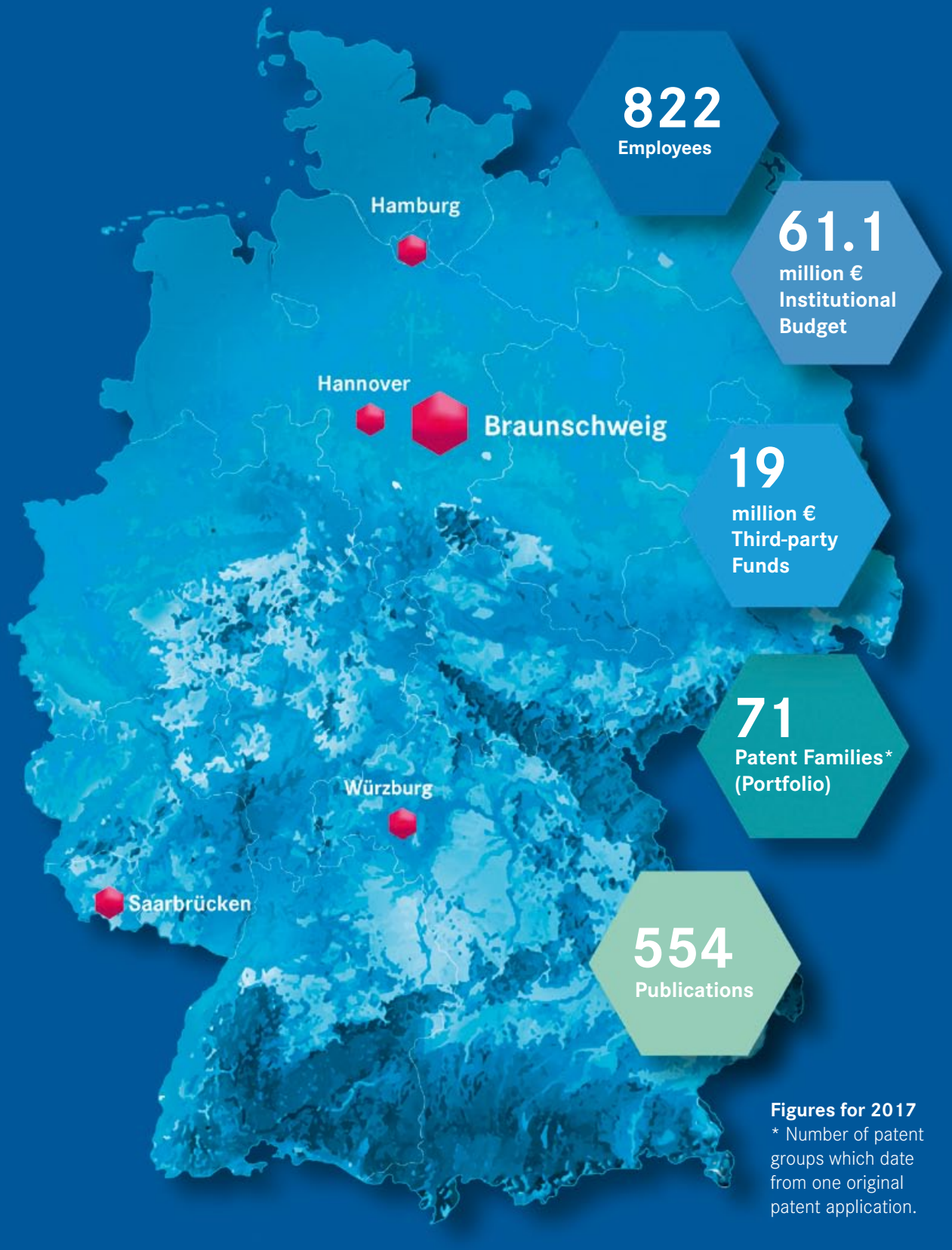
HZI HELMHOLTZ
Centre for Infection Research

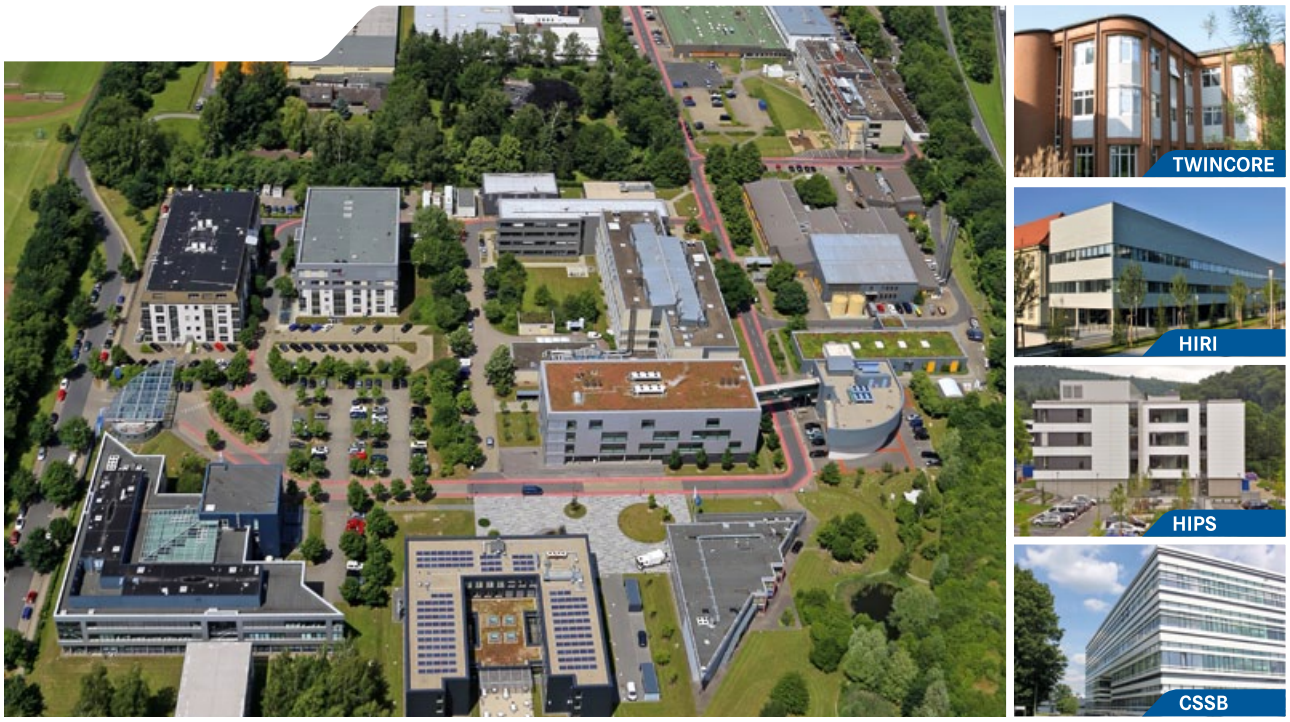
RESEARCH REPORT

HZI 2016 2017

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



Figures for 2017
* Number of patent groups which date from one original patent application.



HZI Campus Braunschweig

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, optimisation for pharmaceutical application
- Bridge between basic research and pharmaceutical industry

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

RESEARCH REPORT HZI 2016|2017

CONTENTS

HIGHLIGHT PUBLICATIONS

PARTNERS AND NETWORKS


T2
START

DEAR READER,

Prof. Dirk Heinz

In scientific research, it is not sufficient to rely entirely on one's own judgement: subjecting one's work and achievements to peer evaluation is a fundamental principle for ensuring scientific quality and impact.

As a research centre in the Helmholtz Association, HZI undergoes critical assessment by expert panels – internal as well as international – every few years. The most important of these regular review processes is the "Programme-oriented Funding" (POF) evaluation organised by the Helmholtz Association, which provides the basis for institutional funding for the upcoming years.

Preparing for the most recent scientific POF evaluation scheduled for March 2018 was an important task on the agenda of HZI's scientists and administration in 2016 and 2017. We were all anxious to learn how the international reviewers'panel would judge our work of the past years. The result – all three Research Topics of HZI's scientific programme were rated "outstanding", the highest possible grade – is a tremendous success and a great encouragement, putting the centre into a good position for its future development.

To assume and consolidate such a leading position in a highly dynamic field, a research centre like HZI needs to constantly re-define itself and its activities. Aiming at the development of cutting-edge fields with a high potential for medical and scientific breakthroughs, HZI has thus aligned itself with excellent partners. Together with these partners we have recently founded new institutes dedicated to RNA-based infection research and individualised infection medicine, respectively: the HIRI in Würzburg and the CiiM in Hannover, both of which are portrayed in this report.

Besides, you will also find detailed accounts of recent developments in core research areas of HZI, highlighted publications from our scientists, awards, partnerships and events that have left their mark over the past two years. Finally, I would like to express my deep gratitude to all HZI employees, our partner institutions and scientific advisory boards as well as our funding bodies for their enormous efforts and contributions towards the continuing positive trajectory of the centre.

We are delighted at your continuing interest in our work and hope you will enjoy reading this report.

Prof. Dirk Heinz
Scientific Director

ABOUT HZI

Portrait of the Research Centre

Mission and Development

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

Despite the huge successes of global vaccination programmes and campaigns and the availability of highly effective antibiotics and antivirals as well as accompanying pathogen control measures, infections continue to pose a severe threat to human health and confront society with enormous challenges.

Increasing mobility of people and goods as well as demographic and climate changes accelerate the spread of pathogens, facilitate their emergence and re-emergence and increase vulnerability to infectious diseases. Furthermore, increasing antimicrobial resistance adds a major challenge to their control and threatens to undo the historically unique progress made in infection control during the last century. The role of infections in the onset of other common diseases – like cancer, metabolic syndrome or neurodegeneration – is becoming increasingly apparent, opening the chance to fight these diseases by preventing or curing the underlying infection.

In line with the mission of the Helmholtz Association to confront major challenges facing society, science and industry, HZI is investigating the basic principles underlying infection processes. It employs latest generation technologies and is developing new approaches to prevention, diagnosis and therapy of infectious diseases.

Scientists at HZI and its partner institutions analyse the fundamental principles of infection processes from both the pathogen and the host sides and develop innovative concepts against infections. The centre pursues an integrated and highly interdisciplinary research concept which is based on profound expertise in the mechanistic explora-

tion 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 of HZI use a multi-scale approach from molecules via cells and organisms to populations. HZI operates at the interface between basic and translation research and, by capitalising on strong partnerships with clinics and industry, transitions the results towards their application. The combination of expertise and interdisciplinary collaboration is unique in the field of infection research, making the centre a frontrunner and technology leader in the struggle against global challenges like antimicrobial resistance or emerging and re-emerging infectious diseases.

The Centre and its Sites

HZI's more than 800 employees work at different locations throughout Germany. The main campus in Braunschweig – with scientists covering all disciplines of infection research – serves as the central research hub. Besides, branch institutes have been established to complement and strengthen the expertise at the main campus with critical mass in specific areas.

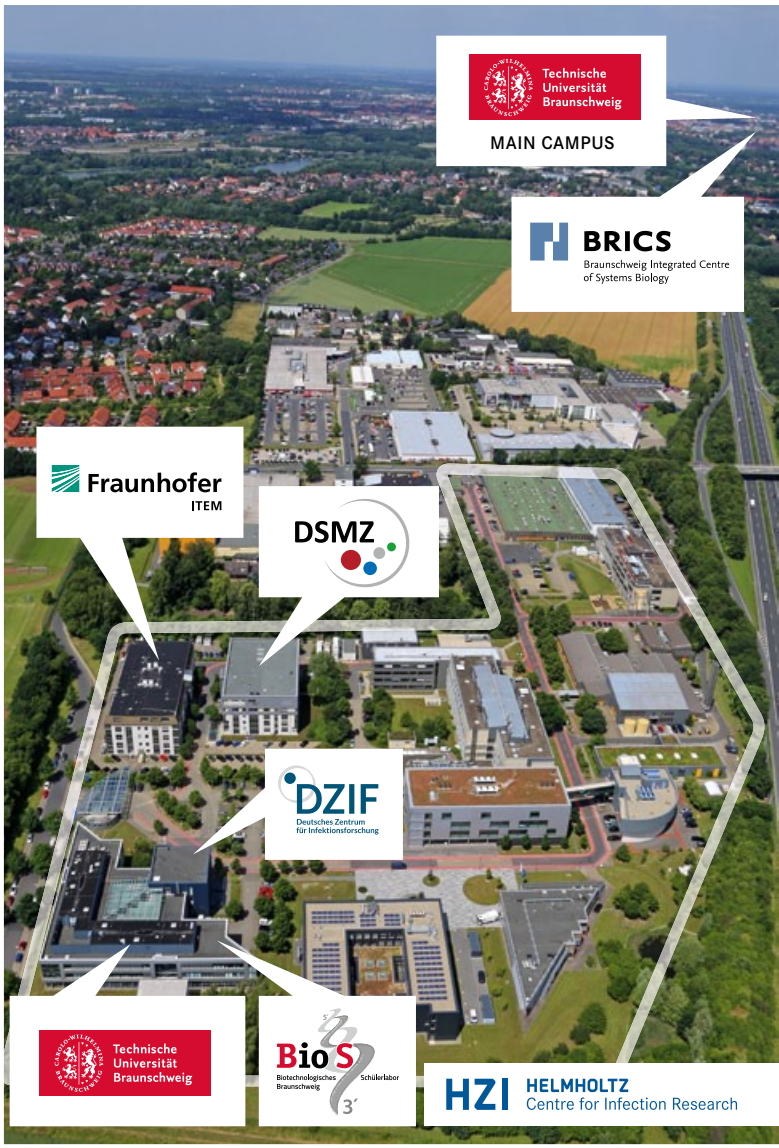
HZI Main Campus

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

The infrastructure on campus comprises, *inter alia*, technology platforms including facilities for fermentative and total synthesis of natural compounds, enabling the identification and development of 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 enable, among others, genotyping of pathogens or expression profiling. A cutting-edge animal facility with 500 different mouse strains allows HZI scientists to analyse virulence strategies and immune modulation concepts in modern, biosafety level 1-3 (BSL1-3) laboratories.

In recent years, the entire campus has been modernised to make optimal use of space and infrastructure: for example, meeting and communication space, library, canteen and administration, formerly spread over the campus, have been relocated to a single new building. This allows both dedication of other buildings entirely to research and optimisation of communication opportunities, to facilitate exchange at an interdisciplinary level.

A new building which will provide capabilities to intensify drug research is currently under construction. The new facility will also host laboratory space for functional genomics



HZI campus overview

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 HZI premises, the centre has recently established the "Science Campus Braunschweig Süd", 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 school lab "BioS" and others.

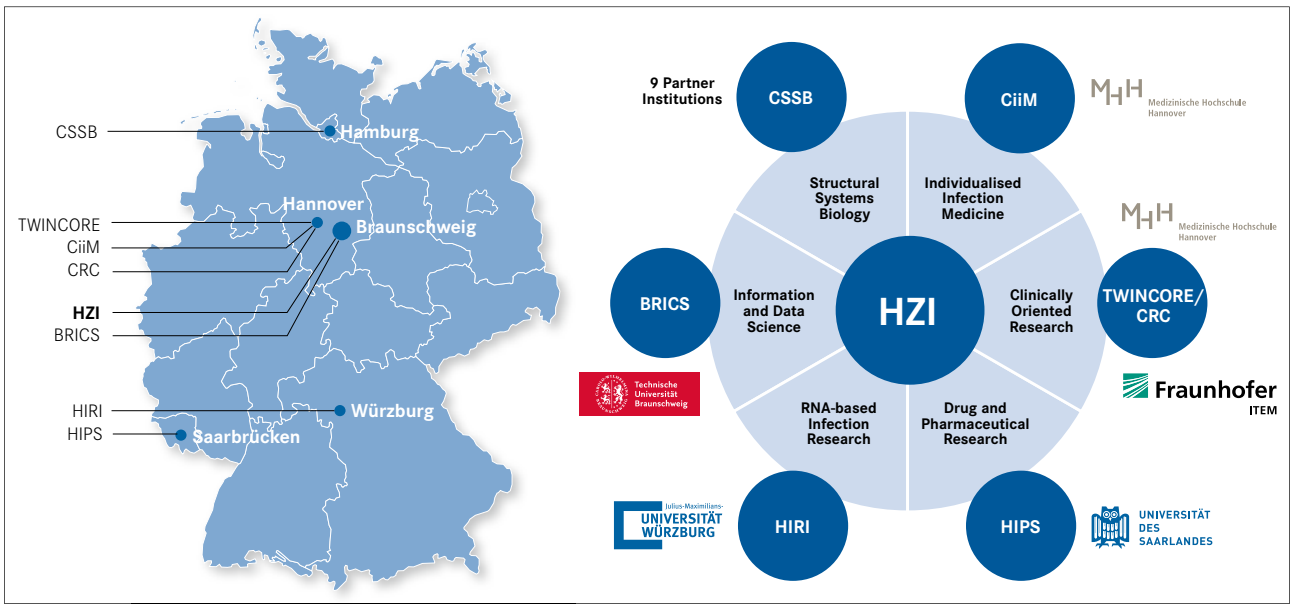
The DZIF main office, translational project management office and funding management are located on the HZI campus. HZI has assumed a pivotal role in DZIF initiated by the Federal Government of Germany in 2012, coordinating the Thematic Translational Units "Novel Antibiotics" and – via its Clinical Director – "Hepatitis", as well as the Translational Infrastructures "Epidemiology", "Product Development Unit" and "Bioinformatics".

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

Fraunhofer ITEM operates a specialised branch on the campus, including a GMP (Good manufacturing practice) facility for the production of biomolecules suitable for clinical testing, offering further opportunities for future cooperation on the campus.

HZI Branch Institutes

HZI partners with sites of excellence – usually universities – in selected fields (see Fig. 1). 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.



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

Within the last 10 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 above for details*).

→ ***Clinically oriented research*** **and** ***individualised infection medicine*****:** The mission of the translation centre TWINCORE in Hannover is to promote clinically relevant, patient-oriented infection research. TWINCORE, with now 160 employees (thereof 50 HZI staff), was founded in 2008 together with 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 initiative jointly developed in a strategic partnership with HZI. At TWINCORE, multidisciplinary teams of physicians and scientists in five research departments – three affiliated to HZI, two to MHH – pursue research motived by clinical needs and observations and translate research results into clinical practice.

Adjacent and complementary to TWINCORE, MHH and HZI will set up the Centre for Individualised Infection Medicine, CiiM, in forthcoming years. The new institute 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. (*see box CiiM*)

→ ***Drug and pharmaceutical research*****:** The Helmholtz Institute for Pharmaceutical Research Saarland, HIPS, in Saarbrücken is focussed 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, including natural compound research, medicinal chemistry and drug delivery. The new HIPS research building now hosts 130 employees (thereof 100 HZI staff) in three departments and three junior research groups, constituting a unique asset for the translational infection research pipeline of HZI.

Clinician scientist at TWINCORE Hannover

JMU Würzburg medical campus: home of HIRI

HIPS Saarbrücken

→ ***RNA-based infection research*****:** The role of non-coding RNAs in infection and immunity represents an emerging and fast-growing field with great potential for innovations. The subject has not been addressed so far with expertise concentrated at a single, dedicated location. In order to develop this field sustainably, the Helmholtz Institute for RNA-based Infection Research, HIRI, in Würzburg was founded in collaboration with the University of Würzburg (JMU) in 2017 (*see box HIRI*). At HIRI, heads of research departments and

DESY campus: home of CSSB

BRICS Braunschweig

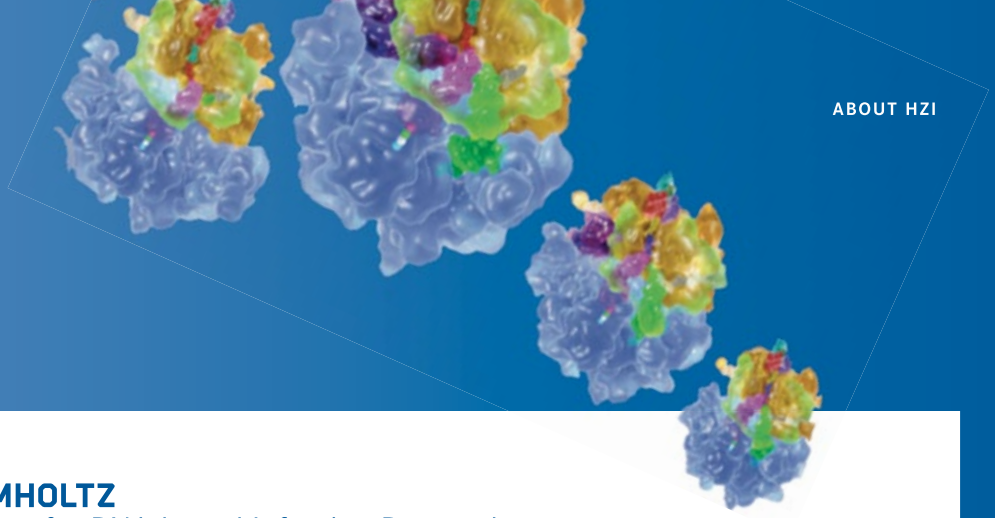
HZI study centre at CRC Hannover

research groups are currently being recruited. A dedicated new 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 have set up the Braunschweig Integrated Centre of Systems Biology, BRICS, in 2016. At BRICS, scientists from both partner institutions (thereof 40 HZI staff) collaborate on bioinformatics and mathematical modelling of infectious diseases and other biological 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 selected "Infections and Therapeutics" as one of its main research fields.

→ ***Structural systems biology***: In Hamburg, HZI has a key role in establishing the Centre for Structural Systems Biology, CSSB, a joint initiative of ten research partners. In the new building on the DESY (German Electron Synchrotron Centre) campus in Hamburg, structural biologists and infection researchers investigate host-pathogen interactions at the highest possible spatial resolution using DESY's high-intensity photon sources (third-generation synchrotron source PETRA III and prospectively European X-FEL). In 2015, a structural biology department of HZI (thereof 10 HZI staff) was relocated to 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 IIa trials) of new medications. CRC was founded in 2014 by Fraunhofer ITEM and MHH together with HZI as a unique translational infrastructure. The CRC also hosts the HZI Study Centre in the framework of the German National Cohort (GNC), where epidemiologists conduct long-term population studies with volunteers.



HIRI HELMHOLTZ
Institute for RNA-based Infection Research

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

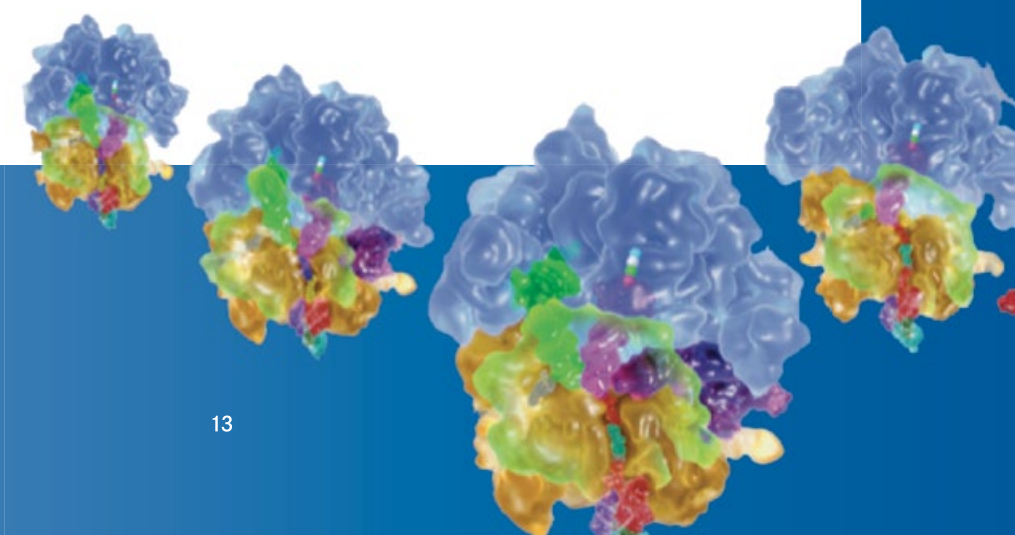
Technological breakthroughs in high-throughput sequencing have led to the discovery of numerous new classes of non-coding RNAs with important regulatory functions in many cellular pathways. It is becoming increasingly clear that non-coding RNAs and other RNA-based control mechanisms also critically affect infection processes on both the host and pathogen side, for example impacting virulence and antibiotic resistance. An understanding of these complex interactions will therefore generate opportunities for the development of new forms of treatment, diagnosis and prevention.

To jointly advance this emerging research area with its high potential for disruptive innovations and breakthroughs in infection medicine, HZI has established a partnership with the University of Würzburg (JMU), whose "Research Centre for Infectious Diseases" (ZINF) is internationally renowned in the field of RNA and infection research. In a highly competitive application process, their concept for a joint Helmholtz Institute was rated "outstanding" by an international panel of reviewers. After consent of the awarding authorities, HZI and JMU inaugurated the Helmholtz Institute for RNA-based Infection Research (HIRI) in Würzburg in 2017. HIRI is the first institute in the world exclusively dedicated to RNA-based infection research.

HIRI will address the roles of RNA in "Bacterial Infections", "Viral Infections" and "Host Responses"; these areas will be complemented with "RNA Delivery". Scientists at HIRI will aim at

- resolving the complexity and heterogeneity of infection processes at the single-cell level
- identifying novel regulatory RNAs with essential roles in pathogenesis
- understanding RNA-based mechanisms in virulence and host defence
- developing innovative delivery techniques for RNA-based interventions
- exploiting RNA-based knowledge for new diagnostics, preventives and anti-infectives.

The internationally renowned RNA researcher Jörg Vogel, recent laureate of the prestigious Gottfried Wilhelm Leibniz Prize, was appointed as founding director of HIRI. In 2017 staff were first recruited to HIRI, and funding successfully raised for a Helmholtz Young Investigator Group. Additional recruitments are ongoing. To promote the implementation of HIRI and foster strong links between HIRI, JMU and HZI, 22 projects with involvement of all partner institutions have received funding since July 2017 within the framework of a competitive seed grant programme.



3D model of ribosomal frameshifting © Wendy Liu

THE CENTRE FOR INDIVIDUALISED INFECTION MEDICINE (CiiM), HANNOVER

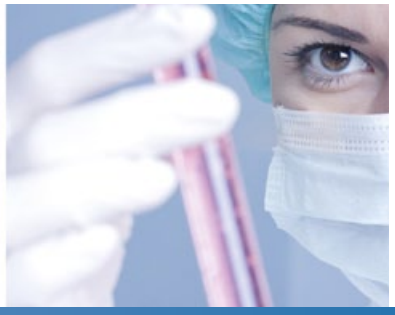
The rapid development of various enabling and disruptive technologies offers radically new possibilities in medicine including systematic diagnostics for precise patient stratification, targeted prevention and tailored therapy specifically addressing the needs of individual patients or patient groups. The resulting potential of individualised (or precision) medicine is widely recognised and already revolutionising cancer medicine. Future approaches in individualised infection medicine will be based on a comprehensive consideration of the specific characteristics of host, pathogen and treatment/prevention and their highly dynamic interactions.

The new Centre for Individualised Infection Medicine (CiiM) is the first of its kind being exclusively committed to providing research evidence for individualised therapy and prevention measures to improve medical care in infectious diseases. CiiM has been jointly set up by HZI and MHH in 2015 and is currently – in its initial phase – operating as a virtual research network. In the near future a dedicated CiiM building in immediate proximity to TWINCORE and MHH will bundle interdisciplinary expertise from the clinic, experimental science, data science and systems medicine as well as state-of-the-art infrastructures under one roof.

Once fully implemented and operational, CiiM units and groups under the leadership of founding director Michael Manns will cover essential aspects in individualised infection medicine. In line with a consistent “bed to bench and back” approach oriented along the needs of specific patient groups, they will aim at

- systematic data collection and in-depth profiling of patients, microbiota and pathogens
- identification of new biomarkers by integrating clinical observations and experimental data
- generation and analysis of sophisticated data sets by means of data science allowing the development of hypotheses and predictive models
- experimental validation of the hypotheses and predictive models by mechanistic research
- transfer of the results towards applied research and clinical trials, which will ultimately allow for optimised recommendations for treatment and prevention.

With its interdisciplinary scientific concept and highly translational mission, CiiM will complement the research portfolios and objectives of both HZI and MHH. It will provide valuable crosslinks to related personalised medicine initiatives within the Helmholtz Research Field Health (*e.g.* iMed), DZIF, and beyond.



THE HELMHOLTZ PROGRAMME "INFECTION RESEARCH"

Dirk Heinz, Scientific Director and Programme Speaker

Infectious diseases still present a global threat to human health and are the cause of more than a fifth of all deaths worldwide. While the industrialised countries overcame many of these diseases during the 20th century with vaccination programmes, improved hygiene and powerful antibiotics, new threats are now causing alarm to society. The rapid spread of newly emerging and resurgent pathogens, chronic infections, increasing resistance to antibiotics, as well as demographic and climate change are lending new urgency to solving the problem.

With its great potential to reduce the global burden of disease, infection research is a cornerstone in the research field "Health" of the Helmholtz Association, Germany's largest research organisation concentrating on the great challenges of our time. In the framework of Helmholtz strategic programmes for integrated research, HZI has developed its programme "Infection Research" to address major challenges in infection medicine. HZI is focussed solely on this research programme and is the only Helmholtz centre involved. The programme lays particular emphasis on *translation* – the timely and effective transfer of results from basic research into medical and pharmaceutical applications and back.

STRATEGY FOR TRANSLATIONAL INFECTION RESEARCH

Controlling infectious diseases requires a fundamentally different approach when compared to other common diseases such as cancer, cardiovascular or metabolic diseases. Infections are a result of complex interactions between pathogen and host. The transmissibility of infectious diseases imposes special requirements with respect to prevention, treatment and control. Every individual treated promptly and effectively, and every infection prevented, will not only serve this individual but will also help prevent other members of society being infected in the first place, thus creating a unique leverage of prevention. However, this requires immediate reaction to novel infectious disease challenges and, thus, a flexible research strategy.

Constantly moving targets pose unique challenges, which demand detailed knowledge of the host, the pathogen and the treatment and prevention options to successfully develop solutions for the control of infectious diseases. Thus, infection medicine and infection research are best characterised by the triad Pathogen – Patient – Prevention/Treatment (see Figure 2). These three entities influence each other in multiple ways. Only a detailed understanding of their complex interactions can be expected to result in advanced diagnostics, improved risk assessment, more effective prevention measures and, ultimately, in safer and more effective therapies.

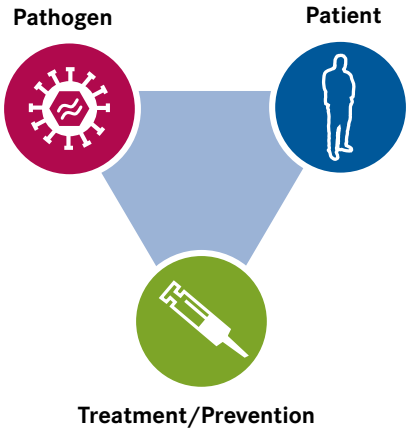


Figure 2: The Triad pathogen-patient-therapy/pervention

RESEARCH TOPICS AT HZI

According to the principles of the triad, research activities of the Programme are structured within a triad of Topics addressing: 1) bacterial and viral pathogens, 2) host immune response and intervention and 3) anti-infectives. These Topics constitute the knowledge base for the centre's infection research. Their interactions provide the "breeding ground" for the translational activities which unfold along defined Research Foci (see below).

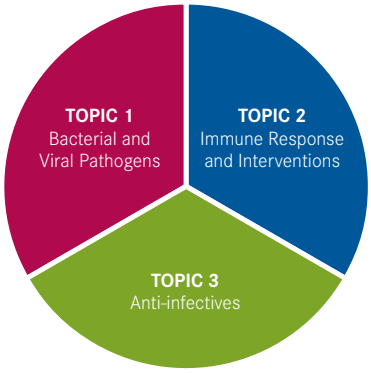


Figure 3: HZI's Research Topics

Topic 1 "Bacterial and Viral Pathogens", spokesperson: Thomas Pietschmann

In collaboration with clinical partners, Topic 1 scientists investigate infections caused by bacteria and viruses. Their aims include understanding the molecular bases of virulence and resistance as well as determining risk factors for the spread of diseases, among them infectivity of and susceptibility to pathogens. They are also striving to identify new targets for antimicrobial therapies.

Topic 2: "Immune Response and Interventions", spokesperson: Carlos A. Guzmán

The focus of Topic 2 is on the host response to infections. Central themes are innate and adaptive immune responses, the transmission of pathogens and their elimination by the host's immune defences. Topic 2 scientists are, furthermore, studying mechanisms which allow pathogens to bypass the host's immune system or even promote their further spread with the involvement of host factors. The insights gained will serve as the basis for new immune-focused strategies for diagnosis (biomarkers), prevention (vaccination) and treatment (immune therapy) of infectious diseases.

Topic 3: "Anti-Infectives", spokesperson: Rolf Müller

In Topic 3, researchers are dedicated to discovering and developing new anti-infectives. Innovative screening methods, microbial genome mining and synthetic biotechnology make it possible to identify new natural compounds with antibacterial or antiviral properties. Working in close cooperation with partners in industry, the researchers study promising substances which intervene in the adhesion, invasion or communication of pathogens and optimise these compounds chemically and pharmaceutically. By means of medicinal chemistry and the development of suitable formulations for optimising drug delivery, the anti-infectives are being made ready for preclinical and clinical trials.

Modern platforms and infrastructures support research in each topic. These include sophisticated technologies for genome and proteome analysis, structural biology and imaging technologies, animal experiment facilities and natural compound libraries.

RESEARCH FOCI AT HZI

Flexibility when addressing new and challenging questions is a key feature of the programme's design. To better focus on clinical questions of major relevance, HZI has developed a dynamic, integrative and interdisciplinary research portfolio merging expertise and technological developments from each Topic. The resulting Research Foci involve basic, pharmaceutical and clinically oriented research. These are ambitious, interdisciplinary projects combining expertise from two or all three Topics to tackle a particular health problem. Currently five cross-topic Research Foci have been established. All of these are driven by the know-how and interests of HZI researchers. Importantly, they also build on the strengths of the centre's cooperation partners. They highlight examples of focal areas addressed by the integrated approach of the programme "Infection Research". Moreover, they are strongly connected to complementary activities funded by the Federal Ministry of Education and Research (BMBF), including the German Centre for Infection Research (DZIF), the German Research Foundation (DFG), as well as international programmes funded by the EU and other funding organizations.

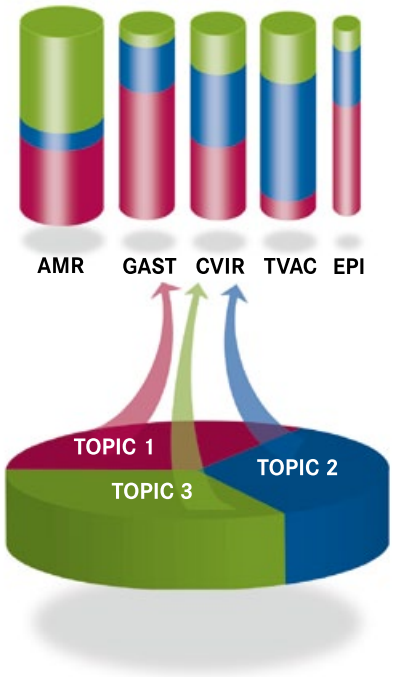


Figure 3: The Topics constitute the "breeding ground" for HZI's Research Foci – interdisciplinary focal projects involving contributions from different Topics. column widths indicate the relative sizes of the Research Foci (staff, infrastructure, funding). Colours indicate the share of each Topic in respective Research Foci.

These five Research Foci (RFs) comprise:

(i) Antimicrobial resistance (AMR)
(ii) Tackling gastrointestinal bacterial infections (GAST)
(iii) Approaches against chronic viral infections (CVIR)
(iv) T-cell targeting and vaccination strategies (TVAC)
(v) Epidemiology for public health solutions (EPI)

Research Focus Antimicrobial Resistance (AMR)

Rising antimicrobial resistance poses a daunting challenge, particularly in light of the scarcity of new 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 molecular mechanisms underlying resistance, explore innovative strategies against pathogens and identify and optimise novel anti-infective compounds.

Long-term objectives include the development of tools for early diagnosis and targeted antibiotic treatment, new methods to selectively block pathogenic mechanisms – thus preventing the emergence of resistances – and the development of novel natural antibiotics exhibiting new modes of action. Sustainable biotechnological production of these novel anti-infectives provides sufficient material for late preclinical studies.

The research of AMR scientists has already yielded a number of novel compounds from natural sources with new modes of action, including potential therapeutics directed against Gram-negative bacteria, which are particularly difficult to treat.

Research Focus Tackling Gastrointestinal Bacterial Infections (GAST)

Only limited options for the effective treatment of acute and chronic enteric infections are currently available. To gain a comprehensive understanding about the course of infection and the complications associated with enteric infections, the Research Focus GAST combines expertise to identify essential virulence mechanisms of enteric pathogens and to characterise the complex interplay between pathogens, the host immune system and the microbial ecosystem of the gut (microbiota). Newly developed technologies enable the discovery of essential virulence strategies and an advanced understanding of molecular mechanisms of bacterial pathogenicity factors leading to the identification of novel promising drug targets.

Research Focus Approaches against Chronic Viral Infections (CVIR)

Chronic viral infections by hepatitis and herpes viruses significantly contribute to the global disease burden. Recently, effective treatment for hepatitis C virus (HCV) infections has become available, but it is challenging to deliver it to all in need. Cytomegalovirus (CMV) therapies are limited by side effects and viral resistance. Vaccines are still lacking for both HCV and CMV, and the understanding of basic mechanisms of pathogenesis, immune control and viral evasion remain incomplete. The Research Focus CVIR dissects principles that govern HCV and CMV persistence including mechanisms of innate immune control and viral evasion. By exploring mechanisms of protective or failing T-cell responses and determinants of protective humoral immunity, CVIR aims to contribute to the development of new preventive measures.

