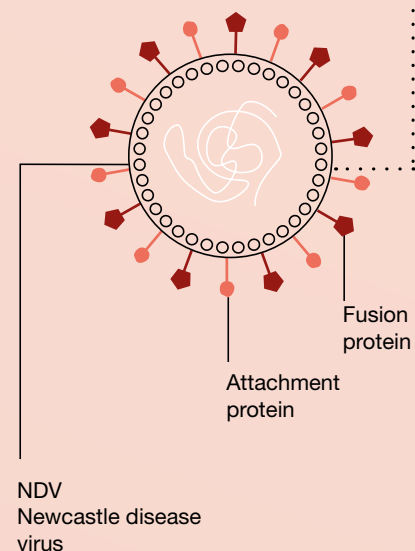
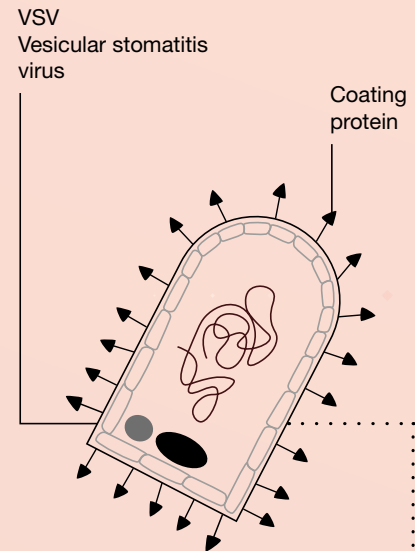


Using viruses as a weapon against cancer? A fascinating idea – and by no means a new one. Back in 1904, American Professor of Medicine George Dock described the case of a leukemia patient whose symptoms diminished following a bout of cowpox. Soon after, reports circulated of a young woman with cervical cancer whose condition had temporarily improved following contact with the rabies virus. “Even back then, some had speculated on the therapeutic potential of viruses. Yet it is only in the last 30 years or so that we have had the knowledge and scientific tools needed to develop viable therapies,” says private lecturer Dr. Jennifer Altomonte, who researches virus-based cancer therapies at the Klinikum rechts der Isar.

Research groups around the world are working to improve virus therapies

Since then, biologists have identified more than two dozen viruses that can multiply in tumor cells and kill them. These oncolytic viruses (onco = tumor, lysis = degradation) include well-known infectious agents like the pathogens that cause polio, herpes and chickenpox, as well as species that would not normally affect humans. In China, a modified adenovirus has been approved as a treatment for carcinomas in the head and neck region since 2003. In Europe and the USA, a modified herpes virus has been marketed as a therapy for specific forms of skin cancer since 2015. Numerous research groups around the world are working to improve the efficacy and safety of virus therapies.

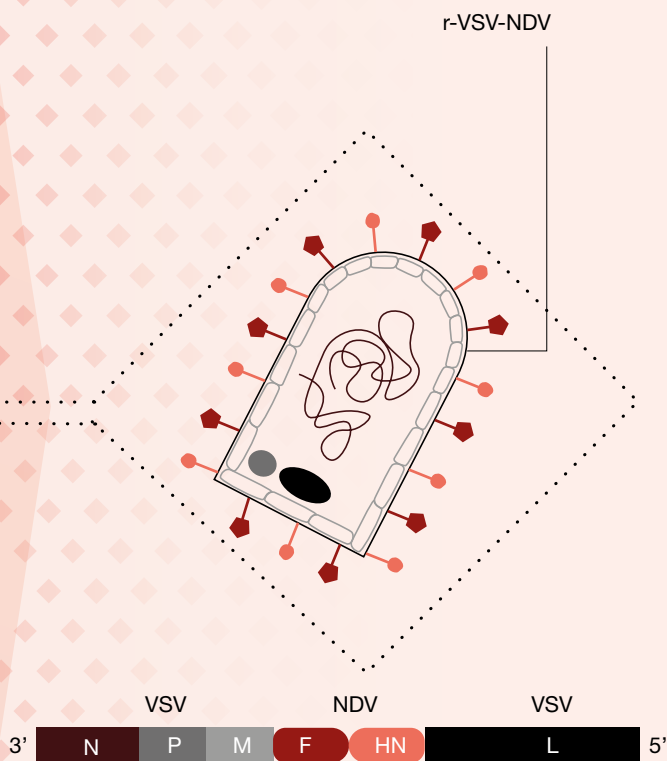
In the 16 years she has devoted to this novel biotherapy to date, Jennifer Altomonte has made decisive advances. Born in the USA, Altomonte focuses her research on the vesicular stomatitis virus (VSV) and the Newcastle disease virus (NDV), both of which are known for their oncolytic effects. VSV usually afflicts hoofed animals such as goats and cattle, while NDV infects birds. These pathogens do not cause illnesses in humans, which presents enormous benefits for their medical application. “Under normal circumstances, the human immune system does not come into contact with VSV or NDV,” explains Altomonte, who holds a doctorate in microbiology. “This means it has no experience handling these viruses. It therefore takes a while for our immune system to detect them in the body and eradicate them. During this time, the viruses can make their way to the tumor cells and destroy them.”



These viruses do not represent a danger to any other organs. Although they also penetrate healthy cells in the human body, these cells are able to identify them as hostile and eliminate them within hours. This defense mechanism is lost during the transformation of healthy cells into tumor cells, which explains why the viruses have an easy job killing these malignant cells – and only these malignant cells.

A virus that kills tumor cells but does not harm humans nor the environment

In principle, this applies to all types of tumor, though Altomonte and her team are currently concentrating their efforts on liver cancer. VSV can amplify itself extremely rapidly in host cells and subsequently spread to surrounding tumor cells to kill them, too. NDV has special attachment and fusion proteins that cause infected tumor cells to fuse with neighboring cells. When employed as oncolytic viruses, the two pathogens work in different ways, but both are effective in the destruction of tumor tissue. Nevertheless, these beneficial qualities are associated with the risk of serious side effects. “We know that VSV can lead to toxic side effects in the brains and livers of mice and rats – and potentially also in humans – when administered at elevated doses,” says Altomonte. “While NDV is not dangerous to humans, it is deadly to birds and can pose a serious threat to the environment and the poultry industry. We have engineered a hybrid of the two viruses, in which the positive qualities of both species were retained, while the unacceptable safety risks were eliminated.” ▶



Best of both: Researchers have combined the features of two oncological viruses to create a hybrid virus. The basic structure of this chimeric virus is taken from vesicular stomatitis virus (VSV). The coating protein of VSV (arrows) contributes to its safety risks; it has been replaced with two beneficial proteins from Newcastle disease virus (NDV). The HN protein allows the virus to attach to cancer cells, while the F protein causes infected tumor cells to fuse with neighboring cells. The newly created construct, rVSV-NDV, possesses enhanced oncolytic characteristics compared to the parental viruses, while eliminating the risks of undesirable side effects for humans and the environment.

