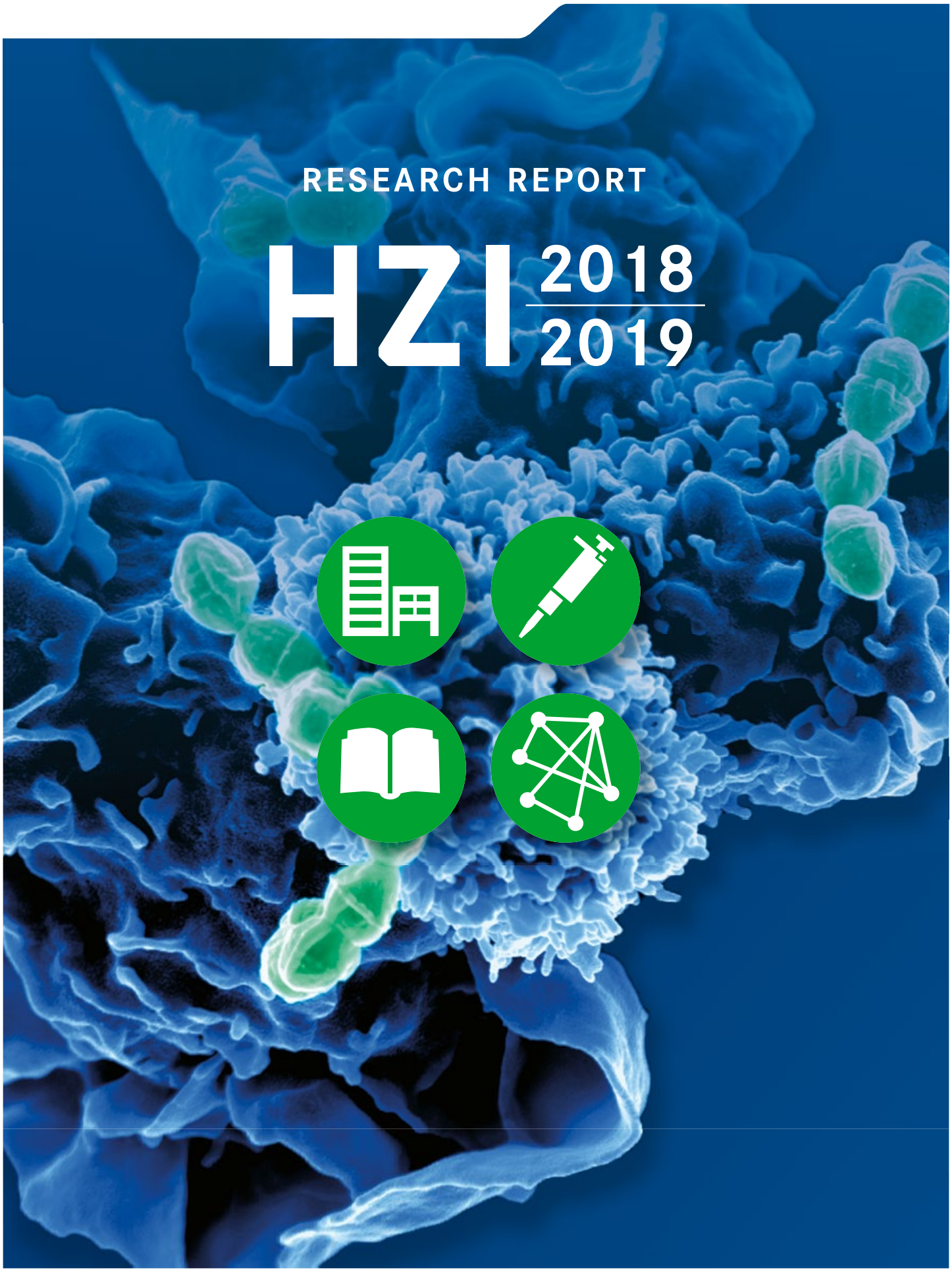
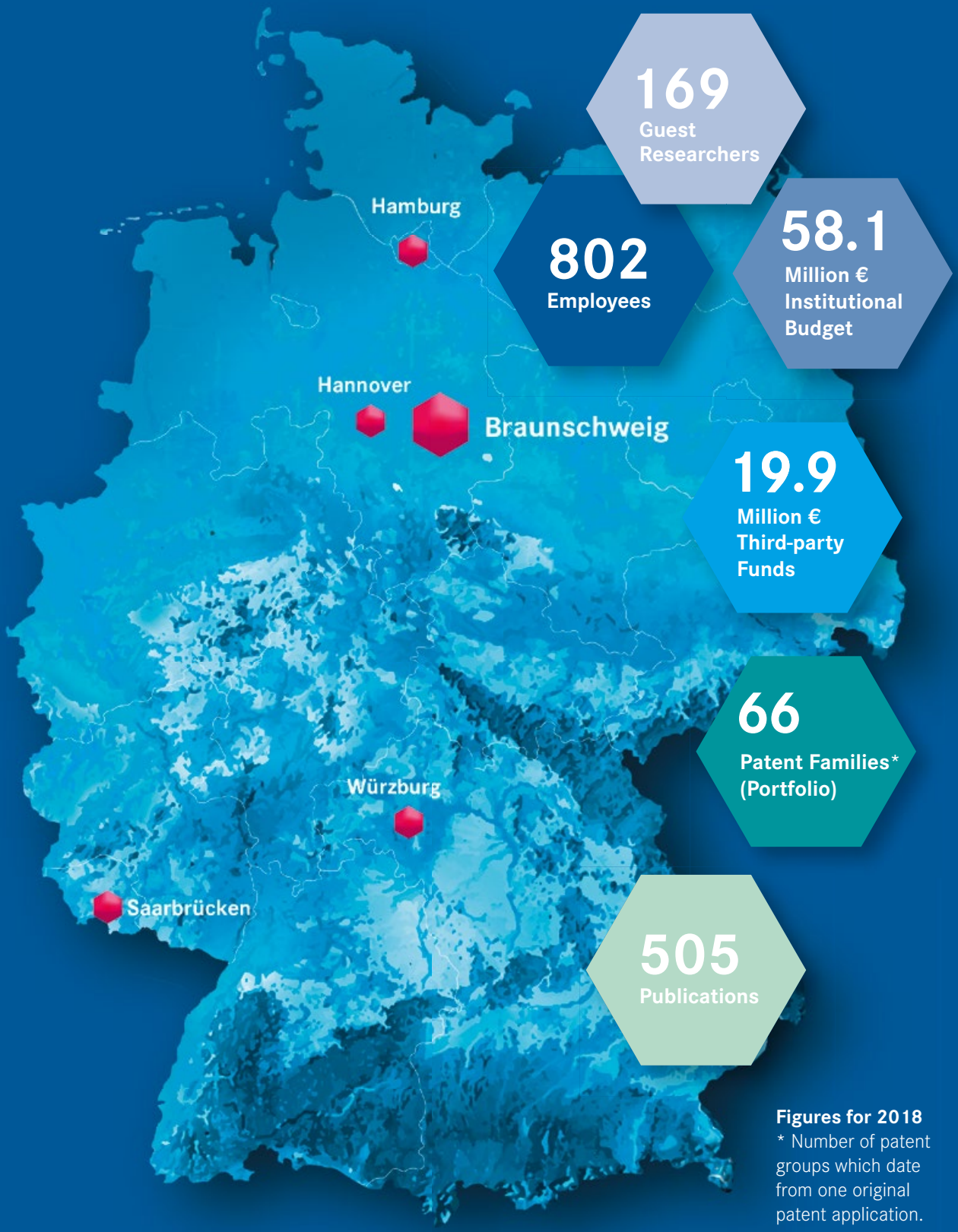
HZI HELMHOLTZ
Centre for Infection Research



RESEARCH REPORT

HZI 2018 2019

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



169
Guest Researchers

802
Employees

58.1
Million €
Institutional Budget

19.9
Million €
Third-party Funds

66
Patent Families*
(Portfolio)

505
Publications

Figures for 2018
* Number of patent groups which date from one original patent application.

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), located on TU main campus

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbrücken

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

Centre for Individualized Infection Medicine (CiiM), Hannover

- Joint venture 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

TWINCORE, Hannover

- Founded jointly by HZI and Hannover Medical School (MHH)
- Translational research by physicians and natural scientists
- Experimental and clinical infection research
- 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 several north German research institutions and universities
- Structural elucidation of molecular processes in infections using uniquely powerful photon sources

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

RESEARCH REPORT HZI 2018|2019

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HIGHLIGHT PUBLICATIONS

PARTNERS, SITES AND NETWORKS


HZI
HELMHOLTZ
Zentrum für Infektionsforschung
HZI
Zentrum für Infektionsforschung
HZI
HELMHOLTZ
Zentrum für Infektionsforschung

DEAR READERS,

The new coronavirus SARS-CoV-2 has shown us how fast and how radically infectious diseases can change the world. The COVID-19 pandemic triggered by this pathogen has developed an alarming global dynamic and is going to have a massive impact on the everyday life of people in most countries of the world.

In 2018/19 – the period covered in this Research Report – SARS-CoV-2 was barely known. It was not until the end of 2019 that a broader public became aware of the first major outbreak of the novel coronavirus infection in the Chinese province of Hubei.

The Helmholtz Centre for Infection Research (HZI) has established a translational infection research programme that enables us to rapidly respond to new challenges, such as the emergence of novel pathogens with pandemic potential. Our research strategy, which we present in this report, is based on a cross-disciplinary approach that brings together infectious disease specialists, immunologists, epidemiologists and drug researchers to address questions of high clinical need such as managing and control of the COVID-19 outbreak.

Our ability to quickly direct research efforts to tackle newly arising challenges is best illustrated by the rapid adaptation of our digital infectious disease control tool SORMAS, which now makes it possible to provide real-time information on the spread of COVID-19. You will find more information on this subject and the possibilities of digital infection control in the relevant chapters of this report. Scientists at HZI will continue to put enormous efforts into COVID-19 research to decipher the mechanisms of disease onset and progression and to develop novel therapeutic options, also keeping in mind future epidemics.

The combination of expertise and interdisciplinary collaborations at a single centre, which is unique in the field of infection research in Germany, positions HZI and its partners in the front line to address the global challenges arising from bacterial and viral pathogens. This was also recently acknowledged during an intense and rigorous two-stage scientific evaluation by international expert panels. As part of the Helmholtz Association's "Programme-oriented Funding" process, HZI proved first its scientific excellence in March 2018 and then, in November 2019, the sustainability of its central strategic concepts. The reviewers attested us a "world-class scientific performance" in areas such as bacterial and viral pathogens, infection epidemiology, RNA-centered infection research, as well as vaccine and anti-infective research. In particular, they highlighted the centre's ability to bridge the gap between fundamental research and the development of potential new drugs and diagnostics, thus contributing to the solution of clinical challenges such as the increasing spread of antimicrobial resistance. These results are a great encouragement, putting the centre into an excellent position for its future development. I would like to give my sincere thanks to everybody who contributed to this tremendous success, both within HZI as well as within our great network of national and international partner institutions.

Detailed information on our cross-disciplinary priority subjects, the so-called Research Foci, is a central part of this report. We also provide up-to-date information about the development of our sites and branch institutes, selected highlight publications, awards, events and partnerships.

I am very grateful for your interest in our research and hope that this report will help to spread news about the huge commitment of HZI staff and cooperation partners in their fight against infectious disease.

Dirk Heinz
Scientific Director

ABOUT HZI

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PORTRAIT OF THE RESEARCH CENTRE

The Helmholtz Centre for Infection Research (HZI) is the largest scientific institution in Germany focusing solely on infection research.

World-wide mobility of people and transport of goods as well as demographic and climate changes continue to accelerate the spread of pathogens, facilitate their emergence and re-emergence and enhance sensitivity towards infectious diseases. Furthermore, increasing antimicrobial resistance of pathogens adds a major challenge and threatens to revert the unique progress made in infection control during the last century. The role of infections as potential causes of other severe diseases – including certain cancers, metabolic syndrome and neurodegeneration – is becoming increasingly evident, offering the chance to fight these diseases by preventing or curing the underlying infection.

In line with the mission of the Helmholtz Association to address major challenges facing society, science and the economy, HZI employs cutting edge research and latest generation technology for new approaches towards prevention, diagnosis and therapy of infectious diseases.

The centre thereby pursues an integrated and highly interdisciplinary research strategy, which is based on profound expertise in the mechanistic exploration of host-pathogen interactions as well as internationally renowned natural product research. 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 population and patient cohorts. HZI focusses on translational infection research and, by capitalizing on strong partnerships with universities, clinics and industry, aims to rapidly transfer results from fundamental research towards clinical application. The unique combination of interdisciplinary expertise and collaboration distinguishes the centre as a frontrunner and technology leader in the continous struggle against global infection challenges like antimicrobial resistance or emerging and re-emerging infectious diseases.

The Centre and its Sites

HZI's more than 800 employees and approximately 160 guest scientists work at different sites throughout Germany. The main campus in Braunschweig – with scientists covering all disciplines of infection research – serves as the central research hub. In addition, branch institutes with dedicated foci have been established to complement and strengthen the scientific portfolio at the main campus.

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 conducted and novel concepts for combatting infectious diseases are jointly developed and implemented via in-house and external collaborations.

The infrastructure on campus comprises technology platforms including facilities for fermentative and total synthesis of natural compounds that make it possible to identify and develop molecules intervening in the infection process. Structural biology permits analysis of interactions between virulence factors, host cell targets and small molecules. Furthermore, units for omics technologies allow genotyping of pathogens and expression profiling. A cutting-edge animal facility with 500 different mouse strains allows HZI scientists to analyse virulence mechanisms and immune modulation concepts in modern, biosafety level 1-3 (BSL1-3) laboratories. A new building that provides capabilities to intensify drug research was still under construction in 2018/19. Starting in 2020, the new facility will also provide laboratory space for functional genomics research to be performed 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 institutions on the HZI premises, the centre has established the "Science Campus Braunschweig Süd" concept, reflecting intense on-site collaborations in research, development and education. Partners in this integrated campus concept 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 startup companies.

The main office of DZIF, which was initiated by the Federal Government of Germany in 2012, is located on the HZI campus, including the DZIF project and funding management units. Scientifically HZI is playing a pivotal role in DZIF, coordinating the Thematic Translational Unit "Novel Antibiotics" as well as the Translational Infrastructures "Epidemiology", "Bioinformatics" and "Translational Project Management Office".

HZI and its partner institutions on the Science Campus Braunschweig-Süd: 1 Technical University Braunschweig (TU-BS) | BRICS, 2 BioS, 3 DZIF, 4 Fraunhofer ITEM, 5 DSMZ, 6 InSCREENeX GmbH, 7 YUMAB GmbH © HZI | Peter Sondermann

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 have been set up to complement each other. Together, they offer a wide range of technologies and expertise, including gene expression analysis. In collaboration with the Braunschweig Integrated Centre for Systems Biology (BRICS), founded together with TU-BS, challenges in the field of data science and bioinformatics are jointly addressed.

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.

HZI Branch Institutes

HZI is engaged in partnerships with sites of excellence – usually universities – in selected fields. These partnerships have provided the basis for establishing branch institutes, which benefit from both their strong thematic foci and their affiliation to HZI with its elaborate infrastructure and long-term perspective.

Within the last ten years, HZI has founded a translational centre, TWINCORE, and two new Helmholtz Institutes, HIPS and HIRI, to significantly strengthen its expertise and critical mass in specific fields. In addition, HZI is part of regional research centres designed to foster specific knowledge and technologies (see figure below for details).

→ ***Clinically oriented research and individualized infection medicine:*** 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, an activity jointly developed in a strategic partnership with HZI. At TWINCORE, multidisciplinary teams of physicians and scientists pursue research motived by clinical needs and observations and translate their findings into clinical practice.

TWINCORE currently also hosts the newly founded Centre for Individualized Infection Medicine, CiiM. This new institute, which was jointly set up by MHH and HZI, is expected to be game-changing by the patient-specific management of infectious diseases and the development of new concepts and strategies for tailored treatments in infection medicine. A dedicated own CiiM building will be constructed adjacent to TWINCORE in the upcoming years.

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

© Oliver Dietze

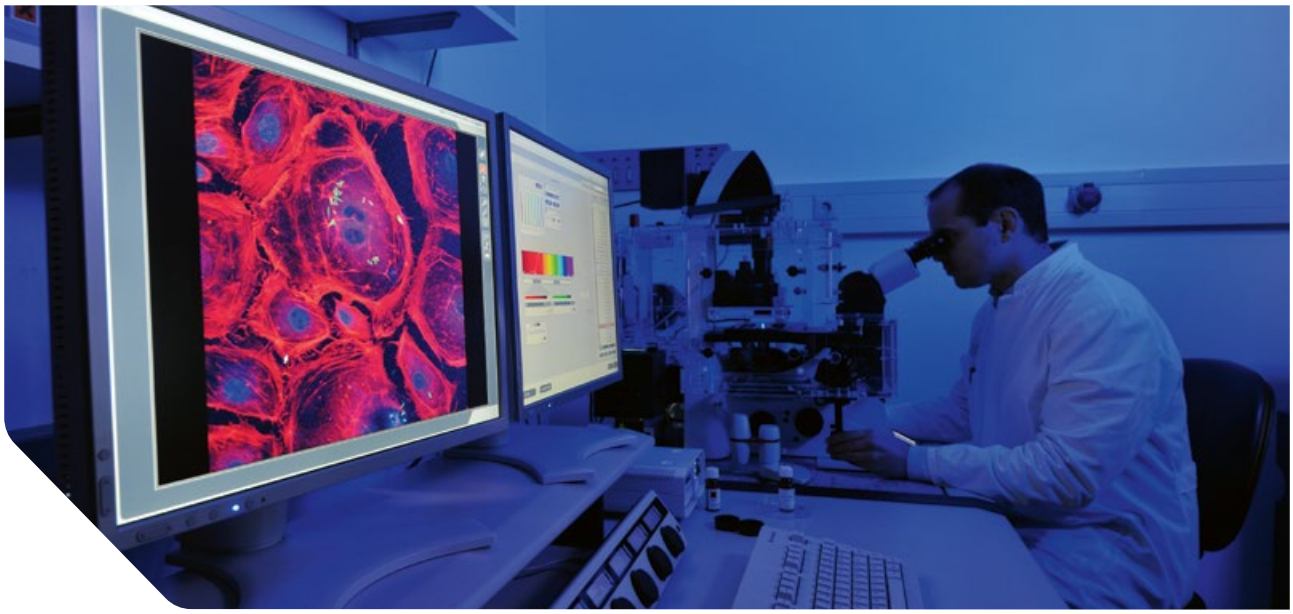
→ ***Drug and pharmaceutical research:*** The Helmholtz Institute for Pharmaceutical Research Saarland, HIPS, in Saarbrücken is focussing particularly on the discovery and development of novel anti-infectives from natural sources like bacteria and fungi. HIPS was established jointly with Saarland University (UdS) in 2009 in order to combine the outstanding expertise of both institutions in pharmaceutical research, especially in the areas of natural compound research, medicinal chemistry and drug delivery. The HIPS research building on the UdS campus now 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 single cell level represent emerging and fast-growing fields with great potential for innovations. In order to develop these fields in a sustainable fashion, 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 one research department and eight research groups. A dedicated HIRI building will be constructed on the medical campus of JMU.

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

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

→ ***Clinically oriented research:*** The Clinical Research Centre, CRC, in Hannover is staffed and equipped for safety and efficacy testing (Phase-I to Phase-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 in the framework of the German National Cohort (NAKO), where epidemiologists conduct long-term population studies with volunteers.



HZI AND ITS SITES

Science Campus Braunschweig-Süd:
HZI Main Campus, Braunschweig

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

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

CRC: Clinical Research Centre Hannover (co-established with Hannover Medical School and Fraunhofer ITEM), 2012

BRICS: Braunschweig Integrated Centre for Systems Biology, Braunschweig (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

CiiM: Centre for Individualized Infection Medicine, Hannover (co-established with Hannover Medical School), 2017

THE HELMHOLTZ PROGRAMME "INFECTION RESEARCH"

THE CHALLENGES

Infectious diseases caused by bacteria, viruses and other pathogens make up the majority of the ten largest global health threats listed by the United Nations in 2019 and are responsible for more than a fifth of all human deaths worldwide. They continue to pose a global threat to human health despite improved hygiene and sanitation measures, global vaccination programmes, and powerful anti-infective therapies introduced in the last century. Even in industrialized countries, infections cause huge socio-economic damage through prolonged hospital stays, sick leave and premature death.

Globalization, increasing mobility, and urbanization are key drivers in the rapid spread of epidemics caused by emerging and re-emerging pathogens such as Ebola or influenza. Epidemic control remains a challenge as we lack sufficient measures for global infection surveillance. The most dramatic example of this threat is the SARS-CoV-2 pandemic outbreak at the end of 2019, leading to a huge number of infections within a few months, with no treatment or prevention options available and necessitating unprecedented global quarantine measures.

At the same time, chronic infections also 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 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 receiving worldwide attention, driving fears of an imminent post-antibiotic era. Antimicrobial resistance (AMR) affects particularly vulnerable groups such as the 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 drug development (*Figure 1*). HZI's unique interdisciplinary approach facilitates the development of innovative, patient-tailored solutions for diagnosis, prevention, treatment as well as surveillance and control of infectious diseases.

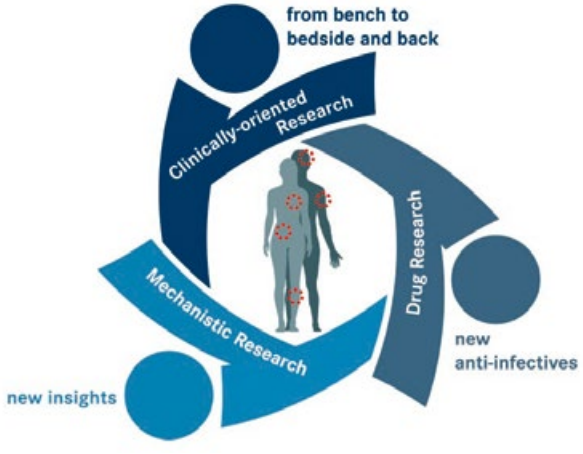


Figure 1: From research to impact: the integrative research strategy of HZI

Recent 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. They offer unique opportunities to obtain a systems-level understanding of infection processes.

The programme "Infection Research" harnesses 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 colonize 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.

RESEARCH TOPICS AT HZI

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

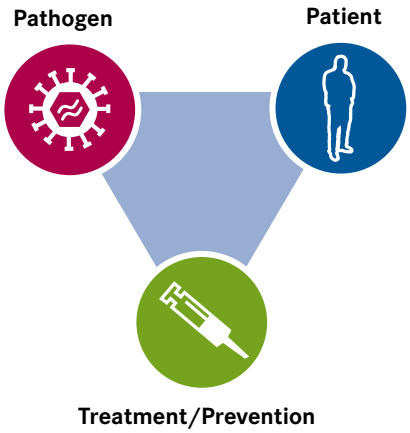


Figure 2: The triad pathogen-patient-therapy/prevention

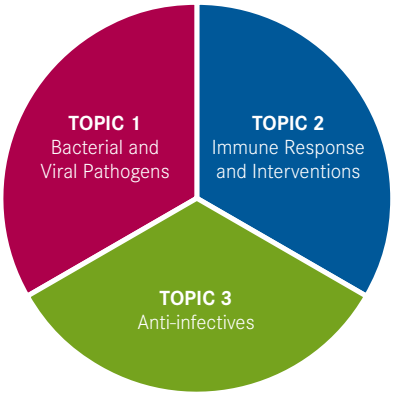


Figure 3: HZI's Research Topics

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 determine 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. Furthermore, Topic 1 implements novel mobile health surveillance systems for rapid outbreak response and investigates associations between infections and non-communicable diseases.

Speaker: Thomas Pietschmann; Deputy Speaker: Theresia Stradal

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

Scientists in this Topic study the detection and clearance of bacteria and viruses by the immune system, the reasons for individual susceptibility towards infections and the mechanisms pathogens use to circumvent the host's immune system. They explore the immune system's ageing, the influence of microbial communities in the body 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 human studies performed in cooperation with clinical partners using patient

and population cohorts. The insights gained serve as the basis for new immune-focused strategies for prevention, e.g. by vaccination, and for the treatment of infectious diseases.
Speaker: Carlos A. Guzmán; Deputy Speaker: Jochen Hühn

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 develops innovative methods to identify, characterize and improve natural products, which could be used to treat infectious diseases. By means of medicinal chemistry, they optimize these compounds as well as 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 where it is needed, as for example the infected organ, tissue or cell. In cooperation with partners from the pharmaceutical industry, the compounds are then planned to be further developed towards clinical trials where their efficacy and safety is tested in small patient groups before they get approved for the daily use in patients.
Speaker: Rolf Müller; Deputy Speaker: Marc Stadler

RESEARCH FOCI AT HZI

On the basis of challenges of high clinical and societal relevance and the special competences of its cooperation partners, HZI has established so-called Research Foci providing a synergistic, dynamic and flexible framework for the 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.

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

→ Antimicrobial Resistance (AMR)
→ Microbial Communities (MICO)
→ Chronic Viral Infections (CVIR)
→ Individualized Immune Interventions (INDI)
→ Digital and Global Health (EPI)

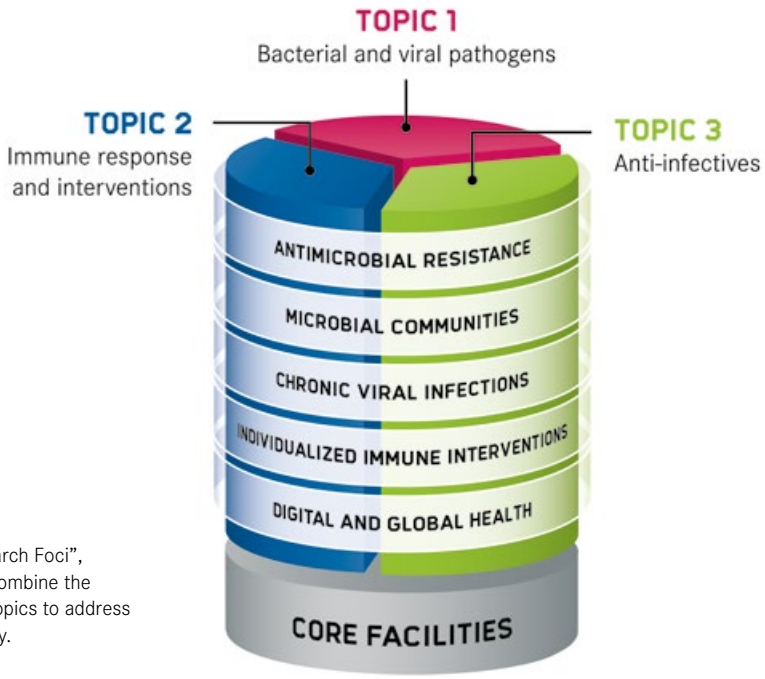


Figure 4: Overarching "Research Foci", supported by core facilities, combine the expertise of HZI's Research Topics to address urgent problems facing society.

The Research Foci are critically supported by state-of-the-art research infrastructure. Specialized 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. DZIF, Helmholtz) 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. Most importantly, 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 six 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 by establishing new Research Foci, in particular dealing with the subjects “Respiratory Viral Infections” and “Infection and Neurodegeneration”
- Transforming infection research through a digital revolution
- Strengthening HZI as a driver of and partner within global networks for translational infection research.

MOST IMPORTANT STRATEGIC PARTNERS

To complement its research portfolio and leverage other existing strengths, HZI has built a network of national and international strategic partnerships. The partners comprise universities, university hospitals, other Helmholtz centres, the German Center for Infection Research (DZIF) and the pharmaceutical industry.

To maximize 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.

IN AND AROUND HZI

HZI-DEVELOPED APP FOR DISEASE MANAGEMENT EXPANDED TO INCLUDE COVID-19

NEW CORONAVIRUS MODULE IN SORMAS

At the end of December 2019, the first cases of pneumonia caused by a novel coronavirus were reported from the Chinese city of Wuhan. In the following months, infections with the pathogen appeared on several continents. Strict measures to control the epidemic were put in place to stop its further spread. The SORMAS app – short for "Surveillance, Outbreak Response Management and Analysis System" – developed at HZI was subsequently further adapted in order to contribute to these efforts. HZI scientists expanded the mobile information system for disease monitoring to include a module for combating the coronavirus epidemic. The new coronavirus module can be implemented in any country that wants to use SORMAS in the future.

Scientists in the Department "Epidemiology" at HZI headed by Gérard Krause had developed the mobile information system SORMAS together with German and international partners. The system operates as an application on mobile phones and other electronic devices. It is particularly suitable for use in regions with weak infrastructure. SORMAS can record local data on disease outbreaks and transmit them to health authorities. Risk assessments can then be made in order to coordinate measures for disease control.

The COVID-19 epidemic has shown how urgently detailed data are needed for risk assessment, and also how great the need for structured management of containment measures is. A systematic review has recently shown that the integration of these two functions is a unique feature of SORMAS.

With the new coronavirus module, the application is available for 20 different infectious diseases including Ebola, Lassa fever, monkey pox, avian influenza, dengue fever, yellow fever, measles, cerebrospinal meningitis, plague, cholera, rabies, and anthrax. SORMAS has already been used successfully in the fight against three large outbreaks that occurred in parallel in Nigeria and is also active in Ghana.

Owing to the flexible 'building block concept' of SORMAS, scientists were able to activate the specific coronavirus module within a few days. The modules for the particular diseases make it possible to detect individual cases early on, even in remote regions, to record clinical details and laboratory confirmations, to approach contact persons prospectively and to offer them therapy at an early stage if they also fall ill. SORMAS regulates these processes and at the same time generates well-validated data in real time for the continuous assessment of risk at national and international levels.

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MEETINGS, PRIZES, PUBLIC OUTREACH

SCIENCE FOR THE PUBLIC

HZI organised the public lecture series **"KrankheitsErregend" ("PathoGenic")** both in autumn 2018 and 2019 for the seventh and eighth time, respectively. In 2018, the lectures focused on influenza infections. Scientists and clinicians informed the audience about historical facts, today's scientific challenges and effective prevention. The 2019 event series consisted of two readings on microbes and hygiene by authors Susanne Thiele and Prof Dirk Bockmühl, respectively, and a Science Slam on health topics.

In October 2018, HZI launched a public discussion on personalised medicine together with the **Haus der Wissenschaft Braunschweig**. Prof Michael Manns, then Clinical Director of HZI and Director of the Centre for Individualized Infection Medicine (CiiM) in Hannover, gave a presentation and participated in the discussion.

Public presentation of scientific subjects attracted interested citizens to various events in Braunschweig's Haus der Wissenschaft (left). © Philipp Ziebart
HZI organized, amongst others, panel discussions about health-related questions. Right: HZI Press Spokesperson Susanne Thiele with science journalist Johannes Kaufmann and HZI scientist Till Strowig. © HdW | Thorsten Witt

According to one estimate, about 33,000 people die every year in Europe from infections with resistant pathogens. Resistance to antibiotics is one of the greatest challenges of our time. Raising awareness of this threat is the main objective of the World Health Organization (WHO) International Antibiotics Week. On the occasion of this event, HZI invited journalists to the workshop **"New Ways in Drug Discovery"** in November 2018, providing information about recent findings in the field. Journalists from various media, in particular from the biomedical press, took the opportunity to discuss with HZI experts the challenges in drug discovery and the fight against resistant pathogens.

During the reporting period, HZI was represented with a stand at two events of the Technical University Braunschweig (TU-BS): In June 2018, HZI took part in the **TU Night**, which had the motto "Vision & Change", and informed the visitors about new vaccination methods. As part of the evaluation of the Excellence Initiative in May 2019, the TU-BS arranged the event **Campus in Motion**, in which HZI contributed with two participatory activities.

Politicians visited HZI and its branch institutes. Above: Federal Minister of Education and Research Anja Karliczek (second from left) and Lower Saxony's Minister for Science and Culture, Björn Thümler (third from left), at TWINCORE Hannover with HZI's Scientific Director Dirk Heinz (second from right) and Ulrich Kalinke, Director of TWINCORE (right). © Ralf Mohr
Below: Saarland's Prime Minister Tobias Hans (middle) with Manfred Schmidt, President of the Saarland University (right), and HIPS Director Rolf Müller. © Daniel Krug

Press Workshop "New Ways in Drug Discovery" on Antibiotic Resistance © Marie Wieczorek

HZI presented its research to the citizens on many occasions. Here: HZI booth at the event "Campus in motion" of the Technical University Braunschweig © HZI | Christine Bentz

Several politicians visited HZI and its sites in 2018 and 2019: Federal Minister of Education and Research **Anja Karliczek** and Lower Saxony's Minister for Science and Culture **Björn Thümler** visited the HZI-coordinated study centre of the NAKO health study in Hannover and the TWINCORE on 31 May 2018. In June 2018, Saarland's Prime Minister **Tobias Hans** visited the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and gave a speech during the "10 years of HIPS" ceremony. As part of his summer trip, **Björn Thümler** visited the HZI campus in Braunschweig on 10 July 2018. In June 2019, **Anja Karliczek** gained insights into key areas of focus at HIPS and Saarland University. She again visited the Hannover Clinical Research Centre in July 2019 and informed herself about ongoing and future clinical studies.

PRIZES AND AWARDS

Selected scientific awards in 2018

Awarded scientist	HZI group or department*	Award	Awarding institution
Neva Caliskan	REMI	Young Leaders in Science Award	Schering Foundation
Petra Dersch	MIBI	Membership in the European Academy of Microbiology	European Academy of Microbiology
Marc Erhardt	IBIS	VAAM Research Award	German Association of Microbiologists, VAAM
Claus-Michael Lehr	DDEL	Listed as “one of the 100 most influential experts in drug development”	“The Medicine Maker“, London/UK
Rolf Müller	MINS	Inhoffen Medal	Association Friends of HZI
Alexander Titz	CBCH	Innovation Award "Medical Chemistry"	German Association of Chemists, GDCH
Jörg Vogel	RABI	Listed as "Highly Cited Researcher"	Clarivate Analytics
Dagmar Wirth	MSYS	Technology Transfer Award of IHK	Braunschweig Chamber of Industry and Commerce (IHK Braunschweig)

Selected scientific awards in 2019

Awarded scientist	HZI group or department*	Award	Awarding institution
Gregor Fuhrmann	BION	Young Investigator Prize of the Horst Böhme Foundation	Deutsche Pharmazeutische Gesellschaft DPhG
Anna Hirsch	DDOP	Runner-up EFMC Prize for a Young Medicinal Chemist in Academia 2019	European Federation of Medicinal Chemistry EFMC
Rolf Müller	MINS	Honorary doctorate at Shandong University, China	Shandong University, China
Rolf Müller	MINS	Honorary professorship at University of Kapstadt, South Africa	Kapstadt University, South Africa
Jörg Vogel	RABI	Feldberg Prize for German-British exchange in the life sciences	Feldberg Foundation
Jörg Vogel	RABI	Highly Cited Researcher	Clarivate Analytics

Selected Grants for HZI Scientists in 2018 and 2019

Scientist	Grant	Granting Agency
Chase Beisel	ERC Consolidator Grant: Horizon 2020: Interrogating native CRISPR arrays to unravel genetic redundancy	European Research Council
Carlos A. Guzmán	Coordinator of project: Vaccine for prevention and treatment of *Trypanosoma cruzi* infection	European Commission
Susanne Häußler	Laureate Research Grant	Novo Nordisk Foundation
Anna Hirsch	ERC Starting Grant: Horizon 2020: Identification and optimization of novel anti-infective agents using multiple hit-identification strategies	European Research Council
Alice McHardy	Grand Challenges: Rational design of a universal flu vaccine using recombinant neuraminidase	Bill & Melinda Gates Foundation
Till Strowig	ERC Consolidator Grant: Horizon 2020: Achieving Spatio-Temporal Regulation of Tissue Regeneration and Inflammation: Studying the communication between the immune system, tissues, and microbiota to develop targeted therapies for immune-mediated diseases and cancer	European Research Council

* see organisational chart for complete names

SCIENTIFIC EVENTS 2018/19

HERRENHAUSEN SYMPOSIUM ON INDIVIDUALIZED INFECTION MEDICINE

How may individualization find its way into care of patients with infectious diseases? This hot topic was discussed during the international Herrenhausen Symposium in June 2018 in Hannover. The symposium aimed at fostering international and interdisciplinary collaboration by bringing together outstanding experts from science, medicine and pharmaceutical industry as well as representatives of important initiatives in the field to address major scientific questions, tackle ethical, social and economical considerations and discuss the requirements for regulation processes in individualized medicine. In seven sessions, two panel discussions and almost 50 poster presentations recent developments in the field of individualized infection medicine were discussed.

Discussing perspectives of Individualized Infection Medicine: Poster Session at the Herrenhausen Symposium © Donnerkeil Fotografenagentur

INHOFFEN MEDAL FOR OUTSTANDING PIONEER WORK

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

In 2018, it went to Rolf Müller, director of HZI's Saarbrücken branch institute HIPS. Müller is specialized in investigating natural products, in particular from soil-dwelling myxobacteria, and developing them into novel anti-infective drugs. In 2019, Phil Baran of the Scripps Research Institute in La Jolla, USA, received the prize. Baran aims to develop the "perfect synthesis"; his pioneering methods and reagents are applied in fundamental chemical research and in the production of pharmaceuticals.

Awardees of the Inhoffen Medal. Photograph on the left side: 2019 prize winner Phil Baran (middle) with (from left to right): Dieter Jahn, Chairman of "Friends of HZI" (Förderverein des HZI), Dirk Heinz, Scientific Director of HZI, Katharina Borst, PhD Awardee 2019, Andreas Kany, PhD Awardee 2019, Anke Kaysser-Pyzalla, President of TU Braunschweig, Hansjörg Hauser (Managing Director of Friends of HZI). © HZI | Verena Meier
Photograph on the right side: 2018 Inhoffen awardee Rolf Müller (right) with HZI's Scientific Director Dirk Heinz. © Kristina Rottig

The awarding ceremony is associated with the "Inhoffen Lecture" in honour of the biochemist Prof. Hans-Herloff Inhoffen, an event organized 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. He died in 1992.

NORDI AND JÜRGEN WEHLAND PRIZE

Since 2010, scientists from across Europe convene regularly at the NoRDI Symposium (North Regio Day on Infection) on the HZI campus.

In November 2018, the topic of the NoRDI VIII symposium was "Anti-infective strategies in the post-antibiotic era". The one-day meeting focussed on innovative and alternative approaches against antimicrobial resistance that go beyond the likewise important screening for new antibiotics or measures such as improved hygiene and antibiotic stewardship. The symposium was divided into three sessions: Epidemiology, Public Health & Anti Transmission Strategies, RNA in Infection, Alternative Therapies.

Embedded in the scientific programme was the awarding of the Jürgen Wehland Prize for young scientists in infection research, with which the HZI has been honouring 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 2016, the prize went to Katherine Beckham, junior scientist at the European Molecular Biology Laboratory EMBL in Hamburg.

"BIOLOGICAL BARRIERS" CONFERENCE AT HIPS

The "International Conference and Workshop on Biological Barriers" is organized every two years jointly by HZI's Saarbrücken branch, HIPS, and the Saarland University (UdS). Target audiences are early stage researchers as well as experienced scientists and professionals from academia and the pharmaceutical industry; the meeting consistently receives more than 200 registered attendees from all over the world. In August 2018, the 12th BioBarriers conference focused on human cell and tissue models for facilitating the clinical translation of new drugs and delivery systems, especially in the context of infectious diseases. Moreover,

Katherine S. H. Beckham, awardee of the Jürgen Wehland Prize 2018, together with (from left) Hansjörg Hauser, Managing Director of the Friends of HZI, Dirk Heinz, Scientific Director of HZI and laudator Matthias Wilmanns.
© HZI | Susanne Thiele

scientists discussed innovative concepts and materials capable to overcome also non-cellular diffusion barriers such as mucus or bacterial biofilms. The conference concluded with sessions on extracellular vesicles and advanced nanomedicines for the non-invasive delivery of macromolecular biopharmaceuticals.

TRANSLATION AND BIG DATA: THE TWINCORE SYMPOSIUM

The TWINCORE Symposium has established itself as an important platform for topics at the interface between basic and clinical research. In the last two years, TWINCORE invited scientists to Hannover for the tenth and eleventh time to share their knowledge. In 2018, the meeting was combined with the celebration of TWINCORE's tenth anniversary and themed "Frontiers in translational infection research". In 2019, the event concentrated on the subject "Infection research meets big data".

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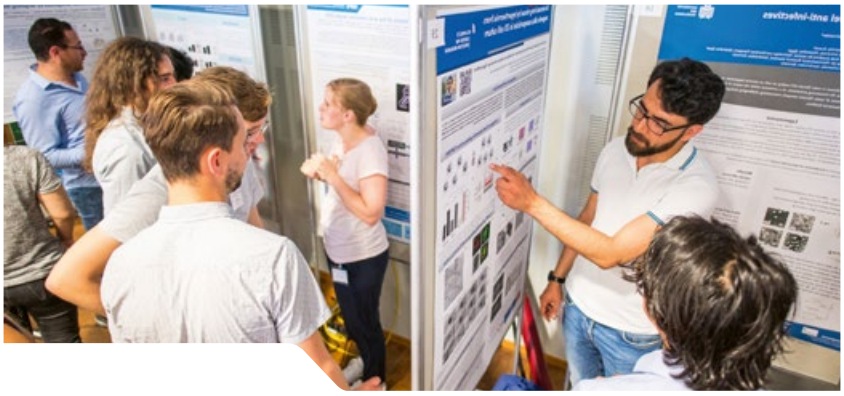
Science and sociality: The celebrations for the tenth anniversaries of TWINCORE in 2018 and HIPS in 2019 were integrated in scientific symposia.

FOCUS ON NATURAL COMPOUNDS: THE HIPS SYMPOSIUM

Every year since 2010, HZI's Saarbrücken branch institute HIPS has provided scientists with a forum for exchanging ideas beyond the boundaries of classical disciplines. The HIPS Symposium brings together researchers from the three pharmaceutical fields of natural product research, medicinal chemistry and drug delivery. In particular, it offers young scientists the opportunity to benefit from the expertise of established, internationally renowned colleagues. The symposium took place in Saarbrücken for the eighth and nineth time in 2018/19. In 2019, it was combined with the tenth anniversary of the foundation of HIPS.

MINI-HERPESVIRUS WORKSHOP IN BRAUNSCHWEIG

In September 2019, HZI hosted the 14th Mini-Herpesvirus Workshop. This meeting provides an opportunity for young herpesvirus researchers to present their data to a knowledgeable audience, meet colleagues and initiate collaborations. Graduate students and postdocs can present their work as short oral presentations which are selected from submitted abstracts. At the 2019 meeting in Braunschweig, keynote lectures were given by David Knipe (Harvard University, Boston, MA) and by Cliona Rooney (Baylor University, Houston, TX).



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"IN MANY CASES, THERE IS NO ONE-PILL-FITS-ALL-STRATEGY"

INTERVIEW WITH YANG LI AND MARKUS CORNBERG, DIRECTORS OF CIIM

Finding tailored strategies for each individual patient to optimally prevent or cure infectious diseases: This is the vision of the Centre for Individualized Infection Medicine (CiiM) in Hannover. Founded jointly by HZI and Hannover Medical School (MHH) in 2015, the Institute has been establishing its first research departments and launched pioneering projects in the recent years. Since 2019, CiiM is headed by a dual leadership team consisting of the renowned data scientist Yang Li and recognized senior clinician Markus Cornberg.

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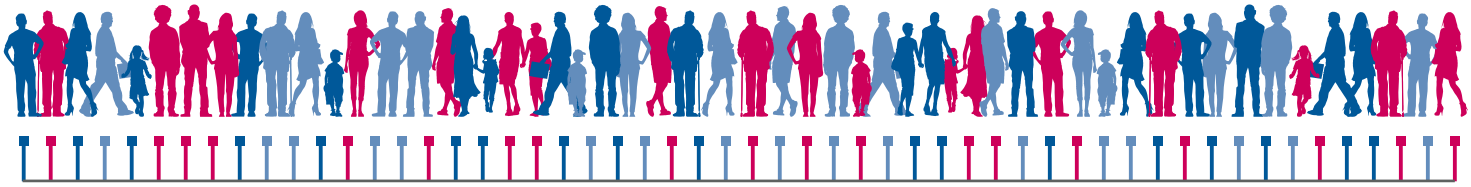
Prof. Cornberg, Prof. Li, what exactly is Individualized Infection Medicine – and why is research into this subject necessary?

