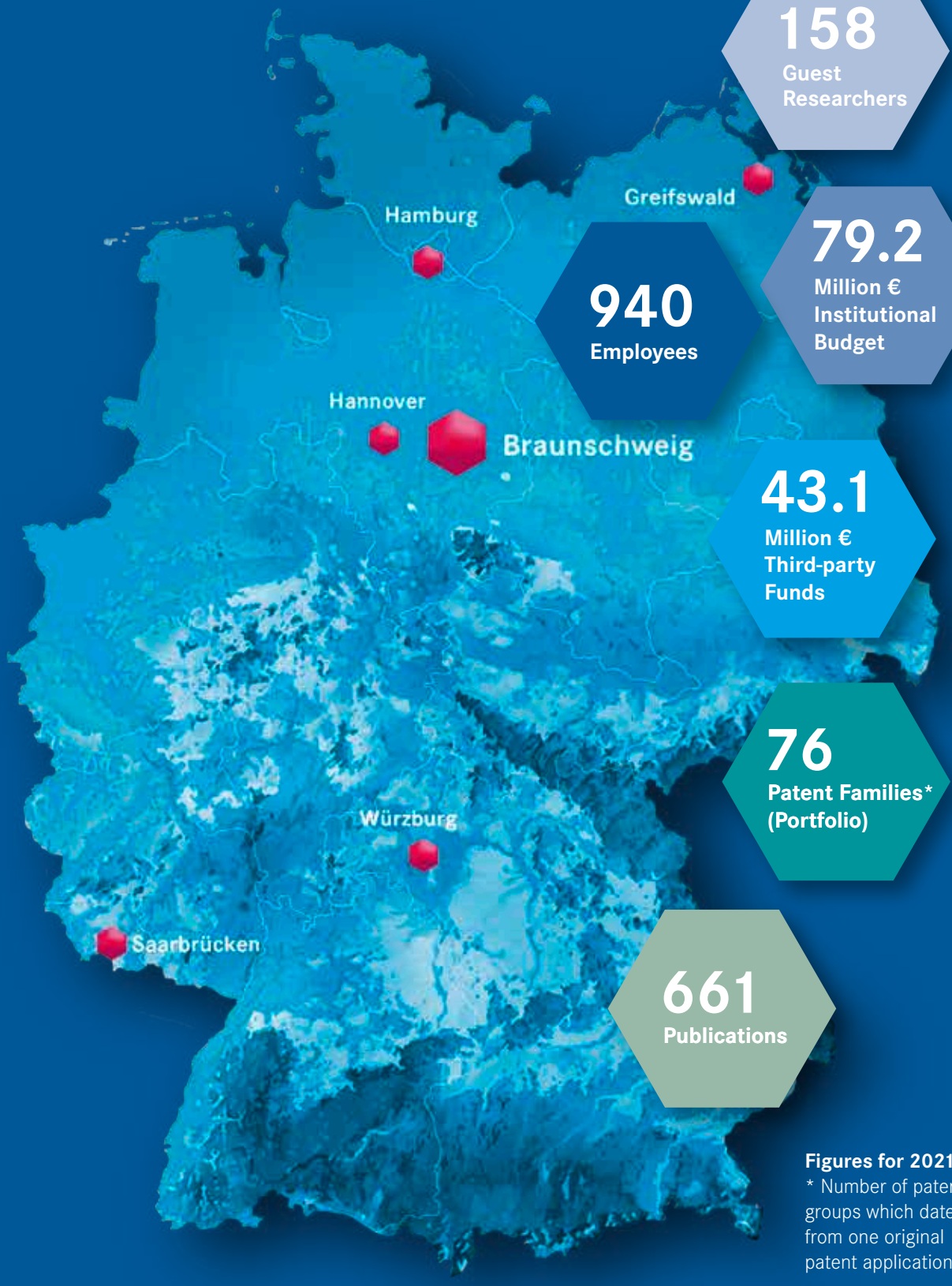
HZI HELMHOLTZ
Centre for Infection Research

RESEARCH REPORT

HZI 2020 2021

THE HELMHOLTZ CENTRE FOR INFECTION RESEARCH (HZI) AT A GLANCE



158
Guest Researchers

940
Employees

79.2
Million €
Institutional Budget

43.1
Million €
Third-party Funds

76
Patent Families*
(Portfolio)

661
Publications

Figures for 2021
* Number of patent groups which date from one original patent application.

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RESEARCH REPORT HZI 2020|2021

Photographs of HZI's sites:
Science Campus Braunschweig-Süd: HZI | Peter Sondermann
CiiM: HDR IMAGINA Visual Collaboration
CRC: Fraunhofer ITEM
CSSB: CSSB | Tina Mavric
HIOH: Jan Meßerschmidt
HIPS: UdS | Jörg Pütz
HIRI: University of Würzburg | Pressestelle
TWINCORE: TWINCORE Collection

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SITES

HZI Campus Braunschweig

- Headquarters of HZI
- Central administration
- Research infrastructure
- Basic research on bacterial and viral infections, immunology, anti-infective agents, epidemiology
- Cooperation with the Technical University (TU) Braunschweig, in particular within the Braunschweig Integrated Centre of Systems Biology **BRICS**

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken

- Founded jointly by HZI and Saarland University (UdS)
- Research on natural compounds, optimisation for pharmaceutical application as anti-infectives
- Bridge between basic research and pharmaceutical industry

Centre for Individualised Infection Medicine (CiiM), Hannover

- Joint initiative of HZI and Hannover Medical School (MHH)
- Elucidation of individual characteristics relevant for infection susceptibility, disease progression and therapeutic outcome
- Bridging clinical practice with state-of-the-art profiling technologies and latest data science technologies

Clinical Research Centre Hannover (CRC)

- Co-established with Hannover Medical School (MHH) and Fraunhofer ITEM
- Safety and efficacy testing of new medications
- Hosts the HZI Study Centre inside the framework of the German National Cohort NAKO

TWINCORE, Hannover

- Founded jointly by HZI and Hannover Medical School (MHH)
- Experimental and clinical infection research
- Translational research jointly performed by natural scientists and physicians
- Bridge between basic research and clinical practice

Helmholtz Institute for RNA-based Infection Research (HIRI), Würzburg

- Founded jointly by HZI and Julius-Maximilian-University, Würzburg (JMU)
- Research on RNA-based mechanisms of virulence and host defence
- Exploitation of RNA research for the development of new diagnostics, preventives and anti-infectives

Centre for Structural Systems Biology (CSSB), Hamburg

- Located on the campus of DESY (Deutsches Elektronensynchrotron) in Hamburg
- Jointly operated by ten North German research institutions and universities
- Structural elucidation of molecular infection processes using uniquely powerful photon sources

Helmholtz Institute for One Health (HIOH), Greifswald

- Founded jointly by HZI, University of Greifswald, University Medicine Greifswald and Friedrich Loeffler Institute
- Addresses the threat posed by the emergence of novel pathogens as well as the evolution of known pathogens
- Holistic research approach, considering human and animal health within their environment

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HIGHLIGHT PUBLICATIONS

PARTNERS, SITES AND NETWORKS

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DEAR READERS,

the past two years covered in this Research Report will undoubtedly go down in history as pandemic years in which SARS-CoV-2 challenged global societies and their healthcare systems in unprecedented ways, quite often pushing them to their limits. Particularly distressing were the great human suffering with many millions of deaths as well as the severe socio-economic consequences of the pandemic, which have not yet been overcome.

Never before, however, has the importance of infectious disease research been so clearly demonstrated, as it was, for instance, a key prerequisite for the rapid development of highly effective vaccines that paved the way for a possible end of the pandemic.

As the largest German non-university institution in this field, the Helmholtz Centre for Infection Research (HZI) was understandably expected to make substantial contributions to the fight against COVID-19. Fortunately, our agile and flexible research programme and organisational structure, which had previously been rated “outstanding” by an international review panel, allowed HZI scientists to focus their research on this emerging threat rather quickly, despite significant pandemic-related constraints.

Within weeks, relevant expertises and resources were bundled and effectively re-directed towards COVID-19-related questions and challenges. Since then, HZI scientists have been able to achieve numerous scientific results and have attracted so far unprecedented levels of third-party funding. They actively participated in national and international research networks and provided expert advice and knowledge transfer to policy-makers and society.

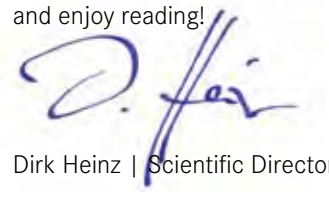
Importantly, our digital outbreak response management and analysis system SORMAS was quickly adapted to COVID-19, providing real-time information on the spread of COVID-19 to the public health authorities in many countries around the world, including Germany. Furthermore, HZI scientists delivered key insights into the pathomechanisms of severe COVID-19 lung disease. They conducted serological studies, invented new diagnostic tools, developed epidemiological models to predict the spread of the disease and took extensive efforts in the search for new small molecular compounds and antibodies active against SARS-CoV-2.

In the meantime, our scientists are already working on strategies to combat or even prevent future disease outbreaks caused by pathogens with high pandemic potential. In view of improving pandemic preparedness, it will be essential to enhance surveillance and prevention as well as to establish and sustain efficient pipelines for the development of rapidly available broad-spectrum therapeutics against emerging pathogens. Further to this, it is critical to better understand the mechanisms that drive the emergence of pathogens, which requires a comprehensive research approach that takes into account the tight interconnection between human, animal and environmental health. This is exactly where our newly founded institute, the Helmholtz-Institute for One Health (HIOH) in Greifswald, comes into play. It deals exclusively with important questions related to One Health such as the evolution and spread of (re)emerging zoonotic pathogens, the role of the ongoing climate change and the development of antimicrobial resistance.

Of course, HZI scientists at all our sites throughout Germany did not neglect their other research activities and projects, achieving impressive progress within our seven Research Foci, as further outlined in this Research Report.

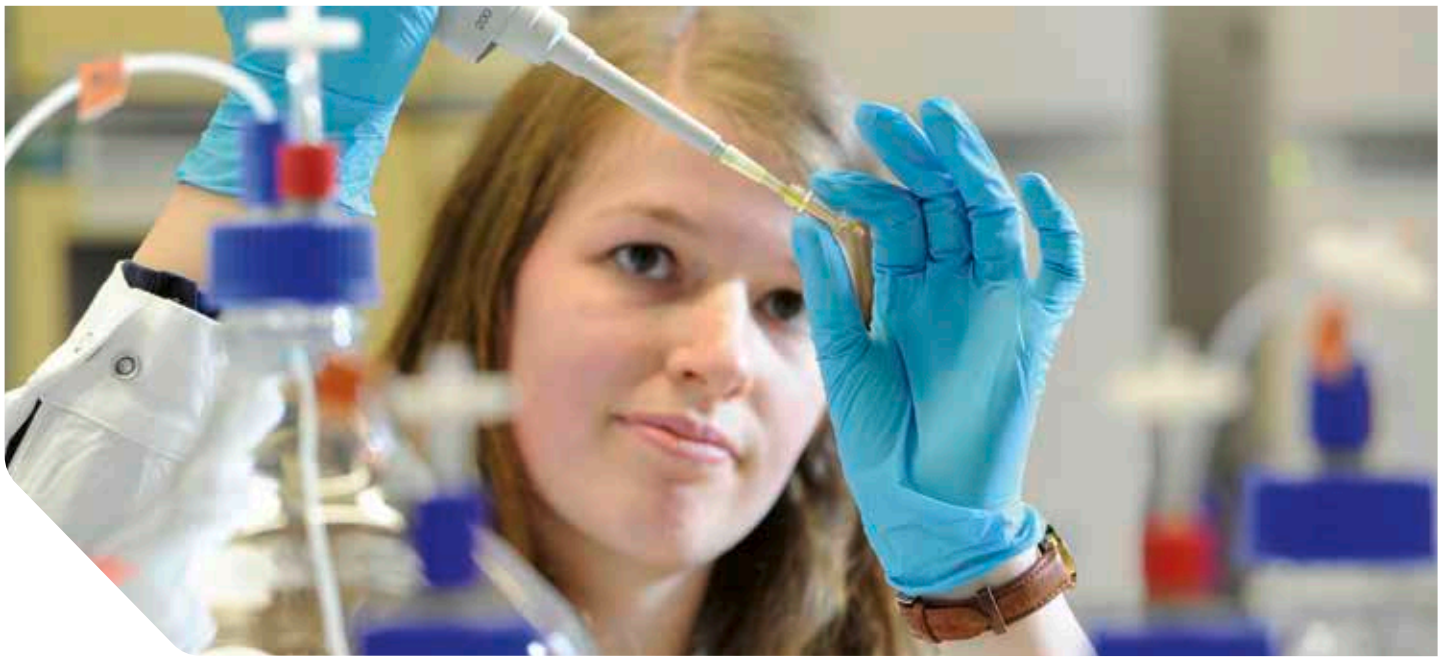
The achievements of the past two challenging years are of great importance for HZI, putting the centre in an excellent position for its future development. I would like to express my sincere gratitude to all who contributed to this team success, including scientific, administrative and technical support personnel, both within HZI as well as our great network of national and international partner institutions.

Thank you for your continued interest in the HZI! Stay tuned and enjoy reading!



Dirk Heinz | Scientific Director

ABOUT HZI



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PORTRAIT OF THE RESEARCH CENTRE

Continued or even increasing deficiencies in global health systems, world-wide mobility as well as demographic and climate change facilitate the emergence and re-emergence of pathogens, accelerate their spread, and enhance susceptibility to infectious diseases. The current COVID-19 pandemic has vividly shown how rapidly a new pathogen can spread all over the world with unforeseen and dramatic consequences for human wellbeing and global economy. The need to define effective strategies to counteract and better contain future disease outbreaks ("pandemic preparedness") has become increasingly urgent.

Likewise, the increase in antimicrobial resistance (AMR) of hospital-associated pathogens constantly poses a major challenge for public health and threatens to reverse the enormous progress made in infection control during the last century.

In addition, the role of infections as potential causes of other severe diseases – including cancer, metabolic syndrome and neurodegeneration – is becoming increasingly evident, offering a chance to overcome these diseases by preventing or curing the underlying infections.

In line with the mission of the Helmholtz Association to address major challenges facing society, science and the economy, the Helmholtz Centre for Infection Research (HZI), Germany's largest scientific institution solely focussing on infection research, employs cutting-edge research and latest generation technologies to investigate new approaches to prevention, diagnosis and therapy of infectious diseases. It pursues an integrated and highly interdisciplinary research strategy based, amongst other strengths, on profound expertise in the mechanistic exploration of host-pathogen interactions, thereby focussing on bacterial and viral pathogens of high clinical relevance. In order to acquire an in-depth understanding of the complex mechanisms underlying infection processes, scientists at HZI use a multi-scale approach from molecules via cells and organisms to populations and patient cohorts.

HZI focusses on translational infection research and, by capitalising on strong partnerships with universities, clinics and industry, aims to expedite transfer of fundamental research findings on to clinical application. The unique combination of interdisciplinary expertise and collaboration distinguishes the centre as a frontrunner and technology leader in the relentless struggle against global infection challenges, including immediate threats posed by AMR as well as chronic and emerging or re-emerging infectious diseases.

The Centre and its Sites

HZI's more than 900 employees and about 160 guest scientists from over 50 countries work at different sites throughout Germany.

The decentralised structure is a direct result of HZI's long-term strategy: the centre is partnered with sites of excellence in selected, future-oriented research fields with a high potential for innovation and of high clinical and societal relevance. In joint initiatives with leading universities, university hospitals and research institutes, HZI is systematically building up the appropriate structure best suited for driving the development of these fields. Thus, the organisational basis for innovative translational research at an internationally competitive level has been established over the past years. This makes HZI attractive for national and international partners, increasingly also from industry.

The main campus in Braunschweig – with scientists covering all disciplines of infection research – serves as the central research hub. In addition, branch institutes with different specialisations have been established to further complement and strengthen the scientific portfolio of the centre.

HZI Main Campus

The centre's main campus in Braunschweig provides a site well suited to HZI's interdisciplinary approach. High-level fundamental research is pursued and novel concepts for combatting infectious diseases are jointly developed and implemented via both in-house and external collaborations.

The infrastructure on-campus exploits technology platforms, including facilities for fermentative and total synthesis of natural compounds that make it possible to identify and develop new molecules intervening in the infection process. Structural biology permits detailed analysis of interactions between virulence factors, host cell targets and small molecules. Units for omics technologies allow genotyping of pathogens and expression profiling. A cutting-edge animal facility with some 400 different mouse strains allows HZI scientists to analyse virulence mechanisms and immune modulation concepts in state-of-the-art, biosafety level 1-3 (BSL1-3) laboratories. Furthermore, infection epidemiology conducts research on the occurence and spread of infectious diseases at the population level.

HZI and its partner institutions on the Science Campus Braunschweig-Süd © HZI | Verena Meier

A dedicated facility provides laboratory space for functional genomics research in collaboration with the Technical University Braunschweig (TU-BS) and the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (DSMZ).

Together with neighbouring institutes and other partners on HZI premises, the centre has established the "Science Campus Braunschweig Süd", reflecting concentrated on-site collaborations in research, development and education. Regional partners in this integrated campus include TU-BS, DSMZ, Fraunhofer Institute for Toxicology and Experimental Medicine (Fraunhofer ITEM), the German Centre for Infection Research (DZIF), the "BioS" lab for school students and a number of start-up companies. The head office of DZIF, which was initiated by the Federal Government of Germany in 2012, is located on the HZI main campus and includes the DZIF project and funding management units. Scientifically, HZI plays a pivotal role in DZIF, coordinating the Research Area "Novel Antibiotics" as well as the Translational Project Management Office. DSMZ is the largest type culture collection in Europe. It offers longstanding expertise in fields like bacterial metabolism and functional genomics and provides HZI researchers with pathogen and compound producer strains. The modern sequencing units of HZI and DSMZ provide complementary services and offer a wide range of technologies and expertise, including gene expression analysis. Fraunhofer ITEM operates a specialised branch on the campus, including a GMP (Good manufacturing practice) facility for the production of biologicals and cells suitable for clinical testing, offering further opportunities for future cooperation on the campus.

Branch Institutes

Within the last fifteen years, HZI has founded the translational centre TWINCORE, and three Helmholtz Institutes, HIPS, HIRI, and HIOH (see below), which significantly strengthen its expertise and critical mass in specific fields. In addition, HZI is an essential part of regional research institutions designed to foster unique expertise and technologies.

→ ***Clinically oriented translational research:*** The mission of the translation centre TWINCORE in Hannover is to promote clinically relevant, patient-oriented infection research. TWINCORE was founded in 2008 by HZI and Hannover Medical School (MHH) - one of Germany's leading university clinics. In line with its clinical focus on transplantation and regenerative medicine, one research pillar of MHH focuses on "infection and immunity", jointly developed in a strategic partnership with HZI. At TWINCORE,

HZI and its sites (left).
The respective branch institutes complement the expertise on the HZI main campus in the strategically relevant fields indicated (right).

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multidisciplinary teams of physicians and scientists pursue research motived by clinical needs and observations and translate their findings into clinical practice.

→ ***Individualised infection medicine:*** TWINCORE currently also hosts the Centre for Individualised Infection Medicine (CiiM). This institute, jointly set up by MHH and HZI in 2015, is expected to be transformative via the development of increasingly patient-specific concepts and strategies in infection medicine. A dedicated new CiiM building is shortly to be constructed adjacent to TWINCORE.

→ ***Drug and pharmaceutical research:*** The Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) in Saarbrücken focusses particularly on the discovery and development of novel anti-infectives from natural sources, like bacteria and fungi. HIPS was established jointly with Saarland University (UdS) in 2009 in order to combine the outstanding expertise of both institutes in pharmaceutical research, especially in the areas of natural compound research, medicinal chemistry and drug delivery. HIPS on the UdS campus currently hosts three departments and five research groups, constituting a unique asset for the translational infection research pipeline of HZI.

→ ***RNA-based infection research:*** The role of non-coding RNAs in infection and immunity and the study of infection processes at the single cell level are emerging and fast-growing fields with great potential for innovation. In order to develop these fields sustainably, the Helmholtz Institute for RNA-based Infection Research (HIRI) was founded in collaboration with the University of Würzburg (JMU) in 2017. HIRI in Würzburg currently comprises two research departments and eight research groups. A dedicated HIRI building is under construction on the medical campus of JMU.

→ ***One Health:*** In 2021, the new Helmholtz Institute for One Health (HIOH) was jointly established by HZI, the University of Greifswald, the University Medicine Greifswald and the Friedrich-Loeffler-Institute. HIOH addresses the ongoing threat posed by the emergence of novel pathogens as well as the adaptation of known pathogens, including the development of antimicrobial resistance (AMR). It will function as a subsidiary institute of HZI on the research campus of the University of Greifswald and will closely link both local partners and other collaborators from the Helmholtz community through joint projects in the spirit of the One Health concept.

→ ***Information and data science:*** On its central campus, TU-BS together with HZI set up the Braunschweig Integrated Centre for Systems Biology (BRICS) in 2016. At BRICS, scientists from both partner institutes collaborate on bioinformatics and mathematical modelling of infectious disease processes. By integrating large data sets, they aim for a systems understanding of infections and immunity using state-of-the-art digital technologies. TU-BS has chosen "Infections and Therapeutics" as one of its main research fields, reflecting its long-term commitment to partnership with HZI.

→ ***Structural systems biology:*** In Hamburg, HZI has played a key role in establishing the Centre for Structural Systems Biology (CSSB), the joint initiative of ten research partners. In the CSSB building on the campus of the German Electron Synchrotron Centre (DESY), structural biologists and infection researchers investigate host-pathogen interactions at the highest possible spatial resolution using high-intensity photon sources, like the third-generation synchrotron source PETRA III and the European free electron laser X-FEL. A structural biology department of HZI is located at CSSB to investigate supramolecular machines involved in bacterial infections.

→ ***Clinical trials:*** The Clinical Research Centre (CRC) in Hannover is staffed and equipped for safety and efficacy testing (Phase-I-to-IIa trials) of new medications. CRC was founded in 2014 by Fraunhofer ITEM and MHH together with HZI as a unique translational infrastructure. CRC also hosts the HZI Study Centre inside the framework of the German National Cohort (NAKO), where epidemiologists conduct long-term population studies with volunteers.

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HZI AND ITS SITES AT A GLANCE

Science Campus Braunschweig-Süd:
HZI Main Campus, Braunschweig

TWINCORE: Centre for Experimental and Clinical Infection Research, Hannover (co-established with Hannover Medical School), 2008

HIPS: Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken (co-established with Saarland University), 2009

CRC: Clinical Research Center Hannover (co-established with Hanover Medical School and Fraunhofer ITEM), 2014

CiiM: Centre for Individualised Infection Medicine, Hanover (co-established with Hannover Medical School), 2015

BRICS: Braunschweig Integrated Centre for Systems Biology (co-established with Technical University Braunschweig), 2016

HIRI: Helmholtz Institute for RNA-based Infection Research, Würzburg (co-established with University of Würzburg), 2017

CSSB: Centre for Structural Systems Biology, Hamburg (co-established with nine North German partners), 2017

HIOH: Helmholtz Institute for One Health (co-established with University Greifswald, University Medical Centre Greifswald and Friedrich-Loeffler-Institute), Greifswald, 2021

THE HELMHOLTZ PROGRAMME "INFECTION RESEARCH"

THE CHALLENGES

Even before the COVID-19 pandemic unfolded in 2020, infectious diseases already made up the major part of the global health threats listed by the World Health Organisation (https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019). Infections are responsible for more than a fifth of all human deaths worldwide. Despite improved hygiene and sanitation measures, global vaccination programmes, and powerful anti-infective therapies introduced in the last century, they continue to pose a global threat to human health. Even in industrialised countries, infections cause huge socio-economic damage through prolonged hospital stays, sick leave and premature death.

Globalisation, increasing mobility of humans, and urbanisation are key drivers in the rapid spread of epidemics caused by emerging and re-emerging pathogens such as SARS-CoV, Ebola or influenza. Epidemic control remains a challenge as we lack sufficient measures for global infection surveillance. The most dramatic recent example of this threat, the COVID-19 pandemic, has led to a huge number of infections worldwide, causing more than 460 million infections and over 6 million deaths (status as of March 2022) and necessitating unprecedented quarantine measures.

At the same time, chronic infections continue to affect millions of people. These infections can promote progressive organ dysfunction that eventually leads to cancer, autoimmune disorders or neurodegenerative diseases. Protective vaccines are still largely missing for many chronic infectious diseases, including HIV/AIDS, hepatitis and tuberculosis.

The combined problems of increasing multidrug-resistant bacterial infections and a concomitant shortage of effective antibiotics are driving fears of an imminent post-antibiotic era. Antimicrobial resistance affects particularly vulnerable groups, such as elderly, newborns, and immunosuppressed patients and poses a systemic threat to modern medicine. Given the declining investment by the pharmaceutical industry in novel anti-infectives and vaccines, publicly funded research must step in to ensure the discovery of urgently needed drugs and foster the development of new tools for the rapid diagnosis and surveillance of pathogens.

HZI'S MISSION AND PROGRAMME

As stipulated by the mission statement of the Helmholtz Association and the Federal Health Research Framework Programme of the German Federal Ministry of Education and Research, HZI pursues long-term and strategic infection research addressing one of the greatest health challenges facing society.

The central mission of HZI is to address the infectious disease threats of the 21st century. To promote clinical and pharmaceutical innovation, HZI scientists have developed the interdisciplinary programme "Infection Research". The programme combines groundbreaking fundamental research with clinically oriented investigation and application-oriented research (*Figure 1*). HZI's unique interdisciplinary

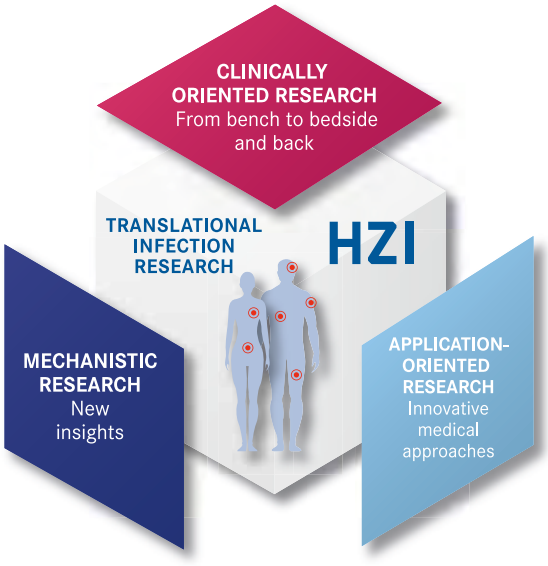


Figure 1: From research to impact: the integrative research strategy of HZI

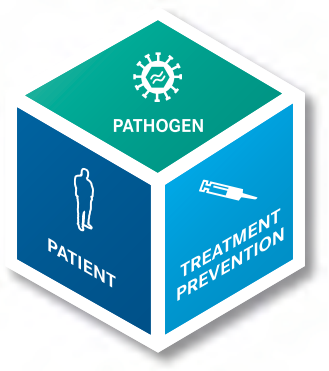


Figure 2: The triad pathogen-patient-therapy | prevention

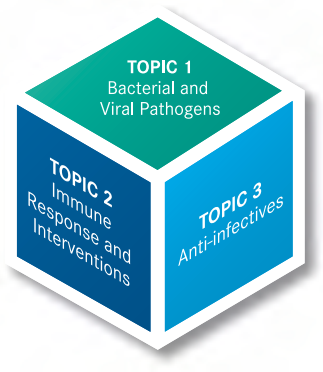


Figure 3: HZI's Research Topics

approach facilitates the development of innovative, increasingly patient-tailored solutions for diagnosis, prevention, treatment as well as surveillance and control of infectious diseases.

Technological advances in biomedicine have opened new avenues in fields like high resolution light and electron microscopy, single-cell analyses, genome editing, and complex data analyses using machine-learning approaches. These state-of-the-art technologies offer unique opportunities to obtain an in-depth understanding of infection processes.

The programme "Infection Research" makes use of these technologies to gain new fundamental insights. Researchers of the programme study the interplay amongst pathogens, the host's immune system and the host microbiota, i.e., the entirety of microorganisms that colonise body sites such as skin and intestine. They investigate the pathogen's response and resistance to existing treatments in order to lay the groundwork for future innovations facilitating patient-specific risk prediction, prevention, therapy and clinical management of infectious diseases. Pursuing the "One Health" approach, which is based on the interconnectedness between humans, animals, and the environment, they contribute to an effective pandemic preparedness in the future.

RESEARCH TOPICS AT HZI

Pathogen research at HZI aims to promote the development of efficient treatment strategies against bacterial and viral pathogens. This endeavour requires a deep understanding of the complex interactions between pathogen and patient, with a focus towards possible treatment options (*Figure 2*). Reflecting this interplay, the HZI programme Infection Research is composed of three integrated Research Topics *(Figure 3)*, namely Bacterial and viral pathogens (Topic 1), Immune response and interventions (Topic 2) and Anti-infectives (Topic 3).

Topic 1 focuses on the role of pathogenic bacteria and viruses in infectious disease processes.
Scientists in Topic 1 study pathogens and their dynamic interplay with the host. They aim to understand the molecular bases of virulence, persistence and resistance, and to de-

PROGRAMME-ORIENTED FUNDING

The Helmholtz Association, Germany's largest research organisation, invests its resources in research programmes that compete with one another for institutional funding provided by the Federal and State Governments.

This "programme-orientated funding" (POF) enables mission-driven and sustainable research and is based on a two-step procedure: A scientific evaluation of the existing programme at the level of the research centre focussing on scientific performance, followed by a strategic evaluation of the new programme proposals, which reviews programme goals, work programme, competences and potential future impact.

After an international expert panel acknowledged HZI's world-class scientific performance in the 2018/19 evaluation, its programme "Infection Research" provides the guidelines for HZI research for the fourth period of programme-oriented funding (POF IV), which started in 2021 and will last for seven years.

termine risk factors for the spread of diseases. They study structures and mechanisms enabling pathogens to infect the host. Their aim is to identify promising targets for new therapies and new diagnostic routes. To this end, they pursue a broad range of research approaches and use comprehensive profiling technologies as well as data analysis techniques to dissect pathogen features relevant for virulence and resistance. Topic 1 studies the interactions between the health of humans, animals and the environment, implements novel mobile health surveillance systems for rapid outbreak response and investigates associations between infections and non-communicable diseases.
(Topic 1, Speaker: Wulf Blankenfeldt; Deputy Speaker: Chase Beisel)

The focus of Topic 2 is the response of the immune system to infections.

Scientists in Topic 2 study the detection and clearance of infectious agents by the immune system. This includes the study of individual susceptibilities to infection and the mechanisms of immune evasion that pathogens deploy to fend off the host defenses. They explore the aging of the immune system, the gut and lung microbiome and virome influences on the course of infections and the impact of infections on neurodegenerative diseases. These activities span the range from fundamental studies in cells and animal models up to studies performed in cooperation with clinical partners and epidemiologists, using patient and population cohorts. This knowledge informs and supports innovative strategies for immunotherapy or for prevention of infectious diseases by vaccination.
(Topic 2, Speaker: Luka Čičin-Šain; Deputy Speaker: Yang Li)

In Topic 3, researchers are discovering and developing new anti-infectives.

Drug research at HZI is concentrating in particular on natural products – substances produced by organisms like bacteria and fungi in great variety that often display intriguing medical properties. At HZI, an interdisciplinary team of leading experts with both academic and industry backgrounds is developing innovative methods to identify, characterise and improve natural products, which could be used to treat infectious diseases. By means of medicinal chemistry, they optimise natural products as well as synthetic small molecules both chemically and pharmaceutically to make them suitable for use as drugs. Topic 3 scientists establish new technologies to ensure the safe and effective delivery of the drug to the site of action, for example the infected organ, tissue or cell. In cooperation with partners from the pharmaceutical industry, the compounds are further developed towards clinical trials.
(Topic 3, Speaker: Mark Brönstrup; Deputy Speaker: Anna Hirsch)

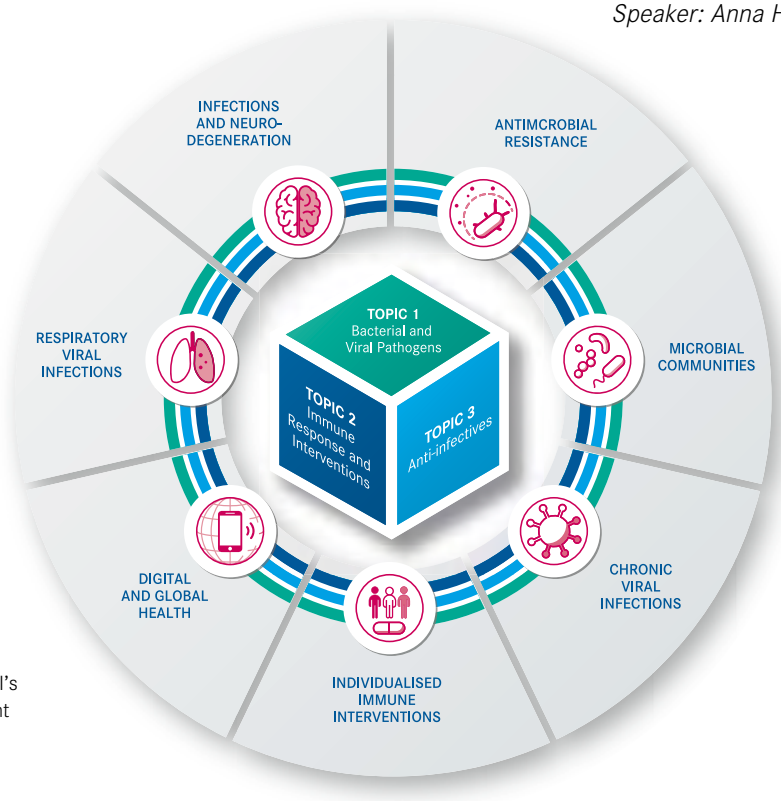


Figure 4: Overarching "Research Foci", supported by core facilities, combine the expertise of HZI's Research Topics to address urgent problems facing society.

RESEARCH FOCI AT HZI

On the basis of challenges of high clinical and societal relevance and the special competences at HZI and its cooperation partners, HZI has established so-called Research Foci providing a synergistic, dynamic and flexible framework for the research programme. The Research Foci aim to integrate expertise from different areas of HZI's research, namely from all three Topics *(Figure 4)*. Within each Research Focus, HZI scientists contribute their expertise and facilitate the transfer of knowledge from the lab to clinical or pharmaceutical application. They offer the flexibility to rapidly adapt the research programme to emerging and future challenges.

Currently, researchers at HZI and its partner institutions cooperate in seven Research Foci addressing the clinically relevant fields of

- Antimicrobial Resistance (AMR)
- Microbial Communities (MICO)
- Chronic Viral Infections (CVIR)
- Individualised Immune Interventions (INDI)
- Digital and Global Health (EPI)
- Respiratory Viral Infections (RVIR)
- Infection and Neurodegeneration (INEU)

The Research Foci are critically supported by state-of-the-art research infrastructure. Specialised enabling core facilities provide access to cutting-edge technologies both for the scientists at HZI and for the wider infection research community. They serve as an important hub for integrating and aligning broader German research efforts (e.g. German Center for Infection Research (DZIF), Helmholtz Association) in this field.

The approaches and achievements of each of our Research Foci are described in detail in this report.

GOALS OF THE PROGRAMME

In recent years, HZI has taken several important steps towards achieving its strategic mission. Key strategic partnerships have been further strengthened to stay at the forefront of infection research and to advance the translational research programme. HZI has achieved high international visibility in fields such as natural product-based drug discovery, data science and modelling, the use of RNA molecules in studying and controlling infections, disease outbreak control as well as integration of precision medicine into infection research.

These achievements enable us to follow seven overarching goals for the upcoming years:

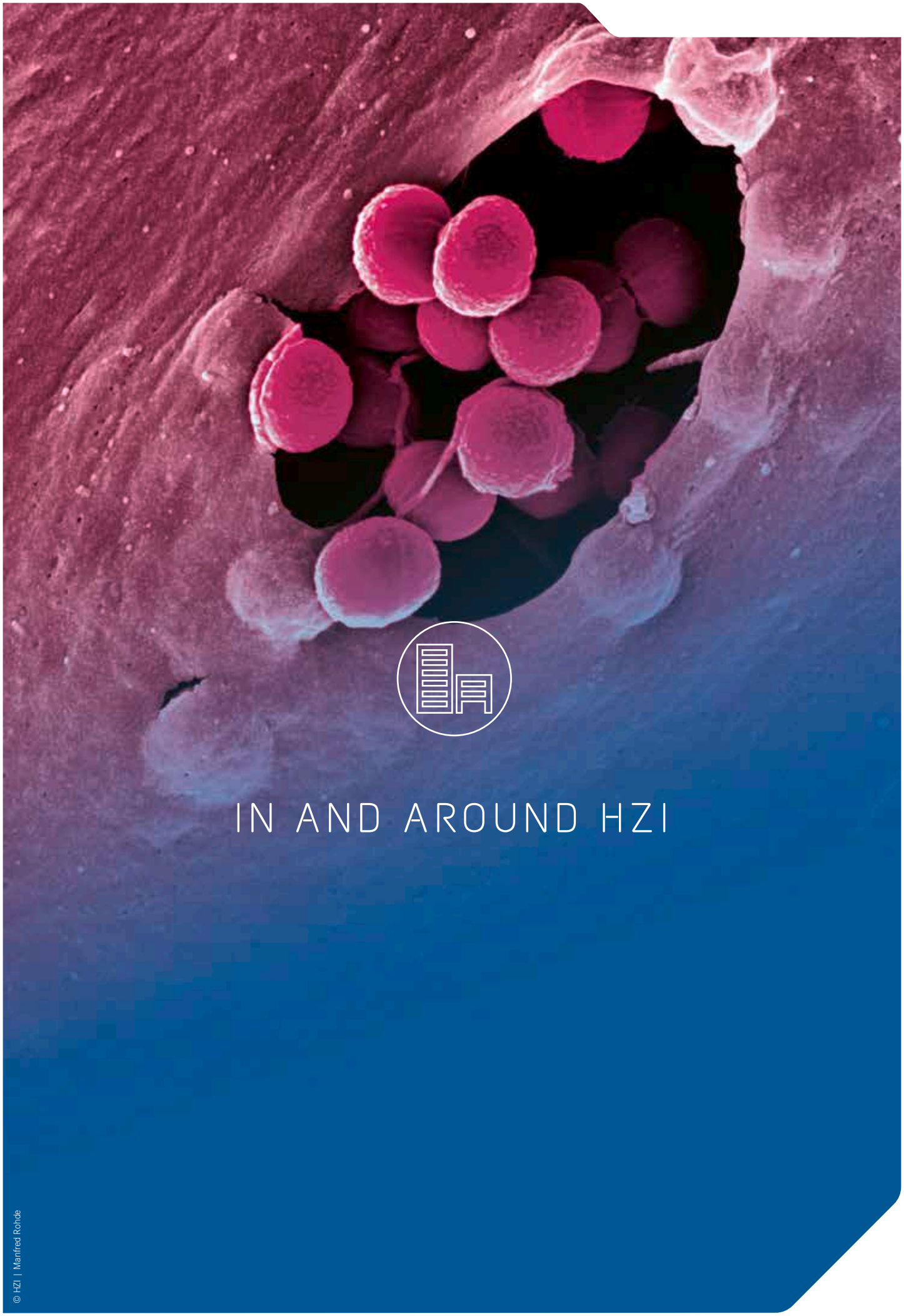
- Establishing HZI as a world-leading academic institution for the discovery and development of anti-infectives
- Positioning HZI as a frontrunner in translating early discoveries into patient-tailored infection medicine
- Pioneering an RNA-centric approach to understand infection processes and microbiomes on the single-cell level
- Addressing global health challenges dynamically by continually adapting the programme, i.e. by establishing new Research Foci
- Transforming infection research through a digital revolution
- Strengthening HZI as a driver of and partner within global networks for translational infection research
- Contributing significantly to the prevention of future pandemics, in particular through groundbreaking research in the field of "One Health"

STRATEGIC PARTNERS

To complement its research portfolio and leverage further existing strengths, HZI has built a network of national and international strategic partnerships. The partners comprise universities, university hospitals, non-university research institutions like, in particular, other Helmholtz centres, DZIF and the pharmaceutical industry.

To maximise synergies in translational and pharmaceutical research and to rapidly integrate new technologies, HZI has founded a number of dedicated research institutes together with academic partners. This network of institutes is unparalleled in German infection research (*see chapter "Strategic Partners all over the World" as well as section "Partners, sites and networks" in this report*).

Author: Thomas Pietschmann, Programme Speaker



IN AND AROUND HZI

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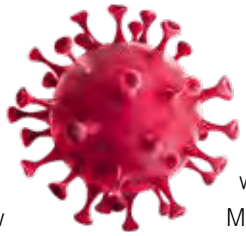
2020 AND 2021: YEARS OF THE PANDEMIC

HZI AND ITS COVID-19 RESEARCH

At the turn of the year 2019/2020, China officially reported the emergence of several cases of a so far unknown lung disease. Physicians of the 8 million-metropolis Wuhan came across patients who suffered from fever, dry cough, breathing difficulties, headache and pneumonia and who did not respond to antibiotic treatment. Some patients developed progressive respiratory failure and even died a couple of weeks after infection. The first cases were all connected to a local seafood market, but quickly more and more people became infected and filled Wuhan's hospitals, so it was clear that there was a human-to-human transmission of this new disease.

Shortly thereafter, it was shown that a member of the coronavirus family was causing the described symptoms, and – more alarmingly – that it was similar to SARS, a virus that had previously infected thousands of people worldwide, killing many of them. At the end of January, this new virus, which was later named SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), reached Europe and Germany. One month later, the WHO declared the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic.



Although coronaviruses were not in their focus before, scientists at HZI reacted fast to the new situation, utilising their expertise and facilities to initiate projects related to research on SARS-CoV-2. They also served as experts right from the beginning; explaining the latest research results to the public in the media and giving advice to politicians as the basis for their actions (*see Chapter COVID-19: A Challenge for Science Communication at HZI*).

The following is a brief summary of the main activities:

E-HEALTH AND SURVEILLANCE

One of the first of HZI's reactions to the emerging COVID-19 pandemic was the implementation of a new module into the eHealth tool SORMAS (Surveillance, Outbreak Response and Management Analysis System). This open-source software was developed in collaboration with African partners at the Epidemiology department of HZI in 2014 during the outbreak of Ebola in western Africa for case management, contact tracing and documentation of symptoms. By using SORMAS, labs, hospitals and medical doctors are able to share data and communicate in real-time, enabling them to react as quickly as possible with protective measures to an outbreak of an infectious disease. After the successful implementation of the COVID-19 module into SORMAS in Nigeria and Ghana, other countries like Switzerland, France, Fiji, Ivory Coast, Nepal and Germany also started to use the system. In Germany, a version specifically adapted for the public health departments was released in April 2020, aiming to ease contact person and case management. Today, with significant funding provided by the German Ministry of Health, more than half of these departments are regularly using this powerful digital tool (*see Chapter "Research Focus EPI"*).

SEROLOGICAL STUDIES

HZI epidemiologist Gérard Krause initiated the nationwide antibody study MuSPAD (full name: "Multilocal and Serial Prevalence Study on Antibodies to SARS-CoV-2 Coronavirus in Germany"), which aimed to identify the number of infections with SARS-CoV-2 in Germany and determine the immunity in the population. The study, generously funded by the Helmholtz Association, started in July 2020 and finished in August 2021. At seven locations all over Germany (Reutlingen, Freiburg, Aachen, Osnabrück, Magdeburg, Chemnitz and Vorpommern-Greifswald), randomly selected citizens were invited to participate. In Hannover, cooperation with the German National Cohort (NAKO) also supplied data for MuSPAD. Blood samples were taken from all study participants, and they filled in a questionnaire about their life situation to identify possible risks for a severe course of a COVID-19 infection. The study showed that, for every reported COVID-19 case, two to five persons are or have been actually infected. Therefore, the considerable under-recording of SARS-CoV-2 infections in different stages of the pandemic could be assessed. The data of this study help to explain the progression of the pandemic in different regions and citizen groups. MuSPAD thus provides information for evaluating the impact of protective measures (*see Chapter "Research Focus EPI" and Chapter "NAKO" in the section "Partners, sites and networks"*).

Start of the antibody study MuSPAD in Reutlingen at the beginning of July 2020 © NMI | Sarah Link

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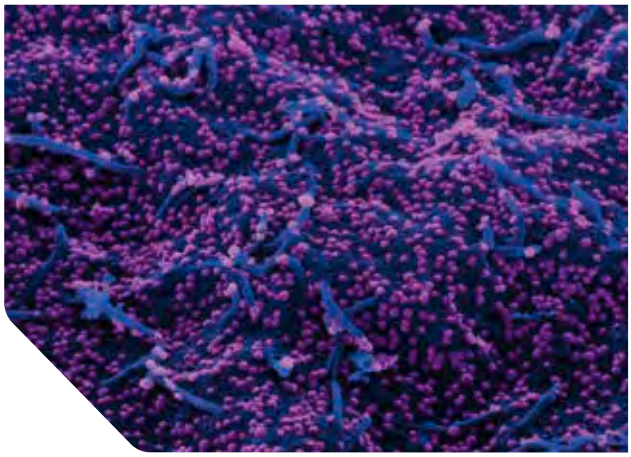
MODELING AND PREDICTION

Data scientists at HZI, like Michael Meyer-Hermann and Alice McHardy, contributed to combating the pandemic with the mathematical modelling of different situations.

The team of Michael Meyer-Hermann studied the spreading of the SARS-CoV-2 virus in Germany in different scenarios. With the mathematical model they developed, they were able to analyse the reproductive number of the SARS-CoV-2 virus, considering non-pharmaceutical interventions and the proportion of undetected cases. The same model was used to investigate an optimal strictness of interventions, taking into account both economic and public health objectives. More detailed modelling approaches allowed to simulate the impact of structures in individual factors like contact patterns and the daily routines of independent humans. Based on both models, the project CoViDec was initiated. This project explicitly aimed to develop models which include human decision making under uncertainty. In the project PANDEMOS, which was funded by the Federal Ministry of Transport and Digital Infrastructure, the impact of mobility aspects on the spreading of SARS-CoV-2 is further investigated.

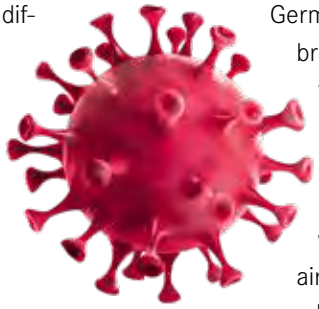
Scientists working in the biological safety level 3 laboratory at HZI © HZI | Susanne Talay



Renal epithelial cells infected with SARS-CoV2.
© HZI | Mathias Müsken

HZI's branch institute HIPS, Saarbrücken, during the pandemic:
HIPS directors Rolf Müller and Stephanie Thomas. © HIPS | Oliver Dietze

Alice McHardy and her team have developed CoVerage. This software analyses the lineage dynamics of SARS-CoV-2 strains and therefore assesses how successfully which virus strain spreads. This helps to analyse the ones currently on the run early on, per country and on a global level. Potentially emerging new variants of concern can then be identified. The web resource uses input from the Robert-Koch-Institute and the GISAID genome database. GISAID is an international initiative that aims to make research data rapidly available to the public. Scientists from all over the world contribute genome sequences of viruses such as H1N1, influenza or SARS-CoV-2 to the database. This provides a broad overview of mutations and their genetic differences. The team is also working on incorporating predictions of the antigenic "immune escape" of these strains into the analysis.



DIAGNOSTICS

A novel tool that might revolutionise the diagnostics of SARS-CoV-2 infections was developed by HIRI scientist Chase Beisel in close cooperation with Cynthia Sharma from the University of Würzburg. As is commonly known, the usual method to confirm an infection with SARS-CoV-2 virus is the PCR test. This test is very reliable as it detects the virus based on one single biomarker. However, if one wanted to know which variant of virus is present in a defined sample, additional tests are necessary, and that might waste valuable time if a result is urgently needed for the treatment of a patient. The new point-of-care diagnostic tool developed by the scientists in Würzburg is called LEOPARD and makes it possible to detect several different pathogens or variants thereof in just one test. The test is based on the CRISPR/Cas9 technique. The technology is very fast and efficient and can also be expanded to cover other disease areas including cancer (*see Chapter "Highlight Publications").*

STUDYING SPREAD AND COURSE OF THE DISEASE

The outbreak of COVID-19 in a meat processing plant in Germany received considerable media attention. This outbreak was studied by HZI scientist Melanie Brinkmann together with researchers from the University Medical Center Hamburg-Eppendorf and the Leibniz-Institute for Experimental Virology. They discovered that the working conditions in this industry – hard physical work, low temperature and air circulation through an air conditioning system with limited fresh air supply – are conducive to promoting the spread and transmission of SARS-CoV-2.

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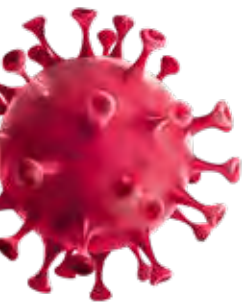
© Pixabay | matthewafflecat

HZI researchers Emmanuel Saliba (HIRI) and Yang Li (CiiM) and their teams have been co-leading a nationwide study on the different immune responses in patients with severe or mild SARS-CoV-2 infections. In order to understand why the virus causes no symptoms in most patients but is also able to induce life-threatening pneumonia, scientists of several institutions all over Germany studied blood samples from convalescent patients in Berlin and Bonn. For comparison, the blood of persons with other respiratory infections and of completely healthy individuals was also analysed. Applying state of the art single-cell-omics technologies, the researchers were able to look at single immune cells in the different blood samples, analysing the transcriptional activities in these cells. They found that certain types of immune cells (such as monocytes and neutrophils) were not acting properly in patients with severe COVID-19, impeding the immune reaction rather than amplifying it. This study paves the way to monitor patients and uncover therapeutic avenues aiming to set a normal immune answer in COVID-19 patients (*see Chapter "Highlight Publications"*).

> *Everyone knows that pestilences have a way of recurring in the world; yet somehow we find it hard to believe in ones that crash down on our heads from a blue sky. There have been as many plagues as wars in history; yet always plagues and wars take people equally by surprise.*
>
> *(Albert Camus, "The Plague", 1947)*



In severe cases of COVID-19, many patients develop an acute respiratory distress syndrome (ARDS) and require the provision of additional oxygen, supportive ventilation or even therapy with an artificial lung. The observed degradation of the lung tissue in these COVID-19 patients is particularly serious and associated with a high mortality. An interdisciplinary study co-coordinated by HZI scientist Emmanuel Saliba (HIRI) investigated the mechanisms of this lung failure, using multimodal single-cell analysis. The scientists could show that macrophages, a type of white blood cells, accumulate in the lung and interact with certain cells of the connective tissue, which are responsible for the formation of scar tissue. This leads to the overproduction of collagen and the observed effects (*see Chapter "Highlight Publications"*).

DRUGS AND ANTIBODIES AGAINST SARS-COV-2

HZI scientists were also involved in the development of possible treatments for COVID-19 right from the beginning of the pandemic. Amongst other approaches, they very quickly started to test already approved drugs for their effect on the SARS-CoV-2 virus. Thomas Pietschmann and his team (TWINCORE), for example, cooperated with scientists from Scripps Research (USA), who provided the so-called Re-FRAME collection of around 14,000 drugs and substances for which extensive safety data are already available regarding their use in humans, in order to identify compounds suitable for treating COVID-19.

The team of Ulrich Kalinke from TWINCORE is following another approach with his project MEMUMAB: here, the recombinant antibodies for the treatment of infectious diseases are being developed. In the blood of convalescent pa-

tients who previously underwent a COVID-19 infection or of vaccinated persons, different antibodies can be found that are able to neutralise the SARS-CoV-2 virus. By isolating specific B immune cells und analysing them on the single cell level, it is possible to decipher the DNA fragments encoding the variable regions of individual antibodies that are responsible for their specificity. After expression of these gene fragments in special cell lines, their effects on SARS-CoV-2 can then be studied. Promising candidates might later be produced on a larger scale and used as therapeutics in the future. This approach is highly versatile and flexible, making it very easy to react quickly to changing conditions, for example the onset of new virus variants.

Virologist Luka Čičin-Šain and his team, together with scientists from the TU Braunschweig and the biotech company YUMAB GmbH, were successful in identifying powerful antibodies against SARS-CoV-2 viruses generated by phage display techniques that are currently being tested in clinical trials.

PUBLICATIONS AND THIRD PARTY FUNDING INITIATIVES

Altogether, HZI scientists have published nearly 100 articles related to COVID-19 during the last two years, about one third of which appeared in top-ranking scientific journals.

They were also successful in securing substantial third party funds for research on COVID-19 and coordinated or participated in several international and national research consortia. Examples include the projects LOKI and CoViPa *(see below)*, which are funded in the framework of the Helmholtz campaign, as well as the consortium "Swift Coronavirus Therapeutics Response" (SCORE). SCORE is supported by the EU and aims to develop new antiviral drugs.

The consortium CORESMA, short for COVID-19 Outbreak Response combining E-health, Serolomics, Modelling, Artificial Intelligence and Implementation Research, also received substantial funds from the EU. Here, data from eHealth systems for the digital case management and from seroprevalence studies, like MuSPAD, are combined and analysed together. The aim is to answer questions related to the assessment of risks and measures to control the pandemic. Research institutions in Germany and several other countries are involved.

The COVID-19 Research Network Lower Saxony (COFONI) aims to pool Lower Saxony's expertise in COVID-19 research, and strategies for dealing with future pandemics are to be developed. COFONI is funded by the Ministry for Science and Culture of Lower Saxony.

PREVENTING/MANAGING FUTURE OUTBREAKS

As new human infectious disease outbreaks are expected to occur more frequently in the future, improved pandemic preparedness is imperative. Targeted agents adapted to the respective infection events are one of the essential components for future successful pandemic management. In this context, HZI together with the German Center for Infection Research (DZIF) proposed the establishment of a novel strategic alliance of academic science, industry, regulatory authorities and politics: the so-called National Alliance for Pandemic Therapeutics (NA-PATH). Once operational and conditional upon additional public funding, this alliance will carry out the necessary research and development of active substances in non-pandemic or pre-pandemic times, right up to the first clinical trials. The focus of NA-PATH will be on viral pathogen groups with high pandemic potential and will include the development of therapeutics with cross-pathogen efficacy, platform technologies and approaches for symptomatic therapy.

Blood sample © Adobe Stock | chompoo

The COVID-19 pandemic has shown us how unprepared we were in the defeat of such a global threat. To learn from this lesson, the Helmholtz Association is funding many projects under the roof of the campaign "The COVID-19 pandemic: awareness, management and preparedness". In interdisciplinary projects, scientists aim to develop solutions for coping with a pandemic, using the latest findings from research in different fields.

At the beginning of 2022, the project LOKI, coordinated by Michael Meyer-Hermann, started in cooperation with partners, including several Helmholtz Centres, with expertise from the fields of epidemiology, data protection, theory and simulation, machine learning, environmental monitoring and earth observation. The local public health authorities Akademie für Öffentliches Gesundheitswesen and the Robert-Koch-Institute also participate in the project. It aims

SUPPORT IN PANDEMIC RESPONSE:

ADVICE AND INFORMATION FOR DECISION MAKERS

With the COVID-19 outbreak, governments and decision makers in companies and non-governmental organisations faced difficult situations. They had to balance effective disease control measures with their undesired collateral effects on health economy and constitutional rights, such as mobility and physical integrity. This includes questions as to what extent governments should close schools, limit mobility and contacts and later enforce vaccinations.

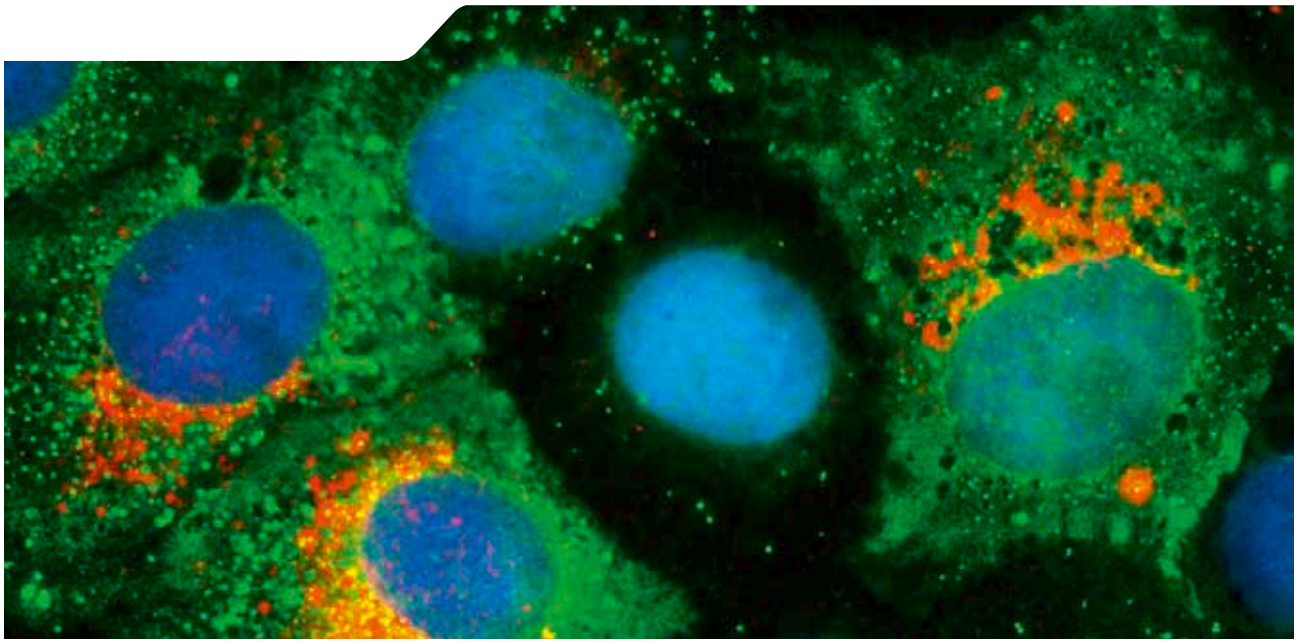
Virologist Melanie Brinkmann, epidemiologists Gérard Krause and Berit Lange, physicist Michael Meyer-Hermann and immunologist Luka Čičin-Šain were among the most visible HZI scientists providing advice, based on intensive contributions from a much larger number of scientists especially in the departments of Epidemiology and Systems Immunology.

HZI scientists repeatedly participated in government advisory bodies and served as invited experts in parliamentary hearings at federal and state level as well as for the Federal Constitutional Court. Together with eleven other experts, Brinkmann and Meyer-Hermann became members of a scientific advisory board established by Germany's newly elected chancellor Olaf Scholz at the end of 2021.