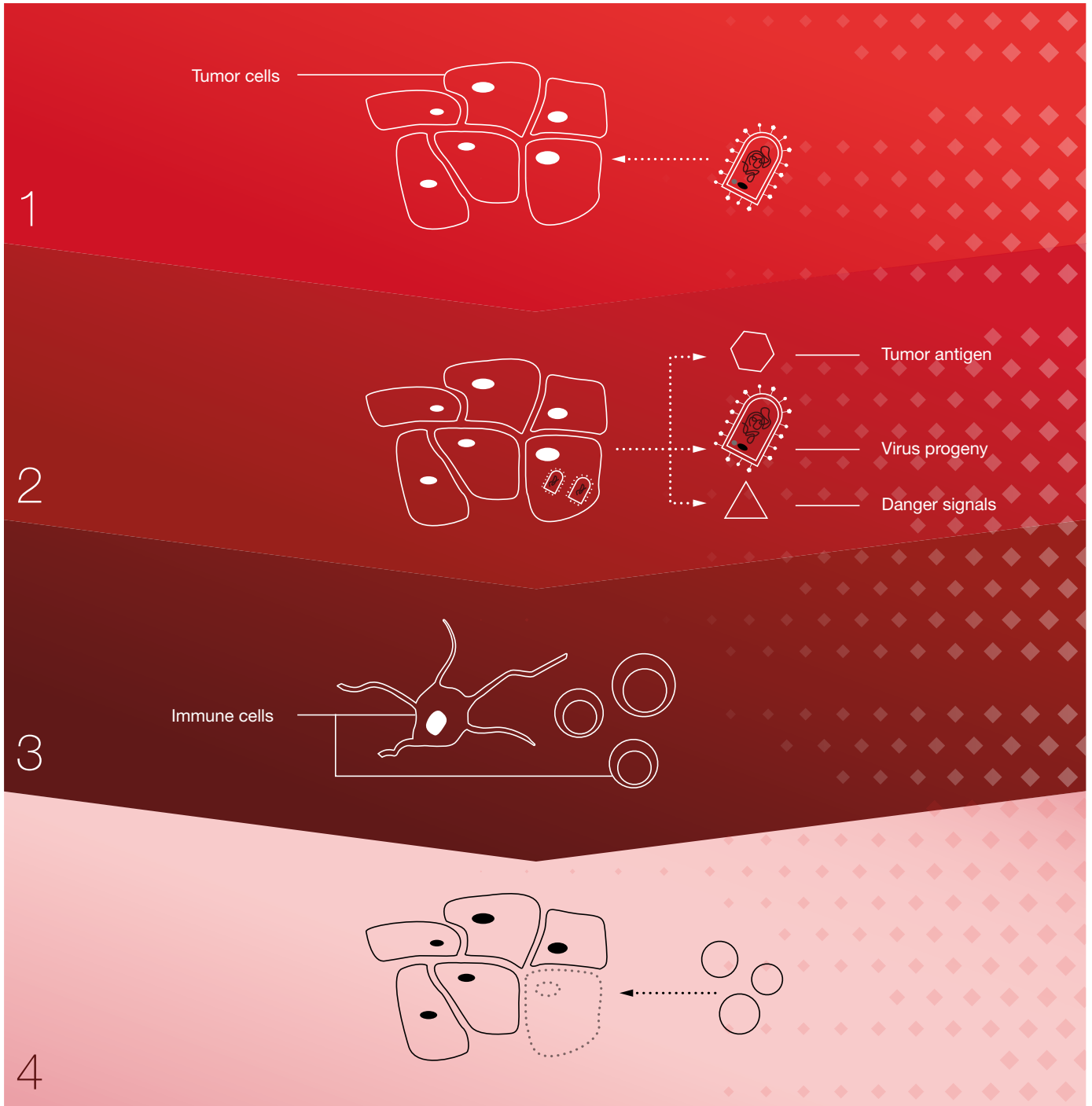


The result is a viral construct called rVSV-NDV (see Fig. 1). This hybrid virus has already demonstrated its proof of concept in cell culture systems and animal models for tumor diseases. The TUM researchers have achieved impressive results in preclinical animal models of liver cancer: In mice treated with the novel virus, the malignant tumors showed a reduction in size, which led, on average, to a doubling of survival time compared to the placebo-treated control animals. Altomonte has now been able to demonstrate an exciting additional aspect of the therapy for the first time in mice with skin cancer. “In most of the animals, even tumors that had not been directly infected with viruses underwent a reduction in size or delayed tumor growth,” she explains. “We can also rule out the possibility that the viruses reached the distant tumors through the bloodstream. The tumor remission must therefore be the result of the body’s own immune system fighting against the cancer.” ▶

“It is only in the last 30 years or so that we have had the knowledge and scientific tools needed to develop viable therapies.”

Jennifer Altomonte

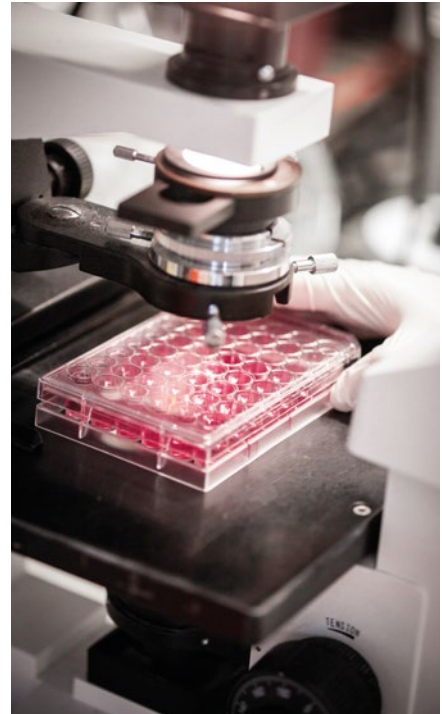
◀ **Jennifer Altomonte and her doctoral student Teresa Krabbe** hope to start a biotech company and eventually develop and market the novel hybrid oncolytic viruses. In 2019, they received the m4 Award from the Bavarian Ministry of Economic Affairs and the EXIST Transfer of Research from the Federal Ministry for Economic Affairs and Energy.



Fusing together with the tumor: Oncolytic viruses specifically infect tumor cells (1) and multiply within them. The dying cancer cells (2) not only release thousands of copies of the virus – which infect further cancer cells – but also tumor antigens and chemical signals, which bring the body’s immune system to the scene. This process activates specific immune cells (3) that also identify uninfected cancer cells and destroy them with the help of cytotoxins (4). This immune response targets both the primary tumor and its metastases – and remains even long after the viral infection has receded.

“We hope to gain approval for our first clinical study in the next two years.”

Jennifer Altomonte



A researcher prepares a dilution of oncolytic virus in saline solution for infection of tumor cells grown in multi-well dishes. The cells are examined under the microscope to assess for changes in cell morphology in response to virus infection.