Cornberg: Infections are very variable and heterogeneous. There is a heterogeneity of the host, thus the patient, but also of the pathogen. Therefore, different forms of treatment may be reasonable and in many cases, there is no one-pill-fits-all-strategy. Individual diagnosis and prognosis and subsequent personalised treatments are needed. In the field of cancer medicine, this approach is very successful – a hot topic that everybody is talking about.

Li: And infections are even better suited for an individualized approach. Both patient and pathogen show significant variations in their genes, their metabolism, on the host side also in age, co-morbidities and other factors. In infections and immunity, personal risk factors play a decisive role at different levels.



How do you exploit these variations in order to develop better cures?

Li: First of all, we try to collect as many layers of molecular and clinical information as possible from different patients. Then we use computational and statistical approaches to integrate these datasets and extract biological information. Based on this, we try to understand why some people develop a disease and others don't, in order to improve prevention in the long run. As for people who have already developed a disease, we ask how we can stratify them into different groups to develop a better treatment, a personalised treatment for each individual.

Can you give an example for the questions you are working on?

Cornberg: When treating Hepatitis B patients, it is a difficult question when to terminate the treatment without putting the patient at risk. On the other hand, we know that in some cases it is worth trying because the patient stays healthy and does not need therapy any more. So, we wanted to know: Can we predict a relapse of the virus infection?

And what did you find out?

Cornberg: Together with Michael Meyer-Hermann, head of the Systems Immunology department at HZI, we developed a machine learning approach to tackle this question. And we found a set of five cytokines, messenger molecules of the immune system, which allowed a good prediction which patient is at risk.

It sounds like you are working more with computers than in the laboratory. Is that so?

Li: In my particular case, this is certainly true. In my research, I am doing 80 percent computation, but I have also set up a wet lab. For one of my projects, I have analysed the transcriptomes, the patterns of gene activation, of more than 50,000 different colonic mucosal cells. These are tremendous amounts of data, you need high-capacity parallel computing to process them. But for the concept of CiiM in general, computation is just one part.

What else do you need?

Li: You need to have access to patients, cohorts, clinical samples and clinical infrastructure. This is key, and it is often not available in basic research.

And how do you make it available at CiiM?

Cornberg: The key concept is to have all kinds of necessary expertise under one roof, so that there is a lot of exchange on a daily basis. Researchers, clinicians, computational biologists sometimes speak different languages. It is crucial that all these experts meet on a regular basis and, even better, also informally during coffee breaks. This allows us to discuss the most urgent medical needs among clinicians, experts on the cutting-edge technologies and computational biologists to develop projects together. I think a place like CiiM was really needed. And there are not many institutes like CiiM in the world.

When there is a need for it, why didn't many others come up with the same idea?

Cornberg: There are many challenges – not every research institution is capable of meeting them. First, you need the patients who give informed consent. And not just individuals, you need really large cohorts of them. You also need dedicated physicians. You need basic research, you need biobanks and expertise in managing them. You need the latest technology in biosciences and data science. And, as I mentioned before: The interaction between the players is most important.

All that is provided in Hannover and Braunschweig?

Cornberg: We have Hannover Medical School, which is one of the leading university hospitals in Germany with a pillar on infection research. We have HZI as one of the leading institutes for fundamental infection research. The two institutions have a long history of intense and fruitful cooperation. There is an excellence cluster, RESIST, and a CRC – a *Sonderforschungsbereich* – on infection. With Twincore, we have already experience with translation, an institute which conducts translational research and has access to patients. This is very important – and one reason why CiiM will be located on the premises of Twincore, until our own building will be completed.

Li: And now we go one step further and bring the patient into the centre of research. By taking samples from patients to CiiM, we will identify biomarkers and pave the way for new, individualized therapeutic concepts.

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

Li: As for technology and methodology for data analysis, I think they are quite general and can be applied or tailored to study any disease, any pathogen or infection. But of course, we try to start with well-defined hypotheses and research questions. So, we are indeed focusing on certain diseases, for example, virus infections in the liver and lung, and also infections of the skin.

Cornberg: MHH is one of the leading transplant centres in Europe. Therefore, there is also a focus on immunocompromised patients that are particularly vulnerable to infections. I mentioned the excellence cluster RESIST before, which investigates the causes of susceptibility towards infections. As for lung and liver infections: the competence network CAPNETZ, which is concerned with pneumonia, and the German Liver Foundation will also be part of CiiM in the future. They will contribute their expertise in conducting clinical trials and their study cohorts, including networks with other national and international experts.

Are physicians interested in CiiM and its research?

Li: Physicians usually come with very clear clinical questions. They are indeed interested in new biomarkers and new treatment options to improve the life of their patients. And, yes, they approach us. I get many emails and telephone calls where physicians ask whether there is a possibility to collaborate with us.

Cornberg: But they want to really participate, not just provide the samples. This is also very important, that we work and do research as one team.

Li: And we give them something in return. I think a unique thing at CiiM is that when physicians participate in research, they immediately get the feedback what is the outcome of the studies. So maybe if we find a biomarker, we can even bring it to the clinic together.

Do clinicians also get the opportunity to work at CiiM?

Cornberg: MHH has already organised Clinician Scientist Programmes in cooperation with HZI, which is a particular benefit for CiiM. We will set up more such programmes in the future. But we also need positions later on – the next step after clinician scientist – which can be ideally embedded into CiiM. To motivate them very early in their career, MHH has established for example the KlinStrucMed programme for medical students, which allows the student to take one year off their studies to perform their medical thesis. The next step then would be the aforementioned Clinician Scientist programme for doctors.

So you also want to provide career perspectives?

Cornberg: We can provide the first step. But in the long run, creating the infrastructure for new positions will be a task which requires the cooperation of several players, including politics.

What is your vision of the future – what would you like to have reached in five to ten years?

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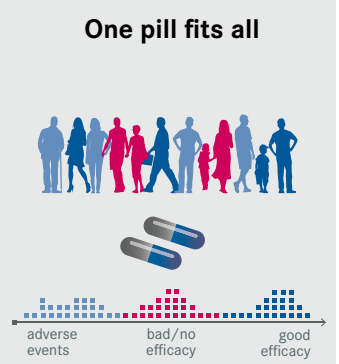
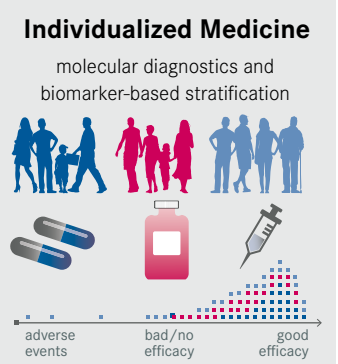
Cornberg: As a very concrete measure, we intend to establish an information centre, where physicians in the region can obtain first-hand information on the latest progress in infection medicine. But above all: CiiM will be recognised as a world-leading institute for its concept of individualized infection medicine.

Li: Ideally, we will already have developed new methodologies to handle different kinds of diseases, and new results that lead to different individualized approaches. We will have demonstrated that our methods work well – and we can attract even more clinician scientists.

Interview: Manfred Braun





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Photos: © Cornberg | CiiM

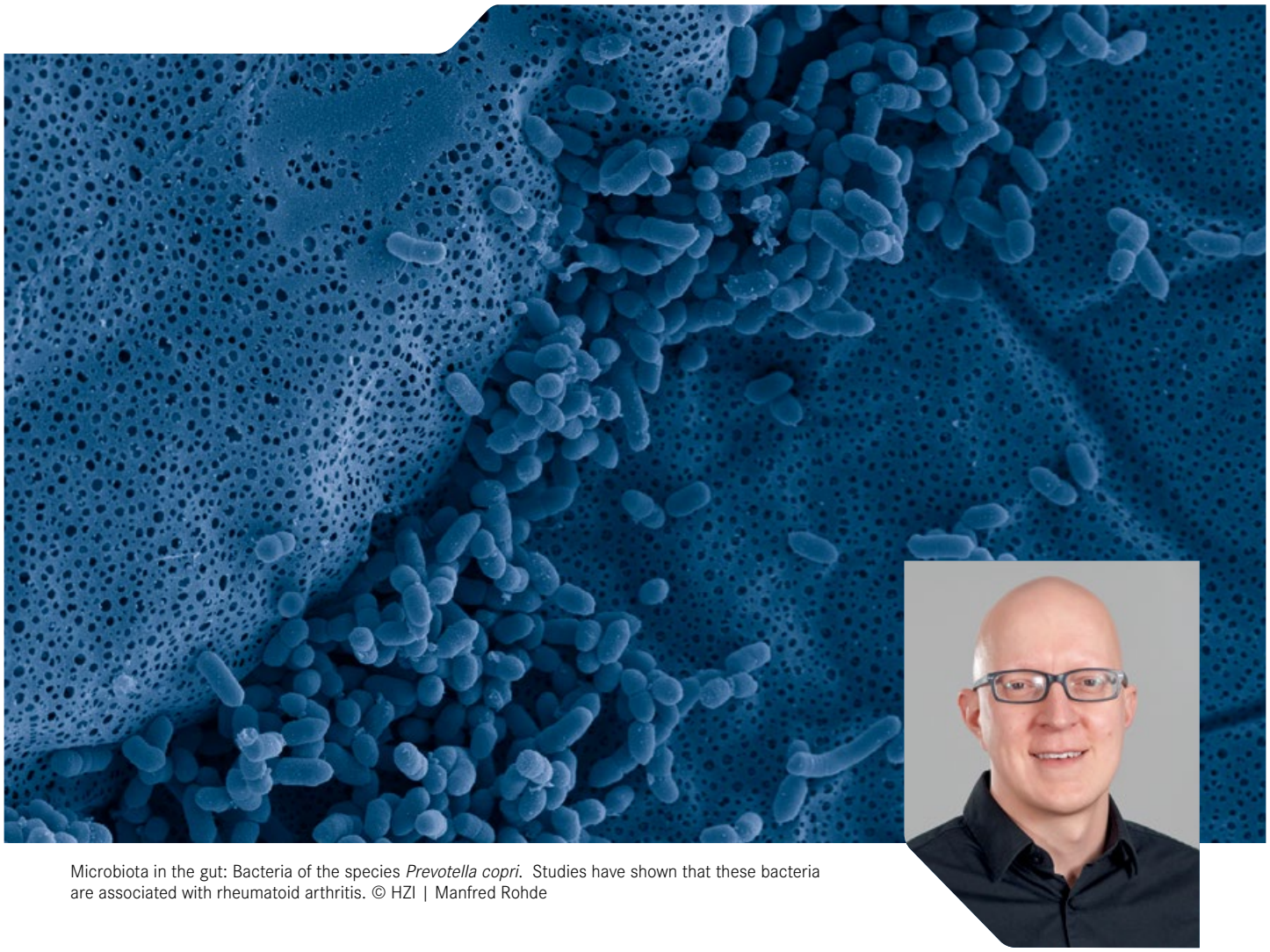
YANG LI heads the HZI Department "Computational Biology for Individualized Medicine" and is appointed Director of CiiM. Her research focuses on understanding the molecular mechanisms of immune-related and infectious diseases by integrating multi-omics data.

Yang Li received her PhD in bioinformatics from the University of Groningen, Netherlands, and continued her career as a postdoctoral fellow at the Bioinformatics Centre Groningen. As independent Principal Investigator at the Department of Genetics of the University Medical Center Groningen, she started to build up her own research team to further her interests in the complex genetics of human diseases using multi-omics datasets. Her successful research in the field of systems genetics of immunological diseases was rewarded with several personal fellowships. She is co-applicant with several large consortia and has published to date about 70 scientific articles, among others in *Cell, Science, Nature Medicine*, and *Nature Immunology.*

MARKUS CORNBERG, MD, is Professor of Medicine for Infectious Disease and senior physician in the Department of Gastroenterology, Hepatology and Endocrinology at MHH responsible for the infectious disease ward and the hepatitis outpatient clinic. He is also appointed Clinical Director of HZI and Director of CiiM.

As physician Markus Cornberg was in charge of numerous clinical studies investigating new drugs for the treatment of viral infections of the liver. He was the first scientific secretary of the German Competence Network for Viral Hepatitis and has been appointed Medical Director of the German Liver Foundation. He was involved in consensus processes for the management of Hepatitis B and is currently member of the scientific committee and the governing board of the European Association for the Study of the Liver EASL. His scientific research focuses on the importance of cellular immune responses for disease progression and therapy response in patients with viral hepatitis. To date, he has published more than 200 scientific articles.

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



Microbiota in the gut: Bacteria of the species *Prevotella copri*. Studies have shown that these bacteria are associated with rheumatoid arthritis. © HZI | Manfred Rohde

"THE MICROBIOTA PLAYS A CENTRAL ROLE IN THE ONSET OF MANY DISEASES"

INTERVIEW WITH TILL STROWIG, MICROBIOTA RESEARCHER AT HZI

Humans – like any other multicellular organism – harbor a large number of microorganisms that colonize body sites such as skin and intestine. The composition of this so-called "microbiota" is highly variable and influenced by nutrition, immune competence, illness and use of medication, especially antibiotics. The recently established HZI department "Microbial Immune Regulation" headed by Till Strowig is interested in enhancing the understanding of how these microbial communities affect human infectious diseases and how they can be manipulated to treat diseases.

Prof. Strowig, how would you explain the function of the microbiota and what has been found out about it to a lay-person?

The microbiota comprises a diverse ecosystem of bacteria, fungi and other small eukaryotes that have a tremendous metabolic potential. They are in a very close interaction with the host and, more specifically, also the host immune system. The functions of the microbiota are diverse. On the one hand, there is the potential degradation of dietary components and the production of a specific type of metabolites that can have health-promoting functions. On the other hand, the microbiome can fight or prevent colonization with bacteria that cause diseases. Recent research suggests that if this community is impaired in its functions, a diverse set of diseases can evolve. They range from inflammatory diseases, for example in the intestine, to even alterations in behavior.

The field of microbiota research is comparatively young …

Yes, when you apply a narrow definition. Microbiota research is a hot topic since about ten to fifteen years. But the existence of the microbiota and part of its function have been known for much longer. As early as in the fifties, it has been shown that transplantation of gut microorganisms from a healthy patient can cure certain forms of severe diarrhoea.

Why has the field been expanding so enormously in the recent years?

This was mainly due to technical progress. Advanced technology for the analysis of genomes and metagenomes has made it possible to determine the exact composition of the bacterial ecosystem in an individual. As a consequence, it became clear that the microbiota plays a decisive role in the onset of many diseases – the metabolic syndrome is just one example. Another example is cancer medication: therapies with so-called checkpoint inhibitors can only be successful when certain defined bacterial species are present in the host microbiota. At least this has been shown in mice. But it can be assumed that there are similar mechanisms in place in humans.

What has led you in your scientific career towards this subject?

I started out as a classical infection researcher, an immunologist. I studied the interplay between a specific pathogen and the immune system. In the course of these projects, we also discovered that the microbiota has an influence on some of the phenotypes we have studied. And I was really fascinated by the complexity and diversity of these microbial communities. How they are actually interacting with the immune system – not triggering classical infections or inflammatory responses, but rather co-existing with the host.

"I was fascinated by the complexity and diversity of these microbial communities"

The name of your department here at HZI, which started last year as a full department, is "Microbial Immune Regulation". What does that mean?

In our department, we are interested in understanding how microbes – not specifically pathogens, but rather the micro-

biota in the intestine – interact with the immune system, and how this regulatory process affects the susceptibility to infections or other immune-mediated diseases.

Are you focusing on certain types of immune cells and on certain gut bacteria?

Yes. In our research, we are, to a certain degree, focused on the interplay between so-called helper T cells with the microbiota. These helper T cells have important regulatory functions, both in the intestine as well as systemically. Their functions range from suppression or regulation of immune responses to antibacterial or antiviral effects. In the worst case, these effects can get out of control and even promote the development of autoimmune diseases.

"There is the option of developing the microbiome into a novel anti-infective"

And on the side of the bacteria?

We are concentrating, among others, on the bacterial species *Prevotella copri*. An interesting microorganism that is present in about 20 to 30 per cent of humans in the industrialized world. In countries where sanitary infrastructure and hygiene standards are lower, a much larger proportion of individuals carries the bacteria in them. *Prevotella* may be associated with diseases like rheumatoid arthritis, but our understanding of its role is still incomplete.

What do you think HZI can contribute to the field of microbiota research?

HZI is an institute with a strong background in microbiology, in microbiological metabolism, and in natural compound research. This opens up a tremendous potential to make a big impact on understanding how microbial functions contribute to different types of diseases. There is, on the one hand, the option of developing the microbiome into a novel anti-infective, or to achieve anti-infective effects by manipulating it in a targeted manner. On the other hand, we can also explore how we can use the microbiome to treat other diseases that are affecting a large proportion of the German population, such as age-related diseases. This is an approach which we are pursuing in cooperation with scientists from other Helmholtz Centres.

What are the most important goals in your research for the next five to ten years?

The goals for the next years are in two big areas. One is trying to get to a functional understanding of specific groups of microbes which have been associated with diseases. And on the other hand, we need to take the step and focus on our research towards application in humans. This is the translational aspect of our research: determining how we can actually modulate the microbiome and thus activate protective functions against infections.

Interview: Manfred Braun

Photos: © HZI | Verena Meier

"PREVENTIVE MEDICINE IS THE MEDICINE OF THE FUTURE"

INTERVIEW WITH CARLOS ALBERTO GUZMÁN, HEAD OF THE DEPARTMENT "VACCINOLOGY" AT HZI

HZI's leading vaccine researcher, Carlos A. Guzmán, plays a central role in several multinational research consortia aiming to develop novel vaccines and immunization strategies. He coordinates projects dealing with vaccination against hepatitis virus and *Trypanosoma* infections, respectively. The establishment of a European consortium for research into a novel universal influenza vaccine is currently being negotiated. Guzmán's interest focuses primarily on adjuvants, i.e. substances that boost immune responses in the host, as well as on understanding the mechanisms behind differential responses to vaccination in humans.

Prof. Guzmán, when did you decide to dedicate your scientific career to vaccine research?

I started as a physician in clinics, and working there, I soon understood: the best medicine is prevention. Even in cases where good therapies are available, we are not always able to prevent the patient from suffering or developing sequelae. Furthermore, there are often significant costs, direct and indirect, which are associated with a disease. So, if we want to apply the best strategy in medicine, we need to prevent diseases from occurring. I am convinced that preventive medicine is the medicine of the future. Particularly considering the ever-expanding costs associated with disease diagnosis, treatment and rehabilitation. Effective vaccines can also prevent the deaths, stagnation of economy, and block of mobility and commerce associated with pandemics like COVID-19.

And in the case of infectious diseases, prevention means vaccination?

Not necessarily. A significant improvement can be achieved by measures that do not seem very glamorous at first glance, but have a tremendous impact: clean water, sanitation, safe food. But more specifically, we can also apply vaccines. Using them, we can – in the most effective scenario – prevent a particular infectious disease lifelong. It is not only a very efficient approach, but it is also a comparatively cheap one, when we consider the costs associated with the therapy of diseases that often will be iteratively taking place during the life of an individual.

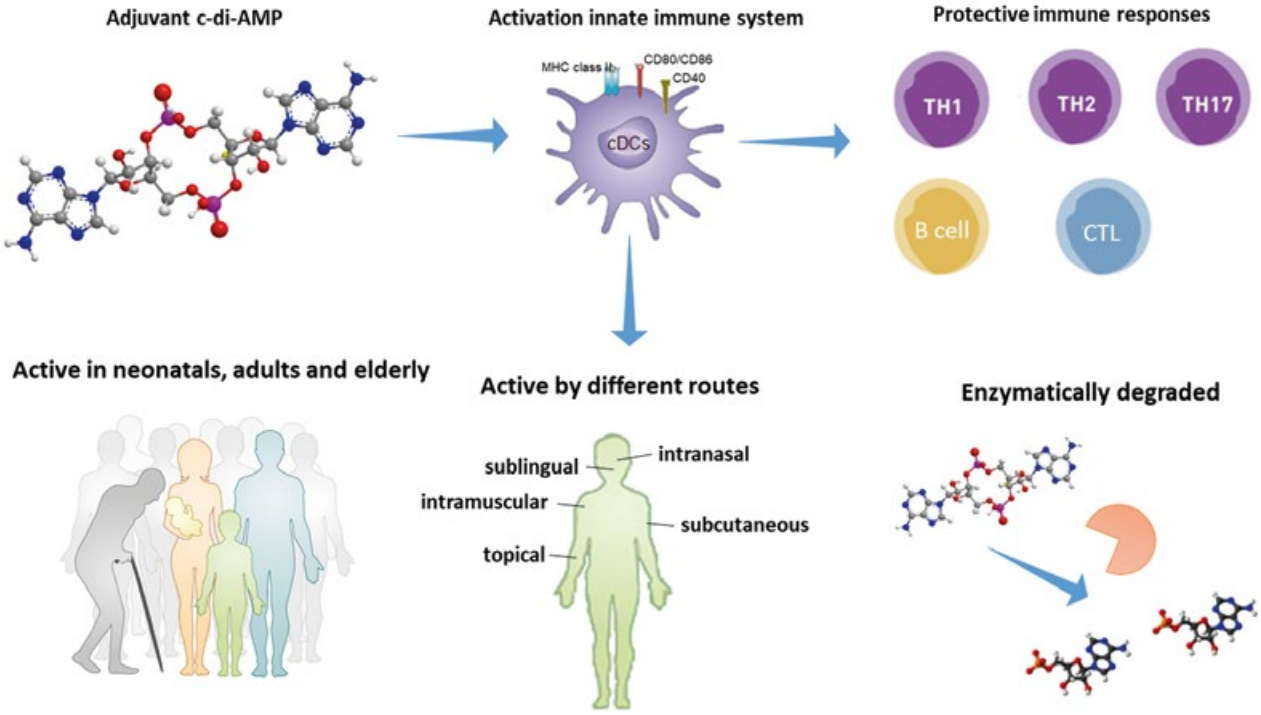
"Vaccination is not only a very efficient approach, but also a comparatively cheap one"

In your research, you are focusing particularly on adjuvants – substances that augment or modulate immune response. Why?

Traditionally, vaccines have been generated by killing or attenuating pathogens. When administered, they evoke an immune response in the host's organism which, in the best case, protects against future infections. Meanwhile there is a trend towards subunit vaccines. Instead of whole organisms, we use only the subcellular components that promote protective immunity. This often requires enhancement by adjuvants. Adjuvants also enable us to modulate the quality of the elicited response, thereby maximizing efficacy and reducing potential risks.

Why are the effects in subunit vaccines so different from those in traditional vaccines?

In inactivated bacteria, for example, we have not only many potential antigens – molecules that can generate a specific immune response – but also structures, such as nucleic acids and components of the cell wall, which have intrinsic adjuvant properties. This means they are able to stimulate so-called pattern recognition receptors of the host's immune system, thereby enhancing immune responses. In subunit vaccines, on the other hand, we only have one or few antigens, which are highly purified and too clean to promote an efficient response. Of course, the safety profile of such a vaccine is increased, because we know exactly what we are administering. Unfortunately, they are lacking components



A versatile compound enhancing the efficacy of vaccinations: the adjuvant molecule cyclic-di-AMP or CDA.

promoting efficacious and long lasting immune responses post vaccination.

So you have to add an adjuvant in order to reach a protective effect?

Even in cases when they are not absolutely indispensable to improve the strength of the response, adjuvants allow us to use less antigen in order to promote protection. That is important for example in pandemics, when there is often only a limited amount of antigen available that can be used for vaccine production. If you can reduce the dose ten-fold, you have ten times as many doses. Adjuvants can also modulate the immune response: Depending on the adjuvant that I am using I can promote, for example, stronger stimulation of either killer cells or antibodies.

So you can selectively trigger the most effective kind of immune response against a given pathogen?

Yes, and you can also reduce the likelihood of adverse events. That is very important, since vaccines are aimed at healthy individuals and therefore there is no tolerance for side effects. Any adverse event will be perceived by the vaccinee as the vaccine not being good enough. And this will lead to a lack of confidence in the intervention and a reduction in the uptake, even if it is working.

"If you can reduce the dose ten-fold, you have ten times as many doses"

What kind of adjuvants are you working on at HZI?

We have studied several substances with a high potential as adjuvants and different modes of action. One particularly promising candidate right now is the cyclic-di-AMP, or CDA. It is a messenger molecule in bacteria and plays a key role in cell-to-cell communication. Our studies have also shown that CDA exerts strong adjuvant activities when delivered via parenteral or mucosal routes.

Why do publicly funded basic research institutes engage in vaccine research? Could it not be left to industry alone?

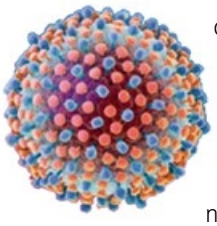
I think that the interaction between academic institutions and industry is a win-win situation. In the area of vaccine development, many of the discoveries that lead to innovative vaccines or efficient technologies for vaccine development are spinning from academic research. In academia, there is a huge potential in terms of early development of vaccine candidates. Industry, on the other hand, has a very strong track-record in bringing these as products into the market. There are many examples in which joint activities in the framework of public-private partnerships, even very early in the process of vaccine development, were extremely successful. They also can de-risk early projects, making them more attractive for industry.

And why is HZI suited to engage in this field?

The knowledge, technologies and infrastructure needed for vaccine development are extremely complex and wide-ranging. At HZI we have a long-standing and broad expertise. We have infrastructure in the area of vaccinology, as well as vaccine candidates that are under development in our own pipeline. We have technologies, such as needle-free delivery systems, adjuvants and live vectors; and expertise in the areas of antigen optimization, preclinical and clinical development. Furthermore, we are partnering with academic and industrial groups for the development and testing of specific vaccine candidates. Finally, we are part of the European Commission-funded TRANSVAC 2 consortium providing technology and services in the area of adjuvants and preclinical validation in small animal models to groups in Europe and worldwide.

You are planning to establish a vaccine research centre at HZI. What would that look like?

We are already providing state-of-the-art infrastructure and technology for vaccine development to academic and industrial partners, as well as our own research groups. We also have strong activities in antigen and adjuvant discovery, antigen delivery based on nanotechnologies, structural vaccinology, immunomonitoring, bioinformatics and systems vaccinology, and identification of biomarkers for vaccine responsiveness. We have a clinical research centre in which we have carried out investigator-initiated vaccination trials. All this can be bundled so that we can establish a value chain from early preclinical development up to first-in-human studies.



Whole virus vaccine

So the vaccine research centre will require cooperation with many partners. Will it nevertheless be located on the HZI campus?

I perceive this centre as having a main core located at the HZI campus, which encompasses the management and strategic planning, as well as critical platforms. These platforms include, among others, animal facilities, sequencing, immunomonitoring, immunoprofiling and protein production. Expertise concerning adjuvants, structural vaccinology and immunology will be located at the HZI campus, which will also host supporting administrative teams for technology transfer, legal affairs and public relations. In addition, there will be dedicated hubs for specific areas and critical infrastructure. For example, nanotechnologies and formulation at HIPS, RNA biology at HIRI, systems vaccinology and artificial intelligence at CiiM, biomarkers, regulatory sciences and trial design at Twincore. Clinical trials and bio-banking activities will be conducted at CRC, metabolomics and mathematical modelling at BRICS, and advanced proteomics at the future Helmholtz institute in Greifswald.

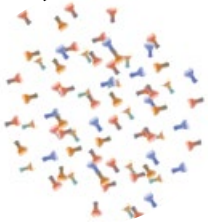
So there will be branches of the vaccine centre all over the country?

Yes, almost by definition the Vaccine Centre will have a dislocated network structure rather than a unique brick-and-mortar building, since many of these fields require their own particular infrastructure or proximity to the clinic. Furthermore, the establishment of the centre will be a dynamic process in which core groups will be set up in the next two or three years. And then, in a modular fashion, we will expand certain key activities, for example, in the area of immunoprofiling of vaccines. And we will incorporate new blocks, such as formulation. It will be a living evolving process, such is the need in this dynamic field. But the core expertise already exists at HZI.

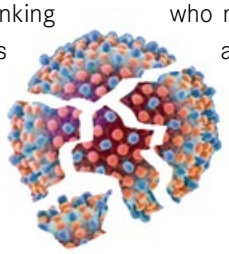
What are the most important goals you want to have reached in the next years?

In the next couple of years, we want to reach first-in-human studies with one of our top adjuvants, CDA, in the context of vaccine candidates against different infectious diseases. We also want to proceed further in the area of nanotechnologies – microcarrier-driven delivery of vaccine antigens or RNA vaccines in a non-invasive form across the mucosa or skin. More generally, we also want to make progress in understanding why certain individuals do not respond well to specific vaccines. For example, the conventional influenza vaccines are effective by 70 to 80 per cent in young adults. But when we are looking at elderly individuals, over 65 years, approximately less than 20 per cent are responders to vaccination. This knowledge is critical to develop diagnostics to know in advance who will truly benefit from the vaccination and who needs to be protected in a different manner. It will also allow to establish new innovative approaches to promote effective immune responses to vaccination in poor responders. This would really be a big step forward in preventive medicine.

"We want to make progress in understanding why certain individuals do not respond well to specific vaccines"

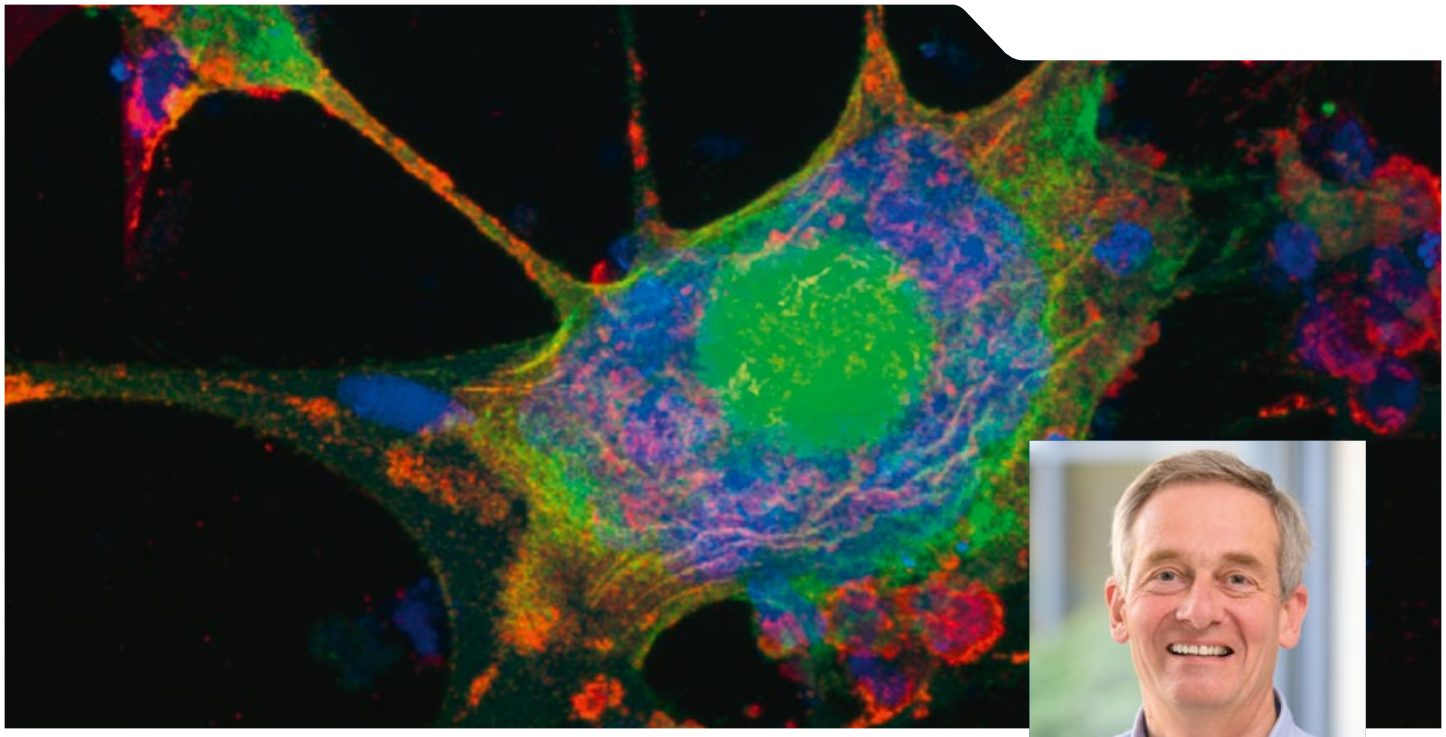


Subunit vaccine



Split virus vaccine

Interview: Manfred Braun



Under attack: Fluorescence microscopy of a human cell infected with Varicella zoster viruses.
Source: Abel Viejo-Borbolla, Carina Jürgens, Shuyong Zhu, MHH-Virologie

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"WE WANT TO UNDERSTAND THE REASONS FOR THE DIFFERENT TYPES OF IMMUNE DEFECTS"

**INTERVIEW WITH THOMAS SCHULZ,
SPEAKER OF THE CLUSTER OF EXCELLENCE RESIST**

Following an infection, some people become only mildly ill, whereas others are affected severely or even killed by the same pathogen. What are the reasons for these differences? Understanding the factors that determine defensive weaknesses is the goal of the Cluster of Excellence RESIST, short for "Resolving Infection Susceptiblity". RESIST started in 2019 and is coordinated by Hannover Medical School (MHH) in close cooperation with HZI research teams. Thomas Schulz, Head of the Institute of Virology at MHH, presides over the RESIST Speaker Team.

Prof. Schulz, why are you so particularly interested in infection susceptibility?

People with an increased vulnerability to infections represent a very important group among our patients. MHH is one of the largest transplant centres in Germany and the biggest lung transplant centre in Europe. So our clinicians deal with immunocompromised transplant patients on a day-to-day basis. In addition, they take care of several other risk groups: patients with rheumatological diseases, with primary immunodeficiencies, premature children, very old persons. All these individuals have mild to severe defects in dealing with infectious agents. Some of them are very specific – the herpes virus infections in transplant recipients for example, or *Pseudomonas aeruginosa* in people with cystic fibrosis. Individually different defensive weaknesses against these pathogens pose an important clinical problem. This is why the idea to apply for a Cluster of Excellence on this subject developed.

"Analysing large and defined patient groups plays a central role"

What are the aims of RESIST?

First, we want to understand the reasons for these different types of immune defects – be they inborn, that is, the result of inherited genetic errors; be they of iatrogenic origin, the result of old age, earlier infections, premature birth or differences in the maturation of the immune system. Then we intend to find new approaches to minimize the risk for these patients. Either by treating the cause of the immune defect, or by tackling the pathogens that play a role in these specific groups, for example Pseudomonas and Herpes viruses, as I mentioned before.

How are you going to achieve this?

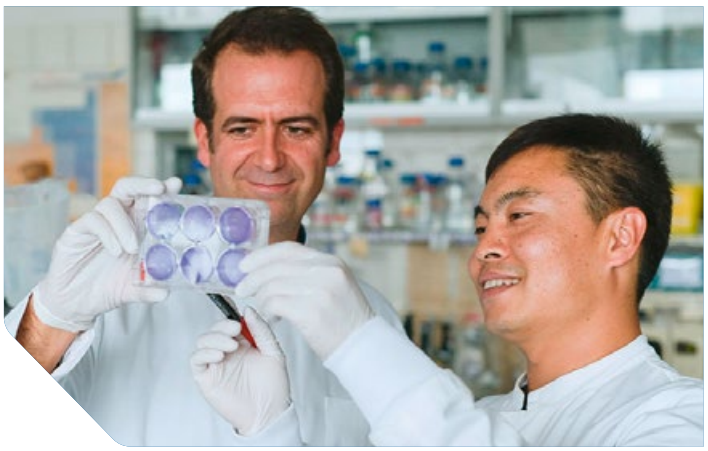
We bring together a group of people with different types of expertise. We involve, of course, clinicians from different fields of medicine, bacteriologists, virologists, immunologists. We also bring in people with expertise and an excellent track record in human genetics, in structural biology – this includes HZI's branch institute CSSB –, in drug research and in primary immunodeficiencies. The principal investigators are drawn from MHH, from HZI, from Twincore, as well as from a couple of neighboring institutions. Some of these colleagues have already collaborated very successfully in our joint collaborative research centre, the *Sonderforschungsbereich* SFB 900, which investigates microbial persistence. This big RESIST consortium will drive forward our programme in pursuing selected specific questions concerning infection susceptibility. The programme provides the best strategy of playing to our local strengths and tackling questions that we are in a particularly good position to solve.

You are addressing a very broad range of problems. How do you make sure that you are not dispersing your efforts?

We start with a broad programme, but we will narrow it down in about two years' time with the help of our scientific advisory board. The idea is that everybody gets a chance to drive forward their original ideas, and we will reassess after two years, and focus on fewer, selected projects. Maybe we will also rebalance the different parts of the programme. But we think that a broadly-based programme in the beginning is a strength. It promotes exchange between disciplines. And it illustrates that there are several fields which we can address particularly well, because we already have expertise and elaborated research infrastructure for it.

Can you give an example?

Analysing large and defined patient groups plays a central role in the RESIST cluster. During the review process, the reviewers commented very positively on the patient cohorts we can make available here. There are, among others, large rheumatology cohorts, a very well characterized cohort of premature babies and large groups of patients with primary immunodeficiencies.



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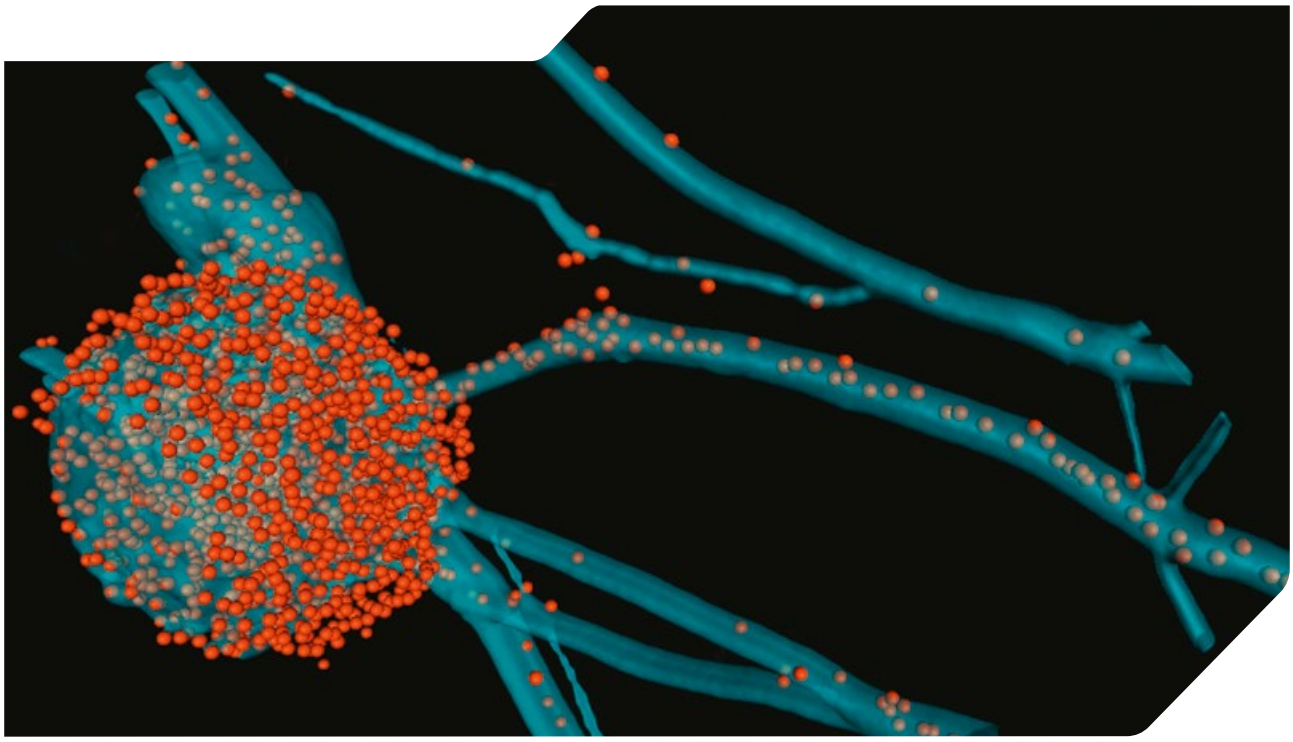

Herpes viruses (shown in red) in neurons (blue), made visible by fluorescence microscopy.
Source: Anna Buch, Beate Sodeik & Rudolf Bauerfeind, MHH

These cohorts already exist since several years. Are you also planning to establish new ones?

Yes. One of them will consist of elderly individuals, drawn from the Hannover/Braunschweig region. These are, in principal, healthy individuals. With them, we will try to understand in what way the elderly immune system differs from the young immune system. Where do the holes in the immune defense appear, and what are the typical weaknesses in an elderly person? Why does a particular person not respond so well to vaccination, for example against influenza? These are questions we address, and on the basis of our results we hope that it will be possible to provide new ways to, for example, change the vaccines so that people might respond better.

You have assembled a range of researchers from different institutions. What is the main asset which HZI brings into the Cluster of Excellence?

HZI is key. Its strengths range from molecular virology in the RNA virus field – here at MHH we work with DNA viruses – to microbiome studies, drug research, structural biology and several other relevant disciplines. Very important is the systems biology and systems medicine approach, particularly in the CiiM which MHH and HZI have founded together and which is still going to be expanded in the upcoming years. HZI provides cutting-edge technologies and complements MHH's clinical and research expertise with its distinguished profile in basic research. We really couldn't do this without the involvement of HZI.

RESIST is funded for seven years – then you can apply for a second round. What is the most important result you want to have achieved after the first period?

I would like to see the group of principal investigators that make up RESIST growing together to a unit – to such an extent that we can capitalize on what we have built. Infrastructures that we have built in RESIST, new cohorts that we have assembled. Then we can think bigger and address new questions in the infection field – correlations with other, non-infective diseases for example. In any case, I am sure that particularly the cohort of elderly individuals I mentioned before will yield important insights – for infection research and beyond.

Interview: Manfred Braun and Hansjörg Hauser ■

HZI AND EVOTEC ESTABLISH A LONG-TERM PARTNERSHIP

JOINING PLATFORMS AND FORCES FOR NOVEL ANTIBIOTICS

Together with the drug discovery and development company Evotec SE, HZI aims to jointly develop derivates of the natural product cystobactamide into a new class of broad spectrum antibiotics targeting high-priority pathogens.

The discovery of antibiotics - substances that specifically intervene in bacterial metabolism and can kill or inhibit bacteria - was undoubtedly one of the most important achievements of medicine in the 20th century. Antimicrobial resistance or AMR is the natural ability of microorganisms to become insensitive to an anti-infective drug. As a result, standard treatments become ineffective, infections persist and new resistance mechanisms may rapidly spread. Therefore, AMR represents one of the biggest threats to global health today.

One strategy to address this major health threat is the development of new antibiotics with novel modes of action that are effective against drug-resistant bacterial pathogens. In order to develop such new drugs and overcome AMR, Evotec SE and HZI established a collaboration in February 2019.

Evotec is a drug discovery alliance and development partnership company focused on rapidly progressing innovative product approaches with leading pharmaceutical and biotechnology companies, academics, patient advocacy groups and venture capitalists. The company operates worldwide and has more than 2,500 employees. Evotec has built a broad and deep pipeline of approximately 100 co-owned product opportunities at clinical, pre-clinical and discovery stages, available for technology transfer.

Research activities in the HZI/Evotec partnership will initially be focused on the optimization of cystobactamides, a family of natural antibacterial products with innovative chemical scaffolds that are active against the most dangerous Gram-negative pathogens on the WHO priority list.

Under the terms of their agreement, Evotec and HZI will collaborate for an initial period of 3 years. The collaboration will combine the unique collection and know-how of natural products at HZI/HIPS as well as the centre's access to *in vitro* and *in vivo* models of bacterial infection with Evotec's leading drug discovery platform, expertise in medicinal chemistry and pharmacology as well as world-leading collection of bacterial pathogens.