Some HZI scientists also served as advisors for the World Health Organisation, Institutions of the European Union and even for national governments outside Germany.

Furthermore, HZI conducted dedicated studies to generate specific evidence needed for developing response strategies to the pandemic. The centre initiated some of these studies, such as Germany's largest non-selective sero-prevalence study (MusPAD), funded by the Helmholtz Association *(see main text)*. Some HZI-studies resulted from direct requests by Government institutions, such as the study on pandemic response in the school setting funded by the 16 state ministries of education, or the implementation study of SORMAS sponsored by the Federal Ministry of Health, both run by the Epidemiology Department *(see chapter "Research Focus EPI")*.

The scientific methods that provided the most direct evidence for pandemic response decision making were primarily epidemiological models, systematic epidemiologic evidence synthesis, population based seroprevalence studies, prospective cohort studies among high-risk individuals, developments of digital health surveillance tools as well as differential serologic diagnostics.



SARS-CoV-2 infected Vero cells. Blue: cell nucleus, bright green: nucleocapsid protein and red: spike protein of SARS-CoV-2.
© HZI | Marco van Ham | CPRO | IMMI

to develop an integrated early warning system for the local detection, prevention and control of outbreaks related to infectious diseases and is focused on integration of the already developed system into daily practice. This includes the testing and the rolling out of the software in pilot projects together with public health authorities.

In addition to LOKI, scientists from seven Helmholtz centres are working together with partners from universities and industries in the project CoViPa (for Corona Virus Pathogenesis). What are the exact mechanisms of a SARS-CoV-2 infection? Which strategies can be used to combat the virus? And what are the risks that other pathogens might spill over to humans? These are the general questions addressed by this project, using SARS-CoV-2 also as a model for possible future outbreaks of infectious diseases. At HZI, several scientists are engaged in sub-projects of COVIPA. Luka Čičin-Šain is coordinating a project that studies the function of several immune cells and their communication via chemical messengers during an infection. He and his team are also testing antiviral substances that are known to block a host protein needed for virus replication. If such drugs are effective, they might be valuable in the current pandemic and beyond.

HZI scientists Dunja Bruder and Andrea Kröger are coordinating another project that tries to shed light on the mechanisms by which pathogens overcome natural barriers in the respiratory tract or the placental barrier. The results are not only important for SARS-CoV-2 infections but might also contribute to the understanding of infections with other viruses of a high pandemic potential, such as influenza A or Zika virus.

During the last two years, the SARS-CoV-2 virus changed the world in a way unthinkable before. It silenced cities, crashed world markets, kept people apart and caused despair in families who lost a member to the disease. However, it also accelerated the development of a new type of vaccine and made the general public aware of the fact that plagues and pandemics are not problems of the past, but can reoccur any time. Through their innovative research activities, HZI scientists will continue to make significant contributions to combating the current pandemic – but also to preparing for the next one.

Author: Kirstin Kleeberg

Explaining the pandemic: HZI researcher Melanie Brinkmann (right) with TV talk show host Anne Will. © NDR | Wolfgang Borrs

COVID-19: A CHALLENGE FOR SCIENCE COMMUNICATION AT HZI

Worldwide, the COVID-19 pandemic brought unwanted restrictions and burdens. Scientists at HZI were, in addition, confronted with completely unexpected professional challenges: many of them re-oriented themselves and contributed their expertise to new research subjects related to the corona pandemic. Almost everyone at HZI, whether researchers or administrative staff, soon realised that COVID-19 had put the Centre in the public spotlight in a way that no one could have imagined before.

SOUGHT-AFTER EXPERTISE

Almost overnight, the number of inquiries to the Centre skyrocketed - citizens turned to HZI, as did politicians and media journalists. From 2020 onwards, around 90 percent of the press office's communication activities suddenly revolved solely around the Corona pandemic. At peak times, the communications department received 60-70 inquiries per day that needed to be addressed. As a result, HZI experts were quoted much more in the media than in previous years: 27,400 mentions in 2020 and 19,200 in 2021 – compared to "only" 2060 in 2019.

The HZI website was accessed six times more often than before; the number of accesses online increased from about 25,000 sessions per month to 155,000 in some months in 2021. Currently, our page views are normalising again to about 40,000 per month. In the social media, the number of users also multiplied: on Twitter, the follower count is currently about 14,300 (as of the end of March 2022, starting from 2,780 followers 2019).

Despite the immense work of the press department, the immediate time and nervous strain on the scientists at HZI was enormous. Scientists such as Professors Melanie Brink-

mann, Michael Meyer-Hermann, Gérard Krause, Luka Čičin-Šain, Berit Lange, Peggy Riese and many others were invited frequently to so many talk shows, TV, radio and newspaper interviews as well as podium discussions or talks that they could only meet a fraction of these requests. Broadcasts included *Tagesschau, Heute Journal, Tagesthemen, Logo,* as well as talk shows like *Anne Will, Maischberger, Maybrit Illner, Hart aber fair* and *Markus Lanz.*

Particularly noteworthy in this phase was significant support provided by Science Media Center Germany (SMC), the independent science editorial office that supports journalists in their reporting. Through virtual press briefings, our experts were able to contribute content and effectively reach 100-150 journalists a time.

CHALLENGE: THE CANON OF SCIENTIFIC OPINION

Another challenge for the communication office was to orchestrate the polyphony of HZI's interdisciplinary experts for the media. We have scientists who can speak about virology, epidemiology and systems biology, as well as about vaccine development, drug development and antibody research. Critical discourse is the normal scientific process, but it sometimes provokes the public reaction that science should speak with one voice. That is why we have integrated higher-level perspectives into our assessments for the media in internal weekly press briefings. The authority to interpret these results was left to politicians. In addition, our experts were often invited to provide policy advice, e.g., in the Chancellor's Roundtable or the Minister Presidents' Conferences. Melanie Brinkmann and Michael Meyer-Hermann are currently members of the Federal Government's Council of Experts. Gérard Krause also advised many boards, e.g., the Bundestag, the Lower Saxony Parliament or the Academy of Sciences in Hamburg.

> *"In the Corona pandemic, mathematical modeling of infection events quickly became very important. The current infection dynamics were analysed and predictions for the further course of the pandemic were made on this basis. These predictions have been noticed at all levels of politics and have influenced decisions about countermeasures. The models have their limitations - but one can get a time advantage from these predictions and use it in policy. We can learn from the experience in the pandemic what mistakes we should not make in the climate crisis."*
>
> *(Michael Meyer-Hermann, Ernst Abbé Colloquium, October 20, 2021)*

© Tagesthemen from 16.12.2021

© Tagesthemen from 18.11.2021

© Tagesthemen from 9.10.2020

HZI experts on air (from left): Berit Lange, Michael Meyer-Hermann, Gérard Krause

© HZI | Verena Meier

FIT FOR MEDIA APPEARANCES

Some researchers had to acquire completely new skills, especially for TV appearances, which they were able to learn by one-to-one HZI media training for talk-shows or investigative interviews with journalists. At the sudden onset of the pandemic, this could often only be achieved through "training on the job", by preparing for and following up on media appearances. In this way, scientists could be primed to spot investigative questioning techniques and answer fluently despite stress before the camera. Limiting oneself to one's own area of expertise and competence is also something that needs to be trained for. The acquisition of media skills will also prepare our young scientists for their possible appearances in future.

NOT ONLY APPROVAL

Unfortunately, those viewed on mainstream TV and social media do not always get positive reactions. Inaccurate and catchy headlines in newspapers exacerbate this problem. All our experts gained this experience in one form or another. While grateful letters from citizens where in the majority, the longer the pandemic went on, more insults and hate mail punctuated the scientists' daily routine. Colleagues were and are shielded by specially briefed staff.

After the BILD campaign "Die Lockdown-Macher" of December 4, 2021 and an over-heated atmosphere with anti-vaccination demonstrations, the topic of personal protection was considered for the first time for one of our experts.

© ZDF | Svea Pietschmann

> *"If negative feedback arrives in my mailbox or on social media after press appearances, my initial reaction is to withdraw from the public eye. But after a short while, when the wave has passed, I feel obliged to continue to reach out to the public - out of a sense of duty and responsibility. Transparency and education is so important. Comments on Twitter are sometimes unbearable – the best way to deal with it is not to read them."*
>
> *(Melanie Brinkmann)*

FACTS VERSUS FAKE NEWS

In science communication, it remains important to take every question from non-experts seriously to engage in proper dialogue - whether about vaccination, anti-corona measures or multi-resistant germs. We try to link scientific results to everyday experience and explain their significance by vivid examples. If we can factually prove that something is wrong, we put it right. But we avoid fierce battles on social media.

What we all have in common is that we like to work at the interface between science and communication. Transfer of knowledge to society is part of our mission and our strategy – and we enjoy it, just as much as our scientists enjoy their exciting research projects.

Susanne Thiele, Press Spokesperson
and Head of Public Relations at HZI

First-hand information on pandemic threats: Lower Saxony's Prime Minister Stephan Weil (middle) and Minister for Science and Culture, Björn Thümler (right), visiting HZI. Left: HZI's Scientific Director Dirk Heinz. © HZI | Verena Meier

SCIENCE FOR THE PUBLIC

HIGHLIGHTS OF THE YEARS 2020 AND 2021

Politicians Visiting HZI

In 2020, several politicians visited HZI and its sites: Lower Saxony's Prime Minister Stephan Weil and the Minister for Science and Culture Björn Thümler visited the HZI campus in Braunschweig on 8th May 2020. They discussed the current challenges in infection research with several HZI researchers, in particular the ongoing COVID-19 pandemic. Federal Minister of Education and Research Anja Karliczek was welcomed at HZI in Braunschweig on 25th May 2020. She was accompanied by Björn Thümler, and received information about the latest findings in SARS-CoV-2 research by HZI. Both visits were well attended by the press. On 23rd August 2021, Lena Düpont, Member of the European Parliament, visited the Braunschweig campus. She was updated on international collaborations involving HZI, and shown the Biosafety Level 3 Laboratory to learn about SARS-CoV-2 research at the centre.

Update on COVID-19: Anja Karliczek, Federal Minister for Education and Research, with Björn Thümler (right) and Dirk Heinz (left).
© HZI | Verena Meier

Soil samples from Saarland's citizens: drug research at HZI/HIPS is supported by volunteers. © HIPS | Uwe Bellhäuser

Public Outreach

In order to kick off the vaccination campaign against SARS-CoV-2, a digital information event organised by the Lower Saxony Ministry for Social Affairs, Health and Equality was held on 21st January 2021, where experts from science, medicine and politics provided information on vaccines. Due to new vaccine technologies and their rapid development, there is a great need for information among the public. HZI's press spokesperson Susanne Thiele moderated the discussion. Dirk Heinz, Scientific Director of the centre, provided information on the mode of action and composition of mRNA vaccines. Other participants were Carola Reimann - Lower Saxony's Minister for Social Affairs, Health and Equality, Matthias Berndt - head of the General Practitioners' Association in Lower Saxony, and Prof Stefan Dübel - head of the Department of Biotechnology at the Technische Universität Braunschweig. Citizens could participate by submitting questions to the experts in advance or live at the event itself.

At the annual meeting of the Alexander von Humboldt Foundation on 26th June 2021, HZI was showcased to several of the approximately 800 international guests during a one-hour virtual "Lab Visit". HZI researchers from the Department of Vaccinology and Applied Microbiology were on tap for an English-language presentation of their work and a question-and-answer session. Focus was on exchange among researchers; the participants came from different fields ranging from life sciences to humanities.

In the "Sample' das Saarland" project, the public are encouraged to help in the search for active, potentially therapeutic, compounds from nature. The citizen scientists take soil samples and thus support HZI at its Saarbrücken site HIPS in the discovery of bacterial strains producing such active compounds. Since the start of the project in 2017, more than 1000 myxobacterial strains have already been newly isolated from the samples analysed so far. The strains identified have been cultivated and screened for novel natural compounds showing antimicrobial activity. Accordingly, several new candidate substances have already been identified using the HIPS well established drug discovery pipeline.

Guided campus tour: Lower Saxony's Prime Minister Stephan Weil (front row, right) and Minister for Science and Culture, Björn Thümler (next left) with HZI scientists. © HZI | Verena Meier

To honour the laureate, HZI named a street on the HZI campus after Emmanuelle Charpentier © HZI | Verena Meier

HIGHEST HONOUR FOR FORMER HZI SCIENTIST

EMMANUELLE CHARPENTIER AWARDED NOBEL PRIZE 2020

Emmanuelle Charpentier, a former researcher at HZI, was awarded the Nobel Prize in Chemistry in 2020. The Royal Swedish Academy of Sciences honoured her pioneering work on the CRISPR-Cas9 gene editing technology. She shared the prize with Jennifer A. Doudna. Charpentier had been head of the HZI department "Regulation in Infection Biology" from 2013 to 2015.

Considered a revolution in the fields of medicine, biotechnology and agriculture, the CRISPR-Cas9 technology is a powerful and versatile tool for targeted and efficient modification of any gene sequence in the cells of living organisms. CRISPR-Cas was originally described as an adaptive immune system in bacteria and archaea to fend off viral attacks.

In 2011, Emmanuelle Charpentier - then working at the Laboratory for Molecular Infection Medicine Sweden (MIMS) at Umeå University - and her laboratory at the Max Perutz Labs of the Vienna University published a seminal article in *Nature*. They described the identification of tracrRNA as an essential component – together with the CRISPR RNA and Cas9 – for the activation of the CRISPR-Cas9 viral defence mechanism in the human pathogen *Streptococcus pyogenes* and other bacterial species.

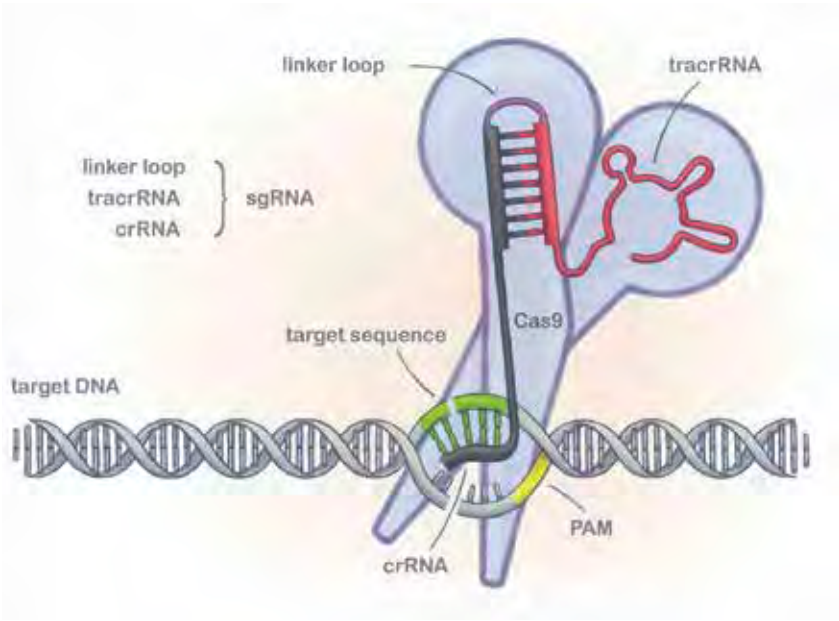
Emmanuelle Charpentier receiving her Nobel Prize medal and diploma © Nobel Prize Outreach. Photo: Bernhard Ludewig.

One year later, in 2012, Charpentier and her laboratory team were able to demonstrate that CRISPR-Cas9 is a dual-tracrRNA-CRISPR RNA enzyme that cleaves DNA in a sequence-specific manner. The system was then developed into a precise gene-editing tool that can correct defective DNA, much like a text editing software can edit or correct typos in a document. The details of the DNA targeting mechanism by the CRISPR-Cas9 system and guidelines for its use as a versatile genetic tool to alter the genome of cells and organisms were published in the journal *Science* in 2012, with the study conducted in collaboration with Jennifer Doudna's laboratory at the University of California, Berkeley.

These fundamental discoveries allowed subsequent applications of the CRISPR-Cas9 gene editing technology in many different scientific fields, including human and veterinary medicine, agriculture and biotechnology. Its broad applicability, versatility and ease of use are the reasons why it was so quickly adopted by the scientific community. The results of the CRISPR research already had a tremendous effect on the life sciences, such as a better understanding of basic mechanisms of life or the development of disease models and screening. While the technology is already in the starting blocks to be marketed in agriculture, the task in the field of medicine in the coming years will be to translate it into safe and effective therapies for severe human diseases for which there are currently no treatment options.

"We are very pleased that Emmanuelle Charpentier's outstanding scientific achievements are now also being honoured by the Royal Swedish Academy of Sciences," says Dirk Heinz, Scientific Director of the HZI. "The CRISPR-Cas9 system has a huge impact on genetic engineering and biomedicine. We are very proud of Emmanuelle and warmly congratulate her and Jennifer Doudna on this tremendous success."

To honour the laureate, HZI named a street on the HZI campus after her *(see photograph)*.



CRISPR-Cas9, the RNA-programmable "DNA scissors": The enzyme Cas9 (blue, represented as scissors) is guided by a single guide RNA (dual-tracrRNA(red)-crRNA(black), linked together with a linker (purple)) programmed to cleave the target DNA in a sequence specific manner. A PAM sequence (yellow; NGG for the Cas9 of *Streptococcus pyogenes*) located downstream of the targeted DNA sequence on the non-target sequence is required. *Deltcheva et al., 2011 (Nature); Jinek, Chylinski et al., 2012 (Science); Drawing by Linnea Holmström Ljung.*

"I am truly amazed at the speed at which CRISPR research and applications in so many diverse areas of the life sciences have developed in recent years. I look forward to seeing new developments in this genome editing and engineering technology, particularly as a gene-based medicine to treat serious human diseases."

Emmanuelle Charpentier

© Halbauer & Fioretti

ABOUT EMMANUELLE CHARPENTIER

Emmanuelle Charpentier studied biochemistry, genetics and microbiology at the University Pierre and Marie Curie (now Sorbonne University) in Paris, where she received her PhD in microbiology for her research performed at the Pasteur Institute. After her studies in France, she spent more than five years working in the United States, where she was a research associate in New York at the Rockefeller University, Skirball Institute of Biomolecular Medicine and New York University Langone Medical Center, and in Memphis, Tennessee at the St Jude Children's Research Hospital.

In 2002, she moved back to Europe to lead her first independent research group as a visiting, assistant and associate professor at the Max F. Perutz Laboratories (now Max Perutz Labs), University of Vienna in Austria, where she completed her habilitation in Microbiology in 2006.

Prior to her current position and until 2017, Emmanuelle Charpentier was Associate Professor at the Laboratory for Molecular Infection Medicine Sweden (MIMS, within the Nordic EMBL Partnership for Molecular Medicine) and visiting professor at the Umeå Centre for Microbial Research (UCMR), Umeå University, where she habilitated in Medical Microbiology in 2013. She was also Alexander von Humboldt Professor and Professor at the Hannover Medical School in Germany. From 2013 to 2015, she led the department "Regulation in Infection Biology" at HZI in Braunschweig. Already during her time in Braunschweig, she was awarded with numerous internationally renowned prizes.

Emmanuelle Charpentier is considered a world-leading expert on the regulatory mechanisms underlying the processes of infection and immunity in bacterial pathogens. She is now Scientific and Managing Director of the Max Planck Unit for the Science of Pathogens in Berlin, an institute that she founded together with the Max Planck Society. She also holds the position of an honorary professor at the Humboldt University in Berlin.

PRIZES AND AWARDS FOR HZI SCIENTISTS

Selected scientific awards in 2020

Awarded scientist	HZI unit*	Award	Awarding institution
Antoine-Emmanuel Saliba	SIGA	EMBO Young Investigator	EMBO
Susanne Häussler	MOBA	DGHM Hauptpreis	DGHM
Claus-Michael Lehr	DDEL	PHOENIX Pharmazie Wissenschaftspreis	PHOENIX group
Alice McHardy	BIFO	Listed as "Highly Cited Researcher"	Clarivate Analytics
Jörg Vogel	RABI	Listed as "Highly Cited Researcher"	Clarivate Analytics

Selected scientific awards in 2021

Awarded scientist	HZI unit*	Award	Awarding institution
Müller Rolf	MINS	Gottfried Wilhelm Leibniz-Preis 2021	DFG
Chase Beisel	RSYN	Medical Valley Award	Bavarian State Ministry of Economic Affairs, Regional Development and Energy
Jörg Vogel	RABI	Appointment as President of the European Academy of Microbiology (EAM)	European Academy of Microbiology
Neva Caliskan	REMI	ZONTA Wissenschaftspreis	International Zonta Club Würzburg
Chantal Bader	MINS	Hans-and-Ruth-Giessen Scholarship	Hans-and-Ruth-Giessen-Foundation
Alice McHardy	BIFO	Listed as "Highly Cited Researcher"	Clarivate Analytics
Jörg Vogel	RABI	Listed as "Highly Cited Researcher"	Clarivate Analytics
Marc Stadler	MWIS	Listed as "Highly Cited Researcher"	Clarivate Analytics

Selected grants for HZI scientists in 2020 and 2021

Scientist	HZI unit*	Grant	Granting Agency
Yang Li	BIIM	ERC Starting Grant	European Research Council
Neva Caliskan	REMI	ERC Starting Grant	European Research Council
Gregor Fuhrmann	BION	ERC Starting Grant	European Research Council
Till Strowig	MIKI	ERC Consolidator Grant	European Research Council
Anna Hirsch	DDOP	Innovative Training Network (ITN)	European Union
Jochen Hühn	EXIM	Innovative Training Network (ITN)	European Union
Anna Hirsch	DDOP	*Pseudomonas aeruginosa* infections in cystic fibrosis patients	Combating Antibiotic Resistant Bacteria (CARB-X)
Mark Brönstrup	CIBO	Small-molecule inhibitor of the *S. aureus* α-hemolysin	Combating Antibiotic Resistant Bacteria (CARB-X)
Martha Böning	IREG	Development Grant Seed Funding for Young Investigators	SFB854 and Health Campus Immunology, Infectiology and Inflammation, OVGU Magdeburg

* see organisational chart for complete names

SCIENTIFIC EVENTS 2020/21

The COVID-19 pandemic led to the cancellation of several planned meetings and events at HZI and its sites, in particular in the early phase in 2020. Nevertheless, scientists found opportunities for exchange among colleagues – on many occasions in a virtual or hybrid format, with video conferences replacing the in-person-meetings partially or in total.

Helmholtz Drug Discovery Conference HDDC 2021

Together with colleagues from Helmholtz Centre Munich (HMGU), scientists of HZI organised the virtual Helmholtz Drug Discovery Conference HDDC in March 25–26, 2021. The meeting focused on novel proteolysis targeting chimeras and new treatment strategies against SARS-CoV-2. The renowned scientists Dirk Trauner (NY University), Fleur Ferguson (UC San Diago), Dan Nomura (Berkeley) and Patrick Cramer (MPI-BPC, Göttingen) were speakers and joined the conference together with 900 participants.

Opening of the new drug discovery building

The newly constructed drug discovery building on the HZI campus, funded by the state of Lower Saxony and the BMBF, combines complementary knowledge from DSMZ and HZI on strain collection, fermentation and synthetic derivatisation to identify and develop future drugs. Seven different research units from the two organisations share the new building. Situated north-east on the campus, it occupies a formerly empty site. The planning started in 2013 and could be finalised after four years of construction in 2020. The building was officially inaugurated in spring 2021.

From molecules to patients to communities

After several common events, HZI and McGill University (Canada) jointly organised a virtual symposium. The event was dedicated to the subject of personalised infection research. It was also the start of the "Infection and Immunity Week", a McGill signature event to celebrate their bicentennial anniversary, and was held in the context of the 50th anniversary of the German-Canadian scientific and technological cooperation. Speakers included scientists from HZI and McGill University as well as the cofounder and CEO of BioNTech SE, Ugur Sahin, who gave the keynote lecture. Around 400 participants mainly from Germany and Canada attended, among them researchers, scientists, medical doctors, dignitaries, industry representatives, embassy delegates as well as HZI Alumni.

HZI/EMBL Symposium

At the end of October 2021, a joint hybrid symposium was held between HZI and EMBL with around 200 participants. Talks focused on "Interfaces" at the organism, intercellular and intracellular level and their specific modulation.

Vaccination Symposium in Hannover

The 25th Vaccination Symposium, a medical education event at MHH, was hosted by the Department of Gastroenterology, Hepatology and Endocrinology of MHH and CiiM in November 2020 and was dominated by COVID-19. Thus, the traditional event did not take place in a full lecture hall as usual, but in the meantime reached more than 300 participants in the virtual format. Besides COVID-19 and the development of vaccines against the disease, talks focused on the prevention of viral hepatitis, vaccination recommendations for patients with chronic diseases, travel vaccinations and vaccinations in children and adolescents.

© HIPS | Yannic Nonnenmacher

Focus on pharmaceutical research: HIPS Symposium

The HIPS symposium, organised by HZI's Saarbrücken branch, the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), brings together renowned scientists and young investigators from three pharmaceutical communities: natural products, medicinal chemistry and drug delivery. It provides a forum for scientists to exchange ideas while crossing boundaries of classical disciplines. At the same time it gives young investigators the opportunity to obtain valuable feedback on their projects by international experts in the respective fields.

In 2020, the conference had to be cancelled due to the COVID-19 restrictions.

In May 2021, the HIPS symposium took place in the form of a one day hybrid meeting, including digital talks, poster and round-table sessions.

Twincore Symposium

The TWINCORE Symposium has established itself as an important platform for topics at the interface between basic and clinical research.

In September 2020, the 12th TWINCORE symposium was held, entitled "SARS-CoV-2, news from Lower Saxony". It was opened by a speech of the Minister for Science and Culture from Lower Saxony, Björn Thümler, and comprised talks from scientists from Braunschweig, Göttingen and Hannover.

In September, the 13th TWINCORE symposium took place as a webinar with a focus on SARS-CoV-2 research in Lower Saxony and in Germany. The meeting reported on "Vaccination and vaccine responses" and offered junior scientists the opportunity to get in touch with renowned experts through workshops. Among the speakers was also Özlem Türeci, co-founder of BioNTech SE.

© TWINCORE | Grabowski

© TWINCORE

Inhoffen Medal for outstanding pioneer work

The Inhoffen Medal, the most prestigious German award in the field of natural product chemistry, is awarded by the HZI Förderverein (Friends of HZI) since 1992. Until 2018, it was endowed with 5,000 Euros. Since 2019, the prize money amounts to 8,000 Euros.

The awarding ceremony is associated with the "Inhoffen Lecture" in honour of the biochemist Prof. Hans-Herloff Inhoffen, an event organised jointly by HZI and TU Braunschweig (TU-BS). Inhoffen was director of TU-BS and co-founder of the Institute for Molecular Biology, Biochemistry and Biophysics (IMB), from which HZI subsequently emerged.

In 2020, the prize was awarded to Christian Hertweck, Leibniz Institute for Natural Product Research and Infection Biology. Due to the pandemic, the award ceremony was postponed. It is scheduled for June, 2022, to be held as an in person event.

In 2021 no Inhoffen Medal was awarded.

Christian Hertweck was awarded with the Inhoffen Medal in 2020
© Leibniz-HKI

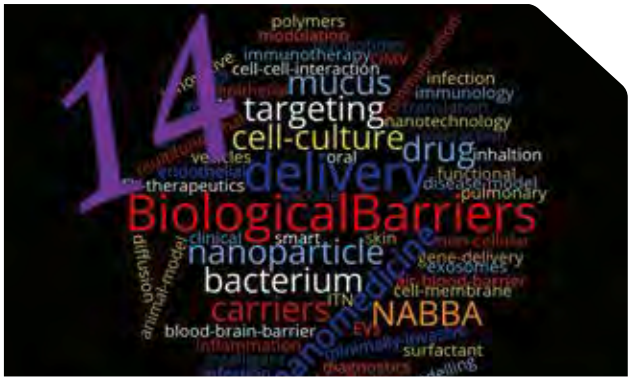
Kilian Schober, awardee of the Jürgen Wehland Prize 2020.
@ Susanne Dürr, TU München

Jürgen Wehland Prize

With the Jürgen Wehland Prize for young scientists in infection research HZI has been honoring outstanding young investigators since 2011. The prize is named after HZI's former Scientific Director Jürgen Wehland, who passed away during his term of office in 2010. In 2020, the prize went to Kilian Schober who just had been appointed as a professor at the University clinics in Erlangen. Due to the pandemics the award was given in an online meeting.

"Biological Barriers" conference at HIPS

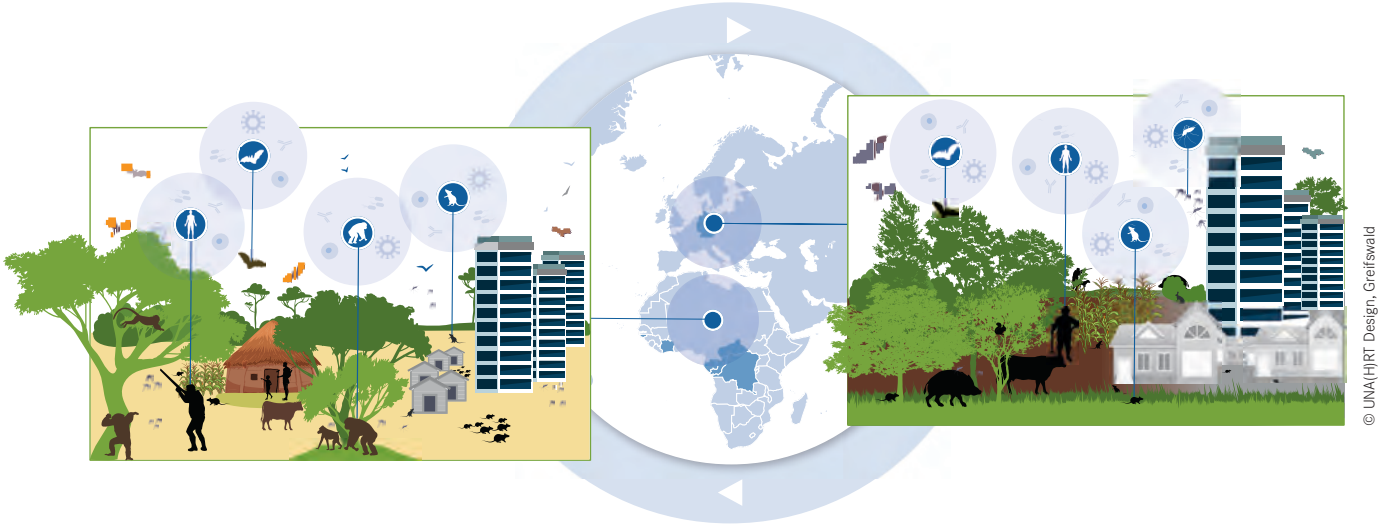
The "International Conference and Workshop on Biological Barriers" is organised every two years jointly by HZI's Saarbrücken branch, HIPS, and Saarland University (UdS). Target audiences are early stage researchers as well as experienced scientists and professionals from academia and the pharmaceutical industry; the meeting regularly receives more than 200 registered attendees from all over the world.



"THE ONE HEALTH CONCEPT IS KEY TO PANDEMIC PREPAREDNESS"

INTERVIEW WITH FABIAN LEENDERTZ, FOUNDING DIRECTOR OF THE HELMHOLTZ INSTITUTE FOR ONE HEALTH (HIOH) IN GREIFSWALD

How likely is it that an event like the COVID-19 pandemic will happen again? According to the "One Health" concept, this depends largely on interactions between humans, animals and the environment. In order to investigate these interactions in an interdisciplinary way, HZI and its local partners in Greifswald have established a dedicated research institute – the "Helmholtz Institute for One Health" (HIOH) *(see also section "Partners, Sites and Networks" in this report)*. Its founding director, Fabian Leendertz, plans to lay the foundation for an effective pandemic preparedness in the future. This includes the continuous surveillance of emerging and circulating pathogens as well as monitoring the increase in antibiotic resistance – ideally on a global scale.



© UNA(H)RT Design, Greifswald

Prof. Leendertz, what exactly is "One Health" – and why is research into this subject necessary?

The One Health concept recognises the interconnectedness between the health of humans, animals and the environment. Traditionally, life sciences pursue a specialist approach, studying different subjects in isolation. We look at animal health in isolation, human health in isolation, and environmental issues in isolation. One Health deals with the interactions between these areas. A very simple example: more trees in a city are better for the climate, and better for your health as well: they have a large variety of outcomes. It is a very broad view.

But you surely have to narrow down your view in some way when conducting research?

Of course. Here at HIOH, we just look at one part of One Health, namely infectious diseases. In particular, we are interested in zoonoses – infectious diseases that can affect both animals and humans – and antimicrobial resistance. But we always take into account that the health of the

How do environmental changes in tropical countries affect the local communities?
This is one of the questions researchers like Fabian Leendertz strive to answer.
© Katrin Nowak

environment, humans and animals are intertwined. That is the basic assumption of this concept.

What questions are you working on?

One important example is the question how the environmental changes we are seeing especially in the tropical countries, like deforestation and the very significant local climate change, impact communities. We look at the abundance and diversity of small mammals in the forests as well as in the villages. We also investigate how these factors impact the risk of the emergence of zoonotic diseases. We would like to understand how and where people come into contact with these animals; and how these changes translate into outbreak or transmission risk.

Can you give an example for a research project related to this issue?

For example, we studied one species of bats, which lives in close interaction with humans in African villages. Many of these animals carry Hanta viruses, and they sleep in the roofs of huts which are covered with palm leaves. And this is where people of the village meet and discuss current affairs: you can thus imagine there is a quite direct interface between humans and bats. The question now is: does the Hanta virus transmit to people via this route and how significant is this route?

Would that not be quite obvious?

You might think so, but in some of these regions the availability of diagnostics is very limited – even in hospitals. You may well notice that someone is sick, but often you cannot diagnose a Hanta virus infection in a reliable manner. Detecting the route of transmission is even more difficult. And this is why we are striving to enhance awareness among the local medical staff, and improve the local diagnostic capacities.

If there is such an obvious need for this kind of research, why haven't many others come up with the same idea and started institutes like HIOH all over the world?

There are actually several One Health institutes, especially in the United States. But most of these have a very narrow and strongly defined focus. They investigate, for example, influenza. This is indeed a very interesting subject, involving research in birds, in humans, and the environment. But the broad diagnostic view in research, which should adopt a longitudinal approach to understand the dynamics of the system is quite rare. I am not aware of any other institute actually following this path.

How does this translate into everyday research practice? What distinguishes you from other institutes?

First of all, it is the long-term perspective. We often have three- or five-year projects or assignments on even longer terms. I myself have previously been working on a long-term project investigating disease spread among primates in the wild – chimpanzees, gorillas, and bonobos. For such a task, researchers need to adopt this long-term approach. For instance, it already takes three to five years until you have habituated these wild animals to human presence. The animals roam about the forest and it takes all this time to show them that they need not be afraid of us. And only once you have achieved this can the real research actually begin. The chimpanzee project in Ivory Coast, for example, has existed for 40 years; that in Gombe, Tanzania, exists 50 years, and

all the projects I am or have been involved in have been going on for a very long time.

Apart from monkeys in the rain forest: How does this work in laboratories and clinics?

There are, for example, long-term clinical studies involving patient cohorts, where samples are taken prospectively and the health of the study participants is observed over a long period of time – like, for example, in the German National Cohort Study, the GNC *(see section "Partners, Sites and Networks" in this report)*, or the Study of Health in Pomerania, the SHIP cohort. We intend to cooperate intensively with those colleagues, and we will pursue this research inside a One Health frame. But most important to us, again, is the big picture. When a new pathogen emerges, we ask where did it come from; what is likely to happen next; and, of greatest concern, which viruses or bacteria are plausible candidates for the next pandemic threat? Again; this depends largely on interactions between humans, animals and environment. I am convinced the One Health concept is key to pandemic preparedness.

Are you going to focus on certain pathogens or medical questions?

No, not on specific pathogens. As for medical questions, respiratory diseases, neurological diseases, fevers that are not caused by malaria: these are the main areas we are interested in. We also have some aspects of severe gastroenteric diseases in our African cohorts at the moment. And of course, antimicrobial resistance is a key issue for us.

What is the role of systems biology and computing in your approach?

The potential of computing, machine learning and modelling for this – if you will – "disease ecology" is enormous. So, I am in very close contact with people engaged in modelling scenarios for different aspects of disease spread and ecology. Many of them admit that a major problem is that the data on which they base their models are rather weak. They use, for example, serological data from decades ago. And when you complain about that, they say: "This is all we have". This clearly shows that we need better data.

And you at HIOH can provide these better data?

Obviously we cannot create a huge impact just by ourselves. We have limited resources and can only do a "deep dive" in two or three regions, but not all over the world. We rather hope to help by creating a template for others in order to obtain more comparative data. However, we are already in contact with the new WHO hub in Berlin. They are very interested in what we will be doing because they also need these kinds of data for the models they will run. And we are also in contact with colleagues at HZI and other institutes who have wide experience in answering these kinds of questions.

Field work in Africa. Members of Leendertz' team in Guinea © Kathrin Nowak

© Kathrin Nowak

What is your vision for the future?

In the next five years, I hope we can prove that our longitudinal One Health surveillance units can become a reality and that they will generate data which are of direct relevance for decision makers – and also for the local communities. And perhaps we will begin to see others emulating this approach. In ten years, we may even see a whole network of "One Health weather stations" around the world, monitoring the important parameters of current and imminent disease outbreaks. They will of course not all be run by us – that would be unrealistic, but we want to be part of such a larger, perhaps global, concept and contribute accordingly. That would really be a goal to go for.

Interview: Manfred Braun

Photos: © Stefan Sauer

FABIAN LEENDERTZ heads the newly founded Helmholtz Institute for One Health in Greifswald and one of its three departments: "Ecology and emergence of zoonotic diseases". In addition, he is also a professor at the University of Greifswald.

His scientific focus lies on understanding the "where", "how", and "why" of microorganism transfer from one species to another, while a central aim is also to elucidate the importance of pathogens and microorganisms for animal populations. To address these questions, he worked on viruses, bacteria, parasites, and more recently also bacteriophages. Each project builds on a One Health framework, including both data on the host (e.g., behavior, ecology, evolutionary history) and the surrounding environment. Each of the projects has a rather practical focus, aiming at translation of research into practice. Fabian Leendertz published more than 170 papers, several in high-ranking journals such as *Nature, Science, Nature Microbiology*, and *PNAS*.

In addition to this scientific focus, the building of long-term cooperations with African partners and the establishment of international networks are key to his research framework; for all his field work, regional partners are involved in the planning and implementation of the projects, including, if possible, laboratory work, analysis, and dissemination of results.

More information about HIOH in the section "Partners, Sites and Networks" of this report.

Campus UdS | © AdobeStock | Gugu Mannschatz
Photos: left: Rolf Müller © Oliver Dietze;
right: Manfred Schmitt © Iris Maurer

"THIS ENVIRONMENT IS CERTAINLY UNIQUE IN GERMANY, IF NOT IN EUROPE"

INTERVIEW WITH MANFRED SCHMITT, PRESIDENT OF SAARLAND UNIVERSITY, AND ROLF MÜLLER, DIRECTOR OF THE HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL RESEARCH (HIPS) IN SAARBRÜCKEN

It often takes many years before a new active compound from basic research hits the market as a drug. In order to accelerate this process, HZI and Saarland University (UdS) will work together even more closely in future. The long-standing partnership of the two institutions took shape in the joint foundation of HZI's branch in Saarbrücken, the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), in 2009. In their new common project, the planned "Research Centre for Bioactive Compounds", HIPS and UdS will interact closely with both the University Hospital in Homburg and the pharmaceutical industry. The federal and state governments will invest 70 million Euro in this new project. The overarching goal is to bring basic research discoveries into clinical application - a process known in pharmaceutical research as translation.

Prof. Schmitt, Prof. Müller, why are you so particularly interested in pharmaceutical research?

Müller: I am a pharmacist by training, so no wonder I am interested in pharmaceutical research. But I think the obvious fascination with this sort of research is its intrinsic interdisciplinarity. In pharmacy, part of the curriculum encompasses medicine, another part deals with chemistry, yet another biology. And that makes it interdisciplinary from the start. In fact, this very interdisciplinarity is what we are now extending with new recruitments in these areas.

Schmitt: At Saarland University, the Department of Pharmacy is part of our life science cluster within our priority research area NanoBioMed. This highly cross-disciplinary field of pharmaceutical and life science research is tightly connected with the Department of Informatics as well as with our Medical Faculty and the Saarland University Medical Centre at the campus in Homburg.

Prof. Müller, you have shown a very clear focus on natural compounds in your research career. Do you still see this as the most promising field for both HIPS and UdS to focus on?

Müller: We built an institute for pharmaceutical research together, and part of it involves natural products research. It is a bastion of antibiotics research, since most antibiotics used in the clinic are derived from natural compounds – substances produced by plants, fungi or bacteria. And we see that here in Saarbrücken, we have a cutting edge advantage in this type of research. Nevertheless, pharmaceutical research goes much further. Our medicinal chemistry and drug delivery departments are also internationally visible, and these are essential for the broad idea of drug development. We do not just want to find interesting molecules, but eventually bring them to medical application. And this requires a much broader scientific base than natural products research alone.

Schmitt: I strongly believe that the exceptionally tight and fruitful alliance between the HIPS and Saarland University resembles a unique scientific environment with a local constellation that can only be rarely found in Germany. Of course, natural product research in the Saarland is in particular driven by Rolf Müller and his colleagues at HIPS. However, the expertise in this field is likewise complemented by leading scientists from Saarland University, for example in the fields of organic chemistry, systems biotechnology or clinical bioinformatics, with whom Rolf Müller and his HIPS colleagues have been working together for many years with great success. By joining scientific forces and excellence in this field, both partners have achieved a high international standing which makes the overall scientific environment, at least in my eyes, unique in Germany, if not in Europe.

Now you have jointly founded the Research Centre for Bioactive Compounds. Why another institute? What are the aims of this centre?

Müller: As a result of our long-standing successful cooperation, the German Bundestag has decided to significantly expand HIPS. We now aim to optimise this process by making use of already well established interactions on site, and further integrate pharmaceutical research with informatics and medicine. We are confident that the synergies we create will not only profit the researchers on site in Saarbrücken, but also translate into new projects and connections with groups at HZI, including its institutes, and within the German Centre for Infection Research, DZIF.

So you want to utilise computer science and clinical know-how to enhance the development of drugs – is that the goal?

Schmitt: This indeed is an important part of it, the keyword here is data-driven drug development. The newly established translational centre for bioactive compounds will address this aspect and likewise strengthen our NanoBioMed focus by close interactions between life sciences, clinical medicine and computer science – our second focus area at Saarland University. I am convinced that conspicuous innovation can be expected from research at the borders between these fields. I would like to add that our State Government in the Saarland strenuously supports the formation and joint extension of this new research focus such that we now have the unique opportunity to combine the substantial expansion of a Helmholtz Institute with a corresponding focus of the partner university.

"We managed to have the recruitments completed. So the brains are already there."

What are the next important steps you need to take?

Schmitt: The key objective right now is to secure and realise the new building as soon as possible. We have already successfully installed additional new professorships which scientifically bridge three faculties of Saarland University with the HIPS – and the Translational Centre for Bioactive Compounds will be a central unit of our joined research campus here in Saarbrücken. Therefore, it is equally important for HIPS as well as for the university that we can start building up this science facility really soon.

Müller: You may be surprised by the President's answer, because typically you would say: we need the right brains first, and then can talk about the bricks. But this actually illustrates how successful our collaboration has been: less than one and a half years after our application for the extension measures was approved, we already managed to have the recruitments almost completed. So the brains are already there.

"It's all about good science and bringing together the best scientists."

Schmitt: And we really look forward to hopefully soon have our new colleagues on board. However, an essential prerequisite for the successful hiring will be the new building on campus. In fact, this new building and joint workspace issue is the greatest challenge that we are currently facing.

Since the know-how is already delivered, can you give an example what concrete projects you will pursue with the new centre?

Müller: One of the major projects started a couple of years ago. We launched a larger programme studying the human microbiota together with a number of colleagues in the clinics and in informatics. We have already obtained more than one thousand clinical samples, which are currently being sequenced. Here, the informatics department is now playing its role. We bring together knowledge about the microbiota and its interaction with the clinical state of the patient, and then want to investigate which factors – in terms of microbes and also metabolites – determine the patient's fate. This is a major programme which we have initiated and want to extend.

Schmitt: We already successfully installed several HIPS/UdS tandem research projects. We use substantial co-funding of the University and HIPS to foster joint research projects between HIPS, clinical medicine and the informatics department, which run very successfully. In total, this amounts to nineteen joint projects.

© Oliver Dietze

Myxobacteria, like *Chondromyces crocatus* shown here, produce a variety of natural compounds which can be further developed into drugs. Pharmaceutical research at HIPS and Saarland University is focussing, amongst other organisms, on these bacteria. © Dr. H. Lünsdorf

As you mentioned, these are ongoing projects already running or in preparation. What will be new in the planned Research Centre?

Müller: The work we do involves quite a number of collaborations with industry. We even have labs on-site which are run jointly with the pharmaceutical industry. With the intended increase in size and expertise, we expect significantly more interactions with industry – and also more joint projects. We have a substantial pile of letters-of-interest for this. Our issue to transform this interest into real-life projects is again related to available space. So, currently, we simply lack the facilities to run these projects.

Schmitt: I think a new quality is given by the high interdisciplinarity which Rolf Müller has already mentioned. These are projects that bridge classical pharmaceutical research with informatics and also with the fields of medicine and life sciences – to a much higher extent than before. This is also highlighted by the new professorships which are currently in the final recruitment process. Here again, to house the new groups and initiate the cooperations with the pharmaceutical industry and with companies here in the Saarland and beyond, even abroad, we need space - lab space - within the next two years.

Besides synergies in joint drug research projects – what are the main benefits of your partnership?

Müller: I think at the end of the day it's all about good science and bringing together the best scientists. So, how do we get hold of the next generation of scientists? I am convinced without excellent education at the universities, keeping pace with the development of the field, there is no next generation of infection researchers, or of pharmaceutical researchers. To me, this argument in itself is so convincing that we need to be close to our universities. We cannot simply rely on technicians and some permanent staff. We need to be involved in education, in the training of master students, postdocs, and graduate students. This ensures the future of our research in Germany.

Schmitt: We have an educational business to conduct. And particularly in highly dynamic and rapidly evolving disciplines, it is crucial to attract new students, also international students and scientists. We need to offer them access to the best expertise available. From the University's perspective, it is essential to have these tight connections to our non-university research partners, like Max Planck, like Leibniz – and like Helmholtz. With HIPS, we have a very tight and highly fruitful connection. And this is an important aspect for the further development and attractiveness of both, our university and of our HIPS/HZI partner.

Interview: Manfred Braun ■

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"THERE IS A GROWING EXPECTATION THAT RESEARCH SHOULD BENEFIT THE BROADER SOCIETY"

INTERVIEW WITH STEFAN SCHERER, NEWLY RECRUITED INNOVATION MANAGER AT HZI

How can a research centre ascertain whether its discoveries really "make a difference": that they benefit patients and public health, drive technological progress, create jobs and improve peoples' lives? HZI's answer is "translation" – the transfer of knowledge and technology into application. To advance and foster this process in a professional manner, HZI has established the position of an "Innovation Manager". Stefan Scherer assumed this position in 2021. For him, translation lies at the heart of his mission.

Dr. Scherer, what exactly is an Innovation Manager – and would you ever have thought that you would become one?

From my perspective, innovation in scientific research means going down new paths, following new directions and making for new destinations; and then, of course, getting results. As for the job of innovation manager: he or she guides this process, and gives advice about venturing into new activities. The main goal is, first and foremost, promoting and driving the transfer of technology and knowledge into application, for the benefit of society. Which means, for example, facilitating the development of spinoffs, or other paths towards making technologies available to the public. Would I have seen myself as an innovation manager from the start? Probably not. It is one of these career developments which arise from opportunities.

And what led you to this point? Can you say a few words about your previous career?

I was educated as an experimental physicist. Having studied in Germany, I worked as a PhD student and postdoc in Switzerland at the University of Bern. There I was engaged in experimental science for space applications – that was a very exciting, motivating, driving subject. I ventured from there into the automotive industry in Germany and North America, and stayed there in middle management for about ten years. Then I had a short stay at the University of Michigan for another space science project in between. Later on, I started at the University of Alberta in Edmonton. That's where I acquired my experience in research management. I also worked there in the regional business development agency responsible for the Life Science sector.

What brought you back to Germany and finally to Braunschweig?

I knew Helmholtz and HZI before, in particular through the Helmholtz-Alberta Initiative, where the University of Alberta cooperated with Helmholtz in some dynamic, future-oriented research fields – amongst them infection research. Moreover, I have always admired Helmholtz as an extra-university research organisation. HZI has some outstanding researchers and an excellent reputation. In addition, I had the impression that it was a very professional, result-driven organisation, and I enjoyed greatly this interaction. Besides, I grew up in Germany and spent many of my formative years there. Somehow, I had started to miss that practical mind-set and more long-term perspectives. So, when HZI announced the position of Innovation Manager, I gladly threw my hat into the ring.

The importance of transfer of technology and knowledge has tremendously increased over the recent years. Why do you think this is so?

"I have always admired Helmholtz as an extra-university research organisation."

I certainly see a stronger attention and focus now on transfer and impact, especially from the funding agencies and from politicians. They provide an open and enabling environment for conducting research, studying and pursuing interesting scientific topics. But at some point, there should be a connection back to broader society. I think that is what is transpiring now: the funders have to answer these questions from the politicians which enable them to do their job, and the politicians in turn have to answer to the voters who put them in power. There is a growing expectation that research results should benefit the broader society. Supporting researchers along this journey within Helmholtz is a fascinating opportunity.

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One of HZI's "breeding grounds" for innovation and translation: The new B2 building for drug research and functional genomics, inaugurated in spring 2021.

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How do you rate HZI's potential for translation, for technology transfer?

HZI has an abundance of fundamental and basic research going on. Most of it is more on the early-stage side, and that presents a challenge in identifying the most promising research topics and areas. The centre also has a number of highly respected, internationally recognised experts. Together, we – scientists, administration and transfer experts – work on increasing the technology readiness levels of promising projects enabled by HZI research findings.

What possibilities do you have in your position to influence the output of a centre like HZI?

I have the opportunity to connect with many groups on the campus and in the various institutes to learn about what's going on, and ask questions around that. Furthermore, HZI has already some instruments at hand to advance projects towards a higher technology readiness level. I should certainly mention the internal innovation fund "Pre4D", which has recently been successfully endowed for the years to come. While Pre4D is not provided with tremendous sums of money, it should be sufficient to help innovative projects at a very early stage until they yield results sufficiently promising to justify the next round of financing from external sources.

"I certainly see a stronger attention and focus now on transfer and impact, especially from the funding agencies and from politicians."

Are there any early results you could mention?

I just started very recently in this position, but I can already give some hints and ideas. To give you an example: last summer I read an article in the *Frankfurter Allgemeine Zeitung* about the Federal Agency for Disruptive Innovation, the "*Bundesagentur für Sprunginnovation*" - SPRIND. I asked some people about it, and everybody said, "Oh yes, I heard about that, but I don't know exactly what they do." As I started looking further into it, it turned out, lo and behold, there was actually a novel funding opportunity arising from that institution which is now helping to advance transfer - at pace.

What happened next?

Having promoted SPRIND within HZI and its research community, we drew the attention of a number of groups who then looked deeper into the details. And building on this relation, after talking to the people within SPRIND, it was rewarding to see that when it came to a competitive call for new antiviral agents, we had five HZI research groups submitting ideas into this portfolio. As far as I can gather, they received around 45 project applications. Nine of those were selected for so-called "pre-commercial contracts" – a novel approach in supporting innovations. In the end, four out of these nine projects, which will now be supported in the first year, include the participation of HZI researchers.

And what will be the next priority project you will pursue?

I would rather not single out one project over the others. There are a number of activities happening in parallel, and when it comes to innovation, it is difficult to tell in advance which opportunities will open up next. But one priority project is the spinoff from SORMAS – the open-source software platform developed successfully by HZI's epidemiologists to manage disease outbreaks. Supporting the department, the inventors and the founders to advance that project is an exciting journey.

You have worked in Canada before. Is there a different approach to transfer than what you can see here in Germany?

I think the entrepreneurial mind-set in North America in general is a little bit more widespread. People are more inclined to go and take a risk, take the path into being the creator of their own destiny, for example with spinning off an idea into a company. What is less developed compared to Germany is this extra-university research world. So that may be a reason why people don't have as many choices: you can have an academic research career within a university, or in very few national research organisations, or you have to go into industry. Then again, in America, if you launch something like a start-up or a spinoff, and it doesn't achieve great success, this is seen as less of a problem. It is more widely accepted and appreciated that you had the courage to do it, and that failure is part of the learning process.

"Supporting the department, the inventors and the founders is an exciting journey."

What results do you want to have achieved five years from now?

I think helping to establish a mind-set where transfer is better appreciated within the research community would be rewarding. And, of course, I would be very glad to see some HZI research results picked up by larger companies, or by a spinoff company which survives the first few years, establishing itself as a partner, or as an employer and taxpayer in the community.

How many spinoffs do you think are realistic in this timeframe?

It is too early for me to say yet. I think we should go step-by-step now, focusing on what is possible at present. And hopefully, within one or two years, we will see the first companies being founded. And by then, the next opportunities will have been identified, and we will pursue these in turn.

Interview: Manfred Braun ■

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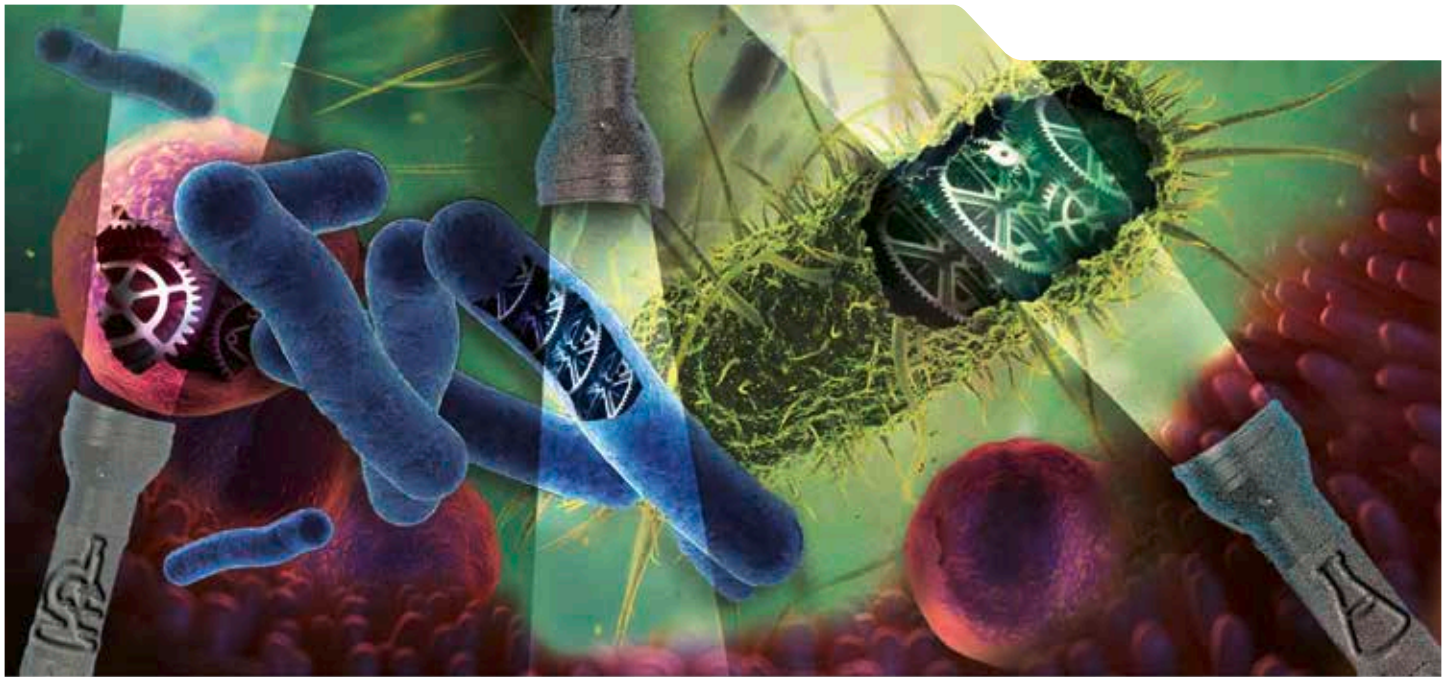


Unlocking the gut microbial functional diversity © Aleksandra Krolik | EMBL

"OUR PARTNERSHIP WILL STRENGTHEN EUROPEAN RESEARCH IN THE FUTURE"

INTERVIEW WITH EDITH HEARD, DIRECTOR OF THE EUROPEAN MOLECULAR BIOLOGY LABORATORY (EMBL) AND DIRK HEINZ, SCIENTIFIC DIRECTOR OF HZI

The European Molecular Biology Laboratory, EMBL, and HZI are now collaborating closely in order to expand the frontiers of health research. Together, they aim to tackle dynamic fields of infection research, focusing in particular on microbial ecosystems.

Prof. Heard, you took over as director-general of EMBL in 2019. Can you say a few words about your career and your motivation?

Edith Heard: I was trained as a geneticist, and I spent the first part of my career in the UK working at the Imperial Cancer Research Fund. Then I moved to Paris to work as a postdoc. There, I started to investigate an epigenetic process called "X-chromosome inactivation", where one of the two X chromosomes in females gets shut down. It's essential for dosage compensation and essential for life. There is also a link to infection: it turns out that many genes on the X chromosome are involved in immunity. And in particular, many genes are involved in autoimmune disease. Then, as you mentioned, in 2019 I became head of EMBL. I felt that it was important that a scientist like me would be able to help Europe through an organisation like EMBL. I also still have a lab and am an active scientist.

What would you define as the main mission of EMBL?

Edith Heard: We have actually committed ourselves to five missions: to perform excellent fundamental research in molecular biology; to offer vital services to scientists; to train scientists, students, and visitors; to actively engage in technology transfer; and to coordinate and integrate European life science research. But I would say the overall mission is to make sure that the life sciences in Europe are delivered

in a very international way. EMBL was set up in 1974 as an organisation to help its member states progress. We have 28 member states which can work together closely thanks to EMBL. We are independent of the European Union, and yet we are behind many of the big collaborative interactions between European countries. Every country benefits from us, but no country can impose itself on us. So I think we are a unique intergovernmental life science research organisation in Europe.

EMBL has defined a new research programme recently. Can you tell us a few words about that?