Research Focus T-cell Targeting and Vaccination Strategies (TVAC)

Effective therapies and vaccines are still lacking for many emerging and re-emerging pathogens. This is particularly true for vulnerable individuals at increased risk for severe forms of infection and poor responders to intervention. Thus, the Research Focus TVAC has established experimental and clinical activities to fill existing knowledge gaps by developing immune-based approaches to prevent or treat resilient infections in vulnerable patients. Research activities contribute towards a better understanding of differential host responsiveness to infection and vaccination (*e.g.* against influenza). New technologies have also been developed from which to derive future immune-based strategies for the prevention and treatment of infections.

Research Focus Epidemiology for Public Health Solutions (EPI)

The Research Focus EPI addresses the societal dimension of infectious diseases in order to develop corresponding public health solutions. The Surveillance Outbreak Response Management and Analysis System (SORMAS) is a notable example of a tool developed by HZI, which is already being used to control disease outbreaks in Nigeria and to understand the spread of infectious diseases. Special infection modules within the German National Cohort (GNC) developed by HZI will allow investigating how infections contribute to non-infectious diseases and vice versa, ultimately leading to novel prevention strategies for these diseases. Furthermore, the Research Focus EPI is developing novel diagnostics, such as differential serology, which allow measuring the effectiveness of vaccination strategies at the population level based on immunological biomarkers.

Across all Research Foci, activities are concentrated on selected clinically relevant pathogens ("focus pathogens"), which present particularly promising research subjects for the HZI and its partners. These comprise, in particular,

hepatitis and herpes viruses, antibiotic-resistant bacterial pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* as well as Enterobacteriaceae and *Clostridium difficile*. Respiratory viral infections, *e.g.* with influenza and respiratory syncytial viruses (RSV), represent an upcoming field in HZI's research which the centre intends to expand in the coming years.

The programme "Infection Research" is set up to promote intensive interdisciplinary collaboration between experts from life and computer sciences, chemistry, pharmacy and medicine. It thereby guarantees that results from excellent basic research are translated into practice as efficiently as possible. In the long term – as the aim of the programme – achieving a profound understanding of the molecular and cellular mechanisms of infection processes will provide a basis for applying innovations. Individualised infection medicine, medical informatics and data sciences as well as upcoming new fields, such as RNA-based infection research, will contribute to innovative strategies to combat pathogens. Novel antibiotics, biomarkers and other diagnostics, vaccines, immunotherapeutics and RNA-based agents discovered or optimised by HZI and its partners will fill empty "development pipelines" and be made available for clinical applications.

IN AND AROUND HZI

NEW ADMINISTRATIVE DIRECTOR: SILKE TANNAPFEL TAKES THE LEAD

On October 1, 2017, Silke Tannapfel assumed the responsibilities of the Administrative Director of HZI. The former head of the Division for Extramural Research Support and Cross-State Bodies in the Bavarian State Ministry for Economy and Media, Energy and Technology based in Munich is the successor to Franziska Broer, who had been appointed Executive Manager of the Helmholtz Association in the year before.

Raised in Nienburg/Weser, Silke Tannapfel studied law at the University of Göttingen and Science Management at the German University of Administrative Sciences Speyer.

She then became head of the division of legal affairs and head of the Chancellor's office of the University of Erlangen-Nuremberg and division head of the Bavarian State Chancellery for Science and Arts at the Bavarian delegation to the European Union in Brussels. As a next step, she was appointed personal consultant of the State Minister of the Ministry for Science, Research and the Arts in conjunction with being the head of the division for science politics and cross-state bodies.

Since 2013, Silke Tannapfel held her most recent position – before coming to HZI – and led the aforementioned division for extramural research support and cross-state bodies in the Bavarian State Ministry for Economy and Media, Energy and Technology.

MEETINGS, PRIZES, PUBLIC OUTREACH

Highlights of the Years 2016 and 2017

A symbol for the research networks in the region: The installation “Science Cloud” at the Burgplatz in Braunschweig.

SCIENCE FOR THE PUBLIC

The public lecture series **“KrankheitsErregend” (“Patho-Genic”)** was organised by HZI in both autumn 2016 and 2017 for the fifth and sixth time, respectively. In 2016, the lectures focused on newly emerging infectious diseases. Scientists and clinicians informed the audience about Zika, Ebola and Hepatitis E. The 2017 lecture series was entitled “Expeditions into the Immune System” and focused on chronic lung infections, the interaction of the immune system with the intestinal flora, and the immune system in old age.

In April 2016 and March 2017, the Northern German qualifying round for the international science communication competition **"FameLab"** was again held at HZI. Young scientists from different disciplines presented their research projects in three-minute talks. Hanzey Yasar from the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) won not only the 2016 regional round at HZI, but later went on to win the German final. In 2017, Panagiota Mamareli from TWINCORE won the qualifying round.

Making Science understandable – and entertaining: The winner of the qualifying round for the 2017 FameLab competition, TWINCORE scientist Panagiota Mamareli, with HZI' Scientific Director Dirk Heinz (left) and moderator André Lampe.

Back in 2007, Braunschweig was elected "City of Science". Since then, more than 800 events have served to familiarise the citizens of Braunschweig more closely with science in everyday life. Ten years on, the city of Braunschweig set up a large installation at the Burgplatz called **"Cloud of Science"** that served as a platform for a ten day programme of events. HZI organised several science talks and activities for children. As part of the anniversary, HZI and its campus partners DSMZ, Fraunhofer ITEM, TU BS and DZIF established a new umbrella brand titled **"Science Campus Braunschweig-Süd"**. In the run-up to the brand development, the mayor of Braunschweig, Ulrich Markurth, found out about the research priorities of HZI and campus developments in Braunschweig-Stöckheim. He was accompanied by the head of Braunschweig's Department of Culture and Science, Anja Hesse.

The founding ceremony for the Science Campus Braunschweig-Süd (from left to right): Dirk Heinz (HZI), Jörg Overmann (DSMZ), Iris Eisenbeiser (Biotechnological School Lab BioS), Anke Kaysser-Pyzalla (TU BS), Timo Jäger (DZIF), Norbert Krug (ITEM), Ulrich Markurth (mayor BS).

The 10th **"Forum Wissenschaftskommunikation" ("Science Communication Forum"**) – the largest symposium on science communication in the German-speaking region – took place in November 2017 in Braunschweig. The main topic was "What reaches whom – techniques and tools of science communication". Participants from Germany, Austria and Switzerland discussed current study results, the use of Twitter, Facebook etc. in times of fake news, crisis communication and the question of which strategies and formats of science communication can be used. HZI participated in the booth of the regional research institutions.

Talking science and informing the citizens: HZI researcher Mark Brönstrup at the "City of Science" anniversary.

PRIZES AND AWARDS

Selected scientific awards in 2016

Awarded scientist	**HZI group or department** (see organisational chart for complete names)	**Award**	**Awarding institution**
Claus-Michael Lehr	HIPS-DDEL	Listed as one of the 100 most influential experts in drug development	The Medicine Maker, London/UK
Gregor Fuhrmann	HIPS-DDEL und HIPS-BION	NanoMatFutur Promotion Award	BMBF
Stephanie Pfänder	EVIR	DZIF-Graduate Student Award of GfV	Deutsche Gesellschaft für Virologie (DGI) zusammen mit dem Deutschen Zentrum für Infektionsforschung (DZIF)
Stephanie Pfänder	EVIR	DZIF-Graduate Student Award of DGI	Deutsche Gesellschaft für Infektiologie (DGI) zusammen mit dem Deutschen Zentrum für Infektionsforschung (DZIF)
Thibaud Renault	IBIS	Humboldt Research Fellowship	Humboldt Foundation
Irene Wagner-Döbler, Helena Sztajer	KOM	Otto-Walkhoff Award	Deutsche Gesellschaft für Zahnerhaltung
Rolf Müller	MINS	PHOENIX Pharmaceutic Science Award 2016	PHOENIX group
Rolf Müller	MINS	David Gottlieb Lecturer 2016	Howard Hughes Medical Institute, University of Illinois, USA
Rolf Müller	MINS	Admission to the Leopoldina (German National Academy of Science)	Leopoldina (Nationale Akademie der Wissenschaften)
Eric Kuhnert	MWIS	Oscar-Brefeld Award	Deutsche Gesellschaft für Mykologie (DGfM)/ German Mycological Society
Melanie Brinkmann	VIMM	STS Science Award	STS = Signal Transduction Society (Gesellschaft für Signaltransduktion)

Selected scientific awards in 2017

Awarded scientist	**HZI group or department** (see organisational chart for complete names)	**Award**	**Awarding institution**
Shuting Xu, Aurélie Ducroux, Christine Goffinet	AIVE	German AIDS Award 2017	Deutsche AIDS-Stiftung
Gregor Fuhrmann	BION	Galenus Technology Award	Galenus Privatstiftung
Maike Windbergs	DDEL	CRS T. Nagai Postdoctoral Research Achievement Award	CRS
Claus-Michael Lehr	DDEL	Listed as one of the 100 most influential experts in drug development	The Medicine Maker
Gisa Gerold	EVIR	Postdoc Research Award	Robert Koch Stiftung
Julia Boehme	IREG	BD Immunology Research Grant	BD Biosciences
Jörg Vogel	RABI	Gottfried-Wilhelm-Leibniz Award	DFG
Rolf Hartmann	DDOP	Portoghese Medicinal Chemistry Award	American Chemical Society
Anna Hirsch	DDOP	Innovation Prize in Medicinal and Pharmaceutical Chemistry	German Chemistry Society

ERC grants for HZI scientists in 2016 and 2017

PI	ERC Grant	Acronym and Title
Luka Čičin-Šain	Proof of Concept	CMVAgVECTOR Mouse cytomegalovirus based vaccine vectors inducing CTL immunity and protection
Alexander Titz	Starting Grant	SWEETBULLETS Sweet theranostics in bitter infections – seek and destroy
Susanne Häussler	Proof of Concept	RAPID Antimicrobial susceptibility testing and phylogenetic identification
Susanne Häussler	Consolidator Grant	COMBAT Clearance of microbial biofilms by advancing diagnostics and therapy
Andreas Müller (OvGU)	Starting Grant	ImmProDynamics Dissecting the interplay between the dynamics of immune responses and pathogen proliferation *in vivo*
Anna Hirsch	Starting Grant	NovAnI Identification and optimisation of novel anti-infective agents using multiple hit-identification strategies

Honoured as one of the world's leading researchers in the field of ribonucleic acid biology: Jörg Vogel (right) received the prestigious Leibniz Award of the Deutsche Forschungsgemeinschaft, DFG (left: Peter Strohschneider, President of the DFG).

BUILDINGS

BRICS Braunschweig

The Braunschweig Integrated Centre of Systems Biology (BRICS) on the campus of the Technical University Braunschweig (TU BS) opened its gates and took up work in August 2016. BRICS is a joint establishment of TU BS, HZI and DSMZ. Biologists, physicists, mathematicians, informaticians, chemists and engineers collaborate here to tackle issues in systems biology, including mathematical and bioinformatic modelling of infections and other biological processes. Five institutes of TU BS and two departments of HZI are located in the new building.

CSSB Hamburg

The Centre for Structural Systems Biology (CSSB) celebrated its grand opening in June 2017 on the DESY campus in Hamburg. In this new interdisciplinary research centre, scientists from ten different research institutions work together on some of the most challenging projects in structural biology: the functioning of viruses, bacteria and parasites. The research focus of CSSB is on the elucidation of structures, dynamics and mechanisms of the infection process of these pathogens. With these achievements, CSSB scientists seek to contribute to the development of novel treatments and therapies. HZI was one of the founding institutions of the CSSB and is represented there by its department “Structural Infection Biology”.

Bricks for the BRICS: Jürgen Hesselbach, President of TU BS, Dirk Heinz, Scientific Director of HZI, Gabriele Heinen-Kljajić, Lower Saxony’s Minister for Science and Culture, Dieter Jahn, Speaker of BRICS, Ulrich Markurth, Braunschweig’s Mayor, and Jörg Overmann, Director of DSMZ.

Prominent representatives from science and politics celebrated the CSSB opening in Hamburg. Fourth from left: Dirk Heinz, Scientific Director of HZI, in the middle with a symbolic key: Matthias Wilmanns, Scientific Director of CSSB, fourth from right: Hamburg’s Mayor Olaf Scholz, second from right: Lower Saxony’s Minister for Science Gabriele Heinen-Kljajić.

SCIENTIFIC EVENTS

"Biological Barriers" conference at HIPS

In August 2016, experts from around the world gathered in Saarbrücken at the "Biological Barriers" conference jointly organised by HIPS and the University of Saarland (UdS). This time, the focus of the biennial meeting was on the body's outer epithelia – skin, lung, intestinal mucosa – as well as on advanced drug delivery systems, preferentially for non-invasive administration via the aforementioned epithelia. Nanomedicine, but also nanotoxicology, and cell- and tissue-based *in vitro* models – including their potential as alternatives to animal testing – were major aspects of the programme. Interactive workshops on Good Scientific Practice and on Bibliometrics completed the event.

NoRDI and Jürgen Wehland Prize

Since 2010, scientists from across Europe convene at the annual NoRDI Symposium (North Regio Day on Infection) on the HZI campus. In October 2016, the seventh symposium, NoRDI VII, revolved around the subject "Infection: Persistence, Resistance and Therapy". Key mechanisms of persistence and resistance employed by pathogens were discussed, and innovative therapies to tackle them were presented. Embedded in the scientific programme was the awarding of the Jürgen Wehland Prize for young scientists in infection research, with which 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 Dr. Luciana Berod of TWINCORE.

Jürgen Wehland Prize 2016: Awardee Luciana Berod with Hansjörg Hauser (Managing Director of the HZI Förderverein, left) and Ulrich Kalinke, Executive Director of TWINCORE.

Inhoffen Medal 2016: Prize Winner Thomas Carell (middle) with Dieter Jahn, President of the HZI Förderverein, and Dirk Heinz, Scientific Director of HZI (right).

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 HZI Förderverein (Friends of HZI) since 1992 and endowed with 5,000 Euros. In 2016, it went to Thomas Carell of the LMU in Munich for his research on DNA repair. In 2017, Helma Wennemers, Eidgenössische Technische Hochschule Zürich (ETH), a pioneer in the development of novel compounds and materials for medical purposes, received the prize. The awarding ceremony is associated with the "Inhoffen Lecture" in honour of the biochemist Hans-Herloff Inhoffen, an event organised jointly by HZI and TU Braunschweig. Inhoffen

was director of the TU BS and co-founder of the Institute for Molecular Biology, Biochemistry and Biophysics (IMB), from which the HZI subsequently emerged. He died in 1992.

Inhoffen Medal 2017: Dieter Jahn, President of the HZI Förderverein, presents the prestigious medal to Helma Wennemers.

VISTRIE Symposia on Viruses and the Immune System

Seven partners from German universities and research institutions and from the Rijeka School of Medicine in Croatia jointly form the virtual institute VISTRIE (Viral Strategies of Immune Evasion). In June 2016 they organised the fourth VISTRIE symposium, focussed on the interaction between viruses and the immune system. Since its beginning, the symposium has been attended by internationally renowned speakers.

Focus on natural compounds: The HIPS Symposium

Every year since 2011, 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 sixth and seventh time in 2016 and 2017.

Immunomodulation and Communication Pathways: 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 eighth and ninth time to share their knowledge. In 2016, the meeting focused on “Immunomodulation and evasion in infection”; the 2017 event concentrated on “communication pathways in infection”.

Stephan Weil (in front), Prime Minister of Lower Saxony, Carola Reimann, Member of the Bundestag, and HZI's Scientific Director Dirk Heinz.

POLITICIANS VISITING HZI

HZI and its branch institutes welcomed politicians on several occasions in 2016 and 2017.

As part of his summer trip, Lower Saxony's Prime Minister Stephan Weil visited HZI in August 2016. He was informed about current challenges and innovative solutions in modern infection research.

In May 2017 Hubertus Heil, member of the Bundestag for the Gifhorn/Peine constituency and deputy chairman of the SPD parliamentary group, came to the HZI campus in Braunschweig. He was accompanied by Carola Reimann, member of the Bundestag for Braunschweig and deputy chair of the SPD parliamentary group.

In the same month, the EU Commissioner for Health and Food Safety, Vytenis Andriukaitis, together with cabinet member Annika Nowak, visited HIPS in Saarbrücken.

In August 2017 Cornelia Quennet-Thielen, State Secretary at the Federal Ministry of Education and Research, came to the HZI campus to learn about newest developments in infection research. During her stay, she confirmed the Ministry's plans to initiate new targeted measures to counter challenges, like the rise of antimicrobial resistance.

State Secretary Cornelia Quennet-Thielen.

Members of the Bundestag at HZI: Hubertus Heil and Carola Reimann.

EU Commissioner Vytenis Andriukaitis (left) at HIPS. In the middle: HIPS Director Rolf Müller.

“I SEE GREAT POTENTIAL FOR NEW APPLICATIONS”

Interview with Jörg Vogel,
Director of the Helmholtz Institute for RNA-based Infection Research (HIRI)

Photo © HIRI/Mario Schmitt

Jörg Vogel is an internationally renowned expert in the exploration of ribonucleic acids (RNAs) as a class of molecules of central importance in the regulation of cellular processes – a field of research that has garnered increasing attention over the past two decades. The 2017 Leibniz laureate has been appointed as director of HIRI. The new institute aims to unveil the numerous roles of RNA molecules during infection processes – and to utilise them for novel therapeutic approaches.

Prof. Vogel, RNA is a key molecule of life. Yet, it has traditionally been considered as a mere storage intermediate for genetic information. This has radically changed. Why?

The breakthrough towards an entirely new perception of RNAs came in the late nineties and in the early years of the new millennium. Since then, many new classes and functions of RNA molecules in addition to the traditional classes of ribosomal, transfer and messenger RNAs have been discovered. New technologies such as high-throughput RNA sequencing have allowed us to explore and analyse the cellular RNA universe. We have learned about phenomena like RNA interference – the shut-down or silencing of genes by small RNA snippets. Studies revealed that RNA molecules with regulatory functions are not rare exceptions but are in fact very abundant in the cell. Meanwhile, entire regulatory pathways based on regulatory RNAs and their interactions with other RNAs and RNA-binding proteins are known – a whole new world of biochemical regulation mechanisms, essential for a tremendous variety of cellular processes.

You and fellow scientists have found out that such regulatory RNA molecules have, in particular, important functions in infection processes. How did you recognise that?

In my years as a postdoctoral researcher in Sweden and in Israel, I initially investigated regulatory RNAs in the non-pathogenic model bacterium *E. coli*. Later, when starting my own laboratory at the Max Planck Institute for Infection Biology in Berlin, I turned to pathogens such as *Salmonella* and Helicobacter. There, we found that non-coding RNA molecules had previously unnoticed functions in the regulation of virulence genes.

Can you describe these functions?

Pathogenic bacteria colonize various niches within the human host. In these niches, they are confronted with the need to compete with other microbes and to evade the immune system. To survive in these different micro-environments, they have evolved complex regulatory networks. It is now well-established that regulatory RNA molecules are important players in these networks. They constitute pathways that enable bacteria to precisely time the expression of their colonization factors, to sense and respond to the host immune defense, and to adapt their metabolism inside the host. Sensory RNAs enable the bacteria to respond to changes in environmental temperature or nutrient supply. All of these factors are important for pathogens.

Why did it take so long to discover these important functions?

For many years, the significance of RNA molecules in infection processes remained invisible. New technology, in particular in the development of high-throughput sequencing, was a game changer in RNA biology. During my scientific career, we often pursued questions that could only be answered by developing or applying new technologies.

Which new and improved technologies did you use?

One example is dual RNA sequencing – which 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. We built the theoretical framework for this method already back in 2012 and experimented quite a lot to gradually improve the lab protocols and advanced these procedures step by step until our first publication in 2016.

But employing dual RNA-seq, you still analyse billions of cells in one sample.

Is that a problem?

It can be a problem, because not all cells of an infected population show the same response. And in some cases, heterogeneous behavior of host or pathogen cells is particularly relevant for the infection process. For example: individual subsets of pathogens can survive and persist in certain host cells. Which genes and which regulatory pathways are active in these cells? To answer these questions, we apply so called single-cell RNA-seq technology. This allows us to study the transcriptome – the entirety of RNA molecules – in every individual cell. We were the first research group in Europe to take this approach to study bacterial infections. And we found, amongst other things, how persistent *Salmonella* bacteria manage to reprogramme macrophages, cells of essential importance in immune defense, in order to survive in the host organism.

“New technologies, in particular in the development of high-throughput sequencing, was a game changer in RNA biology.”

What are the particular challenges of your research field?

Many cellular and biochemical regulation mechanisms, for example gene regulation by transcription factors, tend to display robust phenotypes. Put simply, a gene is either switched on or off. In contrast, regulation by RNA molecules often resembles fine tuning – the effects are not as clearly visible, but nevertheless have significant consequences. Thus, you have to take a very close look to discern these molecular phenotypes, and you have to monitor and analyse expres-

"Research at the interface between disciplines can break new grounds, especially when combined with high-class expertise and cutting-edge technologies."

sion patterns of all transcripts. This requires, as mentioned before, high-resolution techniques, ideally on the single cell level. Moreover, to successfully pursue RNA-based infection research, you need a comprehensive understanding of infection biology in order to interpret your findings on the RNA level correctly.

You are now heading an institute devoted to the subject "RNAs and infection". Why is this a research field on its own, requiring an own institute?

Research at the interface between disciplines can break new grounds, especially when combined with high-class expertise and cutting-edge technologies. Traditionally, infection research has focused on proteins. RNA research, on the other hand, often concentrates more on non-communicable diseases, such as cancer and neurodegeneration. I would argue that its potential for understanding and curing infections has not yet been fully exploited. Working at the interface of RNA and infection requires a special kind of expertise and the advancement and adaptation of current technologies.

What are the specific advantages of the chosen model: an institute jointly operated by a Helmholtz centre and a university?

At JMU in Würzburg, we have built up critical mass in infection research in the recent years. In several fields, we are in a leading position worldwide. Particularly the RNA community in Würzburg is very strong. You could hardly think of a more suitable university partner for an institute like the HIRI in Germany. HZI and the Helmholtz Association, on the other hand, provide excellent infrastructure, like technology platforms and sophisticated mouse models, as well as access to a large research community in important fields like pharmaceutical research and microbiota research. Close interactions with other Helmholtz centres promise synergies and allow access to advanced infrastructures for RNA biology. The combination of these assets puts HIRI in a unique position in its field.

What are, in your opinion, the most important tasks for HIRI in the years to come?

The fact that RNAs play a key role in so many cellular processes means, in turn, that they can also be used to influence these processes in a targeted manner. The development of RNA-based therapeutics in particular: RNA-based antibiotics, is a central long-term goal. With the combined expertise of HIRI, HIPS in Saarbrücken and HZI departments in Braunschweig, we hope to make significant progress towards this objective. Another key subject is RNA delivery – to find suitable techniques to bring therapeutic RNA molecules to infected cells or tissues. In this respect, we can create synergies with HIPS as well. The manipulation of the microbiota by applying suitable RNA agents is another promising field. Around 1000 bacterial species live in the human gastrointestinal tract. It is a fascinating option to selectively remove or eliminate defined species and thus influence the balance of the microbiota for therapeutic purposes.

This can be achieved with RNAs?

RNAs certainly have the potential to provide precision tools for targeted interventions of many kinds. The most promising feature of this class of molecules is their specificity: Via the transcript – the RNA – you can specifically target and eliminate any protein, without the need to interfere with the genome. I see great potential for new applications for the benefit of patients that will harness RNAs – as biomarkers, as regulators, as drug targets and as drugs. And we are determined to make significant contributions to these developments here at HIRI.

Interview: Manfred Braun

“THE CENTRAL QUESTION IS: HOW CAN DISEASES BE CONTROLLED OR – EVEN BETTER – PREVENTED?”

Interview with Gérard Krause, Head of the Department Epidemiology at HZI

HZI epidemiologist Gérard Krause (middle) applied the SORMAS technology successfully to monitor disease outbreaks in Africa.

In the course of his career, epidemiologist Gérard Krause has tracked disease outbreaks in many parts of the world – from the United States and Germany to West Africa. Since 2011, he heads the department of Epidemiology at HZI and teaches as a full professor at Hannover Medical School. In this time he has built a strong epidemiological focus at HZI, adding the in-depth study of the spread of infections to the centre’s research portfolio.

Prof. Krause, how would you explain an epidemiologist’s work to an outsider?

In general, the role of epidemiology is to investigate diseases and their associations with different factors at the population level – in groups of people, not only in individuals. The central question is: how can diseases be controlled or – even better – prevented? When studying infectious diseases in particular, their transmissibility is of key importance and constitutes the main challenge in the management of these diseases.

What is the role of epidemiology at a centre like the HZI?

Within the HZI, the specific role of epidemiology is to help enhance the translation of research results into applications. Therefore, we provide epidemiological research questions and also epidemiological infrastructures in order to validate at the population level what has been investigated at the individual or experimental level. And – vice versa – we generate research questions that emerge from studies at the population level and feed them back into basic research.

What are your most important methods and approaches to achieve this?

We focus on several pillars. One is the development, establishment and maintenance of cohorts: be it patient cohorts or cohorts of the general population. Another is developing novel tools with which to access, collect and connect data for the improvement of disease control. A third pillar is the development of diagnostic tools customized to satisfy needs at the population level. Furthermore, we employ systematic reviews and modelling to collect and extract evidence for addressing important public health questions.

What are the most important projects your department is pursuing here at the HZI?

With respect to the experimental laboratory work, we are pursuing three key projects. One is the development of a differential serology platform to distinguish between individuals who have been vaccinated against a disease and those who have been infected with this disease. This has tremendous public health implications when it comes to measuring the effectiveness of vaccination strategies. The second is the development of a tool which allows us to detect pathogens from the lower respiratory tract and identify those pathogens which cause dangerous infections. Another project we are working on right now is the development of different approaches for the self-sampling of blood. The aim is to enable study participants to take samples from their own blood by themselves. So, we would eventually be able to conduct large-scale population-based studies with blood collections without needing doctors or nurses for every single blood withdrawal.

"We develop tools which allow us to detect pathogens from the lower respiratory tract and identify those pathogens which cause dangerous infection."

You are also exploring new approaches called "digital epidemiology". Can you explain these?

Electronic or mobile health, also referred to as eHealth or mHealth, means employing information and computational technology as well as mobile devices for large-scale disease control. A good example is our mHealth tool SORMAS, which we have developed to detect and control disease outbreaks in Africa.

What is SORMAS doing and how did you invent it?

It originated from a request from a former colleague who was working in Nigeria. She was involved in the response to the tragic Ebola outbreak in Nigeria in 2014. There, the need became evident to obtain modern tools to enable a rapid and effective response to such challenges. We soon concluded that we need to use state-of-the-art IT technology to achieve that. So we designed and conceptualized the Surveillance Outbreak Response Management and Analysis System, in short: SORMAS. It is based on an app for mobile devices which health authorities and medical staff can use to detect and report disease outbreaks early on, and to manage all activities needed to contain the outbreak. The data are transmitted via smartphone and processed centrally by the health authorities. We are very proud that the tool has now actually been implemented in many states in Nigeria.

So SORMAS can help African states to control the next Ebola outbreak more effectively?

More than that. On the one hand, the specific characteristic of SORMAS is that we integrate the regular surveillance and detection, the immediate detection of an outbreak and the response management to that outbreak in one tool. So the tool is not only implemented once the outbreak happens, but it can detect the outbreak to begin with. In that, SORMAS is quite unique. Another important point is: right from the beginning, we were aware that it doesn't make sense to develop such a tool just for Ebola alone. So we have designed SORMAS in such a manner that it can in principle cover all kinds of infections, particularly the epidemic-prone high-priority diseases.

Smartphone App for disease control: the SORMAS system, co-developed by HZI epidemiologists.

Have you already been able to prove that this works?
Yes, definitely. SORMAS has been implemented in response to the monkey pox outbreak, where we were asked by the CEO of the Centre for Disease Control in Nigeria to do that; which was quite a challenge because monkey pox is a very rare disease that was not on the list of epidemic-prone diseases, so we had to quickly programme the process algorithms for that disease. But we did so, and it was then quickly worked out. Then there was a severe meningitis outbreak in Nigeria. We were asked to go to yet another set of states to implement SORMAS there. Then came the big Lassa fever outbreak, and we were again asked to add another number of states to SORMAS implementation. Those were the triggers for the roll-out. And whenever we went there to train people to use the tool, we also trained them to use it for all the other diseases. Meanwhile, we can now cover ten diseases with SORMAS.

And it can yet be expanded to cover further infectious diseases?

Yes, and we will add additional diseases as the need arises. We will also add additional states within Nigeria. The ambition of the CEO of the Nigerian Centre for Disease Control is to roll out SORMAS in the whole country – for all 37 states in the whole country. And of course we are also working on making SORMAS available to other countries in Africa.

So eHealth and mHealth are indeed promising approaches to counter dangerous infections?

We think so, and we are pursuing further eHealth projects besides SORMAS. One is a mobile app called PIA which allows the participants of our great nationwide cohort study, the German National Cohort, to assess and identify acute transient infections. So, we can validate these with laboratory testing and subsequently detect associations with other diseases. Another project in this field is the app that we are developing together with the Paul-Ehrlich-Institute to assess undesired effects after vaccination. We are studying this in a prospective cohort manner which will allow us to achieve a much higher sensitivity for measuring frequent undesired side effects and a better assessment of vaccine safety. A fourth project is being pursued within the HighMed consortium for medical informatics. There, we are developing tools to rapidly identify infectious disease outbreaks within hospitals by medical informatics technology – by using data routinely collected in the hospital process.

When conducting epidemiological research, which advantages can you utilise which are specific to this centre, to the HZI?

One of the big advantages that we have is the very high expertise available in all kinds of laboratory work, in the biochemistry and molecular biology part, in basic research. That helps us a lot in developing our diagnostic tools for epidemiological purposes. It also helps us to conduct joint studies in the framework of our cohorts. Another big advantage is the organisational setup to pursue ideas for a longer time period. I can, within certain limits, explore new approaches for a certain time without being dependent right away on short-term grant funding. Of course it's at my own risk, because it's my budget – so if I fail I have invested resources for nothing. But if I succeed, we can develop something really fundamentally new that was not available before. This is exactly what happened with our differential serology project which I have mentioned previously and which we have submitted for patenting. It is also true for SORMAS. These activities would not have been possible in a setting without sufficient basic funding. The flexibility and the possibility to set the priorities and to invest in high-risk projects: this is really a huge advantage in the setting of a Helmholtz centre.

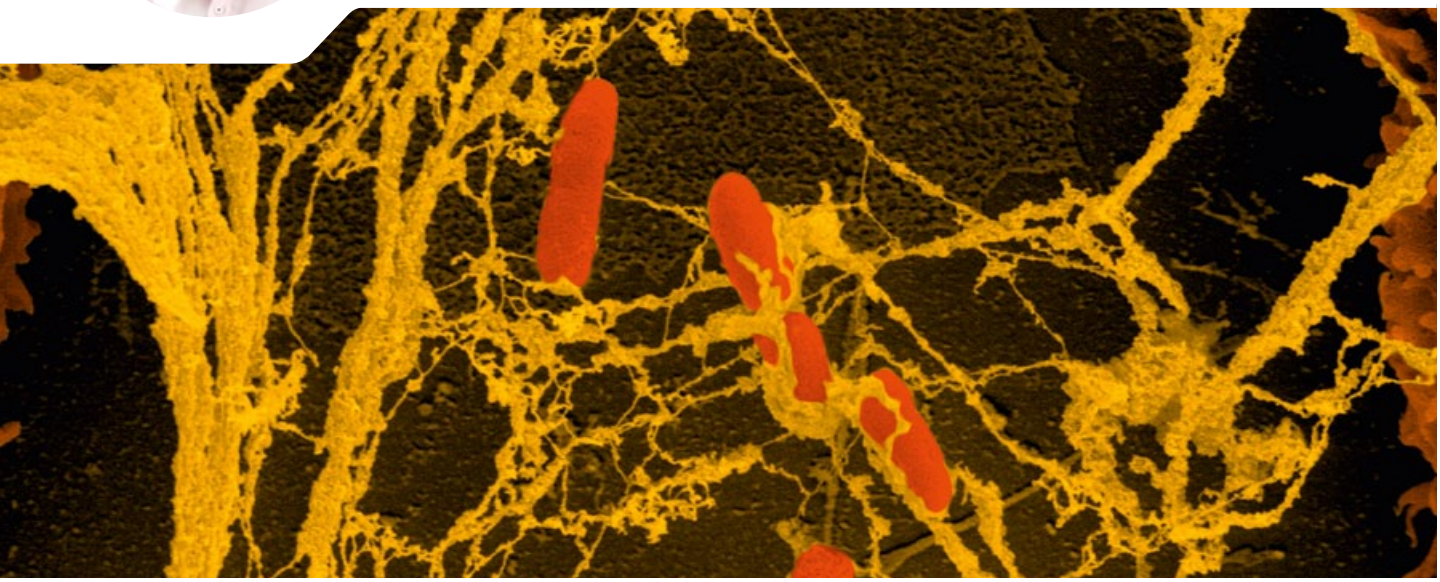
What are your visions for epidemiological research at the HZI in the next five to ten years?

I hope that in the next five to ten years we will have SORMAS implemented in several African states and nations as a routine system. We will have developed a multiplex differential serology platform for a variety of vaccine-preventable diseases. We will hopefully have developed a tool to actually detect lower respiratory tract pathogens from exhalates. And we will have identified at least one or two novel etiological associations between infectious diseases and non-communicable diseases in the framework of our cohorts.

Interview: Manfred Braun

FROM BENCH TO BEDSIDE – TECHNOLOGY TRANSFER AT HZI

Michael Strätz, Head of the department "Third Party Funding"



Pseudomonas aeroginosa

"SEED MONEY" FOR INNOVATION AND TRANSLATION: THE PRE-4D FUND

The transfer of research results into pharmaceutical and clinical application is an integral and vital part of HZI's scientific strategy for innovation-driven research. The centre is dedicated to pursuing this key goal in numerous projects, many of which are embedded in cooperations and brought to fruition with third-party funding. In the recent years, HZI has launched a seed fund in order to advance internal projects and bring them closer to application. Promising initial developments are already visible.

The scientific culture at HZI is closely interwoven with technology transfer management, as reflected by the recruitment of scientific and administrative staff from industry. Technology transfer initiatives at HZI range from regular information and training to raise employee awareness or provide individual advice for inventors and founders to professional management of intellectual property (IP) rights. HZI has set up a structured process for technology transfer that involves IP management, licensing and cooperation with industry partners and fostering the close collaboration of internal scientific and administrative experts with a professional external technology transfer partner, the Ascenion GmbH.

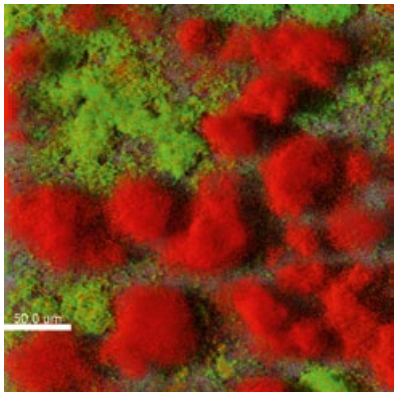
Yet, as innovations in infection research are usually adopted by industry at relatively late and advanced developmental phases, bridging the gap between fundamental research and more progressed stages of development remains a formidable challenge. Early innovations need to be further developed before being transferable to industry. Therefore, HZI has set up the innovations fund "Pre-4D" (with "4D" representing "Drugs, Diagnostics, Discovery and Development") for internal funding of the most promising projects. The Pre-4D fund aims to enhance the development level of drug and diagnostic candidates originating from HZI, thus boosting the attractivity of cost-intensive development

projects for HZI's industrial partners. Pre-4D is funded by the Helmholtz Association (390,000 Euros p.a.) and by the revenues of former license deals (110,000 Euros).

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. So far, six research projects have been selected for funding. In all of these projects, the so-called "Technology Readiness Level" (TRL), meaning the closeness to application in medical or pharmaceutical practice, has been significantly increased. Some of these projects have been developed sufficiently as to be eligible for follow-up funding. Three priority constituting patent applications have been filed, in one case (see below) a first potential commercialization partner could be attracted.

Pre-4D Example Diagnostics: "Rapid antimicrobial susceptibility testing and phylogenetic identification", in short: RAPID, developed by HZI scientist Susanne Häußler, is an assay for identifying antibiotic resistant pathogens early on to provide susceptibility profiles for medical treatment and to enable rapid surveillance for detecting outbreaks in the clinic. The technology, based on the assessment of 50 resistance markers, has shown a sensitivity of more than 98% in predicting resistance of pathogenic *Klebsiella* bacteria. Proof of concept was achieved using clinical samples. For the technological validation in the lab, further studies with *E.coli* are planned. First agreements with an industrial partner have already been settled. *(IP: EP17151531.5 & PCT/EP2018/050890 Rapid antimicrobial susceptibility testing and phylogenetic identification).*

Pre-4D Example Drugs: The bacterium *Pseudomonas aeruginosa* can cause severe lung disease and is particularly dangerous for susceptible patients when it forms biofilms in the lung. Biofilm formation is dependent on the molecule PqsR (regulator of *Pseudomonas* Quinolone Signaling), which HZI scientists aim to inhibit with small molecules, so-called "pathoblockers" (*see also the chapter about the HZI Research Focus AMR*). For the development of PqsR antagonists as a new approach for treating chronic lung infections by *P. aeruginosa*, HZI scientists received seed funding via Pre-4D. This enabled meeting all conditions for significant additional support by the Helmholtz Validation Fund and DZIF flexible funds. Potential candidates for pathoblocker-based drugs are now being tested in animals in order to select lead candidates for further optimization.