In 2019, HZI/HIPS and Evotec have secured substantial project funding from the Bill & Melinda Gates Foundation (project name NP Drug Discovery) and from the BMBF (project name: OpCyBac) for the discovery and optimization of novel antibacterial compounds. The partners are in the process of founding a spinoff company called "Myxobiotics", which is expected to raise venture capital and start operations in 2020.

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FROM BENCH TO BEDSIDE: INNOVATION AND TRANSLATION

SEED MONEY, PARTNERSHIPS, SPINOFFS: TECHNOLOGY TRANSFER AT HZI

HZI aims to turn discoveries from its laboratories into innovations, thus transferring research results into pharmaceutical and clinical application. To achieve this, the centre is committed to sustainable technology transfer to third parties, including small- and medium-sized enterprises as well as industrial companies. HZI has established a structured process for professional technology transfer which is based on internal and external competence. As a specific measure to advance projects at an early stage, HZI has set up an internal innovation fund with substantial co-funding from the Helmholtz Association. Promising developments are already visible. Industry partnerships shall take the projects to the next level. A particularly important partnership of this kind was established in 2019.

The HZI product portfolio comprises compounds for both treatment of and protection against infections as well as novel diagnostic methods and digital tools – e.g. apps – for infection control and prevention. The technology transfer strategy of HZI is designed to reach higher "technology readiness levels" (TRLs) for selected projects. The TRL of a product indicates how close it is to application in medical or pharmaceutical practice.

Innovations in infection research are usually adopted by industry, if at all, at relatively late and advanced developmental stages. Therefore, bridging the gap between fundamental research and more advanced stages of development remains a continuous challenge. Early innovations need to be taken to a higher TRL before being transferable to industry. HZI has established a structured process for technology transfer to meet these requirements. It involves the strategic management of intellectual property (IP) rights, licensing and cooperation with industry partners and also fosters close collaboration between internal scientific and administrative experts. An external professional partner, Ascenion GmbH, supports HZI in this essential part of its mission.

A newly established Technology Development Board (TDB) consisting of internal and external experts meets quarterly to discuss and maintain an active portfolio of six to eight HZI projects. These projects are selected from a pool of competing proposals submitted by HZI scientists. Each selected

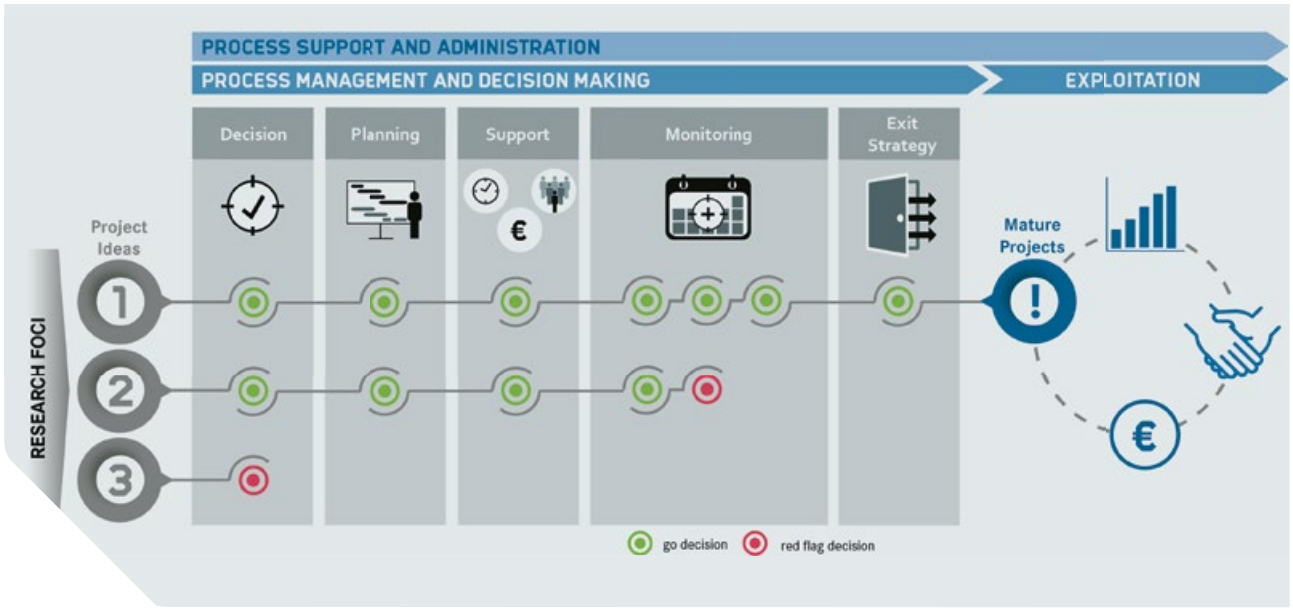
project receives a development plan with a specific target product profile; this plan includes value inflection points jointly defined by board members and project leaders. The Board critically monitors the progress of the projects and makes recommendations regarding further steps. Projects that have reached a certain TRL will be offered to industry.
HZI has set up the innovation fund Pre-4D – "4D" stands for "Drugs, Diagnostics, Discovery and Development" – for internal funding of the most promising projects. Pre-4D is equipped with 0.5 million Euro per year.

After internal application for Pre-4D funding, potential projects undergo a quick and transparent evaluation by internal and external experts. Final decisions about funding are made by HZI management. In 2019, five new research projects were selected for funding. In all these projects, TLRs have been increased.

Some of these projects have even been developed to become eligible for follow-up funding. HZI's dedicated resources for the selected projects are complemented by additional funds from such sources as the Helmholtz Association, the German Federal Ministry of Education and Research, the European Union, the German Centre for Infection Research DZIF, the Bill & Melinda Gates Foundation and the Global Antibiotic Research and Development Partnership (GARDP). In several cases, priority constituting patent applications have been filed and first potential commercialization partners have been identified.

HZI's project exit strategy comprises several elements. In addition to licensing inventions to industry or developing them further through cooperation, the centre sets up new companies (spinoffs) for the acquisition of venture capital. HZI has established a start-up-friendly environment for investment management, with minority shareholdings, tailor-made licensing agreements and personal support for entrepreneurs including return options. For scientists interested in turning their discoveries into spinoffs, Ascenion provides start-up coaching on behalf of HZI. In addition, a start-up roadmap is being developed and has already been implemented in part. It offers educational workshops, the development of standard deal models and management support to lower the hurdles for potential founders.

One particularly successful example of a company co-founded by HZI is Vakzine Projekt Management GmbH (VPM). Originally established to bridge the gap between basic research and vaccine development and to advance selected vaccine candidates towards clinical testing, the company



Scheme of HZI's structured technology transfer process. Innovative projects are regularly reviewed by HZI's Technology Development Board, the most promising projects are transferred to the next stage (see text for details).

has gained international visibility. VPM's main project is a new vaccine against tuberculosis which has reached an advanced state. In 2018, VPM was acquired by the world's largest vaccine development company, Serum Institute of India (SII).

Strategic partnerships with industry enhance HZI's technology transfer, speeding up translation into products. The centre is establishing industry cooperations with a long-term perspective, particularly in the field of anti-infective drug research. To facilitate this process, HZI has systematically recruited scientists from industry and the biopharmaceutical sector.

In 2019, HZI and the drug discovery alliance company Evotec SE jointly raised project funds and entered into a strategic partnership to accelerate the development of innovative antibiotics (cystobactamids) against multidrug-resistant Gram-negative pathogens. In addition, HZI is actively discussing cooperations with other biopharmaceutical companies.

In spring 2019, the start-up company WBC Drug Delivery Technologies GmbH (WBCT) was founded with the participation of Ascenion in order to further develop a drug delivery technology that had been jointly invented by scientists at HZI and its Saarbrücken branch, HIPS. The technology was exclusively licensed to the start-up. WBCT was acquired by the Swedish Klaria Pharma Holding AB in a share deal in September 2019.

Besides spinoffs and joint ventures with industry, participation in big publicly funded consortia is another way of carrying HZI's inventions and discoveries further towards application. The potential use of cyclic-di-AMP or c-di-AMP as an adjuvant to boost the efficacy of vaccines (see also interview with Prof. Carlos Guzmán in this report), for example, was investigated with support from the internal fund Pre-4D. Now, application of c-di-AMP in vaccines is part of the research programmes of multinational EU projects like "Cruzivax".

Wherever possible, part of the revenue from technology transfer is directed back towards fundamental research for public benefit. In particular, the charitable foundation Life-Science-Stiftung uses incomes from Ascenion's IP management to fund research projects. Funds from the aforementioned sale of VPM, for example, are now used to support new projects on infection research.

Michael Strätz

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PhD students in discussion during the break of a meeting at HZI, © HZI | Verena Meier

HZI'S CAREER DEVELOPMENT: SUPPORT FOR SCIENTISTS ON ALL LEVELS

Targeted recruitment of talent and development of careers strengthens the core research fields of the centre and covers relevant new areas. This includes the strategic recruitment of excellent senior scientists as department heads and of promising young researchers as group leaders, postdocs and doctoral researchers.

The new programme "HZI Careers" offers a structured career development process for all employees. Next to continuous appointment of senior and junior scientists, the centre takes responsibility for opening up new career perspectives and supporting all its employees during each stage of their professional lives (see figure next page). HZI offers training opportunities at all competence levels ranging from scientific seminars and workshops to various courses. Moreover, it encourages and supports participation in personal coaching and leadership seminars to empower and prepare employees for positions of responsibility. Specialised programmes for the promotion of junior scientists, such as structured PhD programmes as well as a Clinician Scientist and a Young Investigator Group programme, support HZI's aim to train and foster a new generation of infection researchers.

Appointments of Group and Department Leaders

The Young Investigator programme of HZI is well established and renowned among young researchers in the field. Successful careers of previous Young Investigator Group leaders at universities, other research organisations or in companies serve as a good indicator for success of the recruitment and development strategy at HZI. Since the establishment of the programme, more than ten former group leaders have already obtained W3 professorships. From the start at HZI, Young Investigator Group leaders are fully independent. To support their career development, they are affiliated with a department, whose head serves as a scientific and personal mentor, and are encouraged to develop their own leadership skills. Young Investigator Group leaders are usually co-appointed as W1 professors at HZI partner universities, where they advance their teaching skills. In 2018/2019 seven new Young Investigator Groups have been established, led by Alexander Westermann, Neva Caliskan, Antoine-Emmanuel Saliba, Redmond Smith, Olga Kalinina, Chengzhang Fu and Mathias Munschauer. Based on positive votes of an international review panel and the HZI-internal steering committee, Young Investigator Group leaders may – in accordance with

HZI's internal tenure track guidelines – ultimately receive tenured positions. Tenure has been granted to three former Young Investigator Group leaders, Melanie Brinkmann, Ingo Schmitz, and Luka Čičin-Šain.

During 2018/2019, HZI was able to hire four outstanding senior scientists at full professorship level (Chase Beisel, Anna Hirsch, Till Strowig and Yang Li) and one at assistant professorship level (Luka Čičin-Šain). In most of these cases, competing offers of a university professorship could be countered. The appointments have strengthened core research and help to develop new disciplines.

Postdoctoral Training

For the continuous enhancement of skills and competences of HZI scientists, the centre offers courses and training opportunities, such as the "Management Curriculum" that prepares participants for future steps in their career. An online training course on "patents, inventions and innovations", developed by Ascenion, builds know-how of intellectual property and technology transfer. It is compulsory for all scientific staff and is completed with an online exam.

HZI lays special emphasis on the career development and support of young postdocs. For this purpose, a programme consisting of target-group-specific coaching and seminars is offered. For example, the TRAIN Academy (see box below) offers a 2-year extramural programme on translational research and medicine for postdocs and scientists to widen the participants' career opportunities. The HZI Graduate School also invites postdocs for career workshops, talks and mentoring. Moreover, the management financially supports the postdoc initiative "Did-it", specifically furthering the interests of postdoctoral scientists.

While postdocs usually have transient positions at HZI, the centre has established a tenure system for the most promising talents. Associated with departments, their function is to

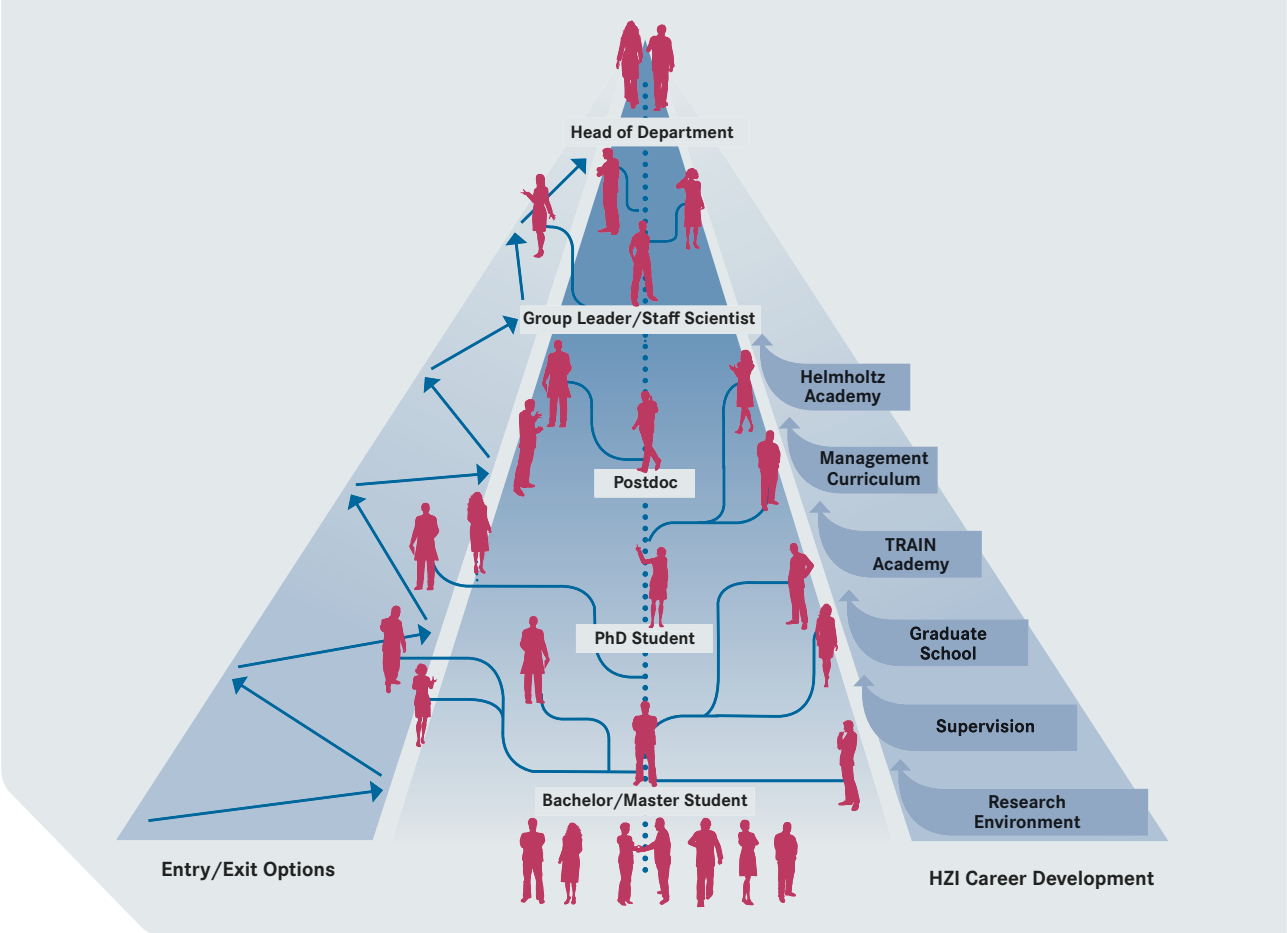


Figure 1: Career opportunities at HZI: A structured process provides development options on every career stage.

keep and develop critical expertise and technologies. Their selection is competitive and performed through a structured process.

PhD Training

Career development is an essential part of the training of young researchers during the completion of their PhD work. To support decision making processes and prepare them for the next step of their career, the Helmholtz Graduate School for Infection Research (GS-FIRE) has developed a structured PhD programme. GS-FIRE offers a variety of lectures, transferable skills courses and several opportunities to establish national and international interdisciplinary networks. The school attracts applications worldwide and, as a consequence, has implemented a competitive three step selection process that ensures the choice of the very best applicants. The management of GS-FIRE is supervised through an executive committee consisting of a permanent member of staff and six scientists representing HZI's research disciplines. GS-FIRE regularly invites renowned international scientists for symposia, workshops and biennial summer schools.

At HZI, all doctoral researchers participate in structured PhD programmes including the GS-FIRE programme or other graduate curriculae, jointly organised together with partner universities. Such curriculae include the joint PhD programme "Infection Biology" (with MHH and TiHo), the Graduate School for Biomedical Data Science (with TU-BS at BRICS), the internationally unique graduate programme RNA & Infection (with University of Würzburg at HIRI) and the PhD programme for epidemiology (with MHH). For all doctoral researchers, thesis committees with annual meetings are compulsory. Structuring of the PhD curricula supports an efficient completion of PhD theses.

A successful self-organising forum of all doctoral researchers at HZI, the doctoral initiative "Do-it", provides a framework for contacts, communication, and dedicated meetings. Do-It is supported by the HZI management.

High-School Student Laboratory

In 2002, the high school student laboratory "BioS" has been established on the campus of HZI. Students from the Braunschweig/Hannover region and all over Germany perform up-to-date experiments in the field of infection research and biotechnology embedded into the environment of a real research centre. 19 different courses reflecting the current state of science have been established. Meanwhile, more than 30,000 students in 1,800 courses have performed experiments in the BioS laboratory. The direct contact to scientific lab work in a large research centre is perfectly suited to stimulate the interest of the next generation for science in general and for life sciences in particular.

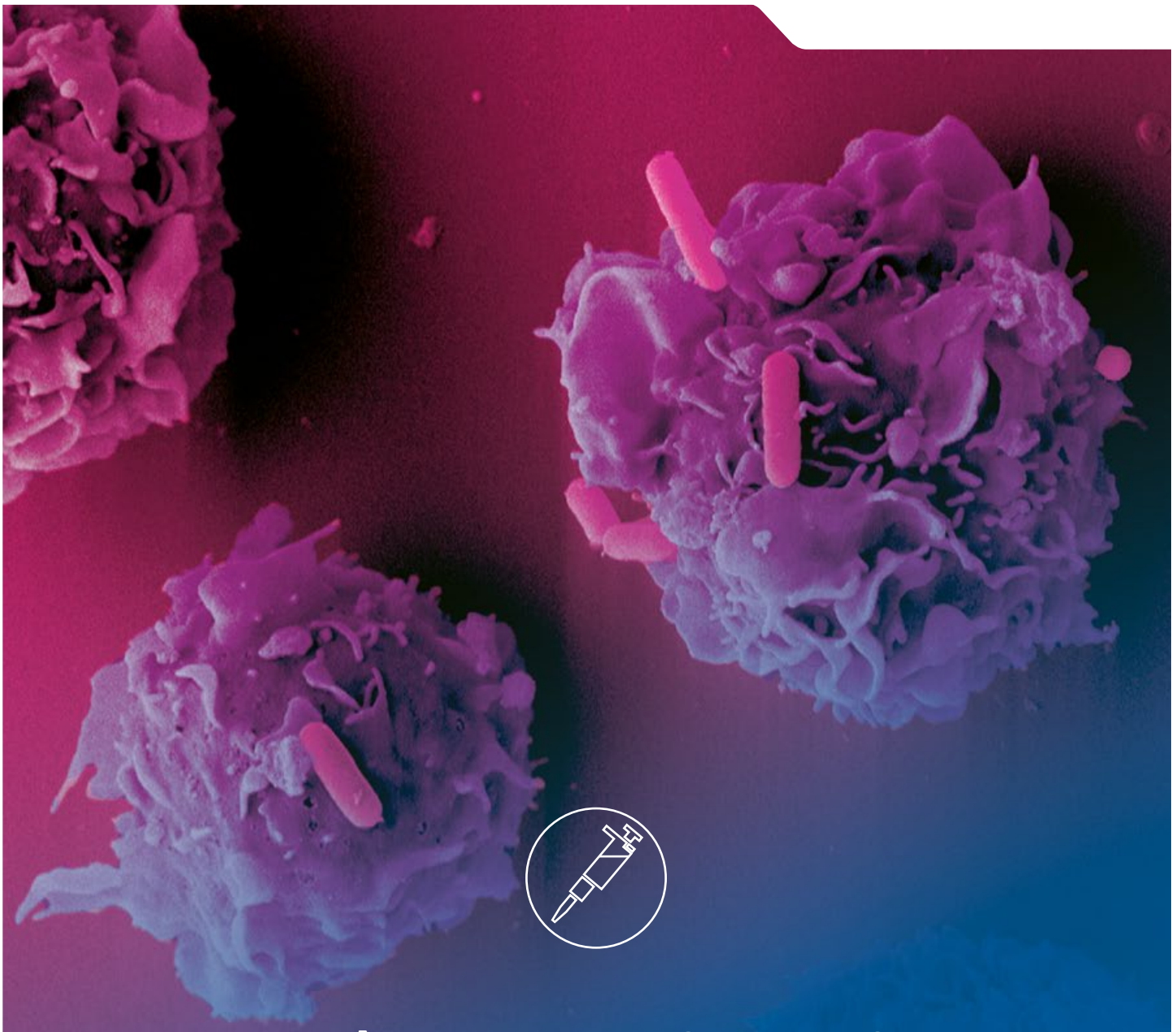
Careers and Families

The option to combine career and family commitments has high priority in HZI's career development concept. HZI has established a range of measures to support families and dual career options at all recruitment levels. Measures to support families include flexible working time, home office, temporary reduction to part-time employment, re-employment after maternity or parental leave, the reservation of places for employees' children in a nearby kindergarten, children's holiday care and child-care services for evening meetings, lectures and other functions.

Equal Opportunity and Diversity

The recruitment and advancement of women into leading positions is a major goal for HZI. Currently, 21% of the W3 professorships and 43% of the W2 professorships are held by women. This is significantly higher than the average at universities. A future goal is to increase the proportion of female scientists in the Young Investigator programme. Therefore, HZI will actively encourage women to participate in the selection process. Gender equality has been successfully established in the group of PhD students and non-scientific personnel.

In every respect, HZI recognises and values individual differences in race, gender, sexual orientation, socioeconomic status, age, physical ability, and religious belief. The high international diversity at HZI is reflected by employees and guest researchers coming from more than 40 countries.



HZI'S RESEARCH FOCI

NEW STRATEGIES AGAINST RESISTANT PATHOGENS

RESEARCH FOCUS AMR – ANTIMICROBIAL RESISTANCE

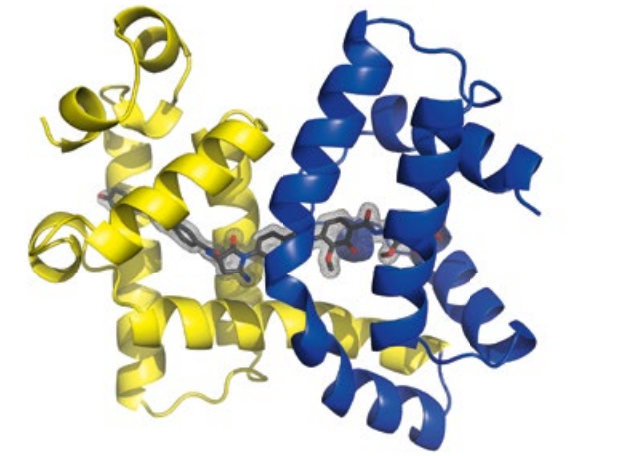
The increasing occurrence of antimicrobial resistance is a severe challenge, particularly in the light of the scarcity of new antibacterial candidates in drug discovery. Scientists in the Research Focus AMR combine expertise in various fields and long-standing experience in industry or industrial-academic 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 optimizing 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 optimize treatment regimens and use approved antibiotics more effectively?
- How can antimicrobial compounds reach their targets more efficiently?

1. PATHOGEN AND RESISTANCE PROFILING

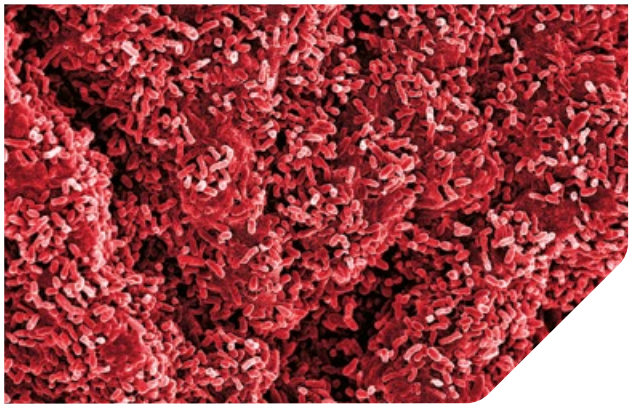
In light of rapidly emerging resistances, our pathogen profiling efforts are aimed at obtaining a comprehensive understanding of pathogenicity mechanisms during infection and colonisation of the human host. Based on the genetic characterization of clinical isolates from infected patients by means of bioinformatic analysis, we aim to advance molecular diagnostics and to detect resistance and other pathogenic traits in bacteria early on, so that suitable therapy options can be chosen in time.



Part of a bacterial resistance mechanism: the structure of the protein AlbA in complex with albicidin was elucidated by HZI scientists © Jesko Köhnke

We introduced BACTOME as a database system that links genetic data from clinical isolates of the pathogenic bacterium *Pseudomonas aeruginosa* with clinically relevant phenotypes of the pathogen. DNA sequences and gene expression values have been recorded for a plethora of clinical isolates to identify common traits shared by most *P. aeruginosa* strains. Deviations from this profile demonstrate the plasticity of the species *P. aeruginosa*.
The database is available at https://bactome.helmholtz-hzi.de.

The Gram-negative pathogen *Klebsiella pneumoniae* is the most common cause of bacterial sepsis and hospital-acquired inflammations of the lung but can also elicit urinary tract infections. Klebsiella bacteria often develop resistance to anti-infectives, for instance the antibiotic albicidin which is based on a natural product. Elucidation of the structure and function of the protein AlbA revealed that it binds albicidin with high affinity. This property of AlbA makes it a decisive component of the resistance mechanism. RF AMR scientists have proposed a possible mechanism for the reaction between AlbA and albicidin.



Biofilm of *Pseudomonas* bacteria. Biofilms make pathogens more resistant against treatments. Promising drug candidates are investigated with the aim to interfere with bacterial biofilm formation. © Mathias Müsken

2. NEW ANTI-INFECTIVES FROM NATURAL PRODUCTS

Bacterial and fungal natural products are the most important sources of novel anti-infectives. It is assumed that the producer organisms synthesize these substances in order to keep unwanted competitors, that is bacterial species in the same habitat, at bay. RF AMR researchers have created a steadily growing, sustainable pipeline of exploratory drug candidates that includes compounds from the bacterial groups *Actinobacteria* and *Myxobacteria* as well as from fungi of the division *Basidiomycota*.

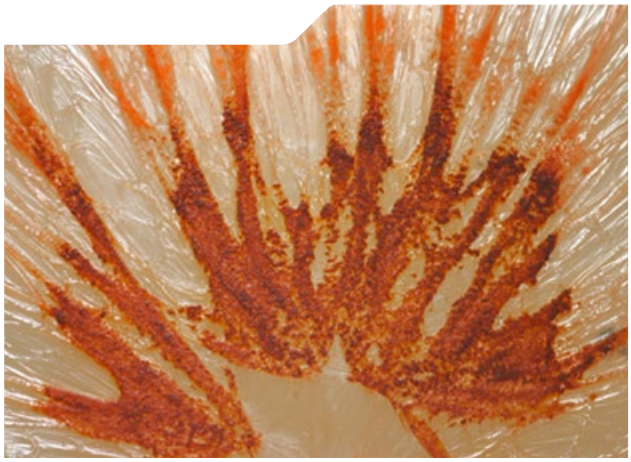
Correlating chemical diversity with taxonomic distance

By screening both known and unknown myxobacterial natural products, RF AMR scientists were able to reveal a striking correlation between taxonomic distance and the production of natural products with different chemical properties. Accordingly, the probability of discovering yet unknown compounds is significantly higher in so far unknown or underexplored bacterial species than in previously known strains. This knowledge will be used to prioritize novel isolates for subsequent analysis regarding the production of interesting natural products (*see also "Highlight Publications" in this report*).

New inhibitors from myxobacteria

Scientists in RF AMR identified the pyxidicyclines, a class of molecules produced by soil-dwelling myxobacteria, as potential antibacterial compounds. Pyxidicyclines act as selective inhibitors of topoisomerases I and IV – two enzymes essential for the survival of bacteria. The myxobacteria themselves are resistant to pyxidicyclines.

As these resistance-conferring genes are often located in close proximity to gene clusters encoding the corresponding antibacterial natural product, RF AMR scientists were able to discover the pyxidicyclines.

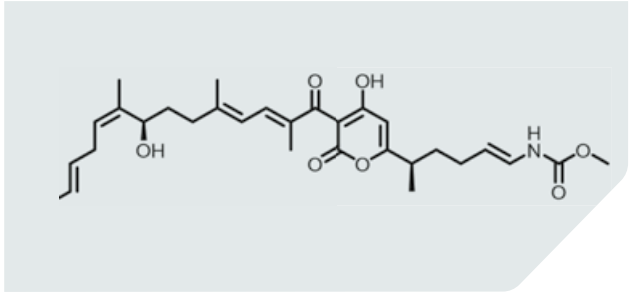


Cystobacter velatus, a myxobayterium producing the compound Cystobactamid which shows promising antibacterial activity.

Corallopyronin A, a potent anti-filarial antibiotic

Corallopyronins are promising lead compounds for the development of novel antibiotics, owing to their ability to inhibit the enzyme RNA polymerase in bacteria, which is essential for protein production and thus for the survival of bacteria. These compounds show activity against nematodes – filarial worms – by killing their endosymbiotic bacteria. These microorganisms live inside the worms' cells and are crucial for their survival.

As chemical synthesis is not a viable option for this highly potent compound class, RF AMR researchers focused on its biotechnological production by growing bacterial strains producing corallopyronins in large amounts. RF AMR researchers are experts in determining the right conditions to grow these strains and established large fermentation capacities for this purpose. In this case, a previously reported production and engineering platform was used and improved resulting in high yields of corallopyronin A.



Corallopyronin A, an antibacterial compound investigated by scientists in RF AMR.

3.OPTIMIZATION OF DRUG CANDIDATES

In order to develop novel anti-infectives, inhibitors of carefully selected drug targets have to be discovered and further optimised. These include "classical" targets – essential biomacromolecules whose inhibition kills the pathogen or stops its growth – as well as antivirulence or "pathoblocker" targets. Interfering with the latter can selectively block certain pathogenic traits of bacteria or viruses, like the formation of biofilms, which make bacteria more resilient against therapies.

Medicinal chemistry in compound optimization

For several natural and synthetic antibacterial compounds we have reached lead status, i.e. these compounds show activity likely to be therapeutically useful but still have to be optimized.

One of the most promising classes of anti-infective molecules discovered and further developed by RF AMR scientists are the cystobactamids. Produced by *Cystobacter*, a genus of myxobacteria, they display promising activity against Gram-negative bacteria, which are particularly difficult to treat. Their mode of action is promising. The cystobactamids are so-called "gyrase blockers" – they prevent the bacteria from unwinding their DNA in a way that is required for reproduction. Gyrase blockers have already been successfully used as antibiotics in clinical settings but many bacteria have become resistant to them. Cystobactamids, on the other hand, are still effective against such microorganisms.

New synthetic derivatives showed high potency against *Acinetobacter baumannii*, a frequent cause of hospital-acquired infections, and other pathogens. This made it possible to further optimise the compound class to produce clinical candidates with the aid of a public-private partnership.

The mechanism of action of another class of natural anti-infectives, the labyrinthopeptins, was identified by RF AMR researchers. Labyrinthopeptins eliminate virus particles by binding to the viral membrane. This leads to activity against a broad spectrum of pathogenic viruses including respiratory syncytial virus, cytomegalovirus and Kaposi's sarcoma virus. Resistance develops only at very slow rates.

PqsR is a molecule essential for biofilm formation by *Pseudomonas aeruginosa*, a pathogen endangering cystic-fibrosis

patients in particular. Successful formation of a biofilm makes the bacteria more resilient to therapeutics and thus more difficult to treat. Optimised lead compounds engage the target PqsR *in vivo* and show a favourable safety profile. To complete their optimization, we are now testing these compounds in three infection models (acute lung, acute thigh and chronic wound infection).

For several other targets, we have identified optimised hit compounds, i.e. molecules that show the desired type of activity in a screening assay.

These include new inhibitors of a major virulence factor of the pathogenic bacterium *Staphylococcus aureus* as well as new selective compounds targeting particular transporter molecules in the bacterial cell membrane of a spectrum of Gram-positive pathogens.

Synthetic variation of an anti-cancer drug (sorafenib) yielded a novel antibiotic which exhibited activity against multi-resistant *Staphylococcus aureus* (MRSA) and could even eliminate established biofilms.

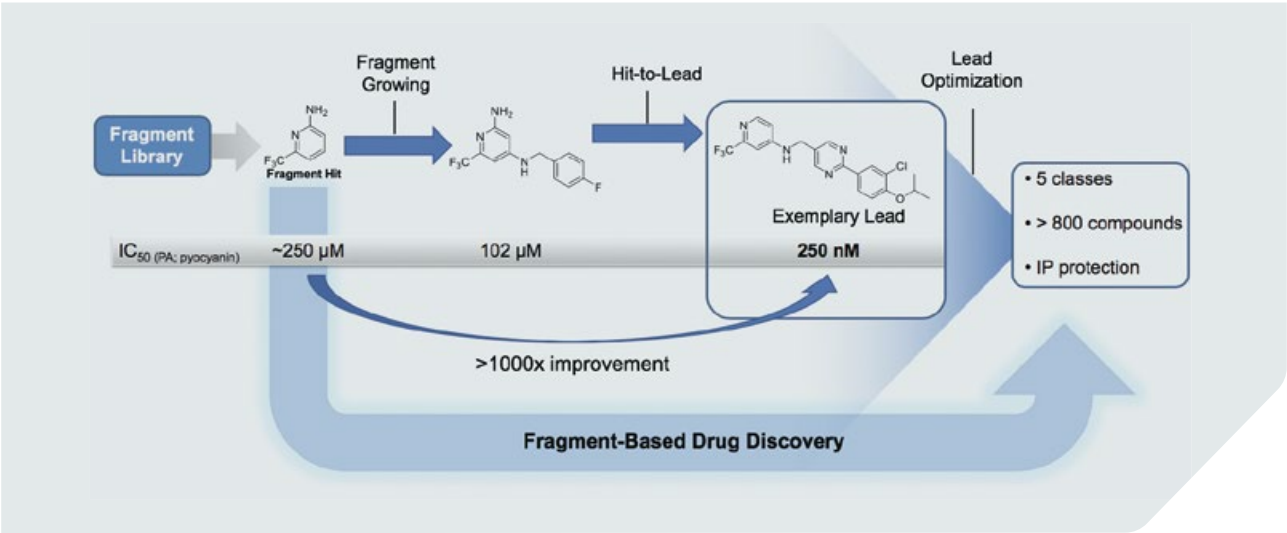
Furthermore, AMR researchers discovered that lignin, a key compound of wood, is a rich source of anti-infective scaffolds. As part of a collaboration with the University of Groningen in the Netherlands, we exploited novel scaffolds generated by a novel catalytic method directly from lignocellulose obtained from woodchips. The resulting lignin derivatives display encouraging initial activity against Gram-positive bacteria.

Establishment and extension of the PK/PD unit

Once new agents and target sites have been identified, the active compound's pharmacological properties must be optimized for clinical use.

HZI has set up a Pharmacokinetics/Pharmacodynamics (PK/PD) Unit with the support of DZIF (see chapter "DZIF" in this report). This infrastructure makes it possible to study the therapeutic efficacy and dosage of novel antibiotics. New drug candidates are thoroughly characterised and tested for their cytotoxicity in cell cultures. Only those candidates that pass these *in vitro* tests are further investigated *in vivo* in pharmacological trials. The data from these trials make a crucial contribution towards the translation of research results into clinical application.

In recent years we have extended the range of animal models studied in our PK/PD Unit. One of the new models simulates lung infections caused by ESKAPE pathogens, a group of bacteria showing increased resistance to commonly used antibiotics. The natural process of airborne infection is mimicked by using an inhalation device.



Steps of drug discovery in HZI's Research Focus AMR: Promising molecules are modified and optimized. The inhibitory concentration ("IC50") is lowered, the efficacy of the compound thus improved.

4. DRUG DELIVERY

In order to be effective, drugs have to reach their site of action. This often necessitates the penetration of biological barriers, be they the mucous membranes of the host or the cell walls and cell membranes of bacteria. The outer membrane of Gram-negative bacteria is particularly difficult to penetrate and protects the bacteria from several drugs and detergents.

We have successfully employed a diverse set of innovative approaches to improve the delivery of lead compounds or preclinical drug candidates across the various biological barriers between the site of administration, e.g. by inhalation, and their site of action. Innovative models mimicking natural biological barriers may also help to reduce the number of animal experiments required to study the uptake of drugs by a host organism.

Extracellular vesicles from myxobacteria were found not only to be biocompatible but also to display inherent activity against Gram-negative bacteria as they are naturally loaded with the antibacterial natural product cystobactamid. We have also shown that these vesicles may overcome the cellular barrier and can kill intracellular *Staphylococcus aureus* infections.

We have successfully developed novel nanocarriers, i.e. nanometre-sized particles used as transport modules for a drug. We harnessed these nanocarriers for the targeted delivery of drugs or antigens in order to combat or prevent infectious diseases and studied their interaction with human immune cells.

Mupirocin is an antibiotic with a unique mode of action. It is active against methicillin-resistant *Staphylococcus aureus* (MRSA) but its clinical use is restricted to topical administration because of its limited stability and rapid degradation in blood plasma. We have demonstrated that encapsulation of mupirocin in lipid nanoparticles (nanoliposomes) allows this antibiotic to be used parenterally for the treatment of MRSA infections.

To understand features that determine uptake of compounds by Gram-negative bacteria, we have built new models that measure drug translocation in a quantitative manner. A new *in vitro* model of the Gram-negative cellular envelope makes it possible to study the transport of drugs and delivery systems across this important biological barrier. This is complemented by a method that quantifies drug concentration in different parts of the cells of pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, or *Proteus mirabilis*.

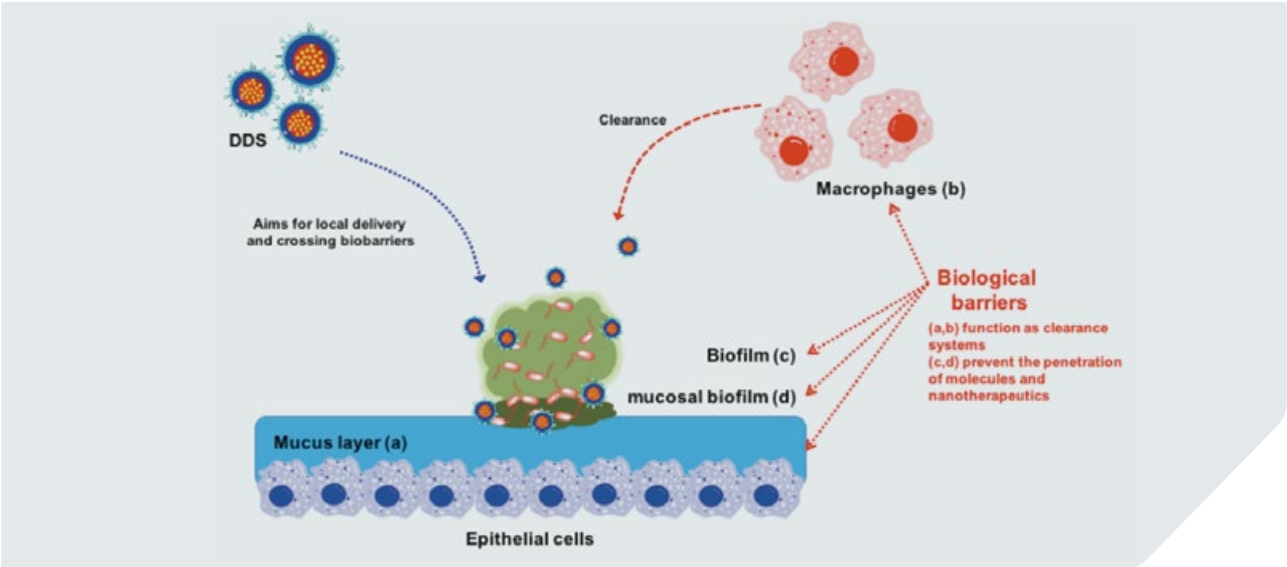


Figure 4: Biological barriers, such as epithelia and bacterial cell envelopes, often prevent drugs from reaching their site of action. Drug delivery systems (DDS) deliver the drug to its site of action, crossing biobarriers if necessary. Source: From Ho et al., Eur. J. Pharm. Biopharm.; 144: 110-124 (2019). Reprinted with kind permission from the publisher.

PERSPECTIVES

In the future, RF AMR will continue to tackle the current AMR crisis from a range of angles. Researchers at HZI will further 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 intends to impact both basic research and clinical translation by 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.

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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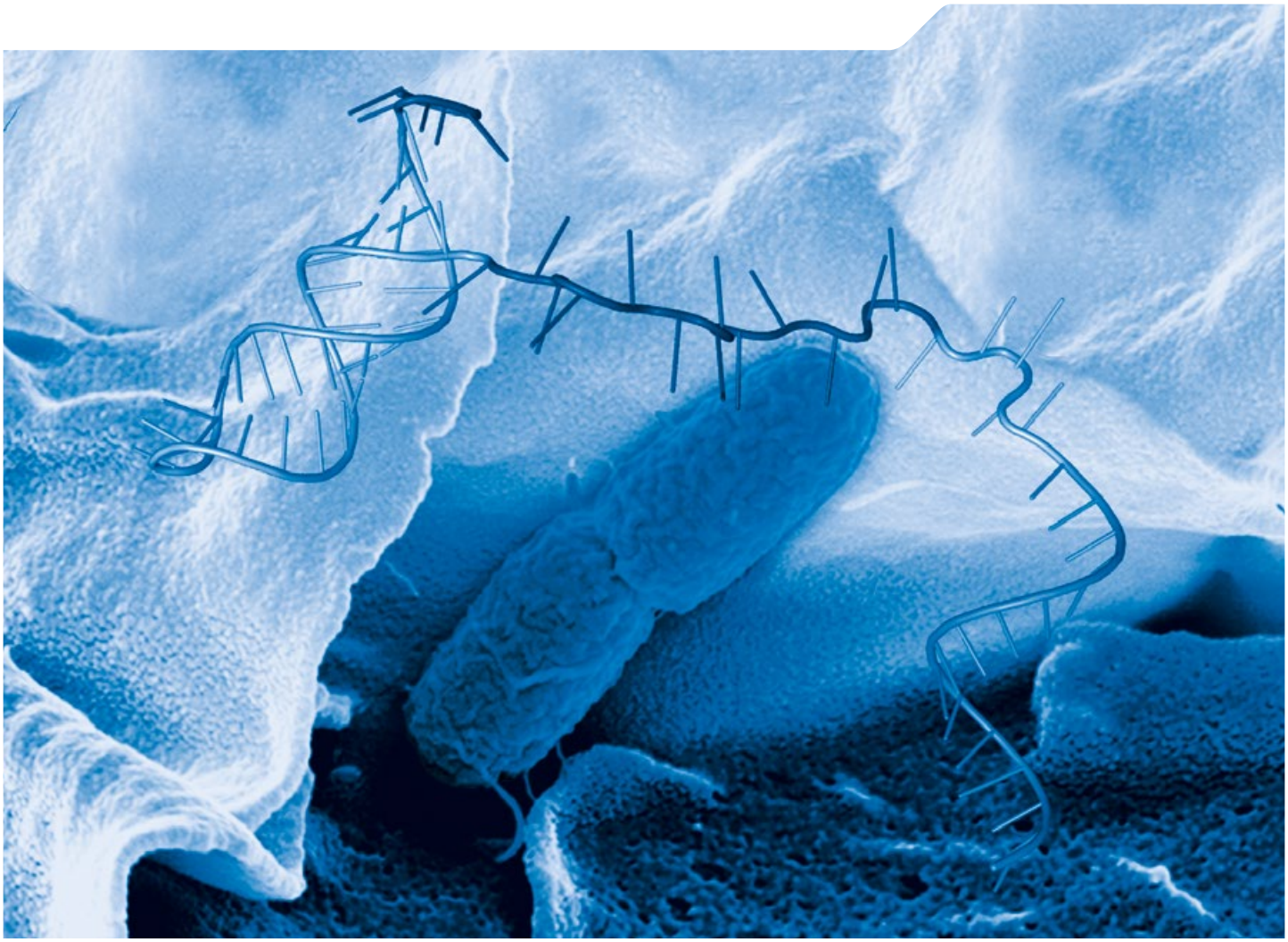


UNDERSTANDING AND INFLUENCING BACTERIAL INTERACTIONS

RESEARCH FOCUS MICO – MICROBIAL COMMUNITIES

The Research Focus MICO combines expertise to characterize and influence the complex interactions between pathogens, our immune system and the large collection of commensal bacteria colonizing the gut and other parts of the human body. This microbiota comprises a vast array of microbes, the gut alone harbors more than 1,000 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, has long been considered 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 visualize 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-centered 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 so far. For example, we have applied it to infected immune cells to study how they are influenced by the

KEY WORDS IN SHORT:

RNA
RNA, short for "ribonucleic acid", is a macromolecule involved in coding and reading genes or in protein synthesis. RNA is 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 an essential role in many regulatory mechanisms in the cell.