Edith Heard: We work in five-year-programmes. The last one was called "Digital Biology", and it just ended last year. When I arrived as Director General in 2019, we discussed thoroughly about the next one, and we decided that the name of this new programme is "Molecules to Ecosystems", and it aims to understand life in context.

"Open Science is one of our central goals."

What do you mean by "context"?

Edith Heard: "Context" means everything from the local environment of a cell, organism or microbial community right through to understanding how a whole ecosystem works, with both the biotic and the abiotic factors. This also includes the environmental changes that take place both naturally and unnaturally, including man-made factors like climate change.

Edith Heard © Kinga Lubowiecka | EMBL

And how do you study these interactions?

Edith Heard: A central part of it is really at the heart of what scientists do - data. At EMBL, we have to do with the whole data cycle, from production to storage, curation, analysis, integration, interpretation, using AI approaches, and so forth. But also, the sharing of data – Open Science is one of our central goals. And the programme is very collaborative. To move us into this new era of ecosystems, we need to link molecular biologists to genome researchers, epidemiologists, ecologists, mathematicians and many other experts. And HZI was actually one of the first partners we linked up to, addressing infection biology and pathogens.

Prof. Heinz, you obviously see EMBL as a partner for HZI's research. What was your motivation for establishing this partnership?

Dirk Heinz: EMBL is a leading international research institution, covering essentially all aspects of molecular biology to study life in-depth. In many ways, HZI and EMBL are quite similar – although we are a bit smaller than EMBL. When you look at the structure, both institutions have a central campus with specialised sites and branches elsewhere. We have research programmes which are regularly evaluated internationally, and which are longer lasting than typical research grants. So we can work strategically on long-term questions. HZI has a strong focus on translational research, EMBL focuses on curiosity-driven research and the translational opportunities this offers. And now of course, with the new programme "Molecules to Ecosystems", there is an almost perfect fit with the HZI programme. When I look at the new programme of EMBL, which Edith has just outlined, it reminds me very much of the HZI research programme.

Where do you see the overlaps?

Dirk Heinz: Just like EMBL, we have infection biology as a central research theme, we study microbial human ecosystems, and planetary health. We are currently building up a new One Health institute in Greifswald, which is part of HZI. All that fits very nicely; there is clearly a strong link. I think we are highly complementary.

Dirk Heinz © HZI | Verena Meier

So now, a long-term strategic partnership is being established. What would you define as its main goal?

Dirk Heinz: As Edith has pointed out, in order to really understand health and disease, we have to study life in context – not in isolation. I think in the past, and this was also due to technological limits, we had to rely on model systems. We gained, of course, quite a bit of knowledge through this approach. But in real life, things often look different, because so many factors play their role and influence each other. Therefore, we have to look at how organisms interact which each other. This interplay is clearly most relevant in infection biology.

Can you name the fields where EMBL and HZI will pursue this approach jointly?

Dirk Heinz: I think it is important that we put a clear focus in this cooperation. There are many areas covered by both our centres, but I think it is wise to start with one specific, defined research field. The partnership between HZI and EMBL will therefore focus on the human microbiome: the ecosystem of microbes in the human body, for example, in the gut. Traditionally, we have been studying the pathogens as well as the host organism. However, the microbiota interacts with both. Alterations in the microbiota have been linked to almost all prevalent human diseases, including the susceptibility and treatment outcome of infectious and immune-mediated diseases.

Edith Heard: This microbial ecosystems area is one of our "transversal themes" as they are called – one of our overarching research themes. We are setting up technologies in state-of-the-art facilities that are relevant for studying microbes and interactions with the host. HZI scientists will be able to use this infrastructure as well.

What kind of technologies are these?

Edith Heard: They include unique high-throughput cultivation, genetics, genomics, and phenotyping of gut microbes and their communities, imaging, and also some cutting edge mass-spec based approaches to measure metabolite and protein levels/activities. And we are moving into machine-learning approaches to do predictive modelling. So we can try to dissect how microbial interactions work, and to understand microbial communities and the context-dependence of pathogenicity, and to contribute to tackling the antimicrobial resistance crisis.

Dirk Heinz: I may add, understanding is one thing – but then intervening with these processes is yet another. The editing of the microbiota may be still science fiction, but I think it is not too far away now. There are already initial promising results showing that we can possibly manipulate the microbiota to our own benefits. We know from medicine in general, that therapies are highly influenced by the microbiota. This is what we want to understand, and then harness this knowledge to intervene and treat patients better in the future, or to prevent infections from occurring altogether.

So is this one of your first key projects together – the editing of the microbiota?

Dirk Heinz: It could be one outcome. There are already approaches going on between Nassos Typas and Till Strowig, for example, where they specifically try to understand the role of certain microbes in the microbiota. The next step will be to know how to interfere with these microbes, and see whether, for example, knocking out some of these microbes has beneficial effects.

"The editing of microbiota is not too far away now."

What are the concrete steps you have in mind for your collaboration?

Dirk Heinz: We will start by implementing a joint postdoctoral fellow programme that allows outstanding postdocs to pursue a research question while spending time at a lab at HZI as well as at EMBL. This year, four postdocs can be

ATC Building on the EMBL campus © Marietta Schupp | EMBL

© Kinga Lubowiecka | EMBL

admitted via this programme. In addition, we will facilitate knowledge exchange and access to core facilities, technology platforms as well as data services across institutions. We also want to have more joint symposia like the one we had at HIRI in Würzburg last year.

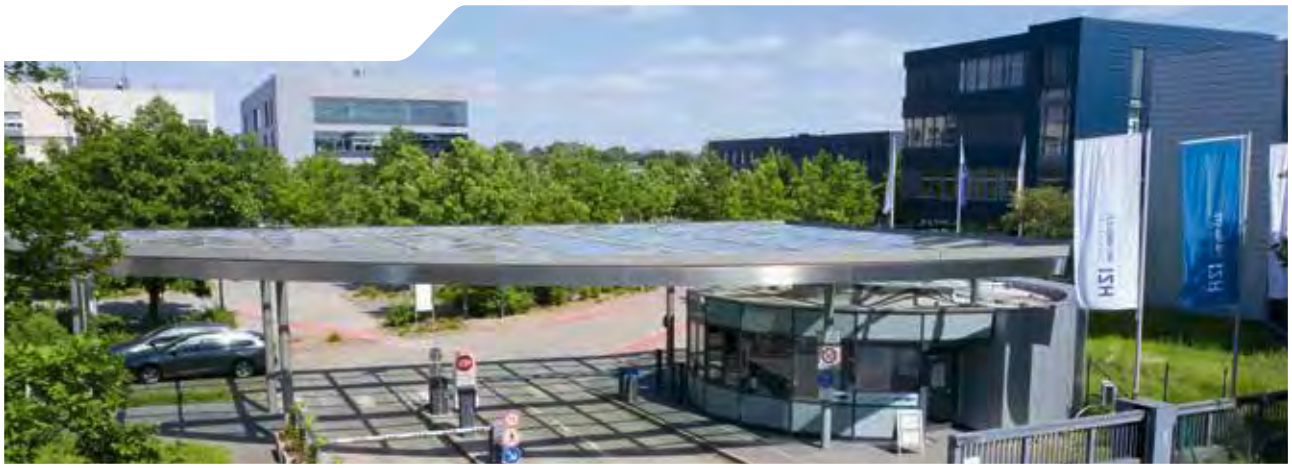
Edith Heard: The postdoc scheme Dirk just mentioned is a perfect example of how we are going to deliver on our joint initiatives. The scientists, in particular the young scientists, are the "life blood" of science. Being able to work on joint research projects between two groups, one at EMBL, one at HZI, and spend three months in the respective partner institute: this is a great opportunity for a young researcher. At EMBL, we already had some of these interdisciplinary postdocs – and they were a huge success.

Is there a key message you want to deliver to society in general?

Edith Heard: I think the COVID-19 pandemic has really shown the tremendous importance of scientific research to a broader public. Research has allowed us to understand basic mechanisms of the pathogen and its spread comparatively soon, and to develop effective vaccines in a surprising speed. Without science, where would we be?

Dirk Heinz: Let me please add the importance of international collaborations. I think recent events in the world have demonstrated how crucial it is that nations cooperate. Science may be just a small part of it, but it certainly can contribute to bring people together. The partnership between HZI and EMBL is a wonderful example of an international collaboration. I am sure it will strengthen European research in the future.

Interview: Manfred Braun



HZI Science Campus © HZI | Verena Meier

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FROM BENCH TO BEDSIDE: INNOVATION MANAGEMENT AND TRANSLATION

TECHNOLOGY AND KNOWLEDGE TRANSFER AT HZI

Transfer of technology and knowledge plays an increasingly important role in HZI's strategic alignment. The diverse past, current and future transfer activities at HZI have been summarised within the framework of a new transfer strategy adopted in autumn 2021. In addition to strengthening the transfer culture at the centre, professionalising innovation management, and further developing existing structures, collaborations, and alliances, the key points of the strategy also include the steady expansion of knowledge transfer.

The centre's attractive transfer portfolio encompasses a broad spectrum of novel active substances, diagnostic procedures and innovative digital health platforms. Through these inventions and products and their transfer into clinical application, the national and international position of HZI in research and development has advanced over the reporting period 2020–2021 in the face of challenges posed by the global COVID-19 pandemic.

One of the five major research policy goals in the Fourth Pact for Research and Innovation – an agreement signed by the Federal Government and Germany's leading research organisations – is to strengthen the transfer of research findings and technologies into industry and society in the decade 2021–2030. To seize this objective, the Helmholtz Association and its centres have developed and adopted a dedicated transfer strategy.

In 2021, HZI established its own transfer strategy with the goal to expand and broaden the technology transfer portfolio in the area of therapeutics, including a set of general and centre-specific indicators to support transparent monitoring. The transfer strategy was adopted by HZI's advisory board in November 2021.

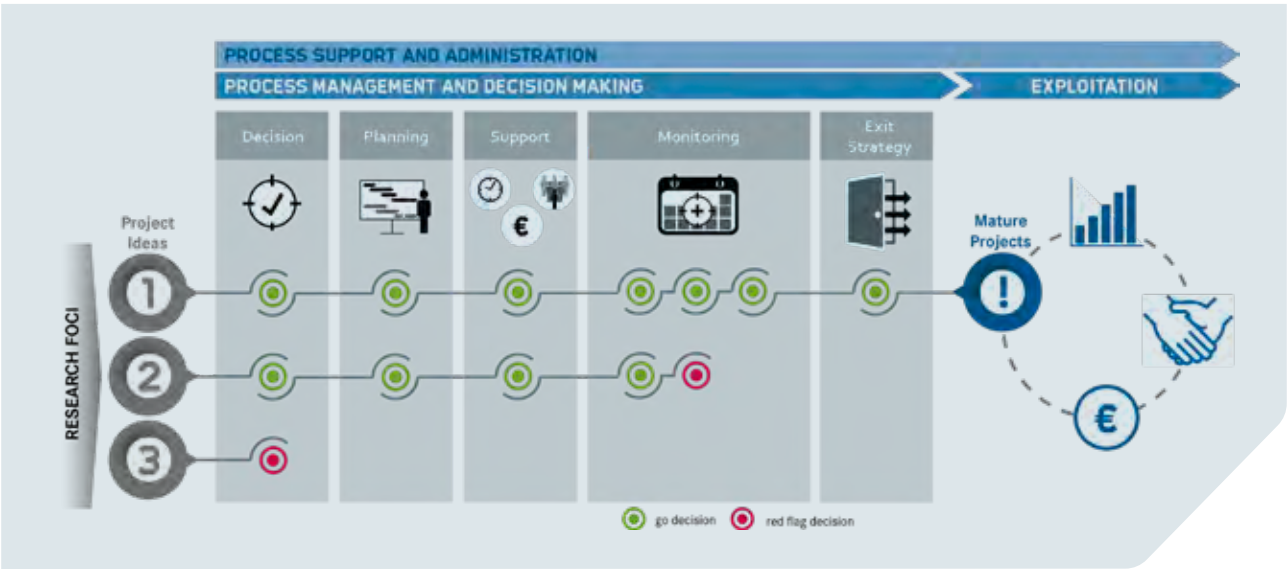
In the same year, 2021, HZI established a department explicitly dedicated to Innovation Management in order to further strengthen its competence *(see also Interview with Stefan Scherer in the section "In and around HZI" of this report)*. The mandate of the new department is to coordinate existing as well as future technology transfer activities related to patents and third-party funding acquisition, and to act as an interface between management and the legal department on the one hand, and external cooperation partners covering all aspects of transfer on the other.

This department is building on the solid expertise and many achievements of previous years to further foster innovation within the scientific community at HZI. With Ascenion GmbH, in particular, HZI has a long-standing technology transfer service partner at its side with regard to invention assessment as well as inventor and spin-off support and the professional management of investments.

The HZI internal Pre-4D innovation fund established in 2016 - where "4D" stands for "Drugs, Diagnostics, Discovery and Development" - has been and continues to be a critical instrument to systematically further develop innovative discoveries and research results reaching into the future. After its resoundingly positive evaluation in 2020 the Pre-4D fund continues to provide support to the scientific community at HZI at an annual level of Euro 500,000. Its objective is to increase the technology readiness levels (TRL) of basic research projects raising their attractiveness with regard to licensing for industrial partners or spin-off companies.

In the 2020-2021 reporting period, in total six projects received funding through the Pre-4D innovation fund. Project topics range from advancing aspects of inhibitors for respiratory viruses and novel compounds blocking bacterial pathogenicity to mouse models of bacterial sepsis. Despite the challenges in execution posed by the global pandemic, all of these projects were able to advance their TRL levels. The innovation fund also expanded its support for digital health tools for epidemiological applications.

Furthermore, various project teams were successful in attracting significant external funding for transfer related activities during 2020-2021. For example, the Federal Agency for Disruptive Innovations (SPRIN-D) – an organisation jointly funded by the German Federal Ministry for Education and Research (BMBF) and Federal Ministry for Economic Affairs and Climate Action (BMWK) – launched a dedicated challenge in 2021 focusing on new antiviral agents with a goal



Scheme of HZI's structured technology transfer process. Innovative projects are regularly reviewed by HZI's Technology Development Board, where internal and external experts meet regularly to discuss and maintain an active portfolio of HZI projects. The most promising projects are transferred to the next stage.

to develop platform technologies. This particular programme follows a pre-commercial contract model with annual competitive evaluations over a 3-year period with the ultimate goal of reaching the proof-of-concept stage by the end of the third year. From a pool of more than 45 applications, nine projects were selected in autumn 2021 to receive funding for the first year. Among these, four projects involve research groups from HZI.

The team developing the diagnostic tool LEOPARD *(see section "Highlight Publications"/Chase Beisel)* at HIRI in Würzburg was awarded support through the GO-Bio initial programme from the BMBF as well as through the Medical Valley Award programme in the state of Bavaria. At the international level, two research groups at HZI were successful in acquiring funding for the first phase of the CARB-X (Combating Antibiotic Resistance Bacteria) programme. CARB-X accelerates global antibacterial innovation by supporting development of new antibiotics and other life-saving products to combat the most dangerous drug-resistant bacteria.

A team at HIPS pursues an innovative approach to treat *Pseudomonas aeruginosa* infections, another team at HZI's main campus in Braunschweig, in close collaboration with the Lead Discovery Center GmbH, is developing a novel active substance preventing the bacterium *Staphylococcus aureus* from colonising the lungs.

The global COVID-19 pandemic provided an unprecedented boost for the Surveillance, Outbreak Response Management and Analysis System (SORMAS), an open-source eHealth platform for processing infection cases, their contacts and managing outbreaks *(see chapter "2020 and 2021: Years of the Pandemic")*. In order to address demand adequately, curation of the open-source software and global dissemination of SORMAS, the spin-off into a non-for-profit entity began in the past year. The SORMAS team was successful in the Helmholtz Enterprise Spin-off programme in autumn 2021, and was awarded funding for a 14-month period to support this ambitious journey. SORMAS is now on its way to establishing a not-for-profit foundation.

A close collaboration to investigate new drug candidates of the cystobactamide family – a group of antibacterial substances derived from natural sources, namely myxobacteria living in the soil – combines HZI's expertise in natural products with the know-how of the industrial partner Evotec which has set up a world-leading translational drug discovery platform in antibiotics. The project is in transition to lead structure optimisation and has made significant progress with support from the Helmholtz Enterprise programme to spin-off the company MYXOBIOTICS.

To increase awareness within the broader research community at HZI regarding innovation and the value of technology and knowledge transfer, several initiatives took place during the reporting period. The transfer service partner Ascenion offered a series of workshops, exclusively for HZI members, related to transfer-relevant topics, for example licensing versus spin-off and introduction to IP strategies.

The continuing education programme offered by the TRAIN Academy is a joint initiative between HZI and regional partners. It focuses on translational research and medicine from idea to product. HZI awarded several scholarships in autumn 2021 for the next cohort (2022-2024) of this accredited training programme on transfer relevant aspects in the biomedical field. A complementary two-day entrepreneurship programme "Young Entrepreneurs in Science (YES)" for graduate level researchers at HZI was offered in autumn 2021 in collaboration with the Falling Walls Foundation. The workshop was attended by over 20 trainees from HZI.

Researchers and scientists at HZI were sought after experts during the COVID-19 pandemic years 2020-2021, and asked to share and transfer their knowledge to society and political decision makers including numerous contributions to expert panels and task forces at regional and national levels. The COVID pandemic has made it increasingly obvious that there is a widespread need for authoritative knowledge transfer from experts, such as HZI has to offer. To effectively combat the coronavirus, the German federal government has set up a scientific council of experts to advise on measures against the pandemic in the future. HZI and the Technical University of Braunschweig are represented by two experts on the panel consisting of 19 scientists from various disciplines.

In forthcoming years, HZI aims to further increase its transfer output, directing additional resources towards Innovation Management and implementing novel concepts, which are currently being developed.

Author: Stefan Scherer



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STRATEGIC PARTNERS ALL OVER THE WORLD

A SPOTLIGHT ON SELECTED INTERNATIONAL COOPERATIONS

HZI addresses 21st century challenges in the field of infectious diseases. It contributes to a better understanding of the underlying principles and to the development of new strategies for early detection, prevention and therapy to reduce the global burden of infectious diseases.

To best fulfil this mission, HZI relies on cooperation with academic, clinical and industry partners as well as on access to state-of-the-art research infrastructures and technologies. This enables the recruitment and promotion of the best minds in translational infection research. Accordingly, promoting and implementing an international perspective in these areas has assumed an increasing priority for HZI. This strategy generates added value for research and is key to advancing scientific knowledge on infectious diseases, and their prevention and treatment in a global context. It also contributes to one of HZI's seven overarching goals, specifically, "strengthening HZI as a driver of and partner within global networks for translational infection research."

The global dimension of the challenges posed by infectious diseases is obvious: pathogens do not stop at national borders, but rather spread rapidly worldwide due to globalisation. This has once more become apparent in the current COVID-19 pandemic.

In 2021, HZI developed an internationalisation strategy – a roadmap that outlines its goals regarding internationalisation and guiding principles for their implementation.
It includes three major strategic approaches:

- Cooperation with top ranking research partners in Europe and worldwide
- Jointly tackling global challenges caused by infectious diseases
- Recruiting and promoting the best talents worldwide

Internationally, HZI scientists are well positioned within numerous bilateral collaborations. These close partnerships are complemented by strategic, thematically focused alliances with internationally leading institutions forged to profoundly strengthen HZI's research impact in key areas.

One example is HZI's long-standing collaboration with Shandong University in China, which resulted in the establishment

Joining forces against infectious diseases: A picture taken from a symposium with scientists from McGill University and HZI on the HZI campus Braunschweig in 2019. Photograph: HZI | Charlotte Wermser

of the "Helmholtz International Lab" dealing with "novel drug candidates for the treatment of bacterial and viral infections with unmet medical need" in 2018. Supported by a five-year grant totalling 1.5 Mio €, scientists can focus on bacterial infections with Gram-negative pathogens, enterovirus-caused hand, foot and mouth disease - highly prevalent in Asia – and bronchiolitis caused by the respiratory syncytial virus (RSV). The Helmholtz International Lab has already produced more than 20 publications and filed three patents. An evaluation in 2021 endorsed the excellence of the International Lab and highlighted this establishment as a paradigm for successful Sino-German cooperation.

HZI can furthermore look back on a close cooperation with the University of Alberta in Edmonton, Canada. Since 2012, both partners have been cooperating in the Helmholtz-Alberta Initiative – Infectious Diseases Research (HAI-IDR), together with Helmholtz Zentrum München. This consortium of internationally renowned virologists (including the 2020 Nobel laureate Michael Houghton), immunologists and drug researchers has focused on infections caused by hepatitis B and C viruses (HBV/HCV). Hitherto, they have concentrated on preclinical studies of a candidate therapeutic vaccine against HBV, and a prophylactic vaccine against HCV, which emerged from this collaboration. Both vaccines were formulated along with HZI's own adjuvant c-di-AMP. Until now, more than 60 publications have emerged from the project. Following the conclusion of the Helmholtz-funded HAI-IDR in 2021, discussions are currently taking place on future collaboration.

Personalised infection medicine is a topic that HZI will pursue jointly with the McGill Interdisciplinary Initiative in Infection and Immunity (MI4) at world-class McGill University, Montréal, Canada. Since 2019, there have been regular events and meetings, most recently a public virtual symposium on the core topic of the strategic partnership, which included a keynote lecture by Uğur Şahin, co-founder and CEO of BioNTech SE. The plan to collaborate in the field of personalised infection medicine was documented by a Memorandum of Understanding in 2020. Collective advantage is clearly anticipated and will take form in 2022 as a joint postdoc exchange programme.

HZI's research interests in the field of microbiota research align closely with those of the European Molecular Biology Laboratory (EMBL), as outlined in its new programme "Molecules to Ecosystems". This cooperation holds great potential

A strong partner in personalised infection medicine: McGill University in Montreal, Canada © Claudio Calligaris

Drone Image of EMBL's campus in Heidelberg, © Massimo del Prete| EMBL

to promote research synergies in this area. A Memorandum of Understanding has been signed between EMBL and Helmholtz Health, encompassing all centres of the Helmholtz Research Field Health. In October 2021, a joint symposium between HZI and EMBL was held in hybrid format with around 200 participants. This symposium was followed by a strategy workshop in which members of HZI and EMBL discussed the next steps of their cooperation. This collaboration, already underway bilaterally, will now move up a gear with an institutionally funded postdoc exchange programme *(see also interview with Edith Heard and Dirk Heinz in this report)*.

High level exchanges involving departmental heads characterise HZI's cooperation with the Rigshospitalet in Copenhagen, Denmark. Accordingly, Susanne Häussler, a HZI department head, has set up a novel molecular diagnostics system to fight antimicrobial resistance and runs it at two sites, MHH and a newly set-up Novo Nordisk Foundation-funded laboratory at the Rigshospital. More detailled information on the antimicrobial resistance profiles of individual bacterial isolates is expected to promote more targeted and more effective antibiotic therapies. This approach will also help determine whether solutions to research problems are sustainable across national borders.

A prime example of cooperation with partners in countries particularly affected by infections is the collaboration between HZI and various countries implementing the Surveillance Outbreak Response Management and Analysis System (SORMAS), an open-source eHealth tool for disease control measures developed by HZI. SORMAS already covers more than 20 high-priority epidemic-prone infectious diseases. Even before WHO classified the current pandemic as a "Public Health Emergency of International Concern", SORMAS had activated a novel COVID-19 specific module in Nigeria and Ghana, supporting their containment measures. In the course of the pandemic, SORMAS has been implemented in Germany, France, Switzerland, Afghanistan, Nigeria, Ghana and Fiji to support control of the pandemic *(see also sections "2020 and 2021: Years of the pandemic" and "Research Focus EPI" in this report).*

Establishment of the new Helmholtz Institute for One Health (HIOH) in Greifswald will add further international partnerships at HZI, notably in sub-Saharan Africa. These areas

International scientists are being trained in safely performing rodent autopsies in Korhogo, Côte d'Ivoire. HZI's new Helmholtz Institute for One Health has strong links to sub-Saharan Africa. © Kathrin Nowak

are hotspots for the emergence of novel zoonotic diseases. Research into the mechanisms of their spread is clearly relevant to global health. HIOH will work closely with local research institutions in Ivory Coast and promote capacity building for disease control there. These long-established networks, which are now added to HZI, represent a valuable opportunity to address further relevant scientific questions at HZI *(see also section "Partners, sites and networks" as well as the interview with Fabian Leendertz)*.

HZI also participates as a partner or coordinator in several large interdisciplinary networks and consortia. Thanks to the focus of its research programme, HZI has thus positioned itself as an active member and conspicuous driving force in the international community. Examples of large EU-funded research projects coordinated by HZI are CORESMA (COVID-19 Outbreak Response combining E-health, Serolomics, Modelling, Artificial Intelligence and Implementation Research) and CRUZIVAX, which aims to develop a prophylactic intranasal needle-free vaccine candidate against *Trypanosoma cruzi* infection.

In the vitally important field of antimicrobial resistance research, HZI is a member of prominent international partnerships, like the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), the Global Antibiotic Research and Development Partnership (GARDP) and the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X).

Author: Birgit Grün

PERSONALIA

SILKE TANNAPFEL TAKES ON NEW CHALLENGES

Silke Tannapfel, Administrative Director of HZI, has left the centre as of December 2021.

The qualified lawyer and science manager has been working at HZI since 2017, after stations in public administration in Erlangen-Nuremberg, Brussels and Munich.

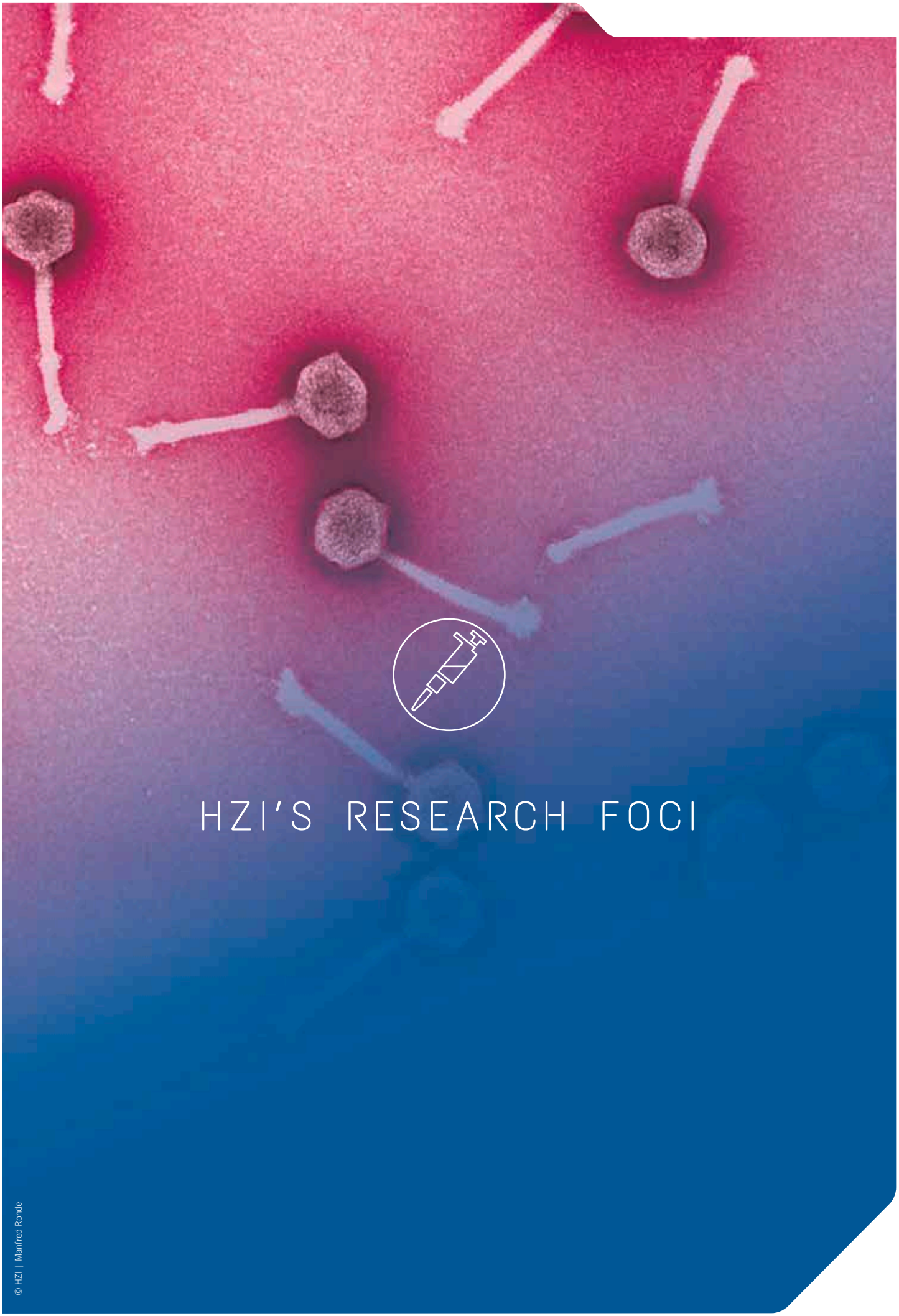
During her time at HZI, Silke Tannapfel was instrumental in successfully tackling a demanding financial consolidation phase. By means of a thorough modernisation of the entire HZI administration, which she actively initiated, she ensured that HZI will continue to fulfill its diverse and complex administrative tasks in an optimal manner in the future.

Silke Tannapfel took up her new position as Ministerial Director in the Hessian Ministry of Higher Education, Research and the Arts in January 2022. The entire HZI sincerely thanks Silke Tannapfel for her important contributions to the centre and wishes her all the best for her future challenging tasks.

HZI MOURNS GÜNTER MAASS

In December 2021, Günter Maaß (1934–2021) sadly passed away. From 1996 to 2000, Professor Maaß had been Scientific Director of GBF, the predecessor institute of HZI. Trained as a biophysicist, as a doctoral student he joined the group of Manfred Eigen, who was later awarded the Nobel Prize in Chemistry. Maaß received his PhD in Göttingen in 1962.

In 1967, he started as head of the Department of Biophysical Chemistry at the Institute for Molecular Biology and Biophysics, later GBF. As one of the first department heads and subsequently as Scientific Director, Günter Maaß played a major role in shaping the centre over several decades.



HZI'S RESEARCH FOCI

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NEW STRATEGIES AGAINST RESISTANT PATHOGENS

RESEARCH FOCUS "ANTIMICROBIAL RESISTANCE" (AMR)

The increasing occurrence of antimicrobial resistance is a severe challenge, particularly in the light of the scarcity of new antibacterial candidates in drug discovery. Scientists in the RF AMR combine expertise in various fields and long-standing experience in industry or industrial-academic collaborations to address these challenges, pursuing a multi-pronged strategy. They investigate the molecular mechanisms causing resistance and explore innovative strategies against pathogens, in particular, by identifying and optimising novel, proprietary anti-infective compounds with unprecedented modes of action. Their approach is focused on, but not limited to, pathogens on the WHO priority list and includes the targeted delivery of drugs to the site of infection. Access to a unique natural product library and the ability to elucidate the mode of action early on make the approach of HZI researchers particularly effective.

MOST IMPORTANT QUESTIONS ADDRESSED BY RF AMR:

- Can antimicrobial resistance be detected early enough to take countermeasures in time?
- How can we find novel drugs against resistant pathogens?
- Can we optimise treatment regimens and use approved antibiotics more effectively?
- How can antimicrobial compounds reach their targets more efficiently?

1. PATHOGEN AND RESISTANCE PROFILING

Knowledge on genetic mechanisms underlying antibiotic resistance has the promise to change the way physicians treat infections. Researchers in RF AMR address a critical unmet medical need and strive to provide the necessary conditions to develop modern molecular diagnostics for early and targeted treatment and for the implementation of effective hygiene measures to control multidrug-resistant infections.

Scientists in the Research Focus AMR obtained new knowledge on the mechanisms of resistance in problematic no-

socomial pathogens. The genomes and transcriptomes of more than 400 clinical *Pseudomonas aeruginosa* isolates have been sequenced and predictive models were generated, which identified biomarkers of resistance to four commonly administered antimicrobial drugs. Modern genome-based diagnostics promises to predict antibiotic resistance in individual clinical isolates with significant improvements to what is currently available and allows the establishment of effective intervention strategies for the control of the spread of multidrug-resistant infections. Research in the RF AMR uncovered the content and composition of microbial genomes which was not only the basis for the prediction of antibiotic resistance, but also for the identification of the phylogenetic relatedness which together with patient movement data lead to the effective identification of patient-to-patient transmissions.

With the aim to improve patient outcome, we store large amounts of genome-wide data on multidrug-resistant pathogens in searchable graph databases that serve as a reference for the automated prediction of antimicrobial resistance phenotypes encoded in any newly to be sequenced bacterial genome. The whole genome sequence (WGS) data is also available for research on other basic research projects, for example, to identify the genetic basis of other infection-relevant phenotypes, such as biofilm-formation capabilities, bacterial virulence or the potential of the individual isolates to produce persister cells. Our established tools include machine-learning algorithms for the identification of phenotype-genotype correlations and can be applied to identify and characterise the genomic composition of pathogens that drive these clinically relevant phenotypes. Knowledge on the genetic components that drive pathogenicity and antibiotic resistance can be used to establish novel molecular diagnostics, they also might serve as novel targets for the development of anti-virulence drugs.

2. NEW ANTI-INFECTIVES FROM NATURAL PRODUCTS

New agents with innovative chemistry and mode of action are urgently needed to combat the global public health threat of antimicrobial resistance (AMR). Microbial natural products (NPs) are one of the most promising sources of novel anti-infectives.

In a Nature Reviews Chemistry article *(doi: 10.1038/s41570-021-00313-1)*, the authors of a Joint Programming Initiative AMR-funded consortium, initiated by HZI researchers, present a strategic plan for the improved discovery and development of new antibiotics. They propose short- and long-term solutions to overcome the most pressing constraints in the various research and funding areas. The goal is to bridge the gap between academic, industrial, and policy stakeholders and bring together interdisciplinary expertise to advance the translational pipeline for the benefit of future generations.

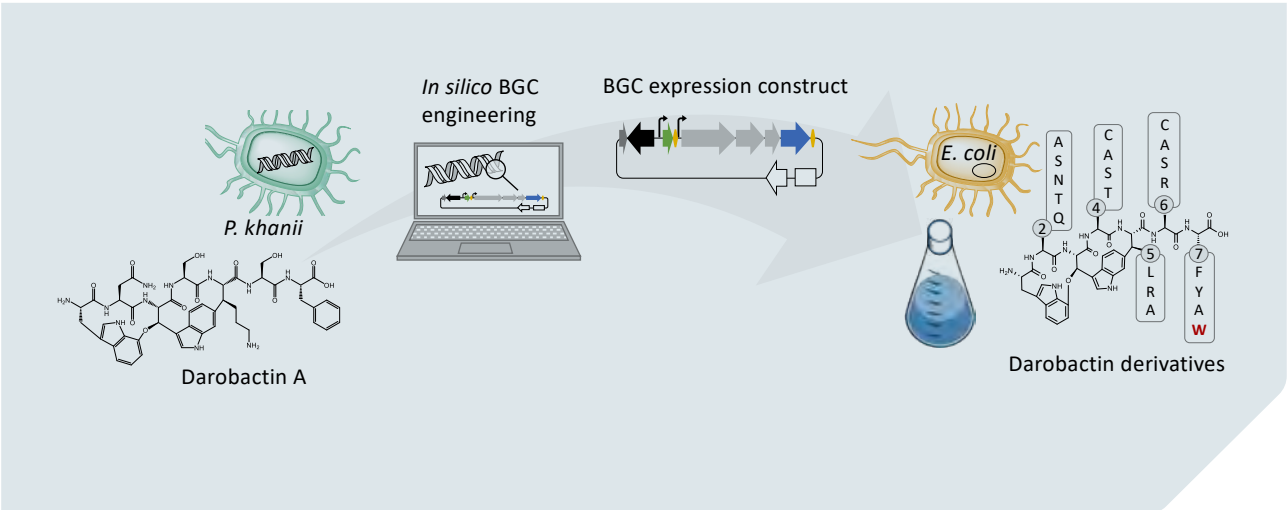

Figure 1: Heterologous expression of the antibacterial natural product darobactin in the host organism *Escherichia coli.* © HIPS | Sebastian Groß

Darobactin shows very good activity against Gram-negative bacteria, but could previously only be produced in relatively small quantities, making in-depth investigation and further development difficult. RF AMR scientists significantly increased the production of darobactin A by heterologous expression of a synthetically engineered biosynthetic gene cluster (BGC) in *E. coli*. Rational design of darobactin variants led to the production of 13 new 'non-natural' derivatives and 4 previously hypothetical natural darobactins. One of the non-natural compounds exerted more potent activity than darobactin A.

Cystobactamides are natural products with promising activity against multidrug-resistant bacteria, including Gram-negatives. RF AMR scientists uncovered the steps of cystobactamide biosynthesis in myxobacteria, allowing for further optimisation of these compounds. Their results provide direct evidence for unique steps in cystobactamid biosynthesis, e.g. the functional description of a new bifunctional domain found in the non-ribosomal peptide synthetase module CysH, albicidin biosynthesis and numerous biosynthetic gene clusters of unknown natural products.

3.OPTIMISATION OF DRUG CANDIDATES

HZI researchers discover and optimise antibiotic hits that were found by an arsenal of discovery techniques, including natural-product research, high-throughput, fragment or virtual screening as well as drug conjugation. The portfolio of drug targets comprises both classical antibiotics and pathoblockers (*i.e.*, compounds that disarm pathogens rather than killing them); both approaches are exemplified in this report.

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The cystobactamide class of natural products (*see above*) has been optimised at HZI in collaboration with the company Evotec and Leibniz University Hannover. The best compounds inhibit panels of drug-resistant clinical isolates with high potency and demonstrated *in vivo* efficacy. The spin-off company Myxobiotics is in preparation, aiming to further develop cystobactamides for clinical application.

A pathoblocker approach is pursued to find better treatments of hospital acquired pneumonia caused by *S. aureus*. The groups at HZI have partnered with the Lead Discovery Center (LDC) to discover small molecules that block *S. aureus'* central virulence factor alpha-haemolysin (Hla) and identified first-in-class inhibitors showing effects even at low concentrations in all relevant cell types. A lead selection, enabled by demonstrating *in vivo* efficacy in four models, was approved by the main funder CARB-X.

Targeting *P. aeruginosa* lectins LecA and LecB with pathoblockers prevents bacterial adhesion to host cells and prevents a major resistance factor, bacterial biofilm formation. In two approaches we identified the first non-carbohydrate glycomimetics that mimic the binding of carbohydrates in the binding site of LecA. Second, we identified optimally spaced linkers to match two native LecA ligands, galactosides, bound in two adjacent binding sites of LecA tetramer. This research led to the most potent bivalent LecA inhibitors reported to date.

The extracellular virulence factor LasB of *P. aeruginosa* is another pathoblocker target. Thanks to a concerted effort enabled by generous funding from CARB-X and the BMBF, we were able to optimise various classes of inhibitors. In addition, the compounds display promising properties for clinical use, such as no inhibition of human off-targets and excellent pharmacological data. Hence, the lead compounds appear suitable for various routes of administration such as inhalation or intravenous delivery.

The pharmacokinetics/pharmacodynamics (PK/PD) unit, supported by DZIF, has become the first port for novel and active agents, as it enables to study therapeutic efficacy and to select the correct dosage. In 2021, we have extended the

range of animal models to study efficacy of compounds in chronic lung infections of *P. aeruginosa*. In addition, a mechanistic *in silico* model for Dengue virus infections predicted the pharmacokinetics and even the pharmacodynamics of soraphens in different species, including humans.

4. DRUG DELIVERY

We are investigating various approaches to improve drug delivery across biological barriers in the context of infectious diseases, such as biofilms and the bacterial cell envelope. Besides, we are working on complex *in vitro* models and *in silico* tools to predict transport and to monitor changes in state of disease and under therapy.

Self-assembling nanocarriers were found most effective to co-deliver pathoblockers along with tobramycin to bacterial cells, enabling complete eradication at 30-fold reduced dose of the antibiotic (*see section "Highlight Publications", M. Empting*). The same carriers could also be used for targeted drug delivery via skin hair follicles when applied topically to the skin. Myxobacteria-derived membrane vesicles could be shown to prevent the formation of and to disrupt existing biofilms. In addition, antigenic streptococci-derived vesicles are currently developed as cell-free vaccine.

Surface-modified liposomes targeting the biofilm lectins LecA and LecB bind with very high potency and specificity and remain firmly attached to lectin-presenting surfaces. This opens new perspectives to overcome the drawback of chemically modifying antibiotics by covalent coupling of lectin-targeting moieties. Targeting of the last resort antibiotic colistin to bacteria was achieved with the help of the bacterial binder ubiquicidin, and its release was triggered by immune cells at the site of infection (*see section "Highlight Publications", Brönstrup*). Antibiotic conjugates with an artificial siderophore were found to be potent agents against Gram-positive and Gram-negative bacterial pathogens. Siderophores, small iron-binding compounds that serve to actively accumulate iron in bacteria, are used as Trojan Horses in this approach.

Two novel bacteria-free membrane permeation assays were developed either by coating the membranes with hydrogel-forming polymers or with bacterial outer membrane vesicles. Machine learning approaches led to novel insights in structure-permeability relationships. Microclusters of *Pseudomonas aeruginosa* biofilms transferred to the air-liquid Interface of bronchial epithelial cell monolayers and a novel microphysiological device allow for repeated deposition of aerosolised anti-infectives for several days and generate readouts for both host and bacterial cell function.

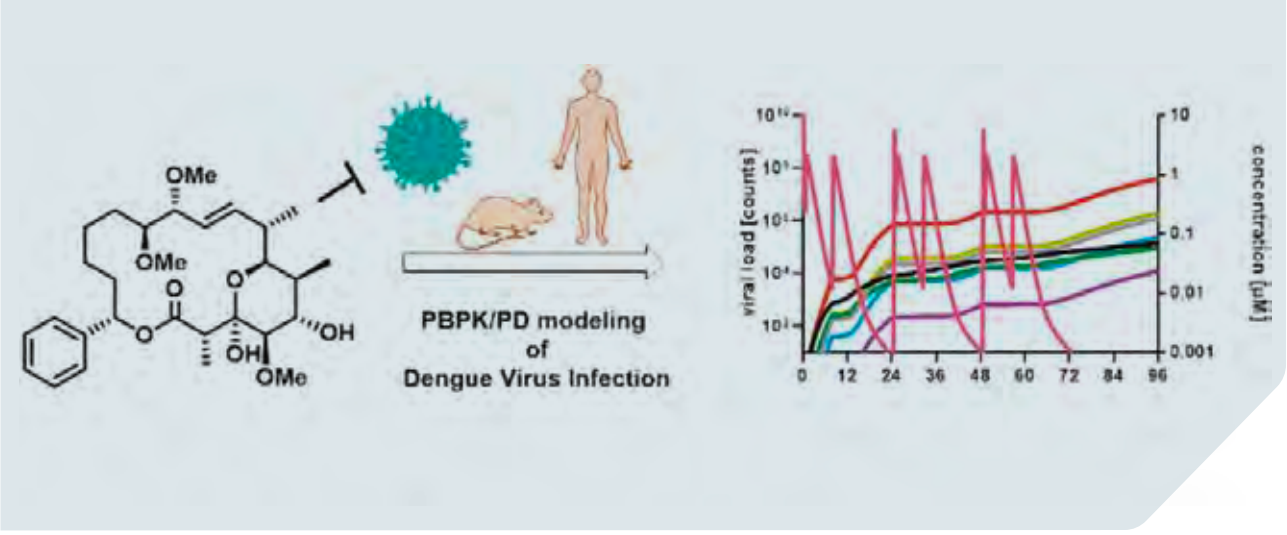

Figure 2: Soraphen A (molecule on the left) inhibits Dengue virus (green, viral structure in the middle of the figure). By using PBPK/PD modeling for both species, mouse and human, plasma levels of soraphen A (pink curve in the graph on the right) can be predicted as well as the viral burden in different organs over time (coloured curves in the graph on the right). The graph was adapted with permission from Rox *et. al.*, ACS Pharmacol. Transl. Sci. 2021, 4;5, 1499-1513, Copyright© 2021 American Chemical Society

PERSPECTIVES

In the future, RF AMR will continue to tackle the current AMR crisis from a range of angles. Researchers at HZI will advance the understanding of bacterial pathogenicity at a systems level and evaluate the impact of novel molecular diagnostic tests for the management of infectious diseases. RF AMR will impact basic research and clinical translation using a small-molecule approach for truly novel anti-infectives. The expansion of our leading natural- product platform is expected to reveal novel anti-infective principles. The most advanced natural products that are now in the pipeline have a realistic chance of reaching early stages of clinical drug development ("clinical proof of concept") in the near future.

In addition to natural products, novel synthetic scaffolds acting on underexplored targets have a high likelihood of circumventing antimicrobial resistance. Using a combination of target-based approaches with rational drug design techniques, scientists at RF AMR will discover and optimise further compounds which attenuate or abolish the pathogenicity of *P. aeruginosa* and *S. aureus*.

Our research on pathoblocker targets has reached a high level of maturity and will approach clinical proof of concept in the coming years. With three first- or best-in-class compounds, HZI has the opportunity to validate the pathoblocker concept clinically.

The prediction and determination of the biological activity of antibacterials and antivirals will be pursued with a "mode-of-action platform" that is expanded with experimental and computational methods that learn from massive profiling data. Furthermore, novel anti-infectives will be developed as pharmaceuticals using tailored delivery solutions, e.g. via small vesicles that deliver them to the site of infection.

Researchers of HZI have now reached the stage when they can put their discoveries into clinical practice, and founding of a spin-off company (*Myxobiotics, see above and chapter "Innovation and Translation" in this report*) is in preparation. If a project reaches the preclinical development phase, founding a spin-out company combined with the acquisition of venture capital or out-licensing are favoured options.

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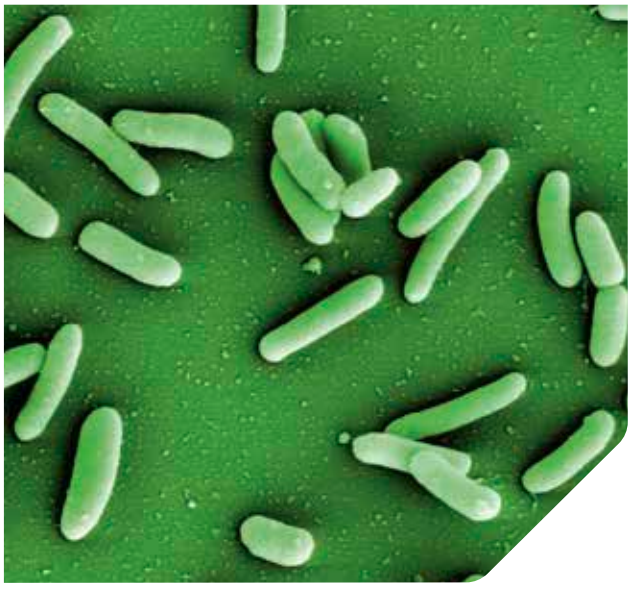

Pseudomonas aeruginosa © HZI | Manfred Rohde

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INVESTIGATORS IN RF AMR:

Olga Kalinina, Susanne Häussler, Ursula Bilitewski, Wulf Blankenfeldt, Mark Brönstrup, Susanne Engelmann, Chengzhang Fu, Rolf Hartmann, Anna Hirsch, Claus-Michael Lehr, Andriy Luzhetskyy, Alice McHardy, Eva Medina, Rolf Müller, Marc Stadler, Alexander Titz, Katharina Rox, Markus Kalesse, Michael Meyer-Hermann, Dietmar Pieper, Joachim Wink.

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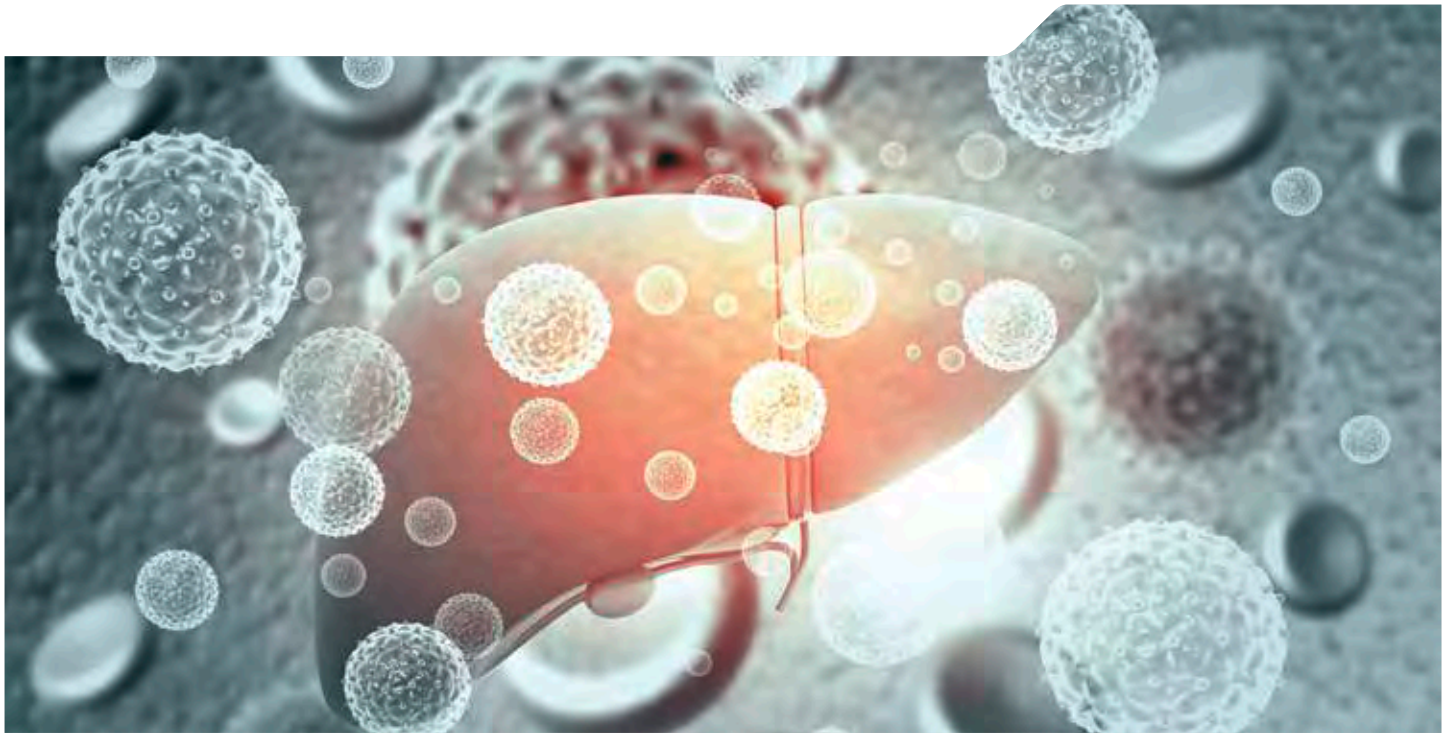
Speakers AMR:
Olga Kalinina, Susanne Häussler



Hepatitis C © Adobe Stock | bluebay2014

COMBATING PERSISTENT VIRUSES

RESEARCH FOCUS "CHRONIC VIRAL INFECTIONS" (CVIR)

Chronic viral infections represent a major problem for human health. They can be established if the host's immune responses are insufficient and/or if viruses escape or actively counteract host defense mechanisms. CVIR researchers aim to elucidate the mechanisms contributing to the establishment of chronic viral infections, with a focus on hepatitis B and C virus as well as herpes viruses. They are examining virus-specific interactions within infected cells and try to shed light on the immune responses of the infected host.

MOST IMPORTANT QUESTIONS ADDRESSED BY RF CVIR:

- Which viral and cellular factors determine the course of infection and what is their mechanism of action?
- Which mechanisms protect from chronic infections?
- How do viruses, in particular herpes and hepatitis viruses, evade, diminish or exploit these mechanisms?
- Which regulatory mechanisms are involved in dampened T cell responses and how can they be exploited?

1. INNATE IMMUNE RESPONSES AGAINST CHRONIC VIRAL INFECTIONS

The intrinsic host responses upon viral infections are mounted by the innate immune system. It represents the first line of defense and critically determines progression and outcome of infections. Innate immune responses are key within the first days of an infection to keep viral replication under control until the adaptive immune system kicks in.

Besides that, the innate immune response is crucial to trigger the adaptive immune response, leading to long-term protection against recurrent infections.

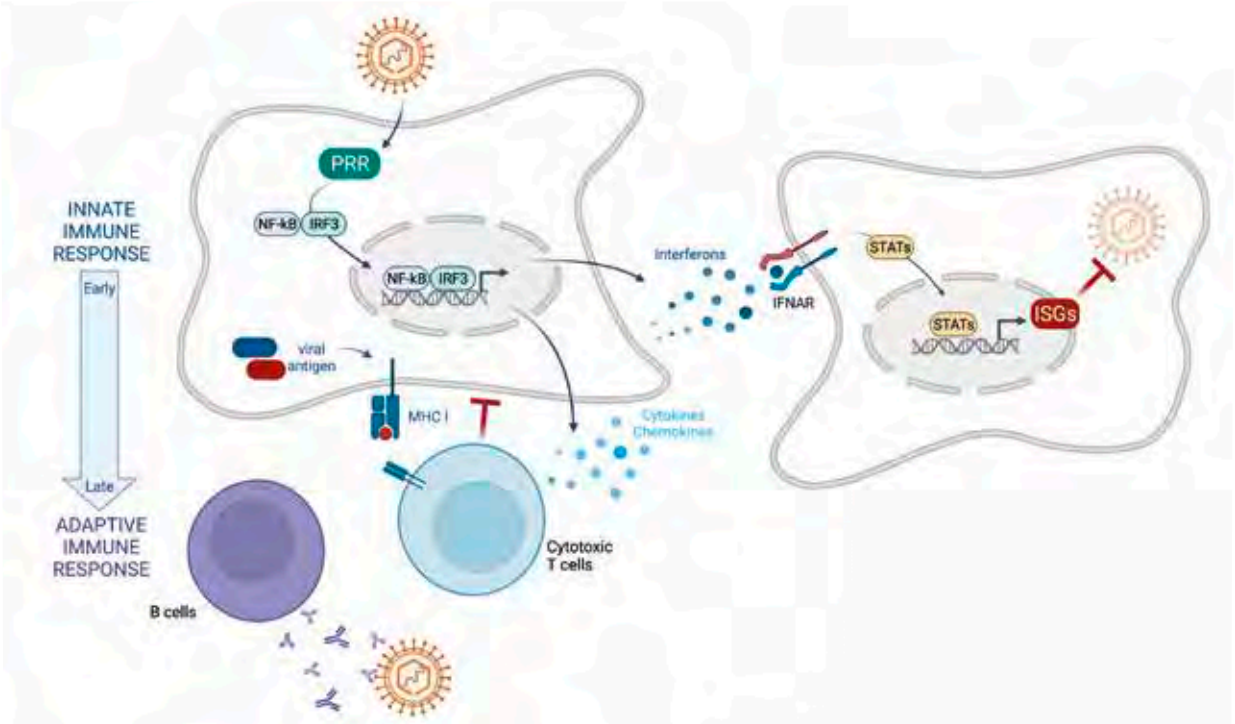


Figure 1: Innate immune response: cellular receptors (PRR) sense incoming viral particles and mount a potent antiviral response mediated by type I interferons and a plethora of interferon-induced restriction factors (ISGs). Adaptive immune response: T cells and antibodies are formed resulting in a specific immune response tailored to the pathogen. © Melanie Brinkmann, created with BioRender.com

Antiviral host proteins

Pattern recognition receptors - proteins capable of recognising foreign molecules - are key sentinels for incoming viruses and activate the type I interferon (IFN) system which plays a critical role in defeating viral infections. The IFN response leads to the expression of so-called "interferon-stimulated gene products" (short: ISGs). More than 300 ISGs can be expressed upon viral infection, and for the majority their exact function to counteract viral infections is not or only partially understood.

CVIR researchers have identified several ISGs that play a previously unappreciated role during infection with the chronic viruses Hepatitis C virus (HCV) and Human Cytomegalovirus

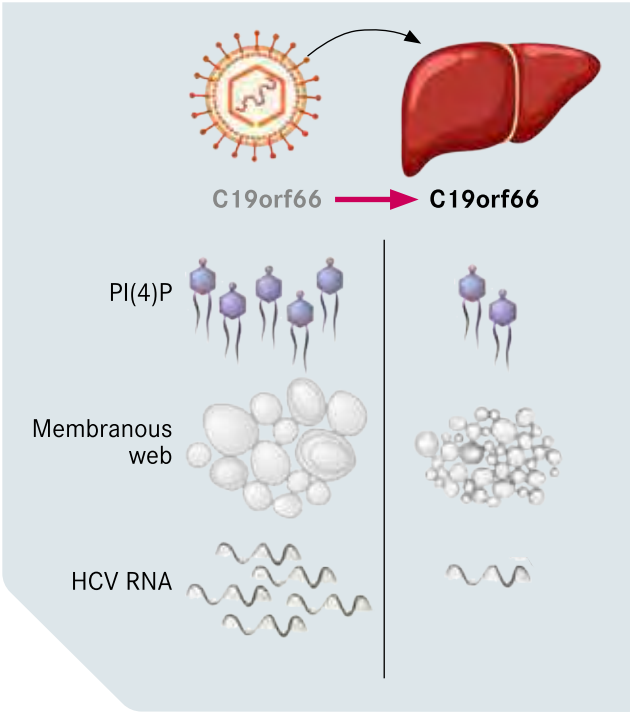


Figure 2A: C19orf66 is a cellular restriction factor upregulated upon HCV infection. It prevents HCV-induced elevation of phosphatidylinositol-4-phosphate (PI(4)P) and alters the morphology of the membranous web, thereby inhibiting viral replication. Adapted from Kinast et al. (2020), Journal of Hepatology 73:549–558 © CC BY NC ND

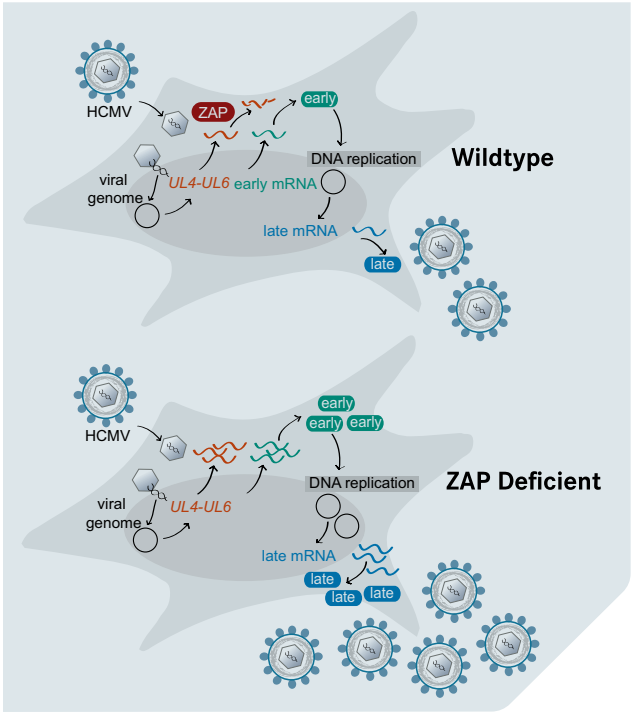


Figure 2B: The cellular restriction factor Zinc finger antiviral protein (ZAP) directly binds and mediates degradation of a specific set of viral transcripts from Human Cytomegalovirus and thereby negatively affects viral replication. © Melanie Brinkmann

(HCMV). Two ISGs – C19orf66 and the Zinc finger antiviral protein (ZAP) – were shown to be upregulated within the first hours of HCV or HCMV infection, respectively.