Dangerous lung infections: biofilm formed by *Pseudomonas aeruginosa.*

Another example is the follow-up funding for the investigation of cystobactamids, a new class of broad spectrum antibiotics with superior activity against Gram-negative pathogens (*see chapter Research Focus AMR*): HZI scientists have received substantial Pre-4D funding to conduct experimental work in cooperation with scientists at Leibniz University Hanover and to generate data to further qualify the project for a subsequent support by European and international funding agencies and attract potential industrial partners.

Altogether, in the short time since its launch the Pre-4D fund has shown very promising results in helping bridge gaps between early research and more advanced stages of drug and diagnostic development. HZI intends to establish this seed funding tool in a sustainable manner to further harness it for the promotion of innovative and translational projects.

COMPUTER SIMULATIONS OF THE ANTIBODY RESPONSE TO INFECTIONS

Michael Meyer-Hermann, Head of the department "Systems Immunology"

MODELLING OF PROCESSES IN INFECTION AND IMMUNITY

In the course of immune responses against viral or bacterial infections, the presence of specific antibodies can decide the fate of the infected individual. This was impressively demonstrated during the last major Ebola outbreak, where the early presence of specific antibodies was a predictive indicator for the survival chances of the patient. Therefore, mounting of germinal centre (GC) reactions, in which high affinity antibodies evolve in a process called affinity maturation, and the speed of affinity maturation of antibodies are critical for successful immune response and the development of immune memory. In GCs, B cells mutate their encoded antibody and enter a competitive evolutionary process of repetitive cycles of mutation and selection. Only the B cells carrying high affinity antibodies survive the GC reaction and are delivered to the organism to produce high amounts of this particular antibody. GC output cells are also the basis for the formation of B cell memory cells, which are reactivated upon receiving a comparable pathogenic challenge. In the elderly population, the onset of the GC reaction is impaired, as well as its shut-down, which induces a chronic inflammatory state. This explains the poor responsiveness of elderly people to vaccinations as known for influenza and contributes to the chronic inflammatory state frequently found in the elderly population.

At the HZI department of Systems Immunology, we contribute to GC research by the development and analysis of computer simulations (Fig. 1) of this particularly central part of an immune response (published in Binder & Meyer-Hermann, *Front Immunol* 7, 2016: 593; Robert et al., *Methods Mol Biol* 1623, 2017: 318). In 2016/17, a particular focus was placed on mechanisms of B cell selection that contribute to the optimisation of the development and production of high affinity antibodies. The knowledge of these mechanisms not only fosters our understanding of how high affinity antibodies are generated on a time scale of 10 days, but also identifies potential targets for immune interventions in patients at risk to suffer from suboptimal GC reactions.

In a cooperation with Elena Vigorito (Cambridge UK) and Rinako Nakagawa (now Crick institute London), we investigated the impact of the microRNA-155 on selection and affinity maturation of GC B cells (published in Nakagawa et al., *J Clin Invest* 126, 2016: 377). It was difficult to isolate the functional impact of microRNA-155 onto B cells in

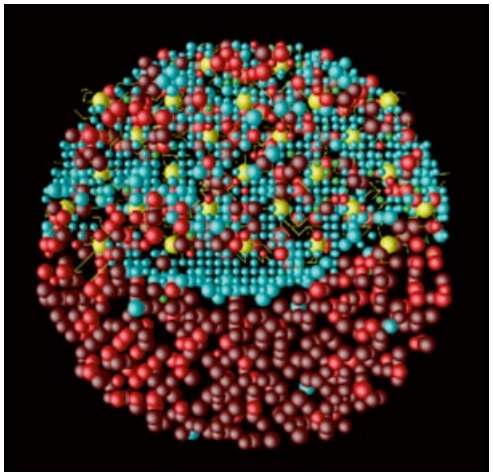


Figure 1: Computer simulation of a GC reaction as published in Papa et al., Nature 547, 2017: 318. A slice of the three-dimensional simulation of the space-time dynamics of the interacting cells is shown at day 8 of the GC reaction. Dividing and mutating B cells (red) form the so-called dark zone. Upon up-regulation of the B cell receptor (cyan), they move to the light zone, where the driving antigen is presented on follicular dendritic cells (yellow). B cells take up antigen from there, internalize and present it to T follicular helper (Tfh) cells (green). Only cells that managed to collect antigen and receive positive signals from Tfh survive and return to the dark zone in order to restart the next round of mutation and division.

experimental settings which mostly showed a phenotype of the microRNA-155 knock-out on the level of the whole GC reaction. We compared the expected GC phenotype for different hypotheses of how microRNA-155 would change the B cell properties, including reduced speed of B cell division, reduced number of B cell divisions, defect of B cell receptor, defect of processing of collected and internalised immune complexes, or increased apoptosis of B cells after interaction with T follicular helper (Tfh) cells. We were able to show that the simulation of most of these hypotheses was in contradiction to the measured GC phenotype. These results suggested that only the deregulation of apoptosis or in B cell receptor signalling are compatible with the data. Thus, the simulations helped to pin down the possible impact points of microRNA-155 onto the GC reaction. This work contributes to a quickly evolving research field about the role of microRNAs in the regulation of cellular function.

In cooperation with Carola Vinuesa (Canberra, Australia) and Michael Dustin (Oxford UK) and in the framework of an Human Frontier Science Program grant that we coordinate, we identified a new mechanism of how B cell selection is controlled, which is specific to humans and has no counterpart in mice (published in Papa et al., *Nature* 547, 2017: 318). Dopamine, well-known from synapses in the nervous system, was shown to be released by Tfh cells to GC B cells. Our collaborators could show that this induced a faster up-regulation of ICOSL (the ligand for the T cell-specific cell surface receptor ICOS which supports B cell signalling and division) in B cells and was involved with a more intense formation of immunological synapses between Tfh and GC B cells. As this mechanism was specific to humans, it was not possible to identify its consequences in experiments. We identified in computer simulations, that the dopamine-dependent faster up-regulation of ICOSL did not change the overall level of affinity maturation. However, high-affinity antibodies appeared a day earlier in the simulations and in higher amounts. These results identified dopamine and ICOSL as potential targets of immune interventions.

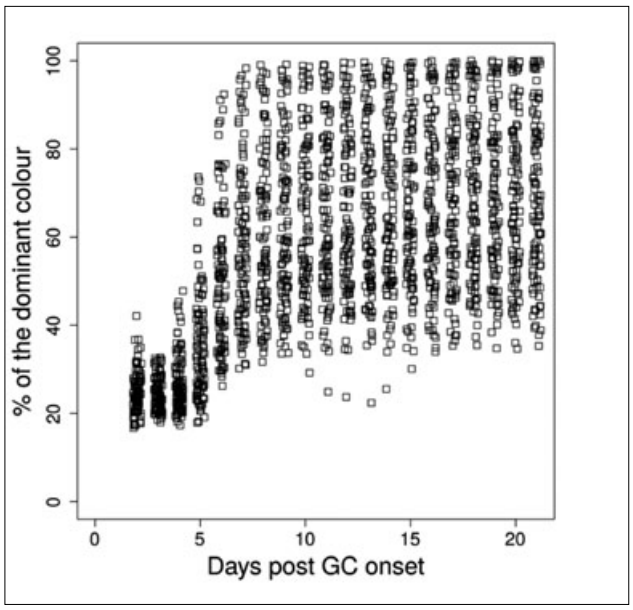


Figure 2: Computer simulation of the evolution of colour dominance, i.e. the fraction of cells carrying the most frequent colour, in GC reactions as published in Tas & Mesin et al., Science 351, 2016: 1048. GC founder B cells were stained in silico with one of 10 random colours, which they kept and transmitted to their progeny. B cell selection has the potential to reduce the clonality of GC B cells to a few cells, which would correspond to a high colour dominance. At each time point of the GC reaction, the colour dominance of 100 simulated GCs is shown. It can be appreciated that the overall colour dominance rises from the starting point by selection of high affinity B cells. However, in agreement with experimental results, the ultimate colour dominance is characterised by an unexpected high diversity of dominances. Thus, the diversity of antibodies is considered an inherent property of GC reaction in addition to affinity maturation.

More generally, the existence of two independent functions of dopamine in the nervous and in the immune system adds to the set of pleiotropic molecules. Also, it shows that affinity maturation is not the only objective of GC reactions but also speed and amount of generated antibodies, which are critical for the success of immune responses, are regulated in GCs.

In cooperation with Gabriel Victora (Rockefeller NY, USA), we further extended the role of GC reactions to the generation of antibody diversity (published in Tas & Mesin et al., *Science* 351, 2016: 1048). By staining of B cells in GCs with various colours, which are kept upon cell division and transmitted to all progeny, an unexpected diversity of colour dominances was found. According to textbook knowledge, the GC reactions starts from a few B cell clones, which are diversified by mutations. Selection reduces the diversity again and only a few clones are kept in the GC after 8-10 days of the reaction. The remaining B cells encode the target high affinity antibody. From this perspective, mono-coloured GCs would have been expected at day 11 of the GC reaction. What was found is a huge diversity of colour dominances ranging from highly multi-coloured to mono-coloured GCs. We recapitulated this diversity of dominances in computer simulations (Fig. 2), incorporated the staining process into the simulations and showed, that the colour dominance found in experiments was, indeed, predictive of the clonal dominance. This revised the general understanding of what GC reactions are for and extended the conviction, that GCs are mainly the source of generating high affinity antibodies by mutation and selection to the notion of GCs generating a high diversity of antibodies. In view of the GCs being the source not only of antibodies for the acute infection but also of immune memory, this suggests the interpretation that the immune memory already prepares for mutated pathogens, which might show up in the next years after the first infection.

The mathematical framework for modelling of GC reactions, as developed by the Systems Immunology department (Fig.1), has evolved to the current state-of-the-art simulation platform in recent years and is being used by many groups world-wide for the planning or interpretation of experimental investigations.

Michael Meyer-Hermann, further affiliations:
Braunschweig Integrated Centre of Systems Biology (BRICS)
Centre for Individualised Infection Medicine (CiiM), Hannover, Germany
Institute for Biochemistry, Biotechnology and Bioinformatics,
Technische Universität Braunschweig, Braunschweig, Germany

TRAINING THE NEXT GENERATION OF INFECTION RESEARCH EXPERTS

Sabine Kirchhoff, Manager of HZI's Interdisciplinary Structured PhD Programme

Figure 1: Visiting "Big Pharma": doctoral researchers of GS-FIRE at UCB (Union Chimique Belge) in Brussels.

TRAINING FOR THE NEXT CAREER STEP

The HZI International Graduate School for Infection Research (GS-FIRE) has developed a basic training programme that complements the practical part of PhD projects. It covers all areas of infection biology and is taught in English. The GS-FIRE recruits internationally, ensures high quality supervision and supports doctoral researchers. They all can profit from research network interactions and infrastructures offered in the Hannover-Braunschweig area.

Attracting talented candidate doctoral researchers interested in HZI's research activities is an essential task for the centre. To help achieve this aim, an international announcement is launched yearly. An elaborate system has been developed to ensure a stringent selection process. Preselection and progress of applications is monitored by an online application tool. Video and on-site interviews complete the selection process. Currently, 170 doctoral researchers actively participate in the GS-FIRE. More than 40 % come from abroad and originate from 38 different countries.

To ensure optimal training GS-FIRE has established high quality standards. This includes accreditation of supervisors, evaluation of PhD projects on offer and assessment of all lectures and courses. Supervision is supported and ensured through thesis committees of scientists who meet regularly

to track the progress of PhD projects and discuss career options of individual candidates.

The training programme aims at optimal preparation of young researchers for their future careers. It complements the individual research activities by training in a broad spectrum of research areas related to infection defence and by offering introductions to relevant classical and novel techniques. To bridge basic research with industrial and clinical application aspects the training programme offers and organizes participation in training measures that catalyse translational infection research.

Curriculum

The Graduate School offers project-oriented postgraduate training for doctoral researchers where they learn how to carry out independent scientific work while doing research in their own scientific project.

Currently fourteen topic lecture series have been implemented, each comprising eight lectures. These lectures are presented by selected researchers from HZI and partner institutes. All lectures are critically assessed by the participating doctoral researchers.

All doctoral researchers present and discuss their work annually during a retreat at which all GS-FIRE participants and supervisors participate. Another occasion involving only young researchers from the GS-FIRE and external PhD programmes is the "Christmas PhD Symposium". This allows them to present their work as posters or via talks. Awards are given for the best presentations in both retreat and symposium.

Biennial Summer Schools focusing on state-of-the-art research in infection and immunity have been organised by the GS-FIRE together with the DZIF academy. Internationally recognised scientists are invited as speakers and mentors for these one-week events, allowing intensive scientific discussions of research projects and networking. The number of participants in these Summer Schools is restricted to 40 doctoral researchers. Six such events have been held to date. (Fig.2).

Mini-symposia, entitled "A Day on..." organised by the GS-FIRE complement the curriculum. For instance, during the "Day on different aspects of translation" nine speakers from basic research, pharma industry and clinic presented their approaches for translating research towards application. In addition, several practical courses were organised in 2016/2017. To see other Helmholtz centres and to meet peers thereat, weekend retreats for newly accepted doctoral researchers have taken place.

Figure 2: Announcement of the biennial GS-FIRE Summer School.

Career and transferable skills supporting events

Career supporting events are an integral part of the curriculum. Monthly career workshops together with invited speakers from different disciplines have been held. Alumni Symposia have offered the possibility to discuss scientific questions, career aspects and personal issues with former HZI members who are now established in their careers. Excursions to large pharma companies are also included in the programme (Fig.1, visit of UCB, Brussels). Transferable skills courses on career planning are offered regularly. Each doctoral researcher has to join a course explaining good scientific practise. All these events alongside the regular curriculum support the young scientists in their career planning and complete the excellent preparation of the next generation of infection researchers.

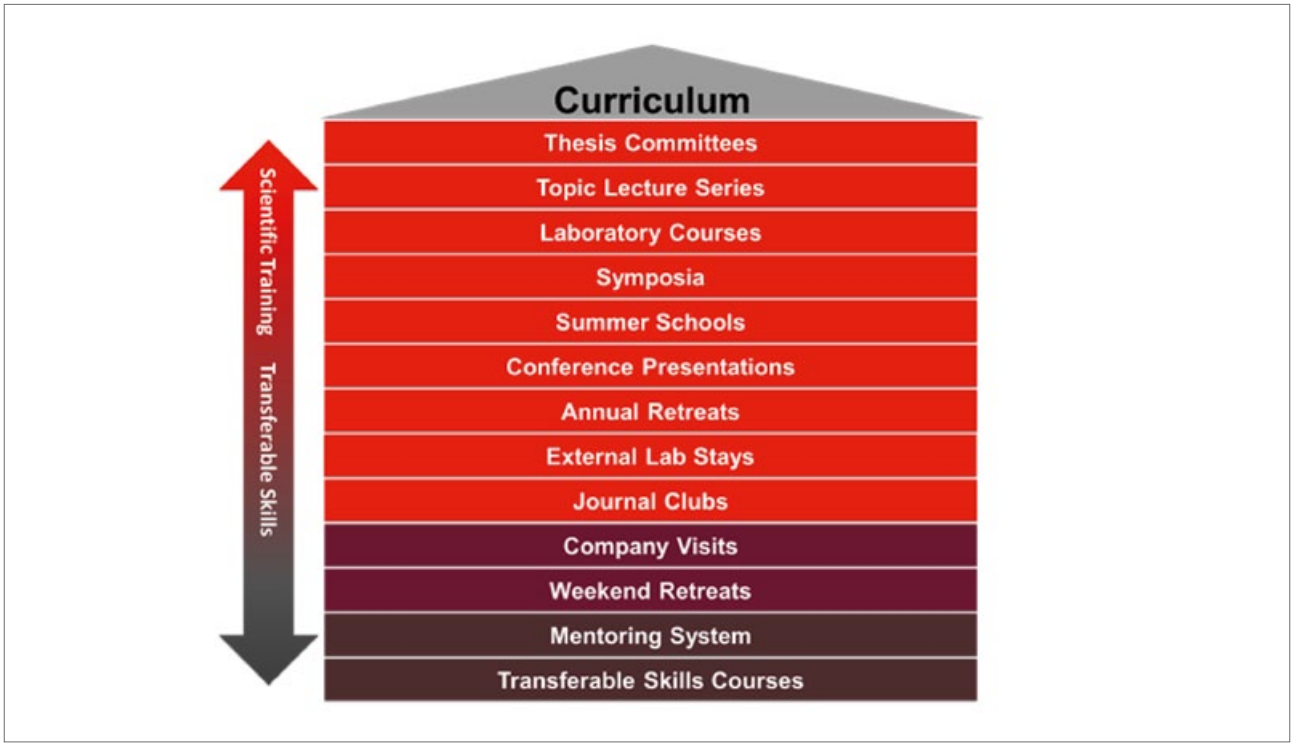
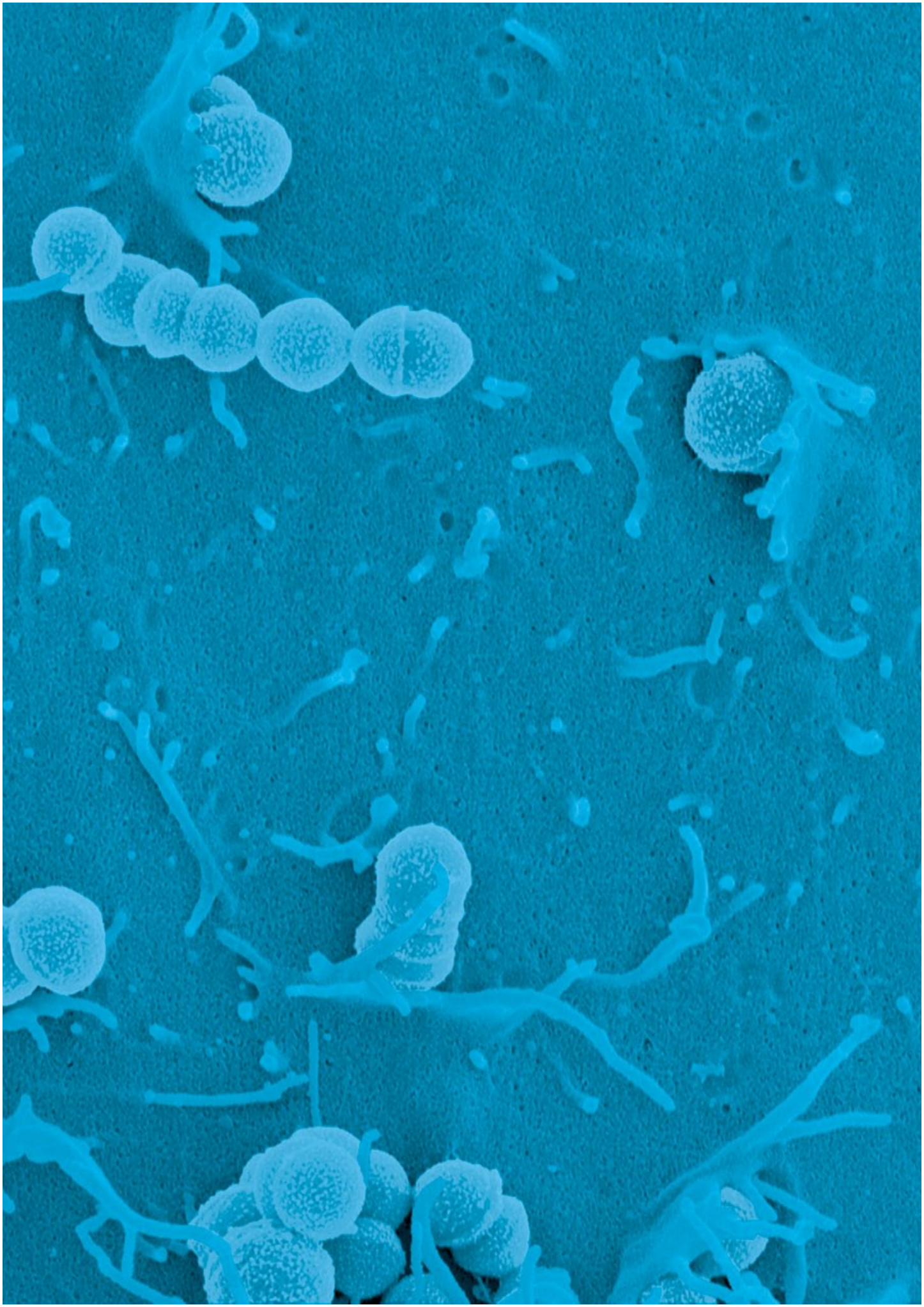


Figure 3: Curriculum of the HZI Graduate School.



HZI'S RESEARCH FOCI

RESEARCH FOCUS
ANTIMICROBIAL RESISTANCE (AMR)

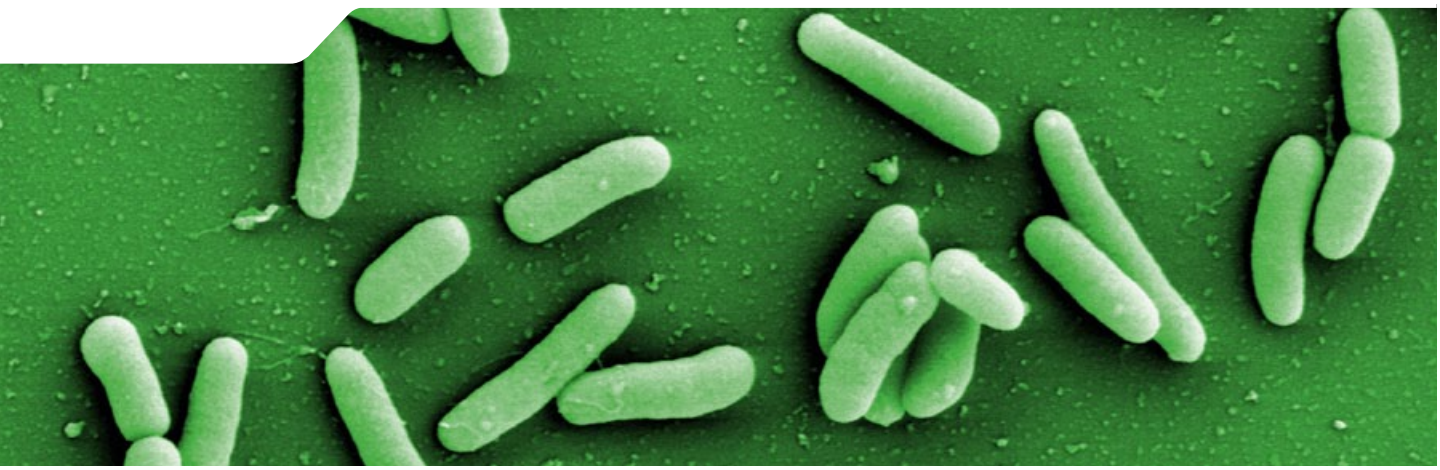


Figure 1: *Pseudomonas aeruginosa*

Hospital-acquired infections caused by antibiotic-resistant pathogens are spreading worldwide and pose one of the greatest challenges to modern medicine. Multidrug-resistant pathogens constitute a threat for individuals and dramatically impact on human health, public health and the principles and practice of clinical medicine.

Management of these infections requires a multipronged strategy that includes not only the rational and responsible use of antimicrobial agents, but also early diagnosis for targeted antibiotic treatment and the implementation of effective infection control measures. Furthermore, increasing resistance calls for research on, and development of, new anti-infectives. This is a high priority challenge particularly in the light of the scarcity of new candidates in the discovery and development pipeline of novel antibiotics exhibiting new modes of action.

In order to tackle these problems, HZI combines expertise in the fields of bacteriology, cell biology, immunology, structural biology, natural products, organic synthesis, medicinal chemistry, pharmacy, bioinformatics and vertebrate model systems.

This allows fundamental research on host-pathogen interactions, innovative anti-pathogenic strategies and novel anti-infective compounds. It also enables the preclinical development of antibiotics and pathoblockers in animal models, the processing and implementation of diagnostic tools for resistance profiling, and epidemiological studies in the clinical setting. Collaborative approaches are complemented by an array of different platforms providing state-of-the-art techniques as well as bioinformatics tools to increase the power of discovery and the ability to interpret large datasets.

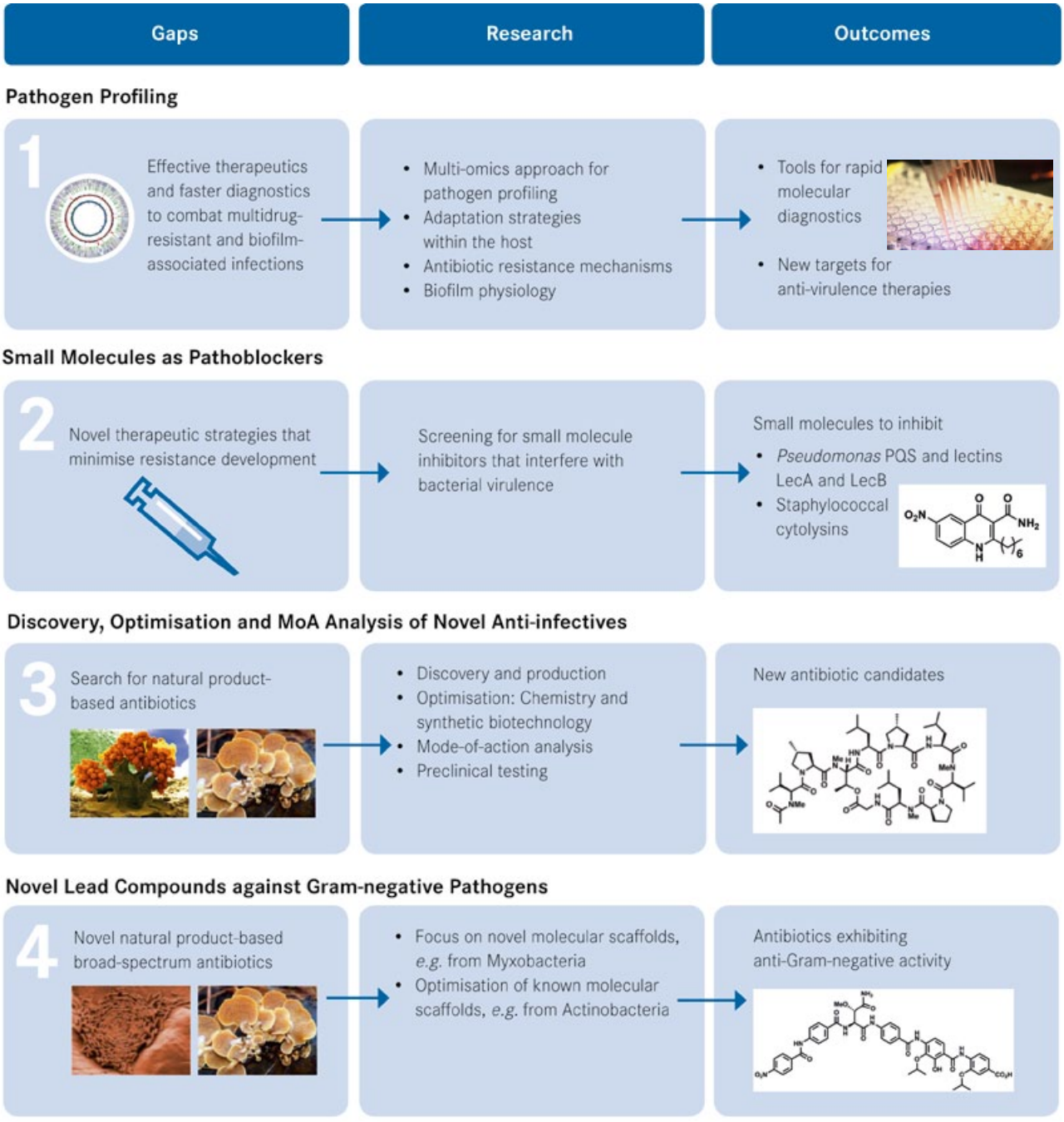


Figure 2: Overview of the research activities of the Research Focus Antimicrobial Resistance.

1. PATHOGEN PROFILING

Staphylococcus aureus and *Pseudomonas aeruginosa* serve as model organisms for clinically relevant Gram-positive and Gram-negative bacteria, respectively. *S. aureus* and *P. aeruginosa* cause severe invasive infections in the hospital as well as in the community setting. They are notorious for their capacity to establish chronic biofilm-associated infections and develop antibiotic resistance. These characteristics make them two of the most dangerous and intractable infectious pathogens worldwide and have prompted the World Health Organisation (WHO) to classify them as high priority for research and development of new anti-infectives. Emerging resistance towards antimicrobials and antimicrobial tolerance of biofilm-grown bacteria does not only call for novel treatment strategies but also underscores the need for optimisation of current diagnostics.

The central goal of pathogen profiling within the Research Focus AMR is to achieve comprehensive knowledge of the pathogenicity mechanisms of these bacteria during infection and colonisation of human hosts. HZI scientists put special emphasis on characterizing bacterial phenotypes under changing and challenging environmental conditions.

To this end, AMR scientists use extensive "omics" technologies to profile the phenotypes of the bacteria. Clinically relevant murine models have been established and patient samples are analysed to uncover bacterial survival strategies. Phylogenetic and resistance markers that are explored in phenotype-genotype association studies are exploited for the establishment of molecular diagnostic tools.

Selected Scientific Achievements

Key to the survival of bacteria during an infection is their capacity to rapidly redirect and fine-tune gene expression to adjust to the host environment and to evade the host defense (1). Scientists at HZI have investigated how bacteria coordinate their gene expression during infection in murine models and in human samples (Fig. 3). They found that pathogens express a completely different set of genes depending

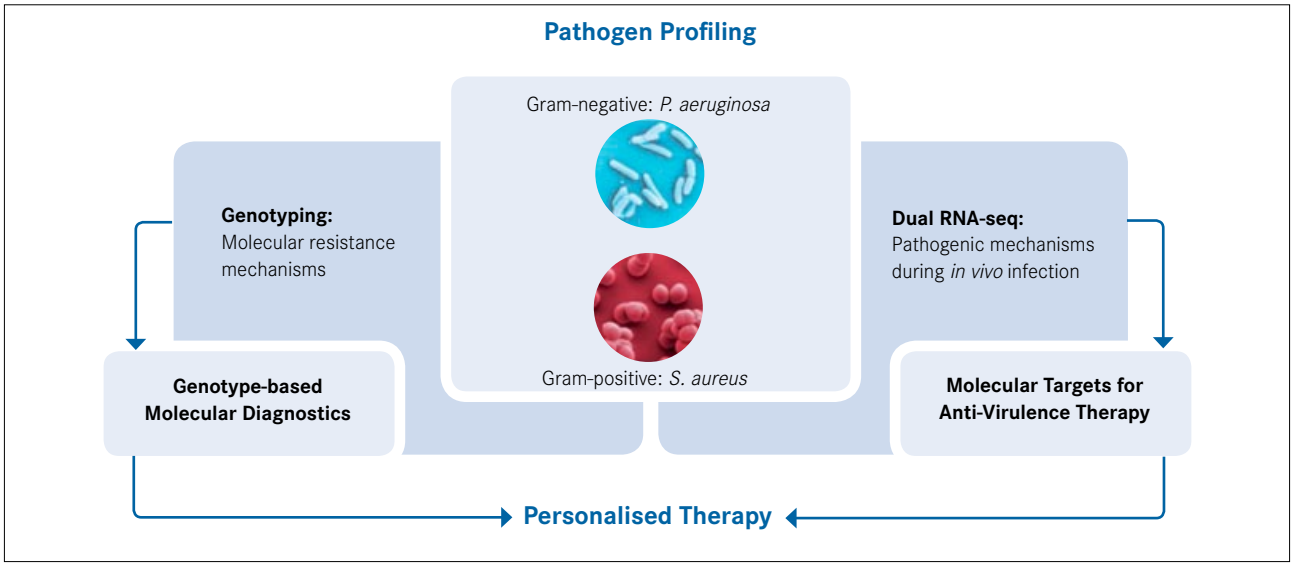


Figure 3: Pathogen profiling of *P. aeruginosa* and *S. aureus*.

on the stage of infection or within biofilms (2-4). The high-resolution mapping of the integral adaptive transcriptional response of bacteria to the host provides the basis for the design of novel therapeutic approaches. Furthermore, animal models of chronic, biofilm-associated infections have also been developed at HZI (5) that can be directly used for the evaluation of treatment regimens.

Individuals vary considerably in the magnitude of their immune response to bacteria, and this variation may affect the expression of virulence determinants by the pathogens and the overall outcome of infection. In this regard, the impact of inter-individual variation of the host response on *S. aureus* expression of virulence determinants during infection has been demonstrated (6). This is important information for the development of anti-virulence drugs, since absence or low expression of the targeted virulence factors could render anti-virulence strategies completely ineffective. Therefore, ideal targets for anti-virulence strategies would be those virulence factors, the expression of which is not influenced by the host background. In addition, the expression of *S. aureus* and *P. aeruginosa* virulence factors under changing environments, including niches within the human host and in a plethora of different strain backgrounds has been evaluated (7). Bacterial profiling in different environments may also provide important information for the future development of strategies for controlling bacterial colonisation and prediction of their virulence potential.

2. SMALL MOLECULES AS PATHOBLOCKERS FOR THE TREATMENT OF BACTERIAL INFECTIONS

Major activities of the Research Focus AMR aim at targeting and disabling bacterial pathogenicity mechanisms with small molecule inhibitors. This so-called pathoblocker concept is an innovative approach towards the discovery of anti-infectives substantially different from common antibiotic agents. Instead of interfering with vital processes, pathoblockers only interfere with infectivity and virulence of a pathogen. Cross-resistances with licensed drugs are avoided due to the novelty of the addressed targets. In general, only low rates of resistance development are expected, as pathoblockers do not impose selective pressure on the bacteria and spare the commensal microbiota.

AMR scientists investigate pathoblockers that interact with novel targets expressed by pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Specifically, inhibitors are developed against the biosynthesis and propagation of the *Pseudomonas* Quinolone Signal (PQS), a unique cell density dependent signalling system of *P. aeruginosa*. PQS is involved in controlling biofilm and virulence factor formation, thereby contributing to resistance and tissue damage. A second promising target class are the lectins LecA and LecB, two adhesins required for host colonisation by *P. aeruginosa*. Both components are relevant for biofilm formation. Thus, lectin inhibitors would not only target acute infections, but could also serve as resistance-breaking agents. Furthermore, cytolysins from *S. aureus* represent promising targets for pathoblockers, since they contribute directly to inflammation, immune evasion and tissue damage. Studies have shown that the inhibition of the alpha-hemolysin alone is sufficient to ameliorate lung infection by *S. aureus*. Drug-like small molecule inhibitors of alpha-hemolysin with strong tissue penetration capabilities do not exist.

Selected Scientific Achievements

Understanding PQS Biosynthesis and Propagation

The enzymes involved in biosynthesis of the PQS signal molecules have been studied to understand their function on the molecular level (8). Very recently, AMR scientists could demonstrate that the heterodimeric PqsBC complex is the key determinant for the variety of these molecules. This knowledge will help to guide future design of *Pseudomonas*-specific PQS pathoblockers.

PqsR-targeting Quorum Sensing Inhibitors as Novel Therapeutics

Different protein targets involved in PQS biosynthesis, reception, or signal transduction were studied, and PqsR proved to be the most favorable one with high translational potential (Fig. 4). Antagonists of this receptor developed by ligand-based approaches are highly active and are able to permeate the Gram-negative cell wall. The most promising compounds also showed potent anti-biofilm activity. In simple *in vivo* models, they completely blocked *P. aeruginosa* pathogenicity without reducing bacterial viability. No toxicity in human cell lines and a high metabolic stability were observed (9).

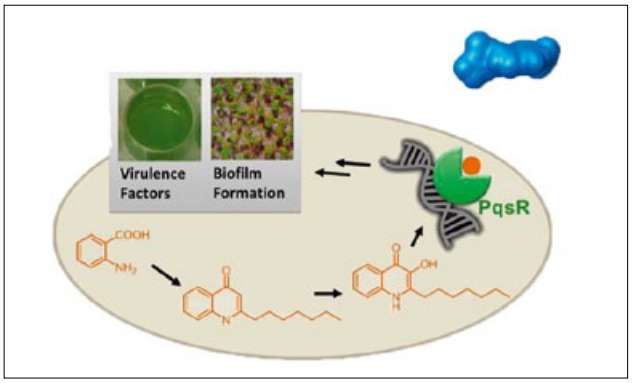


Figure 4: *P. aeruginosa* pathoblocker concept. Figure adapted from Zender et al. J.Med.Chem. 2013. 56, 6761-6774.

Discovery and Optimisation of Novel Lectin Inhibitors

The lectin LecB of *P. aeruginosa* is crucially involved in host cell adhesion and biofilm formation. Despite the known high genomic variability of *P. aeruginosa*, LecB is part of the rather conserved core genome and has been validated as a functionally conserved target in all seventy analysed clinical isolates (10-11). Thus, LecB inhibitors have been developed in an extensive structure–activity relationship studies resulting in nanomolar target affinities. Furthermore, HZI researchers have designed and synthesised the first covalent lectin inhibitor, which was applied in LecA-mediated biofilm imaging.

High Throughput Screening for Staphylococcal-Hemolysin Pathoblockers

Several assays to probe the inhibition of the deleterious effects of *S. aureus* α-hemolysin on host cells have been developed. Following a medicinal chemistry optimisation protocol, several highly potent alpha-hemolysin inhibitors from five different series were identified and confirmed in two secondary assays. Members of the most advanced series exhibit hemolysin-inhibitory activities in the nanomolar range and represent the most potent small molecule inhibitors of alpha-hemolysin reported so far.

3. DISCOVERY, OPTIMISATION AND MODE OF ACTION (MOA) ANALYSIS OF NOVEL ANTI-INFECTIVES

Most of the clinically used antibiotics are natural products or compounds derived from natural products. Biodiversity-oriented natural product research and synthetic biotechnology is expected to continuously play a predominant role in the discovery and optimisation of novel antibiotic classes.