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

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

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

Microbiota
The entirety of microorganisms in an ecosystem, here: in the body of humans or other multicellular organisms. The term "microbiome" refers to the entirety of genes of a bacterial community, but the terms "microbiome" and "microbiota" are often used interchangeably.

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 defense mechanisms of bacteria against viruses and other foreign invaders.

DNA transformation
Process of transferring foreign DNA into a cell.

pathogenic bacterium *Salmonella*. We could show that *Salmonella* modulates immune cells in different fashions leading to a reduced immune response and persistence of the pathogen in the body.

Beyond simple single-cell transcriptomics, it is our ambition to adapt other powerful RNA-sequencing techniques to study the regulation of RNA molecules in single bacteria. The long-term goal is high-throughput RNA biochemistry in single bacterial cells, pathogens and commensals alike.

The grand challenge for microbiology now is to also establish single-cell RNA-sequencing for bacteria, which pose specific problems for the application of this technique such as containing small amounts of RNA. Transcriptomes from single bacteria will help to better understand phenotypic resistance to antibiotic treatment, persister formation under harsh conditions, and phenotypic diversification for bet hedging, to name a just a few applications. Once established, single-cell RNA-sequencing in bacteria would also allow for single-cell dual RNA-sequencing, in which pathogen and host are analyzed simultaneously. Researchers of HZI's branch institute in Würzburg, HIRI, have now 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*.

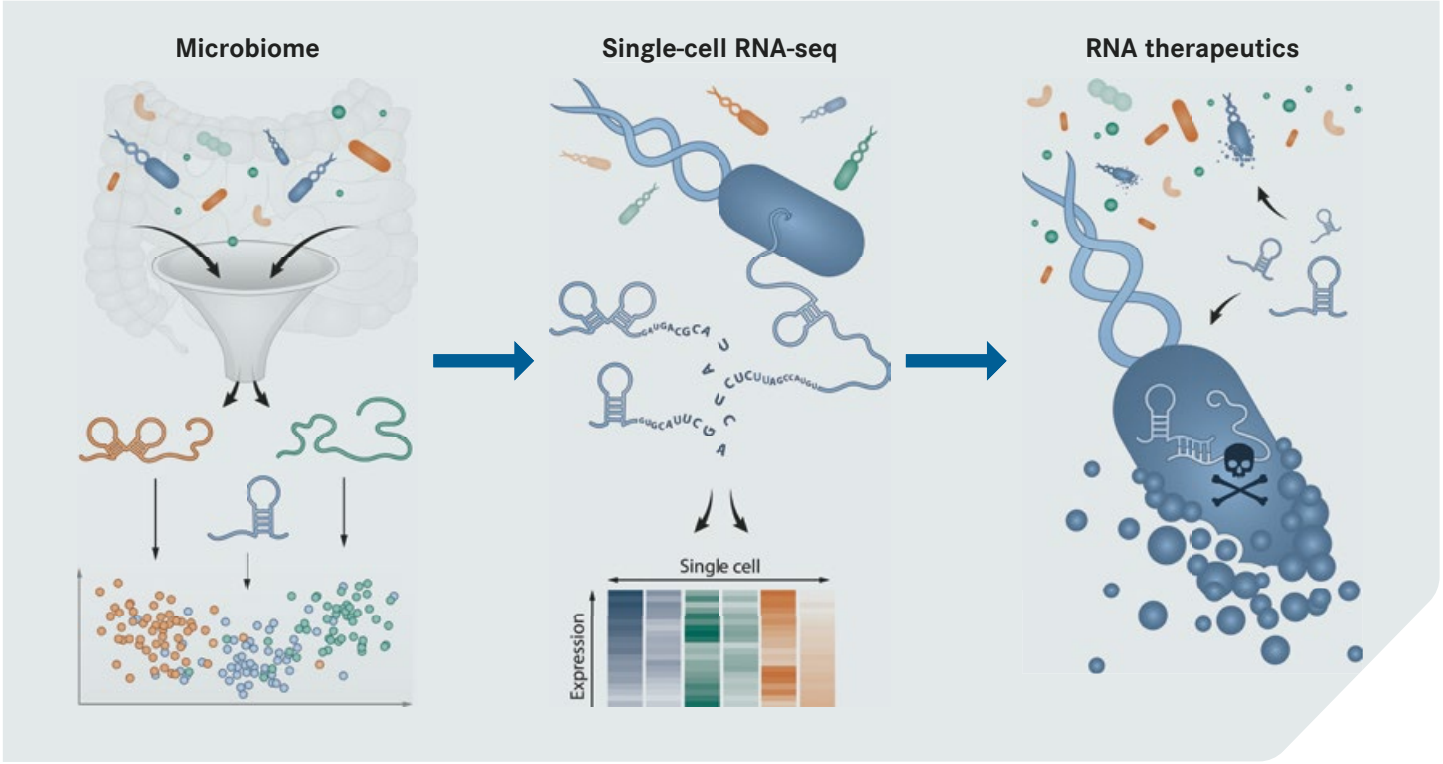


Figure 1: Utilising transcriptomics to counteract pathogens: Studying the microbiota at single-cell resolution using advanced sequencing techniques paves the way to targeting specific species within the large bacterial communities in the gut and other parts of the human body. Source: HIRI

2. INTEGRATED CHARACTERIZATION 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 recognized 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 uncharacterized 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.

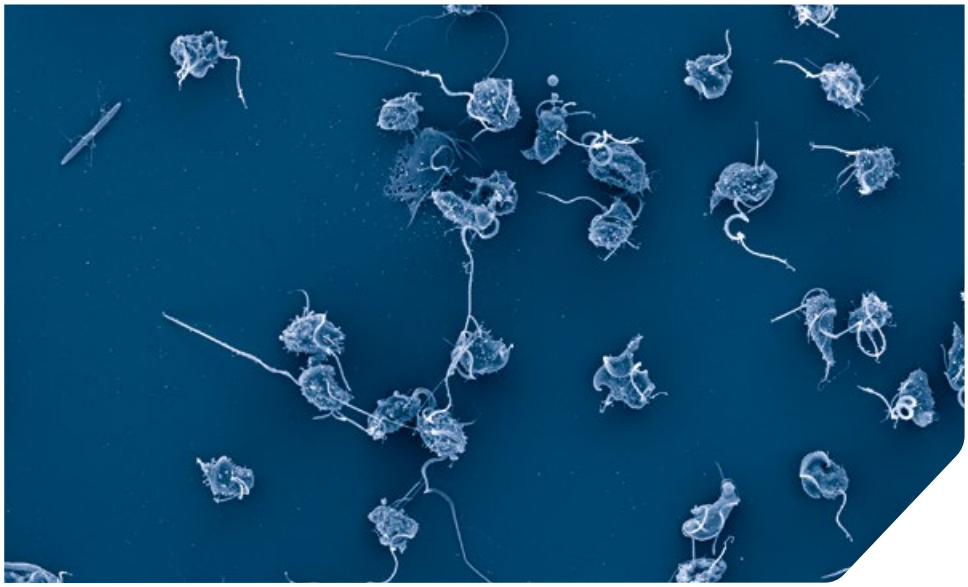
Therefore, HZI scientists have employed bioinformatic approaches to comprehensively identify bacterial species that have been previously associated with diseases. To further develop and benchmark computational approaches to analyze composition and functions of the microbiota, innovative bioinformatic tools were developed, e.g., to simulate large realistic datasets as standards for international community-driven competitions.

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 standardized approaches to characterize the microbiota as well as the integration of clinical data is specifically important in the context of human cohort studies. Using this knowledge, specific patient cohorts were studied in close collaboration with clinical partners to evaluate the influence of infections and disease state on microbiota composition. In order to investigate the relevance of disease-associated microbial signatures, gnotobiotic animal models – germ-free animals selectively colonized with known strains of bacteria – are an essential tool. 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 uncharacterized 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.

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 utilizing 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.

MOST IMPORTANT QUESTIONS ADDRESSED BY RF MICO:

- How does the microbial population in the human body, especially 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 and edit it with single-species resolution?
- 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?



Tritrichomas musculis, a protozoon in the murine microbiota which influences immune responses of the host.

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 characterized, 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 recognize 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.

As a step toward achieving efficient editing in members of the gut microbiota, HZI scientists developed and applied genome editing tools in the probiotic strains of *Lactobacillus plantarum*. The tools were applied to generate critical mutants that helped reveal how adaptation to the host's diet likely played a role in the existing symbiosis between Lactobacilli and fruit flies.

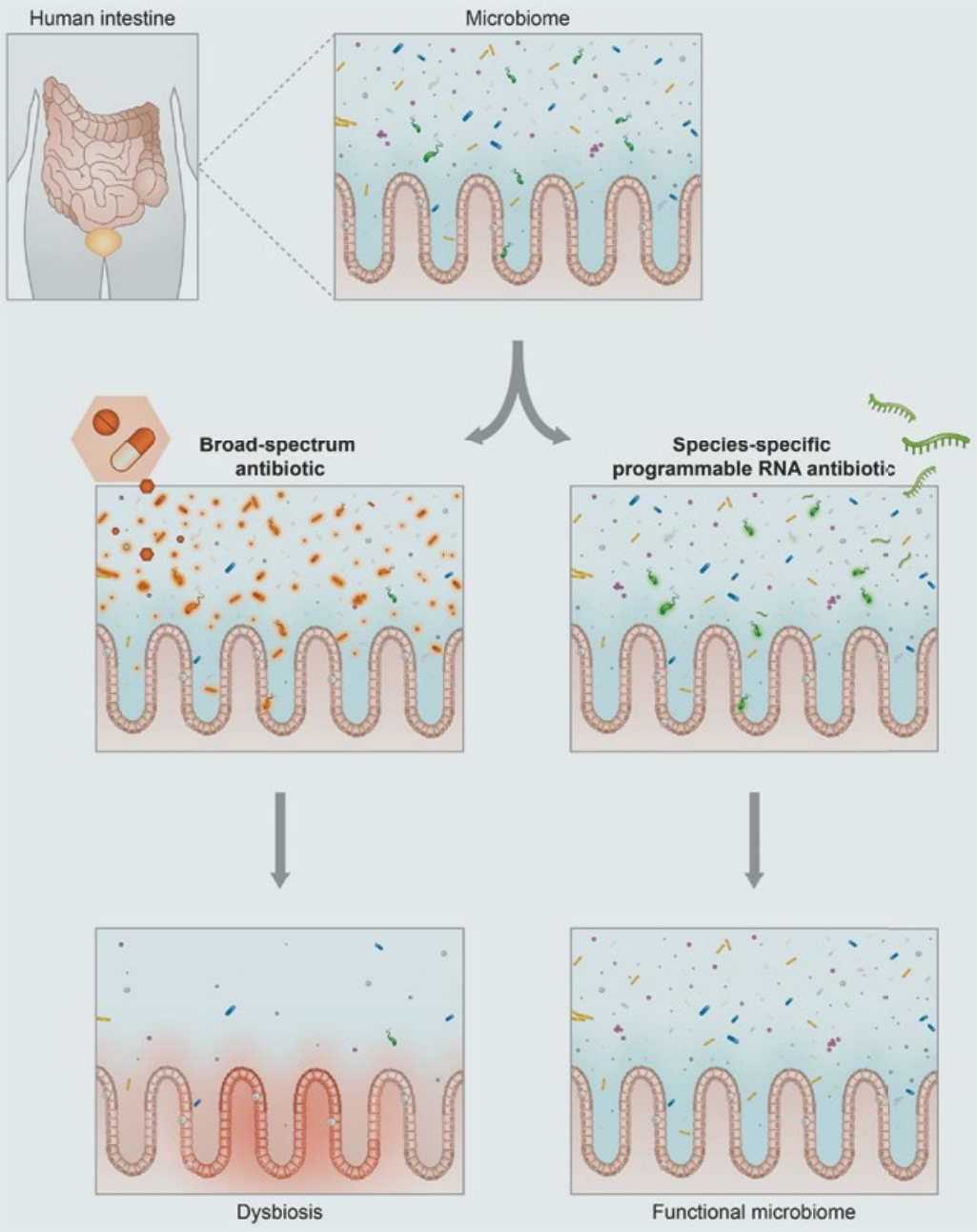


Figure 2: Most current antibiotics are broad-spectrum, meaning that their application to the microbiota (here: intestine, could also be skin or other body part that is populated by microbes) will kill many more bacterial species than just the one of interest (bottom left). Species-specific programmable RNA antibiotics promise true microbiome editing, eliminating only the very species of interest and leaving the rest of the microbiota undisturbed (bottom right). © Wiley , Molecular Microbiology. 2020;113:550–559.

Genetic traits are often conferred not by a single gene but collections of genes that act synergistically. Identifying these genes represents a major challenge given the number of possible combinations of genes to probe. HZI scientists took a major step toward resolving this challenge with the development of a simple and scalable assembly scheme for generating CRISPR arrays. These arrays allow multiplexed targeting of genes with different CRISPR technologies, paving the way for high-throughput genetic screens that can begin untangling genetic interactions and rapidly identify genes for further interrogation and drug design.

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. 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 personalized 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 visualize 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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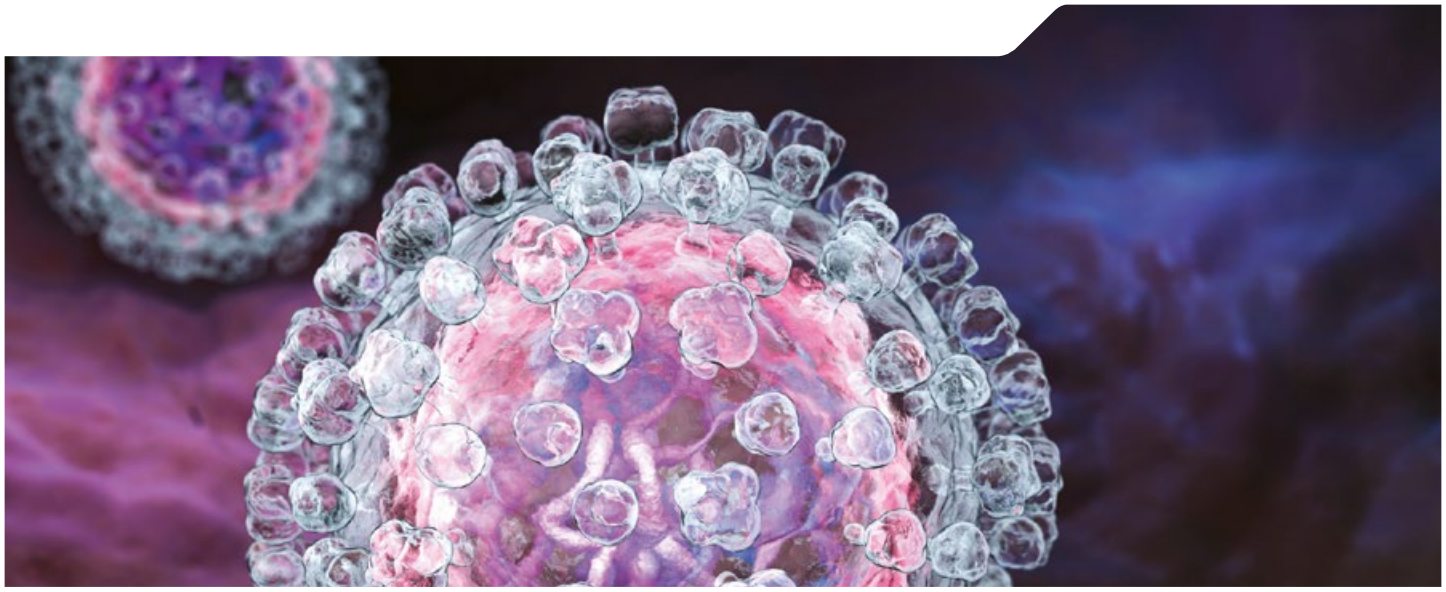


Hepatitis C © Fotolia | Tatiana Shepeleva

COMBATING PERSISTENT VIRUSES

RESEARCH FOCUS CVIR – CHRONIC VIRAL INFECTIONS

Chronic viral infections by hepatitis and herpes viruses are causing a severe global disease burden. Recently, effective treatment for hepatitis C virus infections has become available, but it is challenging to deliver it to all in need. The treatment repertoire for hepatitis E virus is extremely limited. Current therapies against widespread herpes viruses, like Cytomegalovirus and Kaposi's sarcoma-associated herpesvirus, are accompanied by side effects and viral resistance. To improve therapies and develop potent vaccines, the understanding of basic mechanisms of viral pathogenesis is key. This includes immune control and viral evasion, i.e., the mechanisms viruses use to escape the immune system, as well as the development of new model systems to study the aforementioned human pathogens. The Research Focus CVIR dissects principles that are responsible for the establishment of chronic infections with hepatitis and herpes viruses and studies mechanisms of immune control and viral evasion to lay the basis for novel intervention strategies.

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 vaccination approaches induce protective immunity?

1. STRATEGIES TO CONTROL HEPATITIS VIRUSES

Approximately 71 million individuals are chronically infected with hepatitis C virus (HCV). These patients are at risk to develop severe liver disease including cirrhosis and liver cancer. Novel therapies with cure rates greater than 95% are licensed. However, these therapies are expensive, thus access is very limited particularly in those countries with highest prevalence. Since these drugs are used only for a short period of time, it is difficult to predict to which extent drug resistance will necessitate development of salvage therapies with alternative mode of action.

Treatment-induced viral clearance does not protect against HCV re-infection which frequently occurs in populations with high transmission risk, e.g. drug users. A prophylactic vaccine is not available and mechanisms that enable viruses to escape the immune system (viral immune evasion) are incompletely defined. Therefore, analysis of viral evasion strategies and dissection of determinants of protective immunity are important for development of a prophylactic vaccine. Scientists in the RF CVIR characterized the mode of action of novel HCV membrane fusion inhibitors, a class of drugs showing activity against the virus by preventing its entry into the host cell. They identified viral mechanisms of resistance to three classes of molecules. In addition, they profiled a large spectrum of HCV vaccine candidates *in vivo*, providing important information for HCV vaccine development. Details of the latter study are described in the section "Highlight Publications" in this report.

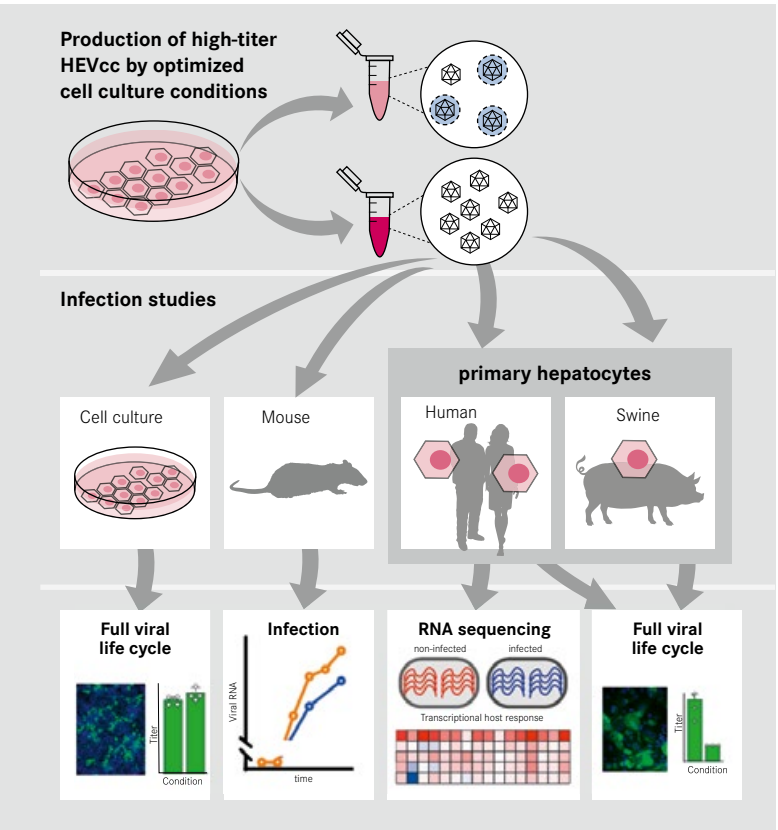


Figure 1: The establishment of a robust HEV cell culture system in human hepatocytes is depicted. These produced so-called HEVcc particles demonstrated replication to high viral loads in mice harboring human liver cells and efficiently infected primary human as well as porcine hepatocytes.

Hepatitis E virus (HEV) is responsible for 70,000 annual deaths worldwide. Recent studies estimated that about 400,000 new infections occur each year in Germany. In most of these cases, HEV infection is self-limiting, but in immunocompromised patients or patients with pre-existing liver diseases, HEV can take a chronic course. The only pharmaceutical intervention possibility is the off-label use of the antiviral drug ribavirin. Cases of treatment failure lead to life threatening, severe complications with liver cirrhosis and hepatocellular carcinoma.

Although HEV was first described in 1983, the first effective *in vitro* model to study viral replication was introduced only in 2011. However, with this model the full viral life cycle could not be analysed, hampering the identification of possible host proteins involved in viral propagation as well as the identification of effective antivirals.

CVIR scientists have now reported the successful establishment of a robust HEV culture system in human hepatocytes, i.e. liver cells, that can be used to study all essential steps of the HEV life cycle. By testing several liver-derived cell lines in combination with specified culture conditions, they were able to produce cell-culture derived enveloped and non-enveloped virus particles called "HEVcc". This differentiation is mandatory to model different transmission aspects of this virus, e.g. the transmission via the fecal-oral route by shedding of non-enveloped particles in the feces, as well as the transmission via contaminated blood products by the blood circulating enveloped form.

The produced HEVcc particles demonstrated replication to high viral loads in mice harbouring human liver cells (human liver chimeric mice). Furthermore, they were able to efficiently infect primary human as well as porcine hepatocytes. Pigs are most probably the largest reservoir for HEV in the natural environment. In-depth analysis of dynamic changes in the gene activity of inoculated primary human hepatocytes allowed detailed insights into the host response to HEV liver cells and provides a unique data set now made publicly available.

This new model system paves the way for the identification of new host factors involved in HEV propagation and pathogenicity. In addition, this system can be the basis for the development of effective HEV-specific antivirals and a potent vaccine.

2. CYTOMEGALOVIRUS AND ITS INTERPLAY WITH THE IMMUNE SYSTEM

Cytomegalovirus (CMV) is a ubiquitous herpesvirus that establishes a life-long infection in a high percentage of the population worldwide. Human Cytomegalovirus infection of immunocompetent individuals goes mostly unnoticed, whereas immunocompromised patients can undergo life-threatening disease. It is therefore important to understand how the host recognizes this virus and how the virus antagonizes the immune response to establish life-long latency and avoid its elimination.

Previously, CVIR researchers had uncovered that human Cytomegalovirus induces an antiviral response mediated by type I interferon (IFN) signaling proteins in human macrophages, a type of immune cells. This response is dependent on two molecules in particular: the cyclic GMP/AMP synthase (cGAS), a receptor for the recognition of pathogens, and its crucial adaptor protein Stimulator of IFN genes (STING). Interestingly, they recently found that STING knockout mice are resistant to infection with murine cytomegalovirus, whereas mice that lack multiple pathogen recognition receptors could not mount type I IFN responses and thus succumbed to infection. By performing sophisticated immunological studies, they could pinpoint a cell-type specific role for STING during infection: While STING is dispensable for survival, it plays an important role in early interferon induction in specialized immune cells in the liver and in the restriction of viral dissemination via myeloid cells. They also discovered that certain cell types of the human immune system (human macrophages and dendritic cells) can limit the spread of Cytomegalovirus by type I IFN dependent as well as independent mechanisms, highlighting the complexity of antiviral responses induced by this virus.

A study by CVIR scientists (*see also section "Highlight publications" in this report*) revealed another novel feature of the cellular STING protein in mice: while the antiviral response mediated by STING is efficiently counteracted by the viral protein m152, another function of STING is used to activate the viral gene programme. This finding explains why murine Cytomegalovirus is not restricted by STING, but rather benefits from it: the virus has evolved a mechanism to specifically antagonize the STING-mediated antiviral response, while preserving its pro-viral function. This mechanism provides an advantage in the establishment of an infection.

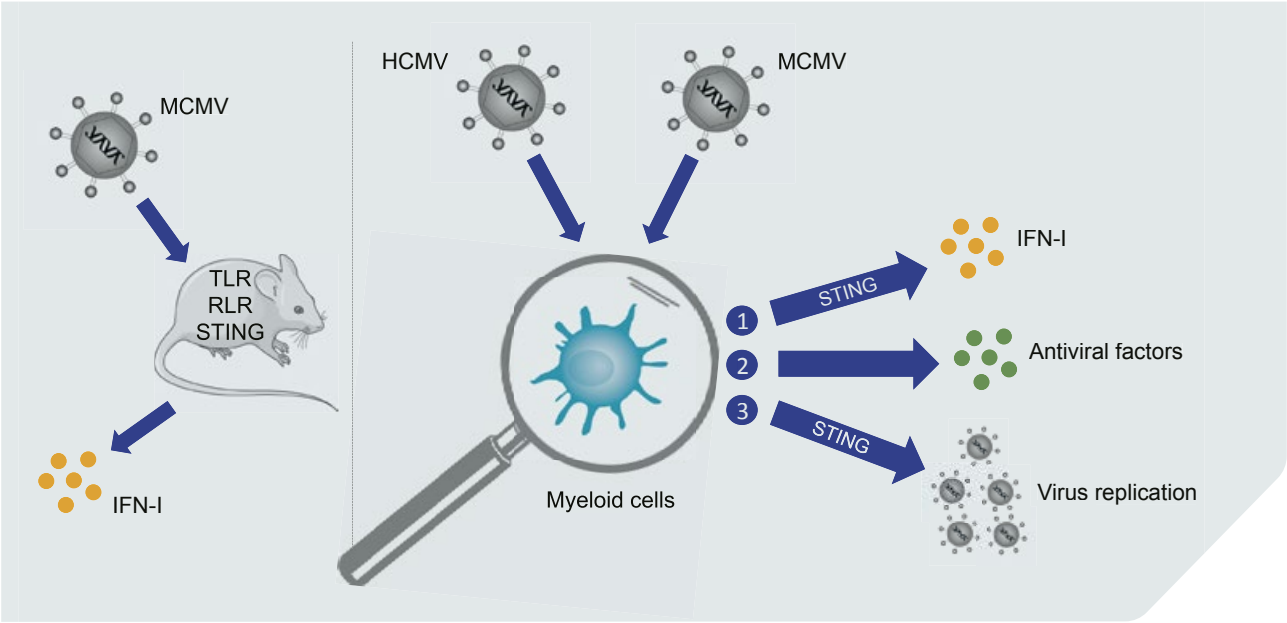


Figure 2: To combat murine Cytomegalovirus infection *in vivo*, three major sensing molecules, TLR, RLR, and STING, act together to mediate protective type I interferon (IFN-I) responses of the immune system (left image). Myeloid cells of murine and human origin deploy similar strategies to respond to Cytomegalovirus infection (right image). (1) Upon CMV infection, IFN-I production of myeloid cells is dependent on an intracellular pathway involving STING. (2) However, due to the diverse strategies of CMV to evade the IFN-I mediated immune response, myeloid cells also produce other antiviral factors in response to infection, which restrict virus propagation and spread. Such factors await their in depth characterization. (3) In addition, CMV exploits a STING-mediated transcription factor response to enhance viral replication, showing once again why these viruses are so successful in establishing life-long infections.

A detailed understanding of the molecular mechanisms of CMV infection allows to develop novel immunotherapeutic strategies, but also to exploit the natural properties of this virus. Cytomegalovirus induces the strongest and most durable immune response involving $CD8^+$ T cells – a type of immune cells playing a central role in combating viral infections – known in human clinical medicine. Due to this unique property, the virus represents a promising candidate vaccine vector for the induction of persistent cellular immunity.

CVIR scientists took advantage of this capability and generated a novel vaccine candidate against influenza based on recombinant murine Cytomegalovirus expressing molecules from influenza A virus H1N1. Immunized mice were capable of controlling influenza A virus infection. The protective capacity of the immunization was associated with specific mucosal immunity in the lungs (i.e., the localized type of immune responses that occur specifically at mucosal membranes and are characterized by distinctive types of immune cells).

3. UNDERSTANDING AND TREATING KSHV INFECTIONS

Kaposi's sarcoma-associated herpesvirus (KSHV) is one of the few oncogenic human viruses known to date. It is the etiological agent of multiple malignancies including endothelial cell-based Kaposi's sarcoma. Infections with KSHV represent a major concern in immunocompromised patients, such as HIV-infected individuals and organ transplant recipients. The strategies how the virus overcomes the various host defense mechanisms are still not fully uncovered. Moreover, no targeted therapy or vaccine is available.

Screening and identification of anti-viral compounds is compromised by the lack of suitable cell culture systems reflecting properties of virus-transformed cells. CVIR researchers utilized a recently developed human endothelial cell line for designing predictive cell culture systems with the aim to identify novel KSHV drug candidates. From a library of 260 compounds that were selected by a recent natural product

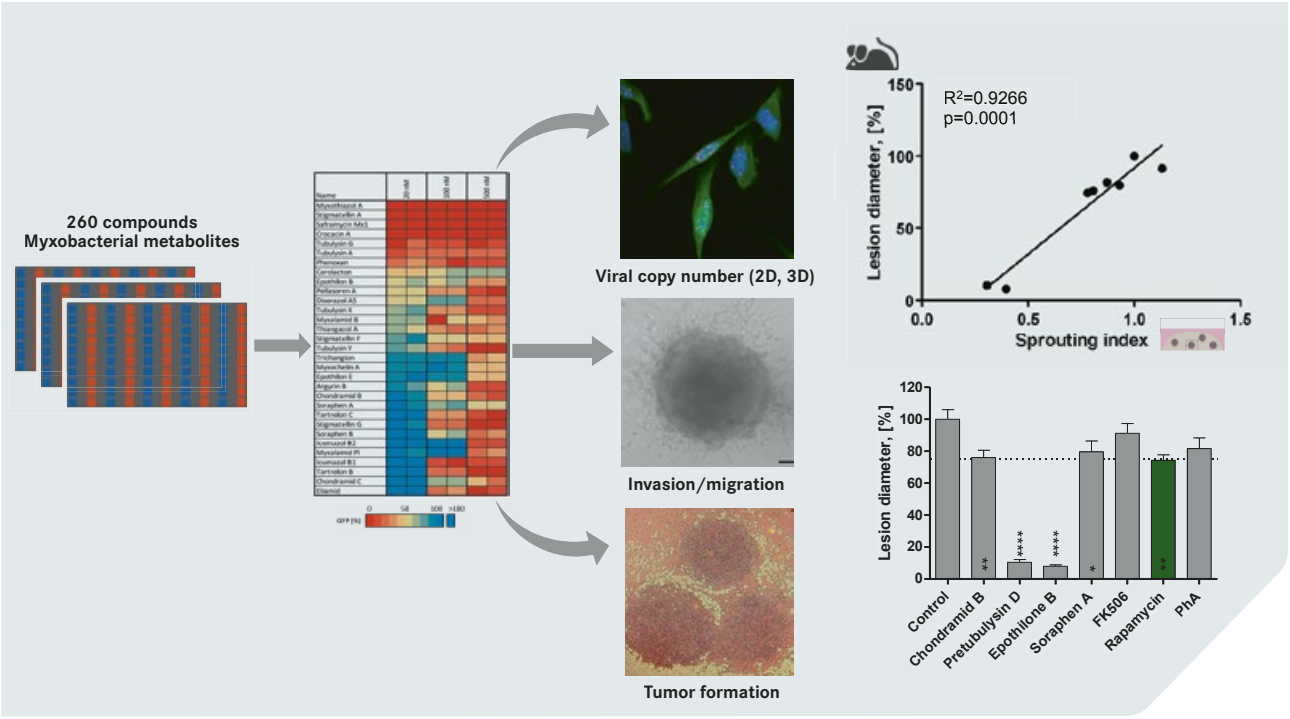


Figure 3: Schematic representation of the screening regimen for identification of novel compounds acting against KSHV. Compounds were evaluated in 2D and 3D cell culture tests. Candidates were validated in a mouse model for reduction of tumor formation by KSHV infected human cells. Reduction of *in vitro* sprouting activity was found to correlate well with anti-tumor effects *in vivo*, highlighting the predictive nature of the *in vitro* screening system.

screening programme, they identified three (Chondramid B, Epothilon B, and Pretubulysin D) molecules which *in vitro* reduce the viral load, tumor sprouting or both. The efficacy of these compounds was demonstrated in a mouse model which resulted in substantial reduction of KSHV tumor lesions. These compounds may offer novel options for specific treatment of this disease.

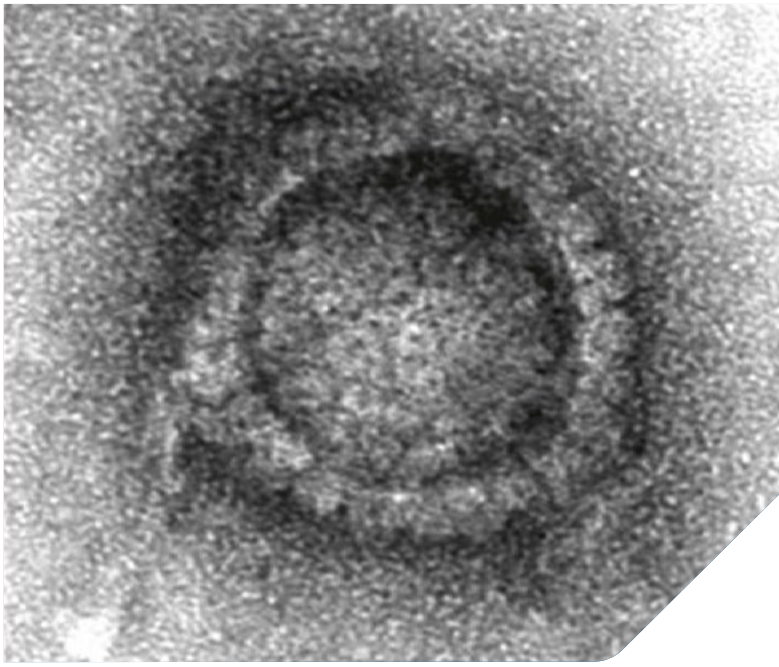
CVIR scientists furthermore gained novel insights into the tight interplay between KSHV and the host's innate immune response. They found that an important antiviral host protein, Oligoadenylate synthetase-like protein (OASL), interacts with the KSHV protein ORF20 and co-localizes with it in particular structures in the nucleus of the host cell, the nucleoli. This interaction and co-localisation was also found in other herpesviruses. Expression of both proteins was induced early after reactivation of cells latently infected with KSHV. It was shown that OASL enhanced infection of KSHV. The findings suggest that the viral ORF20 usurps the function of the host's OASL to benefit KSHV infection. This study contributed to our understanding of the intimate relationship between herpesviruses and their host.

PERSPECTIVES

Researchers of the RF CVIR will continue to build on intra- and extramural collaborative networks of experts involving clinicians, immunologists, structural biologists, medicinal chemists and virologists to address the existing critical knowledge gaps about chronic hepatitis and herpes virus infections. They will 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 Hepatitis C Virus. In parallel, the principles that control cellular immune responses in these chronic viral infections will be explored further.

Cytomegalovirus-based vaccine vectors may elicit systemic as well as mucosa-resident cellular immunity that confers immune protection against numerous viral pathogens, including influenza. Preliminary results indicate that they may also induce protective humoral immune responses, i.e., responses including the formation of specific antibodies. This will be assessed within the Helmholtz EU partnering consortium *MCMVaccine*. In this context, CVIR researchers will also evaluate the potential of murine Cytomegalovirus vectors to act as vaccines against Hepatitis C or chikungunya virus. Finally, we will explore the capacity of herpesviral vectors to elicit immune responses outside of the natural host, where the natural restriction of virus replication ensures against adverse pathogenic effects of the vector.

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



Electron microscopic image of a capsid of the herpes simplex virus.

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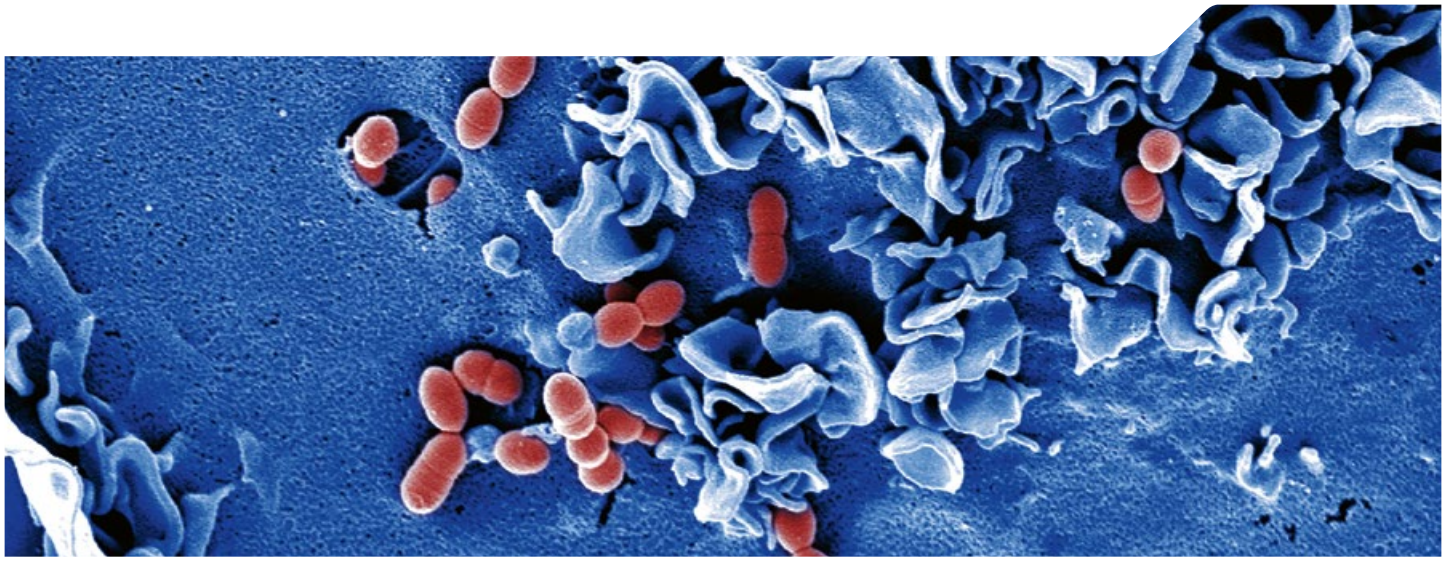


Human Dendritic Cell with *Strepococus gordoni* bacteria © HZI | Manfred Rohde

VACCINES AND IMMUNE THERAPIES FOR HIGH-RISK PATIENTS

RESEARCH FOCUS INDI – INDIVIDUALIZED IMMUNE INTERVENTIONS

For many infectious diseases, effective therapies and vaccines are still missing. This is particularly true for vulnerable individuals that are at high risk for severe forms of infection or poor responders to interventions. Thus, the research focus "Individualized Immune Interventions" INDI deploys experimental and clinical activities to better understand host responses to infections and vaccinations. This research contributes to the development of immune-based approaches 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 of immune responses?
- How to design vaccines and immunotherapies to increase their efficiency of immune protection in vulnerable individuals and populations?

1. VACCINATION IN VULNERABLE INDIVIDUALS

There is a steady increase in the number of vulnerable individuals who are highly predisposed to severe infections – e.g. the elderly, toddlers and small children, immunocompromised, and individuals affected by accompanying diseases. Thus, it is critical to protect them where possible against vaccine-preventable diseases. However, these vulnerable individuals are often also poor responders to vaccination. Hence, it is critical to develop mechanistic insights into the immunological processes underlying responsiveness vs. non-responsiveness to evolve more effective and patient-tailored vaccines. This knowledge would also facilitate the development of point-of-care predictive diagnostics for the stratification and follow-up of vaccines and provide a basis for improved vaccination strategies.

To this end, vaccination studies are being carried out in cohorts of poor vaccine responders, e.g. a birth cohort, cohorts of senior individuals, and patients with decompensated liver cirrhosis, patients treated with therapeutics that affect immune functions. The clinical studies encompass a detailed profiling of the immune system, and the resulting high-dimensional datasets are generated through divergent methods analyzing the microbiota, immune cell subsets, RNA and protein expression profiles as well as genetic variations.

Such datasets are analyzed and integrated using novel computational tools. For example, a prospective, population-based influenza vaccination study was carried out in elderly individuals – 65 to 80 years of age – in two influenza seasons. The obtained results have allowed to identify preexisting diabetes and herpes zoster as risk factors for poor immune responses towards the vaccination, an altered balance between different types of immune cells (regulatory T cells and T follicular helper cells) as one underlying mechanism, and the signal molecules interleukin IL-8 and IL-18 as potential biomarkers for effective responsiveness to vaccination.

Premature babies belong to the particularly vulnerable individuals
Source: Kaiser | MHH

2. PERSONALIZED CELL LINES AS NOVEL TEST SYSTEMS

Susceptibility to infection is often determined by the host's genetic background. Hence, gene variations predicting an increased risk of disease upon infection are scoured in numerous genetic studies at population levels. While such biomarkers allow the identification of vulnerable individuals before disease occurs, they are not sufficient for developing interventional strategies. The latter requires mechanistic understanding of the underlying pathogenesis through experimental studies. However, human biomaterial is often not easily available for research, which limits in-depth studies of personalized immune responses to infection.

We have addressed this gap by developing methods to expand, maintain and characterize human cell populations, and then deploy them to study infection processes. We have generated a library of primary human endothelial cell lines from more than a hundred surgery patients. Some of these cell lines were already used in experiments with a particular class of immune cells, the dendritic cells (DC). These studies showed that DC can limit infection with Cytomegalovirus (CMV) in human cells.

Even more power to this approach was provided by advances in cooperation with *InScreenEx*, a spinoff company of HZI. Employing novel strategies to control cellular gene expression and circumvent the naturally occurring cell death, numerous personalized cell lines could be generated with stable and highly reproducible characteristics, including liver and endothelial cells. These cell systems pave the way for investigation of host-pathogen interaction on a personalized level and support the development of tailored interventions.

Experimental approaches with T cells, another type of immune cells, are now used to identify optimal targets for personalized immunotherapeutic approaches.

3. METABOLITES AS BIOMARKERS

In spite of recent advances in pathogen detection by molecular methods, the timely diagnosis of many infectious diseases still represents a major challenge to clinicians. In many patients, causative pathogens can still not be identified. Therefore, we are focusing on biomarkers – molecules in the host, i.e. the patient, that reflect infection with a specific pathogen or pathogen class. A major emphasis lies on biomarkers to distinguish between viral and bacterial infections, as the treatments differ (antiviral vs. antibiotic).