C19orf66 possesses specific antiviral activity against HCV. It targets the membranous web, which is the replication site of HCV in the infected cell *(Figure 2A)*. The ZAP protein on the other hand restricts the progression of HCMV infection by targeting a specific set of viral gene products, HCMV UL4 and UL5 *(Figure 2B)*. The ZAP protein was also identified as a key player during SARS-CoV-2 infection, acting as a direct regulator of programmed ribosomal frameshifting, which is a crucial process for the replication of the virus. By directly binding to SARS-CoV-2 RNA, ZAP strongly impairs viral RNA folding, preventing the production of the enzymes necessary for its replication. These two ZAP studies nicely illustrate the diverse functions of this antiviral host protein and its capacity to restrict both RNA and DNA viruses in a distinct manner.

Gene expression/activity after HCV infection

Due to the fact that acute HCV infections are often asymptomatic and therefore frequently undiagnosed, not much is known about the early transcriptional events after HCV infection. CVIR researchers could shed some light into this understudied early host response by analysing the transcriptional landscape of primary human hepatocytes (liver cells) after HCV infection. They revealed dysregulation of diverse cellular gene programmes, concurrently promoting both virus clearance and virus persistence. For example, suppression of virus propagation was observed when baseline expression of the interferon regulatory factor 1 (IRF1) coincided with infection-induced upregulation of IFN-controlled effector genes. On the other hand, transcriptional signatures were identified that correspond to severe inhibition of host translation, which likely reduces processing of IFN-regulated gene transcripts and facilitates virus survival. This may ultimately keep HCV under the radar of immune surveillance while initial infection is established, facilitating progression to chronicity.

Mouse models for investigation of HCV

HCV infections are restricted to primates, and the lack of small animal models limits the development of therapeutic strategies. Even when mice express the known human entry factors, they cannot be efficiently infected with HCV. CVIR researchers have now identified two novel restriction factors for HCV in mouse hepatocytes, the transmembrane proteins *Cd302* and *Cr1l*. Combined expression of these proteins in human hepatoma cells impeded HCV uptake and cooperatively mediated transcriptional dysregulation of a noncanonical programme of immunity genes. Notably, ablation of endogenous *Cd302* expression increased HCV infection in entry factor transgenic mice. This finding may be another step towards the development of next-generation murine models for preclinical testing of HCV vaccine candidates.

Exploiting innate responses for design of theranostic cells

Therapeutic strategies that would sense and act against a plethora of viruses are particularly attractive. In this regard, CVIR researchers asked if the broad activation of the innate immune cascades by various viruses can be exploited for visualisation and combating of an infection. In a synthetic biology-based strategy, they rewired the cellular innate response pathway to synthetic modules for expression of visualisation molecules and antivirals. As highlighted in an independent chapter, such 'theranostic' cells can detect and counteract viral infections, even when the viruses dampen this cellular pathway *(see section "Highlight Papers, Dagmar Wirth)*.

New potential antivirals

In a joint effort with researchers from the MHH, labyrinthopeptin A1 and A2 were identified as novel broad-spectrum antivirals that show high efficacy against a wide range of viruses, namely Chikungunya virus, Dengue virus, Zika virus, Kaposi's sarcoma-associated herpesvirus, and HCMV. These compounds target a lipid of the viral envelope that is not present in the membrane of mammalian cells. Thus, the labyrinthopeptins perforate viral particles while the target cells are not affected, resulting in low cytotoxicity. This qualifies these compounds as promising antivirals that can act against a multitude of enveloped viruses.

2. T AND B CELL BASED IMMUNE RESPONSES AGAINST CHRONIC VIRAL INFECTIONS

A few days after infection, adaptive immune responses are established, as characterised by the formation of virus specific immune cells that are key for the combating of the pathogens and for protecting from recurrent infections. This includes T cells that recognise viral antigens presented on the cell surface as well as B cells secreting antibodies that are specifically directed against the virus. In chronic viral infections, these responses are impaired, and researchers of CVIR investigate the underlying mechanisms with the aim to find novel strategies to overcome chronic infections.

Reinvigorating exhausted immune cells

Cytotoxic T cells are a class of immune cells which play a crucial role in preventing or controlling viral infections. They can sense viral antigens that are presented on the surface of infected cells and subsequently kill these cells. However, in chronically infected patients, the cytotoxic T cells are in a non-functional, exhausted state, and cannot eliminate the infected cells. To re-invigorate exhausted T cells, CVIR researchers investigate the mechanisms underlying the failure of T cell responses. In a study *(summarised in Figure 3)*, a synthetic mouse model was utilised which mimics the situation in chronically infected patients with regard to the development of exhausted T cells in the presence of persistent antigen expression in the liver. Using this model, the CVIR researchers identified a small population of profoundly cytotoxic T cells in the liver. These cells displayed efficient metabolic functions, improved effector functions, tissue residency markers and showed reduced exhaustion. In order to expand this cytotoxic T cell subset, the intrahepatic environment was conditioned by activating a crucial innate signaling pathway in myeloid cells. Notably, by expanding the aforementioned T cell subset, the antigen load was completely cleared. The discovery of the cytotoxic activity of these T cells and the possibility to restore exhausted T cells by stimulation of the signaling pathway opens up novel therapeutic options to overcome chronic liver infections in humans.

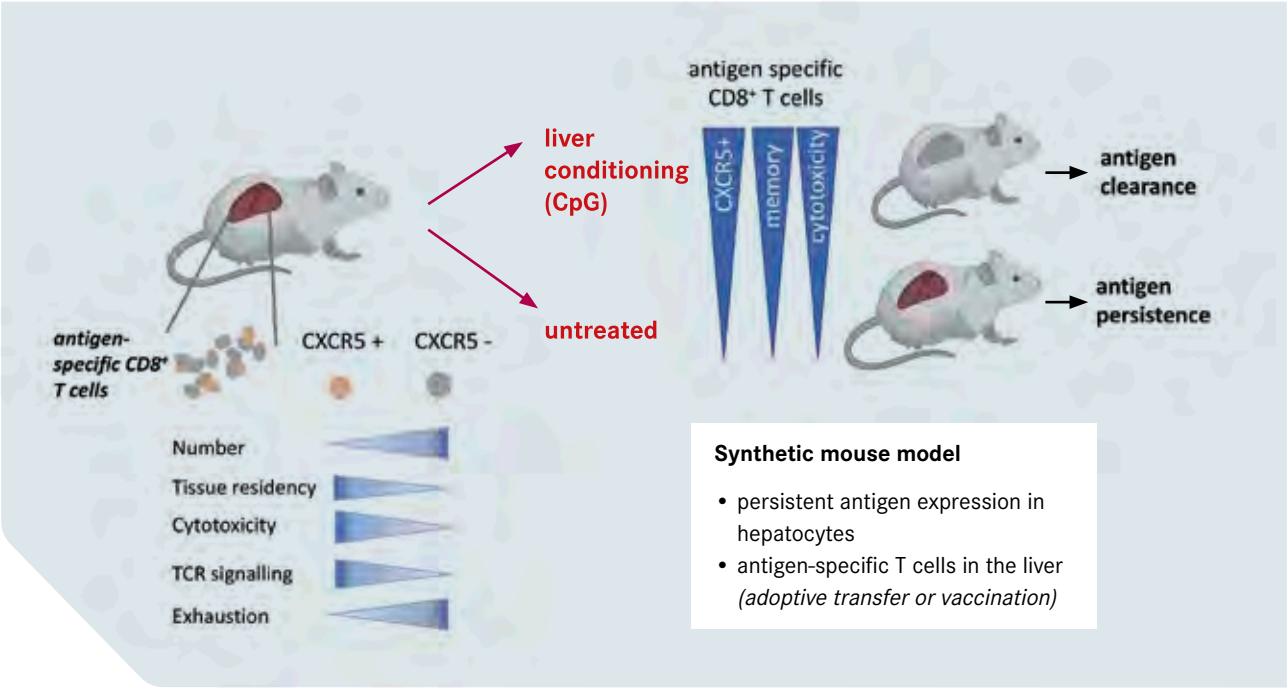


Figure 3: Properties of antigen specific T cells in the livers of mice with persistent antigen expression and the consequences of liver conditioning by CpG oligonucleotide treatment. © Dagmar Wirth

T Cell responses upon blocking checkpoints

Exhausted T cells are also characterised by expressing high levels of inhibitory receptors such as the programmed death protein PD-1. Stimulation of these receptors inhibits the activation of T cells and renders them non-functional. In the field of cancer, therapies that block these inhibitory receptors, so-called "checkpoints", have been shown to result in cytotoxic T cell activation and efficient killing of cancer cells. To elucidate the potential of this strategy for combating chronic viral infections, CVIR researchers aim to better understand the mechanisms and consequences of PD-1 blockage. They raised the question if treatment with checkpoint inhibitors would affect the nature of antigen-specific T cell responses. After treating persistently infected mice with checkpoint inhibitors, the T cell response was reinvigorated. However, the breadth of T cell responses against the virus was significantly reduced. While the consequences of this narrowed repertoire remain to be investigated, this finding may explain so far barely understood adverse effects of checkpoint inhibition strategies, such as viral escape or autoimmunity.

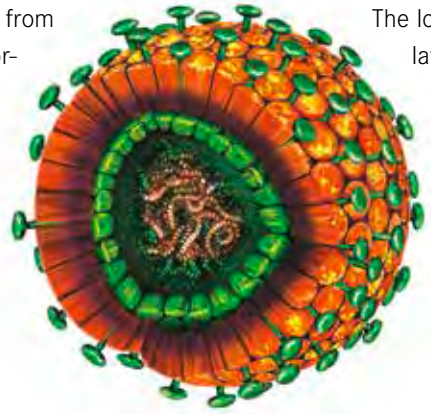
Elite neutralising antibodies against HCV

While cytotoxic T cells have the potential to eliminate chronically infected cells, B cells are relevant for humoral immunity by secreting antibodies that can neutralise infectious particles and/or facilitate uptake by phagocytotic cells. To gain a better understanding of the diversity of the antibody response and to prioritise antigens for vaccines, CVIR researchers generated a reference panel of synthetic viruses. This panel was highly instrumental in identifying various neutralising antibodies from patient sera. By employing bioinformatic tools, individuals with extraordinarily potent and broadly neutralising antibodies could be identified *(for more details of this work, see section "Highlight Publications"/ Thomas Pietschmann)*.

Moreover, in close collaboration with MHH researchers, this reference panel helped to identify various HCV antibodies, including neutralising antibodies with exceptional breadth and potency. Based on their diversity, a machine learning approach was instrumental in designing a *de novo* antibody that efficiently neutralised multiple HCV genotypes. Together, the results of these activities can improve the design of effective vaccine candidates.

PERSPECTIVES

Researchers of the RF CVIR build on intra- and extramural collaborative networks of experts, involving clinicians, immunologists, structural biologists, medicinal chemists, and virologists to address the critical knowledge gaps about chronic hepatitis and herpes virus infections. They elucidate essential molecular mechanisms of immune control and pathogenesis of these types of viruses. To this end, they will further dissect pathways and key principles of innate immune sensing and viral countermeasures. This includes comprehensive studies on selected herpes and hepatitis viruses. Specifically, CVIR researchers aim to investigate alternative strategies for cost-effective hepatitis C therapies and conduct research towards development of a prophylactic vaccine against HCV. In parallel, the principles that control cellular immune responses in these chronic viral infections will be explored further. CVIR researchers also evaluate the potential of murine Cytomegalovirus vectors to act as vaccines against Hepatitis C or chikungunya virus.



The long-term aim of CVIR research is to translate this knowledge into improved intervention strategies and ultimately into optimised and cost-effective management of these infections at the community level.

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INVESTIGATORS IN RF CVIR:

Wulf Blankenfeldt, Melanie Brinkmann, Neva Caliskan, Luka Čičin-Šain, Markus Cornberg, Carlos A. Guzmán, Lothar Jänsch, Ulrich Kalinke, Mario Köster, Andrea Kröger, Alice McHardy, Mathias Munschauer, Thomas Pietschmann, Redmond Smyth, Joop Van den Heuvel, Dagmar Wirth.

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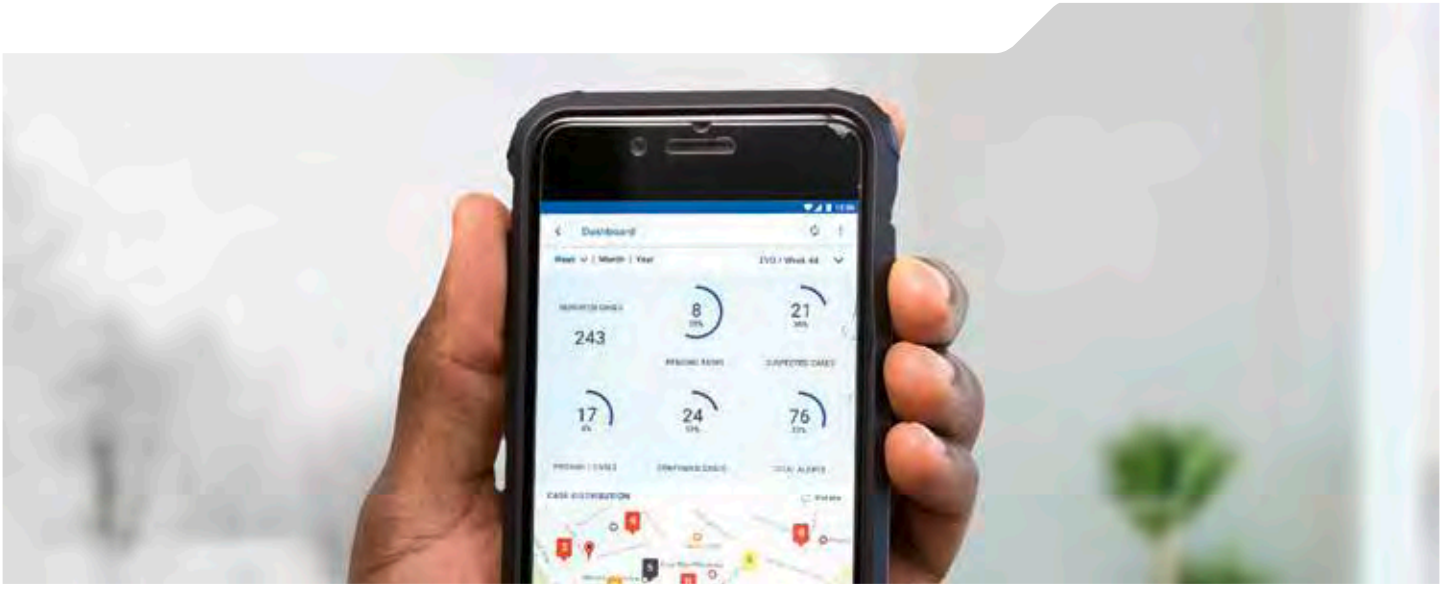
Speakers CVIR:
Melanie Brinkmann, Dagmar Wirth



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STUDYING, PREVENTING AND CONTROLLING EPIDEMICS

RESEARCH FOCUS "DIGITAL AND GLOBAL HEALTH" (EPI)

Research Focus EPI – short for "Epidemiology" – addresses infectious diseases at the level of populations and develops solutions for public health. The development of the Surveillance Outbreak Response Management and Analysis System (SORMAS), coordinated by HZI, improves our understanding of the spread of infectious diseases and has already contributed to the response to epidemics in different parts of the world. In particular, it has played an important role in the surveillance and response to the COVID-19 pandemic. The Research Focus contributes to public health solutions at local, national, and international levels by investigating the burden, determinants and impact of infectious diseases. HZI has intensified the development of electronic Health ("eHealth") and Research ("eResearch") technologies to digitalise infection research. Within the German National Cohort and beyond, the HZI application will allow the discovery of hitherto unknown relationships between infections and non-infectious diseases.

MOST IMPORTANT QUESTIONS ADDRESSED BY RESEARCH FOCUS EPI:

- How can we improve global health security and pandemic preparedness?
- How can applications for (mobile) digital devices enhance the prevention and treatment of infections and subsequent rehabilitation?
- What causal associations exist between infections and non-infectious diseases – and can they offer new approaches for control and prevention?

1. DIGITAL INFECTION SURVEILLANCE: SORMAS

The Surveillance Outbreak Response Management and Analysis System (SORMAS) was launched in 2015 as a research project at the Department of Epidemiology of HZI in collaboration with African partners (Nigeria and Ghana) and now plays a key role in combating the COVID-19 pandemic in low- and high-income countries. Since 2019, the list of countries using SORMAS has gradually expanded to include Germany, Switzerland, France, Ivory Coast, Fiji, Nepal and Afghanistan, among others, which have decided

to establish SORMAS independently of HZI. SORMAS has not only expanded geographically, but has also evolved technically by adding several features and improving the data protection concept. As a result, SORMAS achieved a score of 100 % in the Global Good maturity assessment, making it the first electronic health tool for disease surveillance and outbreak management/response to reach this status worldwide. It currently has 40 disease specific modules, including COVID-19, and is compatible with several digital symptom diaries including the HZI eResearch system PIA (*see below*) and other surveillance systems. Further development of the software includes a statistical analysis application to be deployed alongside SORMAS. It allows users to visualise contact and infection networks, automatically calculate epidemiological parameters, and automatically generate status reports.

2. SEROPREVALENCE STUDY MUSPAD

From July 2020 to August 2021, HZI conducted a nationwide seroprevalence study ("Multilocal and Serial Prevalence Study on Antibodies to SARS-CoV-2 Coronavirus in Germany", MuSPAD) to investigate the SARS-CoV-2 antibody status of more than 39,000 individuals in eight German districts and cities. As part of MuSPAD, we conducted an add-on study within the German National Cohort (NAKO) and examined about 3,000 participants from Hannover in 2021. Among these participants, we observed a – at the time - high vaccine coverage for the first dose (about 90 %) and 53 % for the second dose. The response for our additional questionnaires to be answered from home via PIA was more than 75 %.

Overall, the surveillance detection ratio (SDR), *i.e.,* the number of infections based on our results and the number reported to health authorities, ranged from 2.5 to 4.5, and the estimated infection fatality (the number of reported deaths divided by the estimated number of infected persons) ranged from 0.2 to 2.4 %.

Our anonymised data are available to other researchers on serohub (www.serohub.net). This is a platform for (inter-)national seroprevalence studies on SARS-CoV-2.

Both serohub and the MuSPAD cohort will now be continued within the Team of Clinical Epidemiology and Evidence Synthesis *(see below)*.

3. EVIDENCE SYNTHESIS

Scientists of the Clinical Epidemiology and Evidence Synthesis team have established a hub for evidence synthesis and infectious disease modelling as part of the Research Focus EPI. They are able to perform rapid and scalable systematic reviews and meta-analyses, as well as dynamic models of respiratory infections to better understand the spread of disease in populations. This was crucial during the pandemic to address the need for public information, policy advice, and rapid guideline development. Several systematic reviews and meta-analyses were published, as well as epidemiological studies and comments assessing respiratory infections and pandemic burden, contributing to guidelines and public health policies like the vaccination strategy. In particular, RF EPI researchers have focused on under-detection of infec-

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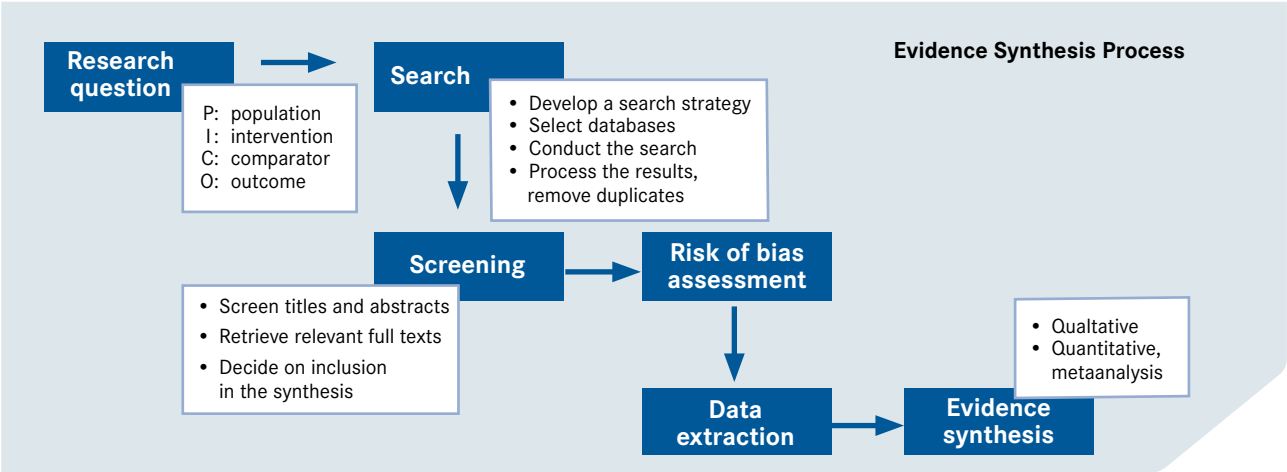
For the seroprevalence study MuSPAD, participants were examined at central locations - left: the Hannover trade fair hall - as well as by mobile teams at home (right).



Outline of the main steps in the evidence synthesis process.

tions, the burden of disease during the pandemic, the indirect effects of the pandemic, and on the role of schools as sites of infection.

For example, they have shown that age modifies the effect comorbidities have on the risk of a severe course of disease and that regions with mask mandates in schools had lower infection rates in schools during 2020 and 2021 in Germany. They have also assessed interventions to reduce disease burden from vaccine-preventable diseases in vulnerable populations and the evaluation of digital health tools for infectious disease surveillance. Dynamic modelling of infectious disease was used to understand the contribution of different population groups to the pandemic. This showed that the contacts of younger age groups contributed relatively more infections to the overall pandemic if age-specific underdetection was taken into account in models.

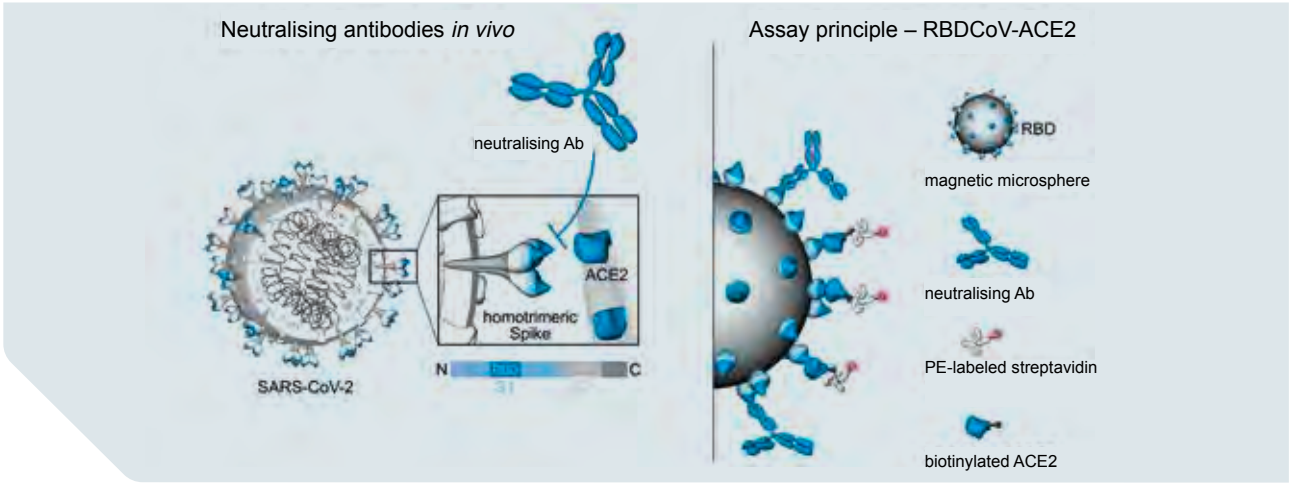
Furthermore, the impact of non-pharmaceutical and vaccination strategies was assessed to evaluate and improve pandemic response, and policy advice was provided to federal and national agencies (*see also Chapter: "2020 and 2021: Years of the Pandemic" in this report*).

4. MULTIPLEX SEROLOGY

Reliable and scalable serological assays are essential tools to investigate previous virus exposure within the general population while simultaneously providing an understanding of the role of antibody responses in disease manifestation, progression and reinfection. In contrast to most currently available serological assays which determine SARS-CoV-2 antibody responses for only a single viral antigen, we – in collaboration with the Natural and Medical Sciences Institute at the University of Tübingen (NMI) – developed a multiplex immunoassay based on the Luminex technology. It includes more than 25 antigens to allow a comprehensive characterisation of antibody-driven immune responses following vaccination or natural infection. Cross-reactive antibody patterns towards seasonal 'common cold' coronaviruses are characterised by including selected antigens of these other coronaviruses.

The rapid evolution of SARS-CoV-2 into "variants of concern" (VoC) has been addressed by the continued expansion of the antigen panel. In addition, we developed a functional analysis of SARS-CoV-2 antibody responses by determining their inhibitory capacity towards the original SARS-CoV-2 Wuhan isolate and selected variants of concern. Both tests have been used in a series of studies, for example, to provide valuable data on vaccine-induced protection against SARS-CoV-2 in at-risk groups such as dialysis patients.

Since summer 2020, a series of COVID-19 vaccines have been approved globally and are widely credited to have reduced SARS-CoV-2-induced severity and mortality. By using the nucleocapsid antigen, we can discriminate between infection and vaccine-induced humoral immunity. Antibody response to all immunisation schemes available in Germany were examined with approximately 3000 samples of MuSPAD *(see above)*. Like others, we found that mRNA or combinations of mRNA and vector-based vaccines elicit higher antibody responses than vector-based vaccines.



© NMI Natural Medical Sciences Institute at the University of Tübingen

Figure: Neutralising antibodies are an important antiviral defense mechanism by inhibiting the entry of SARS-CoV-2 into cells. The RBDCoV-ACE2 assay was developed to measure how well neutralising antibodies present in blood inhibit the binding of the Spike receptor-binding domain (RBD) to the cellular entry receptor of SARS-CoV-2, ACE2. For this, RBDs of SARS-CoV-2 and its emerging variants are immobilised on spectrally distinct magnetic beads. Beads are then incubated with samples where neutralising antibodies, if present, will compete with biotinylated ACE2 for binding to the immobilised RBDs resulting in a reduction of detectable fluorescence from the PE chromophore-labeled streptavididin-biotin-ACE2 complex.

5. INFECTION COHORTS: DIGITAL MONITORING VIA THE APP "PIA"

In all epidemiological infection cohorts of the Research Focus EPI, data collection is performed via the eResearch system "Prospective Monitoring and Management App", PIA. The eResearch system has been developed by HZI since 2017 and is specifically designed for longitudinal epidemiological research. It is free and open source (https://github.com/hzi-braunschweig/pia-system). PIA is adaptable to diverse research fields and topics and allows for complex and varying study designs. As of January 2022, there are more than 1300 participants in four RF EPI cohorts using PIA, namely *App-based Infection Assessment in RESIST (iAR), ZIFCO – Integrated DZIF Infection Cohort within the German National Cohort, DIMI – Digital infection monitoring in persons living with immunodeficiency, and SMARAGD – Sensors for measuring aerosols and reactive gases to deduce health effects.*

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The overall aim of these digital infection cohorts is to investigate risk factors and predictors of infectious diseases and their impact. Using PIA, we conduct syndromic surveillance of acute infections, with a focus on respiratory infections. For certain self-reported symptoms of respiratory infections (including COVID-19), different pathogens can be confirmed by virological PCR analysis of self-sampled nasal swabs.

In addition, ZIFCO focuses on multi-omics layers as predictors, while SMARAGD targets air pollution as a risk factor for respiratory infections. Here, we cooperate with the "Forschungszentrum Jülich", in iAR and DIMI with the MHH. We have also started implementing PIA in SHIP (Study of Health in Pomerania) together with partners in Greifswald with a focus on "One Health".

PERSPECTIVES

Climate change, migration and mobility of food, livestock and people increase the risk of emerging infections and rapidly spreading epidemics while at the same time challenging their control. The resulting societal burdens are threefold.

First, there are no specific treatments for many viral infections and for many bacterial infections the effectiveness of treatments is declining due to antimicrobial resistance. Second, epidemics and endemic infections have destructive consequences for the economy and social wellbeing, as the emergence of COVID-19 continues to show. Third, infections are the causes, triggers and mediators of non-communicable diseases such as cancer and cardiovascular, metabolic and neurodegenerative disease; many of these links have yet to be validated or even discovered.

We are responding to this multifactorial and reciprocal enhancement of societal challenges. We will harness and combine the latest technologies in mobile digitalisation, big data sciences and molecular omics in a truly multidisciplinary symbiosis of digital epidemiology, clinical infectiology and infection biology. We apply our research and development as early and as close as possible to where the need occurs: to patients even before they attend health care facilities, in the midst of the pandemic, and in the design of vaccination campaigns. Our goal is to contribute to the prevention of high-burden diseases, the prevention of future pandemics and the eradication of vaccine-preventable diseases.

The Vision of SORMAS is to be the leading and most sustainable epidemic management system in the world. For this aim we will create the not-for-profit SORMAS Foundation that will curate and promote SORMAS internationally. Anonymised extractions of these data will enable new approaches to epidemiological modelling and other infectious disease research. Research is currently underway to develop a SORMAS One Health module. This will allow the integration of animal and environmental health, which is essential for addressing urgent health threats such as antimicrobial resistance.

We envision PIA as an established eResearch System for implementing epidemiological cohort studies at the national and international scale. Hence, we will focus on further developing aspects that will enhance long-term adherence of study participants to PIA, e.g., by using gamification approaches, while at the same time generating research data from our digital infection cohorts.

The Team on Clinical Epidemiology and Evidence Synthesis will address gaps regarding existing epidemiologal research infrastructures, that also became evident during the pandemic. In our view, three infrastructures are relevant for the future. With our research we have already started to address some of these infrastructure challenges within large collaborative projects funded by BMBF, including RESPINOW, OptimAgent, NUM-PREPARED, NUM-IMMUNEBRIDGE. The MuSPAD cohort and the evidence and modelling hub created and coordinated will be essential in this.

Thematically with the MuSPAD cohort as well as with surveillance data studies and evidence synthesis we will investigate assocations of non-pharmaceutical interventions with infection prevalence on an individual and structural level; assess long-term infectious disease but also mental health outcomes after the pandemic; and look at occupational, personal factors influencing infectious disease outcomes for SARS-CoV-2 but also for other respiratory infections.

Regarding research infrastructure, first, we will support the building of large, rapid and adaptive population panels, which are able to capture infection frequency, immunity, contact structures for different pathogens rapidly.

Second, we will continue to build and support modeling platforms that are able to bring together different modeling groups and their results, combine these with evidence synthesis, present common results, and then disseminate them to decision makers.

And third, in the future we will aim to support the building of a platform for intervention studies of population interventions (such as contact reduction interventions) that can also be quickly reactivated to provide data baselines during a pandemic.

In our epidemiological laboratory, work is currently underway to expand our multiplex bead immunoassay to characterise antibody responses towards other respiratory viruses, such as respiratory syncytial virus or influenza virus, to differentially assess disease burden between those pathogens in the coming years. Overall, the successful development of both assays as novel, high-throughput, resource-efficient analytical research tools designed to capture the complexity of humoral immune responses in a single measurement demonstrates how use of emerging technologies can contribute to guide vaccination strategies, support the development of next generation vaccines, and understand the role of humoral immunity in infectious disease biology.

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INVESTIGATORS IN RF EPI:

Sebastian Binder, Stefanie Castell, Gérard Krause, Berit Lange, Fabian Leendertz, Michael Meyer-Hermann.

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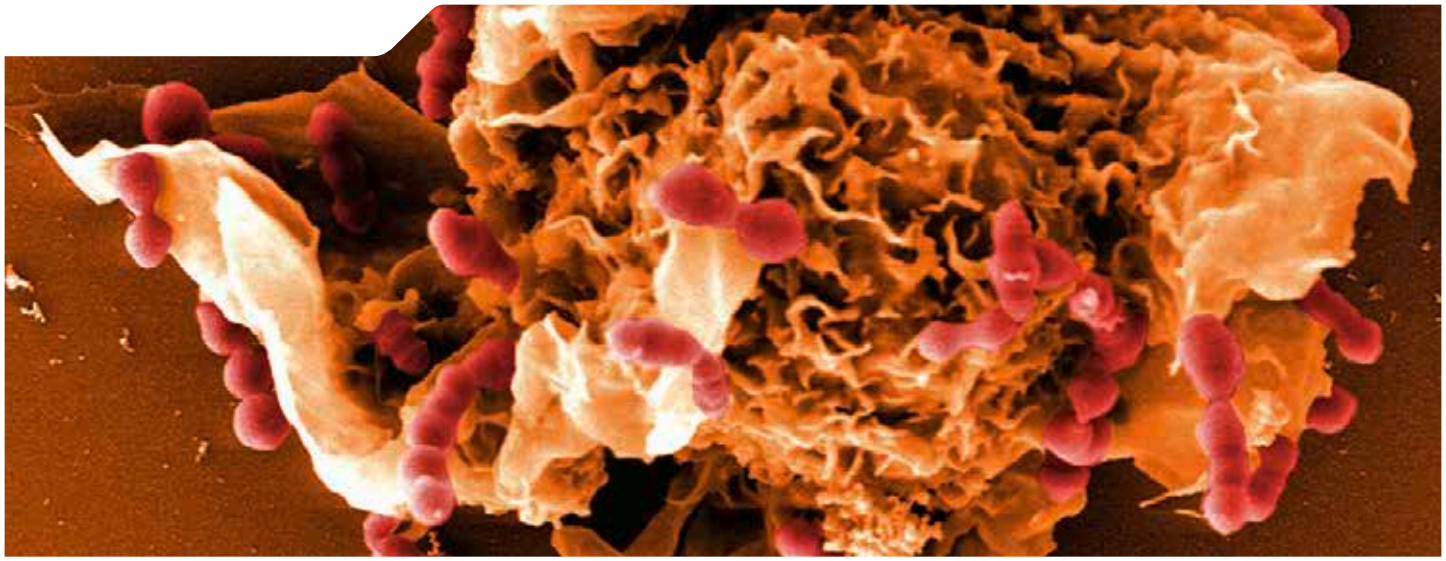
Speakers EPI:
Stefanie Castell and Gérard Krause



Human Dendritic Cell with *Strepococus gordoni* bacteria © HZI | Manfred Rohde

FROM INFECTION SUSCEPTIBILITY TOWARDS TAILORED INTERVENTIONS

RESEARCH FOCUS "INDIVIDUALISED IMMUNE INTERVENTIONS" (INDI)

A steadily increasing number of individuals shows enhanced infection susceptibility, yet the responsible risk factors are often only incompletely understood and for many infectious diseases, effective therapies and vaccines for these vulnerable individuals are still missing. Thus, the research focus "Individualised Immune Interventions (INDI)" deploys experimental and clinical research activities as well as multi-omics data integration approaches to better understand the molecular basis of enhanced infection susceptibility and to unravel risk factors for a severe infection course as well as the mechanisms underlying poor responsiveness to vaccinations or immune interventions. This research contributes to the development of immune-based interventions for the prevention and treatment of resilient infections in high-risk patients.

MOST IMPORTANT QUESTIONS ADDRESSED BY RF INDI:

- Why do individuals respond so differently to infections, vaccinations and treatments? What is the contribution of hereditary traits, and what is environmentally dependent?
- How can studies of vulnerable individuals be used to develop novel immune-based interventions?
- Which parameters predict the efficacy or pathogenicity of immune responses?
- How to design vaccines and immunotherapies to increase their efficiency of immune protection in vulnerable individuals and populations?

1. DYSREGULATED IMMUNE RESPONSE AS CAUSE OF SEVERE COVID-19

Exacerbated immune responses in COVID-19 play a major role in the pathophysiology of SARS-CoV-2 infection. In patients with severe COVID-19, dysregulated immune responses cause severe lung injury, including respiratory failure. Mitigation of immunodysregulation is therefore viewed as a major therapeutic avenue to treat and also prevent severe COVID-19.

However, the large heterogeneity of clinical manifestations and the complexity of immune responses to COVID-19 highlight the need for more detailed analyses using high-resolution techniques and well-characterised clinical cohorts. HZI

scientists contributed to a collaborative study coordinated by the *Deutsche COVID-19 OMICS Initiative* (DeCOI), using single-cell technologies to probe the divergent immune responses in blood samples of mild and severe COVID-19 *(see also section "Highlight Publications"/Emmanuel Saliba)*.

Results from this study revealed that distinct innate immune responses underlie the different clinical trajectories of COVID-19 patients. In particular, certain classes of immune cells – monocytes and granulocytes – were found to be immature and dysfunctional in patients with severe forms of the disease. Consequently, the development of treatments and prevention strategies for severe COVID-19 may benefit from insights gained in other fields, such as oncology, which have successfully applied therapies targeting suppressive myeloid cells.

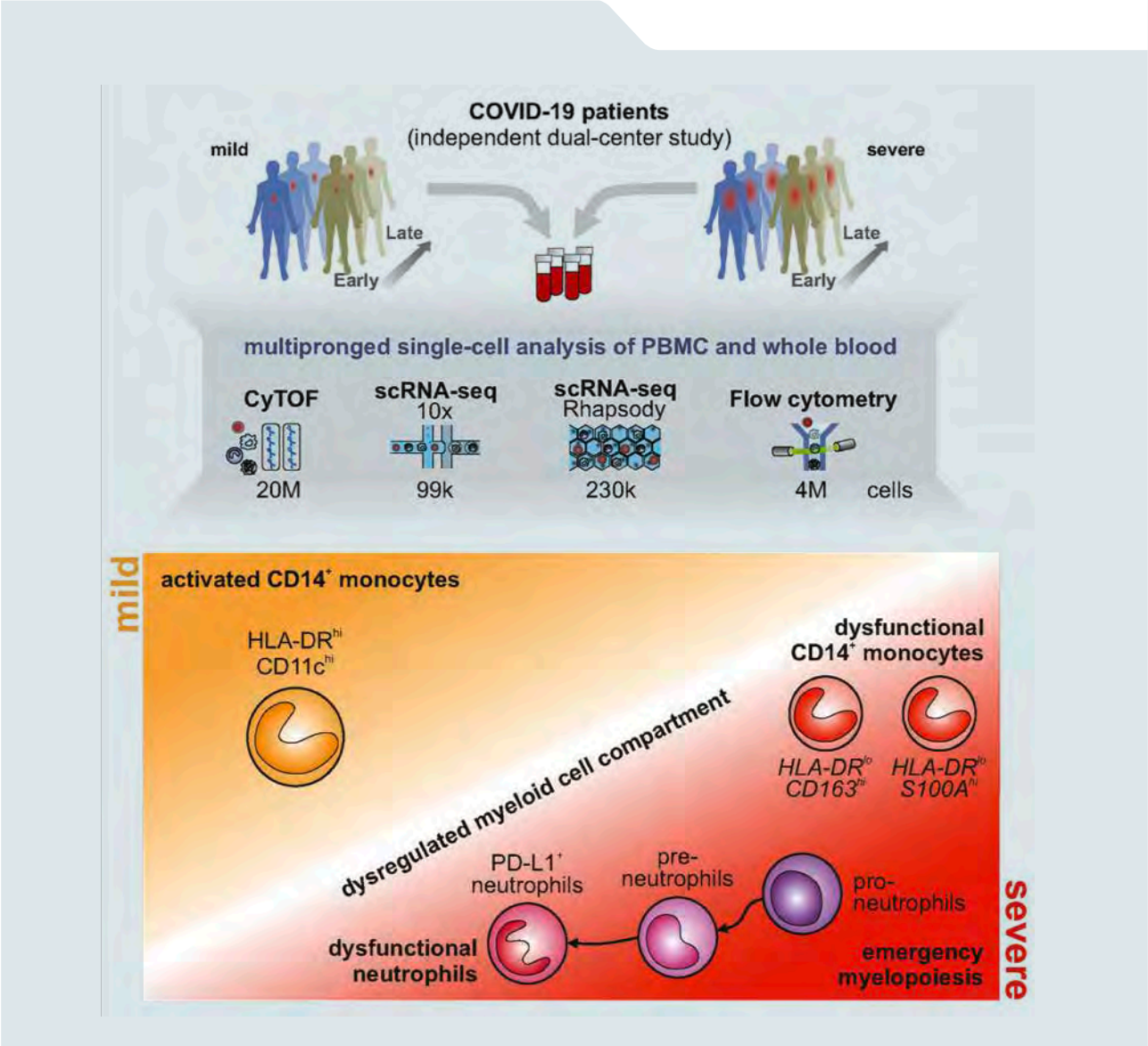


Figure 1: Single-cell transcriptomics and single-cell proteomics analysis in the blood samples of mild and severe COVID-19 revealed profound alterations in the myeloid cell compartment associated with severe COVID-19. While enhanced activated monocytes were found in mild COVID-19 patients, monocytes characterised by low expression of marker genes indicative of anti-inflammatory functions appeared in patients with severe COVID-19. Furthermore, the granulocyte compartment was profoundly altered in severe COVID-19, marked by the appearance of neutrophil precursors due to emergency myelopoiesis (production of bone marrow and of cells that arise from it, namely, blood cells). From: Schulte-Schrepping et al. (2020), Cell 182:1419–1440. Reprinted with permission from Elsevier.

2. IMPACT OF GENETIC FACTORS ON METABOLITES IN PATIENTS WITH IMMUNE-MEDIATED DISEASES

Metabolites – small molecular intermediates from metabolism – play an important role in modulating the immune system in both health and disease. However, most analyses to study the relationship between metabolites and the immune system were performed at "single-omic" levels, *i.e.*, they analyse patterns of only one type of biological molecules – like, e.g., proteins, or metabolites, genes etc. – at a time.

Since biological systems are controlled by complex processes, analyzing only one data type may fall short and fail to provide us with relevant and complete information. Thus, multi-omics studies are needed to unravel causal relationships regarding the contribution of metabolites to immune-mediated diseases and identify novel therapeutic targets. Considering that, computational biologists from HZI performed a study on multi-omics data available from a cohort of 500 healthy subjects. They generated a comprehensive map of blood metabolites, immune phenotypes, and their genetic basis.

Results obtained revealed that baseline metabolites – *i.e.*, metabolites commonly present in a healthy state – have a stronger impact on the innate than on the adaptive immune response. This corresponds to previously made findings that environmental cues, like metabolites, are mainly sensed by cells of the innate immune system. Genome-wide analyses were performed to unmask how genetic factors regulate metabolites related to immune phenotypes. These studies identified 29 significant loci ("mQTLs") that could contribute to the regulation of metabolite levels in the blood.

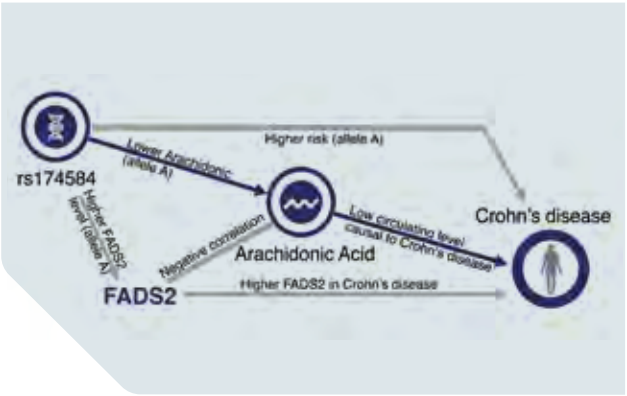


Figure 2: Graphic summary of the regulation network of the mQTL (rs174584-FADS2) in Crohn's disease. From: Chu et al. (2021), Genome Biology 22:198 © CC BY 4.0

A particular mQTL was found to be associated with Crohn's disease. In-depth analyses of this mQTL suggested a a role of FADS2, a biosynthetic enzyme of arachidonic acid, as a key driver of Crohn's disease.

Thus, evaluating the flow of information from one omics layer to another aids in a comprehensive understanding of complex biology processes, and this approach will help to unmask the molecular mechanisms underlying increased infection susceptibility and poor responsiveness to vaccinations in the future.

3. VACCINATION RECOMMENDATIONS FOR TUMOR AND AUTOIMMUNE PATIENTS UNDER B CELL DEPLETION

B cell lymphoma and autoimmune diseases are frequently treated with rituximab – a therapeutic antibody directed against CD20, a protein on the surface of B cells, which constitute a key component of the adaptive immune system. Rituximab confers efficient depletion of recirculating naive B cells. Correspondingly, rituximab-treated patients barely mount *de novo* antibody responses during infections or after vaccinations. Therefore, efficient immune responses of B cell-depleted patients largely depend on T cells, another important class of immune cells.

Scientists from HZI and Hannover Medical School collaborated in a clinical study assessing the immune responses in rituximab-treated rheumatoid arthritis patients upon vaccination against seasonal influenza. Analysis of their influenza-specific T cell responses revealed a less vigorous expansion of influenza-specific $CD8^+$ T cells in rituximab-treated patients than in vaccinated healthy individuals.

Since it is not immediately obvious how B cell depletion impairs the induction of T cell responses, a series of accompanying animal experiments were performed. Very similar to the situation in B cell-depleted patients, B cell-deficient mice infected with mouse-adapted influenza A virus or modified vaccinia virus Ankara (MVA) showed less vigorous expansion of virus-specific T cells than wild type mice. Of note, B cell-

deficient mice do not have an intrinsic impairment of CD8+ T cell responses, as indicated by the comparable expansion of virus-specific T cells in B cell-deficient and wild type mice following vaccinia virus (VACV) infection. A major difference between MVA and VACV is that MVA induces strong type I interferon (IFN) responses, and it could be experimentally proven that type I IFN receptor stimulation of B cells supports the induction of T cell responses during MVA infection. These experiments indicated that depending on the applied stimulus, B cells indeed can promote the induction of CD8+ T cell responses.

What does this new knowledge now mean for B cell-depleted patients? It is well possible that vaccines which induce immune responses including type I IFN lead to less vigorous T cell responses in B cell-depleted patients than vaccines which induce immune responses devoid of type I IFN. This question is particularly relevant in the context of the SARS-CoV-2 pandemic: B cell-depleted rheumatoid arthritis patients are especially vulnerable to SARS-CoV-2 infection and have an enhanced risk to develop severe COVID-19. Since RNA-based vaccines induce stronger type I IFN responses than vector-based ones, it is well possible that B-cell-depleted patients would profit more from vaccination with vector-based vaccines.

This highly relevant question is currently being tested in mouse models and cohorts of B cell-depleted patients.

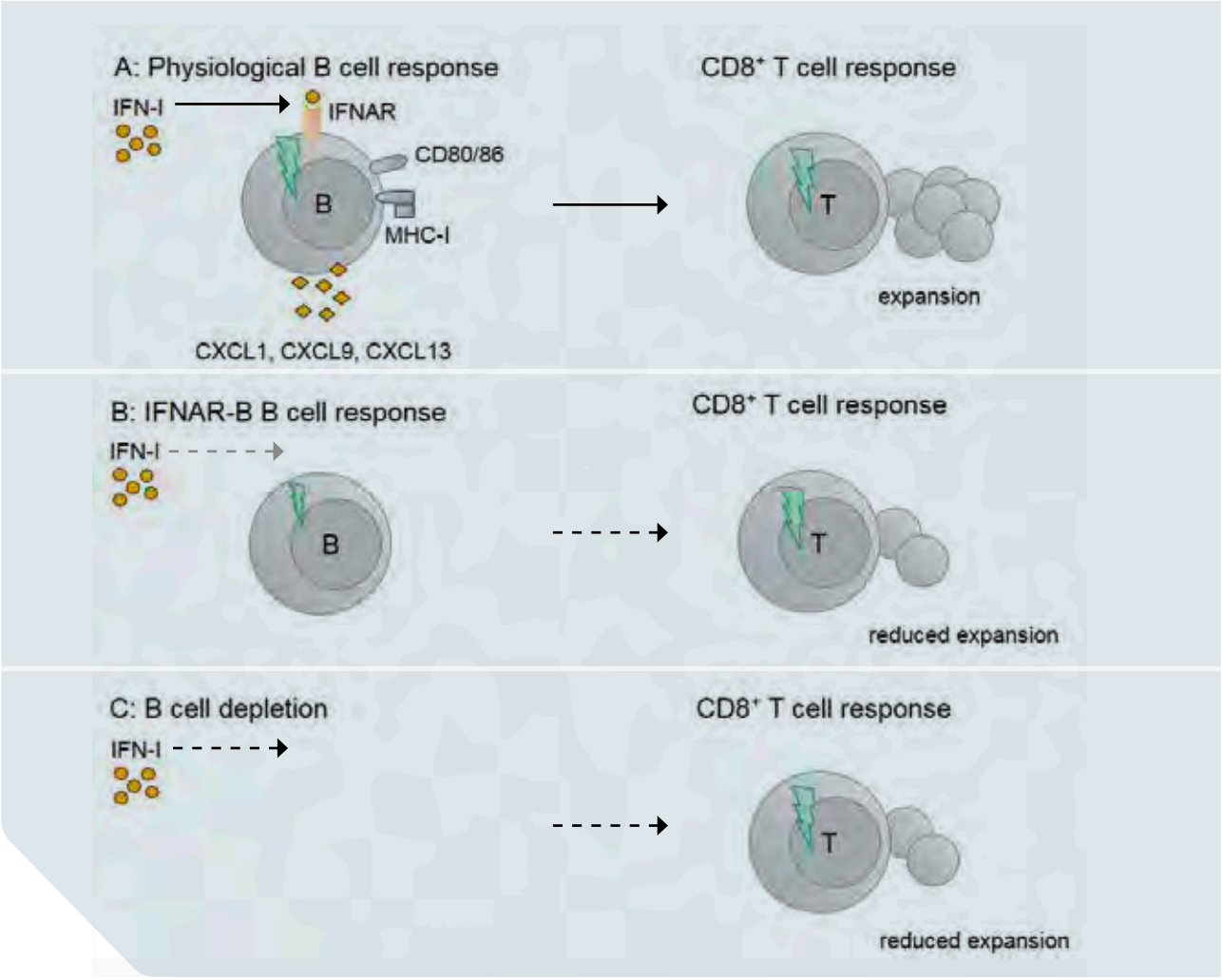


Figure 3: CD8+ T cell responses **A** in wild type mice, **B** mice with a conditional type I IFN receptor depletion in B cells, and **C** B cell-depleted mice.
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4. STRATIFICATION OF PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION

Hepatitis B virus (HBV) infection remains a major global health problem with approximately 257 million chronically infected people worldwide, resulting in 887.000 deaths per year due to progressive liver disease and hepatocellular carcinoma.

Frequently applied treatments (e.g., with nucleos(t)ide analogues or interferon alfa) suppress HBV DNA levels and slow disease progression in patients with chronic HBV infection. However, functional cure – defined as sustained loss of the hepatitis B surface antigen (HBsAg), which is the desired goal of HBV treatment – is rarely achieved with current therapies. Several novel therapeutic strategies, including antiviral and immunomodulatory therapies, that aim for a functional cure are presently being developed.

HBV-specific T cells are considered as key effector cells to control HBV infection, and impaired and exhausted T cell responses are a hallmark of chronic HBV infection. Experts agreed that quantification of HBsAg levels is pivotal to stratify patients for clinical trials with new therapeutic agents. Besides, persistent high antigen stimulation in patients with chronic hepatitis B is associated with impaired cellular immune responses.

However, the impact of circulating HBsAg on T cell responses is discussed controversially, and other viral proteins could potentially have an impact on the immune response as well. Recently, with the ability to determine hepatitis B core-related antigen (HBcrAg) using commercial assays, another HBV marker has become available that may be associated with the immune response. To study the impact of circulating HBsAg and HBcrAg levels, scientists from HZI and Hannover Medical School analysed T cell responses in chronically HBV-infected patients with different levels of both antigens. The main finding from this study is that HBV-specific T cells are not influenced by the level of HBsAg, but rather by the age of the patient. On the other hand, if patients with low HBcrAg levels were analysed, they showed higher HBV-specific T cell responses, irrespective of age. Finally, T cells isolated from patients with low levels of HBsAg and in particular low levels of HBcrAg were more responsive to immune intervention with a checkpoint inhibitor *in vitro*.

This study revealed that age should have a greater importance in the selection of patients for therapy and HBcrAg might be a better marker than HBsAg to discriminate patients with preserved HBV-specific T cell responses. The data also suggest that patients with low HBsAg and/or low HBcrAg are better candidates for immune-based therapies (e.g., checkpoint inhibition) regardless of age, supporting the therapeutic strategy for antigen lowering, for example, with RNA interference. Overall, the data provide new insights that are important for clinical decision making and for the stratification of patients for novel therapies aimed at functional cure of HBV.

PERSPECTIVES

Addressing the challenges of modern infection medicine, researchers of the Research Focus INDI will perform further experimental and clinical studies to better understand protective or pathogenic immune responses to infection and vaccination. These studies will build on recently established unique patient and population cohorts that are available through collaborations with clinical partners at MHH and via the Excellence Cluster RESIST. All activities in the area of immunoprofiling and multi-omics data integration will be bundled in the Center for Individualised Infection Medicine (CiiM) to gain in-depth knowledge of the mechanisms of differential susceptibility to infections, and to identify and functionally validate hitherto unappreciated risk factors. The resulting insights from the molecular profiling of the host response will allow us not only to identify novel biomarkers for a better stratification of patients and for the design of advanced diagnostics, but also to develop more efficient and specifically tailored vaccines and immunotherapies.

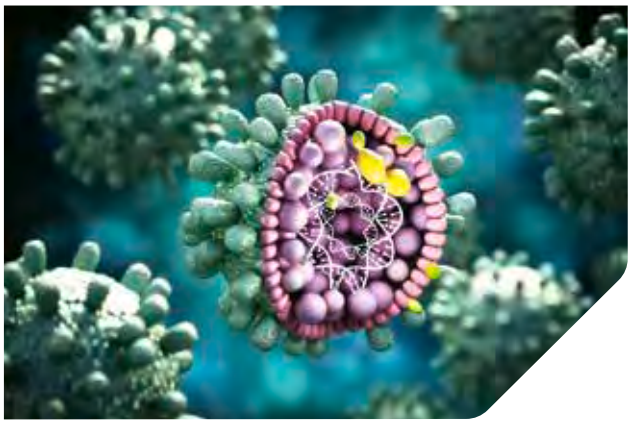

Figure 4: Hepatitis B Virus. © AdobeStock | Destina

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INVESTIGATORS IN RF INDI:

Georg Behrens, Dunja Bruder, Luka Čičin-Šain, Markus Cornberg, Carlos Guzman, Jochen Hühn, Lothar Jänsch, Ulrich Kalinke, Claus-Michael Lehr, Yang Li, Alice McHardy, Michael Meyer-Hermann, Frank Pessler, Emmanuel Saliba, Till Strowig, Dagmar Wirth.

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Speakers INDI:
Jochen Hühn and Marcus Cornberg

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CHARACTERISING BRAIN FUNCTION UNDER CONDITIONS OF INFECTION AND INFLAMMATION

RESEARCH FOCUS “INFECTION AND NEURODEGENERATION” (INEU)

Infections and inflammatory processes can have detrimental effects on the central nervous system (CNS). However, interactions between the immune system and the nervous system are still scarcely understood. The Research Focus „Infections and Neurodegeneration“ (INEU) is harnessing interdisciplinary research approaches to understand how pathogens in the CNS are controlled and how peripheral or central inflammatory processes affect brain functions. Specifically, the complex communication amongst CNS resident cells, peripheral immune cells infiltrating the CNS, and pathogens in the brain is being studied. Additionally, the impact of infections on the onset and progression of age-related neurodegenerative diseases such as Alzheimer‘s and Parkinson‘s disease are analysed. In this context, it is studied how processes in the CNS are influenced by microbiota, e.g., in the intestine and on the lung epithelia, as well as the metabolic status of the cells in the brain. These findings will help to identify early biomarkers for neurodegenerative diseases and to develop personalised therapeutic strategies.

1. IMMUNE MECHANISMS MAINTAINING BRAIN FUNCTION DURING HOMEOSTASIS

Interferons (IFN) provide a first line of host defence when cells detect an invading pathogen. Type I interferons are an important group of cytokines that mediate communication between cells and that are critical for virus control. Even though the role of type I IFN signalling is widely addressed in the context of viral infections, little is known about its potential role under conditions of brain development and homeostasis.

Interestingly, it has been observed that under physiological conditions, a basic level of type I IFN expression is present in healthy individuals. This suggests that tonic type I IFN signalling contributes to the maintenance of physiological conditions that are relevant for regular brain function. A collaborative approach between investigators in the Research Focus INEU addressed this hypothesis by generating conditional mice with a cell type selective deficiency in the type I IFN receptor (IFNAR1) in major CNS resident cells, including neurons and astrocytes. Behavioral traits of such mice, such as learning and memory function, and their brain histology were analysed. Additionally, the neurons of such mice were functionally studied.