A biotech company is planned

Part of this therapy concept involves mobilizing specific immune cells. This makes use of a phenomenon that goes hand in hand with viral infection, namely that when pathogens multiply in tumor tissue and destroy it, the dying cells send out warning signals. At the same time, the virus infection modulates certain mechanisms that cancer cells normally use to evade detection and clearance by the immune system. These two effects essentially remove the tumor's cloak of invisibility and make it detectable by the body's immune defenses. Specific immune cells are promptly activated and attack tumor cells – even those that are distant from the viral infection. This side effect of viral infection, which is exceptionally welcome from a therapeutic perspective, has emphatically manifested itself in Altomonte's studies. “We have some long-term survivors who no longer display any sign of tumor cells,” says Altomonte. “Furthermore, when we re-inject cancer cells into these animals, no new tumors develop. The immune-mediated protection therefore remains even long after the oncolytic virus has been cleared from the body,” she emphasizes.

Altomonte, who now lives in Munich, is also pleased with the results of the studies from a safety perspective. Even very high doses of the engineered virus have not caused any detectable toxic effects in healthy mice. This removes the largest hurdles to the clinical translation of the therapeutic approach. Ongoing and planned projects include the development of new strategies to further activate the immune response, as well as the large-scale commercial production of the hybrid viruses, and testing the new virus therapy on human subjects. These projects all entail significant costs. In light of their highly promising nature and intelligent planning, however, around €3 million of funding has been obtained from various sources. In the fall of 2019, Jennifer Altomonte and her doctoral student Teresa Krabbe received the m4 Award from the Bavarian Ministry of Economic Affairs and the EXIST Transfer of Research from the Federal Ministry for Economic Affairs and Energy. It is hoped that the two prizes, endowed with a combined €1.5 million budget, will enable the founding of a planned biotech company called FUSIX, which aims to develop and eventually market the novel hybrid oncolytic

viruses. “We hope to reach the next major milestone – gaining approval for our first clinical study – in the next two years,” says Altomonte.

Particularly high expectations rest on a project by the name of ONCO-VAX, which the European Research Council is supporting in the form of a Starting Grant from 2020 for a five-year term. The aim is to develop a vaccine that amplifies the immune response triggered by a viral infection in order to secure long-term protection against tumor cells. This gives rise to the following conceivable scenario: First, researchers would take a biopsy from a patient to obtain cancer cells and cultivate in a petri dish. They would then infect these tumor cells with rVSV-NDV – which would kill the cells and reveal the cells’ tumor antigens.

Specific immune cells from the blood of the same patient would be exposed to these antigens, thereby activating them and enabling them to precisely identify the specific antigens of these tumor cells. Immune cells activated in this way would then be injected into the patient as a vaccine, offering an efficient and long-lasting immune protection against the tumor and its metastases.

A bold vision. But will it come to fruition – and, if so, when? Jennifer Altomonte remains realistic. “Even if we are only able to help a few patients with this therapy and manage to slow the progress of their cancer, this would extend their life,” she says. “What’s more, it would do so without the distressing side effects of therapies available today.”

■

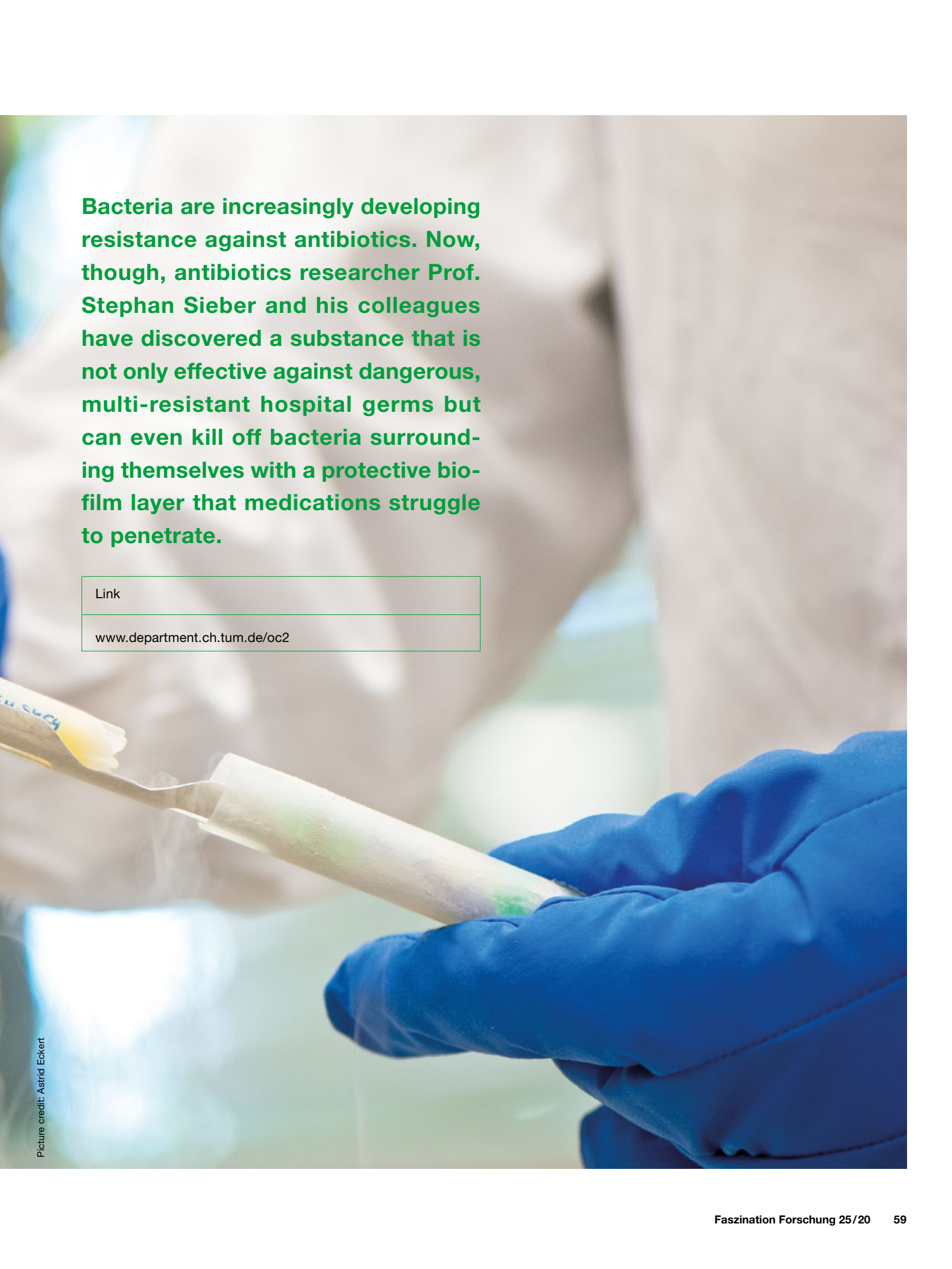
Monika Offenberger



Picture credit: Juli Eberle



Fighting Superbugs with Superdrugs



Bacteria are increasingly developing resistance against antibiotics. Now, though, antibiotics researcher Prof. Stephan Sieber and his colleagues have discovered a substance that is not only effective against dangerous, multi-resistant hospital germs but can even kill off bacteria surrounding themselves with a protective bio-film layer that medications struggle to penetrate.