We focus on metabolites, small molecular intermediates from metabolism, because they often reflect both the early and final stages of an infection process. We have applied a targeted metabolomic screen to cerebrospinal fluid from

patients with infections of the central nervous system (CNS) and several neurologic control groups. Here, we could identify highly specific biomarkers defining the etiology of various encephalitis conditions. These include biomarkers for CNS involvement in the reactivation of varicella zoster viruses. These molecules differ from biomarkers characteristic for patients with enterovirus meningitis without other evidence of neuroinflammation in cerebrospinal fluid. We also described kynurenine and its precursor molecule tryptophan, a naturally occurring amino acid, as biomarkers to distinguish between bacterial, viral, and autoimmune encephalitis/meningitis. A phospholipid called PCaePC44:6 was shown to be a highly sensitive biomarker for bacterial meningitis.

Several plasma biomarkers to diagnose community-acquired pneumonia (CAP) were identified by a similar metabolomic approach. They allow to differentiate this type of pneumonia from infection-triggered exacerbation of chronic obstructive pulmonary disease (COPD). The latter is known as a common disease entity that may have a similar clinical presentation but requires different treatment.

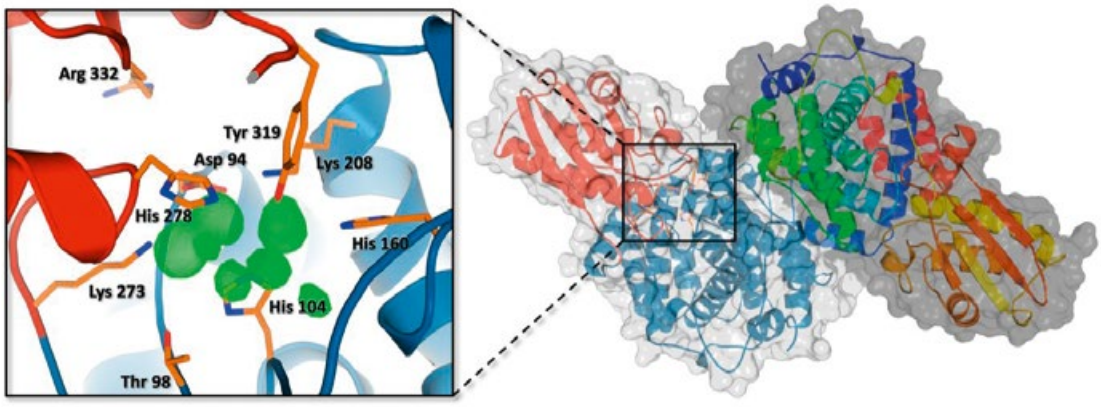
Lastly, a collaboration between immunologists and structural biologists at HZI allowed us to solve the crystal structure of cis-aconitate decarboxylase. This enzyme catalyzes the synthesis of the anti-bacterial and anti-inflammatory metabolite itaconic acid. We identified the active center of the enzyme and naturally occurring human mutations that modulate itaconate synthesis. This enables us to study variations in itaconate synthesis and assess their impact on human susceptibility to infectious and inflammatory diseases. Ultimately, this may lead to the development of genetic biomarkers that predict individual disease risk.

4. IMMUNE-BASED THERAPIES AGAINST HEPATITIS E

Patients who are chronically infected by Hepatitis E virus (HEV) are often vulnerable to develop chronic liver disease. This is particularly true for immunocompromised individuals, such as solid organ transplanted patients. So far, there is no licensed anti-viral therapy for chronic Hepatitis E. Off-label treatments, on the other hand, are associated with severe side effects. Therefore, we propose immunotherapy with virus-specific immune cells as a novel alternative approach. Our research focuses on T cell receptors – molecular structures on the surface of T cells, a particular class of immune cells. T cell receptors play a major part in recognizing the presence of pathogens in the body and inducing immune reactions against them. By screening patients during the acute phase of HEV infection, we identified several T cell receptors specifically targeting cells infected by HEV.

Two of these HEV-specific T cell receptors have been selected due to their strong responses against the virus as candidates for the development of T cell therapies. We characterized them in detail for their target sensitivity, specificity and cytotoxicity. We demonstrated that T cells isolated from patients with chronic Hepatitis E, and engineered to express the candidate anti-HEV T cell receptor, caused strong immune reactions against HEV *in vitro*.

Once the candidate T cell receptors were selected, we also screened them for cross-reactivity to self-antigens, i.e. target molecules present in the host's organism. This is a necessary precaution to address the clinical concern of plausible autoimmune potential. One of the HEV-specific



Structure and binding site of cis-aconitate decarboxylase. Studying the function of this enzyme may help to predict individual disease risks (see text for detail).

T cell receptors showed the ability to cross-recognize such a self-antigen, but it was not functionally active against it.

In summary, we propose that therapy based on engineered T cells expressing an anti-HEV T cell receptor may be a viable option for chronic Hepatitis E treatment. However, the candidate T cell receptors need to be scrutinized thoroughly for their potential undesirable off-target reactivity.

PERSPECTIVES

Addressing the challenges of modern infection medicine, researchers of the Research Focus INDI will perform further experimental and clinical studies to better understand poor immune responsiveness to infection and vaccination. They will, in particular, study immune responses of preterm babies, newborns and senior individuals. Another focus will be on vaccination, especially regarding influenza vaccines in the elderly, vaccination against Hepatitis B, responsiveness to vaccination of transplant recipients and chronic liver disease patients. The resulting insights from the molecular profiling of the host response will allow us not only to identify novel biomarkers for the design of advanced diagnostics but also to develop more efficient and specifically tailored vaccines and immunotherapies.

All activities in the area of vaccinology will be bundled in the incipient Vaccine Center, which will focus on vulnerable individuals and aims to develop vaccine candidates up to first-in-human studies, e.g. for influenza, Chagas, and viral hepatitis. Core activities of the Vaccine Center will include delivery techniques – utilizing, amongst others, nanotechnology, needle-free vaccination and novel vector-based systems –, adjuvants, biomarkers as well as preclinical and clinical vaccination studies.

Further interventional studies will be carried out to assess the capacity of immune-based therapies to enhance responsiveness to vaccines in vulnerable individuals.

Hepatitis E virus © istock | Dr Microbe

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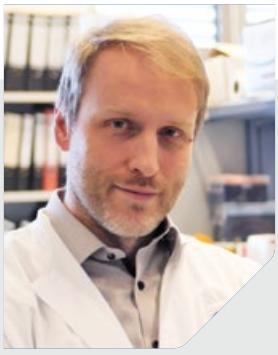

Luka Čičin-Šain

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Application of the SORMAS tool for disease control in Nigeria © Studio 24

STUDYING, PREVENTING AND CONTROLLING EPIDEMICS

RESEARCH FOCUS EPI – DIGITAL AND GLOBAL HEALTH

Research Focus EPI – short for "Epidemiology" – deals with infectious diseases at the level of populations and develops solutions for public health. The HZI-coordinated development of the Surveillance Outbreak Response Management and Analysis System SORMAS has already contributed to the response to epidemics in Africa. It improves our understanding of the spread of infectious diseases. The Research Focus EPI contributes to public health solutions locally, nationally and internationally by investigating the burden, determinants and impact of infectious diseases. HZI has intensified the development of Mobile Health ("mHealth") technologies utilizing mobile electronic devices for infection research. Within the German National Cohort and beyond, these applications will allow the discovery of hitherto unknown associations 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 digital and mobile 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 novel control and prevention approaches?

1. DIGITAL INFECTION SURVEILLANCE AND RESPONSE IN LOW-RESOURCE COUNTRIES

The tragic deficiency of the West African public health system apparent during the Ebola virus disease epidemic in 2014/15 has demonstrated the urgency of developing technology to detect and manage disease outbreaks much more efficiently. A digital surveillance system needs to work in areas of the world where no continuous access to internet, electricity or a reliable mobile or landline phone grid is avail-

able. This evolving field of information and computational technology is generally referred to as electronic or mobile health (eHealth, mHealth). A guiding principle in the development of eHealth/mHealth solutions within the Research Focus "Digital and Global Health" is to develop tools using an open source approach wherever possible to assure free access for the international scientific community and public health institutions in low resource settings.

One such open source tool is SORMAS, the digital Surveillance Outbreak Response Management and Analysis System developed under the guidance of the HZI Department "Epidemiology". Starting from a first version that was created for the Ebola disease outbreak in 2014/15, SORMAS is under continuous development. The digital surveillance tool has already proven its effectiveness in responding to multiple complex epidemics in Nigeria. In the years 2018 and 2019, SORMAS helped to control an outbreak of monkeypox, a disease similar to smallpox that had not occurred in Nigeria for nearly 40 years. In parallel, the seasonal meningitis outbreak in the north of Nigeria and a Lassa fever outbreak occurred. SORMAS was used to monitor all these epidemics and coordinate counteractions against them.

The epidemiological and technological approaches of SORMAS include the integration of case-based clinical process management and disease detection, and also enable it to function offline and to be used by mobile health care workers. These features make SORMAS unique in its field. RF EPI scientists maintain strong cooperation with the Nigeria Centre for Disease Control and the Ghana Health Service, which together are in charge of protecting over 225 million people from infections. International organizations have now started to join the German government in supporting SORMAS deployment in Africa (e.g. World Health Organization, World Bank, Bill and Melinda Gates Foundation, European Union, US Centers for Disease Prevention and Control). In the future, RF EPI will continue to develop SORMAS to provide solutions for emerging challenges like COVID-19, antimicrobial resistance and climate change.

2. DIFFERENTIAL SEROLOGY FOR PUBLIC HEALTH SOLUTIONS

The emergence of novel pathogens such as the virus Sars-CoV-2 and the increasing availability of complex state-of-the-art scientific results to the general public create novel challenges for researchers and public health care professionals when it comes to effectiveness, safety and acceptability of vaccinations.

The successful identification of serum markers that differentiate immune responses to natural infections from immune responses caused by vaccinations is a particular challenge here, as these are important parameters for monitoring the efficiency of global vaccination programmes with the aim of eradicating diseases such as measles.

RF EPI has successfully piloted the identification of these markers by developing a rapid, high-throughput assay using a bead-based multiplexing approach where blood volumes

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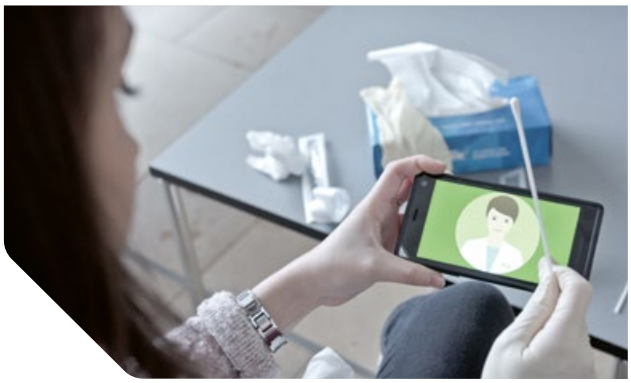
as small as 5 µL are sufficient to screen up to 500 analytes per sample. This "differential serolomics" approach (patent number PCT/EP2016/078, Bohm et al 2018) is currently utilized in a population survey in Columbia to assess the success of a vaccination campaign against the recent upsurge of severe viral hepatitis A.

Hepatitis E is also seen as an increasing public health challenge. Here, RF EPI researchers have established a second assay to facilitate rapid differential diagnosis. This assay is also conveniently usable in population-based surveys while counteracting study fatigue by using only small volumes of blood. In the near future, we plan to further expand our differential serolomics portfolio through dedicated cooperation. The aim is to accelerate the large-scale establishment of a broad serum biomarker panel within the German National Cohort NAKO (see Chapter "Linking Infections to non-communicable diseases / Research within the German National Cohort" in this report).

3. DIGITAL HEALTH IN VACCINOLOGY, COHORT STUDIES AND HOSPITALS

For prospective vaccine pharmacovigilance, it is important to obtain reports from all those who are vaccinated, even the ones without any adverse reaction. For this purpose, digital tools are particularly helpful. They offer low-threshold real-time reporting by the vaccinated persons themselves, and request responses from all of them. Since Germany has no vaccination registry, such an approach uniquely facilitates calculation of the proportion of vaccinated people with adverse events, thus avoiding problems that result from under-reporting of events and non-reporting of vaccinations without events.



In collaboration with the Paul-Ehrlich-Institute, RF EPI scientists developed a novel mobile health (mHealth) tool to prospectively monitor undesired events following vaccination. In a pilot study, feasibility and user preferences were tested. The results were used for a multi-center study with occupational influenza vaccination as the use case. The study was conducted in three institutions in 2018. Study participants reported on occurrence and non-occurrence of adverse events over a period of up to three months after vaccination. Research on transient infections like the common cold also suffers from under-reporting if done e.g. with routine health care data. Again, low-threshold reporting via a mobile application is a solution offered by the digitalization of research. Therefore, HZI has developed a "prospective monitoring of acute infections application" (PIA). As an eResearch system, PIA offers various user interfaces allowing specific access and tasks compliant with data protection regulations, e.g. for participant management, study team, researchers and study participants. We aim to analyse the occurrence and factors for transient respiratory, gastrointestinal and urogenital infections. The long-term goal is to study the impact of such acute infections, especially if they occur repeatedly, on general health and particularly on cardiovascular and metabolic conditions and dementia. The first application of this eResearch system is within the German National Cohort (NAKO), Germany's largest health study (see "Linking Infections to non-communicable diseases / Research within the German National Cohort" in this report and www.info-pia.de).

HiGHmed is one of four German Medical Informatics Initiative projects (www.highmed.org). HZI contributes to the HiGHmed Use Case – Infection Control. Here again, an outbreak detection system is being developed. The "Smart Infection Control System" or "SmICS" focuses on nosocomial infections and clusters that evolve within German hospitals.

Basic and advanced analytical algorithms allowing for cross-institutional analysis combined with smart visualization methods will reveal yet unknown characteristics of clusters and outbreaks to continuously enrich the knowledge base in the context of infection control. For SmICS, RF EPI researchers collaborate with the Robert Koch Institute (RKI), the Peter L. Reichertz Institute of Medical Informatics (PLRI), Hannover Medical School (MHH) and others.

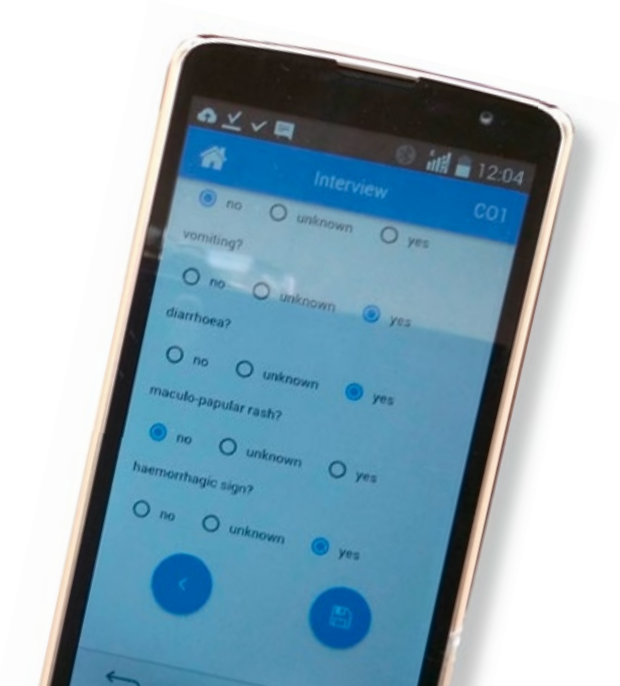
PERSPECTIVES

Climate change, migration, and mobility of foods, livestock and people drive the risk for emerging infections and rapidly spreading epidemics while at the same time challenging their control. The resulting societal burdens are threefold. First, for many viral infections specific treatments do not exist and for many bacterial infections the effectiveness of treatments is decreasing owing to antimicrobial resistance. Second, epidemics and endemic infections have destructive consequences for the economy and social wellbeing, as the emergence of HIV/AIDS, the Ebola epidemic in West Africa and COVID-19 continue to show. Third, infections cause, trigger and mediate non-communicable diseases such as cancer and cardiovascular, metabolic and neurodegenerative disease; many of these associations 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 digitalization, big data sciences and molecular omics in a truly multidisciplinary symbiosis of epidemiology, clinical infectiology and infection biology. We apply our research and development as early and as close as possible to where the need occurs: with the patients even before they attend health care facilities, in the midst of epidemics, and in the design of vaccination campaigns. Our goal is to contribute to the prevention of high-burden endemic disease, the control of emerging epidemics and the elimination of vaccine-preventable diseases.

The vision for SORMAS is to become the established standard surveillance and outbreak control tool in Africa and beyond. The HZI strategy is gradually to pass on the development and implementation of SORMAS to national and international public health institutions and to intensify epidemiological research on the topic. SORMAS will generate unprecedented quality and quantity of clinical and epidemiological data from notoriously understudied regions and diseases. Anonymised extractions of these data will allow for novel approaches in epidemiological modelling and other infectious disease research.

The mHealth applications originally developed by HZI for epidemiological research on infectious diseases have the potential to evolve into platforms for a much broader range of research topics. Further translational steps are likely to include clinical management of immunocompromised patients and standard monitoring systems to comply with novel EU regulations on post-marketing vaccine vigilance.



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HIGHLIGHT PUBLICATIONS

TACKLING LIPID METABOLISM TO CONTROL IMMUNE REACTIONS

LUCIANA BEROD | HEAD OF RESEARCH GROUP HOST PATHOGEN INTERACTIONS AND IMMUNOMETABOLISM

T cells mediate immune defence against invading pathogens and are critical for the development of immunological memory. Yet, uncontrolled T cell responses can lead to pathology. Thus, a particular subset of T cells, the regulatory T cells or Treg, keep inflammatory responses in check. Recent findings have demonstrated distinct metabolic profiles in specific T cell subsets, like Tregs. This suggests that modulating the metabolism of immune cells can open up new avenues for therapy of various medical conditions. We studied the importance of lipid metabolism for T cell development and function. Using genetic tools, we challenged the previous paradigm that differentiation of regulatory and memory T cells depends on long-chain fatty acid oxidation, and propose a new mechanism of metabolic regulation.

In the last decade, it has become clear that cellular metabolism determines the development and function of immune cells. Thus, immunometabolism is turning into a promising new research area for the development of immunotherapies for various diseases. Upon activation, naive T cells convert from a metabolic resting state towards a highly glycolytic phenotype. This metabolic reprogramming allows the generation of macromolecules and energy in the form of ATP, essential prerequisites to meet the high energetic demands of proliferation, differentiation and effector function. ATP is generated from glucose by two integrated pathways - aerobic glycolysis and oxidative phosphorylation (OXPHOS).

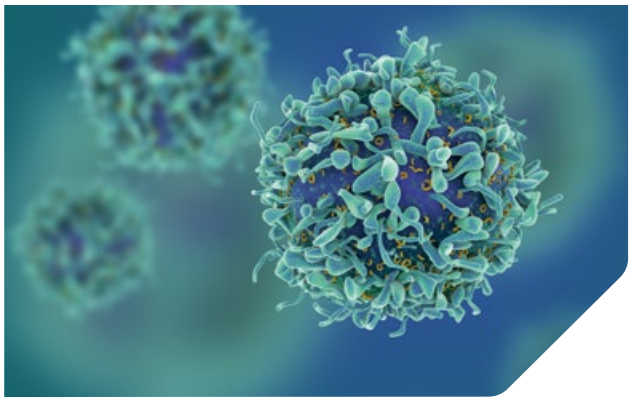


Figure 1: Render of T cells. © fusebulb - stock.adobe.com

In addition to glucose metabolism, T cell activation involves the induction of *de novo* fatty acid synthesis (FAS), generating lipids for the synthesis of new biological membranes. However, T cells can also exploit lipids from the environment as an energy source in a process named fatty acid oxidation (FAO). FAO occurs in the mitochondria and involves the degradation of FAs into acetyl-CoA fueling OXPHOS for ATP production. While short and medium-chain FAs (< C12) freely diffuse into the mitochondria, long-chain (LC) FAs (C14-C18) require transportation across the mitochondrial membrane. The latter process is regulated by carnitine palmitoyl-transferase 1 (CPT1), with the isoform CPT1A being the predominant isoform expressed in lymphocytes.

T cell subsets, including regulatory (Treg), effector (Teff) and memory (Tmem), exhibit distinct metabolic profiles. Previous studies proposed a paradigm in which Teff relied on the engagement of the glycolytic-lipogenic pathway, whereas the differentiation and function of Treg and Tmem depended on LC-FAO. However, the picture of LC-FAO sustaining Treg and Tmem development was primarily based on the pharmacological inhibition of CPT1A by the drug etomoxir. Even though widely used as an inhibitor of LC-FAO, recent studies report off-target effects of etomoxir. Thus, we employed conditional

knockout mice devoid of CPT1 specifically in T cells. We demonstrated that CPT1A-mediated LC-FAO is dispensable for Tmem or Treg homeostasis, development, and survival (Figure 2). Instead, they may be capable of oxidizing not only LC-FAs but also medium- and short-chain FAs to fuel their energy metabolism. Furthermore, we showed that already low micromolar concentrations of etomoxir block CPT1A activity in T cells, indicating that previous studies proposing the requirement of LC-FAO for Treg and Tmem development were due to off-target effects using high doses of etomoxir. Altogether, our study provides insights into the metabolic requirements of immune cell subsets that will be helpful for the development of strategies tackling lipid metabolism for immunotherapy. Also, we propose always to combine the use of chemical inhibitors with genetic models for investigating immunometabolism.

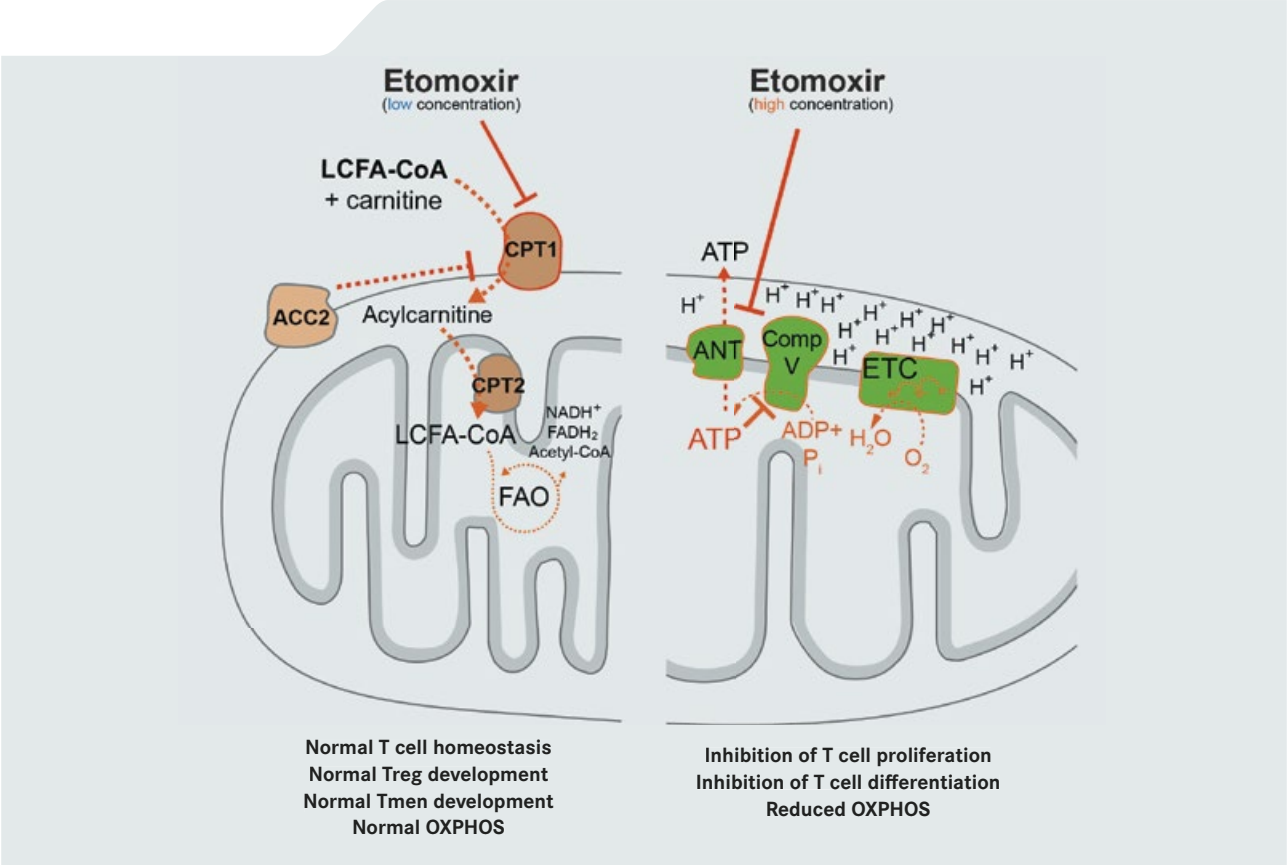


Figure 2: Long-chain fatty acid oxidation (LC-FAs) is largely dispensable for T cell activation and generation of $CD8^+$ T memory (Tmem) cells and $CD4^+$ Treg cells. At high concentrations, the Cpt1 inhibitor etomoxir presents off-target effects on cell metabolism.

Raud B, Roy DG, Divakaruni AS, Tarasenko TN, Franke R, Ma EH, Samborska B, Hsieh WY, Wong AH, Stüve P, Arnold-Schrauf C, Guderian M, Lochner M, Rampertaap S, Romito K, Monsale J, Brönstrup M, Bensinger SJ, Murphy AN, McGuire PJ, Jones RG (2018)

Etomoxir Actions on Regulatory and Memory T Cells Are Independent of Cpt1a-Mediated Fatty Acid Oxidation

Cell Metab. doi: 10.1016/j.cmet.2018.06.002

TAKING OVER CONTROL: CYTOMEGALOVIRUS MANOEUVRES THE IMMUNE RESPONSE

MELANIE BRINKMANN | HEAD OF RESEARCH GROUP VIRAL IMMUNE MODULATION

Host defense against viral infections requires their early recognition by germline-encoded pattern recognition receptors (PRR), resulting in the activation of a potent antiviral defense and, in case of most viruses, clearance of infection. However, through millions of years of co-evolution, herpesviruses have developed sophisticated strategies to manipulate immune control for securing a lifelong infection in their respective hosts. In the work presented below, we highlight a novel immune evasion strategy of the herpesvirus Cytomegalovirus (CMV). The murine CMV (MCMV) protein m152 applies an elegant mechanism to slow down the antiviral interferon (IFN) response mediated by the PRR cyclic GMP-AMP synthase (cGAS) and its adaptor protein stimulator of interferon genes (STING). The study also revealed a novel role for STING during infection: MCMV exploits STING signaling to foster an optimal environment for establishing a successful infection in the host.

Upon binding cytosolic nucleic acids, the PRR cGAS activates the endoplasmic reticulum (ER)-resident protein STING via the second messenger cyclic GMP-AMP (cGAMP). STING then traffics from the ER to the Golgi compartment, where it binds to the kinase TBK1, eventually leading to activation of the transcription factor IRF3, its nuclear translocation and induction of type I IFN transcription (Figure 1). STING is also known to activate the transcription factor NF-kB, which is important for proinflammatory cytokine induction, but the precise nature of STING-mediated NF-kB activation is not well understood. The cGAS-STING pathway is essential for the initial detection of CMV infection. Thus, CMV had to evolve potent inhibitory mechanisms of this pathway to allow successful establishment of chronic infection.

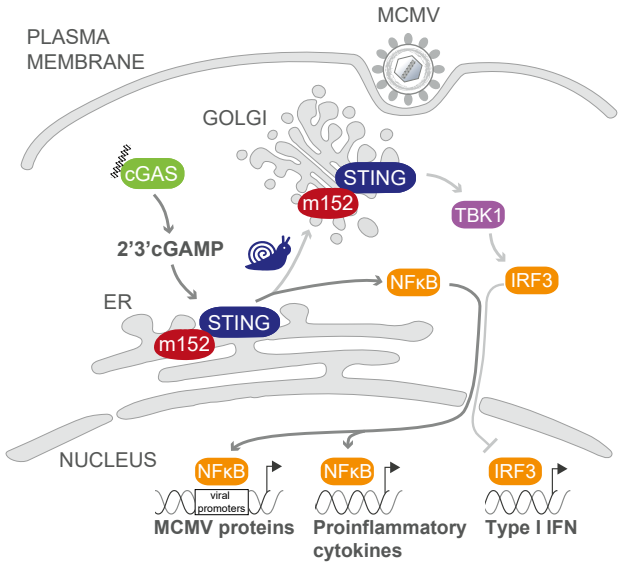


Figure 1: Upon viral infection, the PRR cGAS senses viral nucleic acids and produces the second messenger 2'3'-cGAMP, which binds to the ER-resident protein STING. STING then translocates from the ER to the Golgi compartment, from where it activates a signaling pathway leading to induction of type I IFN via the kinase TBK1 and the IRF3 transcription factor. Moreover, STING induces expression of proinflammatory cytokines via the transcription factor NF-κB. This study revealed several new insights into STING and CMV biology: (1) The viral type I membrane protein m152 specifically binds STING in the ER and thereby slows down its trafficking, leading to a delayed type I IFN response. (2) STING activates the NF-κB signaling pathway from the ER, prior to trafficking, and (3) this function of STING is beneficial for early gene expression of MCMV.

We identified the viral m152 protein as the first MCMV protein that specifically engages the adaptor protein STING. The m152 protein is an ER-localized type I transmembrane protein and efficiently thwarts both Natural Killer cell (NK)- and T cell-dependent immune responses. We have now revealed a third function of m152, which is independent of its effect on NK- and T cell-mediated responses. m152 translocates with STING from the ER to the Golgi compartment upon activation (Figure 2). As a result, m152 perturbs the translocation of activated STING from the ER to the Golgi compartment and thereby delays the type I IFN response to MCMV infection. Interestingly, m152 had no effect on STING-mediated NF-κB activation, which suggested that STING may activate the NF-κB transcription factor prior to trafficking. We observed both *in vitro* and *in vivo* that the inhibitory effect on STING mediated by m152 generates a permissive environment resulting in enhanced transcription of viral genes. However, the absence of STING did not create an advantage for MCMV replication in the first hours of infection, which suggested that STING may exert, in addition to its antiviral role, a proviral role. We made use of the ability of m152 to selectively delay STING translocation from the ER to the Golgi to show that STING activates NF-κB signaling already from the ER (Figure 1) and that NF-kB activity is indeed beneficial for early MCMV gene expression. This study revealed novel insights into the biology of STING and showed that MCMV has evolved a mechanism to specifically antagonize the STING-mediated antiviral IFN response, while exploiting its proviral NF-κB response. This precise modulation provides a crucial advantage at early stages of infection and contributes to understanding of how CMV bypasses the immune system to pave the way to remain in its host for life.

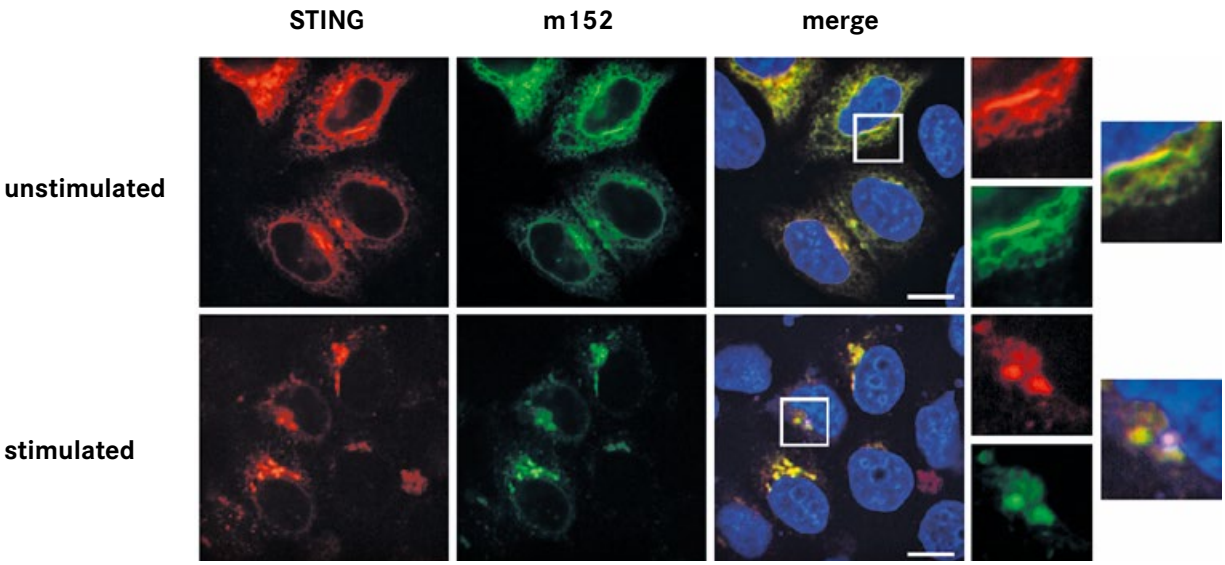


Figure 2: HeLa cells were co-transfected with expression plasmids for STING and m152, and either left unstimulated (upper panel) or were stimulated by co-transfection of cGAS (lower panel). White boxes indicate the region shown at a higher magnification. STING and the MCMV m152 protein co-localize in unstimulated and stimulated cells.

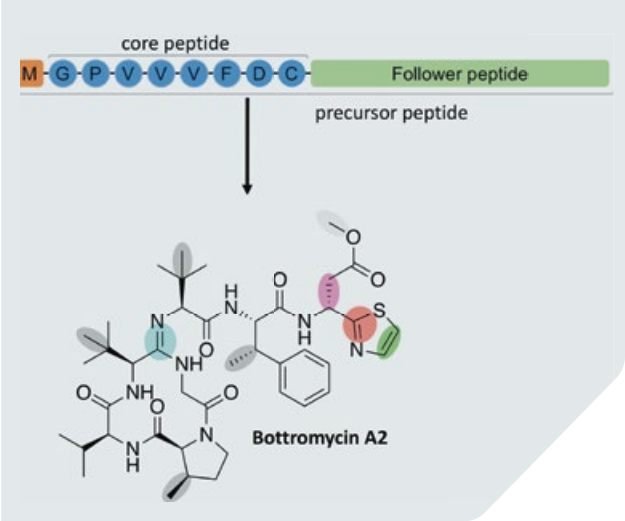
Stempel M, Chan B, Juranić Lisnić V, Krmpotić A, Hartung J, Paludan SR, Füllbrunn N, Lemmermann NA, Brinkmann MM (2019)
The herpesviral antagonist m152 reveals differential activation of STING-dependent IRF and NF-κB signaling and STING's dual role during MCMV infection.
EMBO J. doi: 10.15252/embj.2018100983

INSIGHTS INTO THE BIOSYNTHESIS OF THE NATURAL PRODUCT BOTTROMYCIN

JESKO KÖHNKE | HEAD OF JUNIOR RESEARCH GROUP STRUCTURAL BIOLOGY OF BIOSYNTHETIC ENZYMES

The development of microbial resistance against antibiotics is becoming a global problem - and accelerating. Natural products have been an invaluable source of new antibacterial compounds that often served as a starting point for the development of clinically used antibiotics. Bottromycins are peptide-like natural products discovered in the 1950s and possess antibiotic activity against Gram-positive pathogens, such as the problematic human pathogen MRSA (Methicillin-resistant *Staphylococcus aureus*). The total synthesis of these molecules is very challenging and we have been working towards a detailed understanding of how bacteria make these molecules from simple building blocks. We discovered that the enzyme PurAH plays a critical role in bottromycin biosynthesis and reported its function and crystal structure. Our insights will help to produce bottromycin derivatives that have improved pharmacological properties.

Bottromycins (Figure 1) belong to the fast growing natural product superfamily of ribosomally synthesized and post-translationally modified peptides (RiPPs). Members of the RiPP family have a very peculiar biosynthetic logic – their biosynthesis begins with the expression of a short structural gene via the normal ribosomal route that yields the precursor peptide. The majority of precursor peptides consist of a N-terminal recognition sequence important for processing by parts of the biosynthetic enzymes and a C-terminal core peptide, which ultimately becomes the natural product.



Bottromycins are unique amongst bacterial RiPPs, because their precursor peptide consists of an N-terminal core peptide and a C-terminal follower peptide, which is recognized by several of the biosynthetic enzymes. This inversion results in several biosynthetic peculiarities, one of which is the presence of two YcaO enzymes in the bottromycin biosynthetic gene cluster. YcaO enzymes are extremely versatile enzymes frequently involved in the production of RiPPs. They are, amongst other modifications, able to convert serine, threonine and cysteine residues into five-membered rings (azolines) and frequently have partner proteins that assist them to fulfill their biosynthetic function(s). The modifications introduced by YcaO enzymes endow the precursor peptides with special physico-chemical properties and have been extensively studied. One of the bottromycin YcaO en-

Figure 1: The precursor peptide BotA is enzymatically converted into the natural product bottromycin A2. Post-translational modifications are highlighted.

zymes performs this canonical function, while the second, PurCD, catalyzes the formation of the hallmark modification in bottromycins, the N-terminal macrocycle consisting of four amino acids (Figure 1).

We previously determined PurCD to be an odd enzyme, because it catalyzed the formation of the macrocycle, but also its re-opening. In this study we reported the structure and function of the enzyme PurAH (Figure 2). We found PurAH to be an unusual member of the amidohydrolase superfamily responsible for the separation of core and follower peptides (Figure 3). In doing so, it acts as a novel YcaO helper protein for PurCD by ensuring that the reaction product contains a macrocycle. These data place PurAH as a gatekeeper of bottromycin biosynthesis that determines when the pathway intermediate is "ready" to enter the final phases of bottromycin production. In addition to giving insights into bottromycin biosynthesis that will allow the production of derivatives, it also enables bioinformatic studies to identify novel natural products that employ a similar biosynthetic logic.

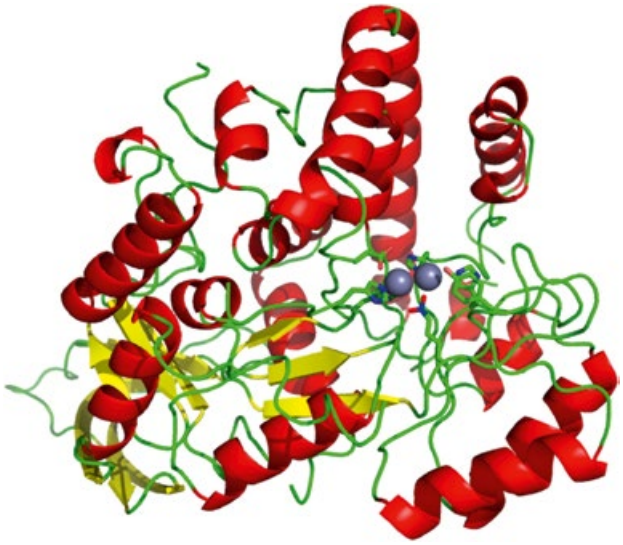


Figure 2: Schematic representation of the structure of PurAH colored according to secondary structure. The two Zn2+-ions bound at the active site are shown as grey spheres.

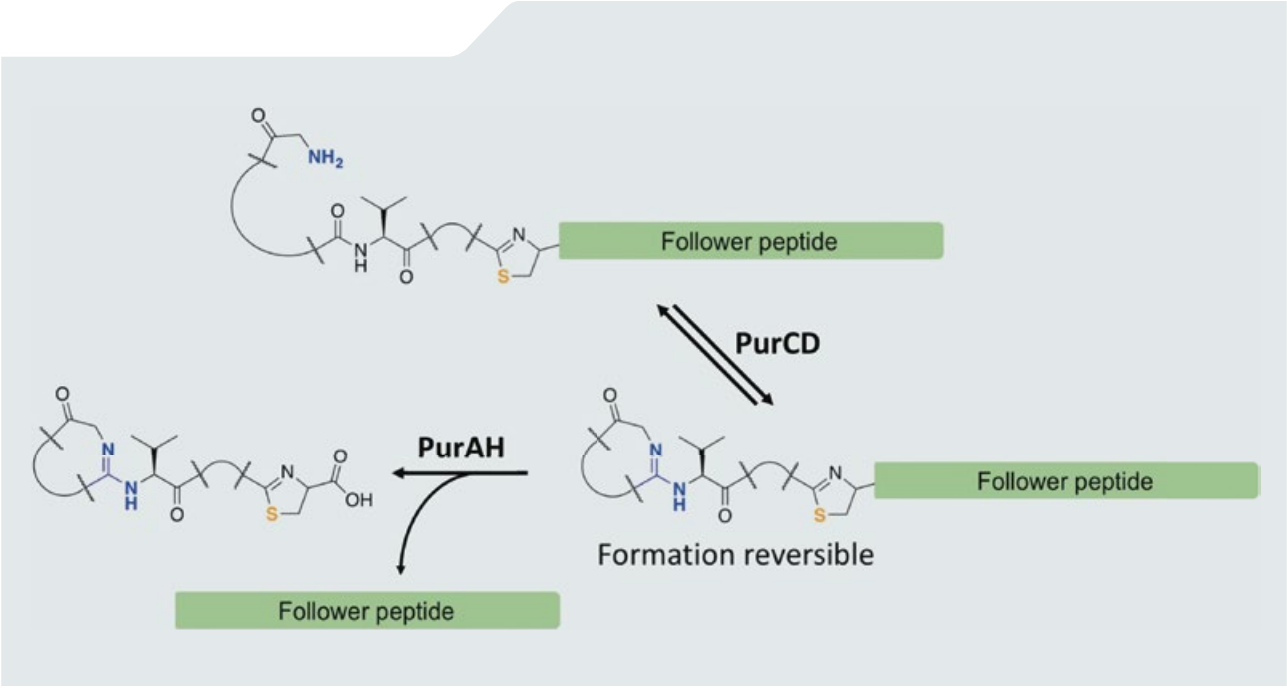


Figure 3: PurAH pulls the equilibrium of macrocyclization in the direction of product by removing the follower peptide, thus preventing PurCD from engaging with the substrate. Adapted from Sikandar et al., JACS 2019.

Sikandar A, Franz L, Melse O, Antes I, Koehnke J (2019)
Thiazoline-specific amidohydrolase PurAH is the gatekeeper of bottromycin biosynthesis
J Am Chem Soc. doi: 10.1021/jacs.8b12231

REPURPOSING ANTI-CANCER DRUGS TO COMBAT ANTIBIOTIC RESISTANCE

EVA MEDINA | HEAD OF RESEARCH GROUP INFECTION IMMUNOLOGY

There is an urgent need for new antibiotics, particularly for those directed against multi-drug resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA). In this regard, repurposing of non-antibiotic drugs for antimicrobial therapy has gained increasing interest in recent years. In this study, the cancer marketed agent sorafenib and its more potent derivative PK150 were identified as repurposed drug candidates against MRSA. PK150 exhibited great antibacterial potency, optimal stability and oral bioavailability as well as suitable *in vivo* efficacy and did not induce *in vitro* resistance. The results of this study support the further development of PK150 for clinical studies.