Therefore, HZI has become a global centre of excellence for research on natural products from bacteria and fungi. The unique collection of myxobacterial strains available in the centre and the screening of these organisms for antibiotics have now resulted in a number of exploratory projects. Several hundreds of new strains of bacteria producing anti-infective compounds have become available via international collaborations. A great number of novel bioactive natural products have been discovered over the past years and are now being evaluated for their potential utility. Substantial funding for these activities was obtained from the German Centre for Infection Research (DZIF) and other third party organisations. HZI natural product screening libraries have been provided to partners, and the platforms for screening and synthetic biotechnology at the branch institute HIPS in Saarbrücken as well as the fermentation and natural products isolation platform at Braunschweig are being modernised. Preparation of natural products at multi-gram scale has become a routine procedure, and these assets are now also increasingly used in cooperation with research partners.

Selected Scientific Achievements

Strain isolation efforts within this Research Focus have yielded numerous representatives of novel genera and six previously unknown families of the myxobacteria as well as unusual actinobacteria and fungi (12), which will be exploited regarding secondary metabolite production. Figure 5 shows the three main steps of HZI's approach for discovery and therapeutic development of novel anti-infectives. Recently, scientists of this Research Focus have studied ~2500 extracts of myxobacterial metabolites using "Myxobase", demonstrating for the first time a direct correlation between chemical novelty of metabolites and phylogenetic distances of producer strains (13).

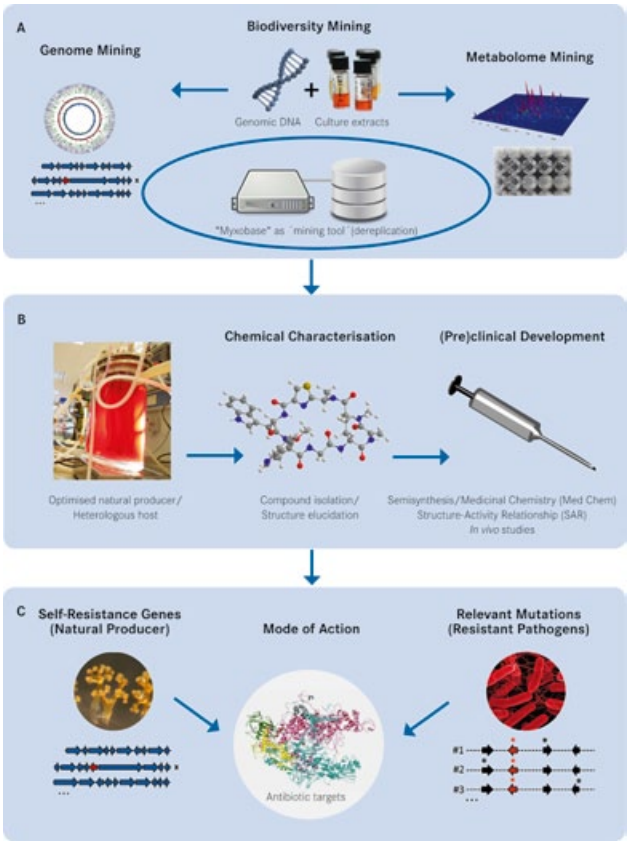


Figure 5: Approach for discovery and (pre)clinical development of novel anti-infectives including three main steps: A) Exploitation of underexplored biologic and genomic resources, B) Optimisation of compound structure and production, C) Mode of action analysis.

Known bacterial metabolites like Griselimycin and Telomycin, whose antibiotic development was stopped in the last century, are being studied in depth applying, inter alia, new methods of synthetic biotechnology to overcome the hurdles that ended their development in the past.

Griselimycin is also one prime example of the expertise of researchers in Research Focus AMR in pathway engineering and MoA analysis in collaborative work with Sanofi, yielding promising novel anti-TB drug candidates (14).

Studies on Telomycin biosynthesis revealed a novel prodrug mechanism and, in collaboration with Sanofi, delivered semisynthetic compounds with improved activities against MRSA.

The HZI platform for discovery and optimisation of natural products forms the basis for future innovation in antibiotic research as new chemical entities exhibiting novel MoA are frequently described for the first time. Production and modification by chemical and synthetic biotechnological means, along with concurrent MoA analysis has set the stage for translation of such compounds into pre-clinical and clinical development. Two of these compounds form the basis of advanced exploratory preclinical projects within the DZIF: the Chlorotonils have been described as potent antibacterials against Gram-positive pathogens exhibiting MICs in the two-digit nanomolar scale. Chlorotonils also show superb antimalaria activity against all growth stages of the parasites and are highly active *in vivo*.

Further, Corallopyronin A and other RNAP inhibitors from myxobacteria are currently evaluated as preclinical candidates for novel anti-infectives. RNAP (DNA-dependent RNA polymerase) is a validated pharmaceutical target for antibacterial drugs, and selective RNAP inhibitors are known to be specific and almost devoid of toxicity against humans.

4. NOVEL NATURAL LEAD COMPOUNDS WITH BROAD SPECTRUM ACTIVITIES AGAINST GRAM-NEGATIVE PATHOGENS

A large proportion of clinically relevant multidrug-resistant bacteria, among them all three 'critical priority 1' pathogens of the World Health Organisation (WHO) priority list, are Gram-negative bacteria. These are particularly difficult to treat, as they possess a highly effective cell envelope composed of two membranes preventing most molecules from permeating into the bacteria and reaching their target. Scientists of the Research Focus AMR are therefore dedicated to discover novel, innovative lead structures against Gram-negative pathogens. To this end, they are using two approaches: (i) identifying novel scaffolds from myxobacteria; and (ii) modifying known scaffolds from another group of bacteria – the actinobacteria – using synthetic biotechnology. These efforts led to the identification of Cystobactamids and Amidochelocardins – novel natural lead compounds with potent activity against Gram-negative bacteria. Cystobactamids were isolated from the myxobacterium *Cystobacter* sp. and possess a unique chemical scaffold composed of p-aminobenzoic acid oligomers. The compounds act as inhibitors of gyrase A, an enzyme essential for DNA replication in bacteria, but at a different binding site than the clinically used fluoroquinolones. Amidochelocardin was generated by genetic engineering of *Amycolatopsis sulphurea*, the producer of chelocardin, which is an atypical tetracycline featuring a distinct mode of action (MoA) and lack of cross-resistance.

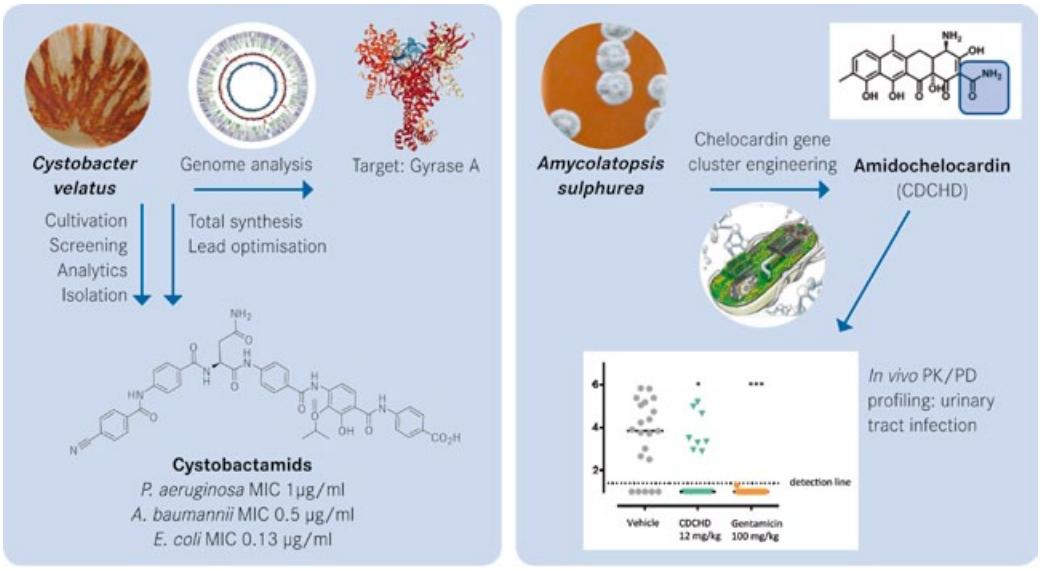


Figure 6: Novel natural lead compounds against Gram-negative pathogens.

Selected Scientific Achievements

Cystobactamids (15) were found to exhibit potent, broad-spectrum antibacterial activities against most major, clinically important pathogens (Figure 6 left). Using the in-house Myxobase dereplication system, numerous producers of this class of antibiotics were identified yielding a derivative with highly improved activity. The compounds inhibit the validated bacterial target gyrase A and exhibit only very limited cross-resistance to known gyrase inhibitors. As biotechnological supply is limited, establishing a chemical synthesis was essential. A 13-step total synthesis of Cystobactamids has been established and used to synthesise >100 analogues with highly improved activities. The project has received funding from DZIF and pre4D. The *in vitro* properties of Cystobactamids have been extensively profiled internally and by IMI ENABLE. The following optimisation objectives have been identified: increase solubility, close gaps in the antibacterial spectrum, decrease serum binding, decrease frequency and understand mechanism of resistance development. A target product profile (TPP) will be defined following the first *in vivo* studies. HZI has a strong IP position in this area.

The atypical tetracyclin Chelocardin was modified by rational genetic engineering of its biosynthetic gene cluster to yield Amidochelocardin (16; Figure 6 right). This work is performed in collaboration with ACIES Bio in Slovenia, and an IP is shared. The compound has strongly improved antibiotic activity compared to chelocardin and therefore represents a novel antibacterial lead structure for which HZI scientists could show *in vivo* proof of concept in mouse infection models. On this basis, an initial TPP was defined for the treatment indication of urinary tract infections.

PERSPECTIVES

In the future, the 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. In this regard, AMR scientists will apply a molecular diagnostic test system (RAPID) in the clinical setting, to uncover previously unrecognised or underappreciated patterns of hospital spread and the emergence of multidrug resistance. Broad application of the assay and

integration of acquired data promise to provide critical information for decision-making with respect to antibiotic use and for improvement of disease surveillance.

Continued advancement of the pathoblocker approaches could lead to a paradigm shift in anti-infective drug discovery, especially within the pharmaceutical industry, which to date solely relies on developing bacteriostatic or bactericidal compounds. If PqsR antagonists will be validated as clinically effective therapeutics, the concept of interfering with bacterial cell-to-cell communication could lead to broader applications such as the development of other narrow or even broad spectrum pathoblockers, depending on which communication system is addressed.

Using a combination of target-based approaches with rational drug-design techniques, scientists of the Research Focus AMR will discover and optimise further compounds, which attenuate or abolish the pathogenicity of *P. aeruginosa* and *S. aureus*.

AMR researchers have built a steadily growing, sustainable pipeline of exploratory drug candidates that now also includes compounds from actinobacteria and fungi. Perspectives to identify truly novel lead structures are clearly realistic as reflected by recent unpublished data. Research has led to the identification of at least five new compound classes exhibiting anti-Gram-negative activity, and numerous novel structures active against Gram-positives. Although these compounds are currently not yet characterised in detail, it is almost certain that they represent completely novel classes of antibiotics. HZI scientists regard this as a superb basis for future efforts towards the development of innovative antibiotics.

Given the *in vitro* microbiological profile and the chemical properties of Cystobactamids, they have the potential to be developed for treating nosocomial infections caused by multidrug-resistant non-fermenters. Amidochelocardins will be positioned for the treatment of urinary tract infections. Both compounds exhibit novel resistance-breaking properties against Gram-negative pathogens and thus have a large translational impact, as the current drug pipeline is particularly thin in this area.


EVA MEDINA


SUSANNE HÄUSSLER


ROLF HARTMANN


ROLF MÜLLER


MARK BRÖNSTRUP

Further contributions:
Ursula Bilitewski, Wulf Blankenfeldt, Susanne Engelmann, Anna Hirsch, Markus Kalesse, Jesko Köhnke, Claus-Michael Lehr, Andriy Luzhetskyy, Alice McHardy, Michael Meyer-Hermann, Andreas Müller, Dietmar Pieper, Ingo Schmitz, Marc Stadler, Theresia Stradal, Alexander Titz, Joachim Wink.

Key Publications Research Focus AMR

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RESEARCH FOCUS TACKLING GASTROINTESTINAL BACTERIAL INFECTIONS (GAST)

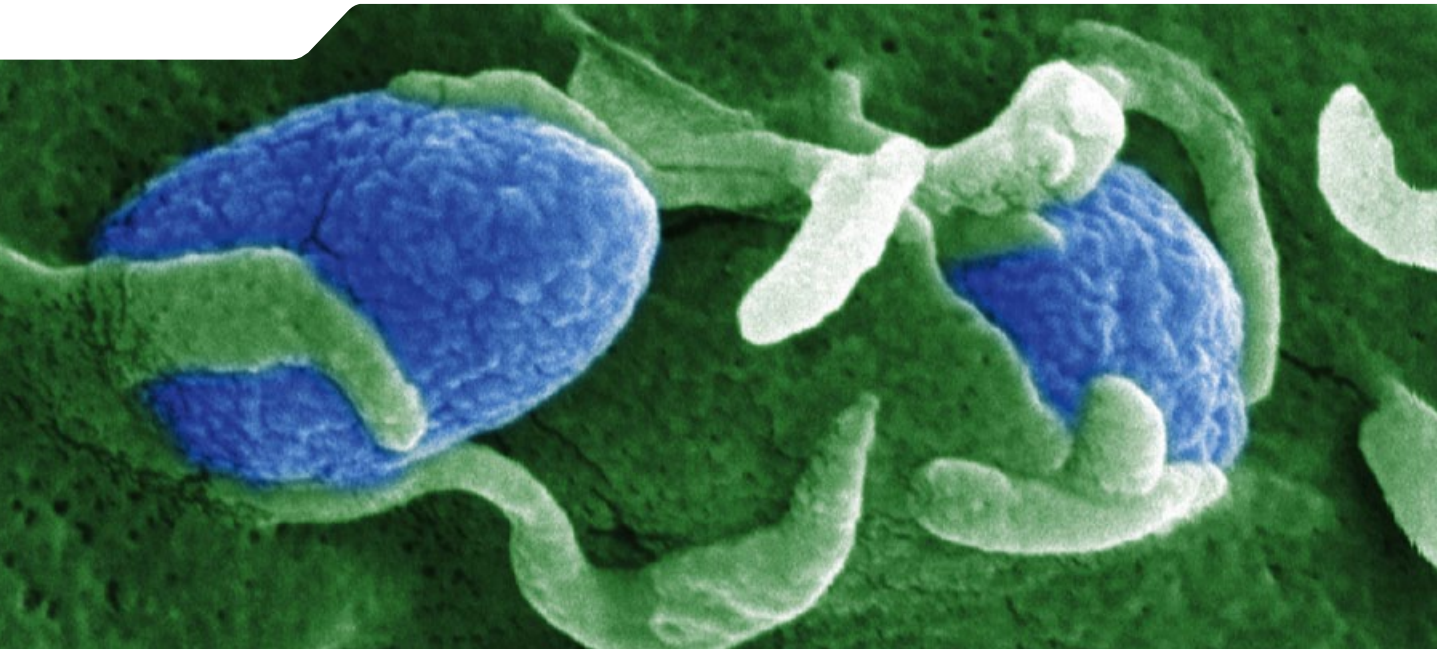

Figure 1: *Yersinia*

Bacterial gastrointestinal infections causing diarrhoea and other gut-associated diseases remain a leading cause of death among children under the age of five worldwide. In 2017, the World Health Organisation (WHO) has rated the gastrointestinal bacteria *Escherichia coli* and *Salmonella* spp. as priority 1-2 ("critical-high") on the global list of antibiotic-resistant microorganisms. A recent evaluation of pathogens threatening public health in Germany ranked the closely related Enterobacteriaceae *Salmonella*, *Yersinia* and *E. coli* among the highest and high priority agents.

The globalisation of food supply, the introduction and persistence of pathogens in unknown environmental niches, the rapid emergence of variants, and the development of antibiotics-resistant Enterobacteriaceae that use the intestinal tract as main reservoir are reasons why foodborne intestinal diseases by these pathogens remain a public health problem. Another important risk factor is collateral damage to the intestinal microbiota after antibiotic treatments, which reduces the ability of the microbiota to prevent colonisation and expansion of enteric pathogens. In turn, infections by enteric pathogens can trigger imbalances in the resident communities of gut microbes.

In light of these challenges, the Research Focus GAST combines experts in molecular bacteriology, cell and structural biology, immunology as well as pharmaceutical research. It aims to unveil the regulation strategies of enteropathogens, analyse the molecular machines essential for their virulence and understand the impact of the microbiota on susceptibility to enteric infections.

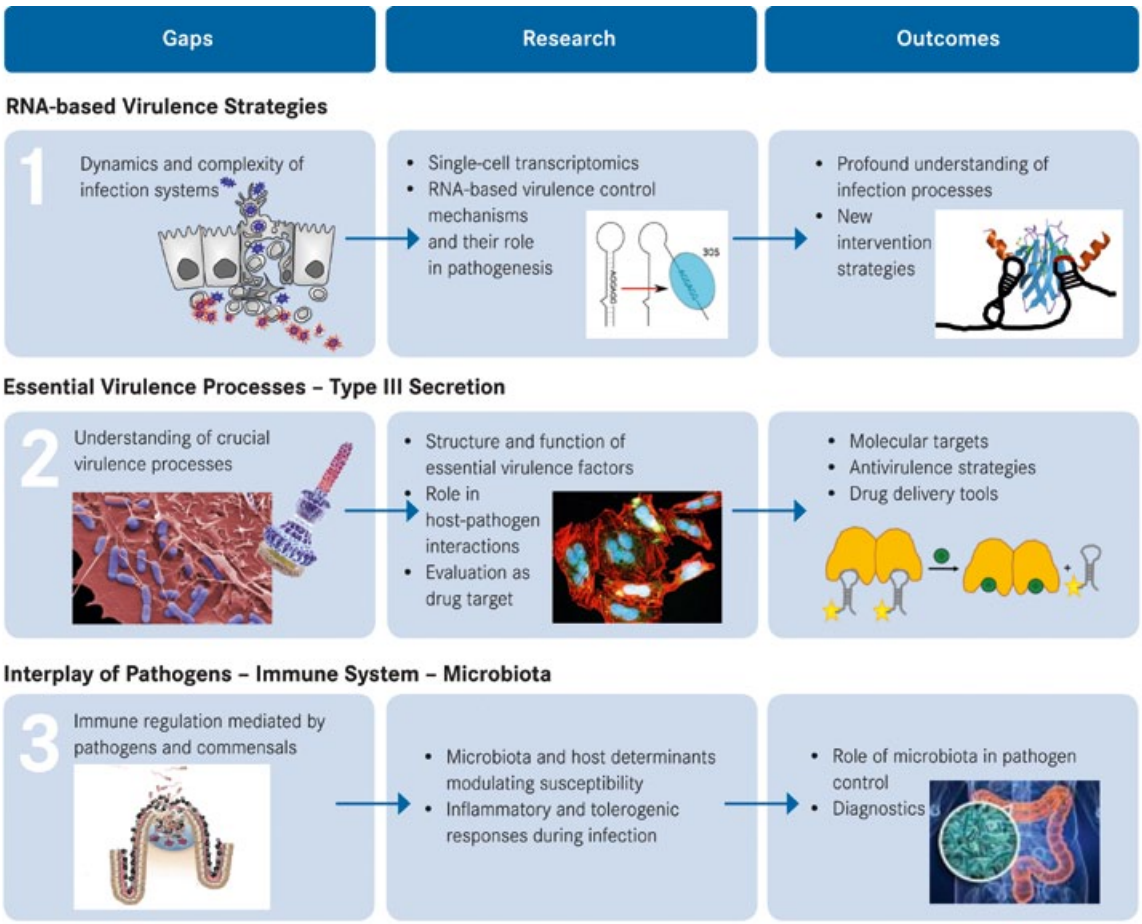

Figure 2: Overview of the research activities of the Research Focus Tackling Bacterial Gastrointestinal Infections.

1. DISCOVERY OF RNA-BASED VIRULENCE STRATEGIES

Treatment of gastrointestinal infections with antibiotics is often ineffective with respect to the reduction of symptoms or resistances, or even harmful because treatment disrupts the protective function of the intestinal microbiota or enhances pathogenesis (*e.g.* in case of enterohemorrhagic *E. coli*, or EHEC). Hence, innovative approaches are required to study and intervene with gastrointestinal infections.

RNA has now emerged as a promising class of molecules to better understand the highly complex and dynamic interplay between a pathogen and its host. Not only has RNA been increasingly recognised as a novel regulator of cellular processes, this class of molecules offers new approaches to high-resolution profiling of host-pathogen interactions and the identification of key virulence control strategies.

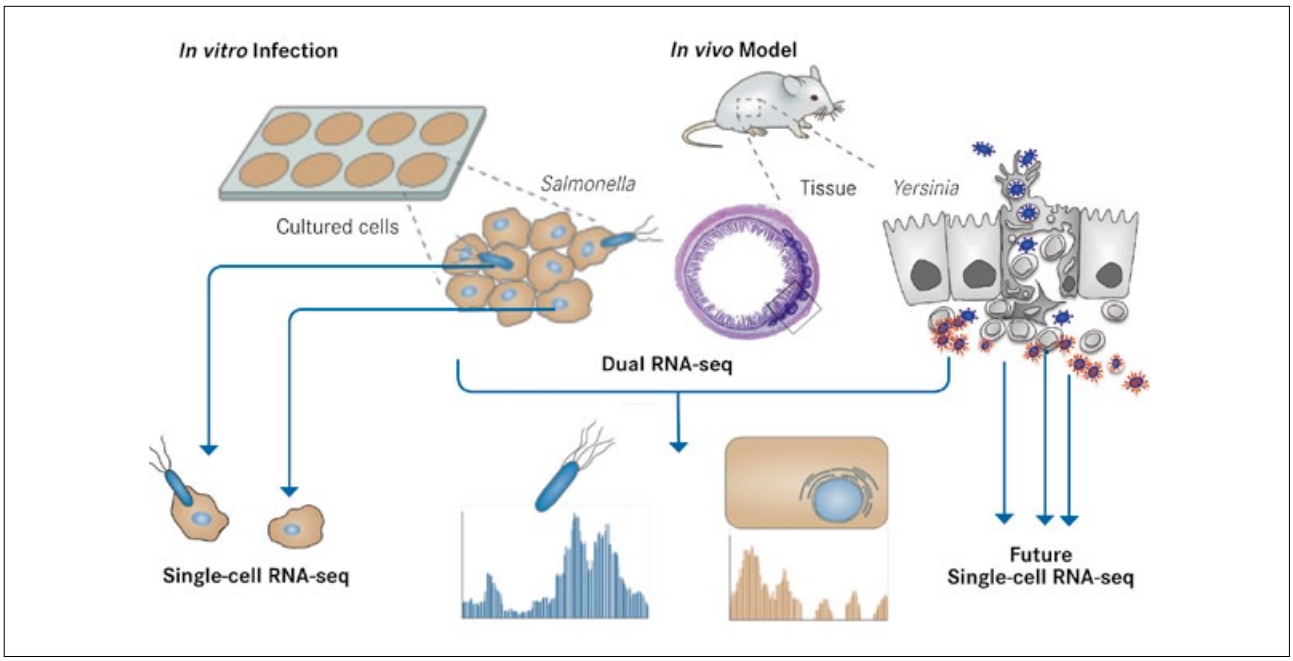

Figure 3: Development of cell and tissue dual RNA-seq, and single cell RNA-seq to investigate host-pathogen responses during the course of an infection by extracellular and intracellular pathogens (1, 2, 10). Adapted from: Dual RNA-seq of pathogen and host, Alexander J. Westermann, Stanislaw A. Gorski & Jörg Vogel. Nature Reviews Microbiology volume10, pages618–630 (2012).

One major goal was to develop RNA sequencing (RNA-seq) approaches that enable a simultaneous capture of all classes of coding and non-coding transcripts in both pathogen and host cells/tissue. In this context, establishment of single-cell RNA analysis promised an unprecedented, spatio-temporal resolution of individual cell properties that dictate the outcome of infections and their treatment. As there is strong evidence that pathogens employ RNA molecules as critical regulators during the course of the infection, scientists of the Research Focus GAST also sought to identify novel sensory and regulatory RNAs with essential roles in pathogenesis, and characterise the underlying RNA-based mechanisms implicated in virulence. The fact that the specific nucleotide sequence of each RNA molecule provides a unique target is reprogrammable, and can be developed into RNA-based drugs, has enabled the development of novel RNA-based intervention strategies for several diseases.

Selected Scientific Achievements

To ensure survival in different host environments, bacterial pathogens possess complex regulatory networks of control systems that coregulate, adjust and fine-tune metabolic functions, stress responses and synthesis of adequate virulence factors in response to host conditions. So far, underlying adaptation processes that drive pathogenesis have mostly been studied in isolation for either the pathogen or the host, neglecting the true complexity of host-induced stimuli acting on the invading pathogen. Researchers at the HZI and the HIRI have recently pioneered dual RNA-seq approaches that permit the simultaneous profiling of the host cells with their colonizing intracellular pathogens (*e.g. Salmonella*) or extracellular pathogens (*e.g. Yersinia*). Using dual RNA-seq, it is now possible to directly correlate host responses to molecular pathways in the pathogen. High-

resolution transcriptional RNA profiles of *Salmonella enterica* replicating within cultured human cells revealed novel insights into pathogen-host cell cross-talk and illustrated the molecular impact of bacterial non-coding RNAs for the precise timing of intracellular pathogen replication (1). Moreover, a fast tissue dual RNA-seq approach was established, allowing comprehensive and simultaneous monitoring of infection-linked gene expression changes. This enables the analysis of gene expression of an extracellular pathogen in the host during the infection process as well as in the context of an ongoing immune response (2). Moreover, several new pathogenesis-related bacterial riboregulators and new auxillary proteins were identified in *Salmonella* (3) and *Yersinia* (2,4,5). In addition, several thermosensitive RNA structures (RNA thermometers), controlling temperature-dependent reprogramming of important virulence factors were identified with a newly developed global RNA structurome analysis (6) and single cell analysis to characterise *Salmonella* growth in macrophages was established (7).

2. ELUCIDATING ESSENTIAL VIRULENCE TRAITS – UNDERSTANDING THE TYPE III SECRETION SYSTEM (T3SS)

In the course of coevolution with their hosts, enteric pathogens have developed sophisticated virulence factors and infection strategies. This includes mechanisms implicated in the adhesion and invasion of host cells and dissemination in the host, and proteins that manipulate or damage host cells or interfere with the host immune response. Only a detailed understanding of the molecular basis of their function will support the development of new antimicrobial therapies and drug delivery strategies.

One key virulence factor for pathogenesis of many important enteric pathogens of the family of Enterobacteriaceae is the Type III Secretion System (T3SS). The T3SS is a widely conserved and highly efficient protein export system that powers injection of effector proteins into the host cell cytoplasm through the needle-like "injectisome", a complex nanomachine that serves as the primary infection and anti-host defense mechanism of many enteric pathogens. A T3SS also drives the secretion of building blocks of the bacterial flagellum. The T3SS generally consists of a basal body, which spans the bacterial cell envelope, topped by a needle-like structure (in case of the injectisome) or a long filament that acts as a propeller (in case of the flagellum) outside the bacterium. The T3SSs of both flagella and injectisome systems of enteric pathogens are evolutionarily related and promote essential virulence processes. Hence, these molecular machines and their translocated factors embody highly promising drug targets. In a combined effort, HZI researchers tackled the open questions to significantly extend the knowledge on T3SSs.

Selected Scientific Achievements

Using single-cell microscopic analyses in combination with mathematical modelling, it could be shown for the first time how the bacterial T3SS/flagellum self-assembly process is controlled and how the flagellum grows outside the cell with remarkable speed through an injection-diffusion mechanism, which is independent of cellular energy sources. In this model, growth of the flagellum is driven by hindered diffusion and proton motive force-dependent secretion of subunits, thus explaining why bacterial flagella do not grow indefinitely (8). Using mutants that bypass the requirement for the T3SS-associated ATPase for protein export, it was found that the T3SS intrinsically is a proton motive force-driven protein export machine with the associated ATPase complex being dispensable for protein secretion (9). Moreover, it was recently revealed that a novel, integral-mem-

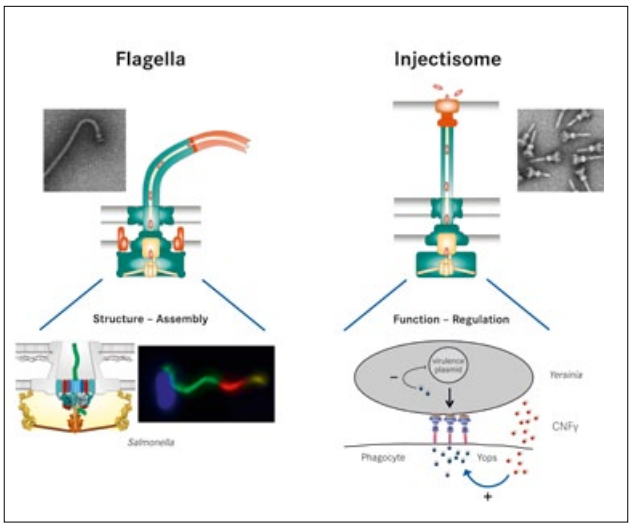

Figure 4: Analysis of the structure, assembly, function and regulation of the flagellar and the virulence-associated T3SS in *Salmonella* and *Yersinia* (9,8). Source: Figure adapted from Renault et al. eLife Sciences Publications Limited; (2017). e23136.

brane chaperone coordinates self-assembly of the multicomponent T3SS of the flagellum and thus facilitates quality control and productive assembly of this complex nanomachine. Research with the T3SS of Yersinia further showed that the efficacy of T3SS-mediated translocation via the pore complex is also regulated by the CNF_Y toxin. It was shown to increase T3SS-mediated translocation of the effectors and leads to a much more severe T3SS/effector-mediated intestinal inflammation and necrosis in gut-associated lymphoid tissues. This activity strongly enhances the severity of infection, whereas reduction of CNF_Y levels initiates an interferon $(IFN)_\gamma$-mediated response comprising non-inflammatory antimicrobial activities and induces tolerogenesis leading to asymptomic, persistent infections.

3. INTERPLAY BETWEEN ENTERIC PATHOGENS, HOST IMMUNE SYSTEM AND THE GUT MICROBIOTA

The gastrointestinal microbiota comprises diverse and dynamic ecosystems that intimately interact with the host in health and disease. A balanced microbiota composition contributes to the normal development of the immune system and to its proper activation during enteric and systemic infections. In reverse, alterations in the immune recognition of the microbiota and shifts between inflammatory and tolerogenic immune responses have been suggested to causally impact the composition and function of the microbiota. Experimental and clinical observations corroborate a decisive role of the microbiota in preventing bacterial infections, as evidenced by enhanced susceptibility to infections in antibiotics-treated individuals. Recent findings suggest that specific microbial members of the microbiota have prominent effects on the course of infections. Moreover, alterations in the microbiota and an imbalance of the mucosal immune system have been observed during enteropathogen infections.

In order to exploit the gut microbiota and the mucosal immune system as biomarkers and therapeutic targets to prevent severe enteric infections as well as associated complications or sequelae, a detailed understanding of the complex interplay between enteric pathogens, the host immune system and the gut microbiota is required. To this end, microbiological approaches, *in vivo* infection models, sophisticated mouse strains, molecular methods and metaomics sequencing as well as bioinformatics methods for microbiome analyses were successfully established and combined over the past years.

Selected Scientific Achievements

Interactions between Microbiota and Host Modulate Susceptibility to Gastrointestinal Infections and Inflammation

Which members of the microbiota protect the host against infection and how exactly they exert this function have remained unknown. Through a combination of gnotobiotic mouse models with anaerobic culturing and immunological analysis a novel mechanism was revealed by which distinct members of the microbiota protect the host against a *Salmonella* infection through modulation of IFN_γ production in intestinal lymphocytes (10; Fig. 5). Additionally, HZI scientists explored the impact of the widely spread bacterium *Helicobacter pylori* on the human microbiome in the upper GI tract in close cooperation with clinicians. They identified the strong influence of *H. pylori* on the global community structures in the stomach and upper GI tract, which may be associated with the development of specific complications (11). These results together show that a more detailed understanding of the microbiota and the host will be crucial to develop novel interventions against enteric pathogens.

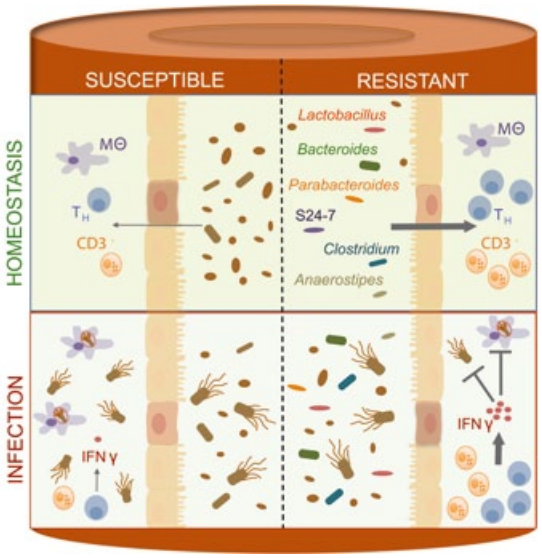

Figure 5: Microbiota alterations and immune responses in the context of an infection. Microbiota composition contributes to colonization resistance against *Salmonella*. Presence of distinct gut commensals determines susceptibility to *Salmonella*-induced diarrhoea by priming the immune system and preventing tissues invasion. Resident bacteria confer protection via enhancing antibacterial IFN_γ production by innate cells and $CD4^+$ T cells during infection (18). Adapted from: Thiemann et al. (2017), Cell Host Microbe 21: 682-694.e5.

Regulation of the Balance between Inflammatory and Tolerogenic Immune Responses in the Context of Infections

Distortion of the crucial balance between tolerogenic and pro-inflammatory immune responses can either result in inefficient clearance of extracellular pathogens or contribute to severe pathology of infected tissues. In this context, the development and functional properties of $Foxp3^+$ regulatory T cells (Tregs) and proinflammatory Th17 cells was thoroughly studied under steady-state and infectious conditions (*see also Research Focus TVAC*). It was observed that *Y. pseudotuberculosis* supports Th17 differentiation while simultaneously limiting *de novo* Treg induction by directly interfering with T cell receptor signalling using the T3SS. Moreover, it was found that the microbiota was required to stably and long-lastingly imprint tolerogenic properties in mLN stromal cells (12), a process that generally takes place in neonates. Single-cell RNA-seq, which was performed in close cooperation with scientists from HIRI, led to the identification of previously unknown location- and microbiota-dependent stromal cell subsets. Based on obtained results, selective targeting of certain T cell populations might be a promising new therapeutic option against enteric infections.

PERSPECTIVES

Scientists in the Research Focus GAST will further develop new techniques to identify principles of virulence that can be exploited for pathogen elimination. A particular focus will be on the discovery of new pathogenesis-related bacterial RNA molecules and auxillary proteins, and the characterisation of the regulation and function of essential pathogenicity factors. GAST researchers aim to reach an in-depth understanding of how pathogens interact with their hosts on the molecular, cellular and organismic/systemic level and resolve the close interplay between these pathogens, the microbiota and the host immune system.

Established dual RNA sequencing technologies and integrated data analyses will be developed further to capture RNA expression patterns at high spatio-temporal resolution of infection processes directly within tissues in infection models and clinical samples.

In parallel, a special emphasis will be put on the structure-function analysis of identified bacterial virulence factors of the selected pathogens alone and in complex with their host targets, which can further be exploited for other purposes. A comprehensive knowledge of the pathogen's physiology and virulence-relevant properties during the different stages will help to reveal potential drug targets.

As the interplay between the host immune system, the microbiota and invading pathogens has emerged as a central determinant in regulating the susceptibility to infections and is tightly linked to numerous human diseases, several initiatives have already been started to identify microbial signatures associated with disease outcome in several human inflammatory diseases.

Together, this will lead to the identification of novel drug targets and optimise the design of antimicrobial therapies.

PETRA DERSCH

TILL STROWIG

Further contributions:
Wulf Blankenfeldt, Emmanuelle Charpentier, Marc Erhardt, Rolf Hartmann, Jochen Hühn, Claus-Michael Lehr, Rolf Müller, Dietmar Pieper, Klemens Rottner, Theresia Stradal, Jörg Vogel.