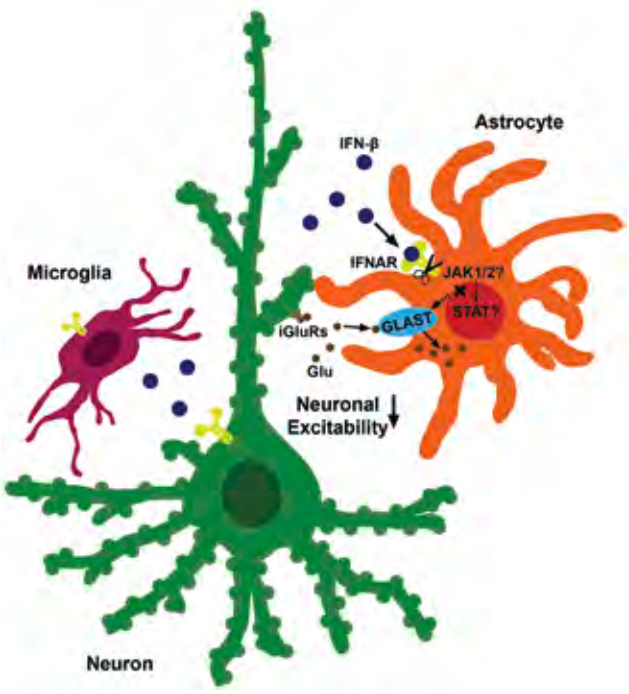


Figure 1: In the absence of viral infection, tonic type I IFN receptor signalling within the CNS is critical for synaptic plasticity and processes of learning and memory formation. We provide evidence that tonic IFN signalling of astrocytes is needed for synaptic plasticity and normal learning behaviour, possibly by regulating synaptic glutamate levels via the expression of the glutamate aspartate transporter GLAST. © HZI/M. Korte, S.Hosseini

The data clearly revealed that basal type I IFN levels in the brain confer tonic type I IFN receptor signalling within the CNS that is critical to maintain synaptic plasticity and to support processes of learning and memory formation. Furthermore, type I IFN receptor signalling of astrocytes, which are characteristic star-shaped cells in the brain and spinal cord that support functions for the neural tissue, modulated this effect *(Fig. 1)*.

These experiments showed that the interferon system is not only essential for pathogen control, but also for optimal CNS homeostatic function, and if it gets out of balance, it might be involved in pathophysiological processes as well. Furthermore, these data suggest that potentially also other cytokine families have an impact on CNS function under homeostatic conditions.

2. IMMUNE MECHANISMS THAT RESTRICT VIRUS DISSEMINATION WITHIN THE CNS

For long time it was believed that the brain is not readily accessible for viruses. Accordingly it was assumed that the brain is immunoprivileged, i.e., it does not show an inflammatory immune response after the intrusion of antigens. It was assumed that in the rare case a virus manages to enter the brain, there is only very little option to control the infection.

This view has entirely changed. Today it is clear that there are several layers of protection against virus infection in the brain. In earlier studies, we discovered that upon intranasal instillation of the vesicular stomatitis virus (VSV), which is closely related to rabies virus, local induction of type I IFN responses is detected early on. We identified astrocytes to be the cell type accounting for early IFN-beta expression. Recently we verified that upon exposure of murine astrocytes to tick borne encephalitis virus (TBEV), early type I IFN responses are induced after sensing of the pathogen. Upon entry of virus into the CNS, dissemination of the pathogen is restricted by activation of microglia, which are tissue resident macrophages of the CNS.

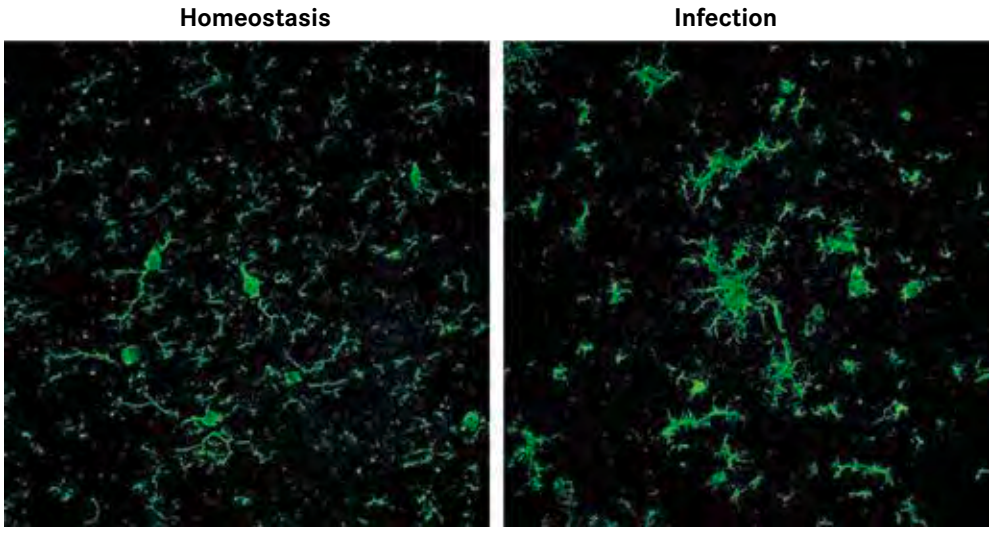


Figure 2: Within the virus infected CNS, microglia are activated and change their morphology. Mice were intranasally instilled with either PBS (homeostasis) or VSV (infection) and 6 days later the brain including the olfactory bulb was extracted. Representative data of confocal microscopy (Olympus FV3000) of the glomerular layer of the olfactory bulb is shown. Microglia were labelled with P2RY12-specific antibodies (green). The data reveals key morphological changes of microglia that are induced by infection. © Kalinke et al., unpulished

Interestingly, upon intranasal VSV instillation, activated microglia *(Fig. 2)* accumulate in peripheral areas of the olfactory bulb, where the highest virus loads are detected. Microglia form a shield of activated innate immune cells that is essential for preventing subsequent virus dissemination to distal parts of the CNS.

MOST IMPORTANT QUESTIONS ADDRESSED BY RESEARCH FOCUS INEU:

- How is the balance between virus control and tissue damage adjusted in the virus infected brain?
- How is the complex communication between brain resident cells and infiltrating immune cells regulated during virus infection of the brain?
- What role do infections and associated inflammatory processes play in the onset and development of neurodegenerative diseases?
- How does the individual microbiota influence processes in the central nervous system (CNS)?
- Which biomarkers can be detected in the early stages of a neurodegenerative disease?
- What are the possible therapeutic approaches and personalised preventive measures for each individual patient?

Surprisingly, type I IFN receptor signalling of microglia was not needed for full microglia activation, accumulation and proliferation. Instead, type I IFN receptor signalling of astrocytes and neurons was required for full microglia activation and protection of the host. These experiments highlighted that excessive communication between different cell types in the brain is required to control brain infection.

Our recent data highlight that also adaptive immune cells are needed to control CNS infection. In-depth analysis revealed that infected neurons produce chemokines, which regulate immune cell infiltration. Neurons sense virus infection by a specific pathway of the innate immune defence *(see section "Highlight Publications")*, which is a potential therapeutic target to treat viral encephalitis. Currently, researchers in RF INEU are focusing on elucidating the cross-talk between innate and adaptive immune cells within the CNS that is needed to control virus infection of the CNS.

3. LONG-TERM CONSEQUENCES OF INFECTION ON BRAIN FUNCTION

Within the last hundred years, novel strains of corona and influenza viruses have caused several global pandemics. Their primary place of action is the respiratory tract, yet, already since the famous pandemic known as the Spanish flu (1918-1920), associated neurological complications have been reported. The symptoms range from mild cognitive deficits to encephalopathy, epilepsy, and dementia.

In the recent SARS-CoV-2 outbreak, patients frequently suffer from headache, dizziness ('brain fog'), nausea, or decreased sense of smell and taste, suggesting that SARS-CoV-2 may indeed affect the central nervous system (CNS). This is underscored by long-COVID, a syndrome that establishes after SARS-CoV-2 infection and continues for weeks or months after resolution of the virus infection. Attention and memory disturbances obviously point towards associated neuronal dysfunction, even with moderate symptoms.

We are just beginning to understand the potential association between respiratory viral infections and chronic neurodegenerative diseases – in particular with a diffused state of consciousness known as delirium, which is associated with intensive care patients of COVID-19, and that is correlated generally with a doubling of the risk to be affected by Alzheimer's disease in later life. Because of the time delay that may occur between the initial infection and the subsequent development of pathology, the nature of this connection will be difficult to elucidate in humans. Coming back to the example of the Spanish flu, patients developed neurological symptoms such as Parkinsonism sometimes decades later. Therefore, we need animal models on which we can test our hypotheses.

Respiratory viruses can induce neuronal damage directly via infection of the CNS or indirectly via peripheral cytokines responses *(Fig. 3)*. Microglia are immune cells of the brain, whereas monocytes, macrophages, dendritic cells and lymphocytes are immune cells in the blood. We recently published that a highly pathogenic influenza virus strain, H7N7 (avian influenza), can invade the brain during infection and cause direct neuronal damage and chronic activation of microglia cells. Yet, virus strains that are most commonly isolated during seasonal influenza outbreaks (H1N1/H3N2) do not infect the brain, but still can cause long-lasting chronic cognitive deficits – in particular in elderly individuals. This emphasises that the direct presence of virus in the brain is not necessary to induce neurological complications. Recovery from such long-term manifestations often take much longer than convalescence from the acute infection.

Furthermore, subclinical infection with tick-borne encephalitis virus can lead to anxiety-like behaviour. Virus replication in the olfactory bulb may induce far-reaching effects on hippocampal neuron morphology and impaired hippocampus-dependent learning and memory formation.

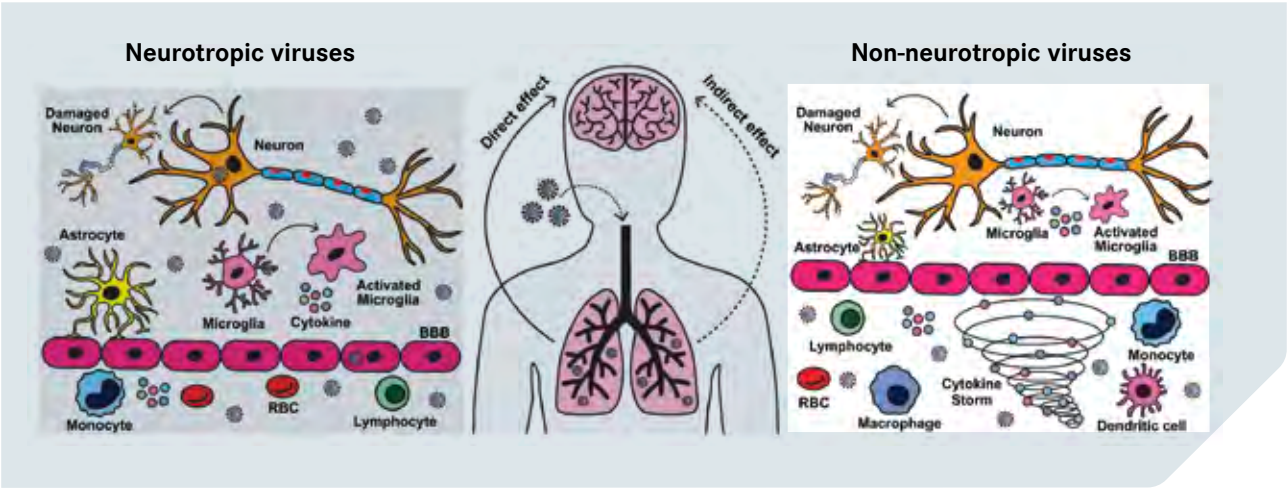
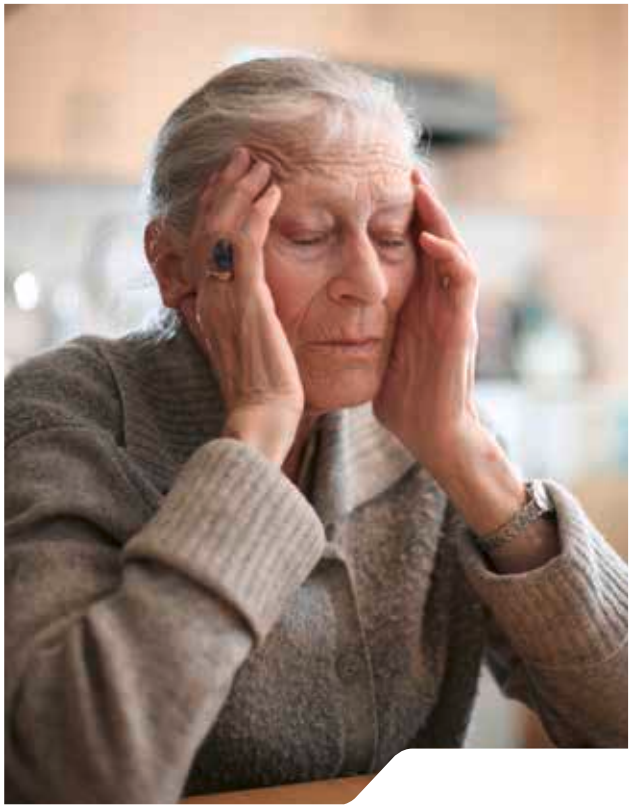


Figure 3: Respiratory viruses can induce neuronal damage directly via infection of the CNS or indirectly via peripheral cytokines. Microglia are immune cells of the brain, whereas monocytes, macrophages, dendritic cells and lymphocytes are immune cells in the blood; BBB: Blood-brain-barrier; RBC: red-blood-cells. © HZI|M. Korte, S.Hosseini

Our research will help to develop strategies for the prevention of long-term effects of future pandemics. Environmental and climate changes presumably will enhance the frequency of the appearance of new viral strains with pandemic potential, which also pose a possible threat to our most important organ, the brain. Strategies are needed not only to acutely fight viral infections, but moreover to protect in particular the highly vulnerable brain that has limited regenerative capacity. Thus, previous pandemics have taught us important lessons about what needs to be considered in future pandemics. We need to understand how immediate and delayed neurological damage is induced by different respiratory viral strains and how this might trigger or enhance neurodegenerative diseases.

This knowledge is crucial in order to identify early biomarkers, to develop better treatment options, and above all, to prevent the downward spiral in the first place via vaccination or specific drugs, especially in vulnerable individuals such as the elderly.



Dysfunction of the central nervous system, as in some forms of dementia, can be linked to infections and immune reactions. RF INEU researchers investigate these interactions. © Fotolia | Konstantin Sutyagin

PERSPECTIVES

INEU focuses on the analysis of immune mechanisms that maintain brain functions during homeostasis, inflammation, and CNS infection. After we discovered that tonic type I IFN receptor triggering of astrocytes is of key relevance for normal learning behaviour, in the future we will study the influence of other immune mechanisms on brain functions under homeostatic conditions. During the last years, we learned a lot about mechanisms that are relevant to control pathogens in the CNS. These studies will be further extended to better understand the difference of pathogen control in the periphery and in the CNS. Specifically we will have to address cell-cell communication during infection, amongst brain resident cells, and between brain infiltrating immune cells and brain resident cells. Of particular interest it will be to delineate how peripheral antigen-specific immune cells maintain their function following recruitment to the brain and how their function is modulated in order to achieve an appropriate balance between maximal pathogen control and minimal brain damage.

Furthermore, we started to better understand the impact of CNS inflammation and infection on complex behaviour. Additional studies are needed to dissect direct virus-mediated effects and inflammation-mediated effects. Such knowledge will help to decipher mechanisms underlying long-term sequelae, chronic fatigue, and potentially long-COVID.

Accumulating evidence points towards infections triggering the onset and progression of neurodegenerative diseases. In initial preclinical studies, we detected clear correlations between infection and the onset of Alzheimer's disease. These studies have to be further refined to address underlying molecular mechanisms. Similarly, in the aftermath of pandemics, the incidence of Parkinson's disease increased, whereas the molecular basis of this is still largely unclear. To address such questions, we have adopted mouse models that spontaneously develop Parkinson's disease to further analyse them under conditions of various different experimental virus infections.

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INVESTIGATORS IN RF INEU:

Luka Čičin-Šain, Carlos Guzmán, Ulrich Kalinke, Martin Korte, Andrea Kröger, Michael Meyer-Hermann, Christian Sieben.

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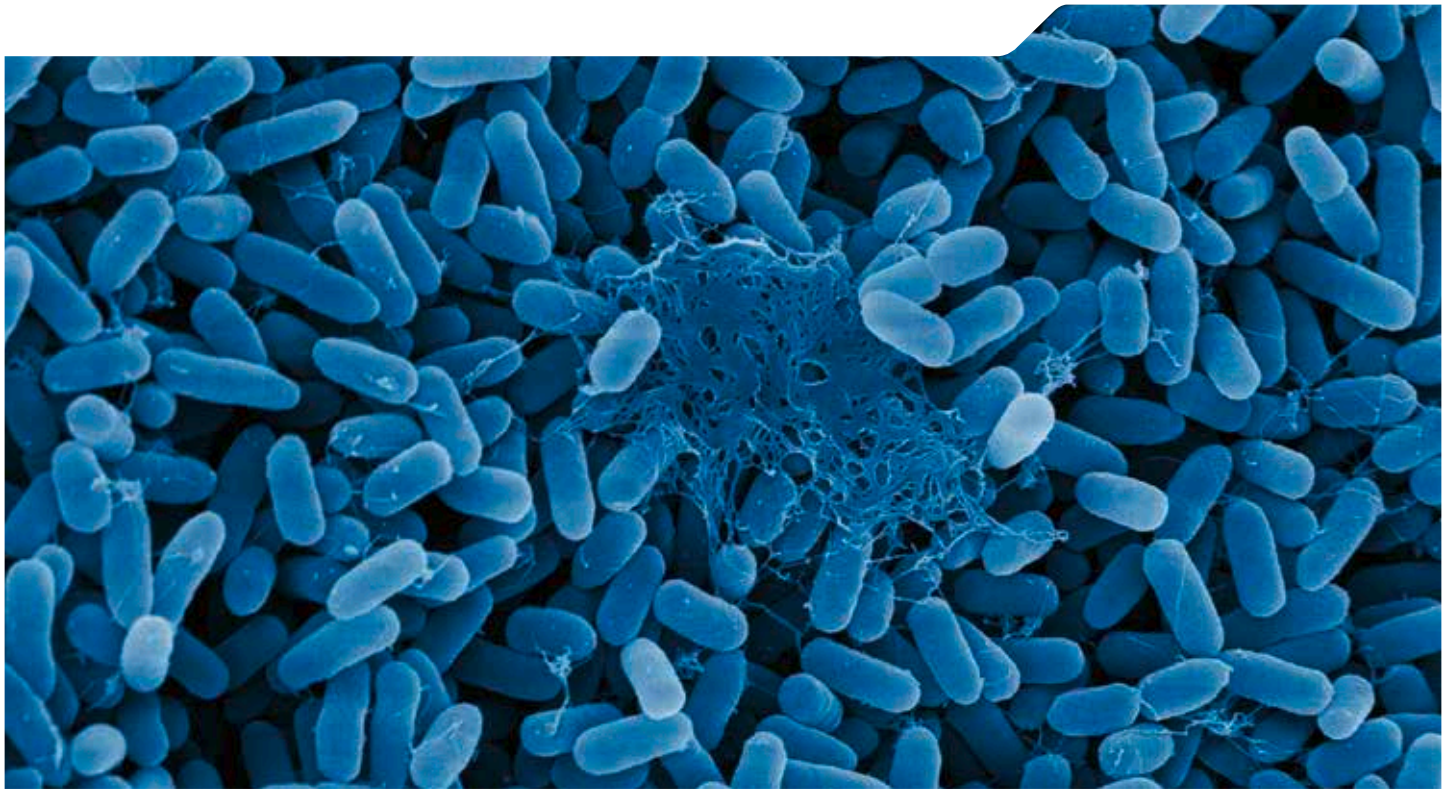
Speakers INEU:
Ulrich Kalinke und Martin Korte



Pseudomonas aeruginosa, a lung-infecting pathogen © HZI | Mathias Müsken

UNDERSTANDING AND INFLUENCING BACTERIAL INTERACTIONS

RESEARCH FOCUS "MICROBIAL COMMUNITIES" (MICO)

The Research Focus MICO combines expertise to characterise and influence the complex interactions between pathogens, our immune system and the large collection of commensal bacteria colonising the gut and other parts of the human body. This microbiota comprises a vast array of microbes.The gut alone harbours several thousand bacterial species. An understanding of their individual or synergistic contributions to human health and disease demands means to modulate their functions on the species level. Gaining this level of understanding demands innovative approaches for profiling microbes, including RNA-based technologies, the establishment of complex host-microbe interaction models, and single-cell analysis.

1. RNA-CENTRIC APPROACHES TO MICROBIOLOGY AND MICROBIOTA RESEARCH

RNA, a key molecule of life, was long considered to serve mainly as a storage intermediate for genetic information. Meanwhile, it is well known that RNA molecules play an essential role in many regulatory mechanisms in the cell.

To date, the RNA-based regulatory mechanisms are unknown for 99 % of the species in the human microbiota. To fully comprehend and rationally modulate the microbiota, methodologies are needed for the rapid identification of the major functional RNA classes of these species and their interaction partners. Making use of high-throughput technologies to detect RNAs and proteins will make it possible to visualise the *in vivo* activities of gut microbial communities

through their functional RNA profiles and networks. These pieces of information will be combined into an RNA-centred atlas of gut microbial activity.

The revolutionary ability of single-cell RNA-sequencing to obtain gene-expression profiles of individual cells rather than to look at the expression averaged for an entire cellular population has led to profound new discoveries in biology, including the identification of new cell types. Single-cell RNA-sequencing is also emerging as a powerful tool to determine cell-to-cell variability in bacterial pathogenesis. This technique, however, has been restricted for technical reasons to larger eukaryotic cells and could not be applied in bacteria until recently. Researchers of HZI's branch institute in Würzburg, the HIRI, have established a routine protocol for global transcriptomics of individual bacterial cells and have applied it successfully to two major human pathogens, the gut pathogen *Salmonella enterica* and the lung-infecting pathogen *Pseudomonas aeruginosa*. Transcriptomes from single bacteria will help to better understand resistance to antibiotic treatment, persister formation under harsh conditions, and phenotypic diversification for bet hedging, to name a just a few applications.

Beyond single-cell transcriptomics, it is our ambition to adapt other powerful RNA-sequencing techniques to study the regulation of RNA molecules in bacteria. The long-term goal is high-throughput RNA biochemistry in bacterial cells, pathogens and commensals alike. By using differential RNA sequencing, we have generated a single-nucleotide resolution transcriptome map of *Bacteroides thetaiotaomicron*, a common member of the human intestinal microbiota and important degrader of polysaccharides in the gut. Our study has thereby advanced the understanding of *B. thetaiotaomicron's* metabolism and contribution towards human health. Furthermore, scientists at HIRI in Würzburg have mapped the RNA molecules of five clinically relevant strains of *Fusobacterium nucleatum*. While *Fusobacterium* colonises the human oral cavity, it is also known to speed up the growth of human carcinomas, for example in the intestine or breast. The findings could thus aid the development of new therapies for various cancers, reemphasizing the power of bacterial RNA research.

KEY WORDS IN SHORT:

RNA
RNA, short for "ribonucleic acid", is a macromolecule made of a chain of building blocks called nucleotides, which "translate" the information contained in DNA into instructions for making a protein. RNA molecules also play essential roles in many regulatory mechanisms in the cell.

Dual RNA sequencing
Dual RNA sequencing is an approach to simultaneously capture and analyse RNA from both host and pathogen from infected cells or tissue. This technique builds on the high sensitivity and resolution of modern RNA sequencing tools.

Transcriptome
The entirety of RNA molecules in the cell at a given point in time. The term "transcriptomics" is used to describe techniques to study the transcriptome.

Single-cell RNA sequencing
Techniques for single-cell RNA sequencing allow researchers to study the transcriptome in an individual cell. For instance, they can detect which genes and regulatory pathways are active in particular bacterial cells, such as persisting pathogens, which survive in host organisms for a long time.

CRISPR technologies
CRISPR technologies represent powerful tools for editing genomes, which allows researchers to alter DNA sequences and modify gene function. They were adapted from the natural defence mechanisms of bacteria against viruses and other foreign invaders.

DNA transformation
Process of transferring foreign DNA into a cell.

2. INTEGRATED CHARACTERISATION OF MICROBIAL COMMUNITIES AND THEIR CROSS-TALK WITH THE HOST

Our understanding of the composition and functional potential of the human gastrointestinal microbiota has made significant progress in the last years. Specifically, it is now recognised that the genetic diversity within these communities largely surpasses the genetic diversity in our own genome. The genetic diversity within the microbiota is the result of the presence of large numbers of previously uncharacterised microbial species in the gastrointestinal microbiota as well as large differences in the overall number of species. Furthermore, the collection of strains varies between individuals as well. However, knowledge is still emerging about which members of these communities encode pathogenic functions and which metabolic pathways are turned on in health and disease. Therefore, HZI scientists have employed bioinformatic approaches to comprehensively characterise commensal bacterial species. A special focus was on identifying phages and anti-phage CRISPR elements that rapidly evolve when microbes and phages meet in the gut.

Different types of medications and disease states actively influence specific members of the microbiota, which potentially results in erroneous assignments of disease-associated microbial signatures in clinical cohorts. Therefore, establishing standardised approaches to characterise the microbiota as well as the integration of clinical data is specifically important in the context of human cohort studies. A key approach to study genetic determinants of microbe-induced host phenotypes is the development of genetic tools for relevant microbes. Through classical and CRISPR-Cas9-mediated approaches novel strategies for characterising gene function in commensal bacteria were established. Another important tool to investigate the relevance of disease-associated microbial signatures are gnotobiotic animal models – germ-free animals selectively colonised with known strains of bacteria. They allow researchers to functionally assess, for instance, the influence of specific bacteria or communities on the development of the immune system. Frequently these approaches involve the isolation of previously uncharacterised bacteria and the study of interactions between microbiota and immune system during specific phases of development, such as during the neonatal phase or during chronic infections.

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Alterations in the microbiota have been linked to numerous diseases, spurring strong interest in the development of microbiota-centric therapeutic interventions for common diseases including infections. In many collaborative efforts, MICO scientists have performed studies utilising a range of microbiological, immunological and sequencing-driven approaches. They apply them to animal models and human cohort studies to gain a functional understanding that will form the basis for novel therapeutic interventions. Yet, scientists still face many challenges, e.g., the evaluation of microbiota manipulation in clinical cohorts, or the establishment of genetically-tractable gut commensals to study their disease involvement, which will be the topic of future research.

3. DEVELOPING AND APPLYING CRISPR TECHNOLOGIES TO INTERROGATE THE HUMAN MICROBIOTA

In contrast to its thousands of bacterial constituents, the human microbiota contains only a handful that are considered well characterised, i.e., their fundamental genetics and physiology are at least moderately understood. These insights have come from the availability of efficient and advanced genetics tools and procedures. Such capabilities have made it possible to elucidate relationships between their genes and their observable traits, whether in isolation or in the context of a complete community. In contrast, these same capabilities are utterly lacking for the multitude of other resident bacteria, restraining our basic understanding of how individual members shape the composition and function of the human microbiota.

HZI scientists are tackling this grand challenge by developing a systematic pipeline for rendering individual bacteria genetically tractable and by developing tools to selectively eliminate individual members of the microbiota, making use of the versatile gene editing tool CRISPR. One challenge of this approach lies in the strain-specific barriers to foreign DNA. Bacteria possess the ability to recognise and degrade DNA of unknown origin. We mimic the specific characteristics of the DNA of the bacterium in order to efficiently integrate foreign DNA. Then, CRISPR technologies are applied to achieve programmable and efficient genome editing and gene regulation to rapidly probe the genetic properties of each bacterium. These technologies are finally scaled to perform genome-wide screens to elucidate the influence of these genetic properties in single experiments.

To study each bacterium in the context of the microbiota, HZI scientists are developing means to selectively eliminate individual bacterial members using CRISPR. This capability provides a distinct means to perturb a community and evaluate how the microbiota and the host respond after removing a bacterium. It furthermore offers the possibility for novel therapeutic approaches to selectively eliminate pathogenic bacteria in the microbiota and allow the introduction of 'beneficial' probiotic strains that can take up residence and provide long-lasting therapeutic benefits.

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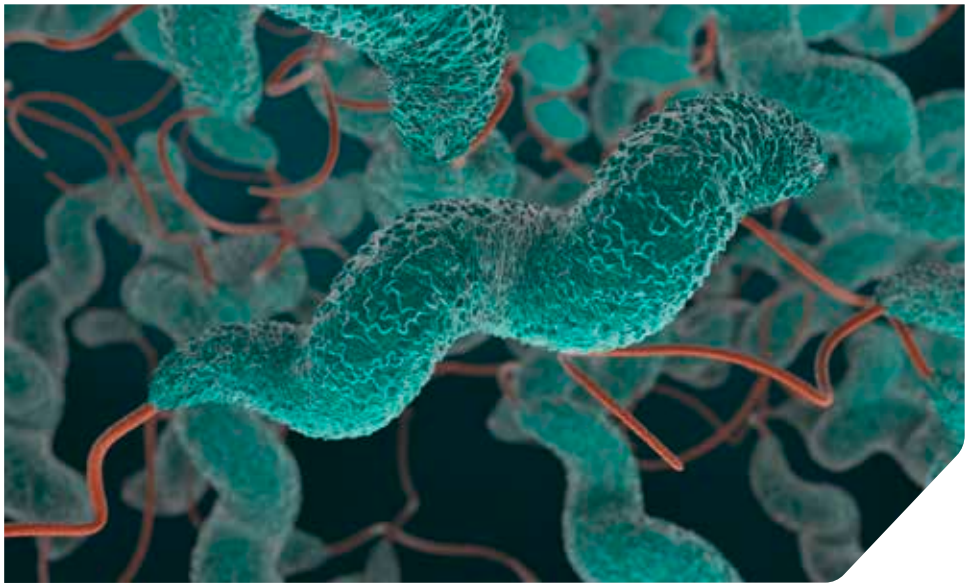
MOST IMPORTANT QUESTIONS ADDRESSED BY RF MICO:

- How does the microbial population in the human body, e.g., in the gut, develop?
- How does it influence susceptibility and resistance toward infections?
- How do commensal and pathogenic microbes communicate with each other and their host?
- How can we read the activity of the microbiota?
- Can we edit the microbiota with species-specific antibiotics?
- Which microbial species are essential for the proper function of the gut?
- How can we prevent damage to the microbiota or restore its function?
- How can we exploit the microbiota to discover new functional RNAs and proteins?

In addition to these systematic approaches to understand and manipulate microbial communities, studies of CRISPR-Cas systems in the food-borne pathogen *Campylobacter* led to a major finding that could be translated into future diagnostics: The technology platform LEOPARD (Leveraging Engineered tracrRNAs and On-target DNAs for PArallel RNA Detection) is based on the finding that DNA cutting by Cas9 could be linked to the presence of a specific RNA. This link allows LEOPARD to detect many RNAs at once, opening opportunities for the simultaneous detection of RNAs from viruses and other pathogens in a patient sample. In the future, LEOPARD's performance could dwarf even multiplexed PCR tests and other methods as the technology has the potential to revolutionise medical diagnostics not only for infectious diseases, but also for cancer and rare genetic diseases.



Campylobacter cells © Adobe Stock | LASZLO

PERSPECTIVES

The identification of markers of bacterial resistance and virulence and the composition of microbial communities in individual organisms enable the discovery of key molecular players that drive bacterial resistance in the competitive and collaborative world of the microbiota. Specifically, a detailed understanding of how microbial communities and microbiota-derived metabolites mediate enhanced susceptibility towards infections and non-communicable diseases is still lacking. To address this challenge, scientists in the Research Focus MICO have jointly established high-throughput platforms allowing the identification of microbial signatures, functional pathways, and microbiota-derived metabolites from large patient and population cohorts.

In the future, we will intensify studies of cohorts of individuals with increased infection susceptibility in collaboration with clinicians at Hannover Medical School (MHH) and the Cluster of Excellence RESIST as well as the German Centre for Infection Research. These studies will be instrumental to understand how variations in the microbiota determine infection susceptibility. For example, they will allow studying the long-lasting consequences of early-life microbiota modulation in preterm infants on the risk for sepsis and respiratory infections that persist into later childhood.

Another important research direction is based on existing comprehensive datasets on the cross-talk between microbiota and host. To this end, scientists will exploit machine-learning approaches to develop mathematical models of dynamic interaction between the microbiota and the local immune system. This will allow gaining novel insights on how this mutual interaction becomes disease-promoting and how microbiota modulation can interfere in a personalised manner. In this regard, the development of novel methods that combine RNA biochemistry with high-throughput RNA sequencing and protein analysis is a key activity, which will enable us to visualise the *in vivo* activities of microbial communities through their functional RNA profiles. This will in turn provide opportunities to edit the microbiota with precision. In particular, the gut microbiota contributes to protection from disease and an increasing number of human diseases have been linked to dysbiosis in the gut. Therefore, novel RNA-based antibiotics are needed that can selectively target a specific species in complex microbial communities, with minimal off-target effects on the host and commensal bacteria. The great potential of antisense oligonucleotides (ASO), in particular, of peptide nucleic acid (PNA), as well as CRISPR-based antimicrobials will be leveraged for microbiota editing.

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INVESTIGATORS IN RF MICO:

Lars Barquist, Chase Beisel, Susanne Häussler, Karsten Hiller, Jochen Hühn, Fabian Leendertz, Yang Li, Alice McHardy, Rolf Müller, Dietmar Pieper, Emmanuel Saliba, Till Strowig, Jörg Vogel, Alexander Westermann.

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Speakers MICO:
Jörg Vogel and Till Strowig

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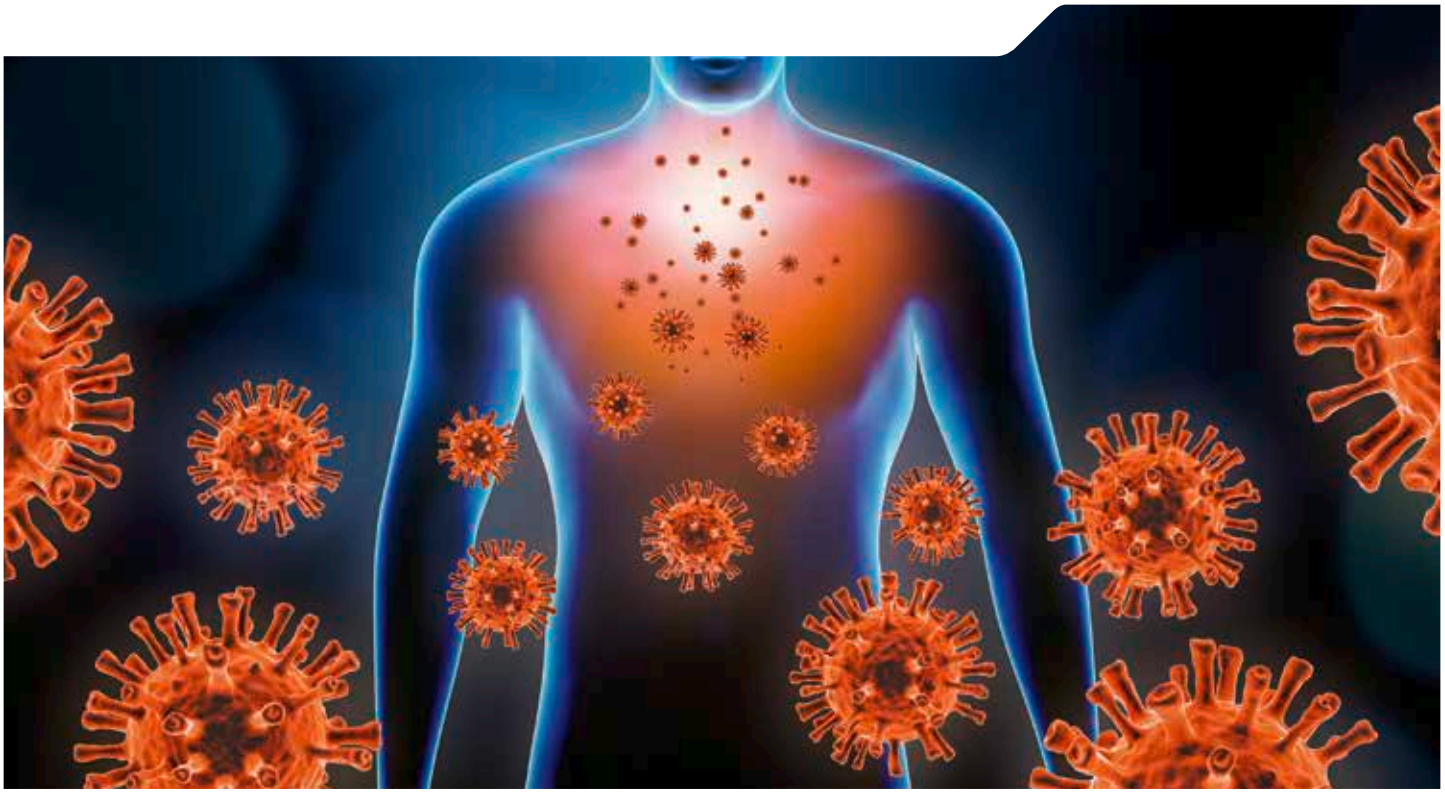

PREVENTING PERNICIOUS LUNG INFECTIONS

RESEARCH FOCUS “RESPIRATORY VIRAL INFECTIONS” (RVIR)

The spread of the coronavirus SARS-CoV-2 strikingly illustrates the global health threat posed by respiratory viral infections. Even before the COVID-19 pandemic, viruses affecting the human respiratory system have caused several global disease outbreaks, such as the annual influenza epidemic.

HZI‘s Research Focus RVIR investigates infections caused by the influenza A virus, corona viruses such as SARS-CoV-2, and the respiratory syncytial virus (RSV). Together with university partners, HZI scientists seek to identify risk factors and markers for severe forms of these infections and use the insights obtained on these types of infection to explore novel approaches for diagnostics and interventions. They are developing antibody and drug-based therapies as well as vaccination strategies. Using bioinformatics and experimental methods, they identify and validate predictive biomarkers and develop recommendations for optimising seasonal vaccines. For this purpose, new digital health tools for monitoring disease outbreaks are being developed and combined with mathematical modelling.

Figure 1: Computed tomography (CT) imaging of the lung of healthy individuals and of COVID-19 patients with a severe course of the disease. Adapted from Wendisch et al. (2021) Cell, 184(26):6243-6261. Reprinted with permission from Elsevier © Elsevier

1. UNDERSTANDING RESPIRATORY VIRUS PATHOMECHANISMS AT SINGLE-CELL RESOLUTION

The COVID-19 pandemic caused by the SARS-CoV-2 coronavirus has been the dominant global public health crisis of the last two years. Clinical presentations of COVID-19 are highly variable, but the reasons for this variability are unknown. The Emmanuel Saliba lab from the Helmholtz Institute for RNA-based infection research (HIRI) along with several other Helmholtz centres from the Health section (DZNE in Bonn, Helmholtz in Munich, MDC in Berlin); the CiiM (Yang Li lab) and Genome Analytics led by Robert Geffers both at HZI; and Universities (Aachen, Charité in Berlin) have joined forces to conduct two multi-centre and multi-cohort studies to unravel the pathology of severe COVID-19 infections. They have used multipronged single-cell technologies to compare samples from patients with mild versus severe COVID-19 *(see also section "Highlight Publications"/Emmanuel Saliba)*.

MOST IMPORTANT QUESTIONS ADDRESSED BY RF RVIR:

- Which pathological mechanisms underlie respiratory diseases?
- How can outbreaks of viral respiratory infections be controlled and contained?
- Which novel prevention and treatment methods are effective against respiratory viruses?

In patients with severe COVID-19, lung damage can be so stark that the body can no longer absorb sufficient oxygen from the air. This condition is referred to as 'acute respiratory distress syndrome', or ARDS. In order to survive ARDS, patients must receive oxygen therapy, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO), whereby a machine assumes the function of the lungs. The study authors analysed pulmonary immune responses and lung pathology in two cohorts of patients with COVID-19 ARDS using functional single-cell genomics, immunohistology, and electron microscopy *(Figure 1)*. Almost all affected patients showed extensive lung tissue damage. The majority of the alveoli had been destroyed and the alveolar walls showed extensive thickening.

We also found ubiquitous deposits of collagen, the main component of scar tissue. The foregoing are characteristics of severe fibrosis. Interestingly, this type of lung failure is not caused by uncontrolled viral replication, but rather by secondary host responses, including those involving the immune system. We therefore analysed the composition and characteristics of immune cells taken from bronchioalveolar lavage and lung tissue. In order to study individual cells in greater detail, we used state-of-the-art single cell analysis. *(For an example of such analysis see Figure 2.)* Using this technology, we were able to show that the pronounced accumulation of macrophages is one of the key features in COVID-19 patients who develop respiratory failure. These

macrophages interact with specific fibroblasts, which in response undergo rapid proliferation and produce large quantities of collagen.

2. UNDERSTANDING INFECTIOUS DISEASE PROCESSES WITH SINGLE-CELL AND MOLECULAR RESOLUTION

Scientists from HZI are developing and using cutting-edge techniques to elucidate intimate contacts between the virus and the host cell. HIRI scientist Mathias Munschauer, together with colleagues at JMU Würzburg and the Broad Institute (Cambridge, USA), is charting the first global atlas of direct interactions between the SARS-CoV-2 RNA and the human host proteome. Mass spectrometry has allowed the scientists to pinpoint host proteins that directly associate with the virus genome. In this particular case, they were able to make quantitative measurements enabling identification of the strongest specific binding partners *(Figure 3)*. Munschauer and colleagues have identified a total of 18 host proteins that have an important function during SARS-CoV-2 infection. In particular, the two host factors CNBP and LARP1 seem to play a key role. As a direct application of this work, the scientists could identify 20 small molecule inhibitors of host proteins that bind SARS-CoV-2 RNA.

Also at HIRI, the Redmond Smyth lab studies influenza virus reassortment, a major mechanism leading to pandemic influenza. To better predict the emergence of potentially pandemic influenza, they are investigating RNA-based restrictions of influenza reassortment. To this end, they developed advanced RNA structural probing assays to measure physical contacts between influenza virus RNAs in cells and in viral particles.

In Braunschweig, the lab of Christian Sieben aims to understand which cellular processes are stimulated by viruses during initial contact at the host cell surface and how in turn host cells interpret infecting virus at the molecular level. To investigate cellular structures and relate how viruses have evolved to use these for infection, they apply super-resolution microscopy in combination with immunolabelling of infected cells.

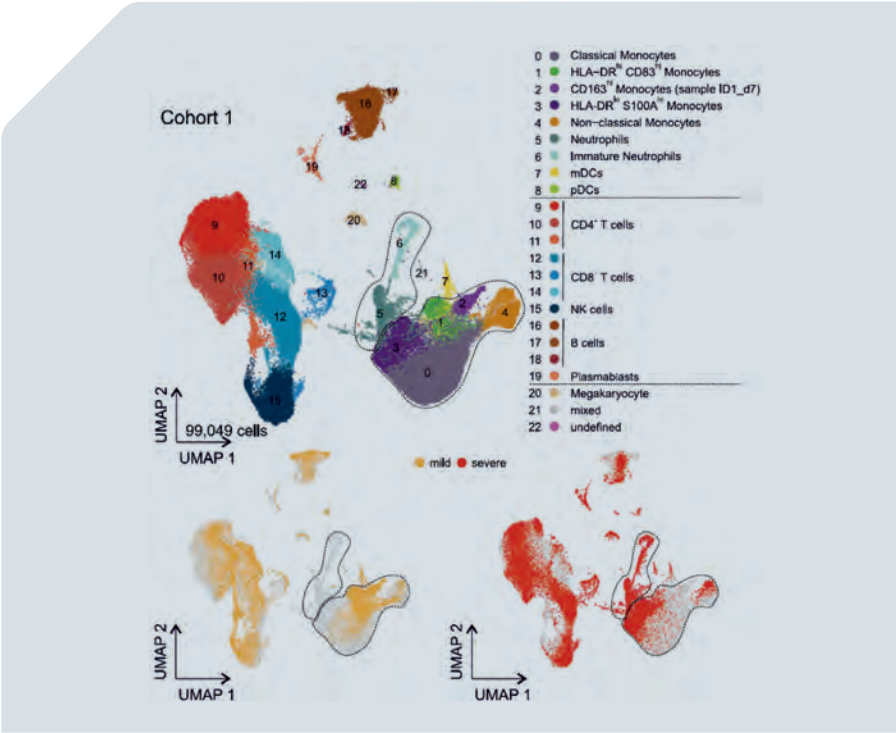

Figure 2: Single cell transcriptomics map visualising 99,049 blood cells from 49 samples (8 mild, 10 severe patients). The map is split according to disease severity (yellow, mild COVID-19; red, severe COVID-19). Adapted from Schulte-Schrepping et al. (2020), Cell 182:1419–1440. Reprinted with permission from Elsevier © Elsevier

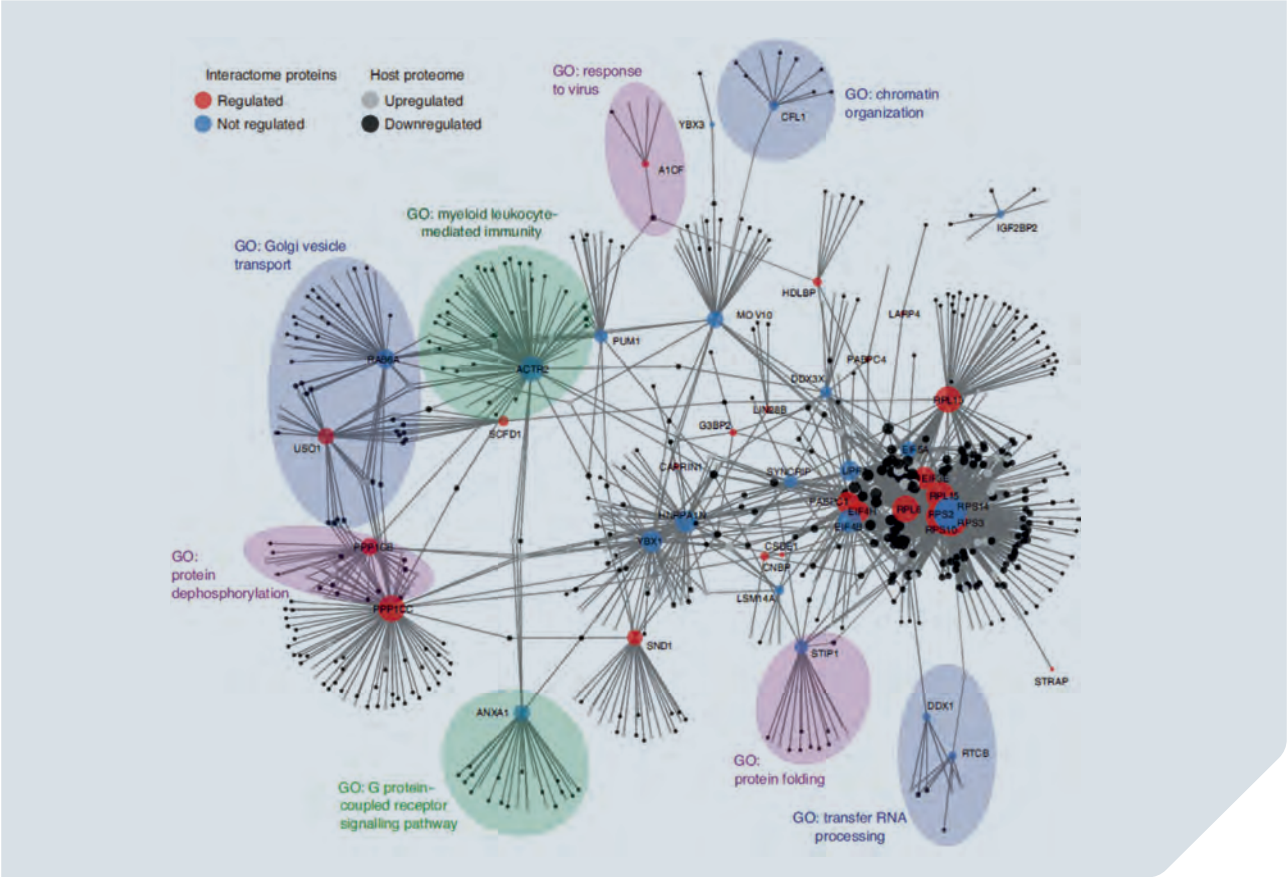

Figure 3: Protein–protein association network of core SARS-CoV-2 RNA interactome proteins and their connections to differentially regulated proteins in SARS-CoV-2-infected cells. Adapted from Schmidt et al. (2021). Nat. Microbiol. 6:339-353 © CC BY 4.0

3. DEVELOPING NOVEL ANTIVIRAL STRATEGIES TO COMBAT RESPIRATORY INFECTIONS:

Previously, scientists of HZI have harnessed the development of new therapeutic strategies to combat RSV and SARS-CoV-2. Against RSV, new hope is emerging with the prophylactic monoclonal antibody (Palivizumab) and vaccine candidates are also in clinical development. However, therapeutic options remain limited, and patients are largely treated with supportive care. The lab of Thomas Pietschmann has characterised novel therapeutic strategies and provided evidence for their antiviral efficacy in an *in vivo* RSV infection model. In a large international effort, they were able to contribute to the discovery of molecules perturbing the fluidity of RSV replication factories, thereby impairing viral RNA replication. This work represents a new paradigm for therapeutic intervention of negative strand RNA virus replication factories and cellular liquid organelles in general. Pietschman's group were able to characterise labyrinthpeptins - lantibiotic natural products - as RSV antivirals via a strong HZI-cross topic collaboration with Mark Brönstrup's lab. They showed that these molecules disrupt RSV virus particles and inhibit their cell entry. Notably, labyrinthopeptins circumvent typical RSV entry inhibitor resistance mutants, and can thus be developed for combination therapies and/or as salvage therapy of entry inhibitor-resistant viruses.

The group of Neva Caliskan at HIRI has studied molecular details of protein-regulated frameshifting by using ensemble and single-molecule analysis tools employing several viruses, including SARS-CoV-2 as a model organism. Most recently, they have identified a host encoded protein called ZAP-S in a frameshift RNA interactome screen. It could be shown that ZAP-S can impair frameshifting by interfering with the folding of the critical RNA-based molecular switch. Without this

stimulatory RNA element, translation of the viral polymerase through the alternative reading grid cannot take place. Accordingly, in collaboration with Luca Cicin-Sain's lab, these scientists also showed that ZAP-S can impact SARS-CoV-2 viral loads by more than 20-fold. Currently, these interactions as well as the function of ZAP are being studied in more detail by structural collaborations. The Caliskan group also aims to develop novel compounds targeting these interactions as a viral intervention strategy.

Also, scientists at the institute benefit from its cutting-edge infrastructure to uncover novel drug candidates. The lab of Katharina Rox together with the Pharmacokinetics/pharmacodynamics (PK/PD) unit with the support of the German Centre for Infection Research (DZIF) have as their mission to facilitate translation from bench to bedside. Therefore, it is actively involved in the thorough preclinical characterisation of novel compounds directed against coronavirus as well as RSV. Within the network CoFoNi (Corona Research in Lower Saxony), the Rox lab aims to provide an infrastructure for the early *in vitro* assessment of pharmacokinetic (PK) para-meters for novel drugs targeting SARS-CoV-2. Similarly, the PK/PD unit also aids in lead selection of novel compounds directed against RSV, in a joint project between HZI, HIPS and Twincore. In the EU-consortium SCORE (Swift COronavirus Therapeutics REsponse), candidate compound selection and design of a proof-of-concept study for repurposed and novel drugs against SARS-CoV-2 are being facilitated, inter alia through *in vitro* characterisations using lung cells, as well as stability testing and PK behavior. As with the SCORE consor-

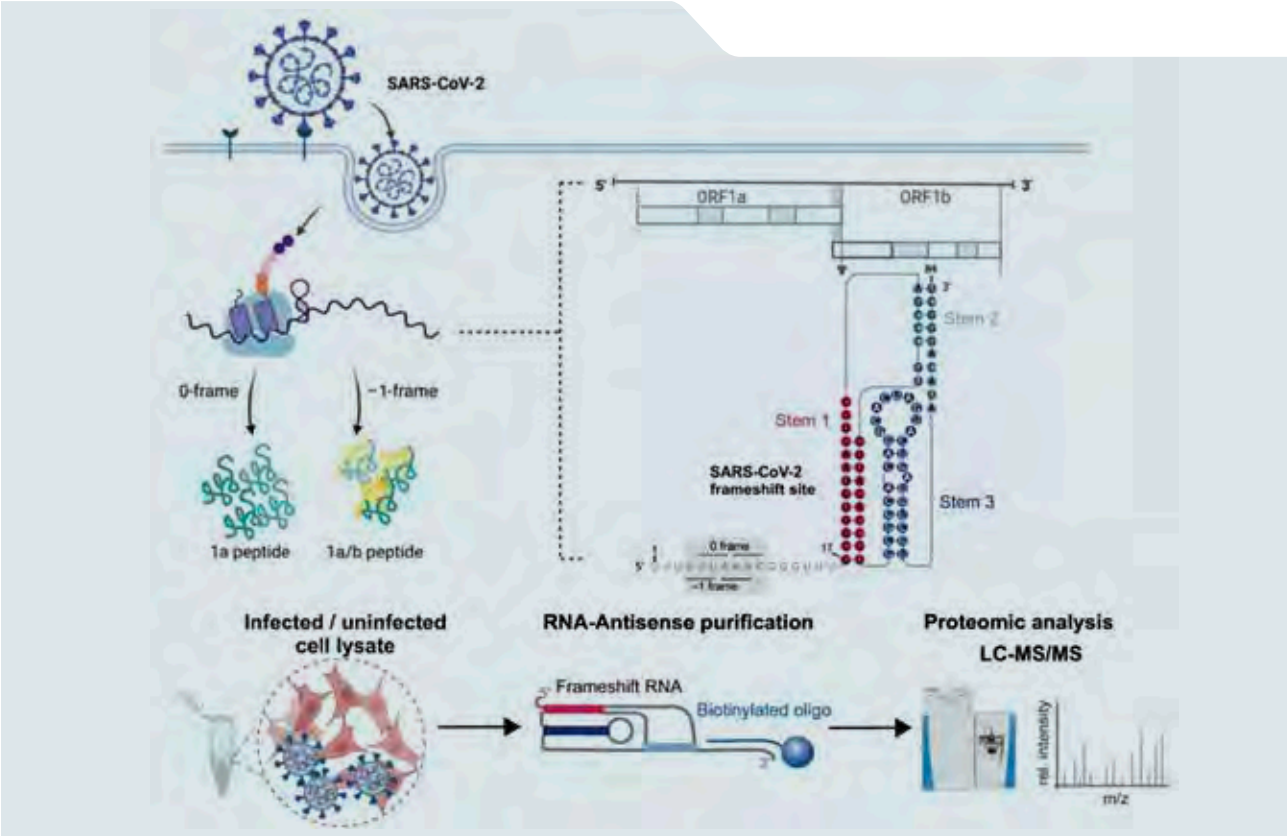

Figure 4: *In vitro* RNA-antisense purification-based discovery of protein interactors of the SARS-CoV-2 −1 programmed ribosomal frameshifting element (PRF) ((Zimmer et al., 2021) lab). *In vitro* synthesised RNA fragment of the SARS-CoV-2 genome, was incubated with lysates of naïve and infected cells. The −1PRF RNA was captured by a biotinylated antisense DNA oligo and isolated proteins were subjected to LC-MS/MS.
Adapted from Zimmer et al. (2021), Nature Communications 12:7193 © CC BY 4.0

tium, compounds are tested for their PK parameters within the EU-IMI-consortium CARE (Corona Accelerated R&D in Europe). Moreover, in silico methods like physiologically-based pharmacokinetic (PBPK) modelling are employed to predict compound levels at target tissues. These strategies are also employed in the project PROTAC-empowered antivirals, funded by SPRIN-D. PROTAC molecules represent a paradigm shift in antiviral drug discovery as they degrade their target protein and, thereby, exert an antiviral effect. Thus, in silico modelling will aid understanding this degradation process. To validate modelling predictions, the antiviral mechanisms of PROTACs are being thoroughly investigated using *in vitro* assays. Thus, the combination of different pharmacological techniques, including in silico modelling, should accelerate preclinical development.

4. MODELING RESPIRATORY DISEASE SPREAD

Non-pharmaceutical interventions (NPIs) are important to mitigate the spread of infectious diseases lacking vaccines or other efficacious treatments. The Michael Meyer-Hermann lab uses mathematical modeling to assess non-pharmaceutical interventions and predict epidemics. Mathematical models are continually revised and extended to accurately represent the dynamics of the spread of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and other infectious diseases. Using reliable models, effective interventions can be determined and used as a basis for informed political decisions. We have assessed non-pharmaceutical interventions and provided a reliable forecast of the COVID-19 pandemic in Germany by considering four important factors: (1) age-dependency of SARS-CoV-2 infection; (2) realistic contact patterns between different age groups; (3) high-quality, spatially resolved information on commuting activities such as incorporation of traveling information based on the social network Twitter; and (4) inclusion of uncertainties by Monte-Carlo Ensemble runs. The software was able to successfully reproduce infection trends in retrospective scenarios and is therefore attractive for NPI testing and prediction in current and potentially future epidemics.

PERSPECTIVES

Building on the infrastructure provided by HZI and on the cutting-edge technologies developed across the Institutes, RVIR scientists will continue advancing our understanding of host-virus interactions at the single-cell level. Using the power of RNA technologies, such as single-cell–omics, ribosome profiling and RNA pull-down, intracellular molecular interactions of the virus and its host will further be investigated. While advancing basic understanding of virus-host interaction, novel therapeutic avenues will be designed and screened. Finally, novel pathogens with pandemic potential will undoubtedly continue to emerge. Research conducted by the scientists from HZI is at the forefront of the pandemic preparedness effort.

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INVESTIGATORS IN THE RF RVIR:

Ursula Bilitewski, Melanie Brinkmann, Mark Brönstrup, Dunja Bruder, Neva Caliskan, Stefanie Castell, Luka Čičin-Šain, Carlos A. Guzmán, Anna Hirsch, Jochen Hühn, Ulrich Kalinke, Martin Korte, Claus-Michael Lehr, Yang Li, Alice McHardy, Michael Meyer-Hermann, Rolf Müller, Mathias Munschauer, Frank Pessler, Thomas Pietschmann, Katharina Rox, Emmanuel Saliba, Klaus Schughart, Christian Sieben, Redmond Smyth.