The rise of *Staphylococcus aureus* "superbugs" resistant to many antibiotics such as MRSA is a serious concern. Therefore, finding new drugs to effectively control and treat this pathogen is paramount. Currently, very few antibiotics effective against MRSA or against other antibiotic-resistant pathogens in general, are being developed, leading to a severe crisis in effective therapeutic options. This is further aggravated by the low interest of major pharmaceutical companies in antibiotic research because of unfavorable economic returns. Drug repurposing – the use of approved drugs for novel therapeutic purposes – has emerged as an innovative strategy to find new antimicrobial agents. Since approved drugs have known safety and pharmacokinetic profiles, repurposing can reduce time, costs and risks associated with the development of novel antibiotics. In a cooperative study between HZI and S. Sieber (University of Munich), it was found that sorafenib, a marketed cancer drug (Nexavar), and its chemical derivative PK150 (Fig. 1) exhibit antimicrobial activity against MRSA. PK150 has even more potent antimicrobial activity than the original drug sorafenib and does not exhibit the kinase inhibitory effect of sorafenib on eukaryotic cells. PK150 was also found to be effective against persisters, which are dormant bacteria highly tolerant to antibiotic killing, and also against *S. aureus* within difficult-to-treat biofilms. Furthermore, PK150 exhibits excellent stability and pharmacokinetic properties includ-

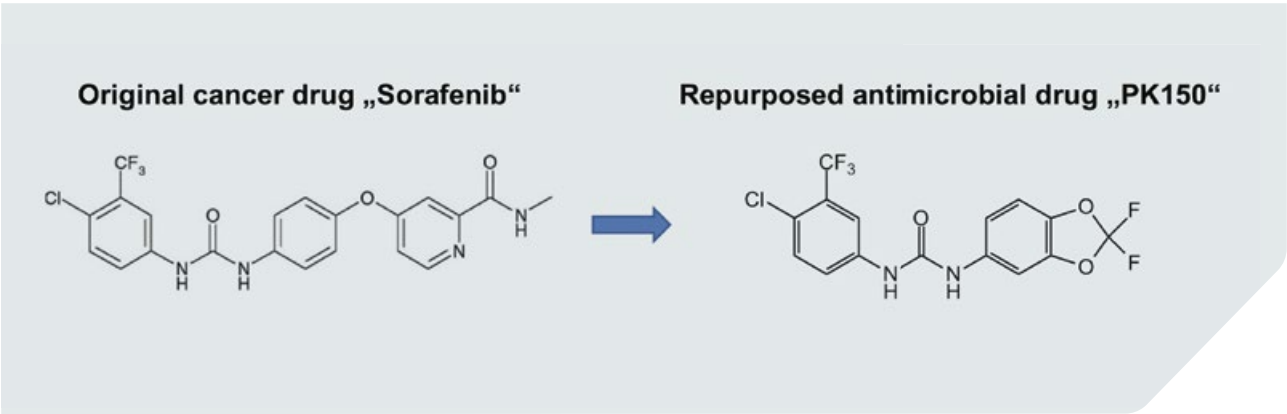


Figure 1: Structures of Sorafenib and PK150.

ing oral bioavailability and is as effective as the marketed antibiotic levofloxacin at controlling *S. aureus* infections in pre-clinical murine models. The mode of action of PK150 against MRSA indicates that this antibiotic targets several bacterial proteins including demethylmenaquinone methyltransferase (MenG), involved in menaquinone biosynthesis, as well as signal peptidase IB (SpsB), involved in the bacteria protein secretion pathway. Thus, by targeting MenG, PK150 leads to alterations in the bacterial energy metabolism; and by targeting SpsB, PK150 induces dysregulation of the secretory machinery, compromising the bacterial cell wall integrity (Fig. 2). Development of resistance of *S. aureus* against PK150 was not observed in this study. Multi-target antibiotics are, in general, less prone to the development of resistance since the most frequent cause of antibiotic resistance are mutations occurring in the target, rendering the antibiotic ineffective. Hence, the likelihood that resistance develops in multiple targets in parallel is extremely rare. In summary, the results of this study demonstrate the therapeutic potential of PK150, a derivative of a marketed cancer drug, for the treatment of MRSA infections, indicating the repurposing of this cancer drug in a new avenue.

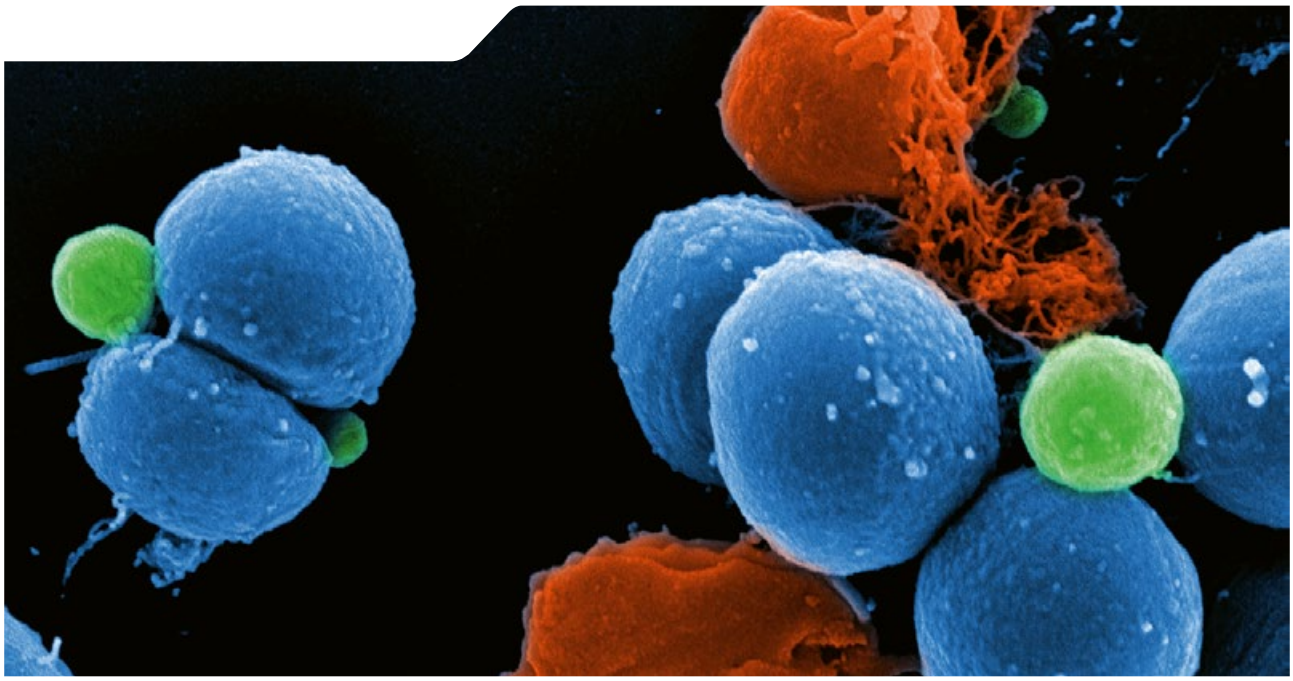


Figure 2: Scanning electron microscope image of intact MRSA (blue), vesicle formation induced by PK150 (green) and MRSA destroyed by PK150 (red). © HZI/Manfred Rohde

Le P, Kunold E, Macsics R, Rox K, Jennings MC, Ugur I, Reinecke M, Chaves-Moreno D, Hackl MW, Fetzer C, Mandl FAM, Lehmann J, Korotkov VS, Hacker SM, Kuster B, Antes I, Pieper DH, Rohde M, Wuest WM, Medina E, Sieber SA (2019)

Repurposing human kinase inhibitors to create an antibiotic active against drug-resistant *Staphylococcus aureus*, persisters and biofilms

Nature Chemistry doi: 10.1038/s41557-019-0378-7

DIVERSE PHYLOGENY FOR CHEMICAL DIVERSITY: EXPLORING MYXOBACTERIAL NATURAL PRODUCT RICHNESS

ROLF MÜLLER | HEAD OF DEPARTMENT MICROBIAL NATURAL PRODUCTS

The increasing resistance of pathogens towards clinically relevant antibiotics poses a growing risk for human health. New treatment options are urgently needed and natural products have long served as a valuable source of new lead compounds for the development of new medicines. In this study we took a close look at the diversity of natural products from soil-living myxobacteria in order to guide our ongoing efforts for the discovery of novel chemical scaffolds from this promising resource. Using high-resolution mass spectrometry coupled to statistical analysis, we show that the isolation of new species, genera and families of myxobacteria is particularly likely to deliver new natural products in the future.

Microorganisms have a long track record as a treasure trove of bioactive natural products, termed secondary metabolites, which are generally seen as evolutionarily optimized molecules and thus constituting interesting starting points for the development of new pharmaceuticals. As part of their search for suitable lead compounds, scientists at HIPS focus on the myxobacteria, a group of soil-living microbes which are nowadays recognized as outstanding producers of secondary metabolites due to their capability to deliver molecules with extraordinary structural complexity and often potent biological activity (Fig. 1).

Extracting new bioactive ingredients from the complex mixtures obtained by cultivation of myxobacteria is a formidable challenge, as the much sought-after candidate molecules are “hidden” within a large number of other compounds that make up the myxobacterial secondary metabolome. A combination of bioactivity-guided isolation, genome-based approaches and metabolomics-inspired methods is nowadays being used for the discovery of novel myxobacterial secondary metabolites. To gain insights into expectable myxobacterial natural product richness, we recently performed a systematic metabolite survey of ~2300 myxobacterial strains

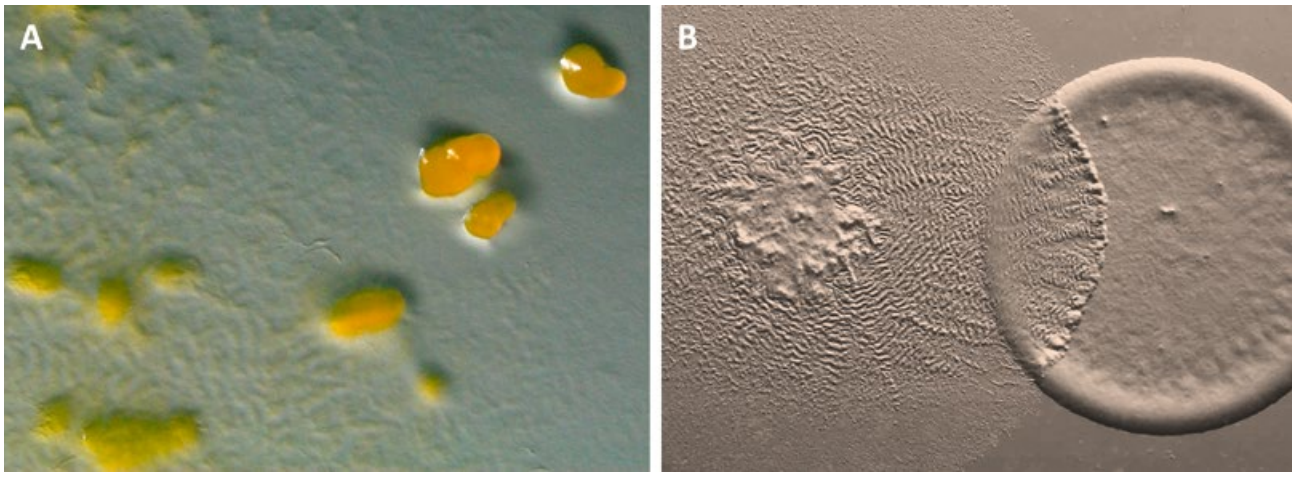


Figure 1: Microscopic images of myxobacteria. **A:** These intriguing microbes are able to move over surfaces as swarms and form multicellular fruiting bodies **B:** Many myxobacterial species also show predatory behavior towards other microorganisms. Here, a swarm of Cystobacter (left) is underway to eradicate an *E. coli* colony (right). Pictures: Ronald Garcia, HIPS

using high-resolution mass spectrometry. Our analysis included both known and previously unidentified metabolites detected under laboratory cultivation conditions, thereby enabling large-scale comparison of production profiles in relation to myxobacterial taxonomy.

We found a correlation between taxonomic distance and the production of distinct secondary metabolite families - thus supporting the idea that the chances of discovering novel metabolites are greater by examining strains from new species, genera and families rather than additional representatives within previously explored clades. This is an important conclusion guiding our ongoing efforts for the isolation of novel myxobacterial taxa and their prioritization with a view to novel secondary metabolite chemistry (Fig. 2).

In our study we also report the discovery and structure elucidation of rowithocin, a myxobacterial secondary metabolite featuring an uncommon phosphorylated polyketide scaffold based on the combined evaluation of biological activity and taxonomic distribution of mass spectrometry signals. We envision that metabolomics-enhanced approaches to natural products discovery will play an important role not only for myxobacteria in the future.

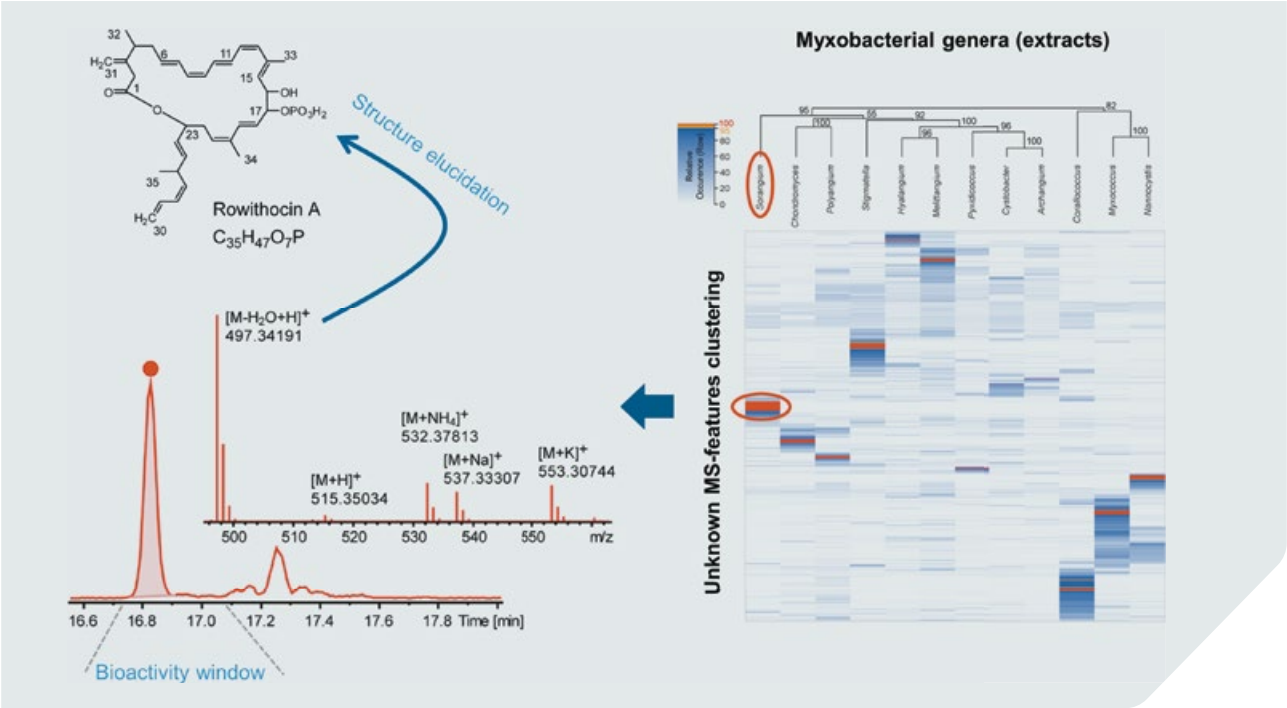


Figure 2: Taxonomic distribution analysis using a high-resolution mass spectrometry "warehouse". Genus-specific signals (circled in red) highlight novel secondary metabolites in bioactive fractions from myxobacterial extracts, enabling the identification of the new anti-staphylococcal compound rowithocin.

Hoffmann T, Krug D, Bozkurt N, Duddela S, Jansen R, Garcia R, Gerth K, Steinmetz H, Müller R (2018)
Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria
Nat. Commun. doi: 10.1038/s41467-018-03184-1

FROM THE *IN VIVO* ANALYSIS OF INFECTIONS TO A RAPID DIAGNOSIS

DIETMAR PIEPER | HEAD OF RESEARCH GROUP MICROBIAL INTERACTIONS AND PROCESSES

Necrotizing soft tissue infections (NSTIs) are rare but devastating infections characterized by extensive necrosis of skin and subcutaneous tissues. A better understanding of the microbiology of NSTIs is important, not only to guide therapy, but also to uncover pathways important in the pathophysiology of the infection that can be targets for development of more rational individualized interventions. Here, we integrated microbial community profiling with analysis of host-microbe interactions by RNA-sequencing to identify substantial differences between the pathophysiology of monomicrobial streptococcal and polymicrobial NSTIs and used this information to identify a specific response that could be exploited as potential diagnostic biomarker.

NSTIs are known for rapid progression and high mortality and often require rapid surgical intervention and amputation (Figure 1). While NSTIs can occur in healthy individuals of all age groups, they are most common in patients with recent surgical interventions, immunodeficiencies or other medical conditions. Their fulminant course necessitates rapid diagnosis and treatment includes drastic surgical debridement of the infected tissue and broad-spectrum antibiotic therapy.

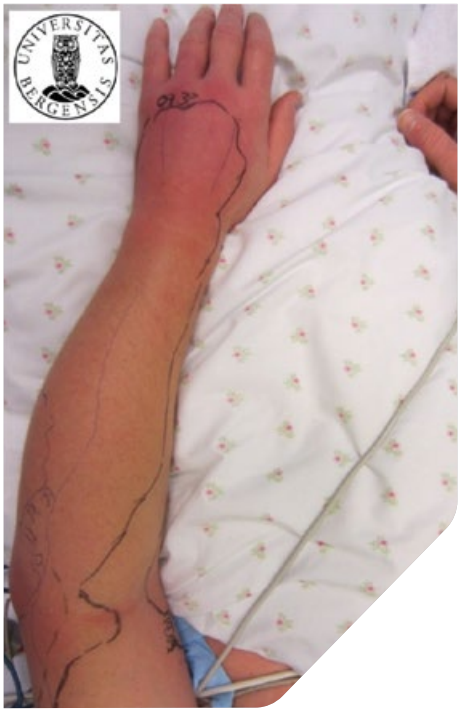


Figure 1: *S. pyogenes* necrotizing soft tissue infection before surgery. © University Bergen

Clinically well-known pathogens, specifically *Streptococcus pyogenes* are frequent causes of NSTIs, however reports on "flesh-eating bacteria" have become increasingly common in the news in recent years. This is probably due to the fact that one of the bacterial species causing these infections (*Vibrio vulnificus*) is commonly contracted during bathing in temperate waters and currently spreads to new areas. Importantly, the majority of NSTIs are caused by polymicrobial infections of otherwise commensal microorganisms. The functionalities that enable them to establish severe infections resembling those caused by specialized pathogens remain poorly understood.

The EU funded 'INFECT' project aimed to improve knowledge of pathophysiology, prognosis and diagnosis of NSTIs and established the world's largest NSTI patient cohort and sample collection. Using this tool, we generated a knowledge base on bacteria involved in NSTIs. We improved our understanding of the subtle differences of NSTIs caused by different bacteria to enable clinicians to promptly and correctly diagnose and treat these infections.

The majority of monomicrobial NSTIs were associated with *S. pyogenes* (Figure 2). The composition of bacterial communities in polymicrobial NSTIs was highly variable, but typically comprised *Prevotella* spp., *Porphyromonas* spp., *Parvimonas* spp., *Fusobacterium* spp., *Peptostreptococcus* spp. and *Bacteroides* spp. bacteria that are common inhabitants of the healthy human intestine, skin and oral microbiota. We were able to show that these cooperating communities (called 'pathobionts') can cause infections that are of similar mortality and destructive potential as those caused by 'professional' pathogens. The molecular mechanisms that cause the widespread tissue destruction, however, varied substantially between polymicrobial infections associated with pathobionts and those caused by *S. pyogenes*.

While pathogenic *S. pyogenes* express a wide range of virulence factors that mediate the different steps of infection, from tissue colonization to evasion of the host immune response, the pathogenic potential of pathobionts in polymicrobial NSTIs is dependent on the co-occurrence of multiple bacterial taxa, which complement each other to enhance the virulence of the bacterial community as a whole.

These unique properties of the infectious agents could be exploited to derive rapid, accurate bacterial diagnosis as different host responses were detected not only within the infected tissue that but also in blood serum. We hope that our work will help to improve rapid microbial diagnosis of NSTIs and ultimately reduce the mortality of these traumatic infections.

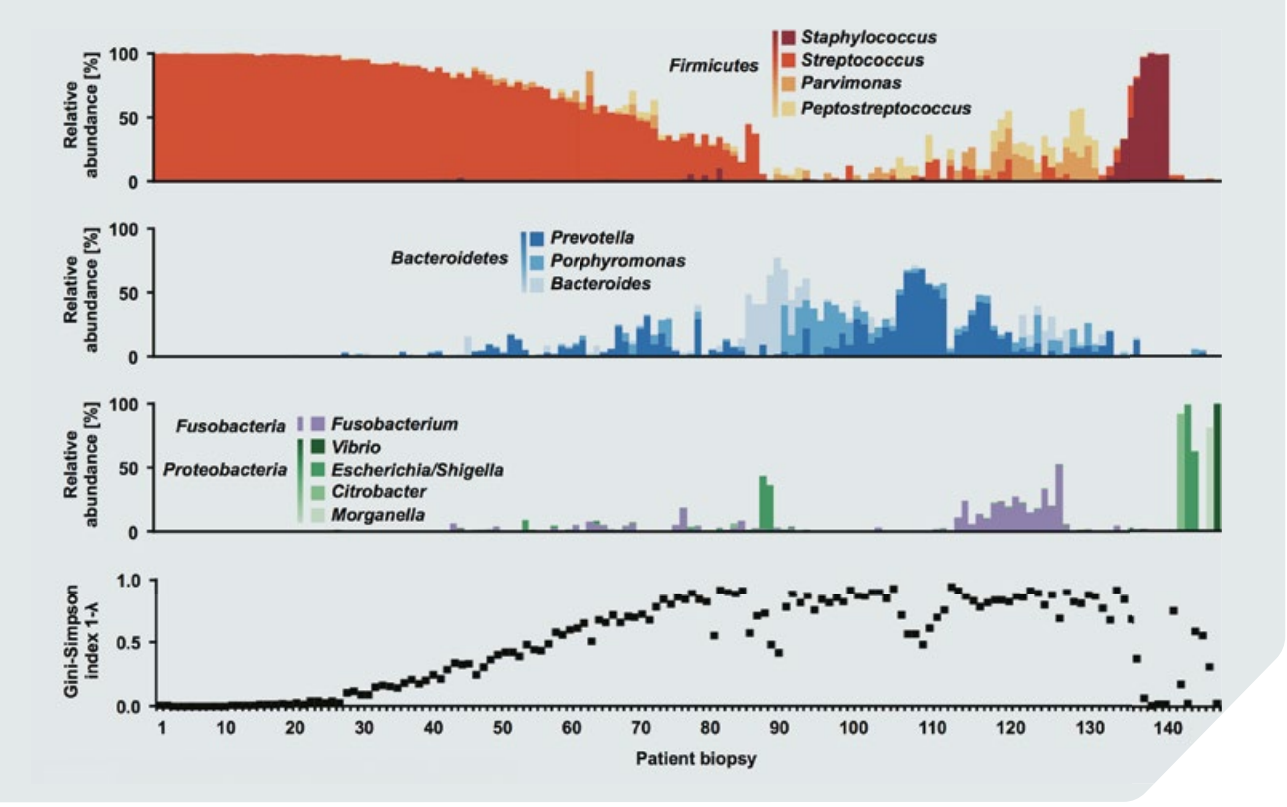


Figure 2: Bacterial composition in tissue biopsies (n = 148) from patients with NSTIs. Bacterial genera with a mean relative abundance of ≥2.5% across all samples or a maximal relative abundance of ≥80% are depicted. The Gini–Simpson diversity index shows genus-level diversity.
From Thänert et al., Nature Commun. 26: 3846 (2019). Reprinted with permission from Nature.

Thänert R, Itzek A, Hoßmann J, Hamisch D, Madsen MB, Hyldegaard O, Skrede S, Bruun T, Norrby-Teglund A, INFECT study group, Medina E, Pieper DH (2019)
Molecular profiling of tissue biopsies reveals unique signatures associated with streptococcal necrotizing soft tissue infections
Nat Commun. doi: 10.1038/s41467-019-11722-8

CHALLENGES AND OPPORTUNITIES IN DEVELOPING AN HCV VACCINE

THOMAS PIETSCHMANN | HEAD OF DEPARTMENT EXPERIMENTAL VIROLOGY

Hepatitis C, a chronic liver disease caused by infection with the hepatitis C virus (HCV), affects 71 million people. It increases the risk of liver cirrhosis and hepatocellular carcinoma, and if untreated, can lead to death. HCV infection is now treatable with antivirals, but viral cure does not offer protection against future re-infections. The development of a prophylactic vaccine would allow containing infections and re-infections and it would possibly lead to the global eradication of HCV. One of the major obstacles to the development of an effective vaccine is the heterogeneity of the different types of HCV strains. In this work, we aimed to develop engineered viral antigens, to focus antibody responses on highly conserved domains of the viral envelope proteins E1 and E2. Ideally, such antigens may induce protective immunity against multiple viral strains.

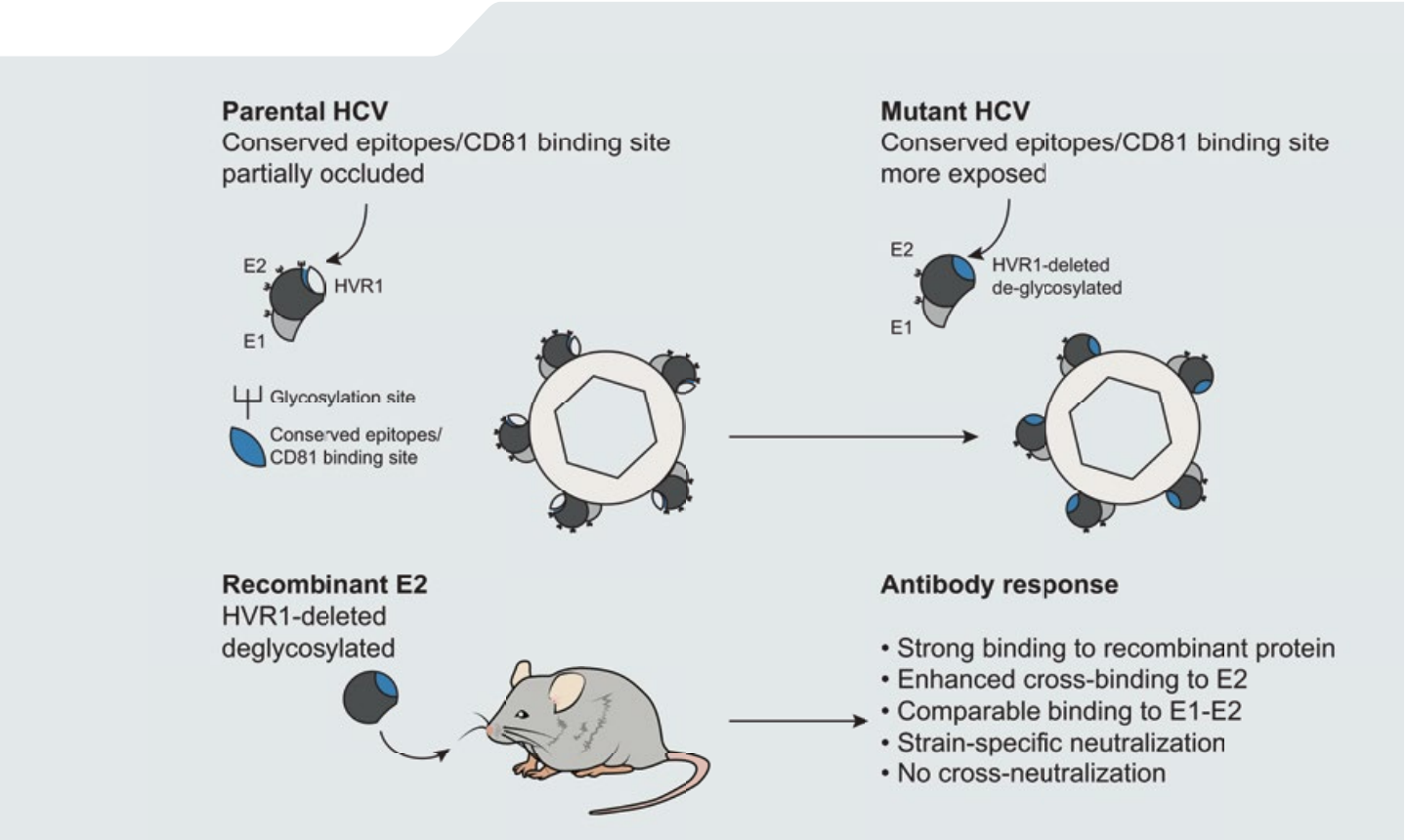


Figure 1: Overview of the experimental procedure and main results. Adapted from J. Hepatol. 2019, 70: 593–602.

In the constant evolutionary arms race between the HCV and its host, the virus has developed defensive mechanisms that constrain the protective capacity of antibodies.

For example, the HCV E1 and E2 proteins form a heterodimer on the virus particle that has the crucial task of interacting with cellular CD81, which serves as receptor for viral cell entry. The highly conserved viral sequence that allows the coupling of E1/E2 with CD81 and that would offer a perfect target to develop antibodies, is however protected by a "cap" – the so called hypervariable region 1 (HVR1). Initial virus cell-surface contacts with host lipoprotein receptors such as the Scavenger receptor class B type 1 (SR-B1) push away this cap. These early contacts are rather flexible and E1-E2 accommodate large sequence variation while maintaining this function. As consequence, the cap domain drifts substantially under antibody pressure making it the most variable genome part of the virus. Cunningly, this two-step mechanism allows for exposure of the much conserved and functionally constrained CD81 binding site just prior to contact with CD81. Hence, it is hardly available to antibodies and the cells of the immune system. Moreover, the mature forms of E1 and E2 are heavily glycosylated. These sugar modifications enhance the protection of the conserved parts of these proteins from attack by the immune system.

Together with our partners from the German Center for Infection Research (DZIF) and in the context of the Helmholtz Alberta initiative, we evaluated the immunogenicity of engineered E2 proteins, which lack the HVR1 cap and specific glycosylation sites. We could show that some of these antigens much more openly present the conserved viral CD81 binding site. By immunizing mice, we obtained vigorous anti-HCV antibodies. In fact, some of the engineered antigens induced antibodies with improved binding to E2 proteins from diverse viral strains. However, these antibodies were unable to cross-neutralize different HCV variants. Taken together with the recent observation that the CD81 binding site itself is structurally flexible, these results suggest that antigen engineering should focus on enhancing exposure of the CD81 binding site and at the same time on constraining its flexibility. In the end, structure-based antigen engineering should help developing antigens that induce broadly cross-protective antibodies.

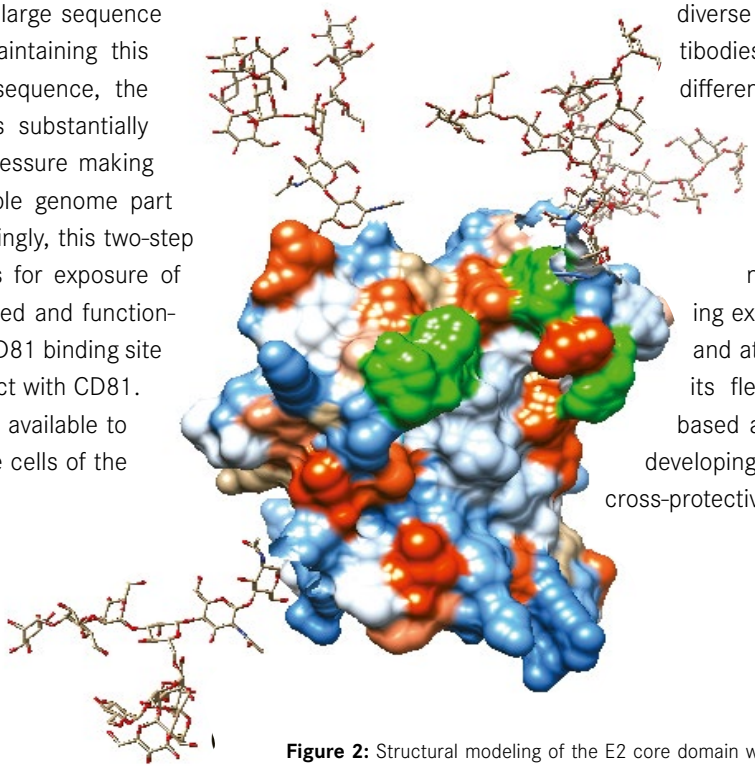


Figure 2: Structural modeling of the E2 core domain with the CD81 binding site highlighted in green. The E2 protein is colored by hydrophobicity of amino acid side chains (red and blue represent hydrophobic and hydrophilic residues, respectively) and green shows antibody or CD81 contact residues.

Khera T, Behrendt P, Bankwitz D, Brown RJP, Todt D, Doepke M, Khan AG, Schulze K, Law J, Logan M, Hockman D, Wong JAJ, Dold L, Gonzalez-Motos V, Spengler U, Viejo-Borbolla A, Ströh LJ, Krey T, Tarr AW, Steinmann E, Manns MP, Klein F, Guzmán CA, Marcotrigiano J, Houghton M, Pietschmann T (2019)
Functional and immunogenic characterization of diverse HCV glycoprotein E2 variants
J Hepatol. doi: 10.1016/j.jhep.2018.11.003.

DISSECTING THE BATTLE BETWEEN PATHOGENS AND HOST CELLS AT THE SINGLE CELL LEVEL

ANTOINE-EMMANUEL SALIBA | HEAD OF RESEARCH GROUP SINGLE-CELL ANALYSIS

Cells are equipped with multiple defense mechanisms to fight off intruders and gene programmes must be activated during an enemy attack in an ordered manner. We have developed a new method, scSLAMseq, which enables us to track the activity of thousands of genes in individual cells over several hours. Here, we were able to explain why some cells are successfully infected by a virus, whereas others are not.

Cytomegaloviruses are widespread, with more than 80 percent of people infected. Generally, healthy people are not harmed by the virus, however an infection in newborns or transplant patients can become dangerous and have serious neurological consequences. When viruses enter our bodies – such as during an influenza or a gastrointestinal infection – cells respond to the intruders to control their spread but sometimes viruses can take over the cellular machinery control. It then produces viral components on a massive scale allowing the intruder to multiply exponentially. But how can it be that one cell is overrun and another succeeds in getting the virus under control?

An experimental method, single-cell RNA sequencing (scRNAseq), that emerged only a few years ago, can be used to determine which genes of a cell are currently active. However, short-term changes in gene activity, such as those that occur during a viral infection, can hardly be detected. In addition, each individual cell can only be examined once. With current technologies it therefore remained unclear, how individual cells react to external stimuli, for example a viral infection. In order to investigate the molecular processes taking place in individual infected cells, we developed a new method, called scSLAM-seq (single-cell, thiol-(SH)-linked alkylation of RNA for metabolic labelling sequencing) which,

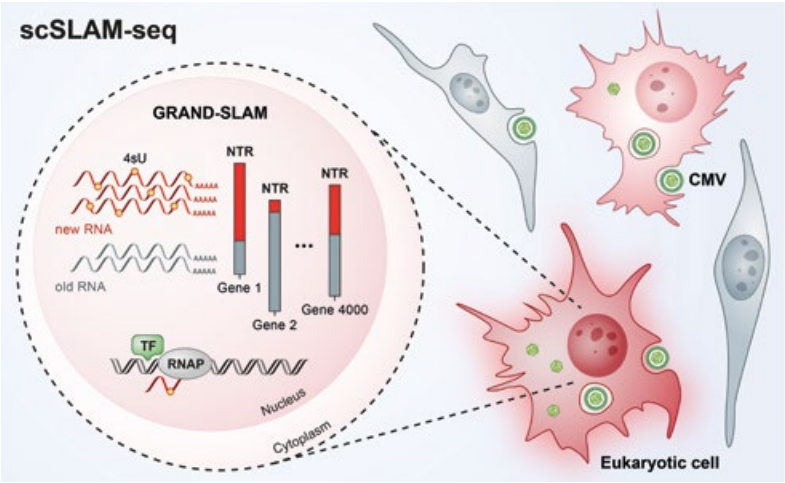


Figure 1: scSLAM-seq captures newly synthetized RNAs in viral infected single-cells. NTR: New-to-total RNA; TF: transcription factor; RNAP: RNA polymerase. Realization: Sandy Pernitzsch. Credit: HIRI

for the first time, enables us to visualize which genes are activated in individual cells within the time frame of a few hours (Figure 1 and 2).

In order to distinguish between RNA that was already present before viral infection and new RNA that was made thereafter, we used metabolic RNA labelling, biochemical nucleoside conversion, and scRNA-seq to record transcriptional activity directly by differentiating between new and old RNA for thousands of genes per single cell. We added a form of the RNA component uracil, which was chemically modified in comparison to its natural variant, to the culture medium of the cells at the same time as the infecting virus. The cells then incorporated the labelled uracil into their newly produced RNA. After extracting RNA from cells two hours later, the labelled uracil was converted into another RNA component, cytosine, via a chemical reaction. In this way, we were able to commonly assess the expression of more than >4000 genes per cell and precisely quantify the relative contributions of new and old RNAs.

scSLAM-seq opens a window on cellular processes that have been hidden so far. The technology integrates the time component on top of transcriptomics data and permits us to visualize and predict infection outcome with an unprecedented resolution.

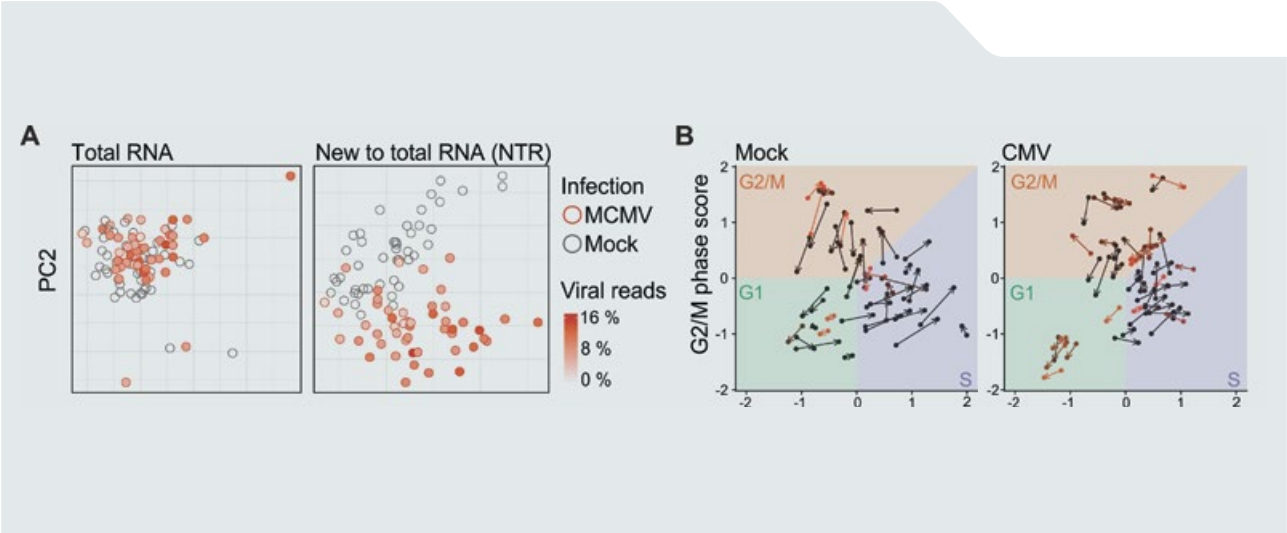


Figure 2:
A: Principal component (PC) analysis of cellular genes does not separate MCMV infected (red-framed circles) from uninfected (grey-framed) cells (left panel), whereas NTR-based gene activity measurement does so successfully (right panel).

B: Although infection was initiated at all cell-cycle stages, cells infected during G1 phase resulted in significantly stronger viral gene expression and cell-cycle disruption. The figure supports this conclusion by analysis of cell-cycle progression (G1, S and G2/M phases) for the uninfected and CMV-infected cells, showing trajectories based on cell-cycle states deduced from old RNA (base of arrows) and total RNA (tip of arrows). Adapted from Erhard et al. 2019 Nature.

Erhard F, Baptista MAP, Krammer P, Hennig T, Lange M, Arampatzi P, Jürges CS, Theis FJ, Saliba A-E, Dölken L (2019)
scSLAM-seq reveals core features of transcription dynamics in single cells
Nature doi.org/10.1038/s41586-019-1369-y

ORALLY BIOAVAILABLE GLYCOMIMETICS BLOCK RESISTANCE-CONFERRING BIOFILMS OF *P. AERUGINOSA*

ALEXANDER TITZ | HEAD OF RESEARCH GROUP CHEMICAL BIOLOGY OF CARBOHYDRATES

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic priority 1 pathogen for infections of cystic fibrosis and immune-deprived patients. It can switch from planktonic life to the so-called biofilms, which are a prime mechanism of antimicrobial resistance. This is why new therapies targeting bacterial biofilm formation and virulence are urgently needed, in addition to new antibiotics targeting bacterial viability. Two lectins, the proteins LecA and LecB, are involved in *P. aeruginosa* biofilm formation. LecB forms non-covalent homotetramers and each monomer contains one binding site for its carbohydrate ligands. One of its proposed roles is to function as a glue in the biofilm matrix by cross-linking exopolysaccharides and bacteria (Figure 1). Because the lectins are located extracellularly, in contrast to many antibiotic targets, they are easy to reach as targets for biofilm inhibition. In this work, a new class of drug-like low molecular weight blockers of LecB with nanomolar affinity and excellent receptor binding kinetics and thermodynamics is reported (Figure 2). The new glycomimetic inhibitors are orally bioavailable and prevent biofilm formation of *P. aeruginosa in vitro* where the natural carbohydrate ligands were ineffective.

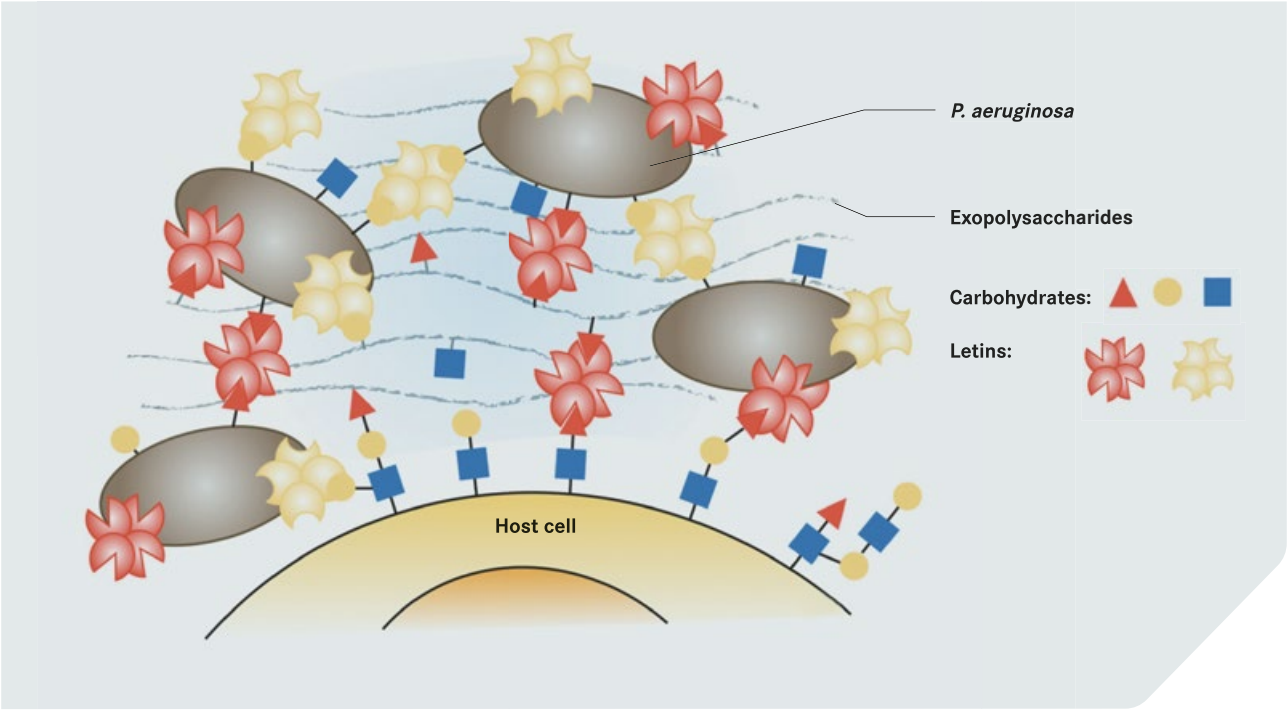


Figure 1: Lectins serve as a "bioglue" in the bacterial biofilm by cross-linking the exopolysaccharides to bacteria and the surface of the host cells.