Link

www.department.ch.tum.de/oc2

Kurzfassung · Langfassung: www.tum.de/faszination-forschung-25

Krankenhauskeime im Klammergriff



Bakterielle Infektionskrankheiten können heute gut behandelt werden, denn seit rund 100 Jahren gibt es Antibiotika. Sie sind gut verträglich, günstig und effektiv. Viele Jahre lang wurden sie gerne verschrieben und großzügig in der landwirtschaftlichen Tierhaltung eingesetzt. Seit Ende der 1990er-Jahre gibt es jedoch ein zunehmendes Problem: Multiresistente Keime, heimisch in Krankenhäusern und lebensgefährlich für geschwächte Patienten. Effektive Antibiotika dagegen gibt es kaum. Stephan Sieber, Professor für Organische Chemie an der TUM, hat mit PK150 eine Substanz entdeckt, die gegen multiresistente Bakterien wirkt. PK150 greift die Bakterien an zwei Stellen an: Es führt zur unkontrollierten Freisetzung bestimmter Proteine, durch die sich das Bakterium selbst von außen auflöst. Und es stört die bakterielle Energieversorgung. So kann PK150 Bakterien sogar im „Persister“-Ruhestadium abtöten, selbst wenn sie sich zusätzlich mit einem Biofilm-Schutzmantel umgeben. PK150 soll jetzt zum Medikament weiterentwickelt werden. □

“The development pipeline for antibiotics around the world is readily estimable.”

Stephan A. Sieber

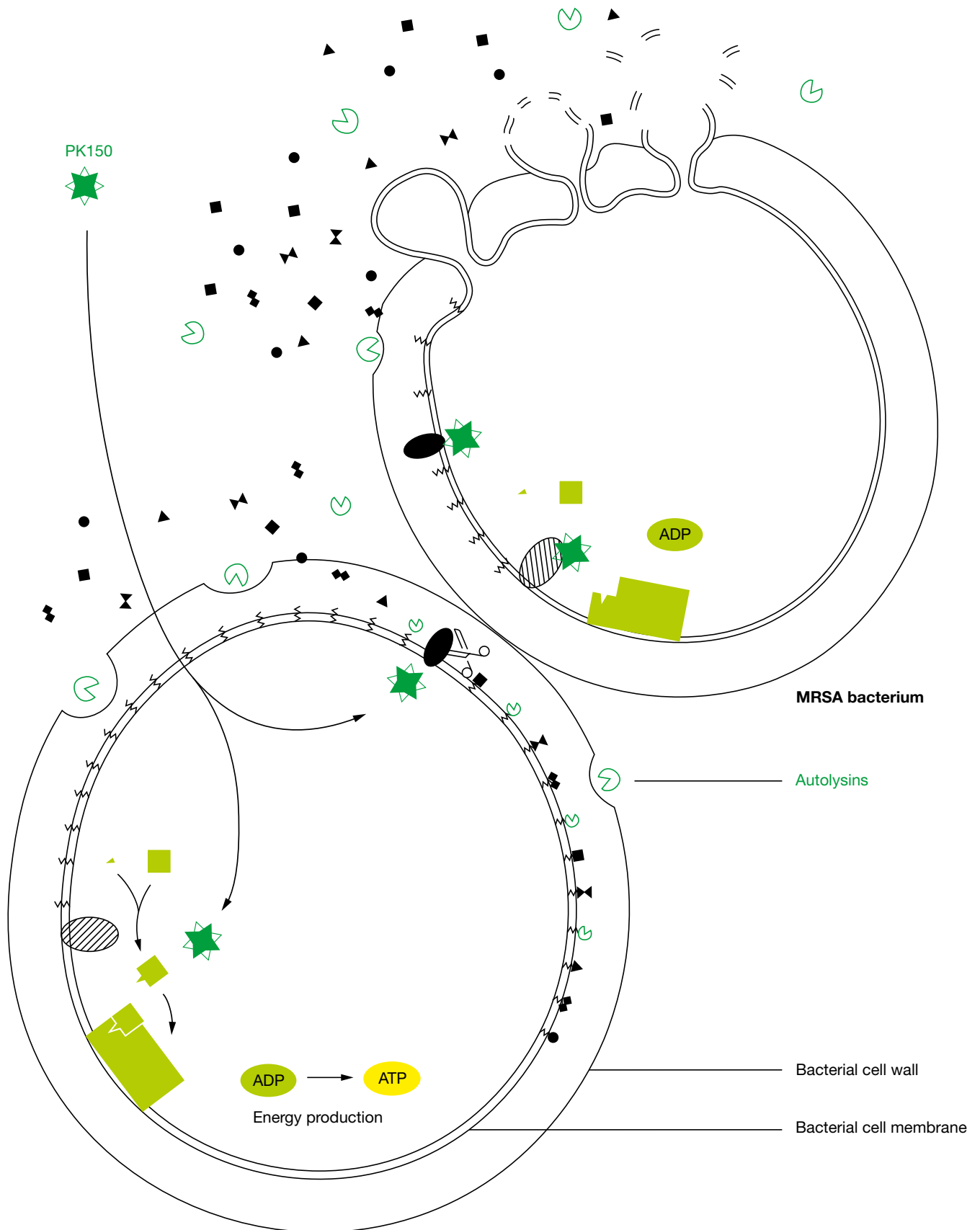
Klaus thought he had been exceptionally lucky when a routine health check uncovered a heart condition. As a slim, athletic man in his late 50s, nobody would have suspected he might have a heart condition. It was diagnosed at an early stage and he underwent surgery, which was successful. Six months later, Klaus was dead. He had become infected with hospital pathogens – multi-resistant bacteria that did not respond to any of the antibiotics that doctors administered. His body, which had been weakened by the operation, would not have been able to fight the infection at all without the support of these medications.

Nowadays, it is rare for anyone in the West to die from bacterial infections. Once-feared illnesses such as plague, cholera and typhus that depopulated entire regions in centuries gone by are no longer present in Germany. Around 1 million people die in Germany each year and around two-thirds of these mortalities are due to

cardiovascular diseases or cancer. In 2018, only around 0.1 percent of deaths were attributed to infectious bacterial diseases, a cause of death that doctors are required to report. We can attribute this to the strict hygiene standards and good quality of medical care in Germany today, as well as the fact that vaccinations are available against some pathogens. Above all, however, we have effective and highly tolerable antibiotics.

Antibiotic-resistant bacteria threaten global health

Since Alexander Fleming discovered penicillin in 1928, antibiotics have been overwhelmingly triumphant in the battle against bacterial diseases. Today, there are around 80 different classes of antibiotics, most of which were developed in the “golden years” of antibiotics research from 1940 to 1970. Antibiotics were seen as a panacea and doctors prescribed them with great regularity. They also came to be used heavily in animal husbandry and fattening. ▶



PK150 blocks two key processes in the bacterium at the same time. It bonds with two enzymes in the bacterial cell. As a consequence, one enzyme (shown black), sets free proteins, including some called autolysins, which break down cell walls. The other enzyme (shown hatched), which is bound by PK150, is important for the bacterium's energy production. All in all, PK150 kills the bacterium by blocking its energy supply and by perforating its cell walls.