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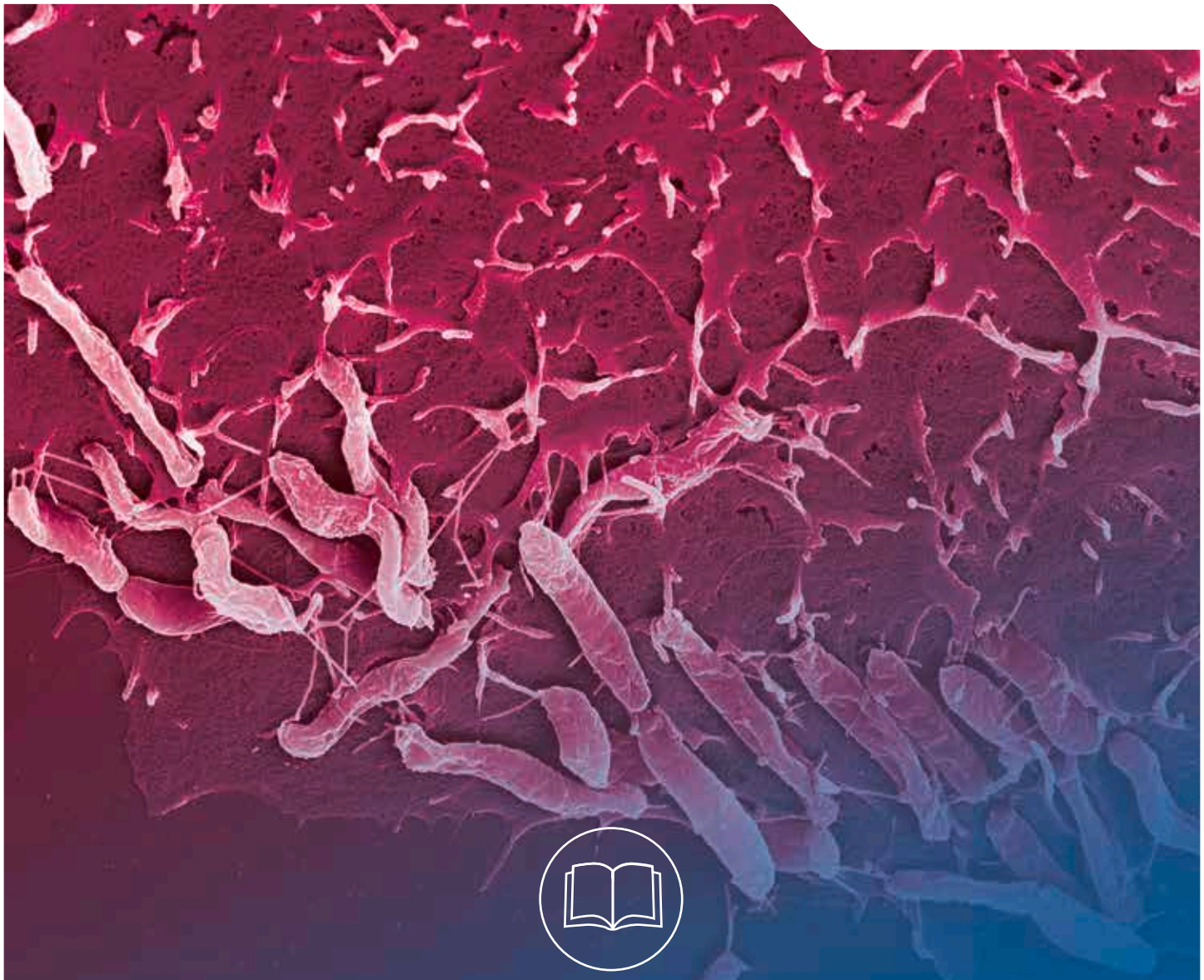
Speakers RVIR:
Emmanuel Saliba and Dunja Bruder



HIGHLIGHT PUBLICATIONS

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CRISPR-BASED TECHNOLOGY COULD REVOLUTIONISE POINT-OF-CARE DIAGNOSTICS

CHASE BEISEL | HEAD OF DEPARTMENT RNA SYNTHETIC BIOLOGY

Am I infected with SARS-CoV-2? Is it one of the more contagious variants? Or could it be the flu? Being able to answer these questions quickly with a single diagnostic test can be decisive for gauging the spread of disease and selecting the right therapy. In this study, we introduce LEOPARD - a whole new CRISPR-based technology, which we have translated into a multiplex diagnostic platform.

Most conventional molecular diagnostics usually detect only a single disease-related biomarker. Great examples are the PCR tests currently used to diagnose COVID-19 by detecting a specific sequence from SARS-CoV-2. Such so-called singleplex methods provide reliable results because they are "calibrated" to a single biomarker. However, determining whether a patient is infected with a new SARS-CoV-2 variant or a completely different pathogen requires probing for many different biomarkers at one time.

Together with colleagues at the University of Würzburg we have paved the way for a completely new diagnostic platform, which we call LEOPARD. It is a CRISPR-based method that is highly multiplexable and has the potential to detect a variety of disease-related biomarkers in just one test. LEOPARD, which stands for "Leveraging Engineered tracrRNAs and On-target DNAs for PArallel RNA Detection", is based on the finding that DNA cut by Cas9 is linked to the presence of a specific RNA. This allows LEOPARD to detect many RNAs at once, opening opportunities for the simultaneous detection of RNAs from viruses and other pathogens in a patient sample.

CRISPR-Cas9 is generally known as a biomolecular tool for genome editing. Here, CRISPR-Cas9 complexes function as molecular scissors that specifically cut DNA. These same scissors are naturally used by bacteria to cut DNA associated with invading viruses. Whether editing genomes or eliminating viruses, Cas9 cutting is directed by guide RNAs. The guide RNAs found in bacteria must pair with a separate RNA called the tracrRNA. This RNA couple then works with Cas9 to direct DNA cutting.

The tracrRNA had always been thought to only pair with guide RNAs coming from the antiviral system. However, we discovered that the tracrRNA was pairing with other RNAs, turning them into guide RNAs: When searching for RNAs binding to Cas9 in the model organism Campylobacter, we surprisingly detected not only guide RNAs, but also other RNA fragments in the cell that looked like guide RNAs. The tracrRNA was pairing with these RNAs, resulting in "non-canonical" guide RNAs that could direct DNA cutting by Cas9 *(Fig. 1)*.

The LEOPARD technology platform builds on this discovery as we figured out how to reprogram the tracrRNAs to decide which RNAs become guide RNAs. By monitoring a set of matching DNAs, we were able to determine, which RNAs were present in a sample based on which DNAs get cut. As part of the ongoing pandemic, LEOPARD could allow a doctor to figure out, whether the patient is infected with SARS-CoV-2, if it is a unique variant, and whether the sample was correctly taken or needs to be repeated–all in one test and in record time.

In the future, LEOPARD could even dwarf multiplex PCR tests and other methods as it works at room temperature and without the need for expensive equipment. In fact, we envisage that LEOPARD can be integrated in lateral flow chambers with results being read out using simple optic devices such as mobile phone cameras. As its application is not limited to infectious diseases, LEOPARD could also be used to identify certain cancers and rare genetic diseases. The technology thus has the potential to revolutionise point-of-care diagnostics and become a game changer in the field.

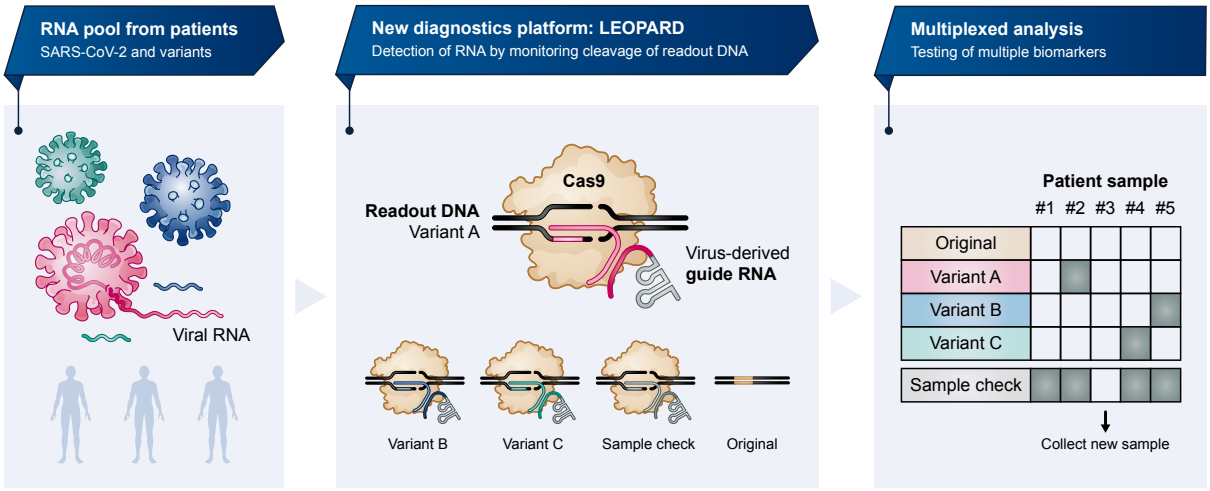

Figure 1: The LEOPARD technology has the potential to revolutionise multiplex point-of-care (PoC) diagnostics. LEOPARD uses patient samples (left panel) to detect any RNA of interest with single-nucleotide resolution. The presence of a certain RNA in the sample leads to cutting of a matching DNA oligo on a chip (middle panel). Cutting of DNA by Cas9 produces a signal that can be read out using a simple optical device such as a mobile phone camera (right panel). The signal pattern provides a precise result in record time and enables medical professionals to rapidly diagnose and treat patients.

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Noncanonical crRNAs derived from host transcripts enable multiplexable RNA detection by Cas9
Science doi: 10.1126/science.abe7106

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ACTIVATING THE LAST RESORT ANTIBIOTIC COLISTIN AT THE SITE OF INFECTION

MARK BRÖNSTRUP | HEAD OF DEPARTMENT CHEMICAL BIOLOGY

Many bacteria show resistance against several antibiotics and, often, so-called last-resort antibiotics need to be used to treat infections. Colistin is one of these last treatment options for multidrug resistant Gram-negative bacteria. Due to the rising antibiotic resistance in bacteria it is used increasingly in clinics, although it has severe side effects like damaging the kidneys (nephrotoxicity) and the nerves (neurotoxicity). We addressed these side effects by synthesising inactivated colistin conjugates that bind specifically to the surface of bacteria *(Figure 1)*. Cells of our immune system – neutrophil granulocytes – are activated by the presence of the bacteria and as part of our non-specific immune defense secrete enzymes that cleave off the modifying group from colistin. This releases the active antibiotic in direct vicinity of the pathogens, leading to their elimination. A proof of this concept has been achieved in our study with a modified colistin in an *in vitro* blood infection model. The concept to mask highly potent antibiotic drugs and subsequently release them selectively at the site of infection serves to improve their tolerability and efficacy.

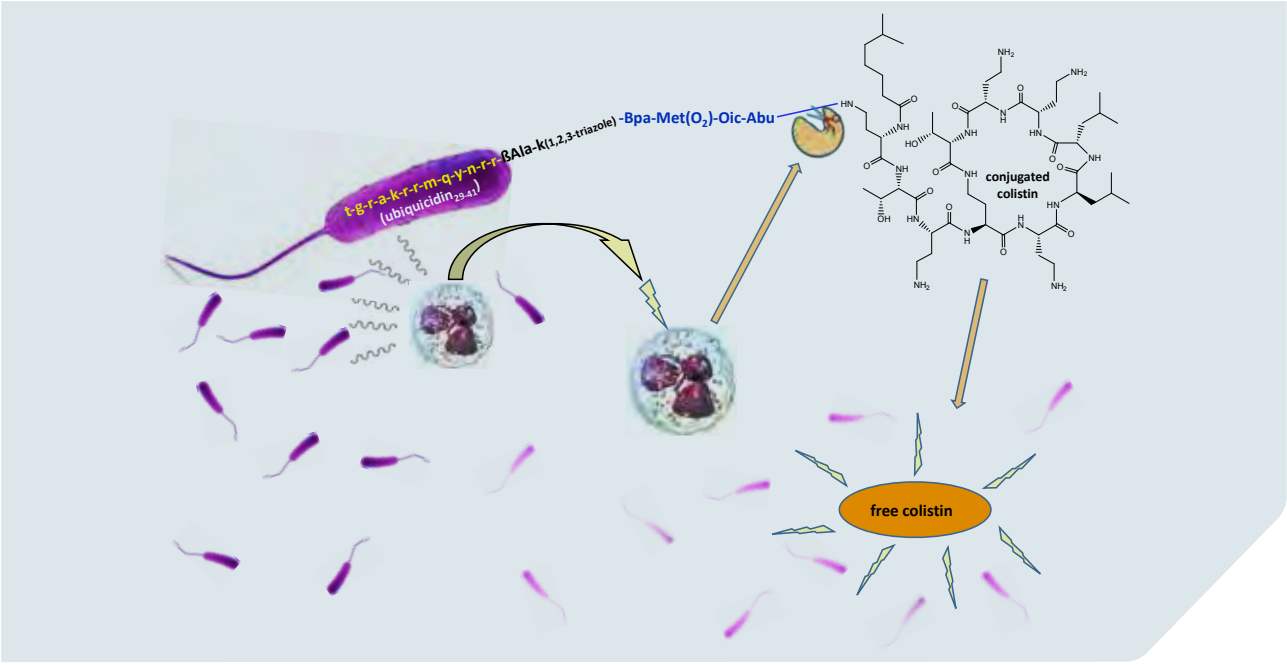

Figure 1: A multifunctional peptide-colistin conjugate for the treatment of bacterial infections binds to *E. coli* bacteria by an all-*D* sequence of the human peptide ubiquicidin$_{29-41}$. Host immune cells, activated upon infection, secrete the enzyme neutrophil elastase that cleaves an optimised linker sequence to release free colistin, a potent antibiotic that kills the bacteria. From Tegge et al. (2021) Angewandte Chemie International Edition 60:17989–17991
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The multifunctional peptide-colistin conjugate has been synthesised by coupling a sequence of the human peptide ubiquicidin$_{29-41}$ (Ubi$_{29-41}$) consisting of *D*-amino acids via a short linker to the antibiotic. Ubi$_{29-41}$ is already known for binding with high affinity to the membrane of Gram-negative bacteria. Ubi$_{29-41}$ was synthesised with amino acids that are all in the *D*- rather than the usual *L*-configuration in order to render the construct resistant to proteolytic cleavage. High-affinity binding of the all-*D* variant to the bacteria was confirmed with fluorescent derivatives. Colistin was modified by two different approaches: semi-synthetically and also fully synthetically with a linker consisting of four non-proteinogenic amino acids, that has been shown before to be cleaved extraordinarily fast by the enzyme human neutrophil elastase. The bacteria-targeting peptide Ubi$_{29-41}$ was coupled to the colistin-linker construct by a copper-catalysed 'click reaction'.

The colistin-peptide construct shows strongly reduced antibiotic activity and is thus a "masked" antibiotic. Upon addition of the colistin conjugate to neutrophil granulocytes, the enzyme neutrophil elastase cleaves the linker, releasing the active antibiotic. This is shown by the reduction of the amount of added *E. coli* bacteria with similar potency as pure colistin. Functionality of the approach was assessed with neutrophil granulocytes that were isolated under mild conditions from fresh human blood. Incubation of the granulocytes in buffer, in which the approach was developed and optimised, and also in the more complex but for clinical application more relevant human blood serum, reduced the amount of added *E. coli* bacteria after addition of the peptide-colistin construct with similar potency as pure colistin *(Figure 2)*.

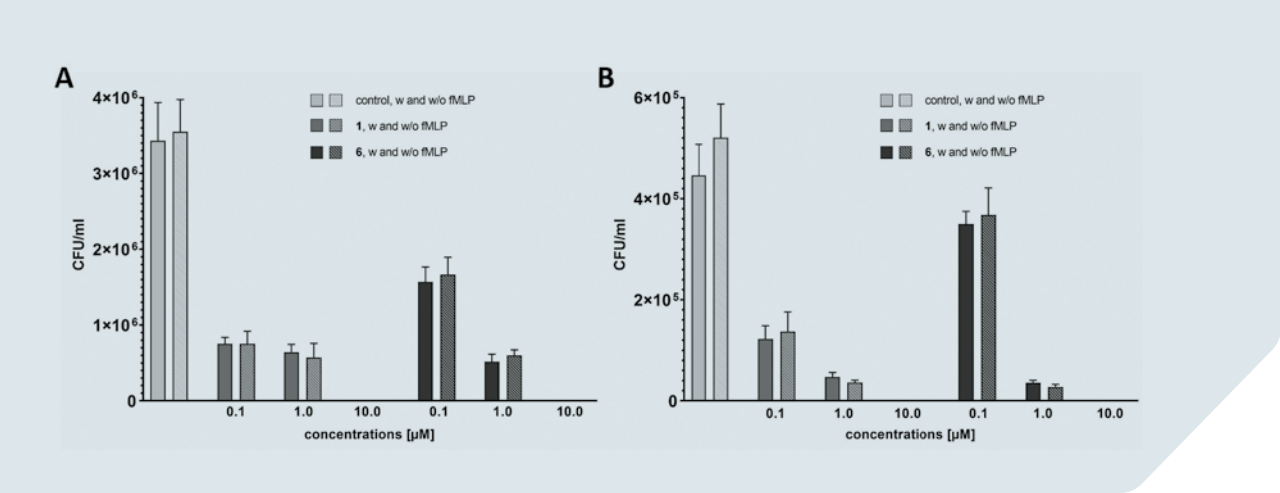

Figure 2: Antimicrobial activity of the peptide-colistin construct against *E. coli* K12 during cocultivation of neutrophil granulocytes with the bacteria in medium RPMI 1640 (**A**) and in human blood plasma (**B**). Colistin 1 and the conjugate 6 were added at three concentrations (0.1 µM, 1 µM and 10 µM), which strongly reduced and at the highest concentration even eliminated the bacteria. fMLP (formyl methionyl-leucyl-proline) is used to activate neutrophil elastase relase.
Adapted from Tegge et al. (2021) Angewandte Chemie International Edition 60:17989–17991 © CC BY 4.0

Tegge W, Guerra G, Höltke A, Schiller L, Beutling U, Harmrolfs K, Gröbe L, Wullenkord H, Xu C, Weich H, Brönstrup M (2021)
Selective bacterial targeting and infection-triggered release of antibiotic colistin conjugates
Angewandte Chemie International Edition doi: 10.1002/anie.202104921

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HOST-FACTOR ZAP-S LEADS TO REDUCTION OF VIRAL LOAD THROUGH IMPAIRED FRAMESHIFTING

NEVA CALISKAN | HEAD OF RESEARCH GROUP RECODING MECHANISMS IN INFECTIONS

SARS-CoV-2 and other viruses with genomes consisting of ribonucleic acid (RNA) use a propagation trick called programmed ribosomal frameshifting (PRF). In doing so, these viruses prove to be masters of manipulation: they invade host cells and hijack their machinery to read the genetic information from a messenger RNA and produce proteins. In addition, the virus uses PRF to alter the reading frame, which allows it to produce all of its own proteins from limited genetic material. In the search for ways to stop this propagation trick used by SARS-CoV-2, researchers at HIRI and HZI have recently identified a restriction factor called ZAP.

A hallmark of SARS-CoV-2 and many other viruses is programmed ribosomal frameshifting (PRF), which allows synthesis of multiple proteins from the same coding sequence. PRF is employed as a way to extend viral coding capacity, ensure correct stoichiometry of structural and non-structural proteins as well as to temporally regulate protein expression. Traditionally, PRF has been described as an RNA-centric gene switch where shift-prone sequences proximal to RNA structures determine PRF efficiency. Pausing at the RNA structure over slippery codons creates the suitable time win-

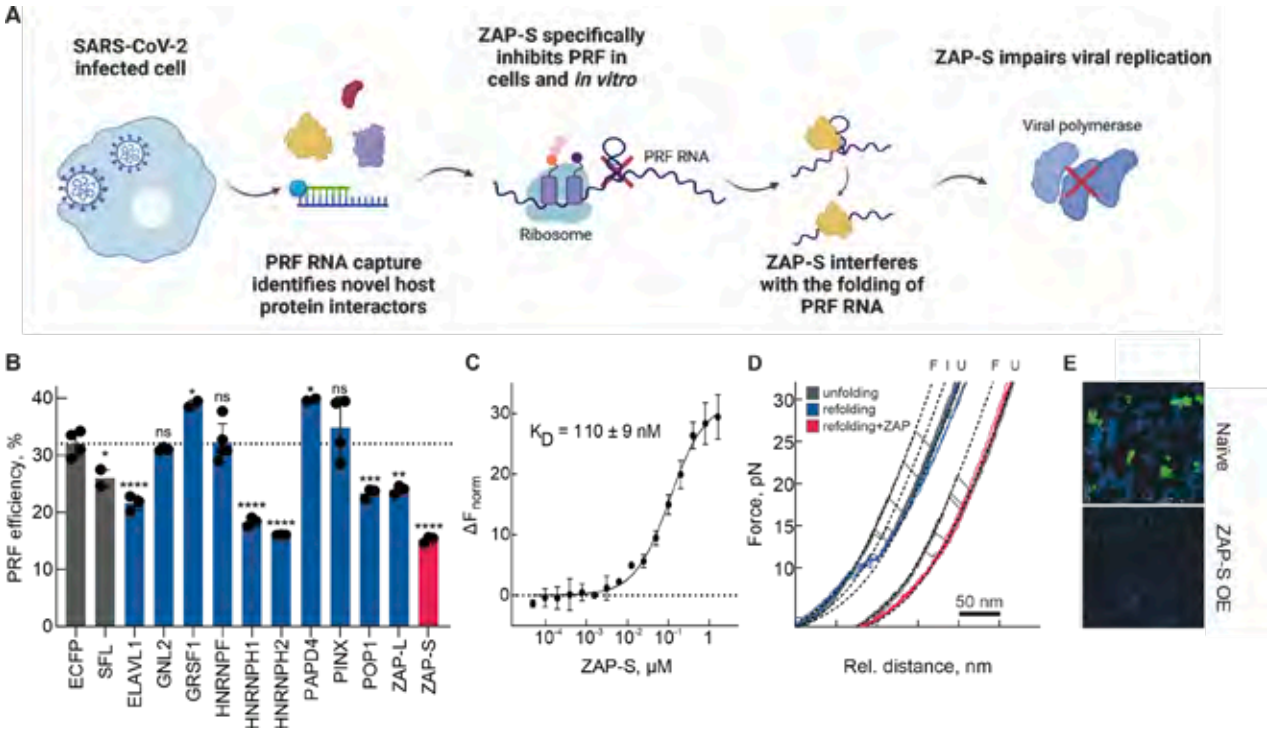

Figure 1: Discovery of ZAP-S as the primary frameshift inhibitory host protein **(A)** Experimental procedure and main results. **(B)** Several SARS-CoV-2 RNA-interacting proteins inhibit PRF. **(C)** ZAP-S binds directly to SARS-CoV-2 PRF RNA, and **(D)** impairs refolding of the SARS-COV-2 frameshift RNA structure. **(E)** Overexpression of human ZAP-S reduces SARS-CoV-2 replication (shown here through detection of viral nucleoprotein via immunofluorescence). From Zimmer et al. (2021), Nature Communications 12:7193 © CC BY 4.0

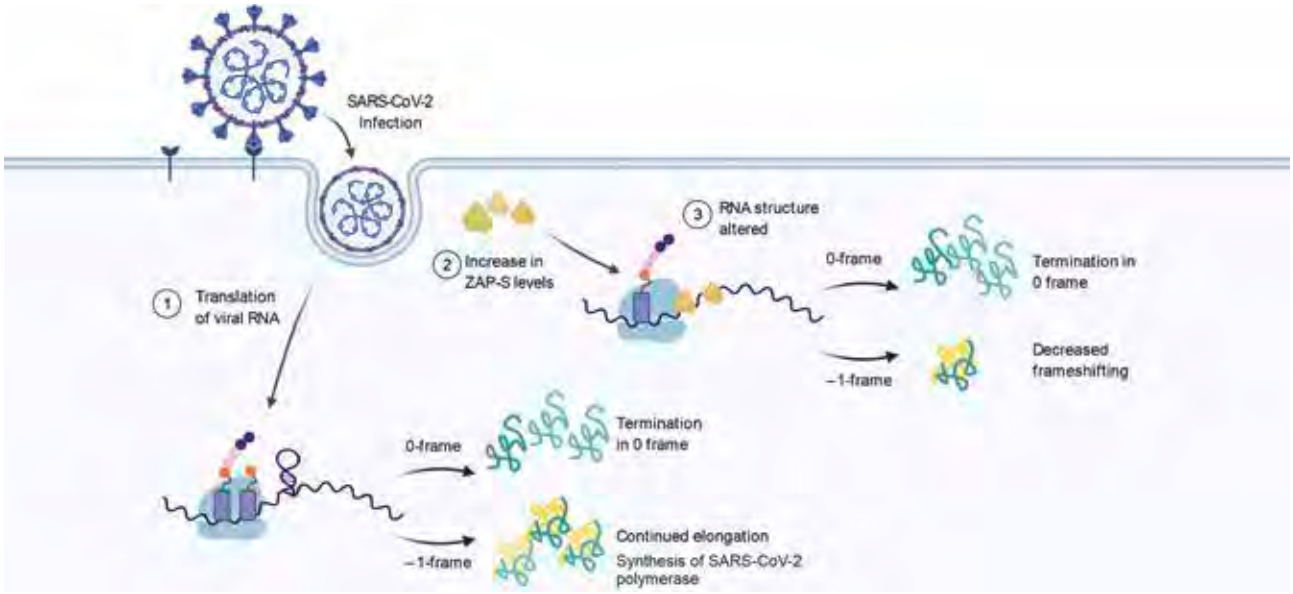

Figure 2: Model for ZAP-S mediated PRF inhibition. 1) Viral RNA is translated by the cellular machinery, and 40% of translation events yield the replicase polyprotein through the alternative reading frame. **2)** Infection leads to increased levels of interferons and production of ZAP-S. **3)** ZAP-S binding to the frameshift RNA alters refolding of the stimulatory RNA, thereby inhibiting PRF, which in turn reduces viral load. From Zimmer et al. (2021), Nature Communications 12:7193 © CC BY 4.0

dow for the translating ribosomes to explore the kinetically more favorable alternative reading frame. Recent work also demonstrated a plethora of regulatory factors affecting PRF, including host and viral proteins, small RNAs or synthetic compounds. Thus, PRF harbors all potential blueprints for antiviral drugs. Yet, whether a specific host encoded factor impairing PRF exists, remains unknown.

In our study, we comprehensively dissected the interplay of the SARS-CoV-2 PRF RNA and the host proteome using RNA antisense purification and proteomics. We identified several proteins with PRF-modulating potential, among which the short isoform of the zinc finger antiviral protein (ZAP-S) had the strongest effect *(Figure 1A)*. Using cellular assays, we were able to show that overexpression of ZAP-S leads to a reduction of PRF by 50%, and reduces the viral load by 20-fold *(Figure 1B)*. Purified ZAP-S was able to recapitulate inhibition of −1 frame translation *in vitro*, indicating that the protein is not acting as a complex with other molecules *(Figure 1D)*. ZAP-S specifically altered PRF in SARS-CoV-2 and the closely related SARS-CoV-1, but not any other viral PRF genes. Therefore, we next dissected molecular details of the interplay between ZAP-S protein and its RNA target in more detail by measuring changes in thermophoresis, which depends on the directed movement of (fluorescent) molecules in a temperature gradient. We showed that ZAP-S binds the coronavirus PRF element directly and specifically and with nanomolar affinity *(Figure 1C)*. Using structural probing coupled to sequencing we were able to investigate changes in the conformation of the RNA element upon protein binding. Furthermore, experiments involving single-molecule optical-tweezers revealed that ZAP-S binding to the PRF RNA delays refolding of the critical stimulatory RNA *(Figure 1E)*. Without this critical stimulatory structure, we envision that the translational pause would be too short for the ribosome to move to the alternative reading frame.

Taken together, in this work, we discovered that a host-encoded factor ZAP-S acts as a specific SARS-CoV PRF inhibitor and provided the first clarification of its mechanism of action using a highly integrative approach. Based on our results, we propose that ZAP-S binding to the frameshift RNA alters the RNA structure and reduces the chance of ribosomes to interact with the stimulatory element of this gene switch. Thus, ZAP-S would likely allow translation to proceed and terminate at the 0-frame stop codon found immediately downstream of the slippery sequence *(Figure 2)*. This highlights the importance of host factors in the regulation of PRF as well as their impact on viral replication. Importantly, our findings may lead to the discovery of novel host-factor mimicking antivirals that so far remain untapped in the cellular proteome.

Zimmer MM, Kibe A, Rand U, Pekarek L, Ye L, Buck S, Smyth RP, Cicin-Sain L, Caliskan N (2021)
The short isoform of the host antiviral protein ZAP acts as an inhibitor of SARS-CoV 2 programmed ribosomal frameshifting
Nature Communications doi.org/10.1038/s41467-021-27431-0

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COMBINING PATHOBLOCKERS WITH NANOCARRIER TECHNOLOGY FOR EFFICIENT BIOFILM ERADICATION

MARTIN EMPTING | HEAD OF RESEARCH GROUP ANTIVIRAL AND ANTIVIRULENCE DRUGS

Pseudomonas aeruginosa has been declared a priority pathogen by the WHO capable of colonising almost every part of the human body and being the causative agent of a wide range of infectious diseases. The occurrence of *P. aeruginosa* strains resistant to commonly used antibiotics is worrisome. Patients with chronic lung diseases like cystic fibrosis, bronchiectasis or chronic obstructive pulmonary disease are at risk of obtaining recalcitrant, recurring and difficult to treat *P. aeruginosa* infections. These are typically accompanied by high morbidity, reduced quality of life, and potentially lethal outcome. The reason for the success of the bacterium lies, among other things, in its ability to produce and settle in biofilms. These biofilms consist of a mixture of different biomolecules including sugars, proteins and lipids and protect the pathogen against the human immune system as well as antibiotics. In this work, we provide an innovative treatment strategy for biofilm-associated chronic infections by employing the synergistic action of Quorum Sensing Inhibitors and the antibiotic Tobramycin on *P. aeruginosa* biofilms in a tailored nanocarrier system.

We pursue a so-called pathoblocker approach, which aims to disable important pathogenicity traits of *P. aeruginosa* (PA) rendering it less virulent and more susceptible for antibiotic treatment. To this end, we identified and optimised Quorum Sensing Inhibitors (QSI) in a medicinal chemistry-driven lead generation and optimisation campaign. The initial hit scaffold was identified by fragment-based drug design and subsequently enlarged into drug-like molecules. X-ray crystallography enabled to gain detailed structural insights on the binding mode of advanced QSI to the target protein PqsR (also called “multiple virulence factor regulator”; MvfR), which is the regulator of the *Pseudomonas* Quinolone

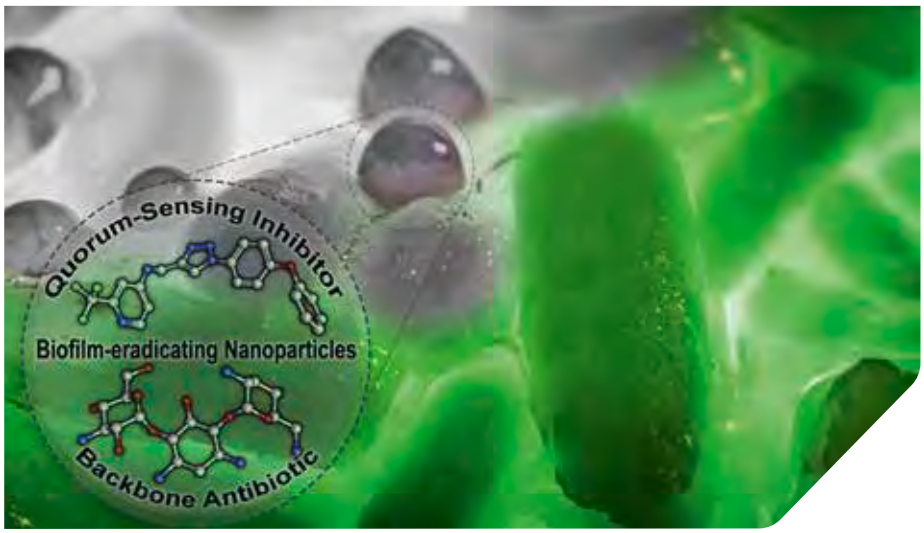

Figure 1: Illustration of dual-loaded nanoparticles eradicating a sessile *P. aeruginosa* biofilm colony via combined action of a Quorum Sensing Inhibitor and a backbone antibiotic.

Signal (PQS) Quorum sensing system. These compounds are capable of disrupting bacterial cell-to-cell communication essential for coordination and regulation of toxin production and biofilm formation. Furthermore, our multi-parameter optimisation efforts yielded a novel inhibitor scaffold *(Fig. 1)* with promising drug-like properties including a decent ADMET and safety pharmacological profile.

In close collaboration between two HIPS research groups, this novel QSI was then encapsulated in a tailored nanocarrier system able to be co-loaded with the aminoglycoside antibiotic tobramycin *(Fig. 1)*. By this means, a dramatic improvement of biofilm eradication efficacy was achieved through synergistic action of both active ingredients *(Fig. 2)*. The concentration of the antibiotic required to fully eradicate the *P. aeruginosa* biofilm was reduced by 32-fold, emphasising the potential of this adjunctive treatment strategy for tackling problematic chronic *P. aeruginosa* infections.

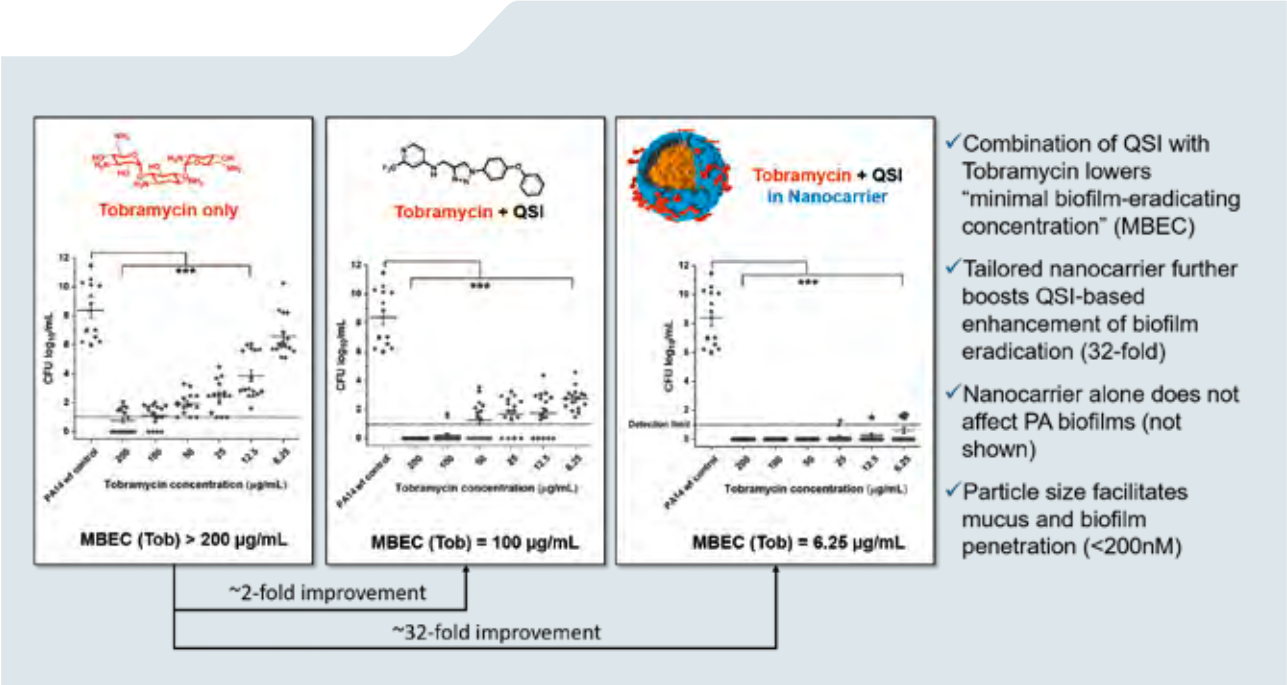

Figure 2: Improvement of biofilm-eradicating efficacy of Tobramycin in combination with novel Quorum Sensing Inhibitor (QSI) and in additional nanocarrier formulation.

Schütz C, Ho D-K, Hamed M, Abdelsamie AS, Röhrig T, Herr C, Kany AM, Rox K, Schmelz S, Siebenbürger L, Wirth M, Börger C, Yahiaoui S, Bals R, Scrima A, Blankenfeldt W, Horstmann JC, Christmann R, Murgia X, Koch M, Berwanger A, Loretz B, Hirsch AKH, Hartmann RW, Lehr C-M, Empting M (2021)

A new PqsR inverse agonist potentiates tobramycin efficacy to eradicate *Pseudomonas aeruginosa* biofilms.

Advanced Science doi:10.1002/advs.202004369

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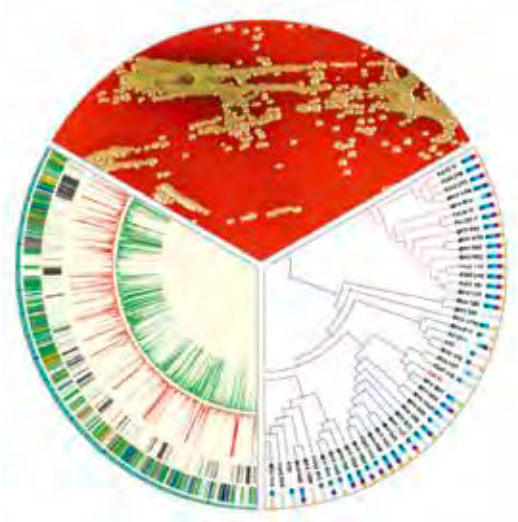
TARGETING ANTIMICROBIAL RESISTANCE WITH MOLECULAR DIAGNOSTICS

SUSANNE HÄUSSLER | HEAD OF DEPARTMENT MOLECULAR BACTERIOLOGY
ALICE C. McHARDY | HEAD OF DEPARTMENT COMPUTATIONAL BIOLOGY OF INFECTION RESEARCH

The increasing resistance of pathogens to clinically relevant antibiotics calls for the development of new treatment options. At the same time, it underlines the need to optimise current diagnostics. We trained machine-learning classifiers on information about the presence or absence of genes, their sequence variation, and expression profiles in a plethora of multidrug-resistant clinical *Pseudomonas aeruginosa* isolates. This led to the generation of predictive models and the identification of biomarkers of resistance to four commonly administered antimicrobial drugs. The implementation of a molecular susceptibility test system in routine microbiology diagnostics holds promise to provide earlier and more detailed information on antibiotic resistance profiles of bacterial pathogens.

Little has changed in antimicrobial susceptibility testing (AST) over the years. It still relies on culture-dependent methods, which means that clinical microbiological diagnostics is labour-intensive and slow. With culture-based AST, it takes 48 hours (or longer) to get a final result, leaving doctors unsure of the best drugs to prescribe for individual patients. This delay also contributes to the spread of drug resistance. It has been shown that antibiotic resistance can be predicted very accurately in a number of bacterial species based on information from the genome sequence. The introduction of molecular diagnostics thus could provide an alternative to culture-based methods and pave the way to combating antibiotic resistance.



In this study, we investigated whether the application of machine learning and feature selection approaches can reliably predict antimicrobial resistance to four common anti-pseudomonal antimicrobials, using not only genomic but also quantitative gene expression information *(Fig.1)*. For this purpose, we sequenced the genomes of 414 drug-resistant clinical *P. aeruginosa* isolates and recorded their transcriptional profiles to generate data on the presence or absence of genes (GPA), genetic variation within genes (SNPs) and gene expression under standard culture conditions *(Fig.2)*. We were able to show that the relative importance of the three types of data differed markedly between the different antibiotics. While ciprofloxacin resistance could be predicted very accurately from genetic variation alone, the inclusion of gene expression data significantly improved the prediction of ceftazidime, tobramycin and meropenem resistance over GPA alone. We also found that a non-accurate categorisation due to uncertainty in testing near the minimal inhibitory antibiotic concentration (MIC) breakpoint could explain failure in the assignment of drug resistance by the machine learning classifiers.

Figure 1: Predicting antimicrobial resistance based on the integration of omics data

In conclusion, we demonstrate that extending the genetic features (SNPs and gene presence/absence) with gene expression values is key to improving performance in antimicrobial resistance prediction. Thereby, the relative contribution of the different categories of biomarkers to antibiotic susceptibility and resistance, respectively strongly depend on the antibiotic. This is in stark contrast to the prediction of antibiotic resistance in many Enterobacteriaceae, where knowledge of the presence of resistance-conferring genes, such as beta-lactamases, is usually sufficient to correctly predict the susceptibility profiles.

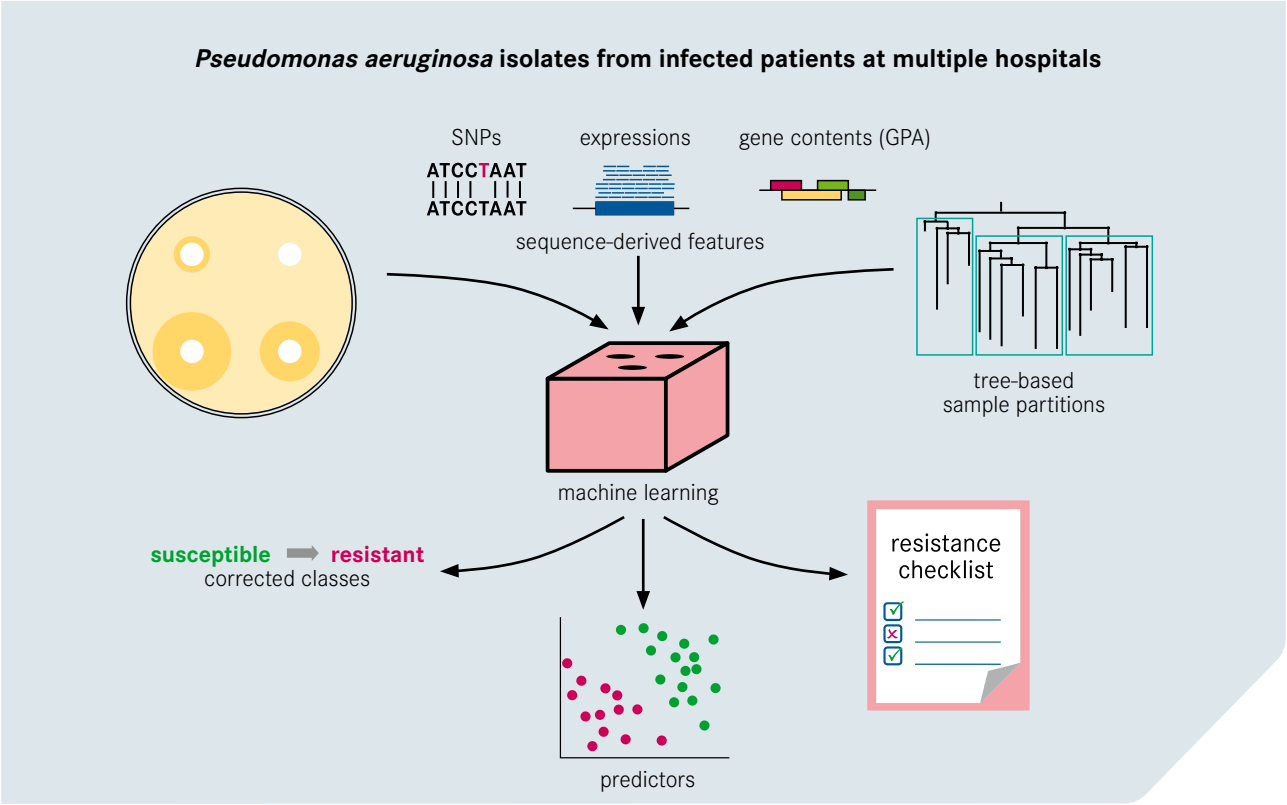


Figure 2: Machine learning models trained to predict drug resistances from Omics data of clinical *Pseudomonas aeruginosa* isolates predict drug resistances and indicate molecular biomarkers.

Khaledi A, Weimann A, Schniederjans M, Asgari E, Kuo T-Z, Oliver A, Cabot G, Kola A, Gastmeier P, Hogardt M, Jonas D, Mofrad MRK, Bremges A, McHardy AC, Häussler S (2020)
Predicting antimicrobial resistance in *Pseudomonas aeruginosa* with machine learning-enabled molecular diagnostics
EMBO Mol Med doi: 10.15252/emmm.201910264

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A "CALL FOR HELP" FROM NEURONS IN THE VIRUS-INFECTED BRAIN

ULRICH KALINKE | HEAD OF DEPARTMENT EXPERIMENTAL INFECTION RESEARCH

Viral infection of the central nervous systems (CNS) occurs rarely. However, if CNS infection happens, e.g., with herpes simplex virus or with tick-borne encephalitis virus, patients can develop severe acute disease and may even die. A high percentage of the surviving patients develop long-term sequelae that may significantly impair quality of life. So far options for causative treatment of viral CNS infection are very limited. Furthermore, it is still unclear how exactly virus infection within the central nervous system is controlled. We found that recruitment of peripheral $CD8^+$ T cells to the virus-infected brain is needed to protect the host. Mostly neurons are infected by virus, which then mount chemokine responses. These chemokines regulate recruitment of peripheral T cells.

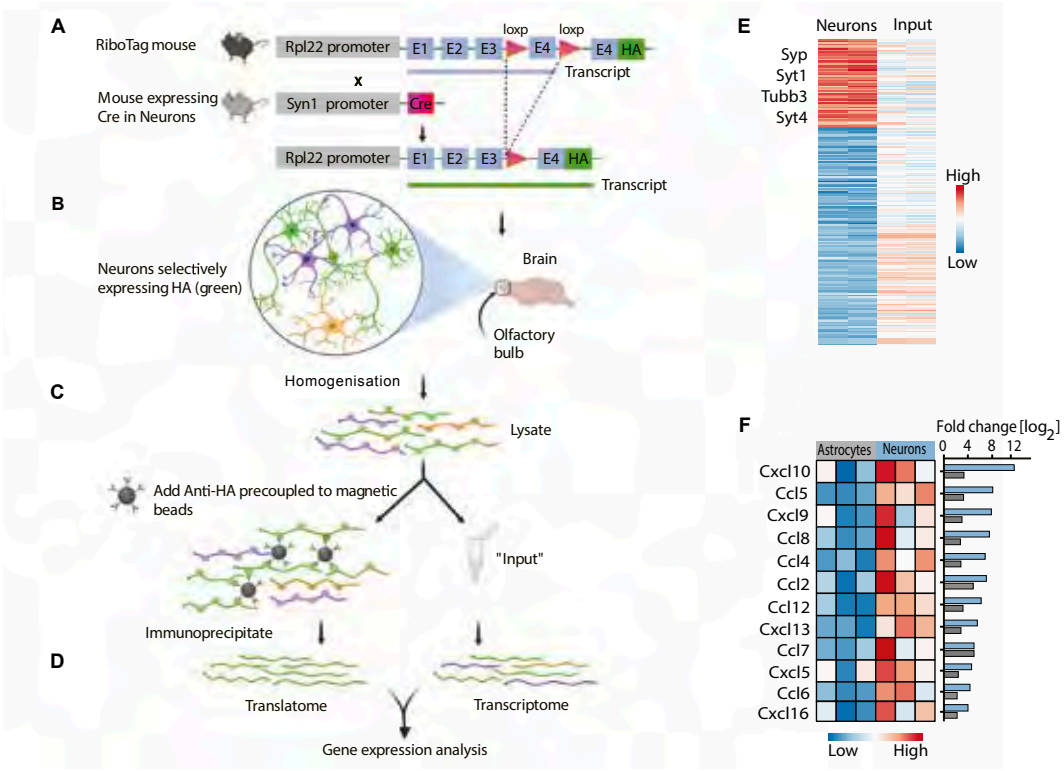

Figure 1: Within the virus infected CNS neurons express chemokines. The RiboTag approach allows *ex vivo* analysis of the translatome of selected cell subsets. **A** RiboTag mice are intercrossed with transgenic mice expressing Cre selectively, e.g., in neurons. As a result, mice are obtained in which ribosomes only of the neurons display a hemagglutinin (HA) tag, as indicated by the extended transcript. **B** Upon isolation of the olfactory bulb and homogenisation of the tissue, **C** neuronal HA-labeled ribosomes are pulled down and **D** used for RNA isolation and sequencing. **E** RNA sequencing is performed from the RNA isolated from the whole olfactory bulb lysate (Input) and the RNA isolated from the pulled-down ribosomes (Neurons). Upon data analysis, the neuron-specific transcriptome is determined as indicated by the enhanced expression of neuron-specific genes such as Syp, Syt1, Tubb3, and Syt4 in the neuronal translatome (Neurons). **F** Analysis of the translatomes of astrocytes and neurons from mice intranasally instilled with VSV revealed that primarily neurons show chemokine expression. Adapted from Ghita et al. Science Immunol. (2021). Adapted from Ghita et al. (2021) Science Immunology 6:eabc9165. Reprinted with permission from AAAS. © AAAS

The concept of the "blood-brain barrier" (BBB) dates back from experiments that Paul Ehrlich and others performed around 1900. The researchers discovered that upon peripheral injection of water-soluble dyes, the entire body was stained except for the brain. These experiments highlighted the existence of a physiological barrier between the periphery and the brain that might impede pathogen invasion and excessive immune cell entry. Accordingly, the scientific consensus outlived for many years that the CNS is well shielded by the BBB to avoid pathogen entry. Today, this concept is outdated. It is well known that viruses and other pathogens may enter the brain via diverse routes. Upon brain infection, local innate immune responses are induced that restrict pathogen dissemination. Several days after onset of the infection, peripheral immune cells are recruited to the brain.

We and others lately discovered that particularly brain infiltrating $CD8^+$ virus-specific T cells are needed to control virus infections within the brain. To address how the recruitment of these cells to the brain is regulated, we analysed mice that were infected intranasally with vesicular stomatitis virus (VSV), which is a highly neurotropic RNA-encoded virus closely related to rabies virus that is broadly used as a neurotropic model pathogen. Upon intranasal instillation, the virus infects olfactory sensory neurons in the nasal epithelium and moves within the axons to the olfactory bulb, which is located in the forebrain. There, type I interferon responses are induced within hours after infection. In contrast, it takes several days until brain resident myeloid cells, the microglia, are fully activated and accumulate at sites of infection in peripheral areas of the olfactory bulb. Simultaneously, peripheral immune cells including $CD8^+$ virus-specific T cells are recruited to the infected brain.

To study responses of the different cell types within the infected CNS, we exploited the RiboTag approach. In brief, transgenic mice that carry tagged ribosomes in selected cell types such as astrocytes or neurons are used for infection experiments. Upon cell-selective pull-down of ribosomes from whole olfactory bulb lysates, the RNA is isolated and bulk-sequenced, which reveals the translatomes, e.g., from astrocytes or neurons *(Fig. 1)*. The detailed data analysis revealed that neurons but not astrocytes were VSV-infected. Additionally, infected neurons mounted chemokine responses, which are essential for the attraction of peripheral immune cells to the infected brain. Furthermore, mice with a deficiency in MyD88, which is the adaptor of a highly relevant cellular virus sensory system, showed reduced production of chemokines in the infected brain as well as impaired immune cell recruitment. In contrast, transgenic mice with a neuron-selective reconstitution of MyD88 showed normal chemokine responses and immune cell infiltration. These experiments verified that indeed chemokines produced by infected neurons regulate the recruitment of peripheral $CD8^+$ T cells to the infected brain.

This newly discovered mechanism might help to develop improved treatment options for virus infections of the brain.

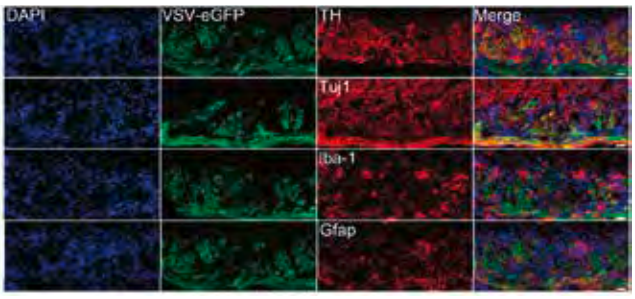

Figure 2: Upon intranasal VSV instillation of mice, neurons in the olfactory bulb are infected. Mice were intranasally infected with eGFP-expressing VSV and 6 days later the olfactory bulb was removed and analysed by immune histology. Images were acquired from peripheral areas of the olfactory bulb, the so-called glomerular layer. Note that tyrosine hydroxylase (TH) and Tuj1 positive neurons, and not IBA-1 positive myeloid cells, co-localise with eGFP. These data show that neuron and not myeloid cells are infected. Adapted from Ghita et al. Science Immunol. (2021)
Adapted from Ghita et al. (2021) Science Immunology 6:eabc9165. Reprinted with permission from AAAS. © AAAS

Ghita L, Spanier J, Chhatbar C, Mulenge F, Pavlou A, Larsen PK, Waltl I, Lueder Y, Kohls M, Jung K, Best SM, Förster R, Stangel M, Schreiner D, Kalinke U (2021)
MyD88 signaling by neurons induces chemokines that recruit protective leukocytes to the virus-infected CNS
Science Immunology: doi: 10.1126/sciimmunol.abc9165#con1

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INTERACTIONS BETWEEN VIRAL RNA AND THE HOST CELL PROTEOME REVEAL POTENTIAL THERAPEUTIC TARGETS

MATHIAS MUNSCHAUER | HEAD OF RESEARCH GROUP LncRNA AND INFECTION BIOLOGY

SARS-CoV-2 infections pose a global threat to human health. While viral proteins are being investigated with great interest, little is known about viral RNAs and their regulation by host factors during the early stages of an infection. This study comprehensively characterises the interactions between the SARS-CoV-2 RNA genome and the host cell proteome for the first time. We find dozens of pro- and antiviral proteins that directly bind viral RNA in infected cells and demonstrate their functional relevance with genetic and pharmacological tools. This work outlines a general roadmap for dissecting the biology of RNA viruses and may improve the rational design of novel antivirals.

As an obligate intracellular pathogen, SARS-CoV-2 co-opts cellular factors for viral mRNA translation and genome replication. The host cell on the other hand encodes many sensors of the innate immune system that recognise foreign RNA, and in turn, trigger antiviral defense mechanisms. Despite the urgent need to better understand the molecular basis of SARS-CoV-2 infections, a comprehensive map of the host cell proteins that bind and regulate viral RNA had not been elucidated previously. This void has now been filled. Taking advantage of an experimental technique known as RNA antisense purification *(Figure 1)*, which was originally developed to study long non-coding RNAs, we capture and identify proteins that directly bind SARS-CoV-2 RNA inside infected cells. In addition to many known binders of viral RNA, we find more than 100 human proteins that directly interact with SARS-CoV-2 RNA *(Figure 2)*.

Among these host-encoded factors are many proteins linked to mRNA translation and stability, along with known antiviral RNA binders and proteins associated with vesicle trafficking and cytoskeleton remodeling. Looking closer at individual candidates, we demonstrate by genetic perturbation that CNBP and LARP1, two of the most strongly enriched viral RNA binders, restrict SARS-CoV-2 replication in infected

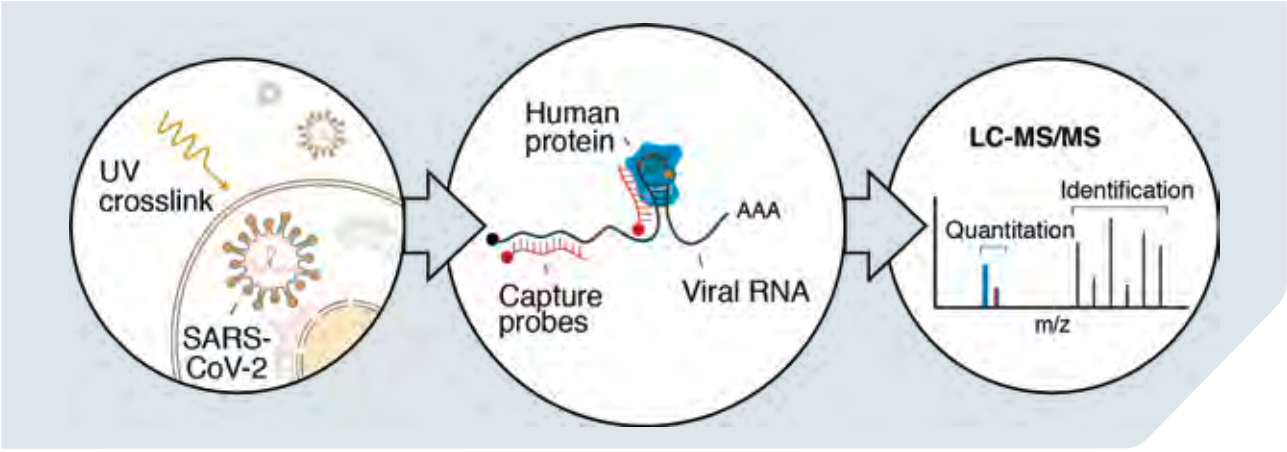

Figure 1: Outline of the RNA antisense purification and mass spectrometry (RAP-MS) method to identify proteins directly bound to SARS-CoV-2 RNAs in human cells undergoing authentic virus infection.

cells. We map the exact RNA binding sites of both of these newly identified antiviral proteins and notice an intriguing binding pattern: LARP1 binds to the 5'-leader sequence that is present in all SARS-CoV-2 mRNAs. This mode of binding is similar to how LARP1 recognises specific host mRNAs to regulate their translation. More work is needed here, but based on our findings so far, it is tempting to speculate that LARP1 might regulate the translation of SARS-CoV-2 mRNA. Beyond CNBP and LARP1, we find dozens of RNA-binding proteins that are known targets of pharmacological inhibitors. We test four inhibitors that target components of our RNA interactome and observe a clear reduction of viral RNA levels and viral titers for three of those compounds in cell lines derived from lung and liver tissues. Combining evidence from our genetic and pharmacological experiments with emerging genome-wide CRISPR screening data, we find clear support for the functional relevance of 18 RNA-binding proteins during SARS-CoV-2 infections.

Overall, our SARS-CoV-2 RNA-protein interactome provides valuable insights into the regulation of viral RNA in infected human cells, which will improve our understanding of viral RNA functions, innate immune response mechanisms and may guide the development of rationally designed therapies against COVID-19.