Natural ligands of LecB inspired the synthesis of glycomimetic anti-biofilm compounds: C-glycosidic small molecules 1 and 2 combine aromatic substituents at C-6 in mannose-based inhibitors with an equatorial methyl group from L-fucose derivatives. The chemically synthesized glycomimetic C-glycosides showed nanomolar inhibition for LecB variants from two strains representing the broad collection of clinical isolates studied before. This suggests that the glycomimetics will be broadly active against LecB-mediated biofilm formation in clinical isolates.

The compounds obtained showed good biophysical properties with kinetic receptor residence times up to 28 minutes and enthalpy driven binding. These glycomimetics are potent inhibitors of biofilm formation without affecting bacterial viability, the former was not achieved with natural glycosides despite also showing high target binding affinity. Due to the absence of an effect on bacterial viability, the rapid resistance development, well-known for traditional antibiotics, is less likely for these pure anti-biofilm substances.

Target selectivity was studied with langerin, a human fucose- and mannose-binding C-type lectin, and in a global approach using stimulated primary murine spleen cells. Both experiments showed no indications for off-target effects and cytotoxicity of the compounds was absent. In *in vitro* pharmacokinetics experiments good metabolic stability against liver microsomes and plasma was observed.

In vivo, the pharmacokinetics of 2a and 2b in mice showed good oral bioavailability resulting in high plasma concentrations and subsequent excretion via the kidneys. The high compound concentrations in urine urge for an application in a *P. aeruginosa* urinary tract infection model. Future work will now evaluate the compounds' anti-infective efficacy in a mono-therapy against biofilms and combined with antibiotics for efficient eradication of the infection.

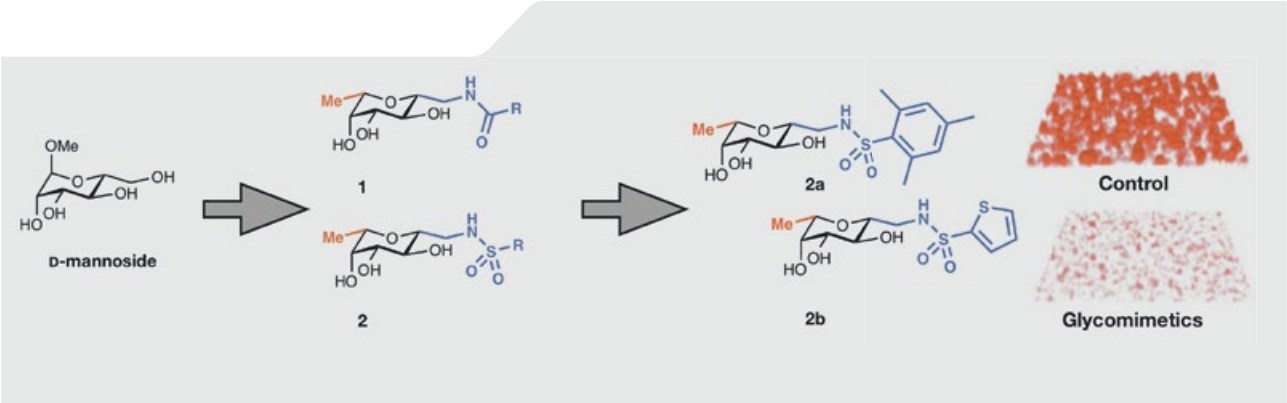


Figure 2: Inspired from natural ligands, glycomimetics 2a and 2b were developed from D-mannosides. They inhibit biofilm formation and show very good oral bioavailability. Adapted from Sommer et al., J Am Chem Soc 2018.

Sommer R, Wagner S, Rox K, Varrot A, Hauck D, Wamhoff EC, Schreiber J, Ryckmans T, Brunner T, Rademacher C, Hartmann RW, Brönstrup M, Imberty A, Titz A (2018)
Glycomimetic, orally bioavailable LecB inhibitors block biofilm formation of *Pseudomonas aeruginosa*
J. Am. Chem. Soc. doi: 10.1021/jacs.7b11133

BACTERIAL 'SLEEPER CELLS' EVADE ANTIBIOTICS AND WEAKEN DEFENCE AGAINST INFECTION

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

So called „persisters" were discovered in 1944 and were thought to be dormant, metabolically inactive bacteria lying low in the body, acting as a "time bomb" for relapse. In the publication presented here, scientists reveal that *Salmonella* persisters hiding in the body's immune cells are actually far from inactive. In contrast, they keep producing and secreting virulence factors to counteract the killing ability of their host macrophages. In other words, unlike in pure bacterial cultures, *in vivo* persisters cannot afford to shut down their metabolic activity completely, but have to maintain protein production and translocation to fight back the host's immune system.

During growth, genetically clonal bacterial populations contain a small fraction of nongrowing, nondividing cells that arise from transient, reversible phenotype switching. These growth-arrested cells are usually tolerant to antibiotics and are called antibiotic persisters. A large proportion of the intracellular pathogen *Salmonella* adopts a nongrowing, antibiotic-tolerant state within macrophages. The first *Salmonella* persister cells that regrow upon release from their host cells are those that maintain metabolic activity during infection.

The authors asked if retention of transcriptional and translational activity might confer an additional physiological benefit to nongrowing bacteria within a host cell. They found that these cells are not dormant but are actively modulating their environment. *Salmonella* within their host macrophage niche deployed a specialized type 3 secretory system called SPI-2 to deliver virulence factors, including SteE, into host cells. SteE changed the cytokine profile of the infected macrophages to reprogramme them into a noninflammatory and

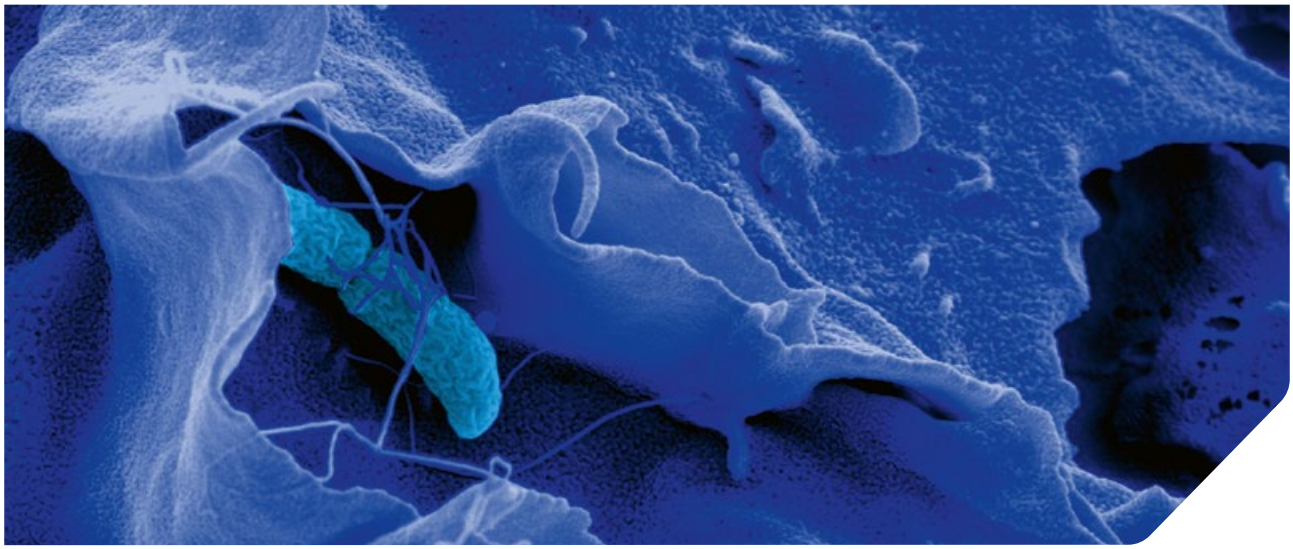


Figure 1: Macrophages, immune cells that have a key role in protecting the body against infections, engulfing *Salmonella* bacteria.

infection-permissive state (M2), thereby undermining host immune defenses. Such reprogramming allowed nongrowing *Salmonella* cells to survive for extended periods in their host. Thus, the bacteria's metabolic activity might confer an advantage to the pathogen during relapse once antibiotic pressure is relieved. In fact, when antibiotics were removed, the *Salmonella* could reemerge and cause disease.

The results of this joint investigation of scientists at the HZI branch institute HIRI together with the lab of Sophie Helaine of Harvard Medical School may help explain why some people suffer from repeated bouts of an illness, despite taking antibiotics, and how this might be circumvented in the future. Although this study investigated *Salmonella* infection of mouse macrophages, many types of bacteria that commonly cause illness are known to form persisters in humans. Such species include pathogenic *E. coli* and the bacterium responsible for tuberculosis, *Mycobacterium tuberculosis*. Researchers are now investigating whether there are ways of turning the tables against these pathogens. The finding that *in vivo* persisters cannot afford to "sleep as tightly" as *in vitro* might be their vulnerability. If one could find means to wake up persisters this would allow our immune system in combination with antibiotic treatment to clear the infection. Thus, a new way of eliminating these bacteria from the body, and thus to preventing recurrence of the infection, would be the result.

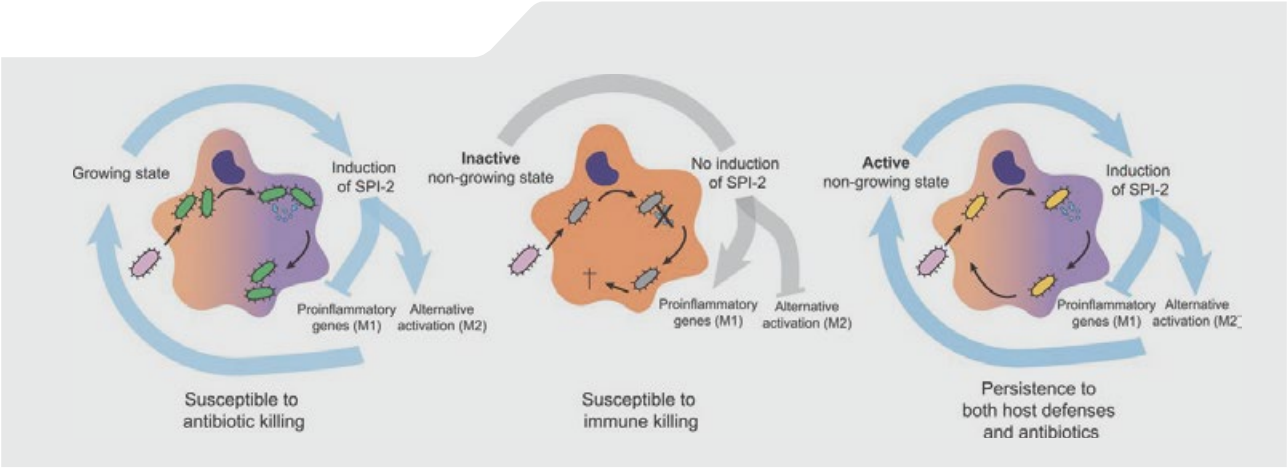
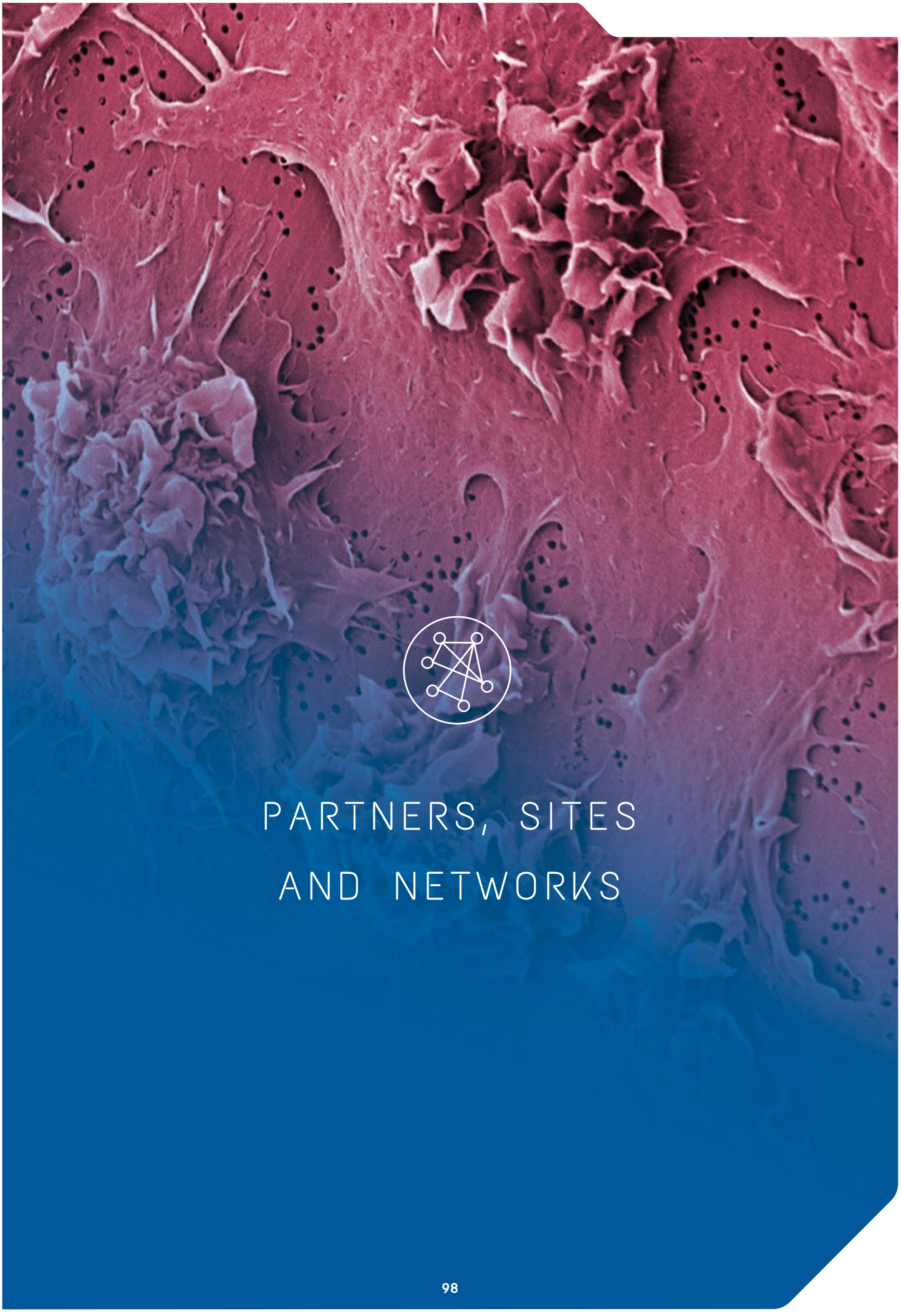


Figure 2: Persisters undermine the host innate immune response and enable long-term survival. Model of macrophage manipulation by *Salmonella* persisters: Growing *Salmonella* bacteria translocate SPI-2 effectors that reprogramme their host macrophage to turn off damaging immune system activation (alternative activation or M2) and create a less hostile environment. Proliferation makes the bacteria susceptible to antibiotic killing (left). Inactive nongrowing bacteria cannot translocate SPI-2 effectors and are killed in the strongly antimicrobial environment (middle). Active persisters manipulate host cell polarization through translocation of SPI-2 effectors, and in turn they retain their activity and maintain the ability to survive in the host while being antibiotic tolerant (right). From Stapels et al., Science; 362: 1156-1160 (2018). Reprinted with permission from AAAS.

Stapels DAC, Hill PWS, Westermann AJ, Fisher RA, Thurston TL, Saliba AE, Blommestein I, Vogel J, Helaine S (2018)
***Salmonella* persisters undermine host immune defenses during antibiotic treatment**
Science doi: 10.1126/science.aat7148



PARTNERS, SITES AND NETWORKS

© scienceRELATIONS

Stem cell transplantation and immunodeficiency is one of the research topics in the DZIF.

TIMO JÄGER | MANAGING DIRECTOR OF THE DZIF

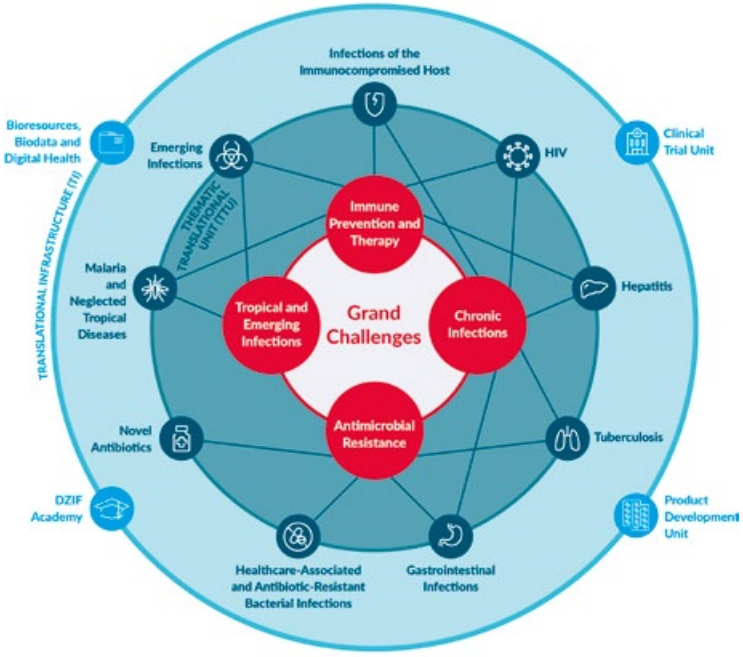
UNITED IN TACKLING MAJOR CHALLENGES

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. Altogether, 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 lately: (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 2018 to 2019, focus was placed on internationalisation which set the course for partnerships with globally active institutions: the Coalition for Epidemic Preparedness Inno-



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

DZIF is an affiliation of 35 research institutes, located at seven sites distributed throughout Germany. Thirteen additional sites are associated partners: Albert-Ludwigs-Universität, Freiburg | Charité – Universitätsmedizin Berlin | Deutsche Leberstiftung/HepNet Study House, Hannover | Goethe-Universität, Frankfurt am Main | Hans Knöll Institut, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Jena | Julius-Maximilians-Universität, Würzburg | Max-Planck-Institut für Informatik, Saarbrücken | Martin-Luther-Universität, Halle-Wittenberg | Otto-von Guericke Universität, Magdeburg – until 2018 | Universität Bayreuth | Universität Erfurt – until 2018 | Universitätsklinikum Essen | Universität Münster

vation (CEPI) and the Combating Antibiotic Resistant Biopharmaceutical Accelerator (CARB-X). In the two DZIF core competence areas "Vaccine Development" and "Averting the AMR crisis", steps have been taken to embed DZIF activities in international consortia. For example, CEPI recognized DZIF's contribution to the early clinical development of the first Ebola vaccine to receive authorization by regulatory authorities in Europe (EMA) and the USA (FDA). Furthermore, CEPI granted funding to a MERS vaccine consortium led by IDT Biologika GmbH. The completion of the phase Ia first-in-man trial for MVA-MERS-S within DZIF was a critical milestone in securing the funds for upscaling and manufacturing of a vaccine stockpile.

Likewise, DZIF incubates the Global AMR R&D Hub (https://globalamrhub.org/), a platform for international coordination of AMR-related research and development activities in Berlin. Through the initiative of the DZIF Product Development Unit (PDU), DZIF has been appointed member of the Global Accelerator Network of the funding body CARB-X (https://carb-x.org/), thereby supporting a portfolio of innovative antibacterial projects around the globe.

Prime examples for such innovative DZIF projects are a new antibiotic against tuberculosis, a new vaccine against the oncogenic Epstein-Barr-virus (EBV) and the broad-spectrum antibiotic Cystobactamid.

BTZ-043, a novel antibiotic active against *M. tuberculosis* was initially discovered in Germany and has now been shown to be active against multiresistant strains of this pathogenic bacterium. With co-funding from DZIF, InfectControl 2020,

BMBF and the European and Developing Countries Clinical Trials Partnership EDCTP, this compound has now advanced from a lead candidate into phase II studies. Since November 2019, the early bactericidal activity of BTZ-043 is being tested in tuberculosis patients in South Africa. The clinical development process is closely coordinated together with the Product Development Unit of the DZIF and the Federal Institute for Drugs and Medical Devices BfArM.

A novel concept, based on recombinant viral genomes with specific deletions, has been developed in DZIF to generate a vaccine against the oncogenic Epstein-Barr-Virus. The development of the EBV vaccine has reached the preclinical stage and a product suitable for clinical testing is being produced with support from DZIF. Assays to determine the composition, biopotency and safety of the final product for vaccine quality assessment have been established.

The natural product-based broad-spectrum antibiotic Cystobactamid has been discovered by HZI scientists and subsequently developed and optimized in DZIF projects; further optimization is now funded by the Federal Ministry of Research (BMBF). The compound has been partnered with the pharmaceutical company Evotec in February 2019. Additional funding was acquired and a spinoff company is in the foundation phase. Making compounds initially developed within DZIF largely independent of DZIF funding, or transferring them into public-private-partnerships is a main criterion for the successful long-term performance of DZIF´s antibiotic development and is exemplified in this project.

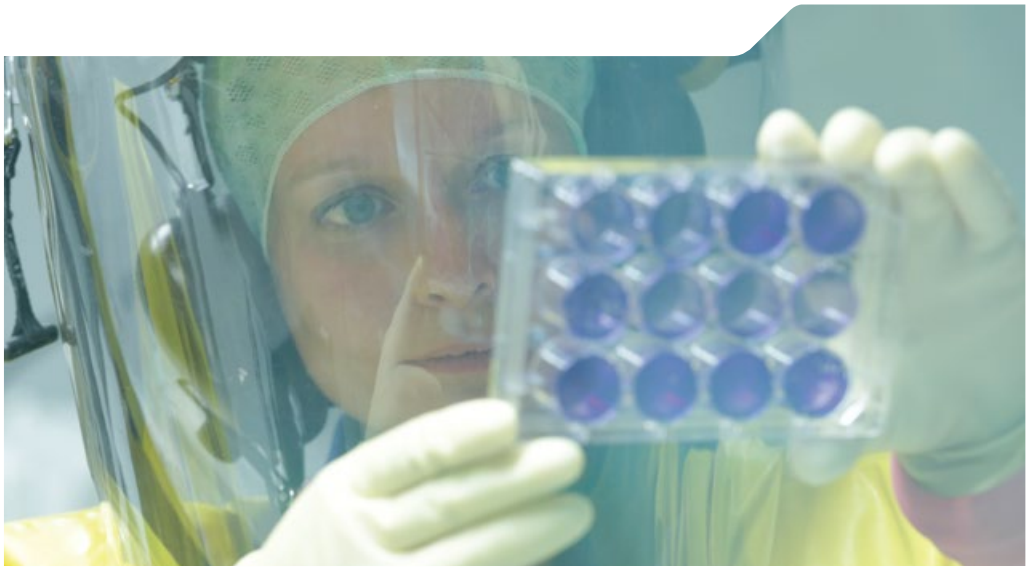
DZIF groups its research activities into research areas and translational infrastructures:

Translational research areas

- Emerging Infections
- Tuberculosis
- Malaria
- HIV
- Hepatitis
- Gastrointestinal Infections
- Infections of the immunocompromised Host
- Healthcare-associated and Antibiotic-resistant bacterial Infections
- Novel Antibiotics

Translational infrastructures

- Product Development Unit
- Clinical Trial Unit
- African partner Institutions
- Biobanking
- Bioinformatics
- DZIF Academy
- Pathogen repository
- Epidemiology
- Novel antivirals



GÉRARD KRAUSE AND STEFANIE CASTELL | DEPARTMENT EPIDEMIOLOGY AT HZI

LINKING INFECTIONS TO NON-COMMUNICABLE DISEASES

RESEARCH WITHIN THE GERMAN NATIONAL COHORT

As is generally known, infections contribute to non-communicable conditions such as cardiovascular, metabolic, malignant and neurodegenerative disease. One important challenge in establishing such associations in the case of acute infections is that they cannot be investigated in humans using traditional paper-and-pencil based survey methods. On the other hand, examining this hypothesis in patients who have already developed such a non-communicable disease carries the problem that the putative contributing infections are likely to have occurred a long time ago and it is no longer possible to detect them without bias. In particular, it is not possible to obtain symptomatic biosamples for microbiological analysis. The German National Cohort (NAKO, www.nako.de) is an excellent tool with which to overcome such challenges.

NAKO is Germany's largest epidemiological health project, having recruited over 200,000 adults from the general population between 2014 and 2019 (baseline examination). Examinations include interviews/questionnaires regarding medical history and e.g. life-style factors as well as tests like spirometry, hearing, ECG and biosamples like blood. This cohort study will conduct follow-ups for several decades. Those individuals who develop cardiovascular conditions, for instance, will then be assessed with respect to information on infections and other risk factors collected at baseline, and will be compared to those individuals who have remained healthy. If it is possible to detect transient infections (years) before the non-communicable diseases occur, they can later be linked to information on these newly occurring conditions.

© NAKO

Establishing causal associations between infections and subsequent non-communicable diseases has immense consequences because it will inevitably open the way for new diagnostic, preventive and therapeutic measures. A good example of this is cervical cancer, of which some forms can now be prevented by vaccination against some human papilloma virus genotypes. The aim of NAKO is to explain the causes of widespread diseases such as cardiovascular disorders, cancer, diabetes and dementia, and hence the contribution of infections to the development of these diseases is one focus of the observational study. A digital add-on project of HZI aims to look at transient infections in more detail using a specifically developed smartphone application that allows NAKO participants to report on episodes of common cold or flu as well as gastrointestinal and urogenital infections. For respiratory infections, laboratory analyses of self-sampled nasal swabs are included (www.info-pia.de).

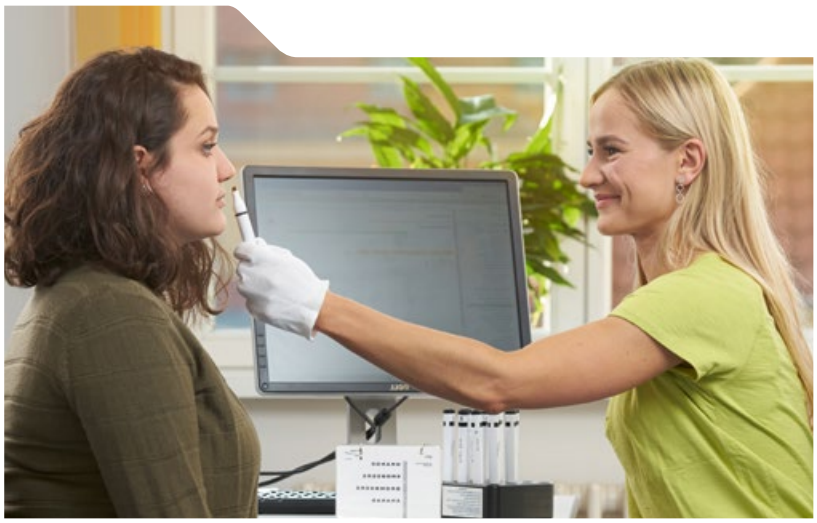
The Hannover study centre

The Department "Epidemiology" at HZI has established the Hannover NAKO study centre and is responsible for vaccination assessment at all 18 NAKO study sites. The facilities of the Hannover NAKO study centre are located in the Clinical Research Center (CRC) in Hannover. It is situated near the Hannover Medical School (MHH) campus allowing a close working relationship with clinicians. The CRC is a joint venture of HZI, Fraunhofer ITEM and MHH. HZI contributes a staff of health-care professionals such as physicians, nurses, receptionists and laboratory technicians, all trained and certified according to the standards of NAKO. The study centre is equipped with examination rooms, interview rooms and touch-screen work stations. Medical equipment includes an echocardiography system, ergometer, spirometry, devices for assessing airway inflammation, automatic detection of arteriosclerotic vascular lesions, equipment for skin autofluorescence measurement of advanced glycation end products and a digital camera.

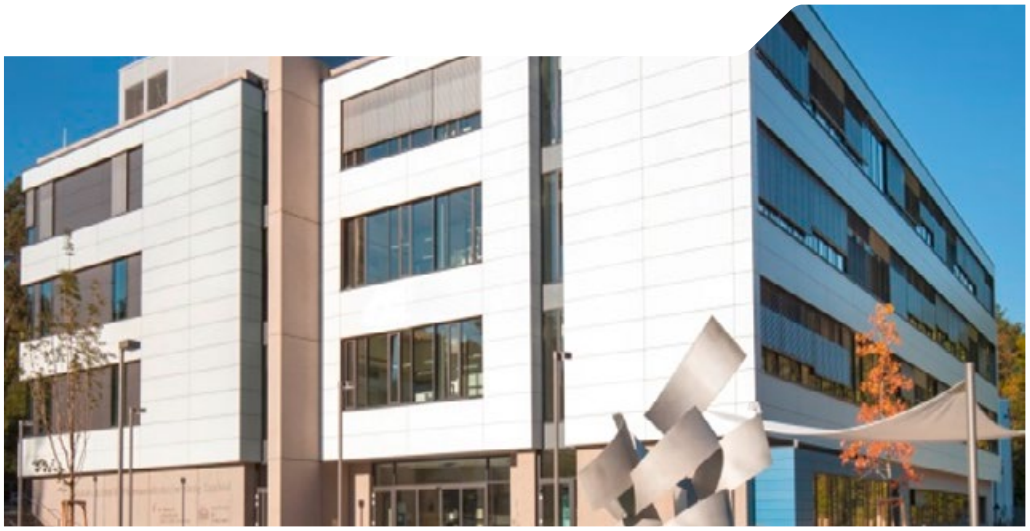
Since December 2019, the study centre in Hannover has been conducting the first re-assessment examinations. These are likely to be finished by the end of 2022.

The examinations will make it possible to investigate the association between frequent acute infections and predisposing risk factors, and also between acute infection types and subsequent non-communicable diseases.

Compared to other large scale cohort studies worldwide, one unique characteristic of NAKO is that infections are part of the main research portfolio. The Department "Epidemiology" at HZI co-coordinates infection-related research activities within the NAKO project.



Photos: © NAKO



ROLF MÜLLER | MANAGING DIRECTOR OF HIPS

IN SEARCH OF NOVEL ANTI-INFECTIVE DRUGS

THE HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL RESEARCH SAARLAND (HIPS)

In 2019, the Helmholtz Institute for Pharmaceutical Research (HIPS) celebrated its ten-year anniversary. Founded jointly by HZI and Saarland University (UdS), HIPS is the first public research institute in Germany that is explicitly devoted to the pharmaceutical sciences. Scientists at HIPS search for novel drugs and ways for their application in the clinic, especially in the area of anti-infectives. Based on its scientific foci HIPS is a key player in the field of "health research", steadily expanding its mission to develop novel drug candidates for therapeutic use. HIPS currently hosts three departments and five research groups, comprising more than 180 people.

To foster and expand scientific collaboration with its local partners at UdS and the Leibnitz Institute for New Materials (INM), the HIPS initiated a research alliance programme between the three parties in November 2019. Thematically, the five-year programme will focus on the development of active compounds. The collaborative efforts within this alliance will include joint research projects, initial financing for further third-party funding, establishing joint professorships, and other strategic measures. To fund the envisaged activities, the participants set up a cooperation fund of 3.3 million Euros.

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 products with antimicrobial properties (primarily from myxobacteria) and the improvement of their pharmaceutical properties. The overall aim is to transfer the scientific findings into clinical practice by specifically optimizing selected compounds for therapeutic use in humans. In early 2019, Evotec joined the preclinical development of the cystobactamids within a public-private partnership. In a project supported by the Bill & Melinda Gates Foundation, scientists are screening for compounds, which could be used to treat patients with tuberculosis and malaria.

In June 2019, the new junior research group ***Genome Mining for Secondary Metabolites*** was established at HIPS within the framework of the Helmholtz International Lab programmeme. Chengzhang Fu, head of the research group, combines bioactivity-based approaches with genome analysis to identify novel bioactive natural products.

Since January 2019, Olga Kalinina holds the Klaus Faber endowed professorship in ***Drug Bioinformatics*** at the HIPS. The Klaus Faber Foundation supports Kalinina's research with a total of 1.6 million Euros until 2022. Her research 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.

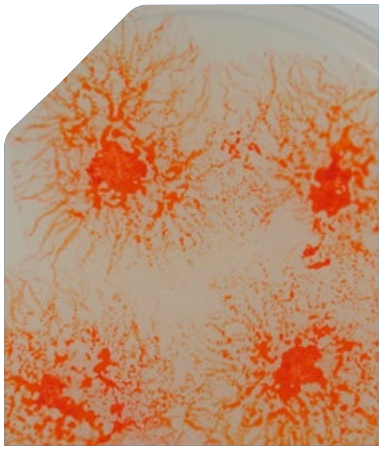
The team around junior research group leader Jesko Köhnke (***Structural Biology of Biosynthetic Enzymes***) provided exciting insights on the biosynthesis of bottromycin, which represent the basis for developing novel derivatives with improved properties.

The department ***Drug Design and Optimization*** headed by Anna Hirsch focuses on the development of novel anti-infectives targeting biologically relevant proteins within bacterial pathogens. The drug targets can be grouped into those that impair vital mechanisms within the bacteria and effectively kill them, and targets for "pathoblockers" that interfere with pathogenicity and virulence without affecting bacterial viability. Various novel classes of compounds were identified and subjected to multiparameter optimization to afford highly effective anti-infectives. The evaluation of the PK/PD profile of the most promising compounds and their testing in preclinical *in vivo* models is ongoing, to bridge the translational gap.

The research group ***Chemical Biology of Carbohydrates*** (head: Alexander Titz) optimized glycomimetics with high affinity towards the *Pseudomonas aeruginosa* virulence factor LecB with oral bioavailability in mice. *In vivo* efficacy studies are ongoing. Further work centers around antibiotic-targeting and identifying new ESKAPE pathogen lectins.

Research at the department ***Drug Delivery*** (head: Claus-Michael Lehr) aims at improving the transport of anti-infectives across the biological barriers that may limit their bioavailability at the site of action. Encouraging new results, also in animal models, have been obtained to proof the concept of bacteriomimetic nanocarriers to combat intracellular bacteria. Self-assembling nanocarriers emerging from a collaborative European Research Project (ITN "Nabba") for simultaneous delivery of tobramycin and novel QSI-pathoblockers developed at DDOP, allow for a significant dose reduction of the antibiotic, which has led to an international patent. Progress was also made in developing human cell culture models for testing drugs against inflammatory and infectious lung diseases, as well as in some high-throughput models to predict drug accumulation in Gram-negative bacteria.

The Junior Research Group ***Biogenic Nanotherapeutics*** led by Gregor Fuhrmann and supported by the BMBF Programmeme NanoMatFutur could demonstrate encouraging first evidence for the potential of myxobacteria-derived extracellular vesicles as novel anti-infectives.



Byssophaga cruenta

Chondromyces crocatus, © HZI | Heinrich Lünsdorf

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) was established in May 2017 as a joint venture between HZI and the Julius Maximilian University of Würzburg (JMU). The HIRI is the first research institution worldwide to focus on the role of ribonucleic acids (RNA) in infection processes. HIRI seeks an integrative approach to exploiting the vast potential of RNA as a diagnostic, drug, and therapeutic target for new strategies to combat infectious diseases and edit the microbiome.

While RNA is increasingly understood to contribute to key regulatory and sensory processes in the cell, the role of RNA in infection biology remains understudied. Hence, the major goals of the HIRI are: (i) Resolve the complexity and heterogeneity of infection processes at the single-cell level. (ii) Identify novel regulatory RNAs with key roles in pathogenesis. (iii) Understand RNA-based mechanisms in virulence and host defence. (iv) Develop innovative delivery techniques for RNA-based interventions. (v) Exploit RNA knowledge for new diagnostics, preventives, and anti-infectives.

Recruitment

By the end of 2019, the institute had grown to 8 research groups and over 60 staff from 17 nations. The HIRI succeeded at recruiting two of these groups through the highly competitive Helmholtz Young Investigator programme.

Fostering local RNA-centric interactions, the HIRI affiliated several JMU researchers, i.e. Thomas Rudel (Chair of Microbiology), Lars Dölken (Chair of Virology), Lorenz Meinel (Chair of Pharmaceutics and Biopharmacy) and Utz Fischer (Chair of Biochemistry). Reaching out further abroad, HIRI supported Peter Fineran, a leading CRISPR researcher from Otago (New Zealand), to receive an Alexander von Humboldt Research Fellowship.

Flow cytometry is a core technology used for single-cell analysis.
© HIRI

HIRI HELMHOLTZ
Institute for RNA-based Infection Research

Publications, funding and awards

Over the reporting period, research by HIRI scientists and HIRI affiliates resulted in 53 publications, many in high-profile journals, and including five experimental papers in Cell, Nature and Science. Jörg Vogel was again named Highly Cited Researcher, acknowledging his exceptional productivity (top 1%) in the field of microbiology. In 2019, he also received the Feldberg Prize for Anglo-German exchange in science.

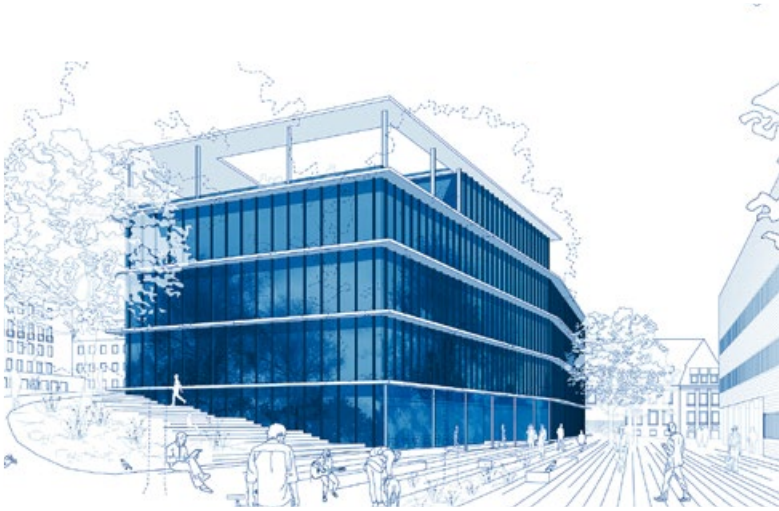
Funding success included an ERC Consolidator Grant (2 M€) awarded to Chase Beisel in 2019, further ERC grants to HIRI affiliates Lars Dölken und Thomas Rudel, as well as a fellowship programme of the Würzburg-based Vogel Stiftung Dr. Eckernkamp to support RNA-centric research of cancer-associated *Fusobacterium* at HIRI.

Training, teaching and networking

HIRI provides an interactive and stimulating environment for students and young scientists of various disciplines. Group leaders at HIRI organize courses on both basic and emerging topics in the fields of RNA and infection biology. Taking place twice a year, these courses are also open to students from Würzburg University. In addition, HIRI scientists have also developed elective courses. A very well-received lecture series "Single-cell Biology" organized by Emmanuel Saliba started in 2019.

HIRI has established an "RNA Seminar" lecture series, which takes place every other tuesday during the semester. International high-profile speakers over the past two years included Anna Pyle (Yale University, current President of the RNA Society), Peter Nielsen (University of Copenhagen), Gigi Storz (NIH) and David Corey (UT Southwestern).

In a collaborative effort with both HZI and JMU graduate schools HIRI established in 2018 a new graduate training programme "RNA & Infection". This programme is a first in the world, and offers young scientists to get specialist training in both RNA biology and infection biology.



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

New building

The new HIRI building is well underway. The designated building ground for the new HIRI building was confirmed and the leasehold contract signed. An architectural competition was officially announced and an interdisciplinary jury ranked proposals to select a winner. In a press conference in October 2018, doranth post architekten (Munich) was announced as the winner of the architectural competition. They have designed the building to fit well into the existing campus of the University hospital. Once finished, the new HIRI will house state-of-the-art facilities and provide space for up to 150 scientific and administrative staff. Building work will start in 2021 at the earliest. The HIRI thus plays a key role in a long-term strategy to boost the impact of Würzburg as a scientific hub.

The Dr. Eckernkamp fellow Falk Ponath working on *Fusobacterium nucleatum*. © HIRI

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ULRICH KALINKE | EXECUTIVE DIRECTOR OF TWINCORE

MOVING INTO A NEW ERA IN TRANSLATIONAL RESEARCH

TWINCORE
Zentrum für Experimentelle und Klinische Infektionsforschung

THE TWINCORE CENTRE FOR EXPERIMENTAL AND CLINICAL INFECTION RESEARCH

In 2018, TWINCORE celebrated its tenth anniversary. In 2008, HZI and Hannover Medical School founded TWINCORE as a joint venture to reinforce cooperative research activities and to establish a translational infection research programme. Since then, the centre has become a prime example of translational research and is well recognized both within Germany and internationally.

At TWINCORE, multidisciplinary teams strive to channel new knowledge into clinical practice and to translate clinical observations back to the researchers. Translational infection research is performed to improve prevention, diagnosis and treatment of human infectious diseases and to enhance the understanding of disease mechanisms.

The anniversary year marked the beginning of a new era. Several researchers were offered interesting positions in other research institutions. Eike Steinmann became head of the Department of Molecular and Medical Virology at the Ruhr-Universität Bochum in April 2018. Tim Sparwasser was appointed Director of the Department of Medical Microbiology and Hygiene at the University Medical Center Mainz in December 2018. Christine Goffinet became W2 professor at the Institute of Virology at the Charité Berlin and the Berlin Institute of Health at the beginning of 2019.

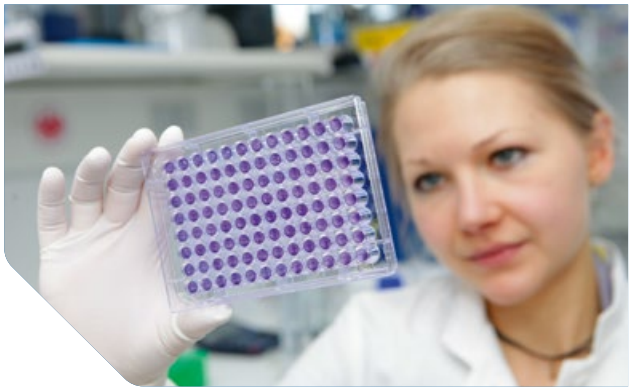
After the departures of these group leaders, new research activities were implemented at TWINCORE. Highest priority was given to the integration of the new group leaders at the Centre for Individualized Infection Medicine (CiiM). These were Yang Li, head of Computational Biology for Individualized Medicine, Chengjian Xu, head of Bioinformatics and Functional Genomics, and Markus Cornberg, head of Immunology of Viral Hepatitis and Infections in Liver Cirrhosis. They and their research teams are based at TWINCORE until the new CiiM research building is complete. It is being built right next to TWINCORE and will be ready within the next few years.

Together with the Department of Gastroenterology, Hepatology and Endocrinology of MHH, TWINCORE established its first Clinician Scientist junior research group in 2019. The Translational Virology group, headed by Patrick Behrendt, will mainly focus on the hepatitis E virus and combine the knowledge gained in the laboratory with the observations made in hospitals. A second clinician scientist junior research group will be recruited in 2020. Furthermore, together with MHH's Institute for Medical Microbiology and Hospital Epidemiology, the junior research group Pathogenesis of Bacterial Infections was established. The group studies the pathogenic bacterium *Staphylococcus aureus* and is led by Volker Winstel.

TEN YEARS: TWINCORE 2008–2018

710 original publications and reviews
31,156 citations
16,715,581 € third party funding

Further new recruitments are focusing on data science. The cluster of excellence RESIST is funding two professorships for bioinformaticians, one at TWINCORE's Institute for Molecular Bacteriology and one at the Institute for Experimental Virology. The recruitment process is ongoing for both positions. Finally, TWINCORE is involved in five projects funded by an initiative of the state of Niedersachsen (Lower Saxony) to investigate the chances offered by big data in the life sciences of the future. Three of these projects are coordinated by members of TWINCORE. The funding allows new data scientists at the PhD and postdoc level to be recruited. Thus, TWINCORE is ready to face upcoming challenges such as the analysis of the pathophysiology of human infectious diseases and the development of new vaccines.