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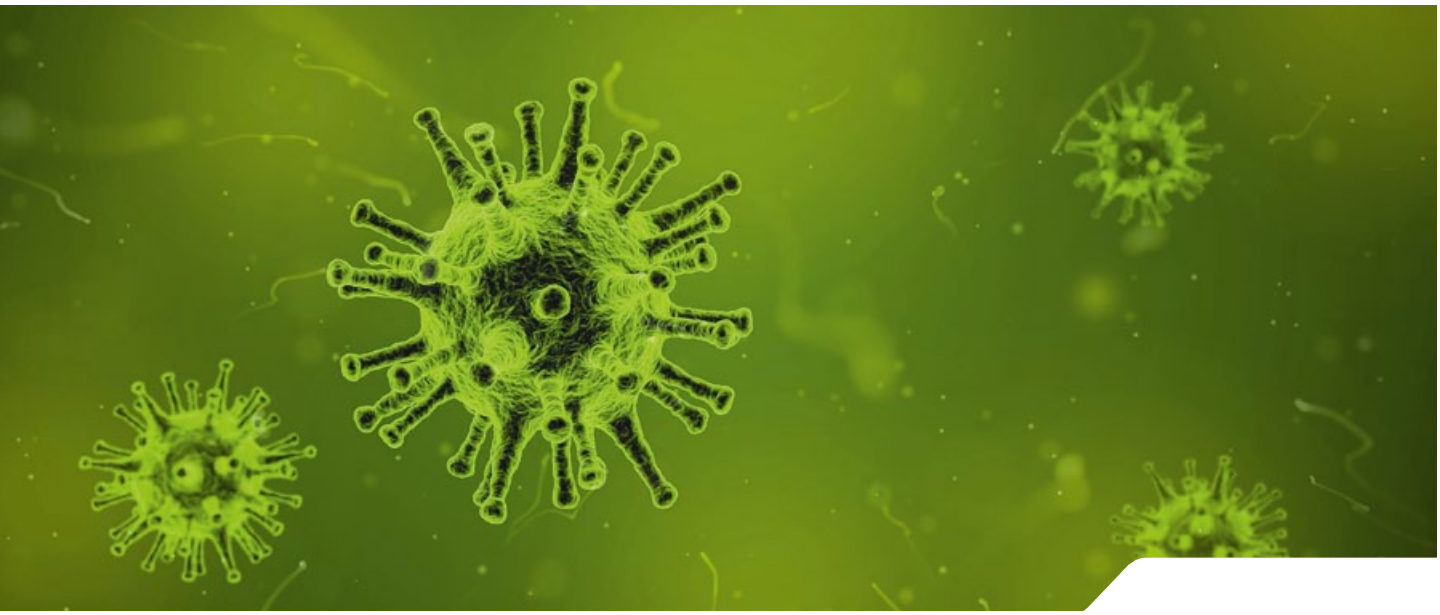
RESEARCH FOCUS APPROACHES AGAINST CHRONIC VIRAL INFECTIONS (CVIR)



Virus particle

Chronic viral infections by hepatitis and herpes viruses cause severe disease in numerous patients – in Germany as well as worldwide. Several hundred million individuals worldwide are chronically infected with hepatitis viruses putting them at risk for severe liver disease. Although highly efficacious therapies for Hepatitis C Virus (HCV) have been developed, it is challenging to deliver them to all patients. Moreover, treatment-induced virus elimination does not protect from re-infections. Cytomegalovirus (CMV) is a ubiquitous herpesvirus, which causes opportunistic and lifelong infections. More than 50% of the European population are latent CMV carriers and reactivation upon immune suppression can result in severe complications, especially in the transplant setting. Therapies are available for treatment of CMV, but they induce the selection of drug-resistant viral strains and are fraught with side effects. Vaccines are lacking for both HCV and CMV, and our understanding of basic mechanisms of pathogenesis, immune control and viral evasion is incomplete.

Researchers of the Research Focus “Approaches against chronic viral infections” (CVIR) address the existing critical knowledge gaps about these diseases.

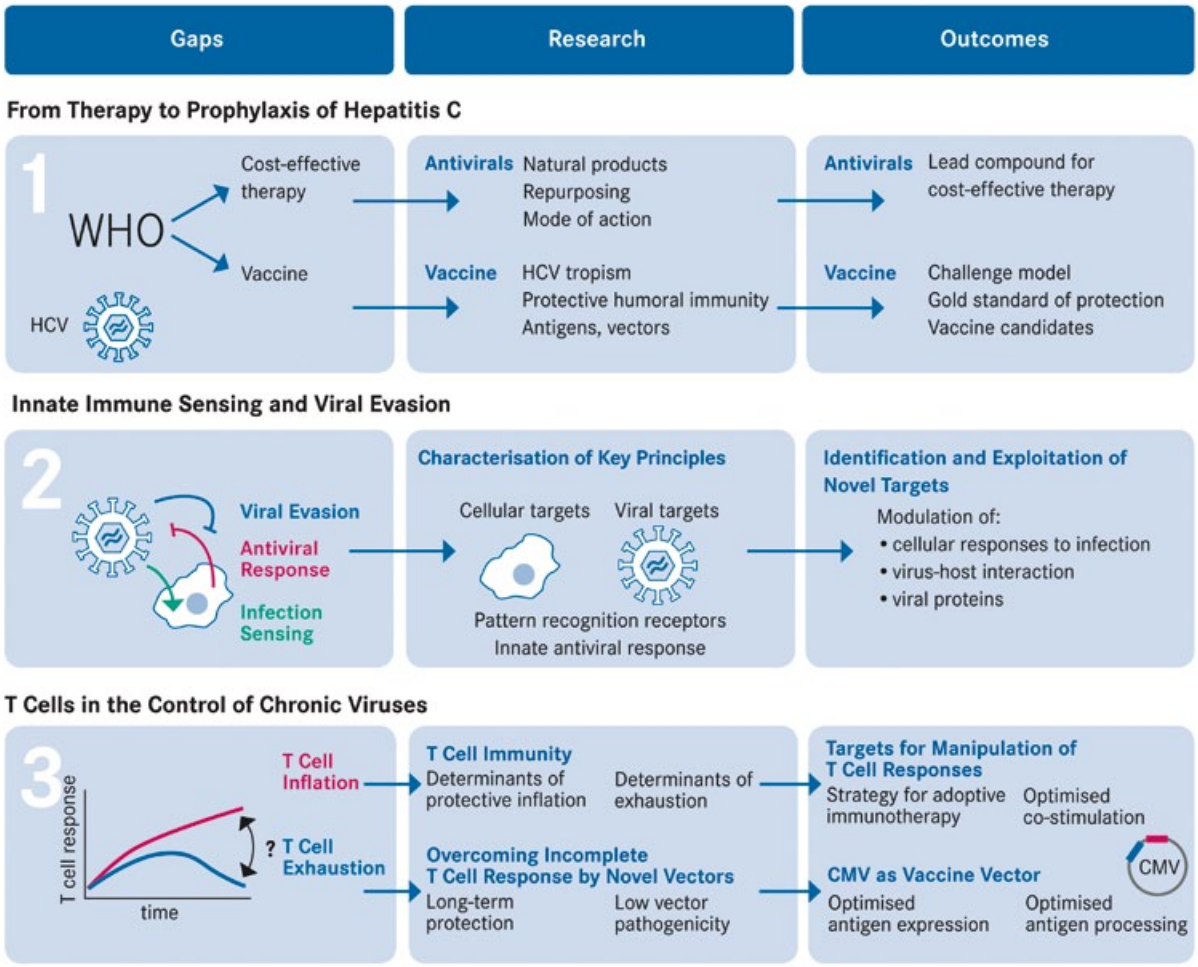


Figure 2: Overview of the research activities of the Research Focus "Approaching Chronic Viral Infections".

PROJECT 1: FROM THERAPY TO PROPHYLAXIS OF HEPATITIS C

Globally an estimated 71 million individuals are chronically infected by HCV. These patients are at risk to develop severe liver disease including cirrhosis and liver cancer. Novel therapies have improved treatment options. However, these therapies are expensive, thus limiting access particularly in those countries with highest prevalence. Therefore, provision of cost-effective treatment is a major challenge and medical need.

Many HCV-infected patients are not diagnosed. Moreover, treatment-induced viral clearance does not protect from HCV re-infection, which frequently occurs in populations with high transmission risk (*e.g.* drug users). Therefore, development of a prophylactic vaccine is an important clinical need.

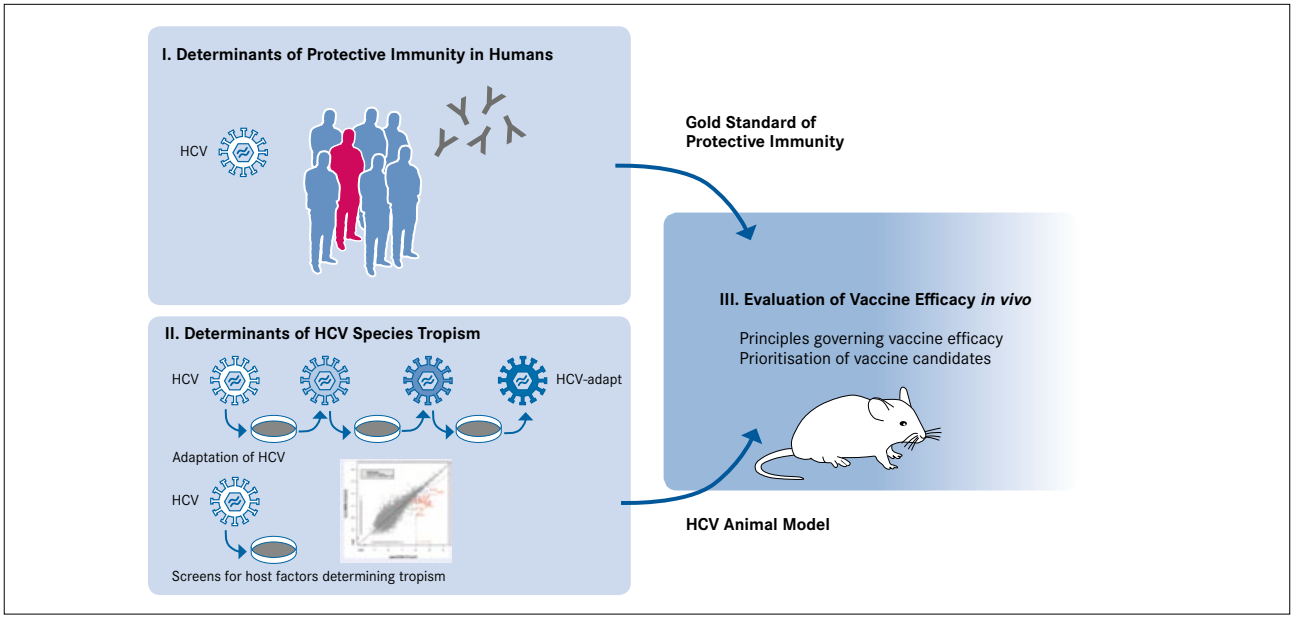


Figure 3: HCV vaccine research. To contribute to HCV vaccine development CVIR researchers explore (I) determinants of protective humoral immunity in humans (*e.g.* 1) which serve as gold standard for vaccine development. To this end large cohorts of patients are screened for rare individuals with elite neutralizing antibodies (red person in panel I). (II) They investigate the determinants of HCV species tropism (*e.g.* 2, P1) to develop an HCV vaccine challenge model. Ultimately, these efforts aim at (III) evaluation of vaccine candidates *in vivo* and studying the principles that govern vaccine efficacy.

Therefore, CVIR develops and uses tissue culture and animal infection models of HCV to discover cost-effective alternatives to currently available HCV combination therapies. Moreover, CVIR dissects principles of HCV species tropism to develop HCV-permissive, immune competent animal models that can be used as vaccine challenge platforms to prioritise HCV vaccine candidates (*see Figure 3*). Finally, under the umbrella of the HCV-vaccine project of the DZIF, which is co-coordinated by a CVIR scientist and coordinated by the Clinical Director of HZI, CVIR teams up with physician scientists to dissect key determinants of protective humoral immunity to HCV in humans. Hereby, the clinical partners offer access to critical HCV patient biobanks. Ultimately, the determinants of natural protective immunity will serve as "gold standard" for the development of a protective HCV vaccine.

Selected Scientific Achievements

Antivirals

CVIR has screened compound libraries to identify new HCV inhibitors. Flunarizine, a licensed neuroleptic, was characterised as first in class HCV membrane fusion inhibitor (3).

HCV tropisms and animal models

HCV vaccine research needs small animal models, but mice cannot be infected with HCV. CVIR scientists have shown that HCV receptor usage is strain-dependent and that ApoE is a critical host factor determining tropism. Novel HCV cell entry and assembly dependency factors with potential relevance for viral tropism were discovered (4 and P2). CVIR scientists engineered mouse liver cells by genetic modifi-

cation so that they allow complete replication of HCV (2). Finally, CVIR researchers discovered and characterised novel HCV restriction factors expressed in mouse liver, and filed a patent to protect usage of this information for development of HCV-permissive animal models (P1).

Humoral immunity to HCV

Infection systems for primary clinical strains were established to assess human cross-neutralizing antibody responses. Circulating apolipoproteins associate with secreted HCV particles and particle maturation facilitates viral escape from neutralizing antibodies (1).

2. INNATE IMMUNE SENSING AND VIRAL IMMUNE EVASION GENES AS MOLECULAR TARGETS

The presence of viruses is sensed immediately after virus entry into host cells by pattern recognition receptors (PRR). PRR then induce a potent antiviral response crucial for efficient control of viral infections. However, viruses that establish lifelong, chronic infections, including HIV-1, HCV and herpesviruses, have evolved efficient countermeasures to disarm this host defense mechanism or even use it for their own benefit.

Currently, only a very limited number of drugs is available for the treatment of HCMV (Human Cytomegalovirus) and KSHV (Kaposi's Sarkoma Herpesvirus) infections. The scientific community is just begins to appreciate the complexity of these viruses, their vast coding capacity and their repertoire for coordinating replication and manipulation of the host. These new insights highlight abundant novel antiviral targets. Therefore, CVIR researchers aim to dissect key principles of innate immune sensing and viral evasion by these human pathogens and to translate this knowledge into innovative interventions.

Selected Scientific Achievements

Innate Immune Sensing via Pattern Recognition Receptors

A better understanding of the role of PRRs in chronic infection and viral counteraction of PRR-mediated immune responses is a crucial prerequisite for the development of novel evidence-based antiviral strategies. On the one hand, understanding the cellular and molecular details of PRR sensing and signaling may improve vaccine formulations by enabling targeted adjuvant activity. On the other hand viral modulators of the innate immune response may provide novel molecular targets for therapy.

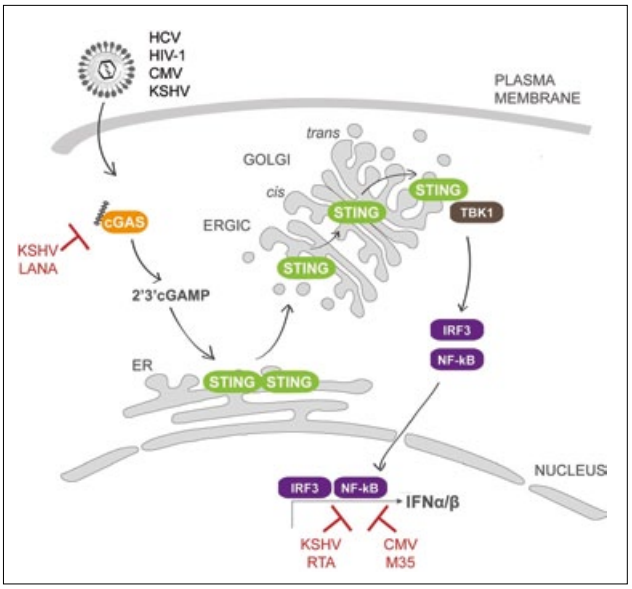


Figure 4: Recognition of viral infection by the innate immune system. Upon detection of viruses, PRR induce a potent antiviral immune response which is counteracted by viruses to allow establishment of chronic infections. CVIR researchers have identified multiple viral proteins that inhibit the antiviral response mediated by pattern recognition receptors (5, 6, 7). These viral proteins and their specific cellular interaction partners may represent targets for the design of novel anti-infectives.

CVIR scientists have shown that the PRR cGAS senses HIV-1 and CMV infection in primary human cell cultures (8). It was further discovered that the cGAS pathway plays a pivotal role to prevent KSHV reactivation from latency, and a KSHV-encoded antagonist of cGAS was identified (6). Finally, CVIR scientists could show that cGAMP, the catalytic product of cGAS, may serve as a suitable mucosal adjuvant.

The contribution of individual cell types and PRRs to the antiviral immune response and their impact on virus propagation was defined, and that PRR sense CMV in a cell type-dependent manner (5). Furthermore, IFNβ alone was shown to be sufficient to reversibly block CMV replication at the level of immediate-early gene expression both *in vitro* and *in vivo*. In the context of HCV, HZI scientists found that murine CD8α-like dendritic cells produce type I IFN in a TRIF-dependent manner.

Immune Evasion Genes as Molecular Targets

CVIR scientists identified M27, a protein of murine cytomegalovirus (MCMV), as a potent inhibitor of the type I IFN response in macrophages and myeloid dendritic cells. M36, an MCMV inhibitor of death receptor apoptosis, was shown to be a critical determinant of pathogenicity in immunocompromised mice. Further, novel HCMV- and MCMV-encoded antagonists of the PRR-mediated antiviral type I IFN response were identified (5).

For KSHV, immune evasion properties of three KSHV proteins were identified (6, 7). Taken together, our efforts have identified several promising targets for antiviral therapeutic strategies at the molecular level, which will be pursued in future studies.

3. T CELLS IN THE CONTROL OF CHRONIC VIRUSES

Ubiquitous human herpesviruses such as CMV elicit uniquely robust T cell responses, which ensure a balance with the pathogen, thus preventing reactivation from latency and viral replication.

Ultimately, a large proportion of memory T cells is devoted to CMV, a phenomenon called T cell memory inflation. This is in striking contrast to T cell responses to chronic-persistent viruses, *e.g.* hepatitis B virus (HBV) or HCV, which become chronic if immunity fails at controlling the primary infection. In this chronic phase, T cells lose the ability to control viral replication and spread – they become exhausted.

CMV specific cells, on the other hand, retain strong antiviral functionality. The reasons for this remarkable difference in clinical outcomes are not well understood. Dissecting the pathways that determine differential T cell behavior in these chronic viral infections will enable the translation of this knowledge into innovative vaccination and immunotherapy approaches.

It is conspicuous that both T cell inflation and exhaustion occur in chronic infections and that antigen persistence appears to be a driving force behind these phenomena. Understanding the mechanisms driving functional or exhausted T cell responses in chronic infection requires suitable experimental models, because clinical studies define correlations, but not cause-effect relationships of observed phenomena. To discover such mechanisms, scientists of Research Focus CVIR juxtapose T cell protection and exhaustion in models of chronic latent and persistent infections, respectively.

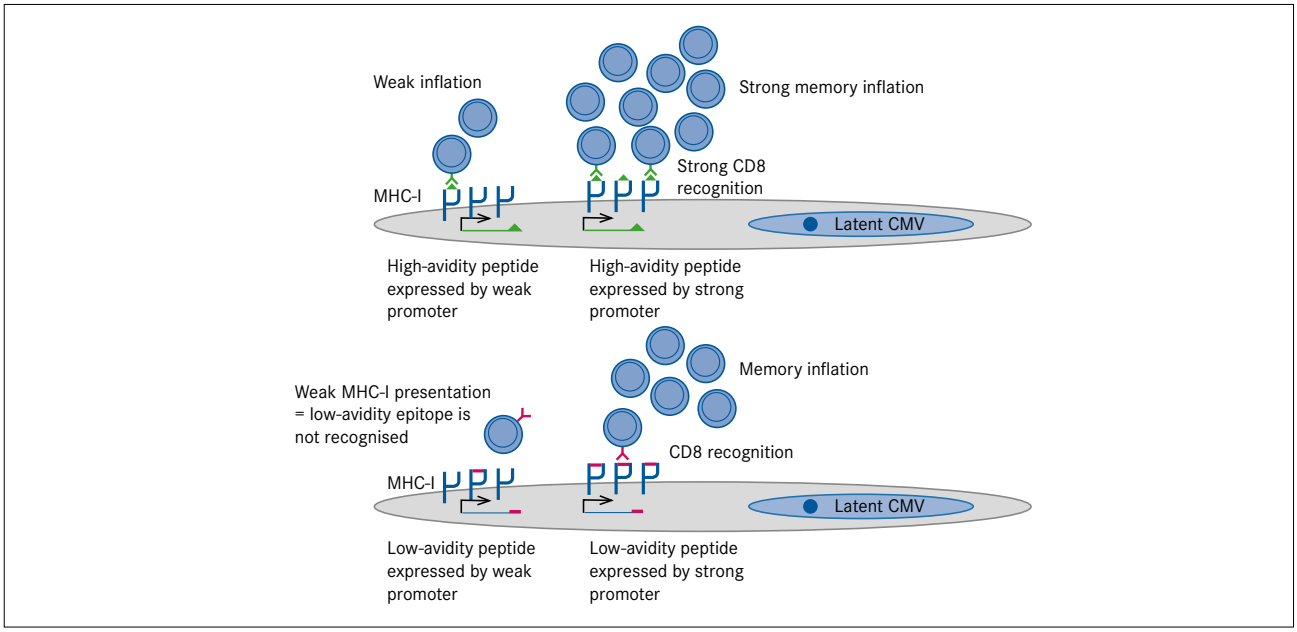


Figure 5: T cell responses to CMV vaccine vectors depend on epitope expression context and on peptide-intrinsic properties. High-avidity epitopes (upper figure panel) induce inflationary responses, and their strength is defined by the promoter strength. Low-avidity epitopes (lower panel) induce inflationary responses only if the epitope is expressed by a strong promoter. Low-avidity epitopes expressed by a weak promoter neither induce memory inflation nor mediate immune protection (9).

Selected Scientific Achievements

Understanding the Cellular Immune Response to CMV Infections

To define long-term effects of latent infections on the cellular immune compartment, CVIR scientists have developed a model of life-long immune monitoring of mice infected with various herpesviruses. While CMV induced drastic changes of the peripheral T cell populations in the latently infected groups, this did not affect the functionality of the naïve T cell compartment (10). This was in line with the observation CD8 T cells against immunodominant epitopes inflated at the expense of cells recognizing subdominant epitopes (9). Hence, the robust and lasting response to CMV does not exhaust the T cell compartment or the TCR repertoire and CMVs may be suitable as vaccine vectors. Mathematical modeling implied that T cell control of CMV infection by cytotoxic mechanisms is an exceptional event, consistent with observations that cytokine-based control of CMV gene expression is sufficient to repress viral transcription and induce latency (11). Antigenic epitopes expressed within immediate-early genes induced the strongest responses, and antigen availability for processing in non-hematopoietic cells was critical for immune protection by CMV-based vaccine vectors (11). Notably, even a low-avidity epitope conferred immune protection if expressed in the appropriate context (9).

Cellular Responses to Chronic-persistent Viruses

To model persistent infections by hepatitis viruses *in vivo*, CVIR scientists have developed genetic methods to induce sustained antigenic expression in hepatocytes. This allowed a comparison of robust and moderate antigen persistence. The robust expression of the model antigen ovalbumin by tamoxifen administration induced a rapid exhaustion of previously primed and functional T cell populations in healthy mice, implying that exhaustion is induced by robust antigenic persistence. This could be overcome, however, by TLR-9 co-stimulation during immunisation, which resulted in protective immunity, even in conditions of high antigen expression.

PERSPECTIVES

Researchers of the Research Focus "Approaches against chronic viral infections" (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 CMV and HCV infections. They will elucidate essential molecular mechanisms of immune control and pathogenesis of hepatitis and herpes viruses. To this end, they will further dissect pathways and key principles of innate immune sensing and viral countermeasures. This includes comprehensive studies on HCV and CMV and related herpesviruses like Kaposi's sarcoma herpesvirus and human immunodeficiency virus 1 (HIV).

Specifically, CVIR researchers aim to investigate alternative strategies for cost-effective HCV therapies and conduct research towards development of a prophylactic HCV vaccine. In parallel, the principles that control T cell responses in these chronic viral infections will be explored further.

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

THOMAS PIETSCHMANN

MELANIE BRINKMANN

LUKA ČIČIN-ŠAIN

Further contributions:
Robert Geffers, Christine Goffinet, Carlos A. Guzmán, Susanne Häussler, Ulrich Kalinke, Andrea Kröger, Michael Meyer-Hermann, Rolf Müller, Andreas Müller, Dirk Schlüter, Ingo Schmitz, Burkhard Schraven, Tim Sparwasser, Eike Steinmann, Siegfried Weiss, Dagmar Wirth.

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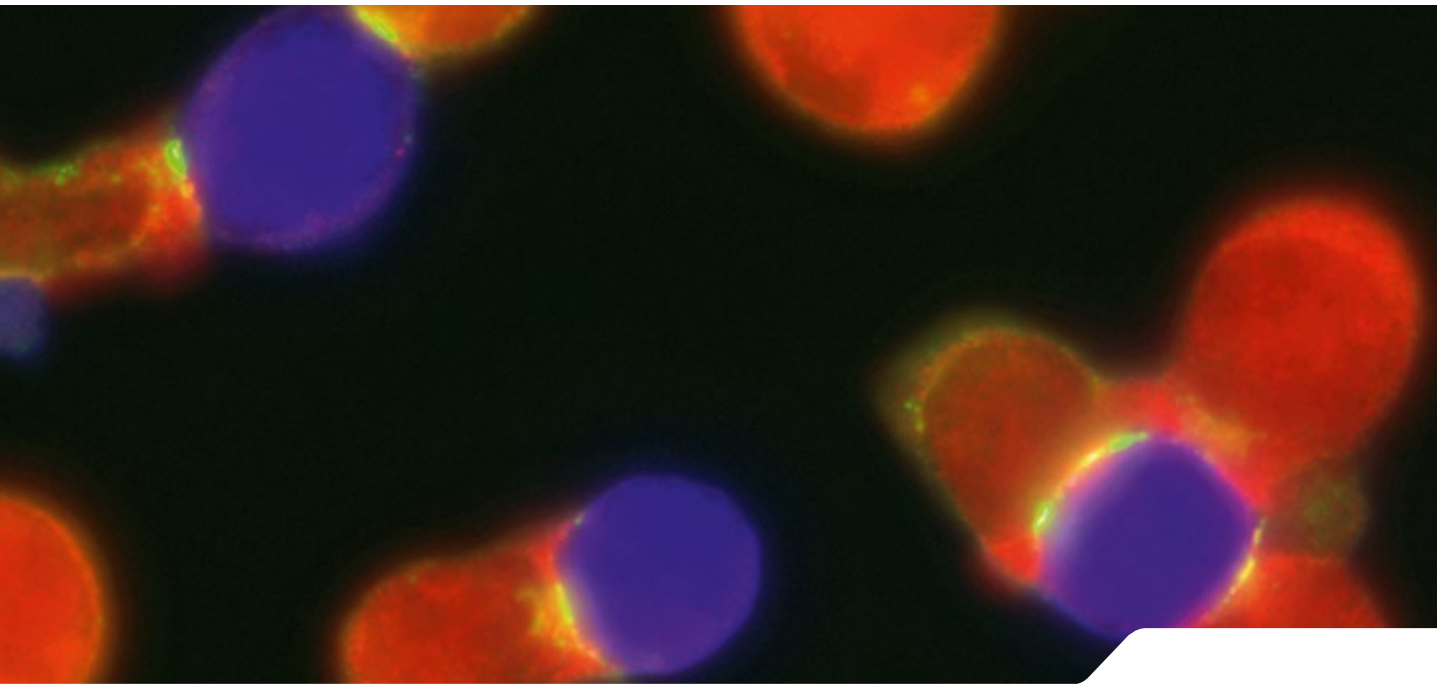
RESEARCH FOCUS T CELL TARGETING AND VACCINATION STRATEGIES (TVAC)



T cells (red) interacting with B cells.

Immune-based interventions are very effective to prevent and treat infectious diseases. This is exemplified best by the success story of vaccination campaigns against several life-threatening pathogens. However, for many infectious diseases either prophylactic vaccines or effective immune-based therapies are missing. Furthermore, new pathogens are constantly emerging or re-emerging, and individuals with enhanced vulnerability to develop infectious diseases are often poor responders to current standard immune-based interventions. These vulnerable individuals not only include the elderly with a senescent immune system and the very young, whose immune system is not fully matured yet, but also patients with primary and secondary immune deficiencies, co-morbidities or undergoing anti-inflammatory therapies for non-communicable diseases.

To tackle these challenges of modern infection medicine, novel individualised or stratified strategies need to be developed. These include advanced diagnostics to guide a personalised management of infected patients, personalised or stratified vaccination approaches, and patient-specific cellular therapies. In order to address these needs, scientists in the Research Focus TVAC aim at increasing the understanding of immune responses to infection and vaccination in humans, obtaining novel insights into helper T cell differentiation and function, and developing advanced vaccination strategies to improve the outcome of immune-based interventions against infectious diseases.

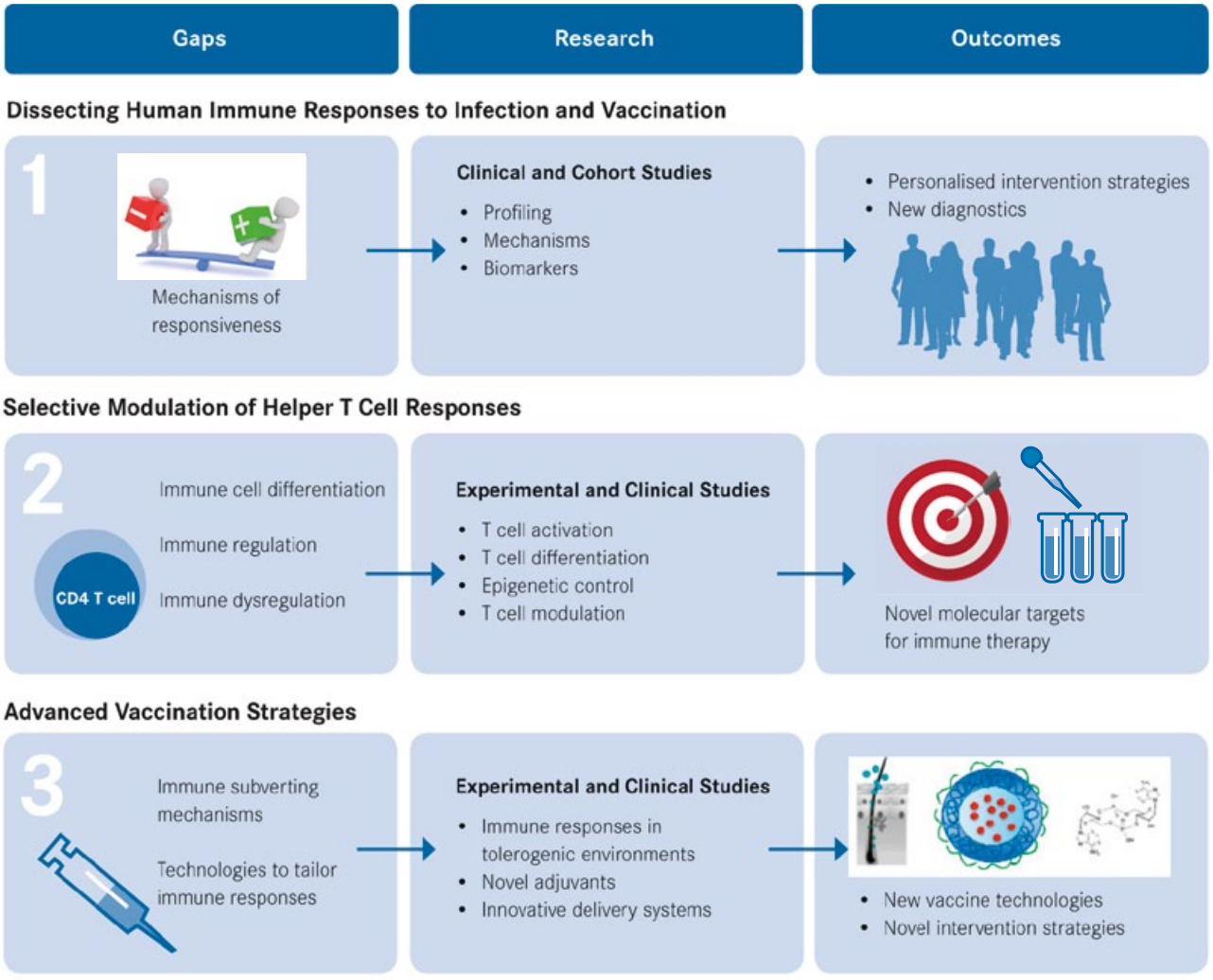


Figure 2: Overview of the research activities of the Research Focus T cell Targeting and Vaccination Strategies.

1. DISSECTING HUMAN IMMUNE RESPONSES TO INFECTION AND VACCINATION

To develop individualised immune-based interventions, it is critical to understand the specific basis of poor or non-effective responsiveness to infection or vaccination in each individual. For example, vaccine efficacy strongly varies amongst vaccinees, as responsiveness is strongly influenced by host-specific factors – age, health status, genetic background, metabolic state, microbiota composition – as well as vaccine-specific factors (*e.g.* antigen, adjuvant, delivery).

For instance, while the overall efficacy of trivalent inactivated seasonal influenza vaccines in individuals under 65 years of age is above 60 %, it drops to less than 20 % in individuals above 65 years.

The precise dissection of the molecular mechanisms underlying responsiveness and non-responsiveness to infection or vaccination will allow devising personalised intervention approaches as well as identifying predictive biomarkers to develop diagnostics for the stratification and follow-up of vaccinees and patients. Thus, observational and interventional studies have been started in different groups of poor responders, who were either recruited or secured through clinical cooperation partners at Hannover Medical School (MHH), Helmholtz Centre Munich (HMGU) and the Influenza Centre in Bergen. These patient groups included elderly, HBV vaccine non-responders, rheumatic patients under immunosuppressive therapy and others. The knowledge generated will be exploited to evolve tailored vaccination strategies based on predictive biomarkers accessible by diagnostics.

Selected Scientific Achievements

Patients with autoimmune disorders suffer from increased susceptibility to infectious diseases. Generally, it was assumed that the immune system of this group of patients shows enhanced frequencies of auto-reactive immune cells, but otherwise is normal. However, clinical studies showed that the entire helper T cell compartment displays an exhausted phenotype. Further interventional studies showed that treatment of patients with the CTLA (cytotoxic T-lymphocyte-associated) 4 fusion protein Abatacept resulted in reversion of the T cell exhaustion (1). It is tempting to speculate that this treatment will have an impact on the susceptibility of patients towards infectious diseases and their responses to vaccination.

Human vaccination studies were also initiated to identify individual immune signatures and early predictors of successful vaccination against influenza in the elderly. Recently, two trials were completed (>230 vaccinees, >65 years old),

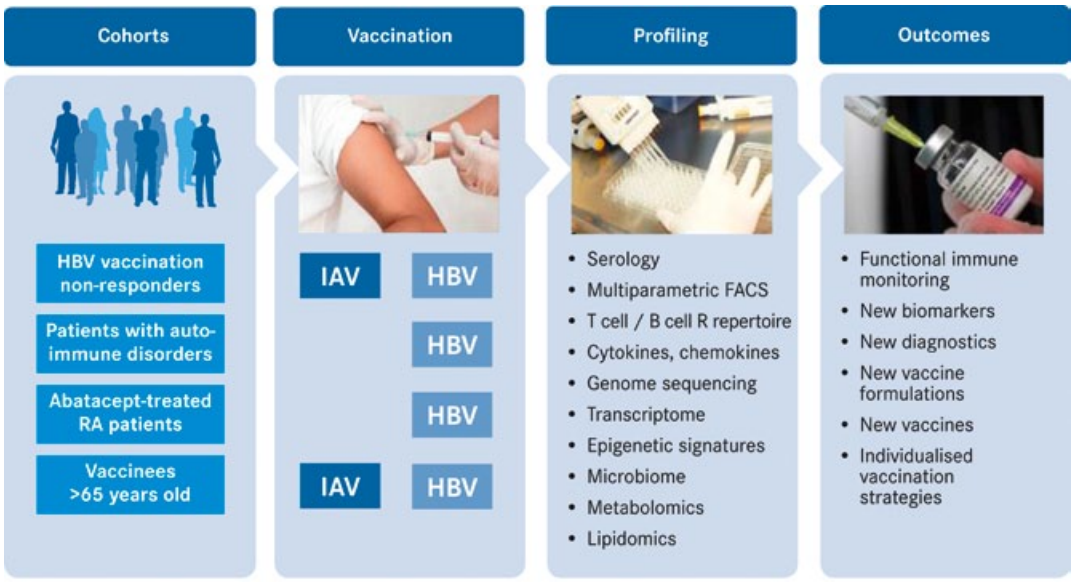


Figure 3: Ongoing and planned vaccination studies. Abbreviations: Abatacept, CTLA 4 fusion protein; IAV FACS, fluorescence-activated cell sorting, vaccine against influenza A; HBV, vaccine against hepatitis B; R, receptor; RA, rheumatoid arthritis.

in which samples were taken before and 1, 3, 7, 21 and 70 days post immunisation with the adjuvanted vaccine Fluad™ (2; Fig. 3). Comprehensive profiling is currently performed, which already led to the identification of two potential predictive biomarkers for non-responders (P1). Preliminary results point towards increased frequencies of follicular helper T (Tfh) cells and NK cells with a memory-like phenotype in responders to influenza vaccine. In this line, an international collaborative research network coordinated by HZI scientists found that the neurotransmitter dopamine released from Tfh cells is time-critical for the efficient generation of high-affinity antibodies (3). Finally, mathematical modelling of immune responses in influenza-infected mice suggested that the less efficient virus clearing in the elderly does not solely rely on a weaker immune response *per se*, but also on a weaker virus replication in aged host cells, which induces a subcritical immune stimulus and delays viral clearance (4).