Since the late 1990s, however, the downsides of antibiotics have become clear. Bacteria are increasingly developing resistance against antibiotics, with some strains even becoming concurrently resistant to several substance classes. This gives rise to multi-resistant germs that become endemic in hospitals. Often referred to in the media as “super germs”, the World Health Organization (WHO) has identified antibiotic-resistant bacteria as one of the greatest threats to global health. In 2017, the WHO published a list of the 12 most dangerous resistant germs and called on governments to create incentives for the development of medications to fight these pathogens. Ultimately, too few new antibiotics have been developed to keep pace with rising resistance.

Big pharma has long since stepped back from the field of antibiotic development, leaving small and medium-sized pharmaceutical enterprises to fill the gap. Developing new medications costs billions and there is a significant risk that bacteria will become resistant once new drugs are launched on the market. What is more, antibiotics with new mechanisms of action are only used when standard methods fail – and their sparing usage generates little revenue. Prof. Stephan Sieber, antibiotics researcher and holder of the Chair of Organic Chemistry II at TUM, recently concluded a study with colleagues on the current state of antibiotics research. “The development pipeline for antibiotics around the world is readily estimable,” he says. “Of the 50 or so substances currently in the clinical phase, there are only a handful of genuinely new developments. The majority are variations of conventional antibiotics that have been once again enhanced to a certain degree.”

A cancer treating drug opens up a path

This makes it all the more gratifying for Sieber that he and his team have identified a candidate antibiotic in his aBACTER project that works differently to most antibiotics. Conventional medications inhibit either the formation of bacterial cell walls, bacterial protein generation or DNA replication. Sieber’s agent, on the other hand, attacks bacteria on two fronts – with both capable of killing the pathogens: His substance causes cells to break down their own cell walls and also blocks energy generation. The aBACTER research project has already been running for five years. In their search for antibiotics with new mechanisms of action, Sieber’s team of scientists came across a drug actually intended to treat cancer but which also has mild antibiotic properties. The TUM researchers created a series of chemical variants to improve the

medication wherever possible before testing it on MRSA – a dangerous multi-resistant bacterium and infamous super germ.

One variant, a molecule given the name PK150, hit the bullseye. Even in minute quantities, the molecule is capable of killing off MRSA bacteria. “It is important to use a low effective concentration so that the substance also has the lowest possible toxicity in the human body,” explains Sieber.

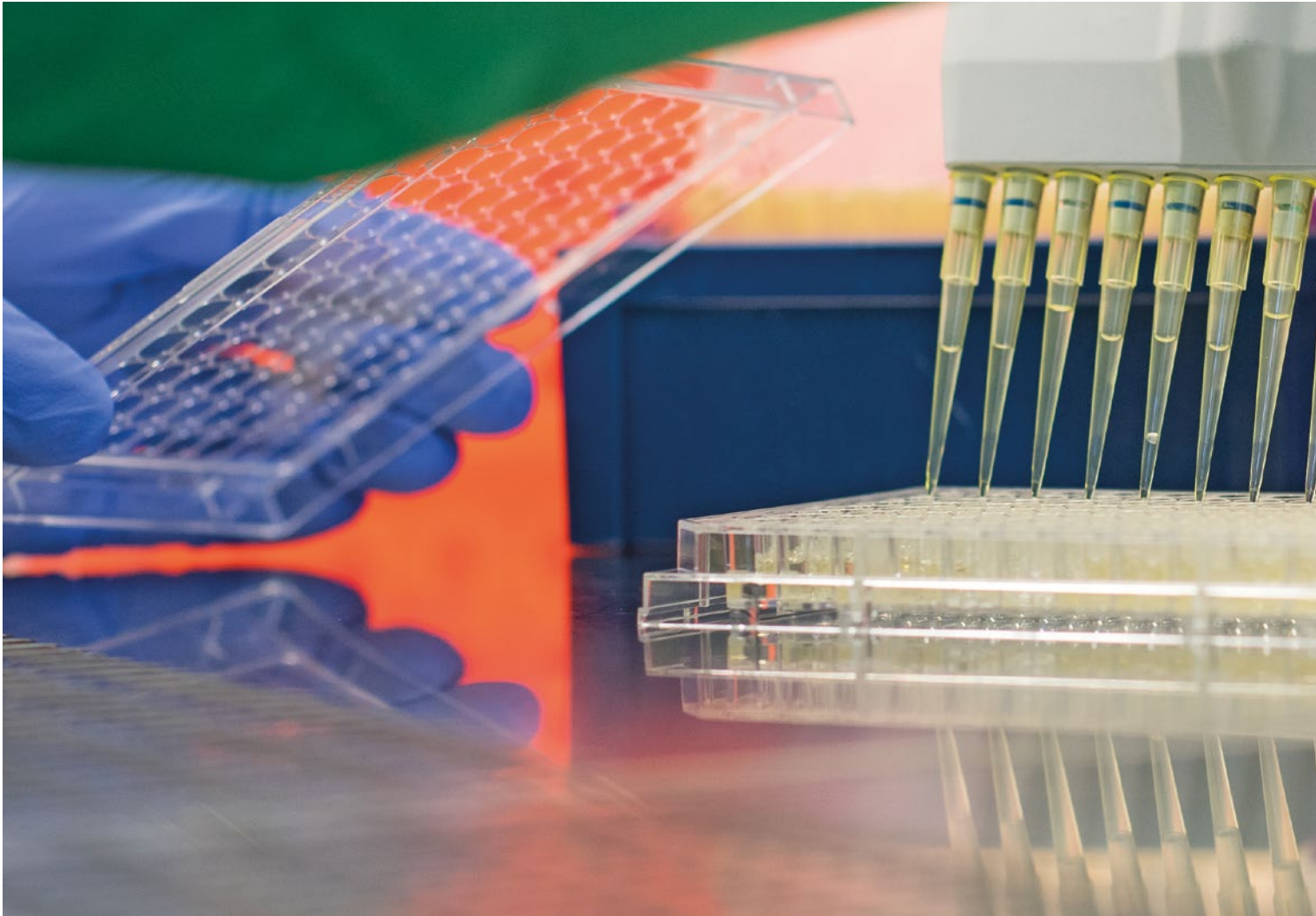
But is there a chance that bacteria might soon become impervious to PK150? Sieber’s team have made every effort to provoke resistance to PK150. The researchers have exposed MRSA bacteria to low concentrations of the substance for prolonged periods – so low that it is only able to kill off some of the bacteria. This gives the surviving bacteria the opportunity to alter their genome so as to render the antibiotic ineffective. In most cases, bacteria need four to ten days for this process. ▶

Prof. Stephan A. Sieber

Born in 1976, Prof. Stephan A. Sieber holds the Chair of Organic Chemistry II at TUM. After completing his doctoral studies in Marburg and Boston (Harvard), the chemist embarked on postdoctoral studies at the Scripps Research Institute in California, where he familiarized himself with target identification approaches. These techniques make it possible to search for binding partners for anti-cancer agents in human cells. As a recipient of an Emmy Noether scholarship at LMU Munich, he began to translate this technique to bacteria in order to shed light on antibiotics’ mechanisms of action. He accepted a position at TUM in 2009. In 2010, Sieber initiated AVIRU, which aims to use a new technique to get to grips with multi-resistant germs. His aBACTER project is funded by the VIP+ Program of the Federal Ministry of Education and Research (BMBF) and also won the Bavarian m4 Award in 2019.