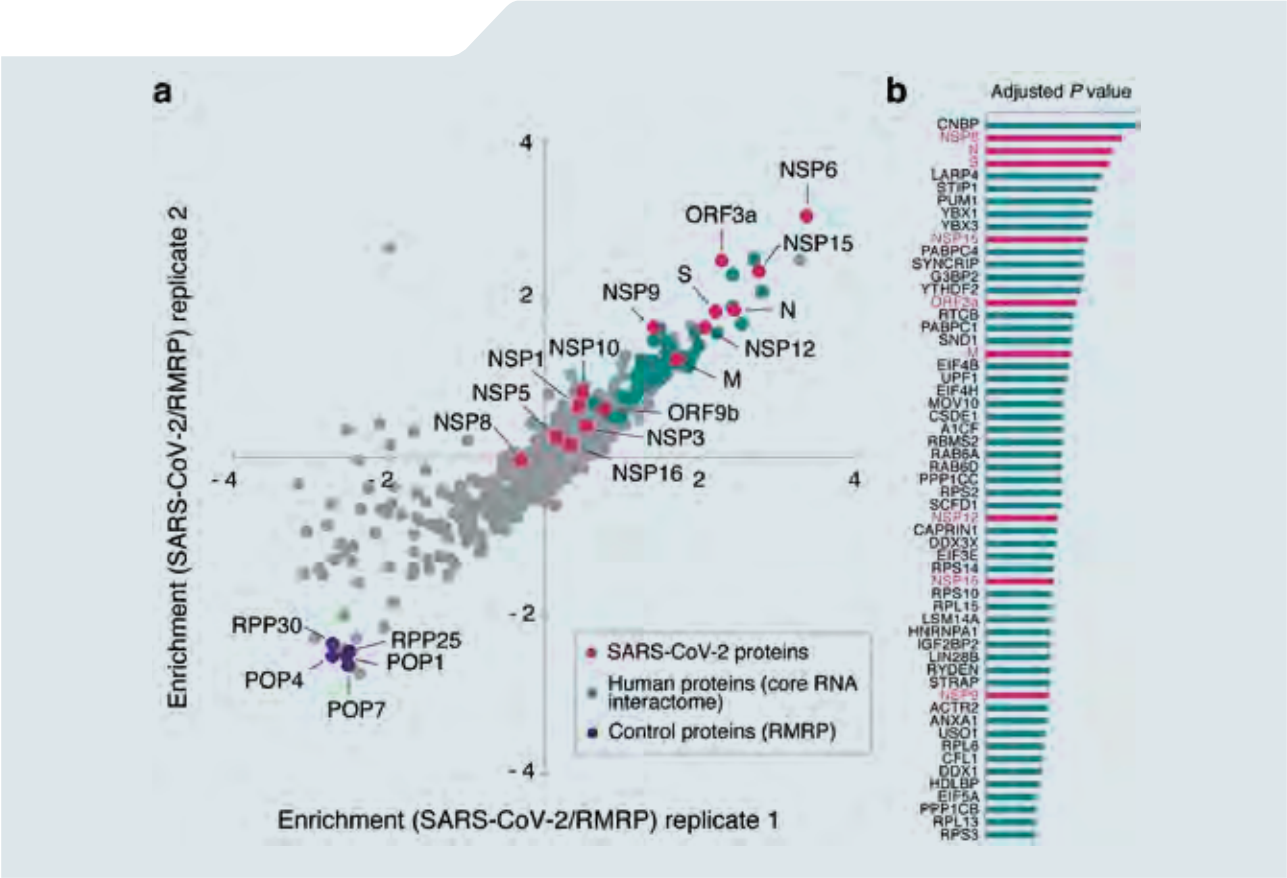

Figure 2: a, Quantification of SARS-CoV-2 RNA interacting proteins relative to proteins interacting with a well-characterised endogenously expressed control RNA (RMRP). Scatter plot of log2-transformed tandem mass tag ratios from two biological replicates is shown. **b**, The core SARS-CoV-2 RNA interactome. Statistically most strongly enriched viral and human proteins are displayed. From Schmidt et al. (2021) Nature Microbiology 6:339-353.© CC BY 4.0

Schmidt N, Lareau CA, Keshishian H, Ganskih S, Schneider C, Hennig T, Melanson R, Werner S, Wie Y, Zimmer M, Ade J, Kirschner L, Zielinski S, Dölken L, Lander ES, Caliskan N, Fischer U, Vogel J, Carr SA, Jochen Bodem J, Munschauer M (2021)

The SARS-CoV-2 RNA–protein interactome in infected human cell

Nature Microbiology doi: org/10.1038 /s41564-020-00846-z

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IN SERO VERITAS: NEW TOOL AIMS TO MAKE HCV VACCINE RESEARCH EASIER

THOMAS PIETSCHMANN | HEAD OF DEPARTMENT EXPERIMENTAL VIROLOGY

Hepatitis C virus (HCV) has infected ca. 71 million individuals worldwide. If untreated, the chronic infection frequently causes severe liver diseases including liver fibrosis, liver cirrhosis and hepatocellular carcinoma. Although effective treatments are available, many infected people are not diagnosed or do not have access to treatment. Therefore, each year ca. 300,000 die due to sequelae of a chronic HCV infection, and HCV causes significant global disease burden. Moreover, each year ca. 1.5 million people infect themselves with the virus and a vaccine preventing transmission is not in reach. Here we developed a new system to quantify the potency of antibody response to HCV. This system will be useful to pinpoint mechanisms of protection and prioritise vaccine candidates.

One reason why it is difficult to develop an HCV vaccine is that this virus is extremely diverse. In fact, virus strains can diverge from each other by more than 30 % at the nucleotide level, posing a formidable challenge to the development of a vaccine protecting against such divergent virus variants. Furthermore, there is no immunocompetent animal model available to test HCV vaccine efficacy. Given this latter point, rigorous and predictive laboratory assays are important to inform about the correlates of protection from HCV and to measures the potency of vaccine-induced immune responses.

Antibodies in general and neutralising antibodies in particular are key immunological effector molecules, which confer immune protection after natural infection. Likewise, many vaccines protect via induction of potent neutralising antibodies. Therefore, test systems for quantification of antibody neutralisation breadth and potency are valuable armamentarium for immunologists and vaccine developers.

In this study, we developed and validated a series of HCV reference viruses, which represent globally sampled, genetically diverse HCV strains. Interrogating human polyclonal antibodies from ca. 400 patients infected with genetically diverse viruses, we show that this reference panel accurately measures cross-neutralisation breadth and potency. Applying this system, we identified HCV patients with elite antibodies and described the properties of particularly powerful HCV cross-neutralising antibodies (Weber *et al.*, Immunity; doi: 10.1016/j.immuni.2021.12.003).

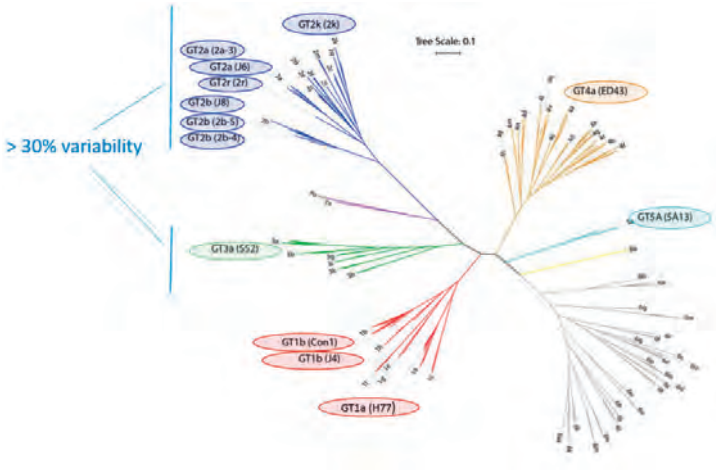

Figure 1: HCV diversity illustrated by a phylogenetic tree of E1E2 amino acid sequences. HCV genotypes are colour-coded and viruses used here are circled. Adapted from Bankwitz et al. (2020) Gut, 70(9):1734-1745 © CC BY 4.0

Initially, we created 13 viruses representing five key branches (genotypes) of the HCV phylogenetic tree. These viruses reflect a broad and diverse range of currently circulating HCV strains *(Fig. 1)*. Next, we subjected these viruses to *in vitro* neutralisation tests with polyclonal antibodies from 100 HCV-positive patients infected by diverse HCV variants. *(Fig. 2)*.

Using these high-resolution neutralisation data and metric-multi-dimensional scaling, the bioinformaticians derived an HCV neutralisation map reconstructing the HCV neutralisation space. This analysis showed that these genetically diverse viruses cluster into six distinct groups, so called viral neutralisation biotypes. Viruses of one such cluster are functionally alike with each other in terms of their interaction with neutralising antibodies. This similarity does not correlate with genetic sequence of the given viruses but apparently represents functionally distinct biological states of the viral envelope proteins, the target proteins of neutralising antibodies.

Taking advantage of this information, we designed and validated a streamlined HCV reference virus panel consisting of a representative strain from each of these clusters. Finally, we show that these selected viruses are as well suited to diagnose breadth and potency of antibodies as the entire panel and we used it to identify patients with elite antibody responses to HCV.

These findings move vaccine development forward in two important ways: First, we now have a rigorous neutralisation test system, and a gold standard of the most powerful antibody responses humans mount against this virus. Both of which help bench marking current and future vaccine candidates. Second, this study pinpoints six neutralisation clusters, which likely represent distinct functional states of the HCV E1/E2 proteins. Rather than combining genetically diverse antigens, it may be better to use a cocktail of antigens representing these distinct functional states to trigger a broadly protective immune response.

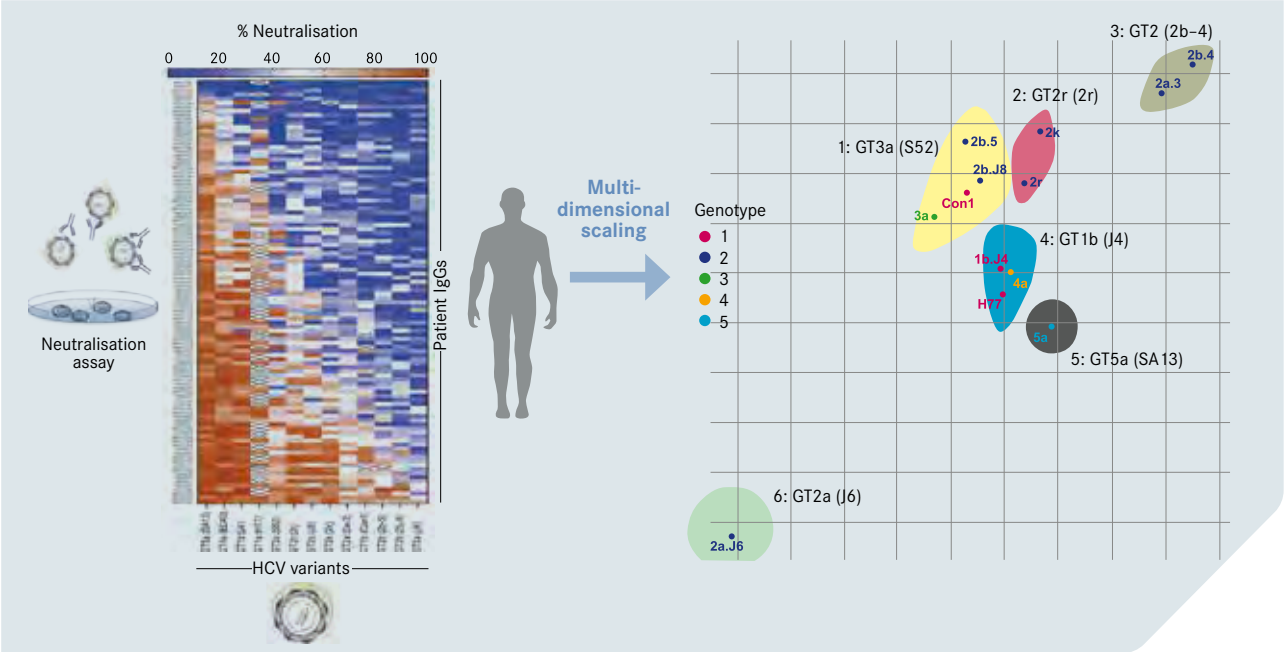

Figure 2: Profiling of human antibodies against HCV by using a bioinformatic method identifies six neutralisation clusters. The virus sequences do not predict mapping to these clusters. Adapted from Bankwitz et al. (2020) Gut, 70(9):1734-1745 © CC BY 4.0

Bankwitz D, Bahai A, Labuhn M, Doepke M, Ginkel C, Khera T, Todt D, Str h LJ, Dold L, Klein F, Klawonn F, Krey T, Behrendt P, Cornberg M, McHardy AC, Pietschmann T (2020)
Hepatitis C reference viruses highlight potent antibody responses and diverse viral functional interactions with neutralising antibodies
Gut doi: 10.1136/gutjnl-2020-321190

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UNDERSTANDING COVID-19 PATHOLOGY - ONE CELL AT A TIME

EMMANUEL SALIBA | HEAD OF RESEARCH GROUP SINGLE CELL ANALYSIS

The COVID-19 pandemic caused by the SARS-CoV-2 coronavirus has been the dominant global public health crisis of the last two years. Clinical presentations of COVID-19 are highly variable, but the reasons for this variability are unknown. In two multi-centre multi-cohort studies, we have used multipronged single-cell technologies to analyse samples from patients with mild versus severe COVID-19. We obtained insights into the systemic immune response to SARS-CoV-2 infection and revealed profound alterations in specific immune cell compartments in the blood and the lung that are associated with severe disease.

Most people infected with SARS-CoV-2 develop mild to moderate respiratory tract infection. Some even show no symptoms at all. However, 10 to 20 percent of patients progress to severe disease, with life-threatening consequences. Assembling an interdisciplinary research consortium that brought together researchers from across Germany, we used single-cell RNA sequencing and single-cell proteomics technology to analyse blood samples (Schulte-Schrepping et al. 2020) and samples of lung tissue (Wendisch et al. 2021). Samples were collected from COVID-19 patients, whose course of disease was defined as mild or severe according to the World Health Organisation classification *(Figure 1)*. Altogether, these studies involved a large group of institutions, including the Helmholtz Health section (DZNE, MDC, Helmholtz Munich) and German universities (the Berlin Charité, Aachen University).

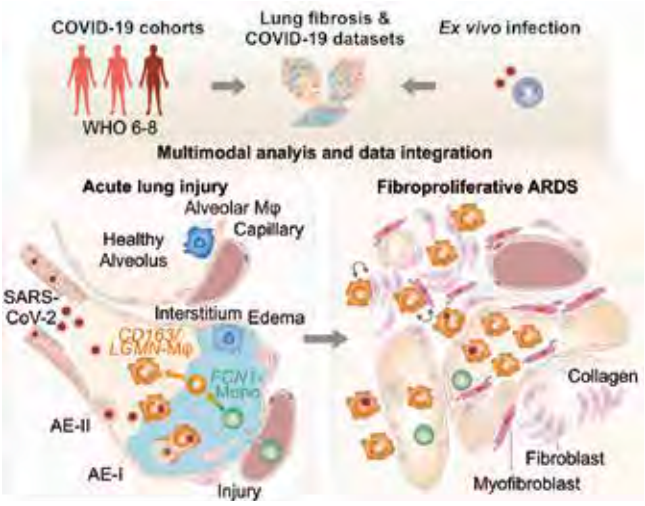

Figure 1: Graphical summary showing how SARS-CoV-2 infection triggers immunological and pathological changes in the lung.
Adapted from Wendisch et al. (2021) Cell, 184(26):6243-6261. Reprinted with permission from Elsevier. © Elsevier

Blood samples were taken from >50 patients from two different cohorts in Berlin and Bonn. The parallel analysis of two independent patient cohorts from two different sites allowed us to directly validate our findings. Blood samples from patients with other viral respiratory tract infections as well as from healthy individuals served as controls. Our approach revealed drastic changes within the myeloid cell compartment that occur during COVID-19, particularly in patients with a severe course of disease. Early activation of a specific type of monocytes with a strong antiviral interferon-signature was a hallmark of mild COVID-19, which receded during the natural course of disease. In contrast, in severe cases of COVID-19, neutrophils and monocytes were only partially activated and did not function properly. We also found considerably more immature cells that can have an inhibitory effect on the immune response. Collectively, these data linked highly dysregulated myeloid cell responses to severe COVID-19.

Figure 2: Computed tomography (CT) imaging of the lung of healthy individuals and of COVID-19 patients with a severe course of the disease. Adapted from Wendisch et al. (2021) Cell, 184(26):6243-6261. Reprinted with permission from Elsevier.

In patients with severe COVID-19, damage to the lungs can be so stark that the body can no longer absorb sufficient oxygen from the air. This condition is referred to as 'Acute Respiratory Distress Syndrome', or ARDS. In order to survive ARDS, patients must receive oxygen therapy, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO), in which a machine assumes the function of the lungs. We analysed pulmonary immune responses and lung pathology in two cohorts of patients with COVID-19 ARDS using functional single-cell genomics, immune histology, and electron microscopy. Almost all affected patients showed extensive lung tissue damage *(Figure 2)*. The majority of the alveoli had been destroyed and the alveolar walls showed thickening. We also found ubiquitous deposits of collagen, the main component of scar tissue. This is characteristic of severe fibrosis.

Interestingly, this type of lung failure is not caused by uncontrolled viral replication, but by secondary host responses, including those involving the immune system. We therefore analysed the composition and characteristics of immune cells taken from bronchioalveolar lavage and lung tissue.

In order to study individual cells in greater detail, we used state-of-the-art single cell analysis *(Figure 1)*. Using this technology, we were able to show that the accumulation of macrophages is one of the key features in COVID-19 patients who develop respiratory failure. These macrophages interact with specific fibroblasts, which in response undergo rapid proliferation and produce large quantities of collagen. Using cell cultures, we discovered that SARS-CoV-2 exerts an effect on macrophages which may, in turn, accelerate this process of fibrosis. As part of this experiment, we isolated macrophage precursor cells from the blood of healthy individuals and subsequently stimulated them with SARS-CoV-2. Curiously, these macrophages display characteristics similar to those seen in idiopathic pulmonary fibrosis, a form of lung disease which causes chronic scarring. This may explain why some of the risk factors for COVID-19 are also risk factors for idiopathic pulmonary fibrosis. These include underlying medical conditions, smoking, male sex and being aged over 60. However, there is one crucial difference between the two diseases: in COVID-19, scarring is – at least potentially – reversible. We were able to follow this repair using CT images *(Figure 2)*. In patients with COVID-19 who had received ECMO support, CT imaging initially showed typical ground glass opacities which, over the course of the illness, became increasingly dense and developed scarring. In patients who were successfully weaned from ECMO support and later recovered, these opacities gradually resolved – although some patients were left with clear evidence of residual scarring.

Schulte-Schrepping J, ... , Saliba AE, Sander LE; Deutsche COVID-19 OMICS Initiative (DeCOI) (2020)
Severe COVID-19 is marked by a dysregulated myeloid cell compartment
Cell doi.org/10.1016/j.cell.2020.08.001

Wendisch D, ..., Saliba AE, Sander LE (2021)
SARS-CoV-2 infection triggers profibrotic macrophage responses and lung fibrosis
Cell doi.org/10.1016/j.cell.2021.11.033

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MINING THE MICROBIOTA FOR A NEW GENERATION OF PROBIOTICS: FROM COHORT TO PRODUCTS

TILL STROWIG | HEAD OF DEPARTMENT MICROBIAL IMMUNE REGULATION

The intestine of a healthy person contains hundreds of different microorganisms, including bacteria, that provide efficient protection against infections. However, if the equilibrium of this community, known as the microbiota, is disturbed – for example, due to antibiotic therapy – pathogens such as salmonella or multiresistant hospital germs such as *Klebsiella pneumoniae* can gain the upper hand – sometimes with severe consequences for those affected. Restoring the microbiota using a new generation of probiotics consisting of specific bacterial cocktails rationally selected for desired phenotypes, e.g., the ability to remove multiresistant bacteria, represents a complementary avenue to the development of novel antibiotics to combat the looming AMR pandemic.

Interindividual difference in the human gut microbiota have been linked to the onset and course of various human diseases ranging from metabolic syndrome to inflammatory bowel diseases. This has prompted clinicians and scientists around the globe to search for strategies to manipulate the microbiota to prevent and treat a wide range of diseases. A well-recognised example is the susceptibility to infections with the pathogen *Clostridioides difficile*, which is strongly associated with the loss of specific functions within the microbiota and can be reverted in individuals with high infection susceptibility by fecal microbiota transfer (FMT). However, transfer of undefined communities can have unwanted consequences, e.g., the transfer of pathogens hidden within the FMT product. Therefore, new approaches based on the transfer of single or small consortia of bacteria are desired to treat *C. difficile* and prevent other infections.

Identification and validation of key species for colonisation resistance

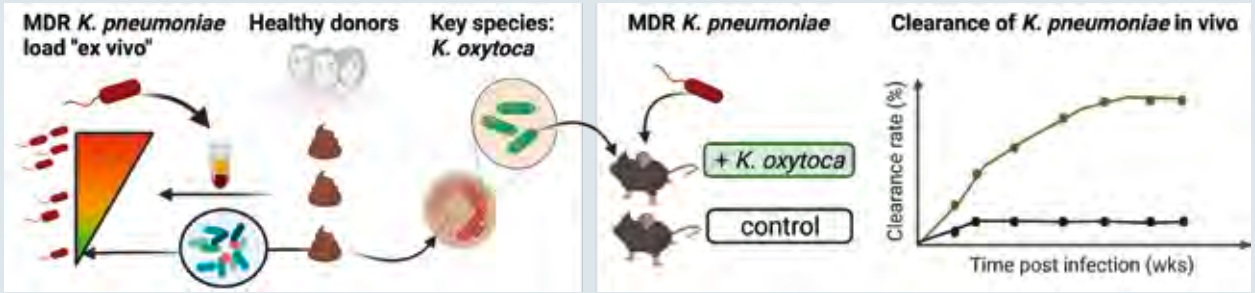

Figure 1: Identification of *K. oxytoca* as probiotic against *K. pneumoniae*.
Left panel: An *ex vivo* assay was utilised to identify patients samples that inhibited *K. pneumoniae* outgrowth. *K. oxytoca* was isolated from these fecal samples. Right panel: Transfer of *K. oxytoca* promotes *K. pneumoniae* clearance from the murine gut.

In our study we characterised the effect of specific members of the microbiota on the pathogen *Klebsiella pneumoniae*. Previous studies had identified that gut coloniation with multi-drug-resistant (MDR) strains of *K. pneumoniae* enhances the risk of bloodstream infections in susceptible individuals. By studying human fecal samples from a cohort of approx. 100 healthy donors we were able to reveal highly variable degrees of ex vivo colonisation resistance against a MDR *K. pneumoniae* strain between individuals. Next, we examined the samples in which *K. pneumoniae* had grown poorly; in many of them a related bacteria named *Klebsiella oxytoca* could be identified. We then tested the behaviour of the two bacteria in different mouse models and found that the addition of *K. oxytoca* can significantly reduce susceptibility to *K. pneumoniae* gut colonisation. Through an in-depth characterisation of the metabolism of the two bacteria specific difference were noted, specifically, the ability to use a distinct group of carbohydrates by *K. oxytoca*. After removing this function from *K. oxytoca*, this bacterial strain was no longer able to protect against *K. pneumoniae*. In additional experiments we were able to show that *K. oxytoca* cooperates with additional commensal bacteria in order to efficiently remove *K. pneumoniae* from the gut. A patent for the preventive and therapeutic use of *K. oxytoca* to remove *K. pneumoniae* from the gut was filed and we are currently characterising the safety of *K. oxytoca* as next generation probiotic in pre-clinical models.

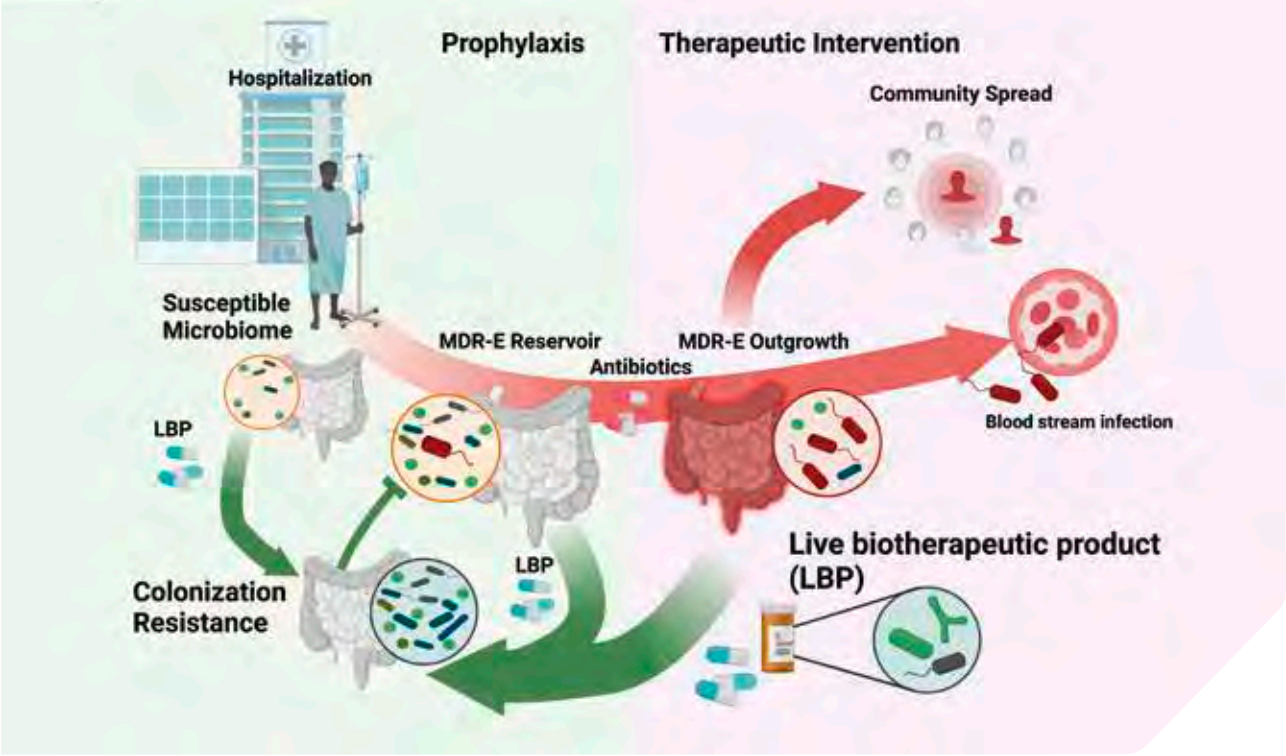

Figure 2: Envisioned strategy to use *K. oxytoca* either as preventive or therapeutic agent to protect patients against *K. pneumoniae*-caused systemic infection.

Osbelt L, Wende M, Almási E, Derksen E, Muthukumarasamy U, Lesker TR, Galvez EJC, Pils MC, Schalk E, Chhatwal P, Färber J, Neumann-Schaal M, Fischer M, Schlüter D, Strowig T (2021)
***Klebsiella oxytoca* causes colonization resistance against multidrug-resistant *K. pneumoniae* in the gut via cooperative carbohydrate competition**
Cell Host Microbe doi.org/10.1016/j.chom.2021.09.003

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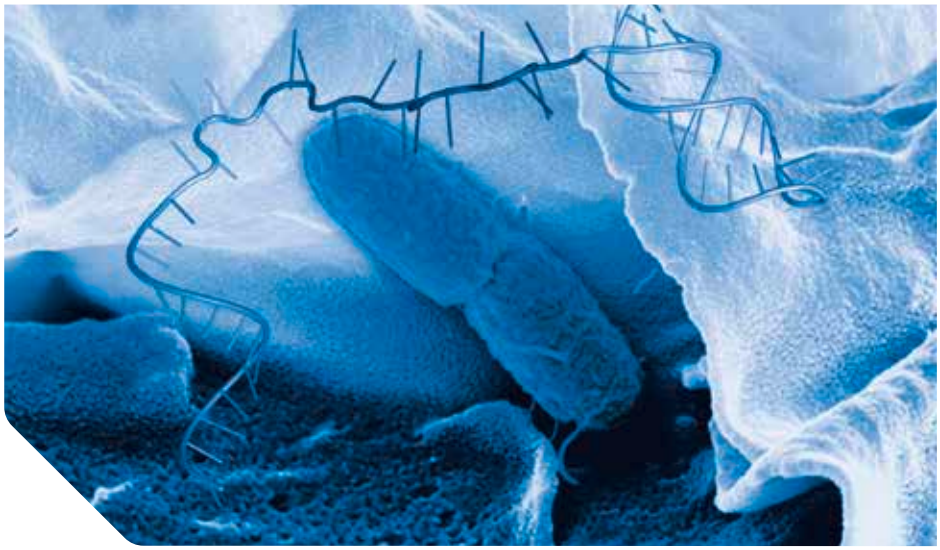
LOOKING INTO SINGLE BACTERIA: THE RNA-SEQ ROUTE

JÖRG VOGEL | HEAD OF DEPARTMENT RNA-BIOLOGY OF BACTERIAL INFECTIONS

Bacteria show a wide range of capabilities in withstanding antibiotic treatment or attack by our immune system, both of which are great challenges in infection research. We have developed a novel approach to reading the activity of genes in an individual bacterium by sequencing its RNA, achieving a 100x increased resolution of such gene expression analysis over previous single-cell techniques. Our pioneering bacterial single-cell RNA sequencing approach promises to elucidate the tricks bacteria use to develop resistance and close these loopholes with tailored drugs.

Even when growing together under the same conditions individual bacteria can behave very differently. Some stand out from the crowd of their genetically identical siblings and defy life-threatening conditions, such as antibiotic treatment. What characteristics make these outliers become bacterial superheroes is not yet understood. However, our study represents a giant leap forward in this field: we have succeeded in profiling individual bacteria using single-cell RNA sequencing, a feat previously only achieved with much bigger cells of other organisms. Why has bacterial single-cell RNA sequencing been this challenging and what information does it provide?

In a bacterium, not every gene is constantly active. However, those genes necessary for the metabolism under given conditions will be switched on. The genetic code of an activated gene is transcribed into RNA. The RNA, in turn, serves as a template for the production of proteins that fulfil certain functions within the cell. The entirety of the RNA present in a bacterium – the so-called transcriptome – therefore indicates exactly which genes are necessary at that point in time and under the given circumstances in order to survive. Thus, by analyzing the transcriptome of a bacterium that resists antibiotic treatment, we can look deeper into its box of tricks.



© SciGraphics | Sandy Westermann

Single-cell RNA sequencing had already been established for cells of eukaryotic organisms, including humans, other animals and fungi, but turned out to be much harder to implement for bacteria, for a simple reason. For the current method of single-cell RNA sequencing, a so-called poly-A tail at the end of the RNA molecule is the starting point for the isolation of RNA from individual cells. However, there is no such poly-A tail in bacteria. Therefore, the analysis of RNA within individual bacteria had previously been impossible.

In addition, a bacterial cell contains an extremely small amount of RNA, namely in the femtogram range (trillionth of a gram). This makes the isolation and handling of bacterial RNA even more difficult. We therefore used a poly-A-independent method known as MATQ-seq (multiple annealing and dC-tailing-based quantitative single-cell RNA-seq). Here, the bacterial RNA is obtained in a different way and propagated in a controlled manner, so that the amount of RNA is sufficient for subsequent analysis.

We then exposed the well-known bacterial pathogen *Salmonella* to different stress conditions. One sample was exposed to a salt shock, the other put under oxygen-free conditions. Using our new single-cell RNA sequencing protocol we created the respective RNA profiles and compared them with those of *Salmonella* cultures from an established database – and this approach worked: the RNA profiles from our tests matched those in the database. The fact that single-cell RNA sequencing now also works for bacteria opens up completely new possibilities in infection biology research. In addition, we could also show that the method worked with pseudomonads, i.e., bacteria that are infamous for colonising the lung. With bacterial single-cell RNA sequencing, we have significantly increased the resolution of gene expression analysis in single bacteria, which is a giant leap forward towards a better understanding of these pathogens and the development of antibiotic resistance. This brings us closer to our ultimate goal: to identify starting points for effective RNA-based antibacterial drugs.

Imdahl F, Vafadarnejad E, Homberger C, Saliba A-E, Vogel J (2020)
Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria
Nature Microbiology doi.org/10.1038/s41564-020-0774-1

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THERANOSTIC CELLS FOR DETECTION AND COUNTERACTION OF INFECTIONS BY REWIRING CELLULAR SIGNALING CASCADES

DAGMAR WIRTH | HEAD OF THE RESEARCH GROUP MODEL SYSTEMS FOR INFECTION AND IMMUNITY

Despite effective mechanisms that allow cells to protect themselves from infection, many pathogens successfully infect their host by suppressing the protective response. Using synthetic biology tools we constructed theranostic cells that sense low concentrations of interferon which is induced by nearly all invaders. These cells react as visualisers of infection. Moreover, implementation of a synthetic amplifier module turns these cells into long-term producers of factors that mediate pathogen defense.

Mammalian cells have developed potent mechanisms to react to pathogens. The release of type I interferons (IFN) represents a crucial first line of defense that is induced immediately after viral and bacterial infections. Upon binding to the cognate receptor, IFNs activate an intracellular signaling cascade that finally results in the activation of genes that contribute to the clearance of the pathogens. We exploited this mechanism to develop theranostic cells that can sense and visualise infections and produce counteracting antiviral factors *(Figure 1)*. To this end, we re-wired the cellular IFN signaling cascade to a synthetic regulatory module. Using Crispr/Cas9 assisted genome editing, we generated cells in which the synthetic transcription factor tTA is controlled by the IFN induced cellular Mx2 promoter. Since tTA specifically activates the synthetic P_{tet} promoter, IFN signaling results in expression of factors of choice. Such Converter cells authentically sense virus-induced IFN in a dose- and time-controlled manner reflecting the trigger pulse *(Figure 2, upper)*.

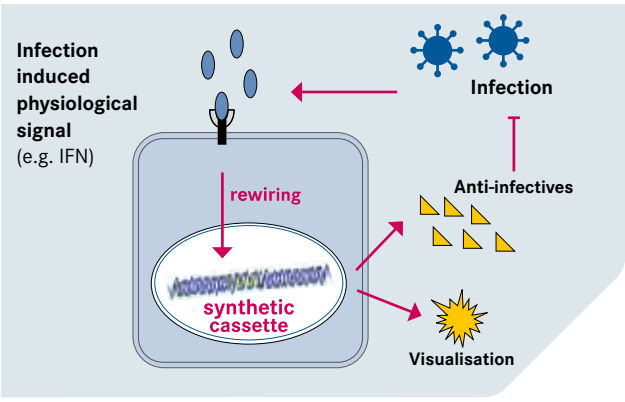

Figure 1: Principle of synthetically rewired signalling cascades (red) for sensing and counteracting infections.

To exploit this concept for the detection of viruses that have evolved mechanisms to block IFN responses, we equipped the cells with a positive feedback module to amplify expression of the synthetic cassettes. Such ConvAmplifier cells respond to a transient IFN pulse with sustained expression, resulting in a 'hit-and-run' expression type *(Figure 2, lower)*. Notably, ConvAmplifier cells – but not Converter cells – sensitively detect infection with Cytomegalovirus, a virus that efficiently undermines the cellular IFN response. This demonstrates that the positive feedback module is crucial for boosting weak IFN triggers *(Figure 3, left)*.

Finally, we asked if these cells can be further refined to produce antivirals and control viral infections. To this end, we modified the murine ConvAmplifier cells to release an antiviral factor (human IFN) that specifically protects human cells. In co-cultures of the murine ConvAmplifier cells with human cells, mouse IFN stimulation activated the synthetic cascade in a hit-and-run phenotype and protected the human cells from viral infection *(Figure 3, right)*. Thus, the study demonstrates that interfacing the cellular IFN signaling cas-

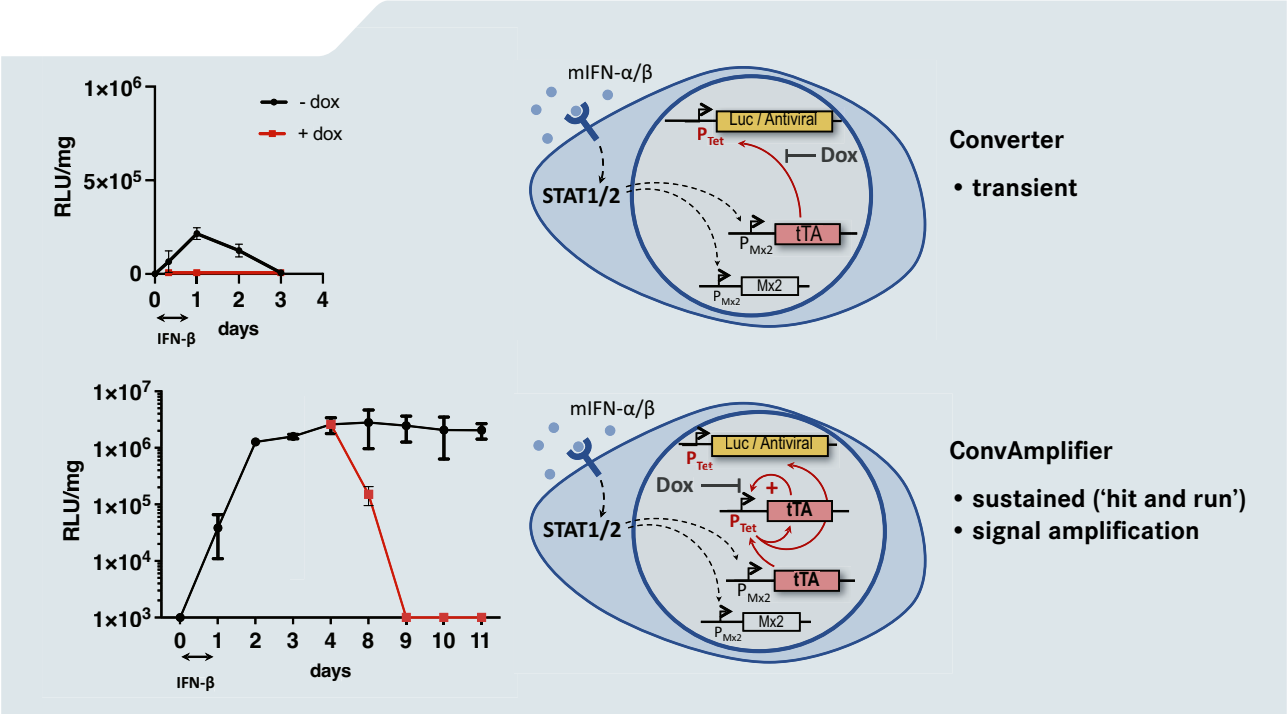

Figure 2: Outline of Converter and ConvAmplifier cells in which a pulse of IFN induces a synthetic cascade to express the reporter luciferase (luc) or a therapeutic gene (Antiviral). Converter cells express luciferase transiently, following the kinetics of the inducer mIFN-α/β. ConvAmplifier cells respond with an amplified and sustained ("hit-and-run") type of expression, as a consequence of the additionally implemented positive feedback module. Expression of the synthetic module can be stopped at any time by gift of doxycycline (dox). Adapted from Gödecke et al. (2020), Nucleic Acids Research 48(20):11799-11811. © CC BY 4.0

cade with a synthetic positive feedback module in theranostic cells provides an attractive strategy to visualise and counteract infections which is even functional when viruses efficiently counteract the IFN response.

The field of synthetic biology and the tools for predictable genome modification are increasingly paving the way for innovative diagnostic and therapeutic concepts. In this context, the application of specifically engineered 'theranostic' cells that on the one hand visualise disease states and on the other hand produce therapeutic agents represents an attractive option. We demonstrate that such cells allow sensitive visualisation of infection. Replacing human IFN *(Figure 3)* by antibacterial peptides extends the application for the defense of bacterial infections. The application of encapsulated theranostic cells may include specific sites and situations of high-risk infection like it is the case in implant medicine.

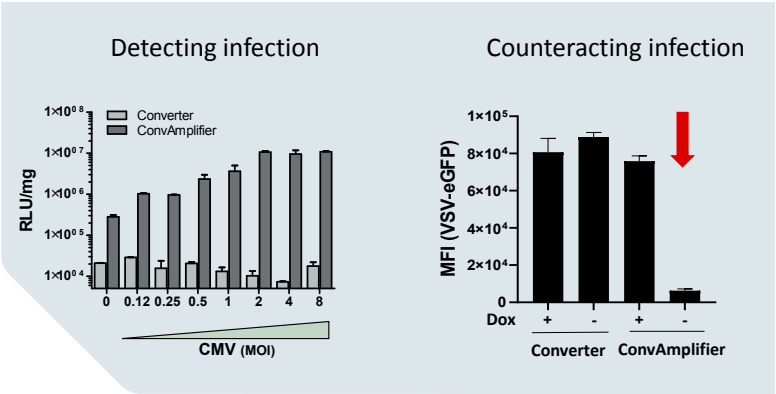

Figure 3: Left: ConvAmplifier cells enable detection of MCMV infection while infection of Converter cells is not sensed. Luciferase activities were determined 24h post-infection at different multiplicities of infection (MOI).

Right: The antiviral activity of the supernatant of murine ConvAmplifier cells inducing an antiviral factor (hIFN) in response to MCMV infection.

Adapted from Gödecke et al. (2020), Nucleic Acids Research 48(20):11799-11811. © CC BY 4.0

Gödecke N, Riedel J, Herrmann S, Behme S, Rand U, Kubsch T, Čičin-Šain L, Hauser H, Köster M, Wirth D (2020)
Synthetic rewiring and boosting type I interferon responses for visualization and counteracting viral infections
Nucleic Acids Res. doi: 10.1093/nar/gkaa961

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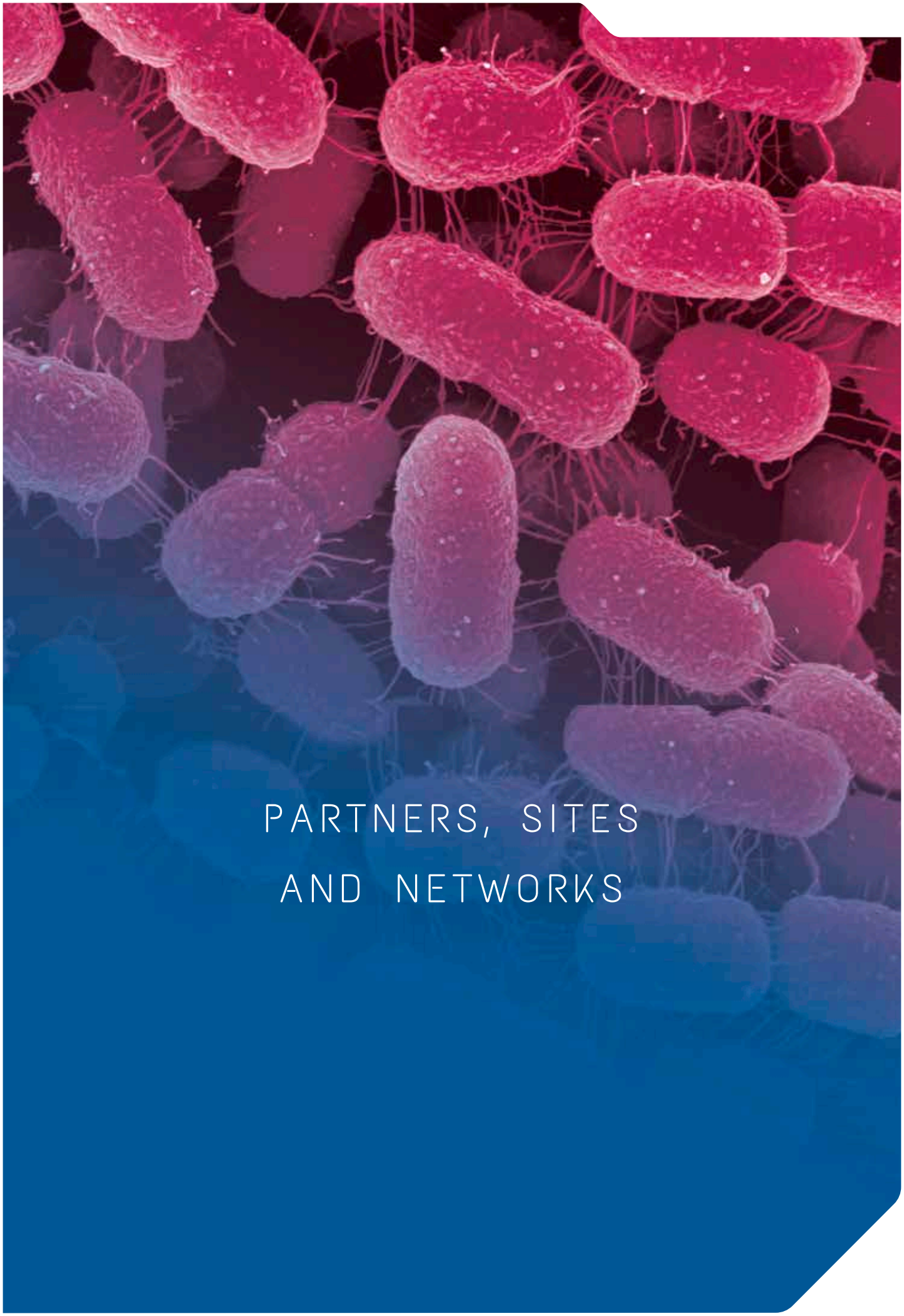

PARTNERS, SITES AND NETWORKS

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ROLF MÜLLER | MANAGING DIRECTOR OF HIPS

IN SEARCH OF NOVEL ANTI-INFECTIVE DRUGS

THE HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL RESEARCH SAARLAND (HIPS)

HIPS was founded in 2009 as a branch institute of HZI in close collaboration with Saarland University (UdS). Its scientists conduct pharmaceutical research with a focus on antimicrobial resistance (AMR) and aim to develop novel bioactive compounds to combat antibiotic-resistant bacteria. To bring these molecules into application, HIPS collaborates closely with international partners from the pharmaceutical industry, as well as academic partners in a highly interdisciplinary setting. In 2020, HIPS acquired 70 million Euro in funding from the federal and state governments for the implementation of new groups as well as a new building.

The acquired funds will allow HIPS to explore new and complementary fields of research, but also strengthen its existing expertise. Within the next five years, HIPS is expected to grow from 200 to more than 300 employees. A focus of the extension will be on drug bioinformatics and clinically oriented microbiota research. All present and future HIPS groups will be members of the joint *Research Centre for Bioactive Compounds*, which was founded together with UdS in 2021. In this centre, scientists and clinicians from HIPS and UdS collaborate closely with industry partners to bring findings from basic research into clinical application as efficiently as possible.

HIPS HELMHOLTZ Institute for Pharmaceutical Research Saarland

The department ***Microbial Natural Products***, led by Rolf Müller, focusses on the isolation and identification of novel natural compounds with antimicrobial properties (primarily from myxobacteria) and their preclinical development. The overall aim is to transfer the scientific findings into clinical practice by specifically optimising selected compounds to achieve proof-of-concept for therapeutic use in humans. The spin-off *Myxobiotics* was incubated within the programme *Helmholtz Enterprise*, aiming to bring the antibiotic class of cystobactamides into clinical application. Together with international partners from the Joint Programming Initiative AMR, a whitepaper on the sustainable discovery and development of novel antibiotics was published in Nature Reviews Chemistry.

The department ***Drug Design and Optimisation*** headed by Anna K. H. Hirsch focuses on the target-based development of novel anti-infectives. The portfolio includes proteins that impair vital mechanisms within pathogenic bacteria and

effectively kill them, as well as targets for so-called "pathoblockers" that interfere with pathogenicity and virulence without affecting bacterial viability. Various novel classes of compounds were identified and subjected to multiparameter optimisation to afford highly effective anti-infectives. To bridge the translational gap, the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) and the BMBF currently support hit-to-lead optimisation of inhibitors of a pathogenicity factor of the bacterium *Pseudomonas aeruginosa*. CARB-X is a global non-profit partnership dedicated to accelerating antibacterial research to tackle the global rising threat of drug-resistant bacteria.

The department ***Drug Delivery and Biological Barriers*** (head: Claus-Michael Lehr) aims at improving the transport of anti-infectives to their site of action. The department investigates both novel (nano-)carrier systems, and complex *in vitro* models, preferentially based on human cells. The developed nanocarrier-systems were successfully employed for co-delivery of antibiotics and pathoblockers to biofilms, as well as targeted delivery of compounds to hair follicles. Cell-free *in vitro* models of the Gram-negative bacterial cell envelope were developed to predict bacterial bioavailability and better understand structure-permeability relations.

The research group ***Chemical Biology of Carbohydrates*** (head: Alexander Titz) developed bivalent glycomimetics with unprecedented high affinity towards the *Pseudomonas aeruginosa* virulence factor LecA, and identified new starting point for non-carbohydrate lectin inhibitors. The frontrunner LecB inhibitor showed efficacy *in vivo* in a mouse model. First antibiotic conjugates have been developed for the targeted delivery to the site of infection.

The Klaus Faber endowed research group ***Drug Bioinformatics***, led by Olga Kalinina, focusses on the mathematical modelling of molecular interactions between proteins and small active compounds, as well as the emergence of antimicrobial resistance caused by genetic mutations using methods of artificial intelligence.

The junior research group ***Genome Mining for Secondary Metabolites***, headed by Chengzhang Fu, is developing innovative tools to utilise CRISPR-Cas to develop genome mining tools for the discovery of novel bioactive natural products.

In 2021, two new groups were installed at HIPS:
The research group ***Antiviral and Antivirulence Drugs*** (headed by Martin Empting) focuses on the target-based identification and medicinal chemistry-driven optimisation of antivirals with novel modes-of-action as well as pathogenicity modulating agents against priority bacterial pathogens.

The junior research group ***Microbiota-Associated Natural Products***, led by Julian Hegemann, focuses on the isolation and characterisation of specific natural products produced by members of the human microbiota. Of especial interest are antimicrobial compounds with host protective features.

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JÖRG VOGEL | MANAGING DIRECTOR OF HIRI

LEARNING THE LANGUAGE OF RNA TO COMBAT INFECTION

THE HELMHOLTZ INSTITUTE FOR RNA-BASED INFECTION RESEARCH (HIRI)

The Helmholtz Institute for RNA-based Infection Research (HIRI), established in May 2017 as a joint initiative between HZI and the Julius Maximilian University of Würzburg (JMU), is the first research institution in the world to focus on the role of ribonucleic acids (RNA) in infection processes. The SARS-CoV-2 pandemic highlighted the vast potential of RNA as vaccine, diagnostic, and therapeutic target for new strategies to combat infectious diseases. With the outbreak of the pandemic in 2020, HIRI scientists immediately contributed their expertise and state-of-the-art technologies to analyse the infection process and the ensuing disease.

Inasmuch as the years 2020 and 2021 were marked by a global pandemic caused by the SARS-CoV-2 virus, it also put the spotlight on a molecule the public had not been very familiar with: RNA. It further showed that RNA-based infection research could hardly be more timely. While work and life in general were deeply affected by the pandemic, HIRI staff stood their ground in order to move the science along and keep the institute running. Thus far, the HIRI has made major contributions to a better understanding of how the virus interacts with the host cell and how COVID-19 can develop into a life-threatening disease.

Recruitment

By the end of 2021, the institute had grown to over 100 staff from about 20 countries. Expanding its local network of collaborative and interdisciplinary research, the HIRI affliliated JMU researcher Dominic Grün (Chair of of Computational Biology of Spatial Biomedical Systems).

Publications, funding and awards

Over the reporting period, research by HIRI scientists and affiliates resulted in more than 120 primary research papers, many in high-profile journals including Science and Cell. Jörg Vogel took over as President of the European Academy of

HIRI group leader Neva Caliskan (left) received the 2021 ZONTA award, which recognises outstanding female researchers. © HZI | HIRI

HIRI HELMHOLTZ Institute for RNA-based Infection Research

Microbiology (EAM) in January 2021. Both in 2020 and in 2021, he was recognised as "highly cited researcher", honoring him as one of the most impactful scientists in the field of microbiology (top 1%). Emmanuel Saliba was selected as EMBO Young Investigator in 2020 and Neva Caliskan received the ZONTA Science Award in 2021.

ERC starting grants (1.5 Million €) were awarded to Neva Caliskan in 2020 and Mathias Munschauer in 2021. Further funding success included an award by the German Federal Agency for Disruptive Innovation (SPRIND) to develop new antivirals (in cooperation with researchers from HIPS and LMU) as well as funding by the Free State of Bavaria for a collaborative research project on SARS-CoV-2 (FOR-COVID). Chase Beisel received funding for the commercialisation of the LEOPARD technology platform through the GO-Bio Initial programme of the BMBF and through a Medical Valley Award of the Free State of Bavaria. LEOPARD ("Leveraging Engineered tracrRNAs and On-target DNAs for PArallel RNA Detection") is a new CRISPR-based technology, which is able to detect the presence of a specific RNA in a patient sample at single-nucleotide resolution. This opens opportunities for the multiplex detection of RNAs from viruses and other pathogens in a patient sample. Future applications in medical diagnostics may target infectious diseases, but also cancer and rare genetic diseases.

Training, teaching and networking

Committed to training students and young scientists, HIRI group leaders organised a lecture series on single-cell analysis, continued the short courses on "Infection and Immunity" and "RNA Biology" and offered workshops on transferrable skills such as science communication. As the pandemic put the RNA Seminar lecture series on hold, HIRI participated in the RNA Collaborative seminar series, a joint project initiated by the RNA Society.

The first HIRI PhD student graduated in 2021: Ehsan Vafardanejad.
© HZI | HIRI

The new HIRI building as envisaged by the winners of the architectural competition. © doranth post architekten

The graduate programme "RNA & Infection" has seen strong international demand and has since grown to 9 PhD candidates over the reporting period. HIRI also launched the Research Career Development Fellowships to support exceptional postdoctoral candidates in their progression to independent researchers.

In 2021, Jörg Vogel secured 3.1 Million Euros for the new international PhD programme *RNAmed*. Funding is provided through the Bavarian Elite Network initiative. This success is a result of a highly competitive selection process. *RNAmed* focuses on training future leaders in RNA-based medicine and goes beyond lab work. Cooperating partners are the universities of Regensburg as well as TUM and LMU in Munich.

New building

Further progress was made towards the new HIRI building. In 2020, the new building was included in Bavaria's high-tech agenda, considerably increasing funds as a result. Demolition work on the designated building site began in 2021. While the groundbreaking ceremony will take place in 2023, completion is not expected until 2026. Yet, HIRI staff are already looking forward to moving in.

Photos: © Stefan Sauer

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HIOH HELMHOLTZ Institute for One Health

FABIAN LEENDERTZ | FOUNDING DIRECTOR OF THE HELMHOLTZ INSTITUTE FOR ONE HEALTH (HIOH)

PANDEMIC PREPAREDNESS AT THE INTERFACE OF HUMANS, ANIMALS AND THEIR ENVIRONMENT

THE HELMHOLTZ INSTITUTE FOR ONE HEALTH (HIOH)

In November 2021, the foundation of the Helmholtz Institute for One Health in Greifswald was completed after a positive review of the research concept by an international, external review panel and the following HZI supervisory board resolution. Its research concept institutionalises the integrative, transdisciplinary "One Health" concept and thus focuses on a holistic approach that considers human and animal health within their environment. The institute is a new location of HZI. The other founding partners of HIOH are the University of Greifswald (UG), the University Medicine Greifswald (UMG) and the Friedrich-Loeffler-Institut (FLI, Federal Research Institute for Animal Health).

HIOH focuses on investigating the intersections of human, animal, and environmental health by establishing comprehensive longitudinal sample and data collection and analysis related to emerging infections and resistance in two model regions:
(1) the African tropics, as "hotspots" for the emergence of new zoonoses; and
(2) Mecklenburg-Western Pomerania with a low population density and a strong agricultural character.

These activities will be significantly strengthened by embedding the HIOH in the existing network of internationally recognised founding institutions. Through these, a broad spectrum of key disciplines is contributed, in particular human and veterinary medicine, microbiology, virology, epidemiology, drug discovery, biodiversity research, anthropology, sociology, evolutionary biology as well as ecology.

The HIOH will work in three research departments:
(1) Ecology and Emergence of Zoonoses,
(2) Epidemiology and Ecology of Antimicrobial Resistance,
(3) Pathogen Evolution.

Two competence units for One Health Surveillance (OHS) and Data Management and Analysis (DMA) and anticipated three junior research groups will complement these departments and provide additional space for innovative and translational approaches. The synergy between HIOH and its

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The founding partners of HIOH at the founding ceremony in April 2022: HIOH founding director Fabian Leendertz (front row, second from right), Katharina Riedel, rector of the University of Greifswald (back row, second from right), Thomas Mettenleiter, president of the Friedrich-Loeffler-Institut (FLI, Federal Research Institute for Animal Health; back row, left), Karlhans Endlich, dean of University Medicine Greifswald (back row, second from left) and Dirk Heinz, Scientific Director of HZI (back row, right) with Bettina Stark-Watzinger, Minister of Education and Research (front row, second from left), Bettina Martin, Minister for Science, Culture, Federal and European Affairs of the State of Mecklenburg-Vorpommern (front row, left) and Otmar Wiestler, president of the Helmholtz Association (front row, right).

founding partners will foster strongly integrative research and its translation into prevention and therapy of infectious diseases.

Fabian Leendertz, who was appointed founding director of HIOH in August 2021, holds a professorship at the University of Greifswald and heads the department “Ecology and Emergence of Zoonoses”. He brings several prestigious projects to HIOH, such as DFG funded cooperation projects focusing on great apes in tropical Africa or ebolaviruses in bats in Tanzania. Another project funded by the ARCUS foundation focuses on SARS-CoV-2 surveillance and expansion of testing capacity at the human and great ape interface in national parks in Côte d’Ivoire, the Central African Republic and Democratic Republic of the Congo.

Currently the new institute is hosted by the founding partners UG and UMG, providing office space and BSL2 laboratories, which are being fully equipped so that HIOH lab work can start in the first quarter of 2022. In cooperation with the UG and FLI, BSL3 as well as BSL4 laboratories are accessible. Also, all HIOH funding partners have jointly initiated 12 seed funding projects. An independent new building is currently being planned. When fully operational, HIOH is expected to host about 120 employees and students.

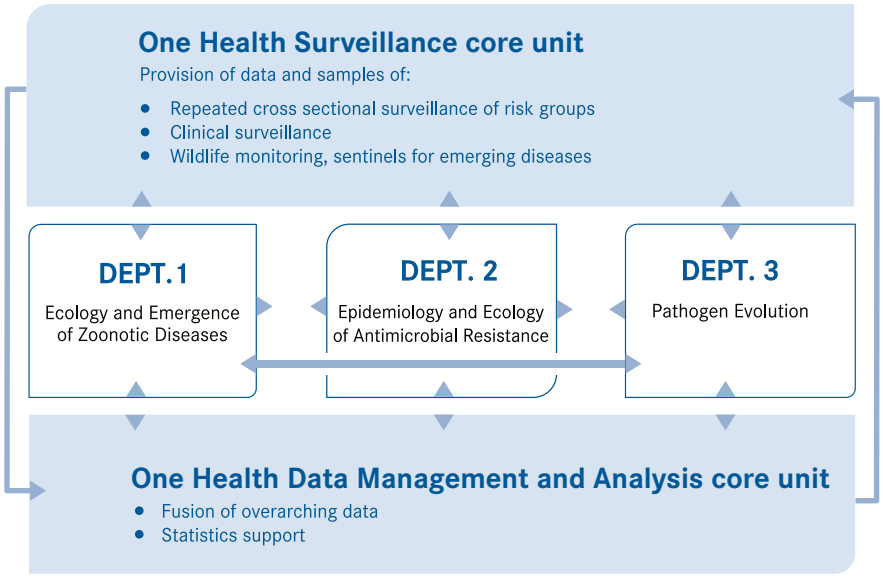

Figure 1: Three departments, supported by specialised core units: The envisaged structure of HIOH.