2. SELECTIVE MODULATION OF HELPER T CELL RESPONSES

Most infections induce immune responses that are able to control and eliminate pathogens. For the orchestration of such immune responses, $CD4^+$ helper T cells play a key role. Accumulating evidence suggests that these cells come in multiple flavours and that highly specialised helper T cell subsets can be generated from naïve precursor cells to mount pathogen-tailored immune responses. However, dysregulated $CD4^+$ helper T cell responses cannot solely be responsible for ineffective pathogen eradication, but might cause over-shooting immune responses resulting in life-threatening

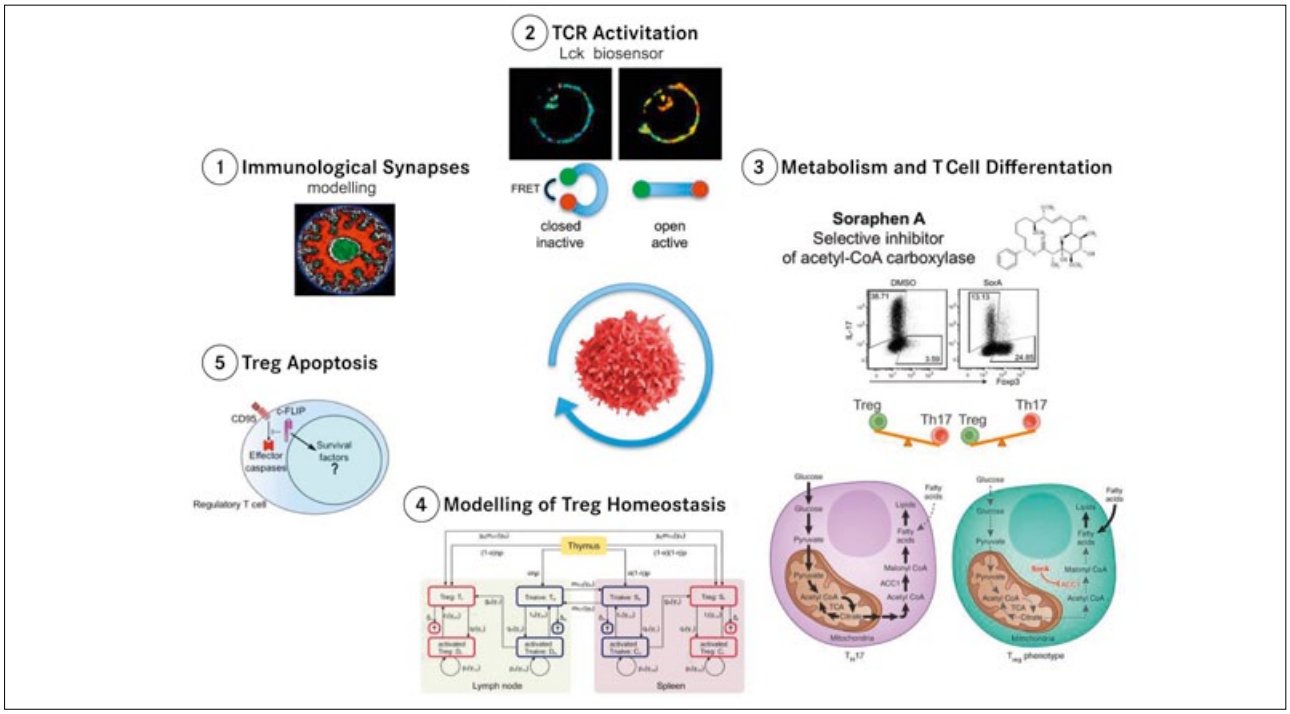


Figure 4: Selective modulation of helper T cell responses at different stages.

immunopathology. Thus, the molecular mechanisms underlying $CD4^+$ helper T cell differentiation and homeostasis were investigated. This is a prerequisite for the development of novel approaches to modulate pathogen-specific helper T cell responses.

Harnessing such immune responses for preventive or therapeutic purposes requires a detailed understanding of cellular players and molecular regulators controlling cell differentiation and function. Comprehensive analyses ranging from high-resolution imaging at the molecular level, proteome, metabolome, transcriptome and epigenome studies to *in silico* and *in vivo* analyses were initiated, allowing the description of basic principles controlling helper T cell differentiation and homeostasis. The use of advanced experimental mouse models enabled ascertainment of new paradigms and generation hypotheses that will be validated in a clinical setting. Overall, insights from these studies led to the identification of novel targets for the selective modulation of helper T cell-mediated immune responses.

Selected Scientific Achievements

Upon activation, naïve $CD4^+$ T cells differentiate into various helper T cell subsets depending on the strength of the TCR stimulus, costimulatory signals and the cytokine milieu. The differentiation process is finalised through epigenetic fixation of lineage-specific gene expression profiles. A unique epigenetic signature of murine Th17 cells was described (5), defining novel biomarkers as well as subset-specific molecular targets for future epigenome-editing approaches. Differentiated helper T cells also change their cellular metabolic programmes to meet the high energetic demands of activated T cells, and recent evidence suggests that metabolites themselves also play a critical role in helper T cell differentiation. It was demonstrated that the antioxidative glutathione can prime T cell metabolism for inflammation by activation of mTOR and support of Myc-dependent metabolic reprogramming, which allows activated T cells to switch to glycolysis and glutaminolysis (6). In a close cooperation of HZI scientists from all three Topics, *de novo* fatty acid synthesis was described as a critical metabolic pathway favouring the generation of inflammatory Th17 cells over regulatory T cells (Tregs) (7). With Soraphen A, a myxobacteria-derived small molecule from the natural compound library of HZI, a potent novel immune modulator was identified, which inhibits acetyl-coA-carboxylase (ACC1), the rate-limiting enzyme in fatty acid synthesis (P2). The use of Soraphen A allowed selective fine-tuning of the Treg/Th17 balance both in the murine and human systems (7). Thus, administration of Soraphen A allows preventing inflammation-driven immune pathology. Taken together, metabolic pathways can dictate T cell fate decisions, and distinct metabolic demands of specific helper T cell subsets make them highly sensitive to pharmacologic inhibitors of metabolism, allowing the development of novel therapeutic strategies.

3. ADVANCED VACCINATION STRATEGIES

The stimulation of a strong immune response following infection or vaccination is not a guarantee for effective immune clearance. The effector mechanisms stimulated could be inadequate to promote efficient clearance or might even lead to immunopathology. Furthermore, mechanisms of immune evasion can render immune responses ineffective. For example, pathogens can survive within a safe intracellular niche during chronic infections or promote immune exhaustion. To achieve efficient protection after vaccination, it is essential to elicit the required type of immune response. Knowledge on infection-mediated hindering mechanisms and technologies to promote tailored immune responses are therefore needed. Thus, a major focus is the understanding of immune subverting mechanisms as well as the develop-

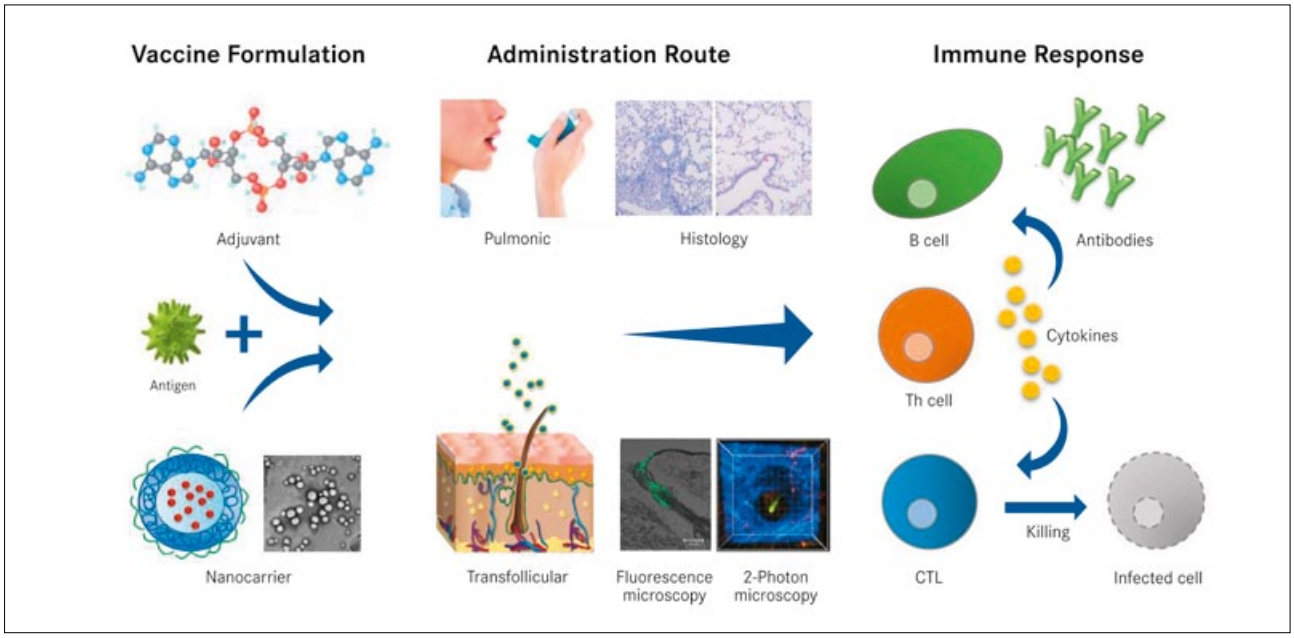


Figure 5: Advanced vaccination strategies.

ment of adjuvants and delivery systems enabling us to address current gaps in vaccinology, *e.g.* promotion of cellular immunity, amenable for needle-free vaccination.

A tolerogenic environment tightly balances, for example, immune responses in the liver, because this organ is constantly confronted with a high load of harmless, food-derived antigens. This may impair the clearance of hepatotropic pathogens, *e.g.* hepatitis viruses, and needs to be addressed in the design of vaccination strategies. Therefore, immune responses in the tolerogenic environment of the liver were studied in detail to close existing knowledge gaps.

Moreover, the choice of adjuvant is critical to modulate immune responses, improve their strength and breadth, and promote efficient memory. Hence, an adjuvant discovery programme to identify compounds with well-defined molecular targets was established.

Selected Scientific Achievements

In the adjuvant discovery programme, new TLR, STING and CD1d agonists were identified. Key observations were validated using human cells and advanced experimental models based on mice humanised for the immune system. Mode of action and structure-activity relationship analysis guided the generation of derivatives with improved biological properties. Among these, Cyclic dinucleotides (CDNs) emerged as the most promising candidate adjuvants, promoting not only humoral, but also polyfunctional Th cell and CTL responses in preclinical animal models (8, 9, P3). In addition, strategies were developed to promote *in vivo* antigen delivery to hepatic cross-presenting dendritic cells (DCs). Vaccine formulations targeting DEC205 or TLR2/6 on cross-presenting DCs were particularly effective in inducing virus-specific CTLs that were able to eliminate virus-infected hepatocytes in the tolerogenic environment of the liver (10).

The development of mucosal and transcutaneous vaccination approaches based on nanocarriers was the main focus in the area of delivery systems. Immunisation studies provided the proof-of-concept for needle-free transfollicular delivery of antigens. The use of antigen-loaded nanoparticles co-formulated with CDN allowed promoting both humoral and cellular responses (11). A complementary approach to target emerging pathogens was based on the use of cytomegalovirus (CMV) live vectors. To this end, gene expression circuits under control of regulatory signals promoting

memory inflation were implemented to stimulate strong cellular responses. CMV vectors (P4) expressing a single immune dominant antigenic epitope provided sterilizing immunity against Herpes simplex virus 1, and allowed growth control of human papilloma virus-induced carcinomas (12), highlighting the potential of this strategy. The context of gene expression determined the size and dynamics of T cell responses, whereas antigen availability for processing determined sustained T cell stimulation and effector phenotype persistence (12).

PERSPECTIVES

Addressing the challenges of modern infection medicine, TVAC researchers will perform further experimental and clinical studies to understand poor immune responsiveness to infection and vaccination. Studies are planned to validate the identified putative biomarkers for responsiveness to influenza vaccines in the elderly, as well as to assess their universal value for other groups of poor responders to vaccination. Additional vaccination studies will unveil the molecular mechanisms responsible for non-responsiveness to vaccination against Hepatitis B and will address responsiveness to vaccination of lung transplanted and chronic liver disease patients. Overall, these clinical studies will provide the knowledge and technologies to evolve an individualised predictive vaccinology.

Selective targeting and modulation of human helper T cell subsets is another key future aim of TVAC. To this end, *in vivo* dynamics of helper T cell differentiation will be dissected in both advanced animal models and 3D human tissue cultures using novel single-cell technologies. Mathematical models of T cell differentiation will be used to understand tissue-specific differences of immune responses, and the molecular mechanisms controlling these processes will be identified. Epigenome-editing of T cells will be developed to improve personalised adoptive cell therapies.

A main goal will be the development of novel intervention strategies for vulnerable individuals or against resilient pathogens. New nanocarrier-based formulations specifically targeted to different subsets of immune cells will be evaluated preclinically. Activities on the delivery of RNAs will be further expanded. GMP-compliant production of CDN will be carried out to be tested in clinical trials. Generated knowledge on biomarkers will also help to evolve tailored vaccination strategies based on predictive diagnostics.

CARLOS A. GUZMÁN

JOCHEN HÜHN

Further contributions:
Wolf-Rainer Abraham, Mark Brönstrup, Dunja Bruder, Luka Čičin-Šain, Karsten Hiller, Lothar Jänsch, Markus Kalesse, Ulrich Kalinke, Claus-Michael Lehr, Michael Meyer-Hermann, Andreas Müller, Rolf Müller, Frank Pessler, Ingo Schmitz, Burkhard Schraven, Tim Sparwasser, Till Strowig, Jörg Vogel, Dagmar Wirth.

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Patents Research Focus TVAC

P1. Pessler F, Guzmán CA, Riese P, Akmatov M. Biomarker im peripheren Blut für die Vorhersage einer unzureichenden Immunantwort auf die saisonale Grippeimpfung beim Menschen**.** [**patent application in 2018**].

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RESEARCH FOCUS EPIDEMIOLOGY FOR PUBLIC HEALTH SOLUTIONS (EPI)

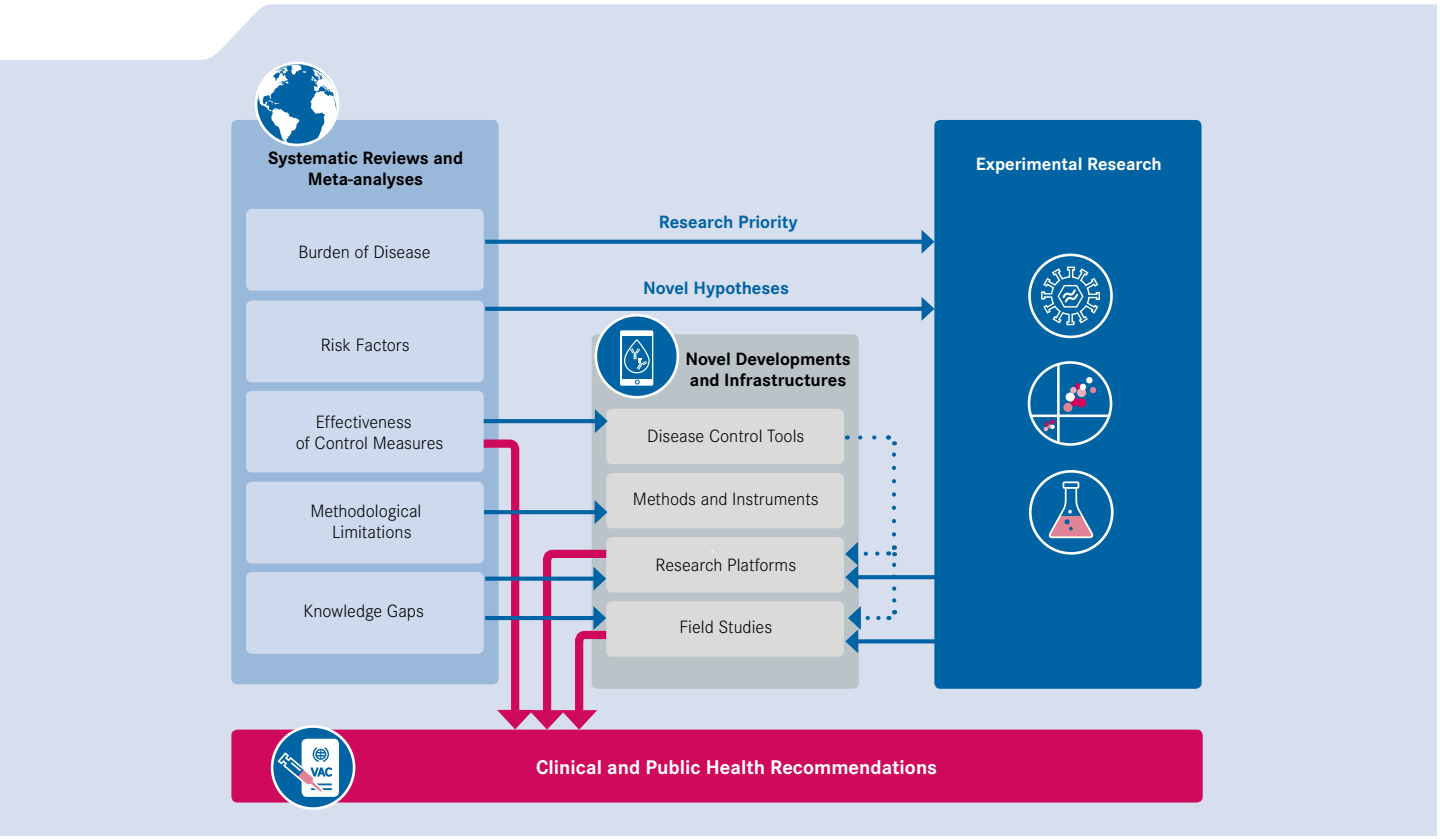

Figure 1: Translational feed-back cycle of epidemiological research linked to experimental research at HZI.

The transmissibility of infectious diseases can rapidly change their epidemiology, but also offers a unique leverage for prevention and control. This, however, requires a societal systems approach, which can also be referred to as Public Health approach. The Research Focus "Epidemiology for Public Health Solutions" (EPI) contributes to Public Health solutions by investigating the burden and the determinants of infectious diseases, by assessing the effectiveness of prevention measures, and by developing novel approaches for the control of infectious diseases at the population level.

In 2011, HZI has established the Department Epidemiology, which has since developed numerous productive interactions with research groups at HZI as well as external cooperations with over 40 research partners in Germany and abroad and has thus become the core of the Research Focus EPI. Fig. 1 shows the cross-link between epidemiological and experimental research at HZI.

Major challenges are being addressed by epidemiological research on viral hepatitis, including the development of differential serology, the establishment of different long term cohort studies, and the development of novel digital tools to support these.

1. BURDEN OF DISEASE AND PREVENTION OF VIRAL HEPATITIS

Acute and chronic viral infections cause a high burden of disease and only limited therapeutic options are available. These characteristics raise the importance for targeted preventive strategies at population level. This requires improved assessments of global burden of disease, and population-specific risk factor assessments in order to provide evidence for novel treatment and immunisation strategies.

So far, serological tests for infections mainly serve diagnostic differentiation for treatment decisions in acute infections. These tests – with the exception of HBV – generally do not allow differentiation between previous natural infections and successful vaccination against a pathogen. Reliable estimates of vaccine effectiveness and burden of disease need a differential serological approach that allows parallel detection of multiple targets in only small amounts of serum in a cost-effective manner.

The Research Focus Epidemiology generates evidence on viral hepatitis using several synergetic approaches, by:

- conducting meta-analyses based on existing scientific work applying systematic reviews and epidemiologic modelling,
- initiating epidemiological field studies in populations for which primary scientific studies are scarce, because of particular infrastructural and socio-cultural challenges,
- developing novel and validating existing methodological tools for conducting such primary field studies where they are needed the most.

These approaches will allow identification of promising targets and target groups that the researchers of the Research Focus CVIR will follow up on. They will also lay the basis for an assessment of the impact of future treatment and immunisation strategies.

Selected Scientific Achievements

Meta-analyses on Hepatitis B Prevalence, Trends and Vaccination Coverage

HZI scientists performed a systematic analysis about prevalence on HBV in 161 countries. In 2010, global HBsAg seroprevalence was 3.6% (95% CI 3.6–3.6) with highest endemicity in African and Western Pacific regions (Fig. 2) (1). An assessment of time trends showed globally reductions in prevalence, but also increases in some African countries (2). Furthermore, coverage of hepatitis B vaccination and its schedules were examined. Results showed substantial delays in vaccination, even in countries with fairly high coverage. Lower odds of delays were seen in vaccinations starting between 6 and 9 weeks of age (3).

Field Sero-Survey on Hepatitis B in Sub-Saharan Africa

The HZI in collaboration with DZIF partners is conducting a sero-epidemiological field study in a representative sample of the urban and rural population in Northern Burkina Faso, one of the regions for which no reliable data on hepatitis epidemiology is available so far. Capillary blood samples from over 4000 individuals have already been collected and are currently being tested.

Field Sero-Survey on Hepatitis B and C in Kyrgyzstan

EPI scientists conducted a sero-prevalence study on HBV, HCV, HIV and *Treponema pallidum* among blood donors in Kyrgyzstan (n = 37,165). HBsAg prevalence was 3.6% (95% CI 3.0–3.8%). HIV prevalence was higher than earlier estimates, with a rising trend, indicating the need for urgent public health actions.

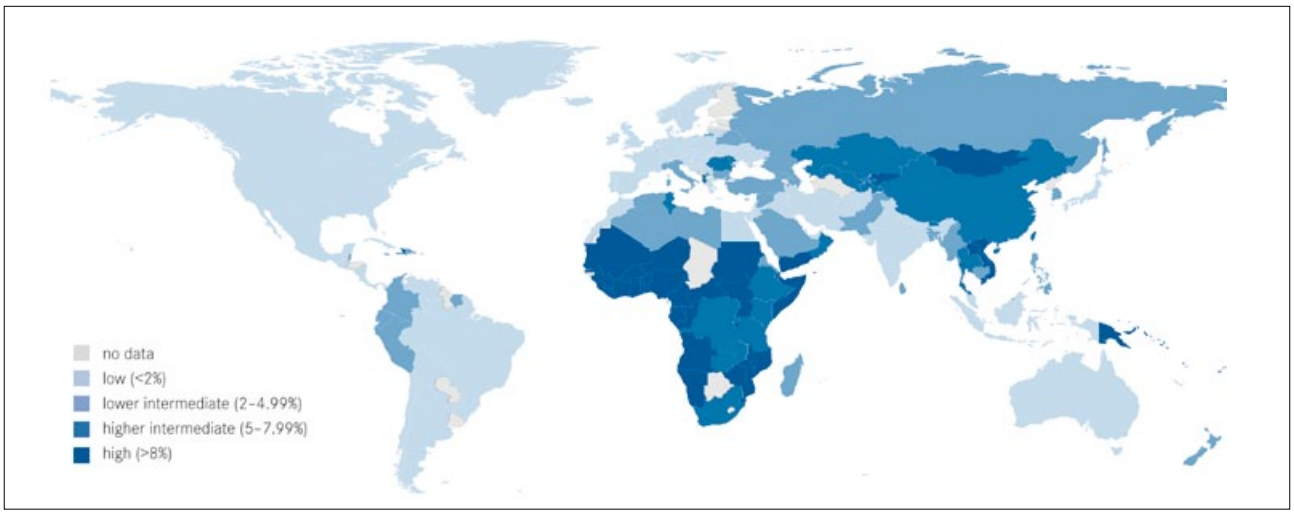

Figure 2: Global HbsAg endemicity (1957–2013). Modified from Schweitzer et al. Lancet 2015.

Differential Serology for Vaccine Preventable Diseases

Within the Research Focus EPI a Luminex®-based differential serology platform has been developed, which distinguishes between antibodies resulting from natural infections and those resulting from vaccination. HZI succeeded in identifying the 2A polyprotein in HAV as the discriminating antigen, leading to a sensitivity of 100 % and a specificity of 95 % with accuracy for identifying vaccinated individuals of 91 % and infected individuals with 74 % (P1, 4-5).

2. LONG-TERM COHORTS ON INFECTIONS CONTRIBUTING TO NON-COMMUNICABLE DISEASES

Prospective long-term cohorts are essential for investigation of predictors and risk factors for incident diseases in the context of personalised medicine. In particular, the role of acute transient or chronic infections in causing or contributing to non-communicable diseases is a broad field of medicine for which knowledge is very scarce. At the same time discovery of such associations carries tremendous potential for effective preventive and therapeutic approaches. The discovery of *Helicobacter pylori* as the causal agent of gastric ulcer and specific forms of gastric cancer in 1983/84 and at the beginning of the 1990s, respectively, has introduced the option for antimicrobial treatment, which has dramatically reduced the respective burden of disease. Another example is the discovery of the causal role of human papilloma virus (HPV) for cervical cancer and the subsequent development of a vaccine against the most relevant HPV strains, which is now an important element of the vaccination schedule in many countries. It is likely that many more such associations between infectious agents and non-communicable diseases still remain unrevealed for a variety of reasons.

Multidisciplinary, prospective cohort studies among humans offer opportunities to detect aforementioned complex associations. Inversely, the cohort approach also allows investigation of risk factors for susceptibility to and severity of infectious diseases, and thus potentially contributes to reducing disease burden directly resulting from them.

Selected Scientific Achievements

The German National Cohort (GNC)

The GNC is Germany's largest Health Study, including 18 study centres throughout Germany enrolling 200,000 adults representatively recruited from the general population (6). HZI runs one of the 18 study centres and hosts the competency unit of vaccination data for all 18 study centres (7, 8; see German National Cohort).

The LöwenKIDS Birth Cohort

The LöwenKIDS birth cohort is the the largest study worldwide that investigates individual history of infections and vaccinations in the first six years of life. In August 2017, 702 participants had been recruited in five German study centres (9).

The DZIF Transplant Cohort (DZIF TxCohort)

The TxCohort enrols prospectively adult patients with organ or stem cell transplantations from the five largest organ transplant centres in Germany. HZI contributes to the epidemiological design of the study and is in charge of routine data analyses of the cohort study as well as of data quality assessment. In May 2018, 758 patients were enrolled into the study protocol. After two pilot phases, TxCohort is currently in the recruitment phase, with the first patients having completed one year of follow up.

The ChilSFree Study

The TxCohort is further complemented by the ChilSFree study, a multinational cohort study focusing on immunomonitoring after paediatric liver transplantation. The aim of ChilSFree is to identify markers and patterns of immune reactivity that will predict the occurrence of acute cellular rejection and infectious complications, with the prospect of guiding immunosuppressive therapy in the future. Patient enrolment for ChilSFree was completed in 2017; data analysis performed by the HZI is currently ongoing.

3. DIGITAL EPIDEMIOLOGY FOR DISEASE CONTROL

The tragic deficiency of the West African public health system apparent during the Ebola Disease epidemic in 2014/15 has demonstrated the urgency of developing technology to detect and manage disease outbreaks much more efficiently.

Such a system needs to be designed so that it still works in areas of the world where no continuous access to internet, electricity and a reliable mobile or landline phone grid is available. This evolving Information and Computational Technology development is generally referred to as electronic or mobile health (eHealth, mHealth), and is an essential element of recent strategies of the WHO, the World Bank, the West African Union and the recently inaugurated African Centre for Disease Control.

mHealth also has the potential to address challenges in today's clinical research and management. It may significantly improve recruitment and retention rates of epidemiological studies. Smartphone-based mobile applications allow capturing acute infections or undesired events after vaccination in real time. Furthermore, digital epidemiology can offer solutions to address the increasing challenge of nosocomial infections in hospitals through modern tools of medical informatics.

Epidemiology at HZI has set a priority in research and development on digital epidemiology and particularly mobile device-based applications. HZI has been and is working with small or medium IT companies as well as with very large ones, such as SAP.

A guiding principle is to develop tools in an open source approach wherever possible, to assure free access for the international scientific community and public health institutions in low resource settings.

Selected Scientific Achievements

Surveillance Outbreak Response Management and Analysis System (SORMAS)

During the West-African Ebola Epidemic in 2014, Nigerian partners, academic institutions from Germany and SAP developed SORMAS. This first version provided seven different interfaces for users (*e.g.* health informant, state epidemiologist) and included four diseases (*e.g.* Ebola). To assure sustainability and independence from software providers, in 2016 an open source version of SORMAS was developed. New functionalities (*e.g.* interoperability with other health systems (*e.g.* DHIS)), user interfaces (*e.g.* laboratory users) and other prone diseases were added into SORMAS. In 2017 SORMAS was implemented in the field in two local government areas of Kano State in Nigeria. Furthermore, SORMAS was used for the response to the monkeypox outbreak in more than 16 states in Nigeria (10-12).

Mobile Application for Prospective Monitoring of Acute Infections (PIA)

HZI researchers developed PIA to prospectively investigate risk factors for acute respiratory infections, acute gastrointestinal infections, and acute urinary tract infections; and second, to investigate the role of infections as risk factors for non-communicable diseases. The app will send regularly notifications to answer questions about signs and symptoms. A pilot study will start in December 2017; the field phase will start in spring 2018.

Mobile Application for Prospective Pharmacovigilance of Vaccines

In collaboration with the Paul-Ehrlich- Institute, the HZI is developing another mHealth tool, a prospective monitoring system for undesired events following vaccination. The tool aims to mitigate multiple biases. Similar to PIA, users receiving questions about occurrence of symptoms after influenza vaccination. This multi-centre study was piloted in 2017 and will be initiated in fall 2018.

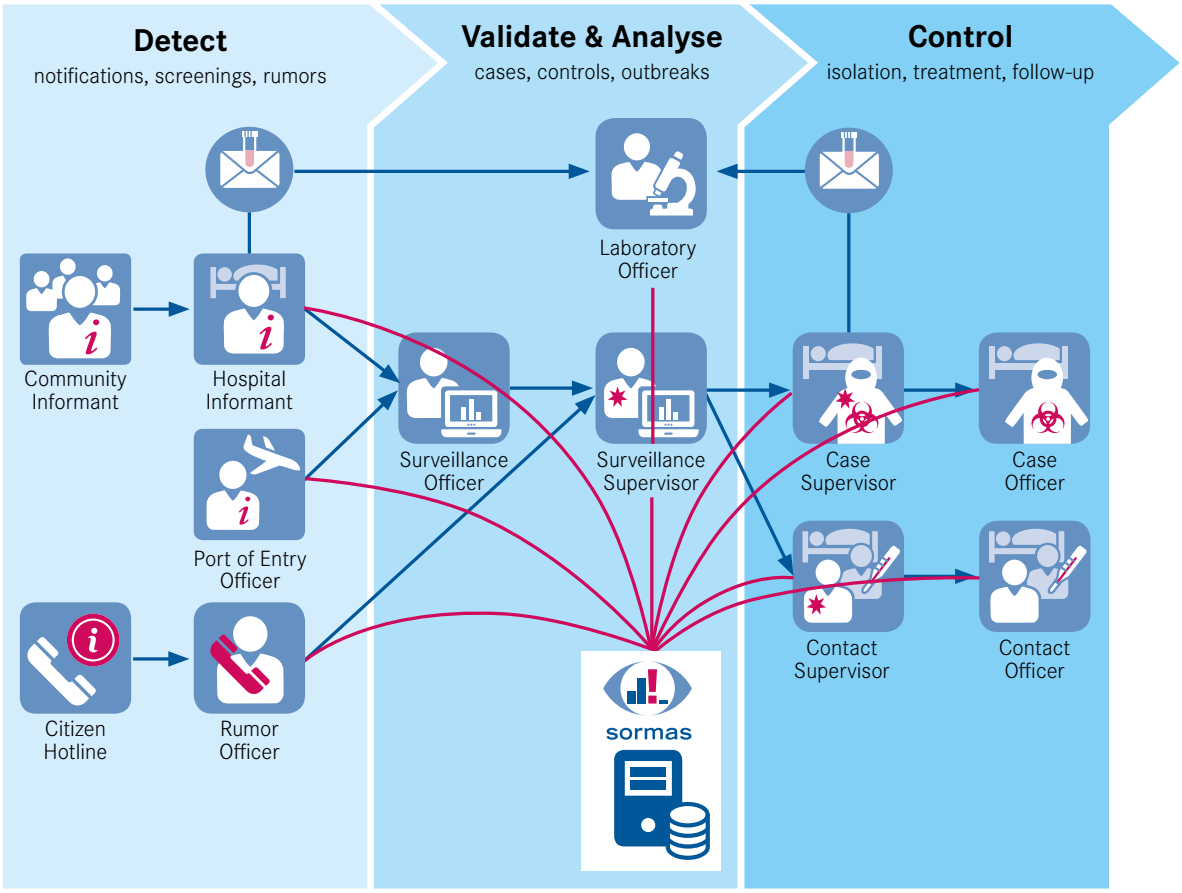

Figure 3: Overview Process Model of SORMAS.

PERSPECTIVES

Current challenges in epidemiological research include increasing hesitance in the population to volunteer for research studies, expanding requirements on data confidentiality, costs of sampling, and storing and analysing bio-specimens for large study populations. Improvement of immunisation strategies requires better diagnostic approaches to distinguish between naturally-infected individuals and those with successful vaccination.

To overcome these challenges, the Research Focus EPI will continue to develop novel research instruments that lower the threshold for individuals to participate in studies, stimulate individual benefits for study participants and develop novel diagnostic procedures that are less invasive, require less bio-material, and generate information to support public health policies. Thus, EPI researchers will contribute significantly to improved clinical management especially in the context of personalised medicine.

In general, HZI aims at addressing research questions that so far – due to methodological constraints – could not be properly addressed. On this basis, the Research Focus EPI will intensify the generation of applied tools and of epidemiologic evidence with direct public health implications at national and global level. In particular, the mHealth applications developed by HZI for epidemiological research have the potential to evolve to platforms for a much broader scope of research topics. Current and future achievements of the Research Focus EPI will become available for the scientific and public health community at large.

With respect to the differential serology, the aim is to expand the portfolio beyond hepatitis viruses, to possibly address pathogens that are targeted for global elimination, such as measles.

GÉRARD KRAUSE

STEFANIE CASTELL

Further contributions:
Mark Brönstrup, Carlos A. Guzmán, Alice McHardy, Rafael Mikolajczyk, Frank Pessler, Eike Steinmann, Joop van den Heuvel.

Key Publications Research Focus EPI

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5. Filomena A, Pessler F, Akmatov MK, Krause G, Duffy D, Gärtner B, Gerhard M, Albert M, Joos T, Schneiderhan-Marra N (2017) Development of a Bead-Based Multiplex Assay for the Analysis of the Serological Response against the Six Pathogens HAV, HBV, HCV, CMV, *T. gondii*, and *H. pylori*. *High Throughput* 6: 14.

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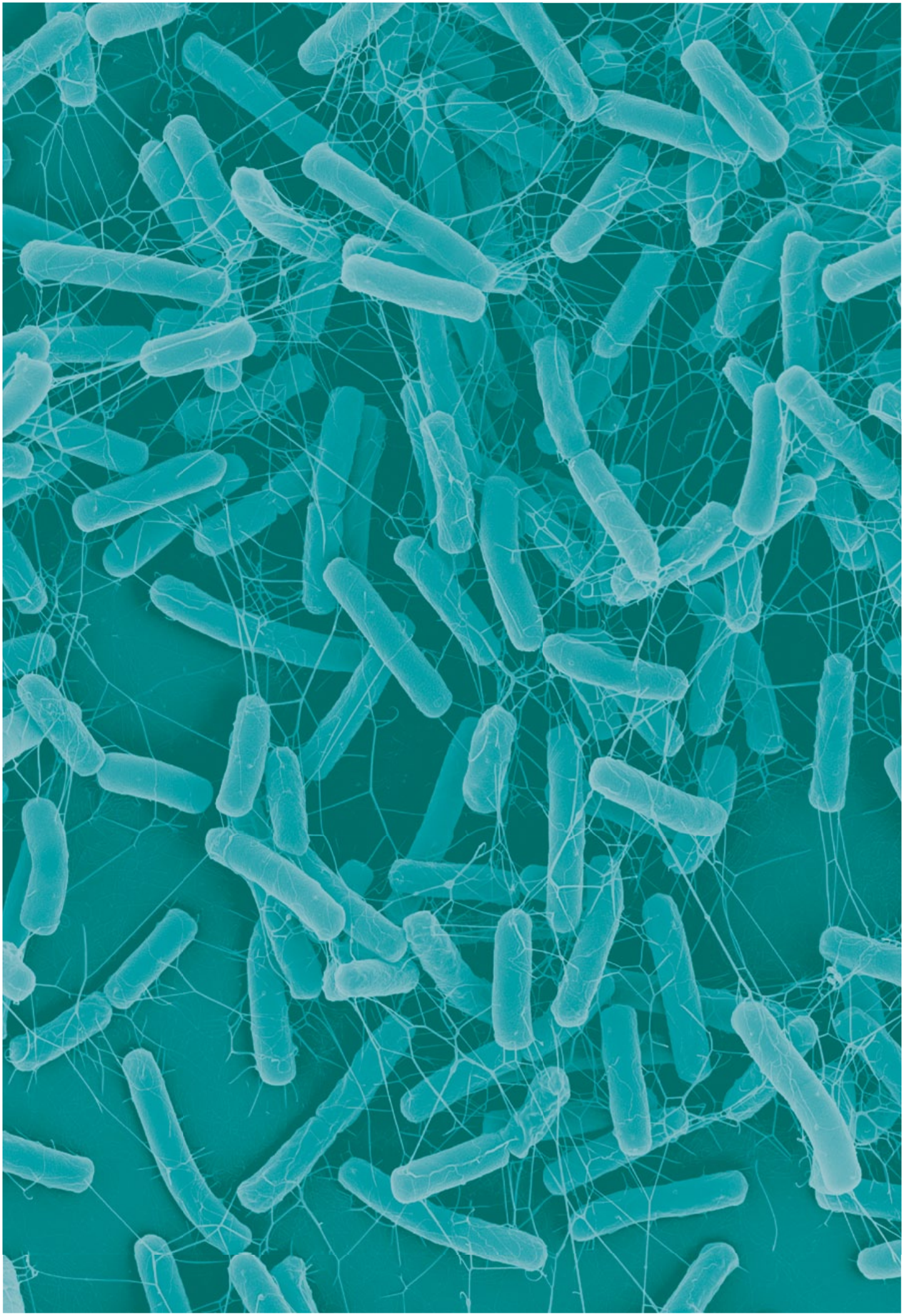
Patents Research Focus EPI

P1. Bohm K, Sievers C, Krause G (2017) Method for differentiation of immune response in an individual. **EP 3173789 A12017.**



HIGHLIGHT PUBLICATIONS

MOLECULAR TROJAN HORSES AS THERANOSTICS FOR BACTERIAL INFECTIONS

Mark Brönstrup, Head of Department Chemical Biology

Infections with multi-resistant Gram-negative bacteria are particularly difficult to control with antibiotics. Drugs must first penetrate a double-hulled cell wall to exert a cytosolic effect. In addition, some infections are difficult to diagnose because they are located deeply inside the body. A promising strategy for the detection and control of these infections is to use molecular probes with theranostic properties: these allow for simultaneous diagnosis and therapy of the infection at an early stage. We recently developed molecular probes that utilise the iron transport system of bacteria to transport antibacterial agents into the bacterial cell. The probes are based on siderophore conjugates with catechol moieties, complexing iron(III), as well as a central DOTAM scaffold and can accommodate a metal ion (*e.g.* for PET imaging), as well as an antibiotic moiety and are therefore suited for theranostic purposes. These "molecular Trojan horses" can also carry fluorescence markers and thus are able to visualize infections in smaller animals.