Picture credit: Astrid Eckert



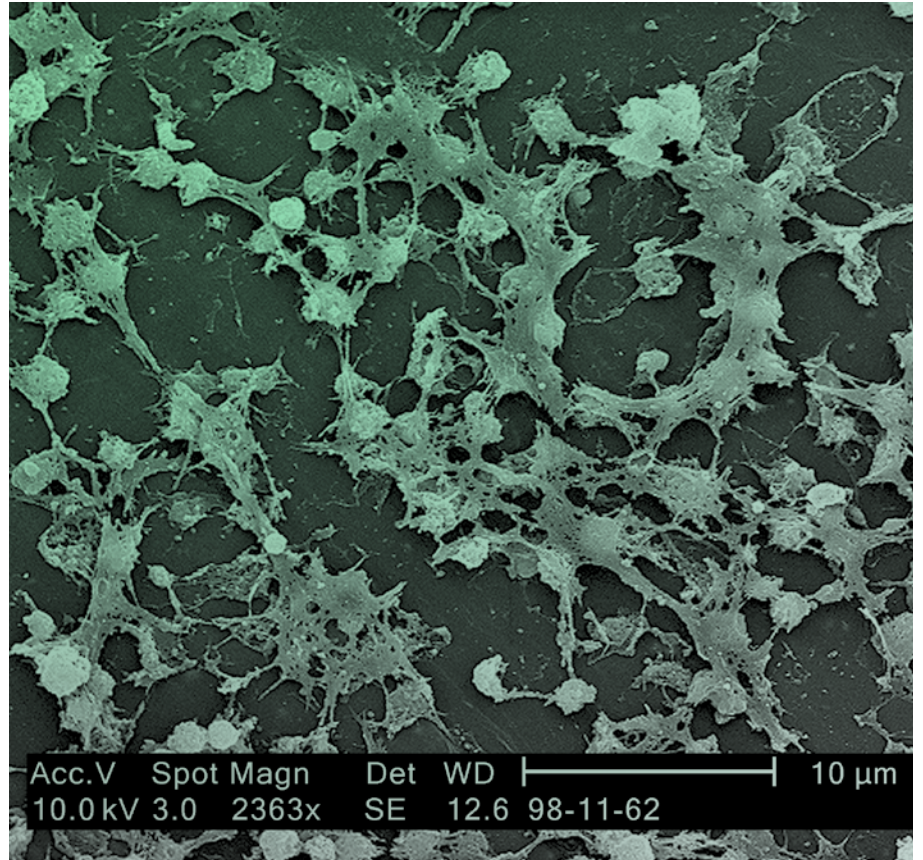
However, PK150 was still able to kill bacteria off after a month – to Sieber’s delight. “We were not able to make bacteria resistant to PK150 in the laboratory,” he said. “Not even when we used special substances to encourage genetic variation in the bacteria.”

Sieber and his team now want to find out precisely how PK150 works. They have identified that PK150 bonds with two enzymes in the bacterial cell. One enzyme regulates protein transport in bacterial cells. PK150 hyperactivates this enzyme, with fatal consequences for the bacterium – it results in the uncontrolled release of proteins, including some called autolysins, which break down cell walls. Normally, bacteria only need autolysins in very small doses to promote cell division. PK150, however, causes them to be released in such volumes that they essentially punch holes in bacterial cell walls.

The other enzyme that PK150 binds to is used by the bacterium for energy metabolism. PK150 blocks this process, which on its own would suffice to kill the bacterium. “The fact that PK150 blocks two key processes at the same time is likely the reason why the bacterium finds it so hard to become resistant to PK150,” surmises Sieber.

Tests with many resistant pathogens

The TUM researcher’s next step took him to the Harz mountains, to Wernigerode in Saxony-Anhalt, home to the Robert Koch Institute’s National Reference Center for Staphylococci and Enterococci. The facility collects resistant germs from across Germany and allowed PK150 to demonstrate that it is effective not only against MRSA but also against a whole host of other dangerous bacteria. Thanks to its novel mechanism of action, PK150 also



Scanning electron microscopic (SEM) image of *Staphylococcus aureus* bacteria, which were found on the luminal surface of an indwelling catheter. The sticky-looking substance woven between the round bacteria is known as biofilm. This biofilm protects the bacteria that secrete the substance from attacks by antimicrobial agents such as antibiotics.