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ULRICH KALINKE | EXECUTIVE DIRECTOR OF TWINCORE

TRANSLATIONAL INFECTION RESEARCH DURING THE SARS-COV-2 PANDEMIC

THE TWINCORE CENTRE FOR EXPERIMENTAL AND CLINICAL INFECTION RESEARCH

At TWINCORE, multidisciplinary teams including clinician scientists, basic researchers and data scientists strive to channel new knowledge into clinical practice. In close collaboration with TWINCORE's founding institutions, HZI and Hannover Medical School (MHH), together with national and international partners, bacteria, viruses and the reactions of the immune system against these pathogens are being studied.

In response to the SARS-CoV-2 pandemic, TWINCORE staff have rapidly developed a wide range of research activities. Altogether, 19 projects in the fields of virology, immunology and bacteriology were launched together with local partners from HZI and MHH as well as with external partners. Two of these projects proved particularly productive: 1) screening of already approved and new compounds for SARS-CoV-2 inhibitory activity ("repurposing"); and 2) characterisation of memory B cells from convalescent COVID-19 patients and vaccinated individuals to decipher their antibody variable regions, and *in vitro* expression of the respective SARS-CoV-2 neutralising monoclonal antibodies.

In September 2020, the 12th TWINCORE symposium was held, entitled "SARS-CoV-2 News from Lower Saxony". It opened with a speech by the Minister for Science and Culture from Lower Saxony, Björn Thümler, and comprised talks given by scientists from Braunschweig, Göttingen and Hanover. In September 2021, the 13th TWINCORE Symposium took place, entitled "COVID-19 vaccination and vaccine responses". The keynote lecture was given by one of the cofounders of BioNTech, Özlem Türeci.

Several scientists working at TWINCORE received attractive job offers at other institutions, e.g., Gisa Gerold accepted the call to a W3 professorship in biochemistry at the University of Veterinary Medicine Hannover (TiHo).

TWINCORE has developed a strategy to fill vacant leadership positions with promising young candidates in a way that clinical collaborations are further reinforced. Patrick Behrendt and Theresa Graalmann were appointed as clinician scientist junior research group leaders at the Institute for Experimental Virology and the Institute for Experimental Infection Research, respectively.

Research activities involving big data were significantly enhanced at TWINOCRE, as indicated by the coordination of three "Big data in life sciences" projects by scientists there.

Two RESIST professors in data sciences, Chris Lauber and Marco Galardini, have taken up their positions at TWINCORE. IT infrastructure was further improved by: 1) establishing direct access to the German Research Network (DFN); 2) obtaining access rights to use the North German High Performance Computing Network (HLRN); and 3) setting up a dedicated High Performance Computing (HPC) system.

TWINCORE organised several international training courses. The DAAD-funded "10th Lower Saxony International Summer Academy (LISA)" took place digitally. An international autumn school in the field of systems medicine ("Systems Medicine for the Development of Novel Theranostic Approaches for Oncological and Immunological Diseases (SyMDROID)") was funded by the BMBF. The module "Translational Medicine" of the international Master's programme "Infectious Diseases - One Health (IDOH)" was funded by the EU's Erasmus+ programme.

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TRAIN
Translationsallianz in Niedersachsen

TRANSLATION ALLIANCE IN LOWER SAXONY (TRAIN):

TWINCORE contains the office of the Translational Alliance in Lower Saxony, which gathers together the translational activities of the region Hannover-Braunschweig. TRAIN - a burgeoning consortium - offers qualifications by the TRAIN Academy and provides technological platforms for translational research. Ulrich Kalinke is branch manager of TRAIN and spokesperson for the TRAIN Academy.

The activities of TRAIN include the coordination of new translational infrastructures, joint implementation platform technologies (TRAIN Facilitation), establishment of joint training formats (TRAIN Academy), and communication of projects between TRAIN partners on translational research (TRAIN Projects).

The University Medical Center Göttingen (UMG) is about to join the TRAIN network and a representative of UMG has been chosen to participate in the coordination of TRAIN Facilitation activities. The recently established COVID-19 Research Network Lower Saxony (COFONI) involves all three TRAIN locations and profits from the research infrastructures established at the different partner sites.

On October 14th, 2021, the kick-off event of the sixth class of the two-year career-oriented education programme "Translational Research and Medicine: From Idea to Product" took place. The teaching events are mostly held in hybrid form to facilitate attendance of participants from the different partner locations. The PhD programme "Biomedical Data Science (BIOMEDAS)" was realised by a TRAIN initiative in 2020. The second BIOMEDAS class started in October 2021.

In the area of TRAIN Projects, a communication project highlights 114 projects on SARS-CoV-2 research in Braunschweig / Hannover / Göttingen.

On April 28th, 2021, TRAIN organised a Parliamentary Evening on the topic of "Infection Research in Lower Saxony in Times of the Corona Pandemic." The event included keynote speeches from the scientific directors of HZI, MHH and TiHo as well as a panel discussion. Both the Vice President of the State Parliament and the Minister of Science and Culture delivered welcoming addresses. Gérard Krause, Head of Epidemiology at HZI, gave a short lecture on the topic "Priorities for Vaccination, Indicators and Digitalisation in Pandemic Response".

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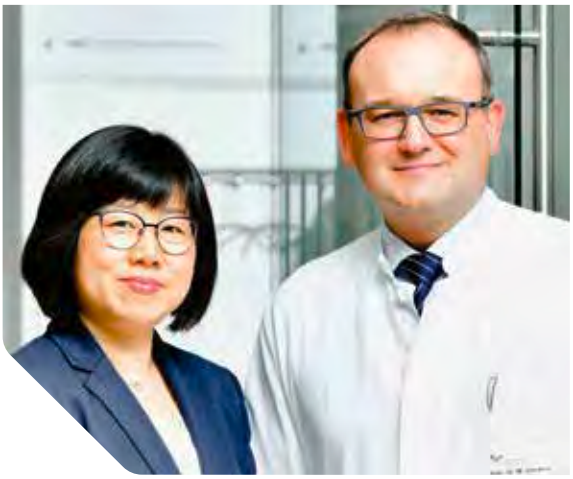

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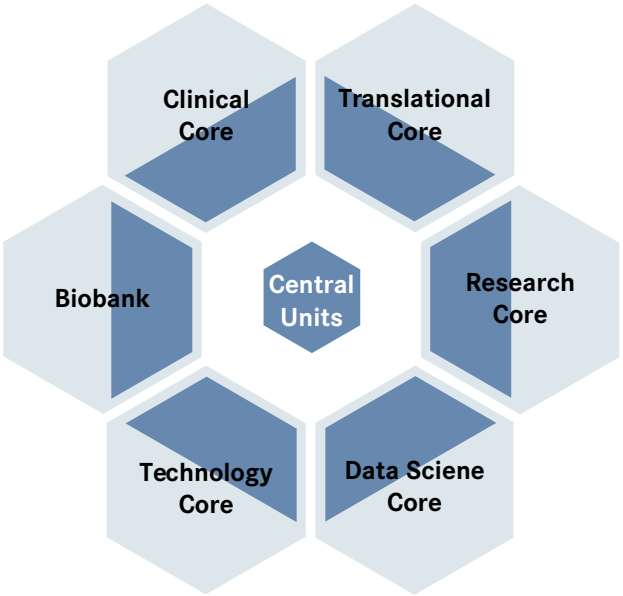
YANG LI AND MARKUS CORNBERG | DIRECTORS CiiM

TOWARDS PRECISION MEDICINE FOR INFECTION PATIENTS

CENTRE FOR INDIVIDUALISED INFECTION MEDICINE (CiiM)

Individual prognosis and diagnosis of infectious diseases and tailored prevention and therapy for the benefit of the individual patient is the declared vision of the Centre for Individualised Infection Medicine (CiiM), a joint initiative of HZI and MHH. As the first institute to apply the idea of precision medicine to infectious diseases, it will pioneer patient-centred and data-based approaches in this field.

The course and severity of infections as well as their optimal prevention and therapy are driven by variable characteristics of the causative pathogens, the affected patients and their microbiota. This holds the potential for individualised approaches in infectious diseases medicine, which has, however, hardly been utilised so far.



Conceptually, CiiM aims to exploit the existing potential and pursue an interdisciplinary approach to better adapt diagnosis, prevention and treatment to current clinical needs in infectious diseases and to the individual patient. This demands a tight linking of disciplines which is supported at CiiM in a targeted manner to set new standards for close collaboration. Clinical data from individual patients or groups of patients are brought together with modern, sophisticated (high-throughput) methods. This requires innovative methods of data analysis and knowledge discovery in the field of Big Data and Artificial Intelligence. The vision is that interdisciplinary teams of basic researchers, bioinformaticians, data scientists and clinicians will bring together complex data sets for each individual patient to generate insights that will facilitate individual patient management and evidence-based medicine. Together with experts in clinical trials, ethics as well as regulatory aspects, these findings are transferred back to the clinics and provide an important guide for

Figure 1: To establish the proposed cross-disciplinary workflow in addition to clinical expertise, a comprehensive overview of the current state of research and technical progress is decisive. Therefore, different expertise cores are planned to be installed at CiiM. © Debarry | CiiM

CiiM
CENTRE FOR INDIVIDUALISED INFECTION MEDICINE

the treating physician in the management of each individual patient *(Figure 2)*.

These conceptual ideas are directly incorporated into the planning of a dedicated CiiM building, in which modern working environments will be created that will promote interdisciplinarity and innovation. The new research building with approximately 2,100 m^2 of usable space is being built in the immediate vicinity of the MHH on the neighboring property of TWINCORE. Until the planned start of operations in 2024, the assigned groups are housed in TWINCORE.

CiiM is headed by the two directors Yang Li and Markus Cornberg, who ensure the needed close link between clinics, research and data science through complementary appointments. Supported by the CiiM coordinaton office (Jennifer Debarry), they develop the scientific programme of the centre in close exchange with assigned group leaders, the management of parental institutions and the Scientific Advisory Board. In this context, the coherent and complementary development of different expertise cores of excellence is particularly crucial and is already being driven forward *(Figure 1)*.

Yang Li and her HZI-department bring their expertise to the Data Science Core in a lead role and also position CiiM at an international level in systems immunology and multi-omics integration for individualised medicine. Within the ERC Starting grant project "ModVaccine", a focus is set on understanding the inter-individual variation of vaccine efficacy and developing adapted personalised vaccination strategies against infections. Further, the group is involved in various SARS-CoV-2 projects leading to pivotal results. Cheng-Jian Xu, an experienced systems geneticist and bioinformatician, and his MHH research group associated with CiiM add the focus on the contribution of environmental influences and resulting chemical modifications of DNA to pathophysiological conditions in infection. Active participation of CiiM in networks such as the PhD programme BIOMEDAS or the International Future Laboratories for Artificial Intelligence further improves the capabilities and integration of CiiM.

Markus Cornberg, an active MHH clinician in the field of infectious diseases, is an internationally renowned expert in viral hepatitis. He strengthens the Clinical and Translational Core and is key for new links into the clinics. In 2021, he coordinated the new clinical S3 guideline on the treatment of hepatitis B virus infections, which increasingly takes individualised aspects into account. These endeavours were preceded by additional measures such as the organisation of specific trainings for clinicians. The network of infectious disease physicians built through such activities will provide CiiM access to samples and patients. Detailed protocols for such patient and control cohorts were implemented and new cohorts were initiated in 2020/2021.

Author: Jennifer Debarry, Coordinator of CiiM

Figure 2: CiiM provides the essential interdisciplinary platform to advance innovative approaches for diagnosis, therapy and prevention of infectious diseases better adapted to the individual patient. © Debarry | CiiM based on AdobeStock | Marina Zlochin, Good Studio, elenabsi

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DIETER JAHN | DIRECTOR OF THE SYSTEMS BIOLOGY CENTRE BRICS

"BRICS 2.0": NEW RESEARCHERS, NEW SCIENTIFIC ROADMAP AND NEW PARTNERS

THE BRAUNSCHWEIG INTEGRATED CENTRE OF SYSTEMS BIOLOGY (BRICS)

The interdisciplinary Braunschweig Integrated Centre of Systems Biology BRICS is jointly operated by HZI, Technische Universität Braunschweig (TU BS) and the Leibniz Institute German Collection of Microorganisms and Cell Cultures DSMZ. BRICS is using state-of the-art high-throughput technologies in combination with bioinformatics -based simulations to make biomedical processes predictable. In this context, the research centre has extended its research focus on the standardisation of corresponding mass spectrometry-based measurements in cooperation with a new partner, the National Metrology Institute PTB (Physikalisch-Technische Bundesanstalt). Further, BRICS is strongly fostering the field of cellular metabolism research and data science with new research groups headed by Thekla Cordes und Tim Kacprowski.

Strategic guidance

In BRICS, biologists, engineers, chemists, physicists and computer scientists are currently working on research topics at the interfaces of different disciplines with the help of systems biology. Systems biology integrates the knowledge derived from high-throughput omics techniques, biochemistry, genetics and cell biology into mathematical models using bioinformatics. These models in turn allow predictions about biological processes. At the same time, a high-resolution microscopic and a spectroscopic characterisation of the underlying cellular processes of interest is carried out with self-developed high-end technology.

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In 2021, BRICS conducted a moderated bottom-up strategy workshop to define fields of action for the establishment of "BRICS 2.0". A scientific roadmap was developed, new communication strategies were implemented, novel formats for the internal information exchange are currently being tested, and a governance structure in concordance with TU Braunschweig´s core research area "Engineering for Health" was recommended.

The National Metrology Institute PTB as novel partner of BRICS

Thus, BRICS offers the best conditions for interdisciplinary, biomedical top research at the TU Braunschweig through the well-coordinated collaboration of natural sciences and engineering. In recent years, the corresponding bioinformatics and systems biology research have been successfully implemented. Standardisation is one of most challenging scientific problems of basic systems biology-based biomedical research oriented towards its translational application. Highly reproducible mass-spectrometry based methods of measurement could serve as one solution in this context. Thus, BRICS needed input at the level of metrology. In order to expand the focus on metrology, it has integrated the Braunschweig localised National Metrology Institute PTB (Physikalisch Technische Bundesanstalt) into BRICS. The PTB is one of the top addresses in the international world of metrology. PTB is dedicated to ensuring progress and reliability in metrology. This scientific expertise, investigating description, preservation and transmission of reference measurement methods mainly in the clinical-chemical research area, e.g., based on mass spectrometry, will complement the research focus of BRICS.

New Scientists at the BRICS

Since October 2020, computer science professor Tim Kacprowski has been working with his research group "Data Science in Biomedicine" at BRICS.

Tim Kacprowski, © Remus | TU Braunschweig

Kacprowski and his group do not only offer analysis tools for many different types of data generated by systems biologists, they also develop new computational methods that allow obtaining more robust and more interpretable results. This will eventually lead to mechanistic insights into complex biomedical problems, e.g., finding out why some patients respond to certain treatments, while others do not.

The new research unit has obtained funding for a BMBF project aimed at investigating the long-term consequences of infectious diseases, using "long-HepC" as an example. This cooperation was established jointly with Karsten Hiller from BRICS, Markus Cornberg (MHH/HZI) and Markus List (Technical University Munich, TUM).

Recently, a new junior professorship in the field of cellular metabolism was filled by Thekla Cordes, coming from the Salk Institute, La Jolla, USA. Furthermore, a PTB junior professorship is currently advertised and will also be located in the BRICS building.

Author: Dieter Jahn, Full Professor of Microbiology at TU Braunschweig and Speaker of the Braunschweig Integrated Centre of Systems Biology (BRICS) ■

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MICHAEL KOLBE | HEAD OF THE DEPARTMENT STRUCTURAL INFECTION BIOLOGY

POWERFUL LIGHT SOURCES FOR INFECTION RESEARCH

CSSB
Centre for Structural Systems Biology

THE CENTRE FOR STRUCTURAL SYSTEMS BIOLOGY (CSSB)

The Centre for Structural Systems Biology (CSSB) in Hamburg is a joint effort of ten partner institutions contributing in total 14 research groups located under one roof on the DESY-Campus in Hamburg Bahrenfeld. Research focusses mainly on the molecular mechanisms used by different pathogens infecting humans. CSSB provides researchers with cutting-edge light sources and state-of-the-art imaging technologies to investigate infection processes over different scales in time and resolution. HZI is represented at CSSB by the research group "Structural Infection Biology" (STIB), led by Michael Kolbe. STIB is working on the architecture and activity of virulence factors that facilitate the invasion of enteric pathogens, causing Shigellosis, Salmonellosis or systemic infections.

For its research, the STIB group has used the highly collaborative atmosphere at CSSB and integrated the powerful synchrotron radiation, X-ray laser and computing sources available at DESY with state-of-the-art cryo-electron microscopy (cryo-EM) techniques and different biophysical and microbiological methodologies established in the institute. Combination of these advanced imaging techniques and methods in cellular microbiology allowed detailed insights into the molecular mechanisms of the bacterial type III secretion system (T3SS), other bacterial virulence factors and human innate immunity receptors.

The T3SS is a nanosyringe-like supramolecular structure that delivers virulence factors into human cells to prepare them for invasion. Researchers in the STIB group solved the structure of this large complex at atomic resolution using cryo-EM on particles isolated from the human pathogen *Shigella flexneri (Fig.1).* This high-resolution study provided important molecular insights into the system suggesting how ion gradients contribute to protein secretion and might enable the development of new therapeutics to block T3SS dependent infections (Lunelli et al, *PLoS Pathogens*, 2020). In collaboration with members of CSSB and the Humboldt-University, the group identified additional interactions with proteins not resolved in their previous cryo-EM studies *(Fig. 2)*. In total, they found that the *Shigella* T3SS is made of more than 15 different proteins, some of them present in multiple copies comprising in total more than 200 subunits.

The group also worked on the function of bacterial flagellar systems. They are essential for motility enabling the directed movement of bacteria towards nutrients or an infection target site. In their recent publication in *Nature Communications* (Horstmann et al, *Nat. Comm.*, 2020), the

STIB group and collaborators have demonstrated that flagella also promote bacterial adhesion and host cell invasion. The flagellums' long, external filament is composed of several thousand copies of the same protein known as flagellin. Methylation of their lysine residues occurs through the enzyme FliB. While this modification of the flagellin's surface published in the 1950's was the first reported protein methylation, the reason behind this process has until this study remained elusive. Based on both *in vivo* and *in vitro* studies, scientists in the STIB group together with collaborators found that methylation of flagella facilitates adhesion of *Salmonella Typhimurium* to hydrophobic cell surfaces and thus plays an important role for the host invasion process.

In another collaboration with Sukrit Srivastava (Indian Foundation for Fundamental Research), HZI researchers outlined a novel strategy for developing vaccines against Coronaviruses. Their multi-epitope vaccine (MEV) and multi-patch vaccine (MPV) target multiple proteins or even the entire proteome of the coronavirus. This approach allows them to cope with future challenges due to the evolutionary adaptation of the virus.

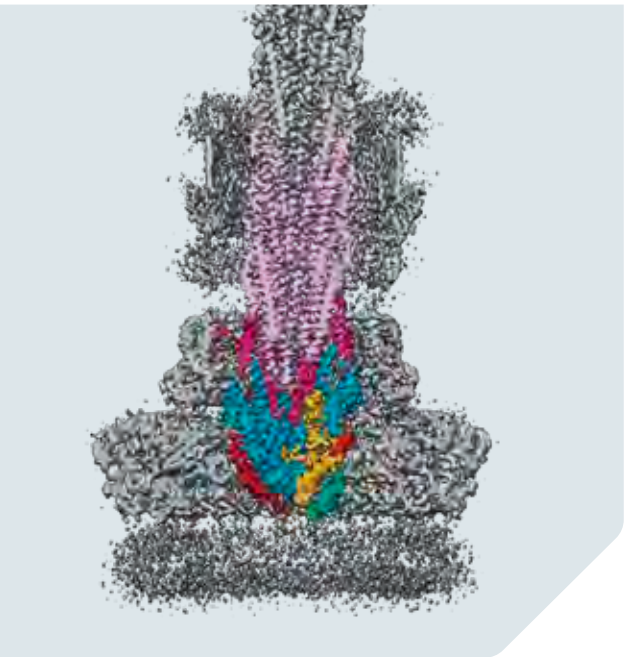

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Figure 1: Cross-section of the cryo-EM reconstruction of the needle complex. Cryo-electron microscopy map sliced along the vertical axis of the Shigella T3SS. The export apparatus core and the proximal end of the needle are colored according to the different constituting proteins (SpaP light blue; SpaQ red; SpaR dark yellow; SpaS turquoise; MxiI magenta; MxiH pink).

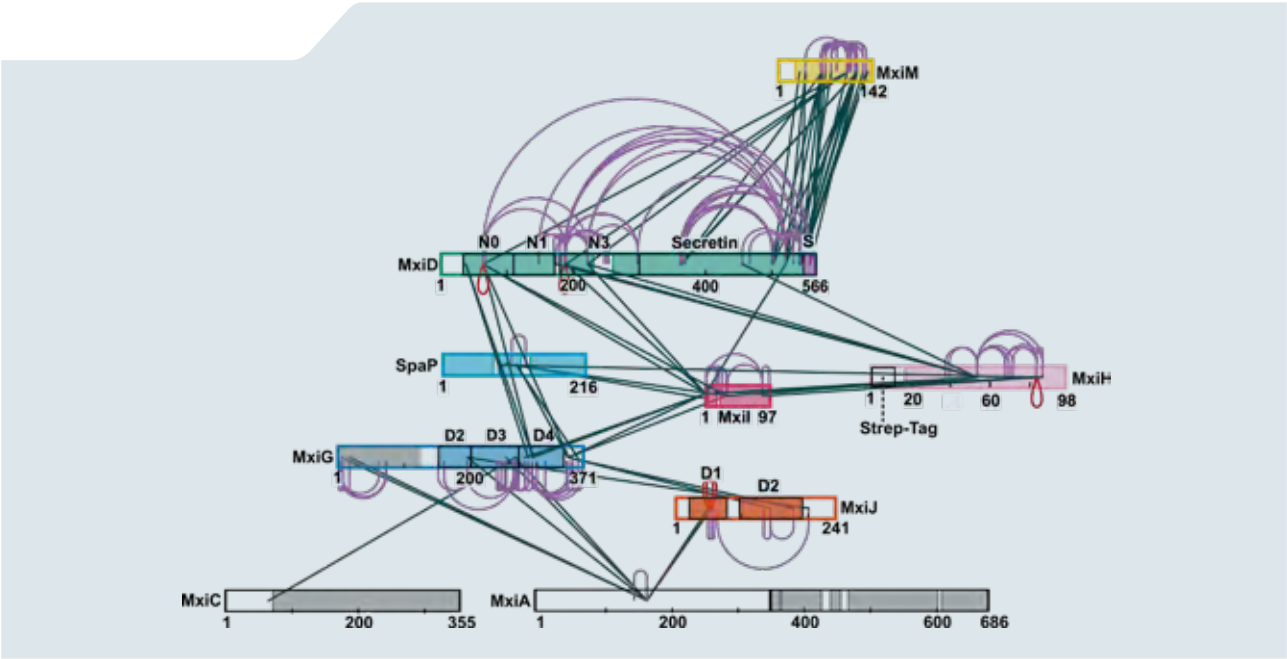

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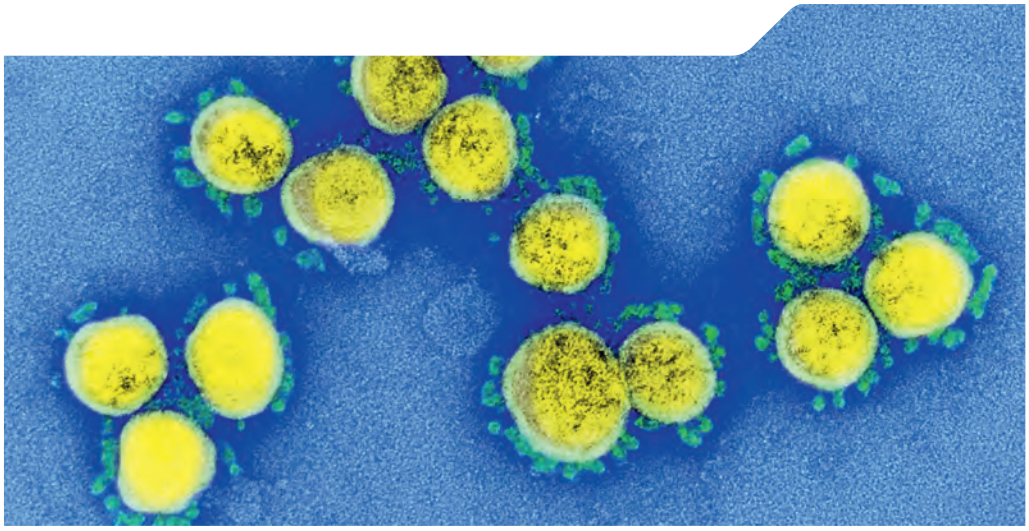
Figure 2: Cross-linking network of the type 3 secretion system proteins.
Different proteins are displayed as bars. Regions that are structurally solved are colored according to Fig.1.

Timo Jäger © DZIF



In 2020 numerous DZIF scientists started research projects on SARS-CoV-2 © NIAID

TIMO JÄGER | MANAGING DIRECTOR OF THE DZIF

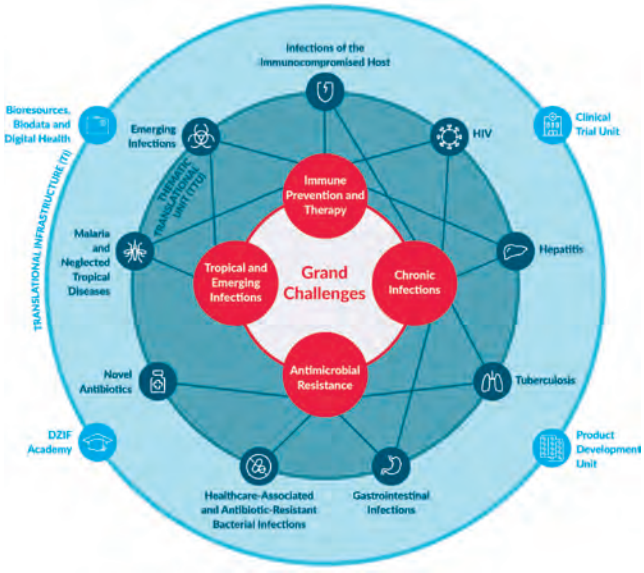
UNITED IN TACKLING MAJOR CHALLENGES

THE GERMAN CENTER FOR INFECTION RESEARCH (DZIF)

Infectious diseases pose major challenges for science, medicine and politics in Germany and throughout the world. The German Center for Infection Research (DZIF) tackles these challenges through translational research. It aims to rapidly advance basic research discoveries towards pre-clinical and clinical development. In all, 35 institutions have come together under the DZIF umbrella and over 500 doctors and scientists work closely together in order to curb infectious diseases. As one of 35 member institutions HZI plays an important role in different research areas, especially at the partner site Hannover-Braunschweig.

Firmly established themes within the DZIF include the development of new antibiotics, the fight against multidrug-resistant bacteria, research into infections that are prominent on a global scale such as HIV, malaria, tuberculosis and gastrointestinal diseases as well as newly emerging pathogens and how to combat them. Together, the research areas address the four Grand Challenges in Infection Research that have been defined as DZIF focal areas: (i) Tropical and Emerging Infections, (ii) Chronic Infections, (iii) Immune Prevention and Therapy and (iv) Antimicrobial Resistance (AMR). The membership of two regulatory agencies in DZIF, the Federal Institute for Drugs and Medical Devices BfArM and Paul-Ehrlich-Institut PEI, allows the full integration of both in scientific and regulatory advice needed for the development of novel anti-infective drugs and vaccines.

In order to ensure that the DZIF is well positioned and sets the right priorities, it underwent an evaluation by its Scientific Advisory Board and independent, external experts in 2020. All research areas and their plans for the coming



© DZIF research areas and infrastructures addressing the Grand Challenges in infection research.

SITES

Hannover – Braunschweig
Hannover Medical School
University of Veterinary Medicine Hannover, Foundation
TWINCORE–Centre for Experimental and Clinical Infection Research
Helmholtz Centre for Infection Research
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures
Robert Koch Institute

Hamburg – Lübeck – Borstel – Riems
Bernhard Nocht Institute for Tropical Medicine
University Medical Center Hamburg-Eppendorf
Universität Hamburg
Universität zu Lübeck
Leibniz Institute for Experimental Virology (HPI)
Research Center Borstel–Leibniz-Center for Medicine and Bioscience
Friedrich-Loeffler-Institut

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Bundeswehr Institute of Microbiology

Heidelberg
Heidelberg University
German Cancer Research Center

Gießen – Marburg – Langen
Giessen University
Philipps-Universität Marburg
Paul-Ehrlich-Institut Langen

ASSOCIATED PARTNERS

DZIF is an affiliation of 35 research institutes, located at seven sites distributed throughout Germany. Twelve additional sites are associated partners: University Hospital, Freiburg | Charité – Universitätsmedizin Berlin | German Liver Foundation / HepNet Study House, Hannover | Goethe-Universität, Frankfurt am Main | Hans Knöll Institut, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Jena | Julius-Maximilians-University, Würzburg | Leibniz University Hannover | Ruhr-Universität Bochum | University Bayreuth | University Medicine Greifswald | Düsseldorf University Hospital | University Münster

years were examined in detail. The result of the evaluation is encouraging: the successes so far were rated as “impressive”, the plans for 2021 to 2024 as “outstanding” and “extraordinary”.

An example for a successful translational project is Hepcludex (formerly known as Myrcludex B) jointly developed by researchers from Heidelberg University Hospital (UKHD) and the Medical Faculty of Heidelberg, the DZIF and other partners. Hepcludex is a first-in-class entry inhibitor for the treatment of hepatitis D and prevents hepatitis D and B viruses (HDV/HBV) from entering liver cells. On 28 May 2020, EMA recommended Hepcludex for approval and the European Commission has approved it for prescription in Europe.

Further prime examples for innovative DZIF projects and global partnerships are a project for a new antibiotic against tuberculosis, the Incubator for Antibacterial Therapies in Europe (INCATE) and the “Combating Antibiotic-Resistant Bacteria Biopharmaceutical (CARB-X) Accelerator”.

Within the framework of “Academia and industry united innovation and treatment for tuberculosis”, or UNITE4TB for short, the DZIF, together with the University Hospital Munich (KUM), has been an associated partner on the industry side of the Innovative Medicines Initiative (IMI), a major programme of the European Union and the European Federation of Pharmaceutical Industries and Associations (EFPIA), since the beginning of 2020. The aim of the programme is to accelerate the testing of new combinations of active substances against tuberculosis. BTZ-043, an active substance against tuberculosis funded by the DZIF, is also to be tested within this framework. BTZ-043 was discovered by researchers at the Leibniz Institute for Natural Product

Research and Infection Biology – Hans Knöll Institute (HKI) – and has been further developed in partnership by the HKI and the LMU University Hospital in a research collaboration within the DZIF and the research consortium "InfectControl" since 2014. The new drug has already successfully passed the early clinical trials.

With INCATE (Incubator for Antibacterial Therapies in Europe), in 2021 a consortium was launched to boost the development of antibiotics. Partners from the academic, industrial and public sectors are working together to accomplish this task. One of the founding members is the DZIF.

In addition, DZIF, BfArM, and PEI are cooperating within the "CARB-X Accelerator". DZIF actively advises and accompanies German academic groups on their way to successful CARB-X funding and takes on an advisory function for international companies or working groups in the already existing CARB-X portfolio. In 2020, two German research projects received CARB-X funding for the development of new drugs against antibiotic-resistant pathogens.

In 2020 and 2021, numerous scientists and physicians at DZIF flexibly adapted to the need for research on COVID-19. Shortly after the SARS-CoV-2 virus was identified, DZIF scientists were able to develop and publish a diagnostic test that is still considered gold standard today. DZIF scientists started to develop neutralising antibodies against SARS-CoV-2 as well as a vaccine using a modified vaccinia virus Ankara (MVA) vector. For a number of years, in a collaboration between the DZIF and the IDT Biologika GmbH, a MERS corona virus vaccine has been developed on basis of the MVA. The international vaccine initiative CEPI (Coalition for Epidemic Preparedness Innovations) supports the development of the vaccine against the MERS coronavirus with up to 36 million $. In 2021, the clinical phase Ib study started at the University Hospital Hamburg-Eppendorf.

Furthermore, the ongoing COVID-19 pandemic has shown an unprecedented need to establish infrastructures and product-directed projects to expedite the translational process towards compounds that could be useful to combat future pandemics. This consideration has led to the concept for a National Alliance for Pandemic Therapeutics (NAPATH), jointly proposed by DZIF and HZI as a strategic alliance of science, industry, regulatory authorities and politics *(see also chapter "2020 and 2021: Years of the Pandemic" in this report)*.

DZIF groups its research activities into research fields and translational infrastructures:

Translational research fields

- Emerging Infections
- Tuberculosis
- Malaria
- HIV
- Hepatitis ** (co-coordinated by HZI)*
- Gastrointestinal Infections
- Infections of the immunocompromised Host
- Healthcare-Associated and Antibiotic-Resistant Bacterial Infections
- Novel Antibiotics ** (coordinated by HZI)*

Translational infrastructures

- Product Development Unit ** (HZI involved)*
- Clinical Trial Unit
- African Partner Institutions
- Biobanking
- Bioinformatics
- DZIF Academy
- Pathogen Repository
- Epidemiology
- Novel Antivirals

From January 2021, the African Partner Institutions were integrated into the Malaria and Neglected Tropical Diseases research area and the Novel Antivirals were transferred to the research area Infections of the Immunocompromised Host. The Biobanking, Bioinformatics, Epidemiology and the Pathogen Repository were brought together into the new infrastructure Bioresources, Biodata and Digital Health ** (coordinated by HZI)*

Development of vaccines. © Hartmut Bösener

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STEFANIE CASTELL AND YVONNE KEMMLING | DEPARTMENT EPIDEMIOLOGY AT HZI

RESEARCH FOR BETTER PUBLIC HEALTH

THE GERMAN NATIONAL COHORT AND ITS INTEGRATED INFECTION RESEARCH PROJECTS AT HZI: AN UPDATE

As the largest epidemiological health study in Germany, the German National Cohort (NAKO Gesundheitsstudie) is well suited to determine risk factors for and pathways to diseases such as cancer, diabetes, and infectious diseases. HZI conducts additional integrated studies focusing on public health relevant infectious diseases. These studies benefit from the NAKO infrastructure and investigate risk factors and – in the long term - the impact of infectious diseases on non-communicable diseases. In the last two years, a special focus has been on investigating the seroprevalence of SARS-CoV-2 within the framework of the MuSPAD study.

The German National Cohort (NAKO, www.nako.de) is a multidisciplinary, population-based cohort study that aims to investigate the causes of widespread diseases, identify risk factors and improve early detection and prevention of disease.

The study is supported by grants from the German federal government, German state governments, and the Helmholtz Association during two funding phases that cover the years 2014 to 2018 and 2018 to 2023. Between 2014 and 2019, 205,217 persons aged 20–69 were recruited and examined in 18 study centres across Germany. Participants are followed up for incident diseases via e.g., questionnaires on a regular basis. In addition, currently, all study participants are invited for re-examination at the study centres, enabling the analysis of gradual or continuous changes over time, e.g., regarding vascular, cardiac, metabolic, neurocognitive, pulmonary, and sensory function, but also in terms of changes in exposures. This first re-examination of study participants started in October 2018 and is expected to be completed by the end of 2023. It is expected that about 135,000 study participants will take part in the first re-examination after accounting for deceased participants and dropouts. As of December 2021, NAKO has re-examined 66,870 participants.

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The Hannover study centre

The facilities of the HZI NAKO study centre in Hannover are located in the Clinical Research Center (CRC) and situated near the Hannover Medical School (MHH) campus allowing a close working relationship with clinicians. Healthcare professionals such as physicians, nurses, receptionists, and laboratory technicians work at the study centre - all trained and certified according to NAKO standards.

It is planned to examine 6,750 participants in the second phase of NAKO. The re-examinations started in 2018. By the end of December 2021, 3,627 participants had successfully completed the re-examination programme at the Hannover site.

ZIFCO – Integrated DZIF Infection Cohort within the German National Cohort

This add-on project of HZI investigates transient infections in detail using the specifically developed smartphone- and web application "Prospective Monitoring and Management App (PIA)" (www.info-pia.de) that allows NAKO participants to report on e.g., symptoms of respiratory infections like common cold or flu as well as of gastrointestinal and urogenital infections. For respiratory infections, laboratory virological analyses of self-sampled nasal swabs are included. Digital infection symptom monitoring via the eResearch system PIA can help to avoid recall bias and assure sample collection at an early stage of disease, facilitating detection of viruses. By the end of 2021, about 1000 NAKO participants had been recruited for ZIFCO at the Hannover study centre, and over 300 nasal swabs have been analysed by the Institute of Virology of the Hannover Medical School (MHH), including testing for SARS-CoV-2.

The MuSPAD Study in NAKO

Scientific data on the prevalence of SARS-CoV-2 is important to guide measures in order to fight the pandemic. Many political decision making processes rely on modelling approaches and modelling is dependent on assumptions that can be informed by serological data.

From July 2020 to August 2021, HZI conducted a nationwide seroprevalence study (MuSPAD) to investigate the SARS-CoV-2 antibody status of more than 39,000 people in eight districts and cities *(Gornyk et al. , Dtsch Arztebl Int 2021)*. As add-on study "MuSPAD in NAKO", we examined about 3,000 participants from Hannover in June and July 2021. Here, the SARS-CoV-2 seroprevalence, weighted by age and gender, was about 20% in August 2021 in those unvaccinated. The ratio of underdetection was between 3 and 4, meaning that about every fourth infection was discovered *(for further information, see chapter "Research Focus EPI" in this report)*.

Borreliosis Serology

During the NAKO baseline-examinations in Hannover from 2014 to 2018, study nurses collected blood samples, making later serological investigations possible. From the study centre Hannover, 8,009 samples underwent analysis to detect Borreliosis antibodies. The presence of both IgG and IgM antibodies was screened using Enzyme-linked Immunosorbent Assays (ELISA) and confirmed with line immunoblots. Current seroprevalence estimates play a critical role in monitoring the spread of Borreliosis, which may be accelerated by climate change. Furthermore, the extensive survey of the participant characteristics allows for the determination of vulnerable groups and risk factors for seropositivity – a key for re-focusing public health interventions.

Authors: Stefanie Castell / Yvonne Kemmling
Co-Authors: Gérard Krause, Jana Heise, Max Hassenstein, Manuela Harries

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(Status: End of 2021)

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RG **ZEIM**
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N.N.

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RG **IREG**
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Other locations | branch offices

HIPS, Helmholtz Institute for Pharmaceutical Research Saarland
Managing Director: Prof. Dr. Rolf Müller | Administrative Management: Dr. Stephanie Thomas

HIRI, Helmholtz Institute for RNA-based Infection Research
Managing Director: Prof. Dr. Jörg Vogel | Administrative Management: Alice Hohn

BRICS, Braunschweig Integrated Centre of Systems Biology

CiiM, CiiM, Centre for Individualised Infection Medicine
Managing Directors: Prof. Dr. Yang Li, Prof. Dr. med. Markus Cornberg

CSSB, Centre for Structural Systems Biology

TC, TWINCORE, Centre for Experimental and Clinical Infection Research GmbH
Scientific Director: Prof. Dr. Ulrich Kalinke / Administrative Management: Dr. Albrecht Goez

HZI HELMHOLTZ Centre for Infection Research

KD
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Prof. Dr. med. M. Cornberg

BR
Staff Council
T. Twardoch, Chair

Helmholtz Centre
for Infection Research GmbH
Inhoffenstraße 7
38124 Braunschweig

Topic 3: Anti-Infectives
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Microbial Interactions and Processes
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Drug Delivery
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JRG **BION** HIPS
Biogenic Nanotherapeutics
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Drug Design and Optimization
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Dep. **MCH**
Medical Chemistry
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Microbial Natural Products
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RG **AMEG** HIPS
Actinobacteria Metabolic Engineering Group
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JRG **GEMS** HIPS
Genome Mining for Secondary Metabolites
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Microbial Drugs
Prof. Dr. M. Stadler

RG **MISG**
Microbial Strain Collection
Dr. J. Wink

Location currently being established

HIOH, Helmholtz Institute One Health
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Administrative Management: Carl Friedrich Keding

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Dep.: Department
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JRG: Junior Research Group

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Animal Welfare Officer
Dr. M. Pils

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H. Ohrdorf

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A. Anfang

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E. Gerndt (Deputy Director)

FMM - Funding Management DZIF
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JUR – Legal Affairs and Licences
Dr. Ch. Kügler-Walkemeyer

ORG – Organisation
R. Lomberg
(Anti-Corruption Commissioner)

PA – Human Resources
J. Schinkel

PE – HR Development
Dr. S. Kirchhoff

BEM - Occupational Re-entry Management
C. Körner

RZ – Computer Centre
Dr. J. Metge

SU – Safety and Environmental Affairs
Dr. E. Grund

S3-Platform
Dr. S. Talay

TB – Technical Services
O. Rabe

Staff Units

BIB – Library
A. Plähn

FASI – Occupational Safety Specialist
C. Strömpl

IMM – Innovation Management
Dr. St. Scherer (Technology Transfer Commissioner)

DA – Third Party Funds Acquisition
Dr. B. Gerstel

PS – Patents
D. Meseke

IR – Internal Auditing
Ch. Beth

PuK – Press and Communication
S. Thiele

QM – Quality Management
Dr. H. Kollmus

VIT – Administration-IT
H. Eggers

WCB – Scientific Controlling and Reporting
N.N.

WST – Scientific Strategy
Dr. B. Manno
(Knowledge Transfer Commissioner)

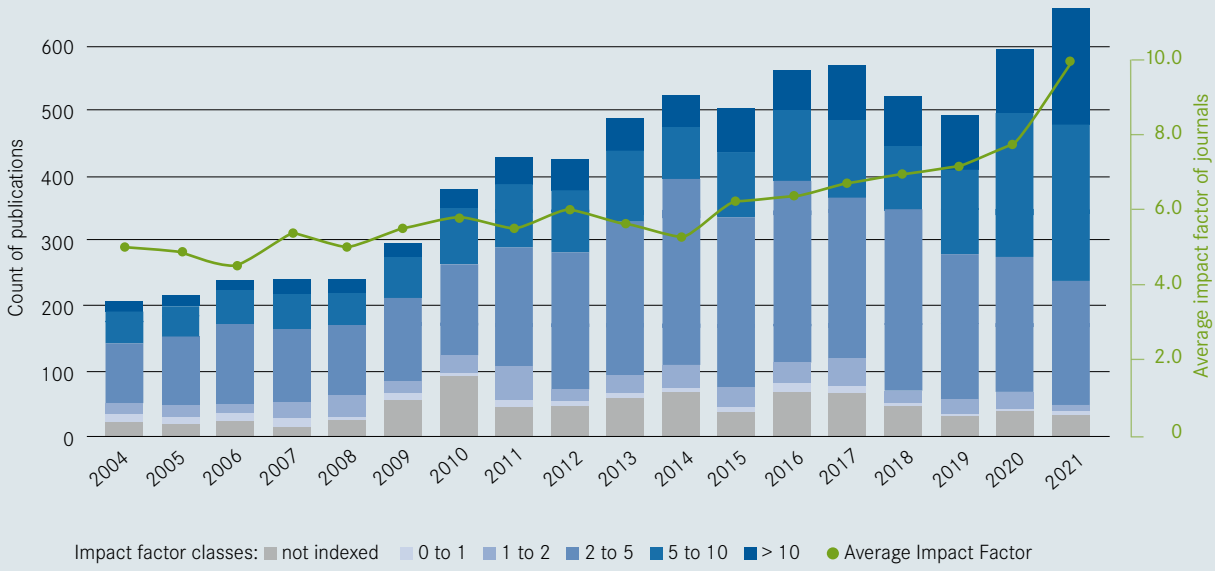
SKO – Strategic Communication
M. Braun
(Knowledge Transfer Commissioner)

Version: July 2021

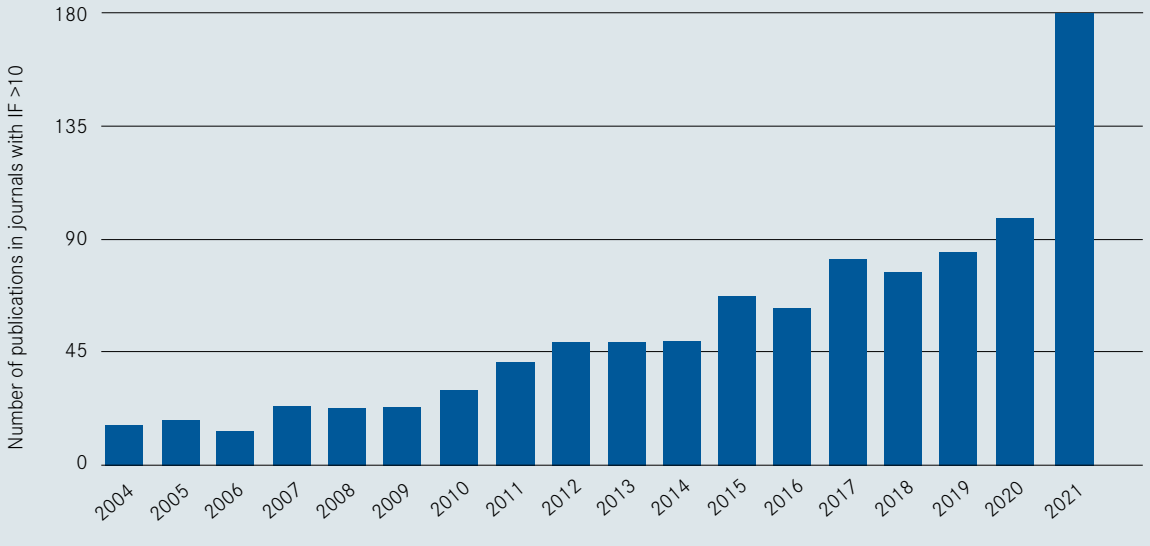
PUBLICATIONS

In 2020 and 2021, HZI scientists published more than 1200 scientific articles, a high percentage of which appeared in high-ranking research journals (> 20 %).

Number of HZI publications over time



Publications of the HZI in high-impact journals

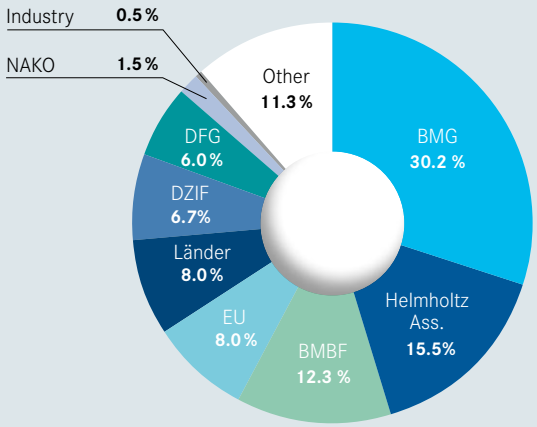


FINANCING

For the year 2021, the institutional budget of HZI amounted to 79.2 Mio. € plus 43.1 Mio. € from third-party financing. Most of the third-party funds originated from national programmes (>90%) and about 8.5 % from EU programmes and industry.

THIRD-PARTY FUNDING OF RESEARCH IN 2021

Source	Spending (M€)
BMG *(Federal Ministry of Health)*	12.93
Helmholtz Association	6.68
BMBF *(Federal Ministry of Education and Research)*	5.30
EU	3.45
Länder	3.45
DZIF	2.91
DFG	2.58
NAKO	0.65
Industry	0.23
Other	4.89
Sum (M€)	**43.07**



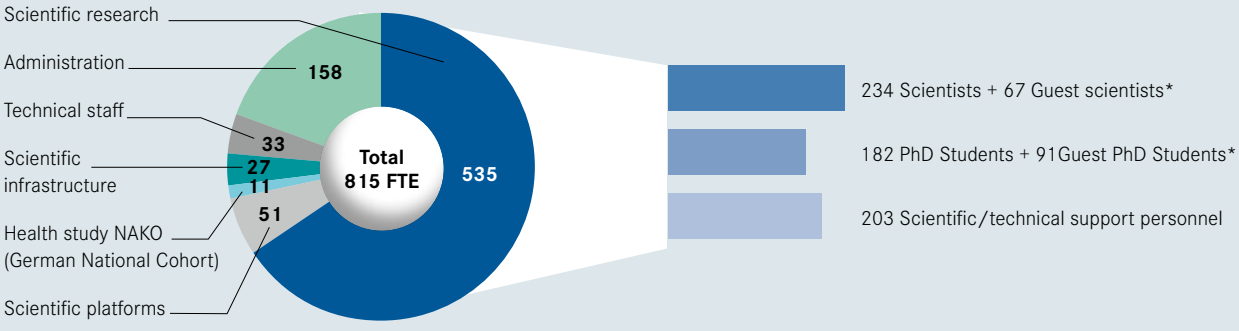
PARTICIPATION IN RELEVANT RESEARCH NETWORKS

The Centre participated in 2021 in 34 DFG Programmes (including Clusters of Excellence and Collaborative Research Centres), 27 EU projects (including ERC Starting, Consolidator and Proof-of-Concept Grants) and 64 BMBF / BMG / BMVi projects.

PATENTS, PROPERTY RIGHTS AND LICENSES

	2020	2021
Priority based applications	16	11
Total number of held property rights	272	265
Licence agreements to others	24	25
Licence proceeds (thousand €)	447	400

PERSONNEL



At the end of 2021 the HZI staff comprised 940 full and part time employees, amounting to 815 Full Time Equivalents (FTE). Scientific personnel represents the majority of HZI staff (597 FTE). *) In addition, 158 guest researchers worked on a variety of projects. As they received their stipends from third parties, they are not included in the chart.

RESEARCH REPORT 2020/2021

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Inhoffenstraße 7 | D-38124 Braunschweig, Germany
Telephone +49 531 6181-0 | Fax +49 531 6181-2655
info@helmholtz-hzi.de | www.helmholtz-hzi.de
Member of Helmholtz Association of German Research Centres (HGF)

Responsibility of:	Prof. Dirk Heinz (Scientific Director of HZI)
Editorial Team:	Manfred Braun (Editor in Chief), Hansjörg Hauser (Deputy Editor), Kirstin Kleeberg (Image and Text Editing), Andreas Holz (Facts and Figures)
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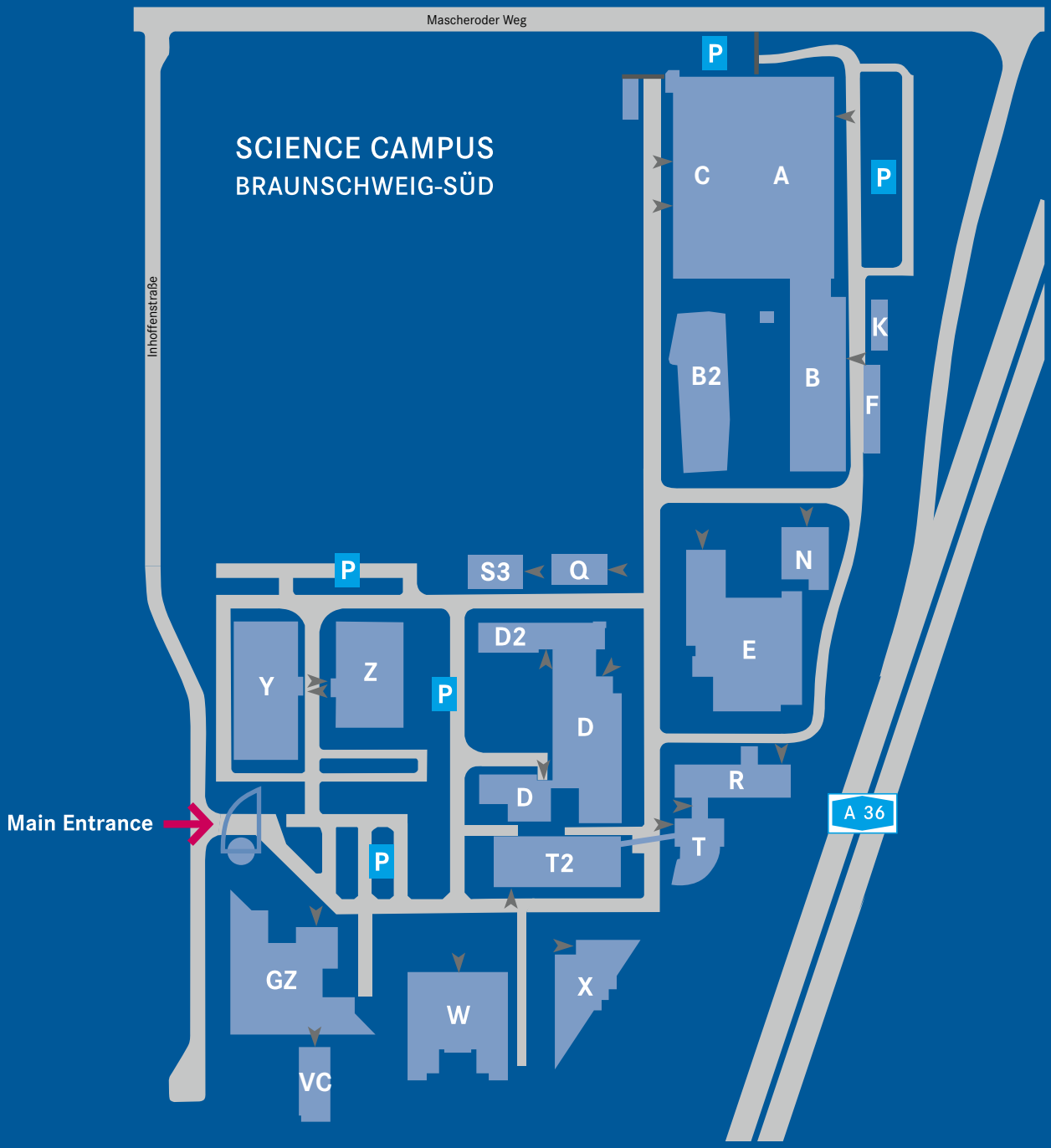

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B	Research Labs
B2	Research Labs, Drug Research & Functional Genomics
C	Research Labs
D	Research Labs, InSCREENeX GmbH
D2	Offices
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GZ	Gründerzentrum Building – DZIF Office, School Lab BioS, Research Labs
S3	S3-Facility
T, T2	Animal Facility
N	Infrastructure & Administration
VC	Research Infrastructure / Epidemiology
W	Administration, Library, Canteen
X	Forum – Event & Seminar Building
Y	Research Labs, Fraunhofer ITEM
Z	Leibniz Institute DSMZ

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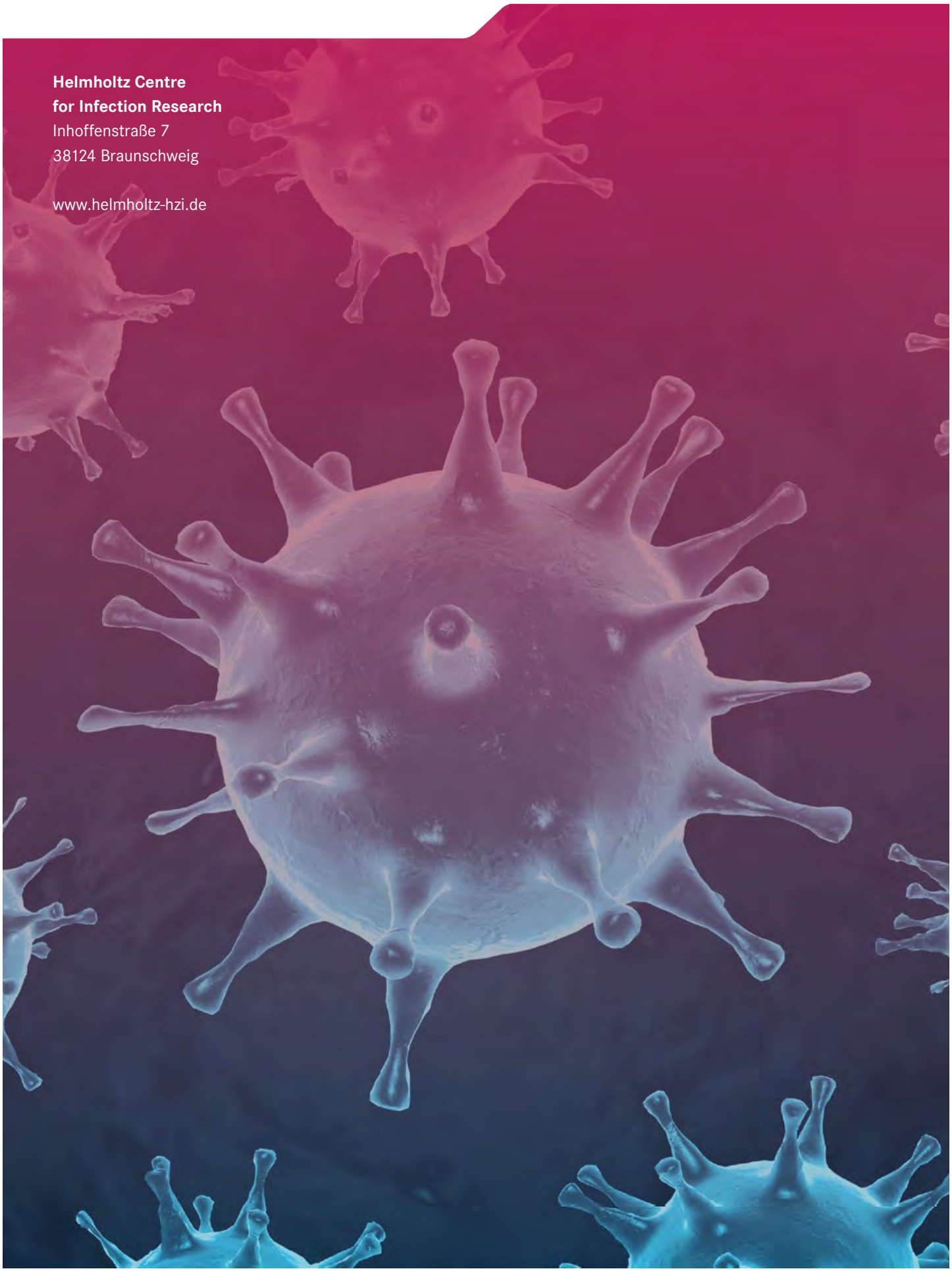

Helmholtz Centre
for Infection Research
Inhoffenstraße 7
38124 Braunschweig

www.helmholtz-hzi.de