Infections caused by multidrug-resistant Gram-negative bacteria result in significant mortality and morbidity worldwide. The lack of knowledge on how to assure a sufficient translocation of bioactive molecules across the complex Gram-negative cell wall represents the main scientific hurdle that hampers the discovery and development of effective agents versus Gram-negative pathogens. Also the diagnosis of infections needs technological innovations – this concerns *inter alia* novel methods to detect infections at sites that are unknown or inaccessible for sampling, *e.g.* biomaterial-associated infections or endocarditis. An innovative approach to efficiently translocate bioactive small molecules as well as imaging compounds over the Gram-negative cell wall uses biomimetic conjugates that embark on essential bacterial transport systems. We developed a multifunctional agent that exploits the iron transport system of bacteria. The amount of iron required by bacteria is covered by the excretion of siderophores. These low molecular weight compounds scavenge iron even from host proteins like ferritin and are actively taken up by bacteria. This is a great opportunity for us to design artificial agents, which the bacterial cell will take up readily. Our strategy is reminiscent of the Trojan horse: we can easily append an antibiotic effector to artificial agent complexes and covertly transport the effector into the bacterial cell, where it can exert its antibacterial effect. The tetrapodal DOTAM (1,4,7,10-tetraazacyclodode-

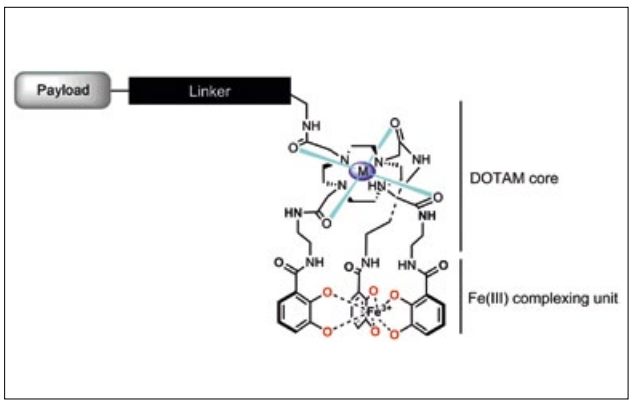


Figure 1: General structure of siderophore – dye/antimicrobial conjugates with a payload connected by a variety of linkers to a DOTAM moiety, able to complex metal ions *e.g.* for PET imaging approaches, which in turn is connected to three Fe-(III)-complexing catechol moieties.
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cane-1,4,7,10-tetraaceticamide) moiety was selected, as it fulfills all functional and synthetic requirements and in addition confers several advantages such as high water solubility, low toxicity and proven biocompatibility. Three of the four arms branching from the DOTAM core were functionalized with catechol moieties for iron binding. The fourth arm was either coupled to flurophores for siderophore-based imaging approaches or functionalized with antibiotics for treatment of bacterial Gram-negative pathogens (Figure 1).

Their ability to cross the Gram-negative cell wall was evaluated by growth-recovery assays in siderophore-deficient strains (*E. coli* Δ*entA* and *P. aeruginosa* Δ*pvd*/Δ*pch*) as well as by imaging experiments with a FAP/MG system adapted to *E. coli*, proving the broad acceptance and applicability of the developed Trojan horses by Gram-negative and Gram-positive strains. An ampicillin-DOTAM conjugate inhibited the growth of siderophore-deficient *E. coli* strains with IC_{50} values comparable to those of ampicillin (*E. coli*: Δ*entA* 2.1 to 2.1 μM). Furthermore, fluorescent-labelled bacteria were further analyzed by confocal microscopy, flow cytometry and stained with propidium iodide to exclude an antibacterial effect of the employed siderophore-dye conjugate. No enhanced amounts of dead cells upon treatment with the DOTAM-Cy5.5 probe could be observed, underlining the validity of uptake into live cells. Co-localization of bioluminescence signal in *P. aeruginaosa* (PAO1) infected IFN-β luciferase reporter mice with the fluorescence induced by the DOTAM-Cy5.5 probe could demonstrate correct labelling at the site of infection (Figure 2). In summary, siderophore conjugates based on a DOTAM scaffold have been characterised as multifunctional agents that are applicable for the diagnosis and treatment of bacterial infections. Future studies are focused at improving antibacterial properties of the molecular probes further, and at their applicability for the molecular imaging of infections in larger animals, and finally humans.

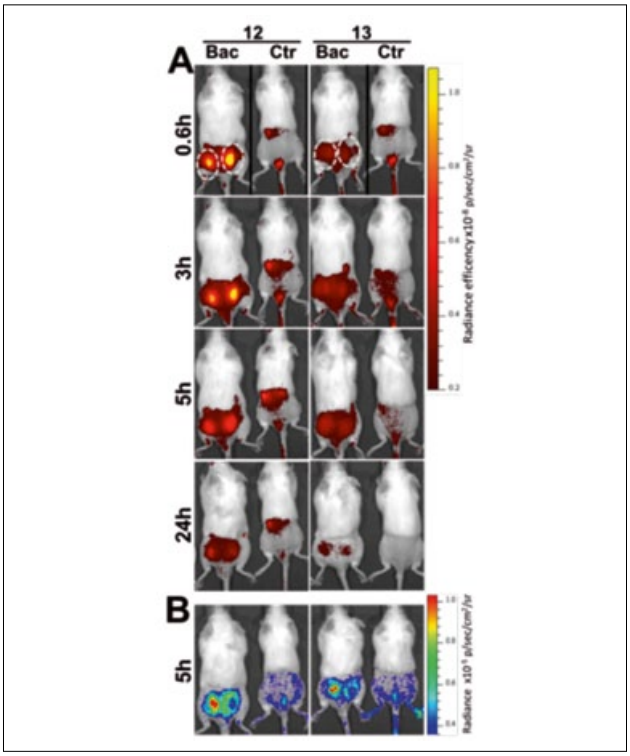


Figure 2: Small-animal model for the diagnosis of bacterial infections by siderophore conjugates. a) A suspension of bacteria (*P. aeruginosa* PAO1) was injected subcutaneously into the back (left and right) of interferon-β luciferase reporter mice at the positions indicated by dashed white ovals (Bac), followed by intravenous injection of 12 (DOTAM-Cy5.5) or the control compound 13 into the tail vein. Uninfected animals (Ctr) were treated likewise. The fluorescence images were recorded after 0.6,3, 5, and 24 h. b) A luciferin solution was injected intraperitoneally, and the animal bioluminescence induced by the host immune response was recorded after 5 h. Printed with permission from John Wiley and Sons.

Ferreira K., Hu H.-Y., Fetz V., Prochnow H., Rais B., Müller P.P., Brönstrup M. (2017) **Multivalent Siderophore-DOTAM Conjugates as Theranostics for Imaging and Treatment of Bacterial Infections**. *Angewandte Chemie – International Edition.* 56 :8272–8276

DISCOVERING HOST-PATHOGEN INTERACTIONS BY TISSUE DUAL RNA-SEQ

Petra Dersch, Head of Department Molecular Infection Biology

A detailed knowledge of virulence-relevant genes of pathogens and pathogen-induced host responses is required to obtain a comprehensive understanding of host-pathogen interactions that allows us to design effective treatment strategies. For this purpose we developed an unbiased probe-independent RNA sequencing approach (*Tissue dual RNA-seq*) to simultaneously monitor genome-wide, infection-linked transcriptional alterations of the host tissue and the colonizing pathogen.

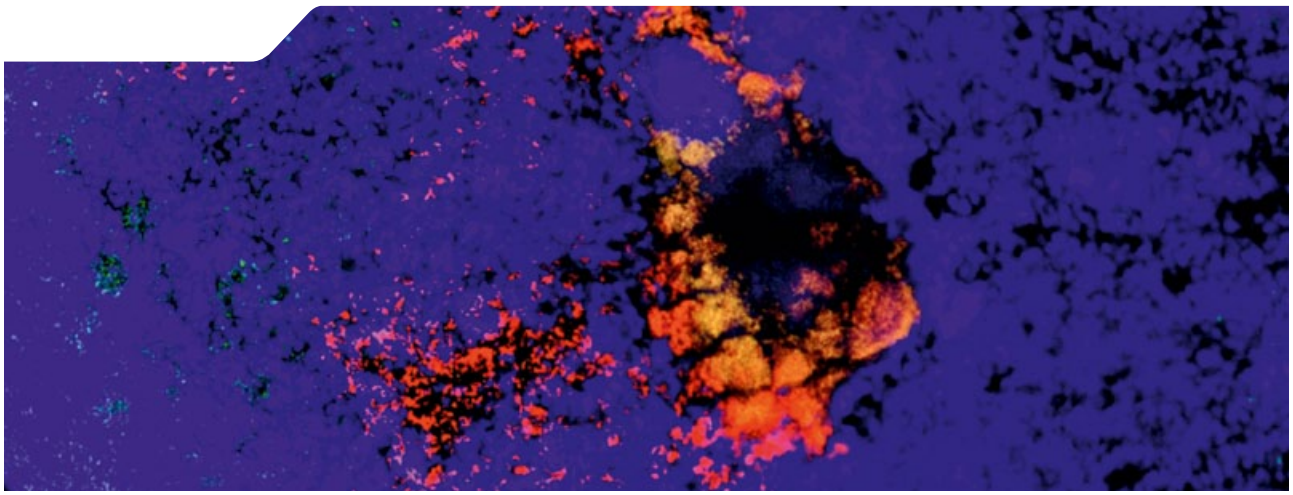


Figure 1: Attack of infecting Yersinia pathogens (labeled in red) by host immune cells in a Peyer's patch.

The interplay between enteric pathogens and their host is highly complex and very dynamic. Both interaction partners must rapidly reprogramme their expression profile in order to adapt to the conditions experienced in the different host niches for survival and proliferation. In the initial infection phase, the pathogens express flagella to cross the mucus layer and multiple adhesion factors to colonize the intestinal tract. Moreover, they induce certain metabolic pathways, which allow them to import and utilise nutrients that are available in the intestinal tract. Invasive enteric pathogens such as *Yersinia pseudotuberculosis* can also pass through the intestinal epithelial layer and colonize underlying lymphoid tissues, in which they are attacked by immune cells, in particular neutrophils. To prevent their eradication, they have to respond by inducing efficient immune evasion strategies. To capture virulence and host defense traits as well as regulatory processes steering host-pathogen interactions during the infection, we established a probe-independent RNA-sequencing approach termed *Tissue Dual RNA-seq*.

For this approach, we lysed *Yersinia*-infected lymphoid tissue of the ileum of mice. Total RNA of the bacteria and host cells was isolated, the ribosomal RNA was depleted and the remaining RNA pools converted into cDNA libraries and subjected to Illumina sequencing. Sequencing reads were mapped to the Yersinia genome and virulence plasmid, and the mouse core genome. Data normalization and analysis of the aligned reads allowed us to simultaneously profile the transcriptome of *Y. pseudotuberculosis* and its infected host. A detailed differential expression analysis between in-

fected and uninfected mice revealed known, but also many unknown host immune responses. *Y. pseudotuberculosis* triggered a very strong IL-6 promoted inflammatory and acute phase response in the lymphoid tissue, induced an activation of the coagulation cascade for fibrin matrices formation around the bacterial colonies and initiated a T_H1/T_H17 response to eliminate the bacteria. The pathogen itself was found to increase the gene and expression dose of important pathogenicity traits (type III secretion system, antiphagocytic Yop effector proteins) and induce virulence-related functions counteracting phagocyte-triggered Fe^{2+}, Zn^{2+}, and Mn^{2+} deprivation, radical stress and nutritional restraints. Moreover, numerous non-coding RNAs and ribo-regulators of the pathogen, influencing this response, were identified. The strongest impact was found for the RNA binding protein CsrA of the carbon storage regulator system, which was shown to upregulate the type III secretion system and the antiphagocytic Yop effectors upon host cell contact.

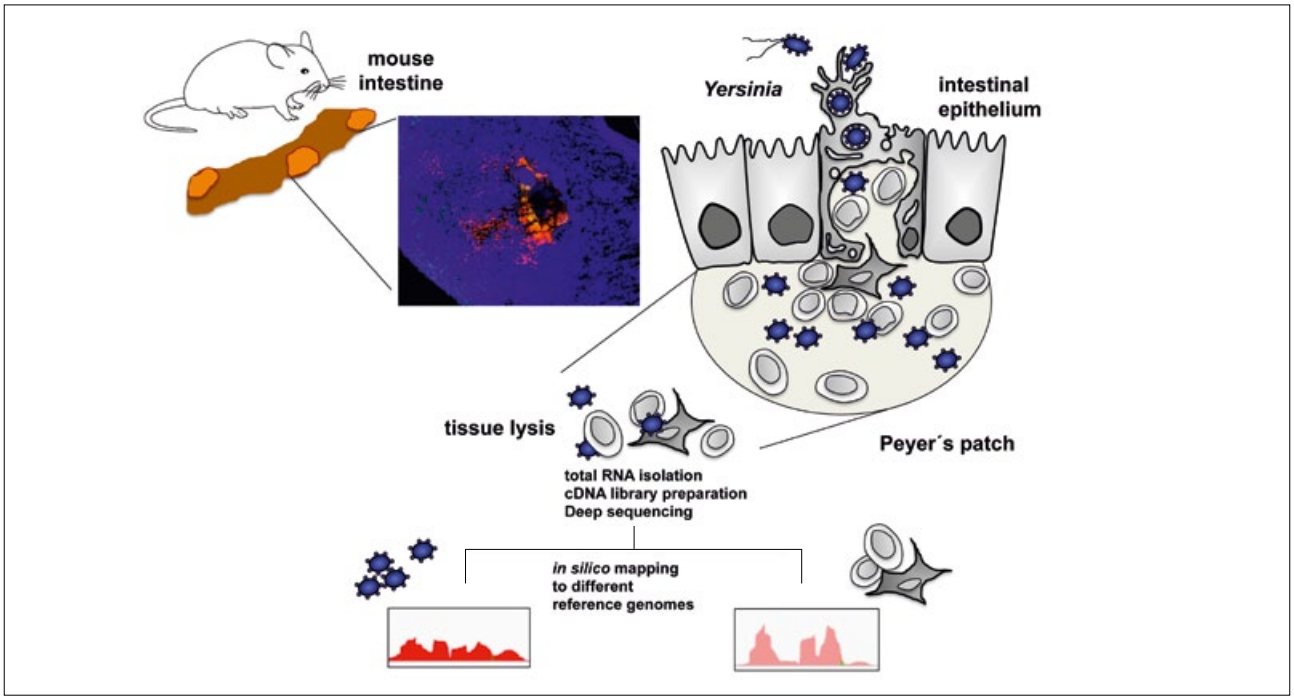


Figure 2: Scheme of the *Tissue dual RNA-seq* approach. Infected mouse ileum was prepared in which Yersinia replicates as microcolonies (red-labelled colonies) in the gut-associated lymphoid tissues, the Peyer's patches. Peyer's patches were isolated, total RNA was prepared and cDNAs produced which were sequenced using a Illumina Hiseq2500 sequencer. Obtained reads were mapped onto the *Yersinia pseudotuberculosis* and the mouse genome.

Nuss A. M., Beckstette M., Pimenova M., Schmühl C., Opitz W., Pisano F., Heroven A.K., Dersch P. (2017) **Tissue dual RNA-seq allows fast discovery of infection-specific functions and ribo-regulators shaping host-pathogen transcriptomes**. *Proc. Natl. Acad. Sci. USA.* 114(5): E791-E800

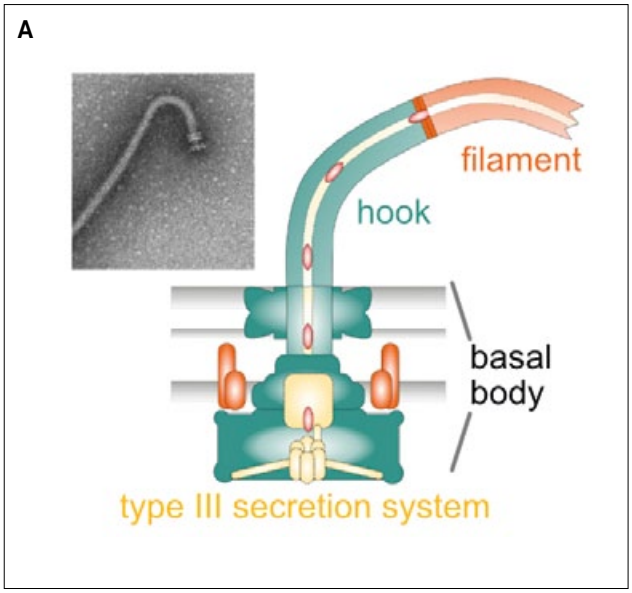
BACTERIAL FLAGELLA GROW THROUGH AN INJECTION-DIFFUSION MECHANISM

Marc Erhardt, Head of Junior Research Group Infection Biology of *Salmonella*
Since 2017: Prof. for Bacterial Physiology Humboldt-Universität, Berlin

***Salmonella enterica* employs a wide variety of virulence factors, including flagella, adhesins and effectors secreted via the injectisome to establish a successful infection. These molecules are needed to propel the bacteria towards target cells, attach to and infect host cells, as well as survive in intracellular environments. The bacterial flagellum and the injectisome are self-assembling nanomachines. The external filament of the flagellum is several times longer than a bacterial cell body and made of a few tens of thousands subunits of a single protein: flagellin. A fundamental problem concerns the molecular mechanism of how the flagellum grows with remarkable speed outside the cell, where no discernible energy source is available.**

Many bacteria move by rotation of a helical organelle, the flagellum. The external flagellar filament is several times longer than a bacterial cell body and is made out of up to 20,000 flagellin subunits. A type III export apparatus located at the base of the flagellum utilises the proton motive force (pmf) as the primary energy source to translocate axial components of the flagellum across the inner membrane. Exported substrates travel through a narrow 2 nm channel within the structure and self-assemble at the tip of the growing flagellum. It has been a mystery for several decades how bacteria manage to assemble the building blocks of flagella outside of the cell, where no discernible energy source is available.

Here, we monitored the dynamic assembly of individual flagella using *in situ* labelling and real-time immunostaining of elongating flagellar filaments. We first monitored, in real-time, the dynamic assembly of individual filaments by employing a continuous *in situ* immunostaining approach to visualize growing flagella. A *Salmonella* strain was grown in a microfluidic device in the presence of labelled anti-flagellin antibodies. We observed an initial filament growth rate of ~100 nm · min^{-1}, which decreased over time. This corresponds to a translocation speed of ~1,700 amino acids per second, making the type III export apparatus located at the base of the flagellum a protein export machine of remarkable speed.



We further measured the growth kinetics of individual filaments of *Salmonella enterica* by site-specific labelling of flagellin subunits containing an exposed cysteine residue using sulfhydryl-specific (maleimide) fluorochromes. Multiple exchanges of maleimide fluorochromes *in situ* during normal culture growth allowed us to determine the growth kinetics of single filaments with high spatial resolution. These experiments demonstrated that the extension length of the filament is inversely proportional to its initial length,

until the growth rate for long filaments decreases to a point where it becomes minimal, and allowed us to *in fine* derive a growth curve. The best fit of the growth curve is based on an injection-diffusion model where flagellin monomers, which are at least partially α-helical inside the channel, are pushed by a pmf-driven export apparatus into the channel and move diffusively in one dimension through the length of the flagellum.

In summary, the combination of experimental and mathematical evidence demonstrates that a simple, injection-diffusion mechanism controls filament growth outside the cell. This flagellum growth model is based on simple biophysical parameters, where growth of the flagellum is driven by both hindered diffusion and pmf-dependent secretion of subunits, and thus explains why bacterial flagella do not grow indefinitely.

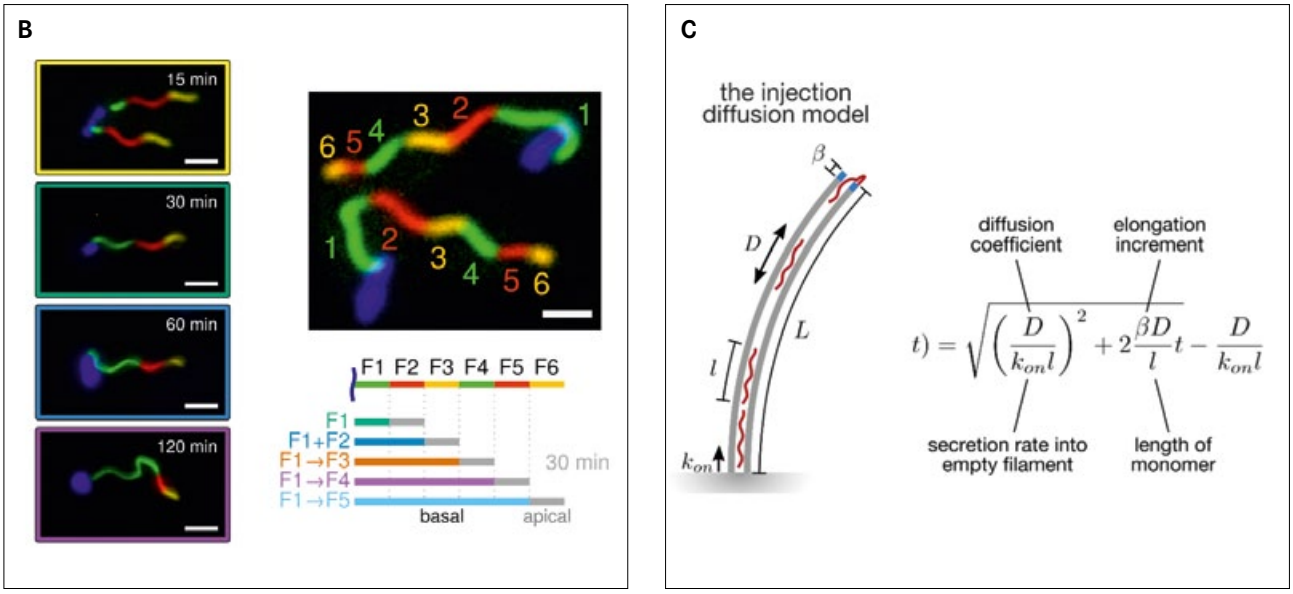


Figure 1: A: Schematic depiction of the bacterial flagellum. **B:** *In situ* filament labelling reveals a negative correlation between filament length and elongation rate. **C:** Equation and biophysical parameters of the injection-diffusion model. Figure adapted from Renault et al. eLife Sciences Publications Limited; 2017. e23136.

Renault T., Abraham A.O., Bergmiller T., Paradis G., Rainville S., Charpentier E., Guet C.C., Tu Y., Namba K., Keener J.P., Minamino T., Erhardt M. (2017) **Bacterial flagella grow through an injection-diffusion mechanism**. *eLIFE*, doi: 10.7554/*eLIFE.* 23136

INTERCELLULAR TEAMWORK ENABLES INDUCTION OF ANTIVIRAL RESPONSES

Christine Goffinet, Head of Research Group Innate Immunity and Viral Evasion

Host cells are equipped with pattern recognition receptors which sense viral components and alert the cell, resulting in the induction of an antiviral state that contributes to antiviral defense and clearance of the viral infection. On the other hand, invading viruses have evolved sophisticated strategies to counteract or evade these cellular alarm mechanisms. In our paper, we describe how T cells and macrophages cooperate to establishing an antiviral response against HIV-1.

Cyclic GMP-AMP (cGAMP) synthase (cGAS) is an intracellular cytoplasmic DNA sensor that senses DNA that aberrantly localizes in the cytoplasm. Upon DNA binding, cGAS catalyzes the sysnthesis of cGAMP, a cyclic dinucleotide and small messenger, which binds to STING and thereby induces a TBK-1/IRF-3-dependent signaling cascade, culminating in the transcription of interferon and interferon-stimulated genes. Retroviruses, which retrotranscribe their RNA genome into a viral double-stranded DNA, and DNA viruses, have evolved to evade or counteract cellular sensing mechanisms that lead to the establishment of an antiviral state.

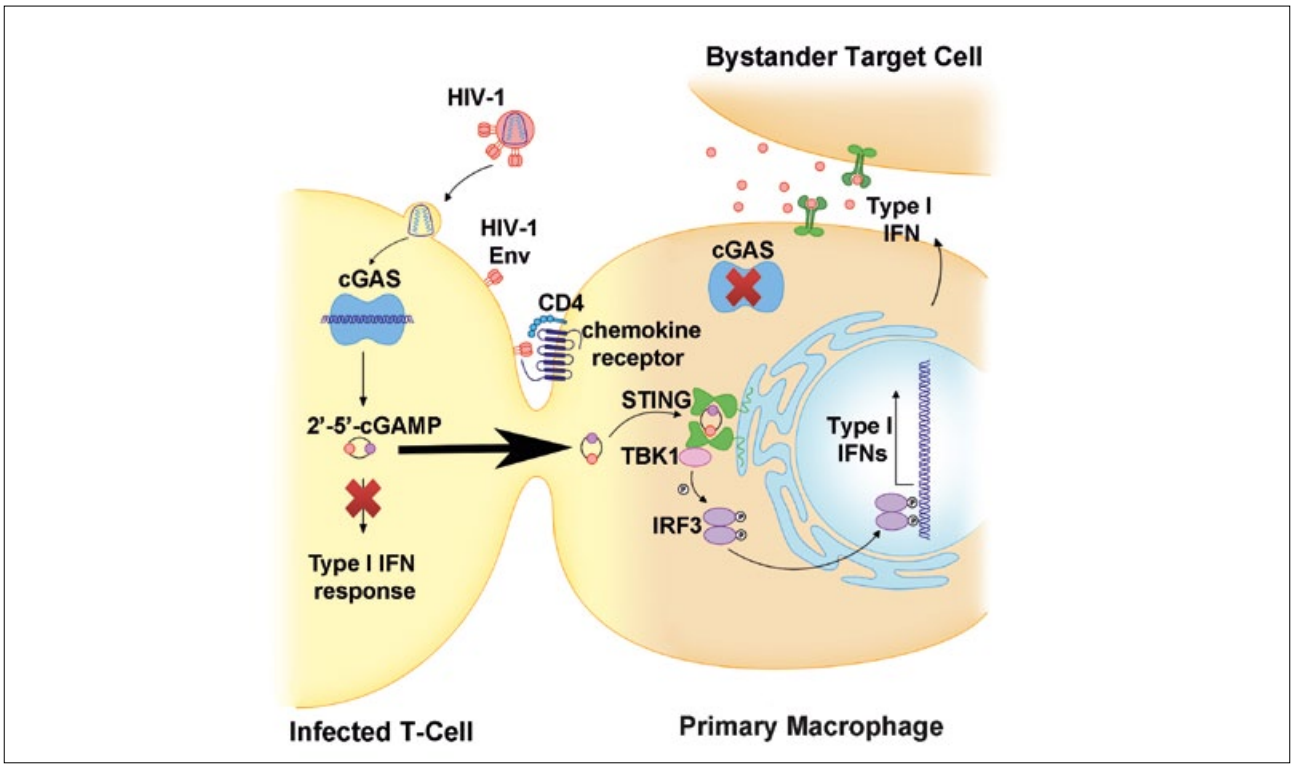


Figure 1: HIV-1 infection triggers cGAMP synthesis in primary human T cells. HIV-1 Env expression at the surface of infected T cells enables the formation of membrane microfusion sites with neighboring HIV-1 target cells which encode the HIV-1 receptor/co-receptor complex. cGAMP is delivered horizontally to the neighboring cell where it binds to STING and triggers the downstream cascade, eventually leading to type I IFN and induction of ISGs. Printed with permission from Elsevier.

These viral evasion mechanisms consist in shielding the reverse transcription product and the DNA genome, respectively, in a protective viral capsid structure that reduces the accessibility of those DNAs to cellular DNA sensors, and/or expressing specific proteins that antagonize cGAS/STING-mediated DNA sensing.

The main target cells in which human immunodeficiency virus I (HIV-1) propagates are CD4+ T cells and macrophages. HIV-1 has perfectly evolved to invade these target cells "silently", without triggering substantial antiviral responses. However, we observed that this silent phenotype is drastically changed in HIV-1-infected cocultures of T cells and macrophages. Using a combination of genetic, functional and imaging approaches, we found that HIV-1-infected T cells indeed undergo cGAS activation and synthesize cGAMP, which however appears to be a dead-end product in this cell type in the context of HIV-1 infection. Cocultured macrophages, which naturally express the HIV-1 receptor/coreceptor complex, form HIV-1 Env-mediated fusion pores with HIV-1 infected T cells, which display HIV-1 Env glycoproteins on their surface. These communication tunnels serve as intercellular conduits allowing the horizontal transfer of cGAMP from T cells to macrophages, where it directly activates STING and induces an antiviral state in the coculture. In conclusion, T cells and macrophages, despite being unable to mount an antiviral response on their own, seem to cooperate by exploiting the inherent ability of HIV-1 Env to mediate cell-to-cell fusion.

Given the high density of HIV-1 target cells in lymphoid organs and tissues, the high frequency of cell-cell interactions during systemic patrolling and migration of immune cells, and expression of HIV-1 Env at the cell surface of infected cells, it is likely that intimate cell-cell contacts, some of them inducing membrane fusion events, occur *in vivo*.

We propose that horizontal spread of cGAMP via membrane fusion pores induced by viral glycoproteins might also occur during other viral infections which promote cell-cell fusion and replicate through DNA synthesis. Future studies are required to decipher whether this mode of intercellular small molecule exchange is beneficial or detrimental for the host *in vivo* and to develop how these findings could be harnessed for therapeutic or protective antiviral purposes.

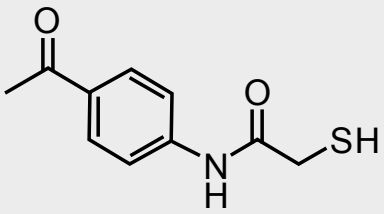
Xu S., Ducroux A., Ponnurangam A., Vieyres G., Franz S., Müsken M., Zillinger T., Malassa A., Ewald E., Hornung V., Barchet W., Häussler S., Pietschmann T., Goffinet C. (2016) **cGAS-mediated innate immunity spreads intercellularly through HIV-1 Env-induced membrane fusion sites**. *Cell Host and Microbe.* 20: 443-457

A NOVEL CLASS OF INHIBITORS TARGETING CLOSTRIDIAL COLLAGENASES

Rolf Hartmann, Head of Department Drug Design and Optimization

Clostridia are ubiquitously occurring, Gram-positive pathogens responsible for severe diseases like tetanus (*C. tetani*), gas gangrene (*C. perfringens, C. histoly-ticum*) or pseudomembranous colitis, a complication of antibiotic therapy (*C. difficile*). These diseases still have high mortality rates and remain challenging to treat. In a time when mankind is facing a threat imposed by the increasing emergence of antibiotic-resistant pathogens, it is of utmost importance to develop new anti-infectives with novel modes of action. Towards this end, the concept of developing 'pathoblockers' targeting bacterial virulence has gained significant attraction. Since bacteria are 'disarmed' rather than killed, this approach should lead to reduced resistance formation. In this context, clostridial collagenases represent highly attractive targets. Clostridia produce and secrete these extracellular zinc-metalloproteases as pivotal virulence factors favoring disease progression by facilitating host invasion and evasion from the host immune response. Considering their extracellular localization, targeting these proteases is conceptually attractive since there is no need to permeate the bacterial cell wall. By combining biophysical approaches with an *in vitro* inhibition assay, we discovered a class of fragment-like thiols as potent clostridial collagenase inhibitors with selectivity toward a range of human matrix metalloproteases (MMPs).

In order to discover novel collagenase inhibitors, the biophysical screening of a focused protease-inhibitor library was combined with a functional Fluorescence Resonance Energy Transfer (FRET) based assay. These efforts led to the discovery of a class of *N*-aryl mercaptoacetamides with promising activity on collagenase H (ColH), a highly efficient virulence enzyme of *C. histolyticum*. Interestingly, these compounds were generated *in situ* from a pro-drug-like thiocarbamate by hydrolysis in assay buffer. Further investigation of derivatives of this class led to the discovery of *N*-(4-acetylphenyl)-2-mercaptoacetamide (1) as the most active inhibitor, with remarkably low nanomolar potency (Figure 1). Structure-Activity Relationship (SAR) analyses highlighted para-substitution to be highly favorable for inhibition, in particular by hydrogen-bond accepting moieties.



1 IC_{50} 0.017 ± 0.002 µM

Figure 1: Structure and *in vitro* activity of ColH inhibitor cp. 1.
Source: Schönauer et al., J. Am. Chem. Soc. 139, 12696-12703.

We generated an X-ray crystal structure of the best inhibitor in complex with ColH, revealing, for the first time, a non-primed binding mode of a ColH inhibitor (Figure 2, A). The observed preference for hydrogen-bond acceptors is most likely due to a distinct interaction of the para-acetyl moiety with the protease edge strand (Tyr428, Glu430).

In metalloprotease drug design, selectivity toward human protease antitargets is of crucial importance. Therefore, we investigated the activity of our inhibitors on a broad range of six MMPs. Importantly, the *N*-aryl mercaptoacetamides displayed high selectivity toward all MMPs tested, while efficiently inhibiting several other clostridial collagenases (Figure 2, B). Using computational methods, we rationalized the unprecedented selectivity by a tilted orientation of the edge strand in MMPs, which does not allow productive interactions with the inhibitor as in ColH. Together with the low cytotoxicity of our compounds toward HEP G2 cells, these findings pave the way for the rational development of highly selective, broad spectrum clostridial collagenase inhibitors as novel pathoblockers to fight clostridial infections.

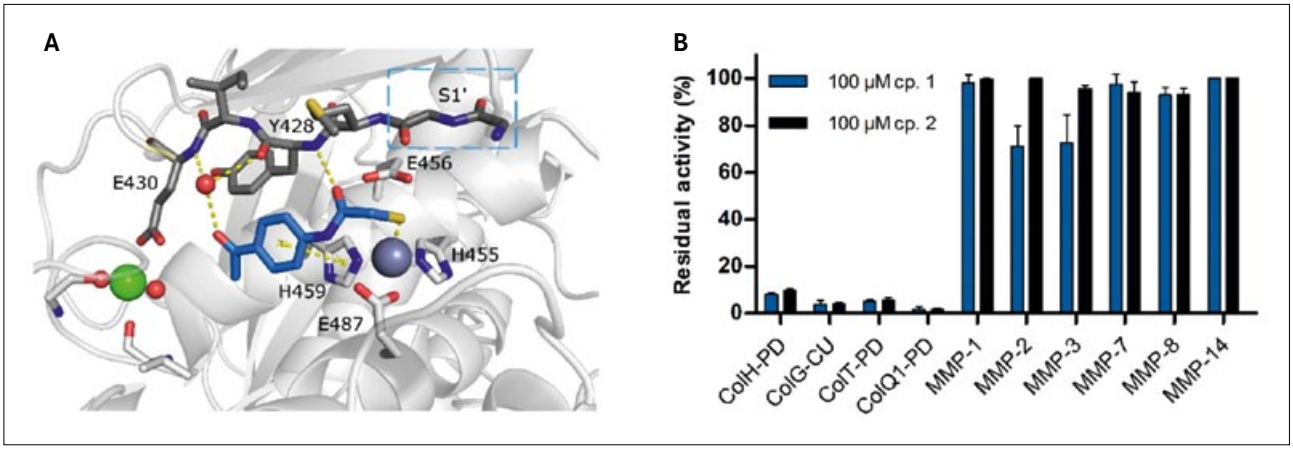


Figure 2: Peptidase domain of ColH in complex with cp.1 (blue) (A). Inhibition of selected MMPs and bacterial collagenases by cp. 1 and 2 (*N*-(4-chlorophenyl)-2-mercaptoacetamide). Source: Schönauer et al., *J. Am. Chem. Soc.* 139, 12696-12703.

Schönauer E., Kany A.M., Haupenthal J., Hüsecken K., Hoppe I.J., Voos K., Yahiaoui S., Elsässer B., Ducho C., Brandstetter H., Hartmann R.W. (2017) **Discovery of a Potent Inhibitor Class with High Selectivity toward Clostridial Collagenases**. *Journal of the American Chemical Society.* 139, 12696–12703

ESTABLISHING A ROADMAP FOR SOFTWARE SELECTION IN MICROBIOME RESEARCH

Alice C. McHardy, Head of Department Computational Biology of Infection Research

Metagenomics studies the genomic information retrieved directly from a microbial community found in a certain habitat, which enables studies of microorganisms that are difficult to obtain in pure culture. Computational methods for assembly, taxonomic profiling and binning are key to interpreting metagenome data, but a lack of consensus about benchmarking complicates performance assessment. The Critical Assessment of Metagenome Interpretation (CAMI) challenge has engaged the global developer community to benchmark their programmes on highly complex and realistic data sets, generated from ~700 newly sequenced microorganisms and ~600 novel viruses and plasmids and representing common experimental setups. Assembly and genome binning programmes performed well for species represented by individual genomes, but were substantially affected by the presence of related strains. Taxonomic profiling and binning programmes were proficient at high taxonomic ranks, with a notable performance decrease below family level. Parameter settings markedly affected performance, underscoring their importance for programme reproducibility. The CAMI results highlight current challenges but also provide a roadmap for software selection to answer specific research questions.

Metagenome data includes genome sequence information from different microbial community members that are present at varying abundances and degrees of similarity to each other. From these data, researchers would like to infer which taxa are present in the community, and to reconstruct the genomes of the individual community members, to generate hypotheses about who might be doing what, to study their microevolution or changes of taxon abundances in response to environmental changes. Since the advent of metagenomics, a true avalanche of computational methods for these tasks has been described, however, it is often unclear which software is best for a particular question or data set. However, there is a lack of standards in the field regarding their evaluation, in terms of data sets and quality measures used. Therefore, it is oftentimes difficult or impossible to compare the results of different method papers in a meaningful way.