Picture credits: Astrid Eckert, Janice Carr: phil.cdc.gov/details.aspx?pid=7484

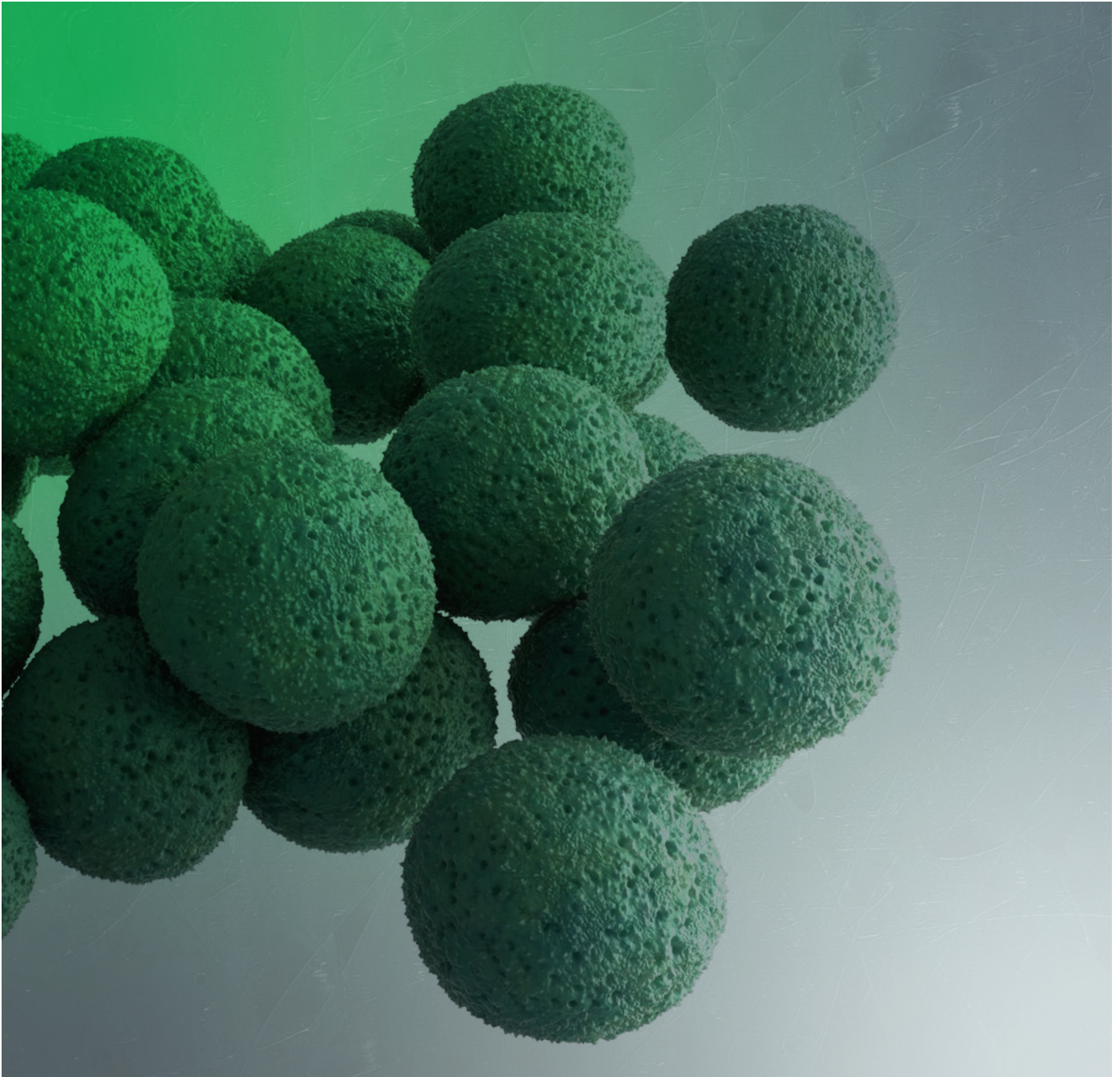
eliminated bacteria that other antibiotics cannot. This is because some bacteria tolerate antibiotics by, in effect, playing dead – they shift into an idle state in which they hardly divide and their metabolic processes are reduced to a minimum. A bacterium in this idle state is known as a “persister”. Given that many antibiotics target the division mechanism or an active metabolism, this state renders persistent bacteria safe from such agents. Groups of persistent bacteria sometimes surround themselves with a protective biofilm, a sort of thick mucus. This allows them to survive on an artificial hip or a catheter in the human body while protected against the body’s immune system and many antibiotics – and thus become the starting point for severe infections. Sieber’s team has observed that PK150 can both kill persisters and break down biofilms. ▶

Petri dish showing growing bacteria (left) as well as non-growing bacteria (right), which lack an essential gene. The protein encoded by this gene represents an attractive antibiotic target.



Bacterial colonies (small spots) are selected for subsequent studies.





3D illustration of *Staphylococcus bacteria*

Graphics: edlundsepp, Christian Scheck (source: turbosquid); Picture credits: Astrid Eckert

“That is the most exciting of the substance’s qualities,” believes Sieber, “because there is no antibiotic on the market today capable of permanently breaking down biofilm.”

In the coming years, PK150 is to be developed into an active agent that can prove its credentials on patients in clinical studies. The TUM researchers have fixed their sights on infective or bacterial endocarditis – a condition

in which biofilms and hospital germs play a major role – and hope to have PK150 approved as a treatment. Before being approved as a drug, PK150 will need to prove that it is effective and tolerable in the human body. If this testing proves successful, there will be an entirely new class of antibiotics with which to fight many multi-resistant germs.

■

Markus Bernards

Freunde zu Verbündeten gemacht

D

Phagen statt Antibiotika: Das Spin-off Invitris der TUM entwickelte ein neues Herstellungsverfahren für Bakteriophagen. Das sind hochspezialisierte Viren, die sich gegen bakterielle Infektionen einsetzen lassen. Es ist deutlich schneller und kostengünstiger als bisherige Methoden. □

Turning Enemies into Allies

A team of students has developed a new production method for bacteriophages. These are highly specialized viruses that can be used to counter multidrug-resistant bacterial infections. This discovery at the TUM Chair of Biophysics has led to a spin-off called Invitris. The novel production method could soon be used in practice for the first time and there are plans to found a company to promote the concept.

Bacterial infections can occur anywhere in the body, from the lungs to the stomach to the intestines. While infections of this type are frequently treated with antibiotics, bacteria are swiftly becoming immune to these substances. Very specific groups of viruses could represent an alternative to antibiotics. Known as bacteriophages, these viruses infect and kill bacteria but are not harmful to human cells. As the natural enemies of bacteria, they are highly suitable as a treatment for bacterial infections. Although their existence has been known for almost a century, the use of phage therapy has been limited to date. This is due to the lack of safe and efficient production methods for therapeutic bacteriophages.

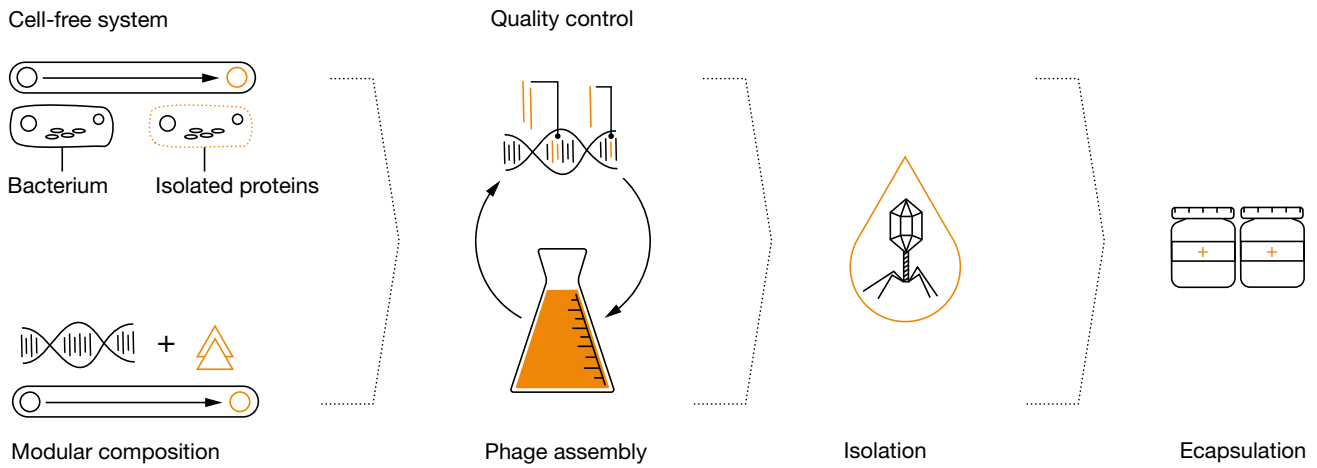
Back in 2018, a team of students from TUM and LMU Munich decided to take up the gauntlet and address this problem. Their work on the topic formed their entry to an international contest for students of synthetic biology called iGEM (International Genetically Engineered Machine Competition). TUM and LMU students have successfully collaborated on iGEM entries for years. In 2018, the 16-strong team brought together students from a range of disciplines, including molecular biology, biotechnology, bioinformatics and electrical engineering.