© CSSB | Tina Mavric

MICHAEL KOLBE | HEAD OF THE DEPARTMENT STRUCTURAL INFECTION BIOLOGY

USING POWERFUL LIGHT SOURCES FOR INFECTION RESEARCH

THE CENTRE FOR STRUCTURAL SYSTEMS BIOLOGY (CSSB)

CSSB
Centre for Structural
Systems Biology

The Centre for Structural Systems Biology (CSSB) in Hamburg opened its doors on June 29, 2017. The centre is the result of a joint effort of in total ten partner institutions located in North Germany. It employs cutting-edge light sources and state-of-the-art imaging technologies to investigate how pathogens infect humans. HZI is represented at the CSSB by the group "Structural Infection Biology" (STIB; led by Michael Kolbe) working on the architecture and activity of protein transport systems that facilitate the invasion of enteric pathogens, more specifically of bacteria that cause diarrhoea, one of the main causes of infant mortality in the world.

For its research, the group has used the highly collaborative atmosphere at the CSSB and integrated the powerful synchrotron radiation and X-ray laser sources available at DESY with biophysical and microbiological methodologies to elucidate the molecular mechanisms underlying the activity of the bacterial type III secretion system (T3SS) and of human innate immunity receptors. The T3SS is a nanosyringe-like supramolecular structure that delivers pathogenicity factors into human cells to prepare them for invasion. In collaboration with members of the CSSB, the group used small-angle X-ray scattering and crystallography to determine at the nanoscale resolution the precise composition of the regulatory cytosolic apparatus that in concert with the T3SS facilitates the secretion of these pathogenicity factors into host cells. Furthermore, the STIB lab conducted cryo-electron microscopy studies of purified T3SS to disclose unforeseen conserved substructures within the membrane-embedded components of the core structure of the T3SS (Fig.1). These high-resolution studies provided important molecular insights into this essential bacterial virulence factor and might enable the development of new therapeutics to block Gram-negative infections (Lunelli et al, PLoS Pathogens, 2020).

In another long term collaboration with the groups of Stefan Kaufmann (MPI for Infection Biology) and Pedro Moura-Alves (Oxford University) the HZI researchers found that the human Aryl-hydrogen receptor (AhR) senses the presence of several important bacterial pathogens including *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* (Moura-Alves et al Nature. 2014). More recently they also showed that the transcription factor AhR acts as a sort of cellular secret agent spying on the QS molecules sent by *P. aeruginosa*. AhR is unique in that it is able to recognize three distinct types of QS molecules (Moura-Alves et al Science. 2019). CSSB scientists developed an assay to help identify and quantify these small molecules sensed by the AhR (Puyskens et al, Cell Host & Microbe, 2020).

The group moved to the CSSB building in 2017. Together with this group, the CSSB hosted the laboratories of four group leaders with expertise in structural biochemistry of membrane protein complexes. This exquisite combination of expert partners will provide the group with many opportunities to improve even further the quality of their research addressing the molecular mechanisms of protein transport associated with infection.

The CSSB provides space for four core facilities (Protein Production, Protein Characterization, Protein Crystallization and Light Microscopy). STIB is heading the protein production core facility as part of HZI to support all CSSB researchers and partner institutions in producing recombinant proteins more efficiently. In spring 2018, the protein characterization and crystallization facility started user operation. Additionally, HZI and the group will benefit greatly from access to the S2 laboratories equipped with last generation cryo-electron microscopes and high speed fluorescence microscopes located in the basement of the CSSB. Another S3-laboratory which is also located in the basement is currently in the construction phase. Future cryo-electron microscopy analysis studying the detailed architecture of infected cells and the organization of protein complexes involved in virulence will provide valuable insights into the interaction of the pathogen with its host at the near-atomic resolution.

In 2017 and 2019, the CSSB underwent its first scientific evaluations by independent scientific advisors with an overall very positive outcome. The council reaffirmed the full support for the "Hotel Research" concept and the future research directions of the Centre. As part of the CSSB commitment to enhancing opportunities for collaboration, the CSSB hosted an Opening Symposium entitled "Frontiers in Structural Systems Biology of Host-Pathogen Interactions" organized by Michael Kolbe (HZI) in cooperation with Dr. Kai Grünewald (HPI) and Prof. Thomas Marlovits (UKE). This experience resulted in a great success as new collaborative activities between the renowned invited speakers from the fields of structural and infection biology with the CSSB members were built. Another CSSB symposium is currently being planned for November 2020.

In the global effort of developing novel methods for fighting infectious diseases, the Helmholtz Association and the CSSB organized a workshop on modern methods in structural biology in autumn 2018, as part of the cross-programme activity "Helmholtz Structural Biology". The course brought together postgraduates at the early and late stage of their doctoral studies with experts from different disciplines to find solutions for the today's global challenges in infections biology.

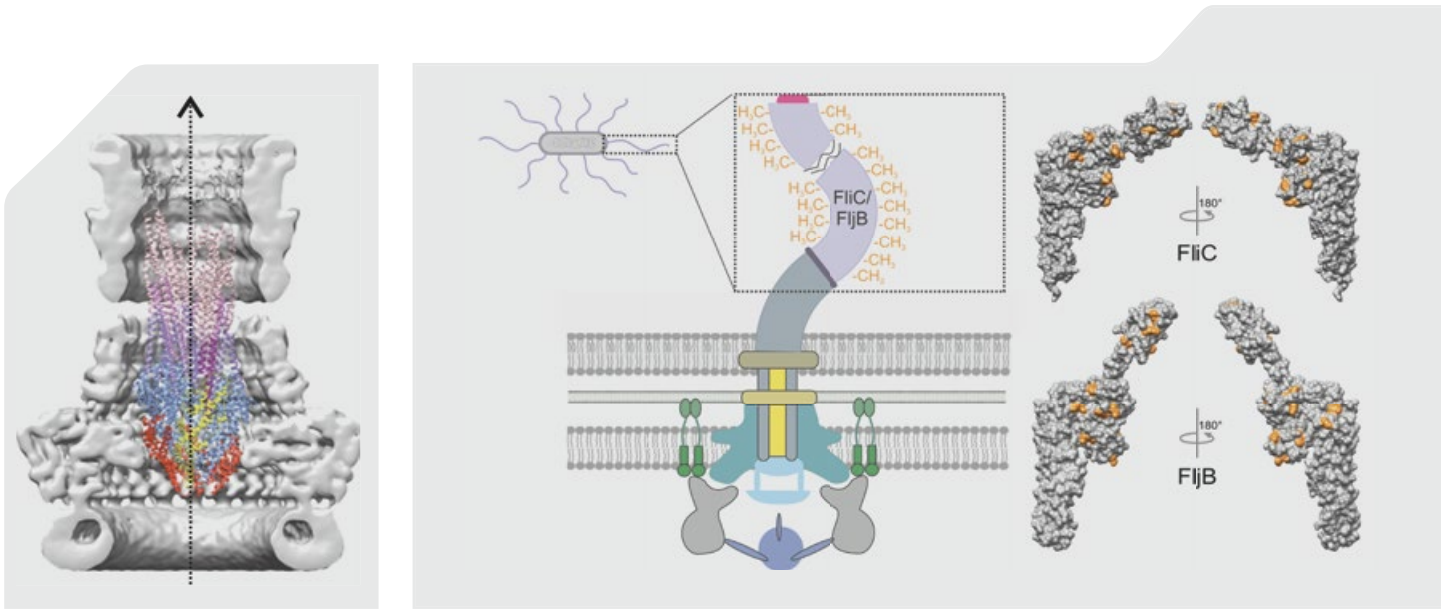
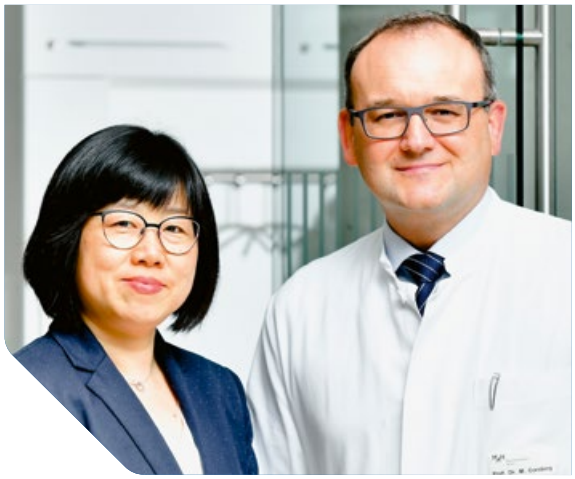


Figure 1: (left) cross section of the *Shigella* needle complex: The proteins forming the inner core of the needle complex are highlighted as coloured schemata. Passage of the secreted effector molecules is indicated by an arrow. (right) Methylation of flagellin subunits changes the surface properties of bacterial flagella.

© Tom Figiel



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YANG LI AND MARKUS CORNBERG | DIRECTORS CIIM

TOWARDS PRECISION MEDICINE FOR INFECTION PATIENTS

CENTRE FOR INDIVIDUALIZED 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 Individualized Infection Medicine (CiiM). CiiM is a joint venture of HZI and MHH and was founded in 2015, initially as a virtual network. 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.

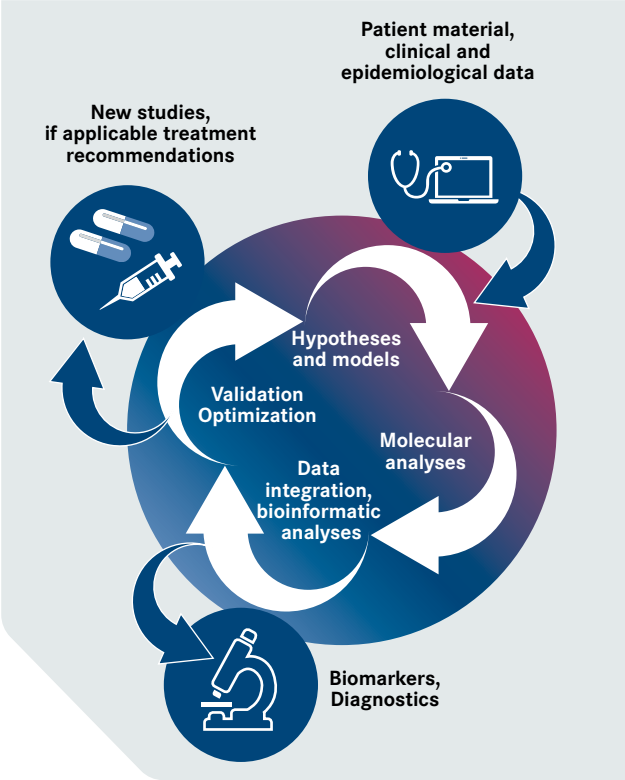
Newly occurring and recurring pathogens, chronic infections and increasing resistance to approved drugs are constantly presenting physicians with new challenges. The CiiM team is working to address those challenges by pursuing the vision of treating infection patients in a way that is adapted to the specific needs of each individual. In order to achieve this, CiiM is dedicated to investigating individual characteristics and their influence on susceptibility to infection and the success of available therapies. In the planned CiiM building, interdisciplinary teams of scientists, bioinformaticians, data scientists and clinicians will apply computer-assisted methods to patients data. This will include analysing existing routine clinical data and combining it with newly collected molecular data of individual patients and pathogens to predict the course of infection and the success of therapy. Their findings will provide attending physicians with important guidance for tailored prevention and therapy, thus facilitating individual patient management and evidence-based medicine.

CiiM
CENTRE FOR INDIVIDUALISED INFECTION MEDICINE

The operating and organisational concept for the CiiM building, which will be constructed directly adjacent to Twincore and accommodate around 150 employees in an effective area of about 2,100 m², was finalised and approved in 2018. At the beginning of 2019, the construction measures were initiated with a coordination meeting; the planned services were put out to tender and evaluation took place at the end of 2019. Until the new building is commissioned, research groups of HZI and MHH directly assigned to CiiM are housed at Twincore.

Yang Li, an internationally renowned expert in systems genetics and multi-omics integration for individualized medicine, was appointed co-director of CiiM and head of the HZI/CiiM department “Bioinformatics of Individualized Medicine”. She took up her new positions in May 2019. The research group of Markus Cornberg, an active MHH clinician in the field of infectious diseases who took over the positions as clinical director of HZI and co-director of CiiM from Michael Manns at

the beginning of 2019, also moved in next door to Yang Li at Twincore. As part of a dual career offer for Yang Li, her husband Cheng-Jian Xu, an experienced systems geneticist and bioinformatician, was appointed group leader at MHH and started the new "Bioinformatics and Computational Genomics" CiiM group in June 2019.

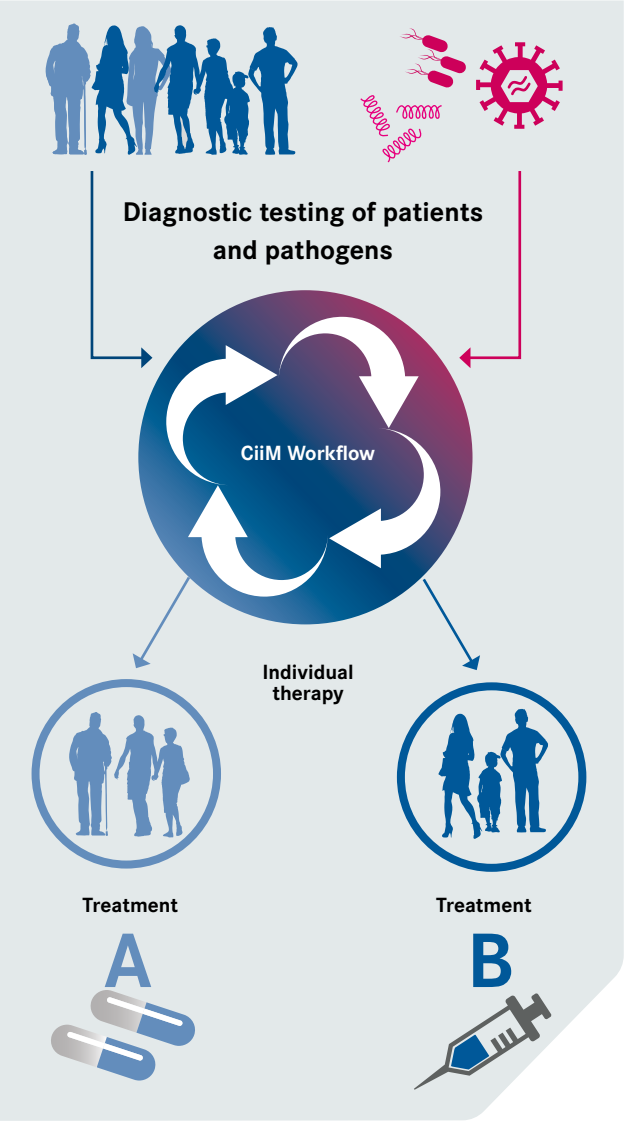


Integrating findings from clinical and basic research: CiiM's workflow is designed to enable the development of novel diagnostics and treatment options.

Those groups, in cooperation with the CiiM faculty and further research groups planned for the institute, started developing various aspects of the CiiM workflow and establishing the research field. Relevant patient and control cohorts with available multi-layered data sets are of central importance. Thus, in addition to already existing cohorts, the team helped to implement more detailed protocols and initiated new cohorts in 2019. In order to further establish the necessary structures and initiate pilot projects, acquisition of third-party funding is actively being pursued.

Bridging the different disciplines is key for CiiM. The CiiM projects offer participation opportunities for clinicians of various educational levels (from students to hospital directors) and thus promote exchange. As the demand for health data science experts is exceptional, CiiM has initiated a Biomedical Data Science Research Training Group (BIOMEDAS) that will become operational in 2020 as part of the TRAIN initiative. These endeavours were preceded by additional measures such as the organisation of the international Herrenhausen Symposium "Individualized Infection Medicine - The Future is Now" in 2018 and are now accompanied by frequent CiiM Lectures from internationally renowned experts. The aim is to expand the network, support interaction, attract experts and increase the visibility of CiiM.

Author: Jennifer Debarry, Coordinator of CiiM ■



The most suitable therapy for each patient: Individualized medicine aims to categorise patients and define groups based on the most promising treatment for the respective individual.

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Information and Data Science at the heart of Braunschweig:

The BRICS building on the campus of TU Braunschweig comprises laboratories, offices and meeting rooms which are used for interdisciplinary experimental and theoretical work on four floors. A training laboratory, a computer pool and large meeting rooms are available for student education.

DIETER JAHN | DIRECTOR OF THE SYSTEMS BIOLOGY CENTRE BRICS

SYSTEMS BIOLOGY FOR HEALTH RESEARCH AND BIOTECHNOLOGY

THE BRAUNSCHWEIG INTEGRATED CENTRE OF SYSTEMS BIOLOGY (BRICS)

BRICS is a joint interdisciplinary bioinformatics and systems biology research centre of Technische Universität Braunschweig (TU-BS), HZI and the Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ). Systems biology integrates the knowledge derived from high throughput omics techniques, biochemistry, genetics and cell biology using bioinformatics into mathematical models. These models in turn allow predictions about biological processes.

By December 2019, BRICS comprised 33 principal investigators from TU-BS, HZI, DSMZ and TWINCORE, thereby providing a state-of-the-art technical infrastructure analysing genome, transcriptome, proteome and the metabolome with strong bioinformatics based modelling in infection research.

Investigating the Networks of Metabolism

Our major scientific focus is on the metabolism of selected organisms as one of the major readouts of cellular adaption processes. We use a combination of experimental mass-spectrometry-based methods and computational approaches to understand the dynamics of metabolisms derived from underlying gene regulation and protein networks. At BRICS, our Metabolomic Research Unit represents a close collaboration of researchers from TU-BS, HZI and DSMZ.

In May 2019, scientists at BRICS founded the German Society for Metabolome Research e.V. (DGMet) to strengthen funding of research on metabolism and foster better networking in order to promote collaborative research.

Key project: *Clostridioides difficile*

The Gram-positive bacterium *Clostridioides difficile* (formerly: *Clostridium difficile*) causes a high number of gut infections with thousands of deaths per year in Germany. The *C. difficile* associated diarrhoea is one of the most dangerous infections acquired in hospitals. The research consortium CDInfect is highly interdisciplinary with scientists from HZI, TU-BS, DSMZ, MHH and the Universities of Göttingen and Greifswald. It examines the role of bacterial adaptation mechanisms, the effect of toxins and of the microbiome on *C. difficile* infections in different project areas. The aim is a basic molecular understanding of adaptation strategies of the organism in its environment, including the communication of its metabolism with the metabolism of other microbiome bacteria and of the host cells, to develop better therapies and diagnostics of *C. difficile* infections.

Key project: Protein complexes of *Pseudomonas aeruginosa*

The Gram-negative bacterium *Pseudomonas aeruginosa* is the major cause of death for patients suffering from cystic fibrosis. Essential for the colonization of the lungs is the formation of biofilms in an anaerobic environment, as within the highly viscous mucus, a microaerobic/anaerobic milieu prevails. In the DFG funded Research Training Group GRK2223 "Protein Complex Assembly (PROCOMPAS)", a team of scientists from TU-BS and HZI focusses on the dynamics and function of large protein complexes of anaerobic respiration (denitrification) involved in energy generation during infection. Besides protein-protein interaction dynamics, the resulting metabolic adaptation processes are of central interest. In a second international research consortium, financed by the CF Trust of UK, we investigate *P. aeruginosa* from cystic fibrosis patients using systems biology methods.

Key project: *Roseobacter*

Bacteria of the *Roseobacter* group are among the most common prokaryotes in marine ecosytems. They are extremely physiologically versatile and play an important role in the global cycles of the oceans. In the DFG funded Collaborative Research Centre (SFB) TRR 51, an interdisciplinary consortium from TU-BS, DSMZ and the University Oldenburg is working on the comprehensive understanding of the successful evolution and adaptation of the *Roseobacter* group in marine ecosystems. A major focus lies on the investigation of the metabolism as members of this group harbor distinct metabolic capacities that enable survival in certain ecological niches. In the last years, a detailed characterization of the metabolism of two model organisms belonging to this group, *Dinoroseobacter shibae* and *Phaeobacter inhibens*, was achieved. Next, the metabolic crosstalk with algae will be studied.

The President of the Helmholtz Association Otmar Wiestler (second from right), together with TU President Anke Kaysser-Pyzalla (second from left) and HZI's Scientific Director Dirk Heinz (left), visited Research Units at BRICS. Karsten Hiller, Head of the Department "Immunometabolism" (right), explained his research. © K.Rottig | TU Braunschweig

Collaboration Projects at BRICS

- **CF Trust:** Gas, food and lodging; understanding the physiological and metabolic requirements of *Pseudomonas aeruginosa* in the cystic fibrosis airways. UK CF Society.
- **Out of the dark into the light:** Picking electrogenic microorganisms from the human intestinal microbiome. VW Experiment/VolkswagenStiftung.
- **CDInfect:** Adaptation strategies of *Clostridioides difficile* during host infection. Niedersächsisches Vorab.
- **PROCOMPAS:** Protein Complex Assembly, DFG Research Training Group.
- **Roseobacter:** Ecology, physiology and molecular biology of the *Roseobacter* clade: towards a systems biology understanding of a globally important clade of marine bacteria. DFG collaborative research centre.

Technologies and Resources at BRICS	
Genome Analysis	HZI Genome Analytic platform (Geffers, HZI)
	PacBio DNS Sequencing (Overmann, DSMZ)
Proteome Analysis	Microbial Proteomics (Engelmann, HZI/TU-BS)
	Cellular Proteomics (Jänsch, HZI)
Metabolome Analysis	Immunometabolism (Hiller, HZI/TU-BS)
	Pathometabolism (Wegner, TU-BS)
	Microbial Metabolism (Schmidt-Hohagen, TU-BS)
	Bacterial Metabolism (Neumann-Schaal, DSMZ)
Databases	BRENDA-The comprehensive Enzymed Informaton System (Jahn, TU-BS)
	BacDive- The bacterial diversity metadatabase (Overmann, DSMZ)
	PRODORIC2- Gene regulation in prokaryotes (Jahn, TU-BS)
Biotechnology	Human antibody engineering and phage display (Dübel, TU-BS)
Bioengineering	Systems biotechnology and fermentation (Spieß/Krull, TU-BS)
Nanomicroscopy	Physical and theoretical chemistry (Ebbinghaus/Walla TU-BS)

Author: Dieter Jahn

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Prof. Dr. med. M. Cornberg

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Helmholtz Programme Infection Research

Topic 1: Bacterial and Viral Pathogens
Spokesperson: Prof. Dr. Th. Pietschmann

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Genome Architecture and Evolution of RNA-Viruses
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RG **HOPI** — HIRI
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JRG **LRIB** — HIRI
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RG **RANA** — HIRI
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RG **REMI** — HIRI
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RG **RSYN** — HIRI
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RG **SIGA** — HIRI
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Dep. **SFPR**
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RG **MPRO**
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RG **NBSC**
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RG **RPEX**
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Michael.Kolbe@...

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RG **MZBI**
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RG **VIMM**
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RG **SIME**
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Ulrich.Kalinke@...

RG **BIOM** — TC
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Mircrobial Immune Regulation
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RG **IMCI**
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RG **MSYS**
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Legend

Dep.: Department
RG: Research Group
JRG: Junior Research Group

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GB – Equal Opportunity Commissioner
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IT-Safety Officer
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VPS – Represent. Body for Disabled Employees
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Harald.Ohrdorf@...

Animal Welfare Officer
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Spokesperson: Prof. Dr. R. Müller

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RG **CBCH** HIPS
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Dep. **MINS** HIPS
Microbial Natural Products
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RG **AMEG** HIPS
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JRG **GEMS** HIPS
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RG **MISG**
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JRG **SBBE** HIPS
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RG **WIBI** HIPS
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Dep. **MWIS**
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ORG – Organisation
Richard Lomberg
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Joachim.Metge@...

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Erwin.Grund@...

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Axel.Plaehn@...

DA – Third-Party Funds Acquisition
TT – Technology Transfer
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Michael.Straetz@...

PS – Patents
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Dagmar.Meseke@...

FASI – Occupational Safety Specialist
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IR – Internal Auditing
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N.N@

PuK – Press and Communications
S. Thiele
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QM – Quality Management
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Heike.Kollmus@...

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WST – Scientifc Strategy
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(Knowledge Transfer Commissioner)
Birgit.Manno@...

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

Version: January 2020

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Universitätscampus E8.1, 66123 Saarbrücken
Managing Director: Prof. Dr. Rolf Müller | Adminstrative Management: Dr. Stephanie Thomas

HIRI, Helmholtz Institute for RNA-based Infection Research
Josef-Schneider-Straße 2, 97080 Würzburg
Managing Director: Prof. Dr. Jörg Vogel | Adminstrative Management: Alice Hohn

BRICS, Braunschweig Integrated Centre of Systems Biology
Rebenring 56, 38106 Braunschweig

CiiM, Centre for Individualized Infection Medicine, c/o TWINCORE,
Feodor-Lynen-Str. 7, 30625 Hannover
Managing Directors: Prof. Dr. Yang Li | Prof. Dr. med. Markus Cornberg

CSSB, Centre for Structural Systems Biology, Notkestraße 85, 22607 Hamburg

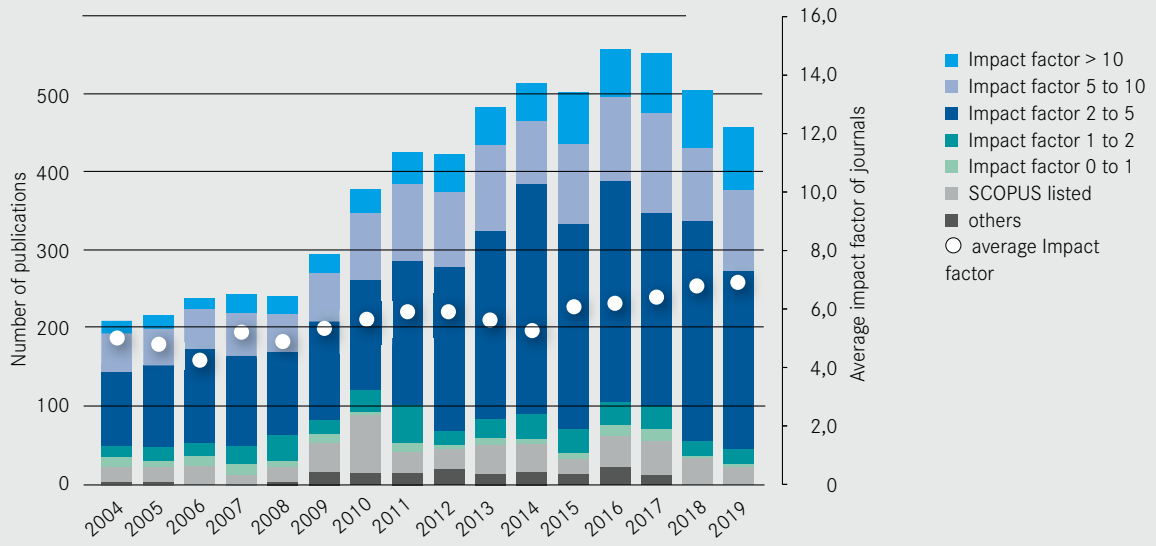
TC, TWINCORE, Centre for Experimental and Clinical Infection Research GmbH,
Feodor-Lynen-Str. 7, 30625 Hannover
Scientific Director: Prof. Dr. Ulrich Kalinke | Adminstrative Management: Matthias Fiebag

FACTS AND FIGURES

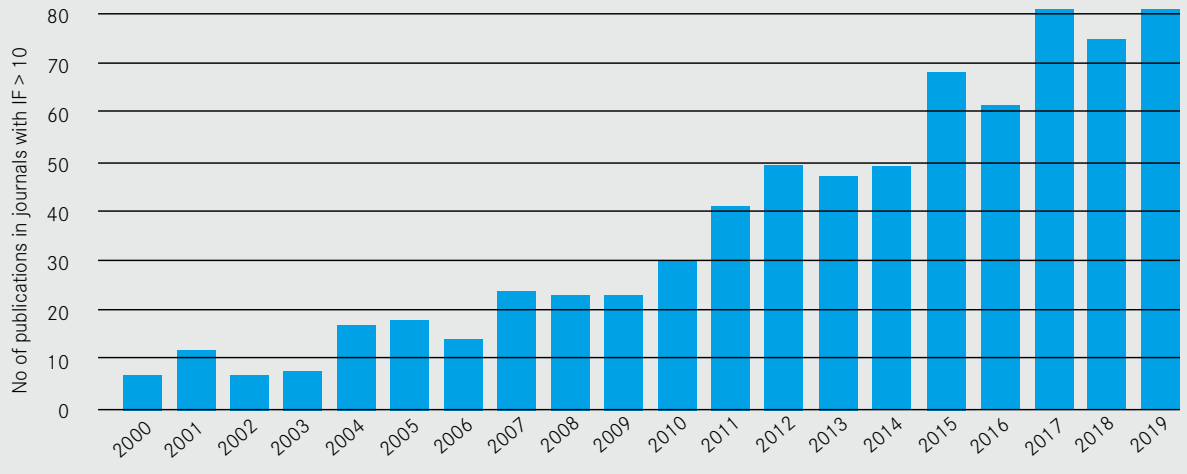
PUBLICATIONS

In 2018 and 2019, nearly 1000 scientific articles were published by HZI scientists. Over the recent years, the percentages of articles in high impact journals have been increasing.

Number of HZI Publications in different ISI-Impact classes



HZI publications in ISI- journals with impact factor >10



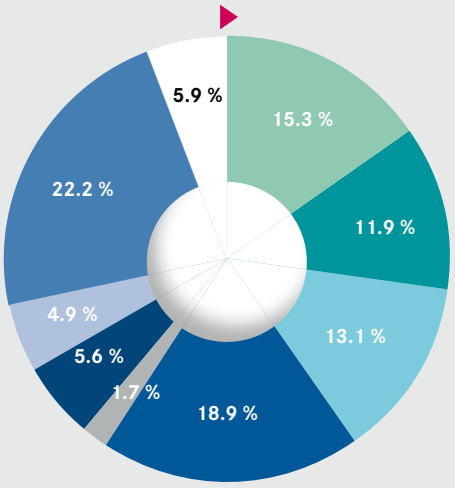
FINANCING

In 2018, the complete budget of HZI amounted to 78 Mio € including 19.9 Mio € of Third Party Funding.

More than 85 per cent of the external funding came from national programmes, about 15 per cent were from EU programmes and industry.

Third party funding of Research in 2018 (in 1000 €)

Source	Full cost
BMBF	3,050
DFG	2,382
EU	2,609
Helmholtz Association	3,765
Industry	345
Länder	1,117
NAKO (LKII)	990
DZIF	4,429
Others	1,189
Total Sum	**19,876**



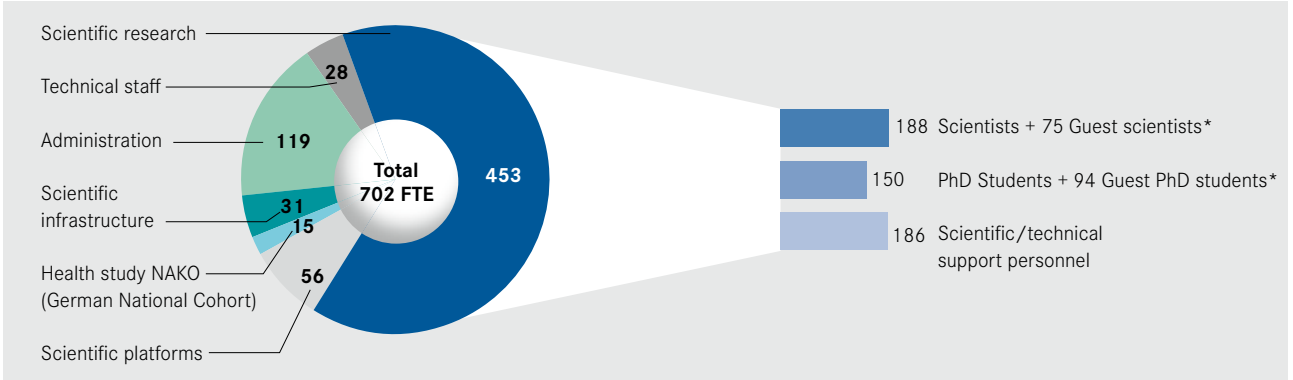
PARTICIPATION IN RELEVANT RESEARCH NETWORKS

In 2018, HZI and TWINCORE participated in 12 DFG Programmes (including Clusters of Excellence and Collaborative Research Centres), 23 EU projects (including ERC Starting, Consolidator and Proof-of-Concept Grants) and 30 BMBF / BMG / BMWi projects.

Patents, property rights and licenses

	2018	2019
Priority based applications	2	4
Total number of held property rights	306	237
Licence agreements	21	23
Licence proceeds (thousand €)	476	454

PERSONNEL



At the end of 2018 the HZI staff comprised 802 full time and part time employees, amounting to 702 full time equivalents (FTE). Scientific personnel constitutes the majority of HZI staff (524 FTE). * The 169 guests who worked in various projects, received their payment from third parties and are thus not included in the diagram.

OFFICIAL BOARDS AND COMMITTEES OF HZI

Members of the Supervisory Board (SB) and the Scientific Advisory Committee (SC), including the Clinical Board (CB) as a subcommittee (Status: Autumn 2019)

Members of the Supervisory Board (SB)

Function	Name, Titel	Organisation	Location
SB	Prof. Dr. med. Jan Buer	Universitätsklinikum Essen	Essen
SB	Prof. Dr. Luka Čičin-Šain	HZI	Braunschweig
Vice-Chair SB	MinDir Rüdiger Eichel	Niedersächsisches Ministerium für Wissenschaft und Kultur	Hannover
SB	Prof. Dr. med. Petra Gastmeier	Charité – Universitätsmedizin Berlin	Berlin
SB	Prof. Dr. Wolf-Dietrich Hardt	ETH Zürich	Zürich | Switzerland
SB	Prof. Dr. Caroline Kisker	Julius-Maximilians-Universität	Würzburg
SB	Prof. Dr. Christine Lang	MBCC Group	Berlin
SB	Prof. Dr. med. Michael Manns	MHH	Hannover
SB	Christian Mees	Staatskanzlei Saarland	Saarbrücken
Chair SB	MinDir'in Prof. Dr. Veronika von Messling	Bundesministerium für Bildung und Forschung	Berlin
SB	MR'in Dr. Andrea Niedzela-Schmutte	Bayerisches Staatsministerium für Wirtschaft, Landesentwicklung und Energie	München
SB	Prof. Dr. Ingo Schmitz	HZI	Braunschweig
SB	MinR'in Dr. Antina Ziegelmann	Bundesministerium für Gesundheit	Berlin

Scientific Advisory Committee (SC) Including Clinical Board (CB) as a subcommittee

Function	Name, Titel	Organisation	Location
SC/CB	Prof. Dr. Marylyn Addo	University Clinic Eppendorf	Hamburg
SC	Prof. Dr. Ingo Autenrieth	Eberhard-Karls-Universität	Tübingen
SC	Dr. Harald Dinter	Bayer Pharma AG	Wuppertal
SC	Prof. Dr. Irmgard Förster	Universität Bonn	Bonn
Chair SC	Prof. Dr. Wolf-Dietrich Hardt	ETH Zürich	Zürich | Switzerland
SC	Prof. Dr. Jutta Heim	Basilea Pharmaceutica International Ltd.	Basel | Switzerland
SC/CB	Prof. Dr. Susanne Herold	Universitätsklinikum Gießen und Marburg (UKGM)	Gießen
Vice-Chair SC	Prof. Dr. Caroline Kisker	Julius-Maximilians-Universität	Würzburg
SC	Prof. Dr. Percy A. Knolle	Technische Universität München, Klinikum rechts der Isar	München
SC	Prof. Dr. Antonio Lanzavecchia	Institute for Research in Biomedicine	Bellinzona | Switzerland
SC	Prof. Dr. Melanie Ott	University of California	San Francisco| USA
SC	Prof. Nataša Pržulj	University College London	London | UK
SC	Prof. Dr. Marianne van der Sande	Institute of Tropical Medicine	Antwerp | Belgium
SC/CB	Prof. Dr. Norbert Suttorp	Charité Universitätsmedizin	Berlin
SC	Prof. Dr. Paul Wilmes	Luxembourg Centre for Systems Biomedicine, University of Luxembourg	Esch-sur-Alzette | Luxemburg

RESEARCH REPORT 2018/2019

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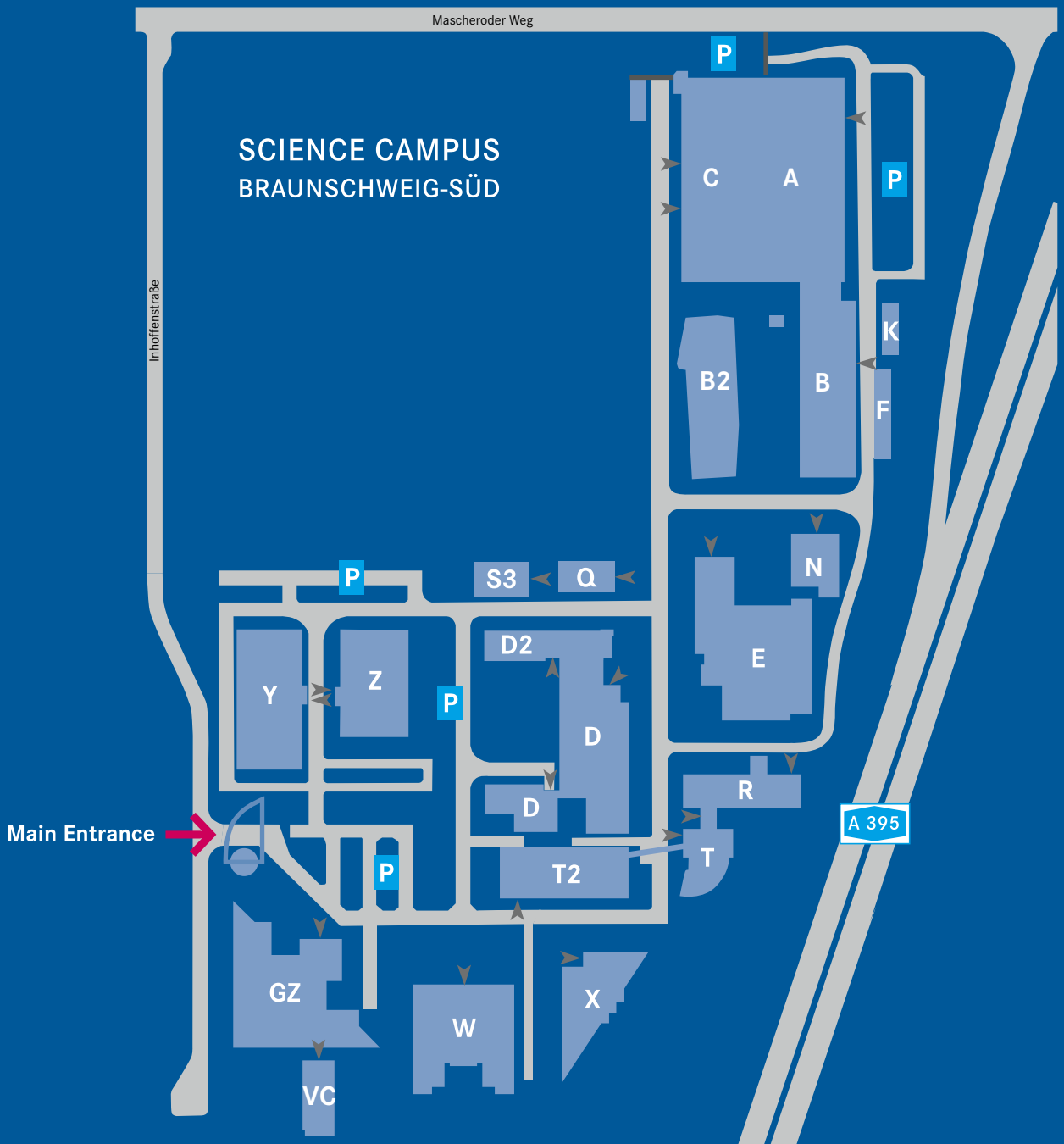

A	Research Labs, YUMAB GmbH
B	Research Labs
B2	Research Labs, Drug research & Functional genomics
C	Research Labs
D	Research Labs, InSCREENeX GmbH
D2	Offices
E, F, K, Q, R	Infrastructure
GZ	Gründerzentrum Building – DZIF Office, School Lab BioS, Research Labs
S3	S3-Facility
T, T2	Animal Facility
N	Infrastructure & Administration
VC	Administration
W	Administration, Library, Canteen
X	“Forum” – Event & Seminar Building
Y	Research Labs, Fraunhofer ITEM
Z	Leibniz Institute DSMZ

HZI HELMHOLTZ
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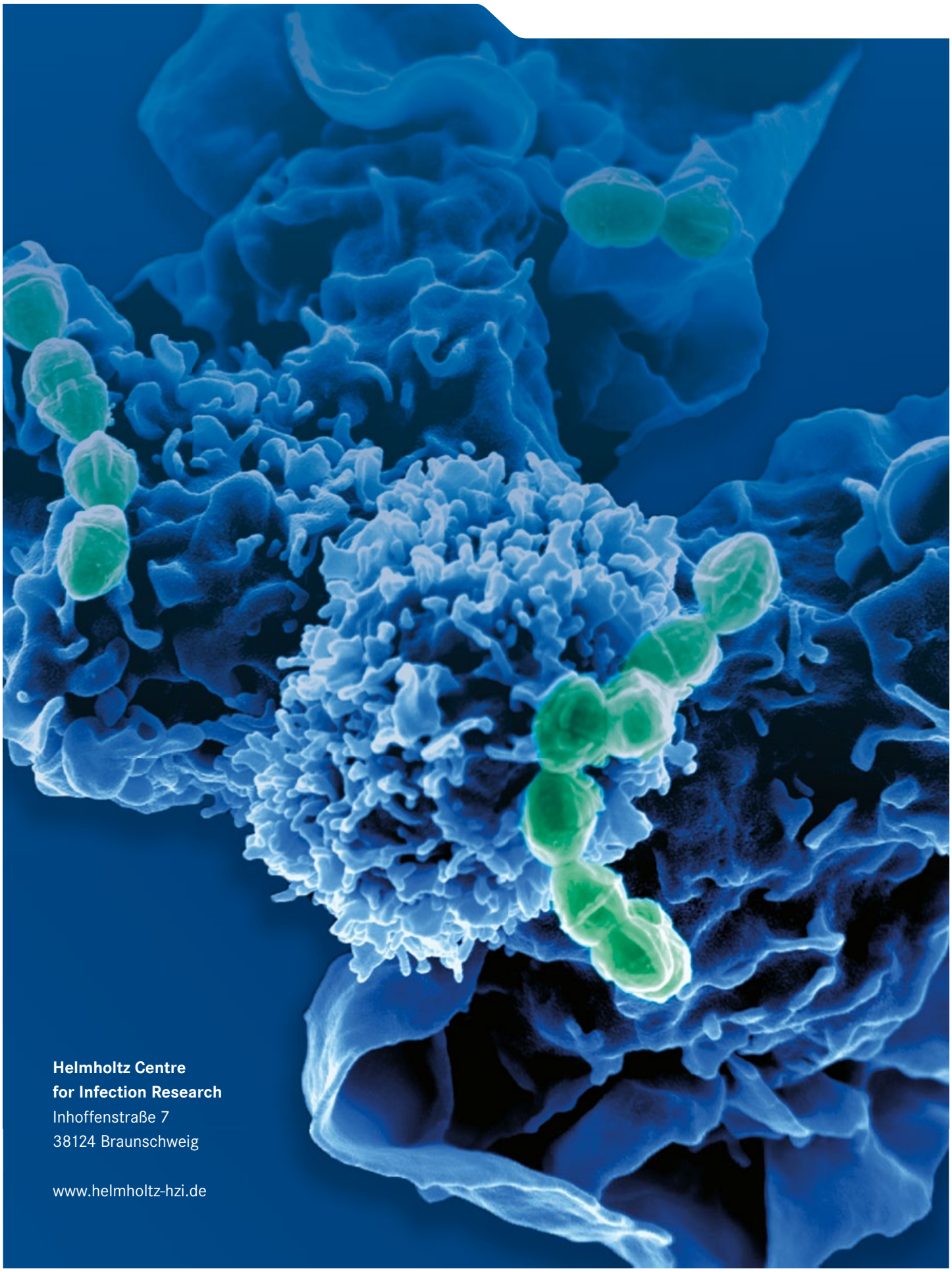

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