The cell-free production of phage is faster and requires less effort in the laboratory than conventional methods. Proteins are isolated from non-pathogenic bacteria. DNA of the desired phage is added. The quality or purity of the phage DNA is ensured before isolating and encapsulating the phage.

Cell-free production system proves promising

The team collaborated to develop the idea for a new phage production method. An important aspect to bear in mind is that, in order to multiply, phages require a host cell – a bacterium. Production systems developed to date involve removing all pathogenic endotoxins secreted from a bacterium before using it to produce phages. This method entails cost-intensive and time-consuming cleaning procedures and the use of high-grade cleanrooms. The iGEM team, on the other hand, have based their approach on a cell-free system. First, they isolate the proteins in non-pathogenic bacterial cells ordinarily responsible for the production of proteins and phages. They obtain an extract and add the DNA of the desired phage. The major benefit of this cell-free system is that it avoids the need for laborious cleaning procedures, thereby enabling phages to be produced significantly faster and more safely. At the iGEM final in Boston in October 2018, the joint TUM-LMU project won through against 350 other teams to claim second prize overall alongside other awards, such as the Best Entrepreneurship Project.

“According to current estimates, ten million people will die as a result of antibiotic resistance each year by 2050 [...].” Franziska Winzig



The spin-off Invitris was set up to continue developing the cell-free method after the competition. Three students and a supervisor from the iGEM team have been joined by a graduate bioinformaticist/business administration specialist as they strive to improve the technique further. In principle, it would be a suitable treatment for all bacterial infections. “As each bacteriophage only infects a specific species of bacteria, the Invitris method can compose any desired variety of phage, typically with an incubation period of just a few hours,” explains Invitris’ Franziska Winzig. Currently in the sixth semester of her biology studies, she plans to write her bachelor’s thesis on the method. “According to current estimates, ten million people will die as a result of antibiotic resistance each year by 2050 – more than the sum of deaths caused by all cancers. Hopefully, we will be able to counteract this trend,” says an optimistic Winzig.

Set for deployment

Plans to found a company to promote the method are also in the pipeline, reveals Winzig. To prepare for this step, the team has called on funding from TUM and also took part

in the UnternehmerTUM Medtech Bootcamp 2019, which is geared to preparing entrepreneurs for the incubation and initial financing phase of their new company. Invitris aims to bring the method to market as soon as possible. “We will likely start in Belgium,” says Franziska Winzig. Thanks to the country’s adoption of magistral preparation (also known as compounding pharmacy), phages can now be applied as a therapy in Belgium without having to pass through clinical studies. Germany is examining this approach at present. The Invitris team is currently working to obtain certification to apply its phage medicines. In fact, they are already in contact with a Belgian military hospital where the cell-free production method for phage medicines to fight wound infections could be used for the first time.

■ Gitta Rohling

Graphics: edlundsepp (source: Invitris)

Link
www.invitris.com

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Masthead

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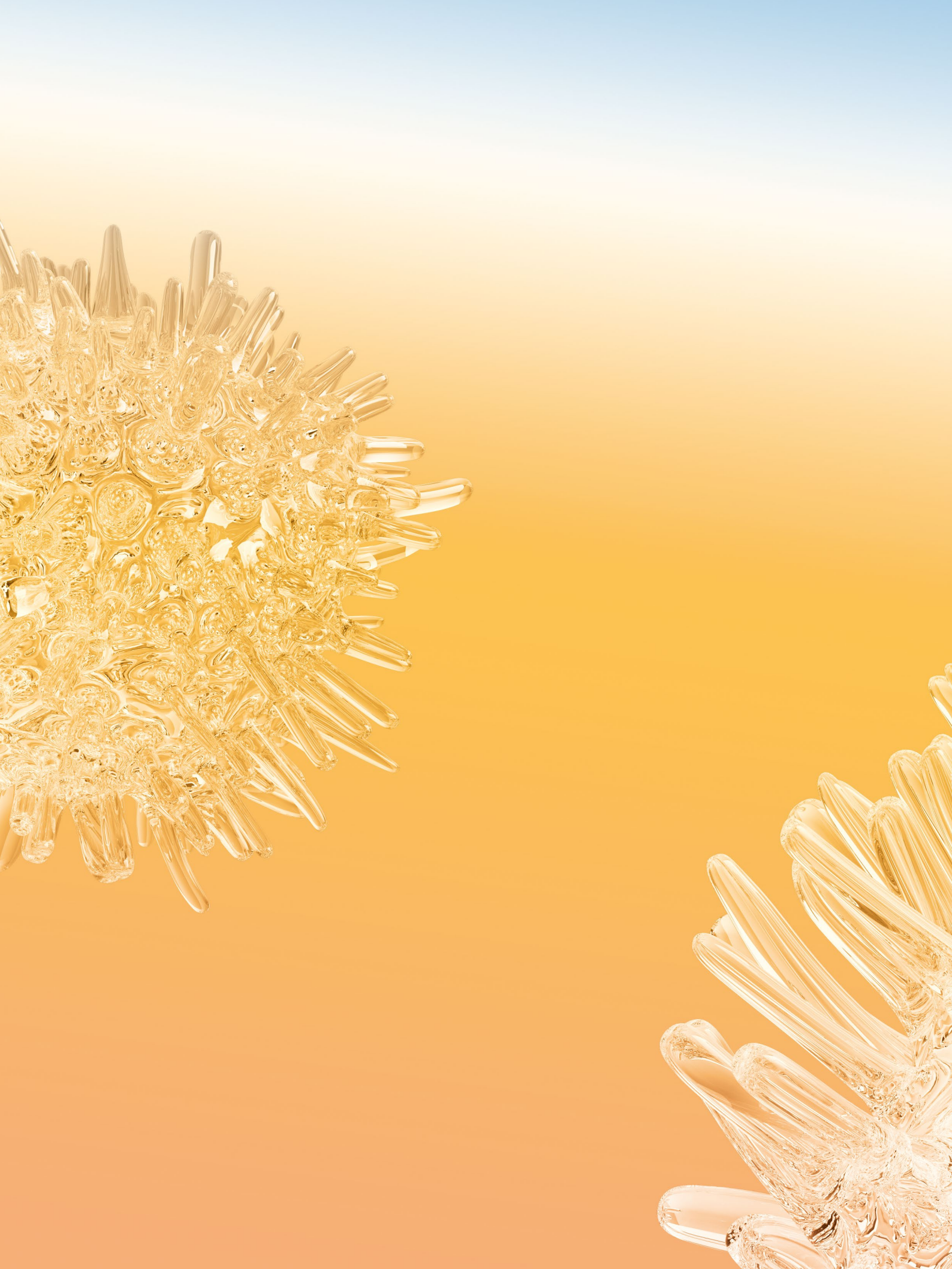


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