For this reason, together with colleagues from the University of Vienna and the University of Bielefeld, an initiative called the "Critical Assessment of Metagenomics Interpretation" (CAMI) was founded. CAMI strives to involve the global scientific community in contests on realistic metagenome data sets and in establishing standards on how to best assess software performances. The aim is to generate more objective, reproducible and realistic assessments of software performance, to identify the best methods and data sets for different research questions and to facilitate method evaluations for software developers and microbiome researchers. This is important to further improve microbiome research using metagenomics in the future.

The first CAMI benchmarking challenge analysed the performance of four common software categories for metagenome analysis, namely of taxonomic profilers, metagenome assembly software (Figure 1), of taxonomic and of genome binning programmes. Anybody interested could participate in the contest. These results were analyzed by a panel of experts from the community, again including everyone interested, who jointly defined the best quality measures for this purpose.

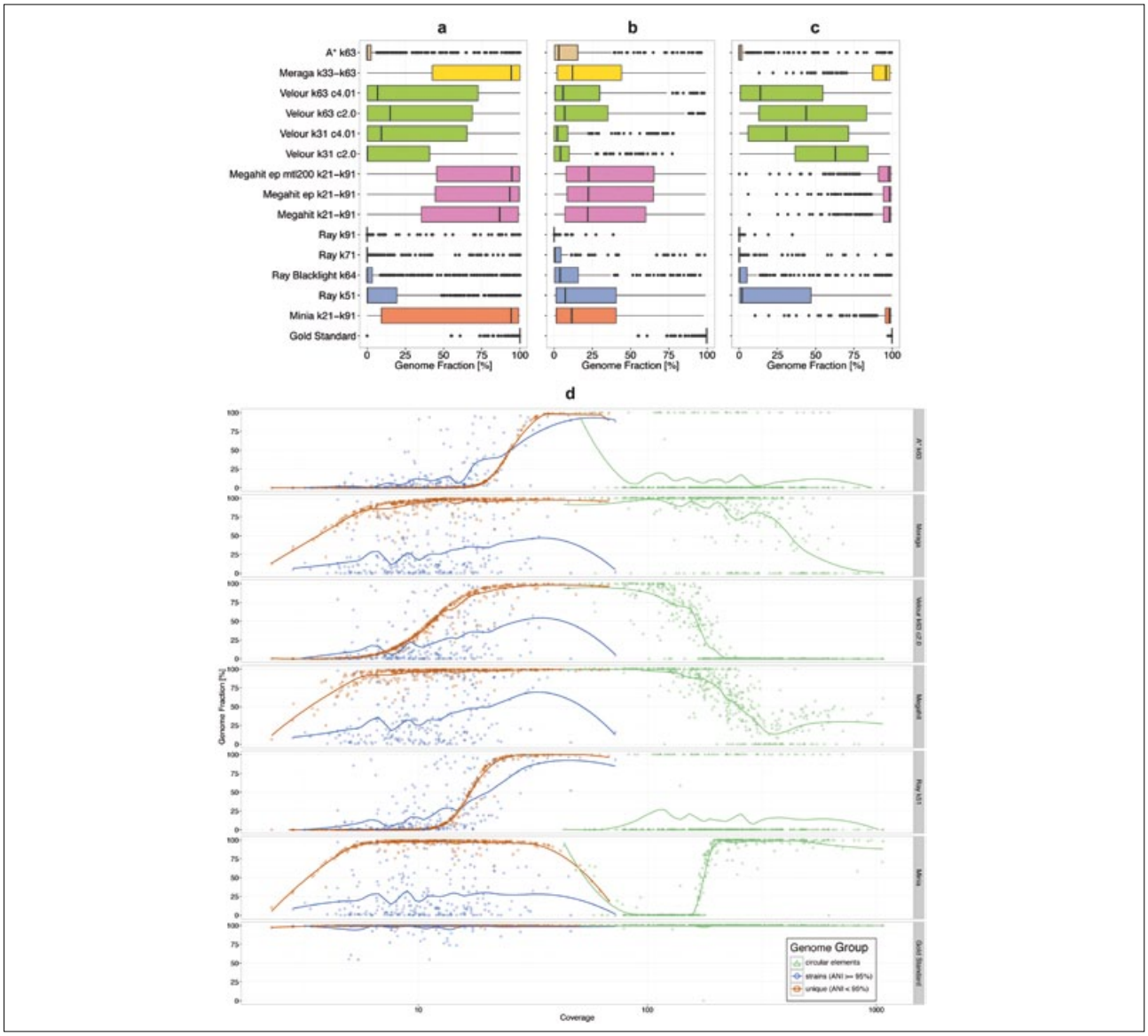


Figure 1: Results of evaluating metagenome assembly software on data set from the CAMI challenge. (**a–c**) Fractions of genomes for individual community members assembled by each assembler for all genomes (a), genomes with Average Nucleotide Identity (ANI) < 95% (b) and genomes with ANI ≥95%, representing closely related organisms (**c**). Dots indicate individual data points (genomes); colors indicate results from the same assembler incorporated in different pipelines or parameter settings. Boxes reflect the interquartile range and center lines the median. (**d**) Genome recovery fraction versus genome sequencing depth (coverage). Data were classified as unique genomes (ANI <95%, brown), genomes with related strains present (ANI ≥95%, blue) or high-copy circular elements (green). The gold standard includes all genomic regions covered by at least one read in the metagenome data set. Some assemblers do well across a wide range of genome coverages, *e.g.* Megahit and Meraga, and some (Minia) also assembled high-copy circular elements well. Printed with permission vom Springer Nature.

Sczyrba A., Hofmann P., Belmann P., Koslicki D., Janssen S., Dröge J., Gregor I., Majda S., Fiedler J., Dahms E., Bremges A., Fritz A., Garrido-Oter R., Jørgensen T.S., Shapiro N., Blood P.D., Gurevich A., Bai Y., Turaev D., DeMaere M.Z., Chikhi R., Nagarajan N., ..., McHardy, A.C. (2017) **Critical Assessment of Metagenome Interpretation – a benchmark of metagenomics software**. *Nature Methods*. 14: 1063–1071

HOST-INHERENT VARIABILITY INFLUENCES *S. AUREUS* TRANSCRIPTIONAL RESPONSE DURING INFECTION

Eva Medina, Head of Research Group Infection Immunology

The rise of antibiotic resistance calls for alternative strategies to treat bacterial infections. One attractive strategy is to attenuate bacterial pathogenesis by the specific inhibition of virulence factors essential for the pathogen survival during infection. Considering that the expression of virulence traits by pathogens is not constitutive and may vary from person to person, we used dual RNA sequencing (RNA-seq) and experimental models of *Staphylococcus aureus* infection to demonstrate that intrinsic variability in the levels of host resistance to infection greatly affects the pathogen expression of virulence factors *in vivo*. We furthermore provide evidence that differential expression of virulence factors by the pathogen in different hosts strongly influence the efficacy of anti-virulence strategies, highlighting a potential limitation for the implementation of these strategies.

Anti-virulence strategies based on attenuation of bacterial pathogenesis rather than directly targeting bacterial growth or viability have received increasing attention as novel treatment options for bacterial infections. One important aspect that should be considered carefully when designing anti-virulence strategies is that the expression of virulence traits by the pathogens can vary from person to person. Consequently, absent expression of the targeted virulence factors could render anti-virulence strategies completely ineffective.

We used dual RNA sequencing of host and pathogen (dual RNA-Seq) and two mouse strains that exhibit different levels of resistance to *Staphylococcus aureus* (A/J are very susceptible and C57BL/6 are very resistant) and therefore mimic the human inter-individual variation in the response to infection (Figure 1), to demonstrate that host variability strongly influenced the expression of virulence factors by *S. aureus* during *in vivo* infection (Figure 2). Specifically, genes encoding important virulence factors such as toxins and extracellular proteases are expressed to a greater extent by *S. aureus* during the infection of resistant C57BL/6 than during infection of susceptible A/J mice. We also investigated the consequences of this host-dependence of bacterial virulence factors expression on the effectiveness of anti-virulence strategies by neutralizing a virulence factor that was expressed by *S. aureus* to a higher extent during infection of C57BL/6 than during infection of A/J mice. The

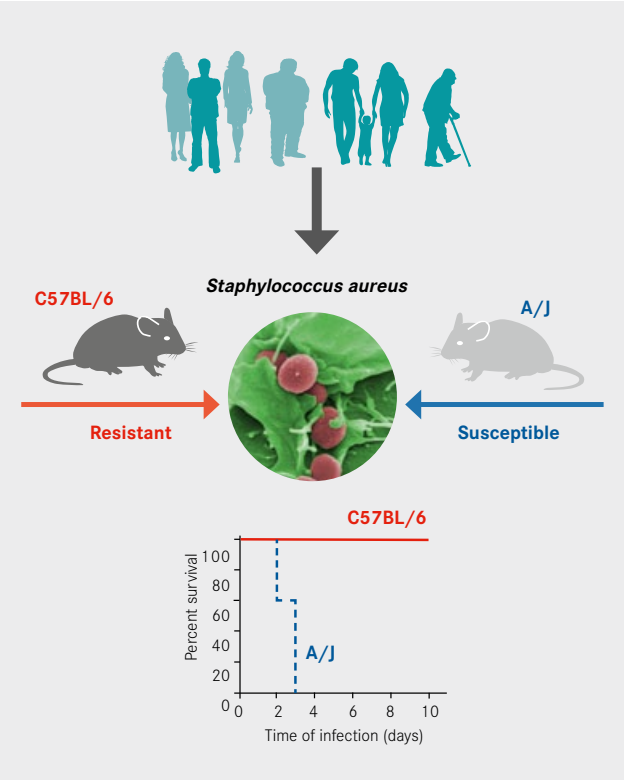


Figure 1: Inter-individual variation in the response to infection can be mimicked by mouse strains of different genetic backgrounds. In case of *S. aureus* infection, C57BL/6 mice are prototype of resistant hosts and A/J mice of susceptible hosts. This difference in infection resistance is illustrated by the greater mortality of A/J than C57BL/6 mice after intravenous infection with *S. aureus* (lower panel). Adapted from Thänert et al., Nature Communications 8: 14268.

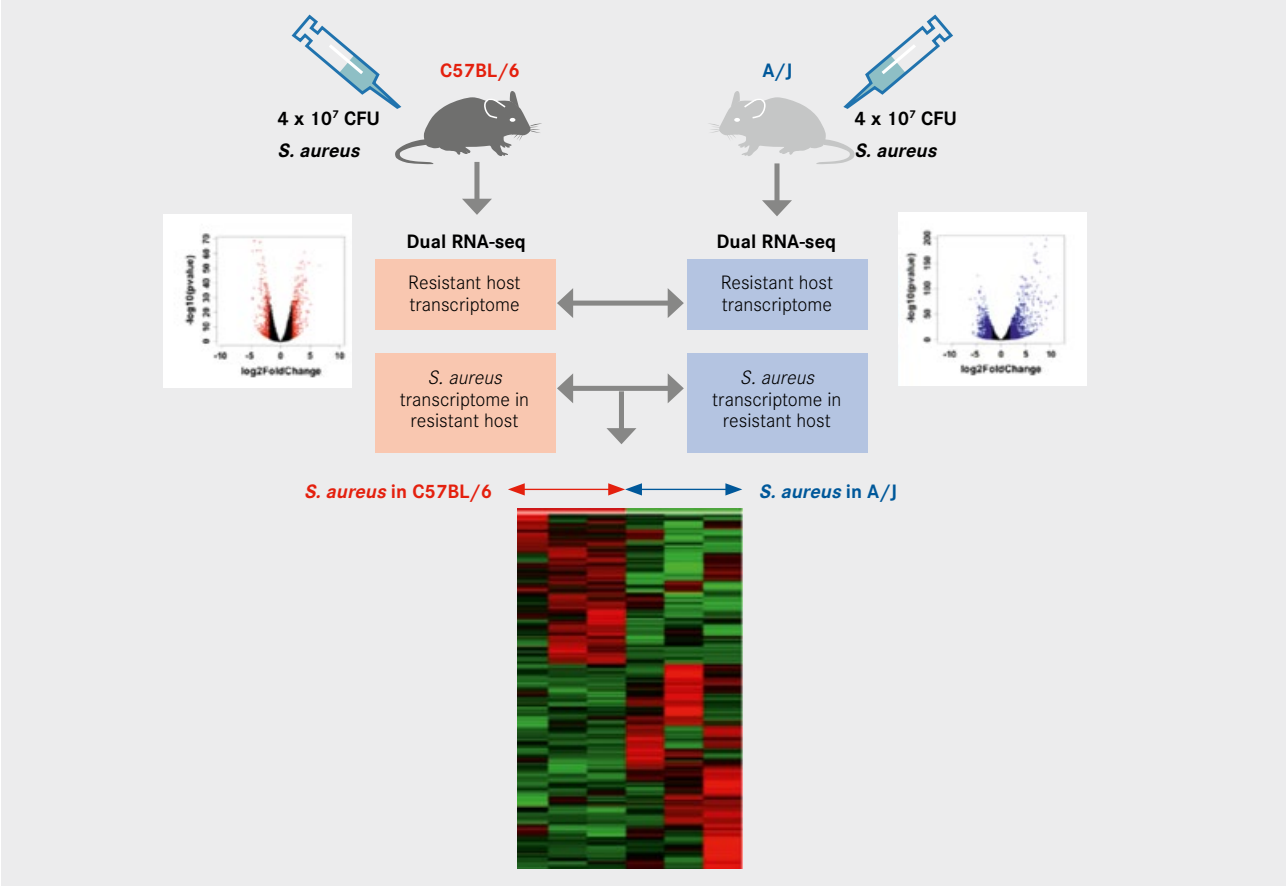


Figure 2: Summary of the experimental approach and results after dual RNA-seq analysis. Susceptible A/J and resistant C57BL/6 mice were infected with *S. aureus* and dual RNA-seq analysis was performed to simultaneously determine the gene expression profile of the host and pathogen in the infected tissue. The genes differentially expressed by *S. aureus* in A/J and C57BL/6 mice were identified and related to the infection-associated transcriptional response of the corresponding mouse strain. Adapted from Thänert et al., Nature Communications 8: 14268.

results showed a significant reduction of *S. aureus* fitness in C57BL/6 mice but did not affect the bacterial fitness during infection of A/J mice.

These findings highlight that the efficacy of an anti-virulence strategy will depend on the level of expression of the targeted virulence factor, which can be in turn influenced by the intrinsic variability in the host resistance to infection. This information is essential when searching for novel anti-virulence targets since optimal targets would be those virulence factors which are highly expressed by the pathogen during infection, independently of the host background.

Thänert R., Goldmann O., Beineke A., Medina E. (2017) **Host-inherent variability influences the transcriptional response of *Staphylococcus aureus* during *in vivo* infection**. *Nature Communications.* 8: 14268

NOVEL CYSTOBACTAMIDS AGAINST GRAM-NEGATIVE PATHOGENS

Rolf Müller, Head of Department Microbial natural Products

Microbial resistance against existing drugs is increasing worldwide, while our antibiotic arsenal is shrinking: for more than thirty years, no new antibiotic class with broad-spectrum antibacterial activity has been approved to the market. This is due to the fact that antibiotic development is challenging, especially since fully synthetically derived molecules often lack required pharmaceutical properties and the discovery from natural sources is tedious and often hampered by very low product titers. Nevertheless, natural products are highly valuable sources for antibacterial drug development, since such molecules have been evolutionarily adapted to reach and address their biological targets inside bacterial cells. In the work presented below, we show possible ways how the above-mentioned obstacles can be overcome by applying multidisciplinary screening approaches.

The application to natural product research of new cultivation techniques, minimized and automated assay systems, analytical equipment with higher sensitivity, and sophisticated data mining tools impressively show that nature still harbors promising lead structures such as the recently discovered cystobactamids. In the course of screening extracts from novel myxobacterial isolates, we identified a sample inhibiting growth of *Pseudomonas aeruginosa* and other bacterial pathogens. Bioactivity-guided fractionation and dereplication using our in-house database 'Myxobase' revealed the presence of the known cystobactamids 919-1 and 919-2 in those extracts, which however did not explain the activity towards *P. aeruginosa*. By applying the abovementioned techniques, several target molecules could be identified with chemical properties similar to the known cystobactamids. MS fragmentation of these target compounds and comparison with the structural data from the known derivatives lead to the hypothesis that more potent cystobactamids with broad-spectrum antibacterial activity are produced by myxobacteria. A different substitution pattern on only a few loci on the cystobactamid scaffold (see Fig. 1), plus a connection moiety formed by five different linker types (A-E, Fig. 1), ultimately led to a high number of different congeners, much like a combinatorial library. Finally, eleven new cystobactamid derivatives containing at least one of these structural variations were purified, chemically characterised and subsequently tested to compare their bioactivity against different bacterial pathogens.

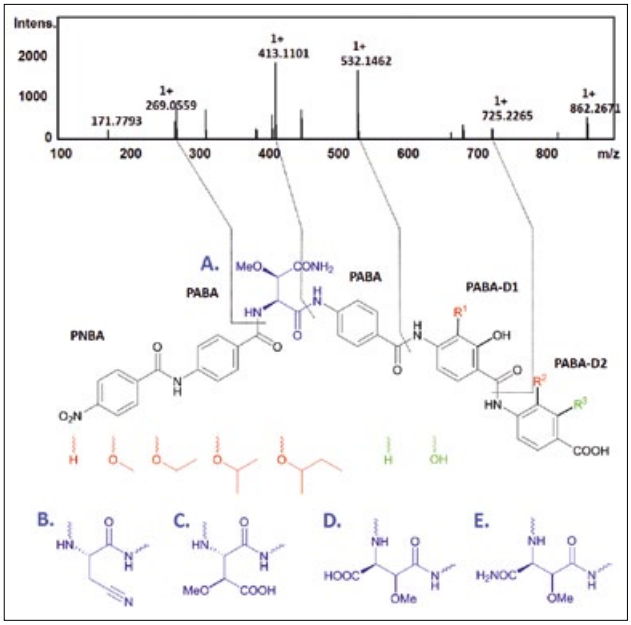


Figure 1: Structural diversity of natural cystobactamids from Myxobacteria. New derivatives were initially identified by MS fragmentation. These contain different linkers (blue) and differ in their substitution pattern of *para*-aminobenzoic acid (PABA) units (red, green). Adapted from Hüttel et al., (2017) Angewandte Chemie - International Edition 56, 12760.

Figure 2: Structure activity relationships drawn from the naturally derived cystobactamid library. Shown is the most active variant cystabactamid 861-2, which was the template to establish the total synthesis and the most important positions were derivatizations alter the activity towards different Gram-negative pathogens. The microtiter-plates illustrate the different susceptibility of *E. coli* and *P. aeruginosa* on different structural variants in crude extracts separated via UHPLC, observed during the activity guided identification of the molecules. Adapted from Hüttel et al., (2017) Angewandte Chemie - International Edition 56-12764.

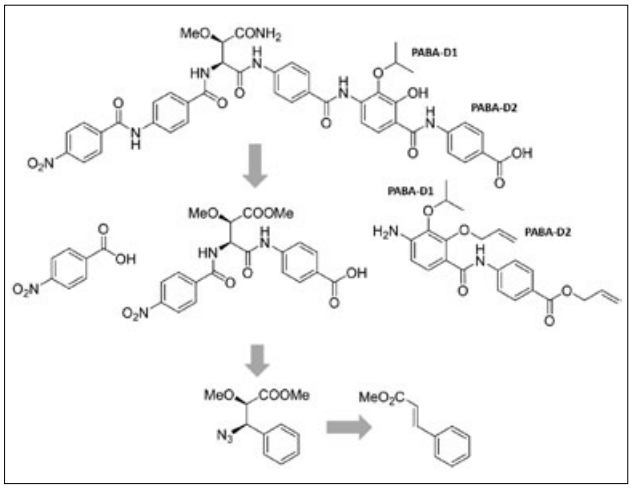


Figure 3: Structure of cystobactamid 861-2 and its retrosynthetic analysis. Adapted from Hüttel et al., (2017) Angewandte Chemie - International Edition 56, 12760.

This comparison of biological activity revealed strong differences between these very similar molecules that were caused by only minor variations on the positions R^1 to R^3 and different linker types (see Fig. 1). Generally, derivatives with linker A were most active, whereas the substitution pattern of PABA-D1 and D2 (see Fig. 1) seemed to fine-tune the activity and render some of the novel cystobactamids active against commonly multidrug-resistant (MDR) pathogens such as *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Acinetobacter baumannii*. Furthermore, these variations also seem to be beneficial to overcome cross-resistance with fluoroquinolones in *Escherichia coli* and efflux-related resistance in *P. aeruginosa* (Fig. 2). The derivative cystobactamid 861-2, which consists of linker A and exhibits R^1=*iso*-propoxy, R^2=H and R^3=H, was selected as the best starting point for further optimization and was thus targeted by total synthesis. The currently best route, involving a Sharpless dihydroxylation, a Kuhn-Roth type oxidation, and phosphorylchloride-mediated amide bond formation, gave access to 861-2 in 13 steps (longest linear sequence) and 2.3% overall yield (Fig. 3). It also helped defining the stereochemistry at the methoxy-asparagine unit. The work described here is an important step to enable cystobactamid's lead optimization and preclinical development.

Hüttel S., Testolin G., Herrmann J., Planke T., Gille F., Moreno M., Stadler M., Brönstrup M., Kirschning A., Müller R. (2017) **Discovery and Total Synthesis of Natural Cystobactamid Derivatives with Superior Activity against Gram-Negative Pathogens**, *Angewandte Chemie - International Edition.* 56(41): 12760-12764

THE ACTIVE UPPER GI TRACT MICROBIOTA AND HELICOBACTER INFECTION

Dietmar Pieper, Head of Reasearch Group Michrobial Interactions and Processes

***Helicobacter pylori*, a documented carcinogen, infects a significant portion of the human population. As its interaction with the surrounding microbiota and the effect of this microbiota on human health are poorly understood, we analyzed in detail the active global bacterial communities from five distinct sites of the upper gastrointestinal (GI) tract allowing the differentiation between host and niche effects and obtaining an understanding of microbiota/*H. pylori* interactions.**

Until the discovery of *H. pylori*, the human stomach was considered to be sterile due to its acidity. However, in recent years culture-independent analysis revealed the presence of a complex microbiota. *H. pylori* is usually acquired during childhood and persistent throughout life. Infected individuals develop chronic gastritis and a subgroup progresses to gastric cancer. *H. pylori* infection was also indicated as a cause of duodenal ulcer.

Despite the importance of *H. pylori* and the gastric microbiota for human health, the knowledge on microbes in the upper GI tract is quite limited. Even though saliva is indicated as a source for bacteria present in stomach aspirates, there is a lack of knowledge on whether these are able to colonize the gastric mucosa. It is also not known to what extent the microbiota differ at distinct niches, to what extend *H. pylori* influences these microbiota and, thus, how they affect host health.

To better understand the interaction of *H. pylori* with the surrounding microbiota, we aimed to characterise the non-*H. pylori* bacteria, to identify whether they are transients or residents and specifically to assess in how far *H. pylori* affects the biodiversity. To achieve this, the microbiota of saliva, stomach aspirates, stomach biopsies, duodenum aspirates and duodenum biopsies of 24 individuals were analyzed by high-throughput sequencing. In contrast to most sequence-based studies, which analyze DNA and comprise dead cells, we analyzed the biodiversity based on RNA profiles and describe the active microbiota component.

We showed that each subject had an individual microbiota, which was consistent throughout the investigated regions. This underlines the concept of a continuous bacterial migration through the upper GI tract with the oral cavity as the dominant source of active bacteria. We also showed the stomach environment to select against various bacteria, which are active in either saliva or in the duodenum. We further evidenced a major difference between the luminal microbiota, probably adapted to a low pH value and the mucosa adherent microbiota of the stomach. Only minor differences were evident between the mucosal communities of the duodenum and stomach, contrasting with the clear

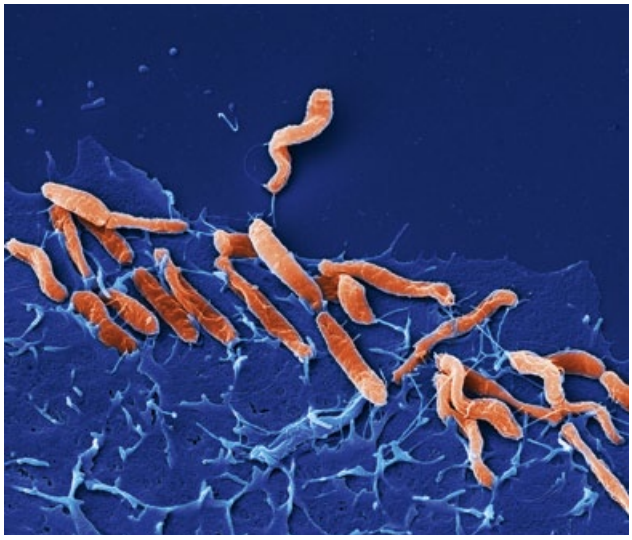


Figure 1: *Helicobacter pylori* colonizes the human stomach and is the etiological agent of peptic ulcer disease.

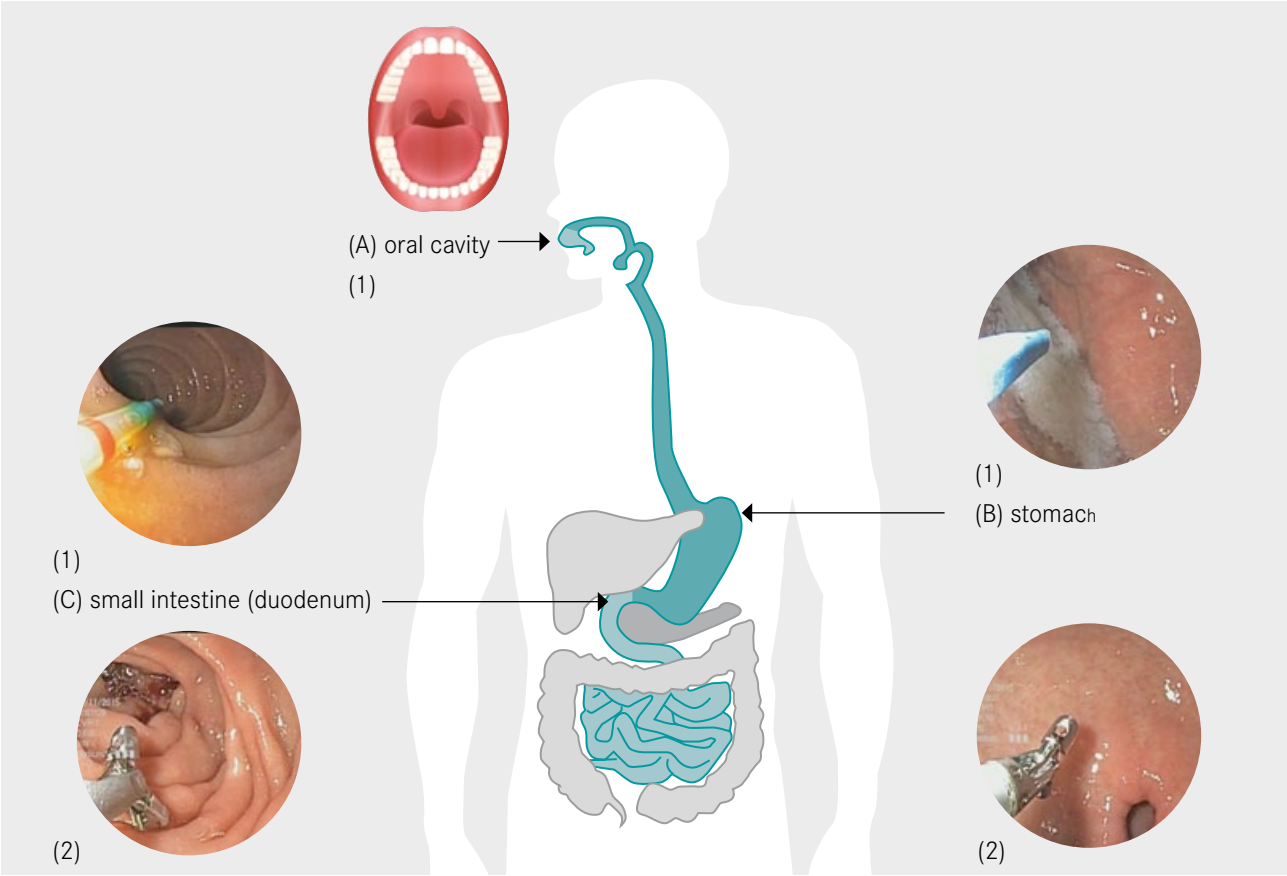


Figure 2: Samples were taken from different sites of the upper gastrointestinal tract. [A] oral cavity, [B] stomach and [C] duodenum. To differentiate luminal and mucosal microbial communities aspirates [1] were taken under sterile conditions to represent l the uminal content, and mucosal biopsies [2] were taken reflecting the mucosa adherent bacterial communities.

preference of *H. pylori* to colonize the stomach mucosa. Strikingly, the influence of *H. pylori* on the community was also very evident in the duodenal samples. Duodenal ulcers and gastric cancer have been reported to coexist in a number of patients and it is assumed that undetected factors such as different compositions of the microbiota contribute to this pathology. Interestingly, infection with *H. pylori* influences the composition of the oral microbiota and/or vice versa. Assuming an oral to oral transmission route of *H. pylori*, differences in the active oral microbiota might influence the susceptibility to *H. pylori* infection and a better understanding on the interaction between *H. pylori* and the oral community might give insights into the mode of transmission.

Schulz C., Schütte K., Koch N., Vilchez-Vargas R., Wos-Oxley M.L., Oxley A.P.A., Vital M., Malfertheiner P., Pieper D.H. (2017) **The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection**. *Gut.* 67: 216–225

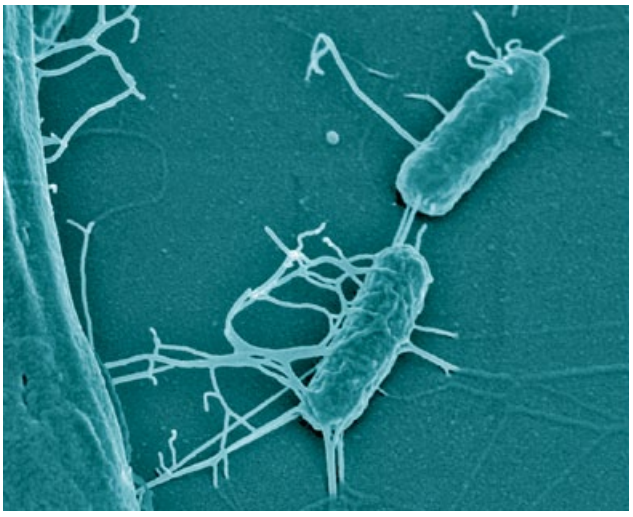
THE REGULATION OF SUSCEPTIBILITY TO INFECTIONS BY THE MICROBIOTA

Till Strowig, Head of Junior Research Group Microbial Immune Regulation

The human intestine harbors a dense and diverse microbial ecosystem, the microbiota, which contributes to the physiology of the host during health and disease. Among others, the microbiota contributes to colonization resistance against invading pathogens. However, the identities of the microbes that modulate host resistance have remained largely elusive. Utilizing mice colonized by distinct microbiota communities, we demonstrate that severity of disease induced by enteric *Salmonella* Typhimurium infection is strongly modulated by microbiota composition. In this study, we specifically show that distinct microbiota members protect against intestinal *Salmonella* infection via enhancement of antibacterial IFNγ responses.

Non-typhoidal *Salmonella* (NTS) species, including *Salmonella enterica* spp. serovar Typhimurium (*S.* Tm) belong to the family of Gram-negative Enterobacteriacea. Infections with NTS species are one of the leading causes of foodborne disease outbreaks and are the major bacterial cause of acute gastroenteritis, causing approximately 93.8 million cases and resulting in 155,000 deaths per year. No effective therapies are currently available to reduce disease burden.

Upon ingestion *Salmonella* has to compete with the resident intestinal microbiota and cope with the mucosal immune system to colonize the host (Figure 2). While mucosal inflammation is required to eventually clear infection, *Salmonella* intriguingly benefits from it to outgrow commensal bacteria; *e.g.* utilizing specialized metal-uptake systems and inflammation-associated metabolites. Vice versa, commensal bacteria are able to confer resistance against *Salmonella*. Specifically, protection against pathogens can derive directly from commensal-pathogen interactions or indirectly by enhancing protective immune-mediated antibacterial pathways. Disturbance of the resident microbiota and thus, altered susceptibility to infections can be induced by changes in diet and intake of medication. In particular, antibiotic use is associated with an increased susceptibility to several enteric infections, including *Clostridium difficile* infections and non-typhoidal *Salmonella* disease, which is at least partly mediated by altering the composition and functionality of resident bacterial populations.



In this study, we employ mouse lines with distinct microbiota compositions and a mouse model of *Salmonella*-induced diarrhea that requires antibiotic induced disruption of direct colonization resistance. We demonstrate that severity of disease induced by *S.* Tm is strongly dependent on the composition of the intestinal microbiota (Figure 2). We show that resident intestinal bacteria from protected mice present before antibiotic treatment induce enhanced

Figure 1: *Salmonella* is a major human pathogen and a model organism for bacterial pathogenesis research.

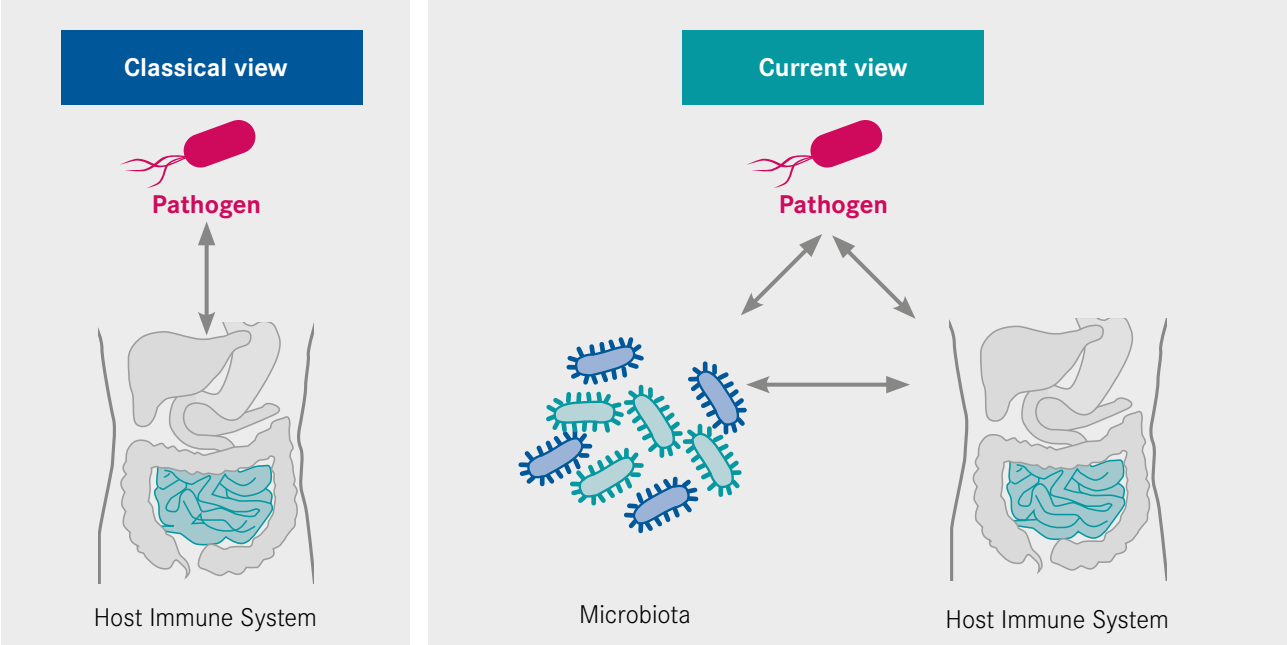


Figure 2: Microbiota alterations and immune responses in the context of an infection. Presence of distinct gut commensals determines susceptibility to Salmonella-induced diarrhea by priming the immune system and preventing tissue invasion. Resident bacteria confer protection enhancing antibacterial IFNγ production by innate cells and CD4+ T cells during infection.

production of IFNγ in immune cells in the lamina propria and demonstrate that modulation of IFNγ production by the protective microbiota is necessary for augmented protection against *S.* Tm. Finally, we show that transfer of small communities of cultivable bacterial species isolated from protected mice to susceptible mice recapitulated the protective effect by attenuating tissue invasion of *S.* Tm. These results show for the first time that modulation of IFNγ production by specific intestinal bacteria reduces the severity of *Salmonella*-induced disease and thus potentially guide the development of novel probiotic interventions against enteric infections.

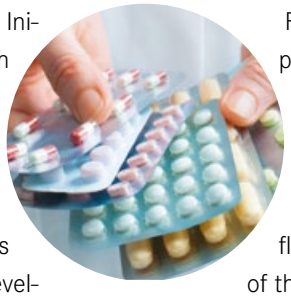
Thiemann S., Smit N., Roy U., Lesker T.R., Gálvez E.J.C., Helmecke J., Basic M., Bleich A., Goodman A.L., Kalinke U., Flavell R.A., Erhardt M., Strowig T. (2017) **Enhancement of IFNγ Production by Distinct Commensals Ameliorates Salmonella-Induced Disease**. *Cell Host Microbe* 21, 682–694

MIGRAINE MEDICINE AGAINST HEPATITIS C VIRUS

Thomas Pietschmann, Head of Institute for Experimental Virology

Worldwide, more than 70 million people are chronically infected with the Hepatitis C Virus (HCV). This infection causes severe liver disease and can ultimately lead to hepatocellular carcinoma. Since the introduction of direct acting antiviral drugs (DAAs), HCV infection can be cured. The therapy is very expensive though and not available for the vast majority of patients in developing countries without efficient health care systems. Thus, availability of cost-effective alternative therapy options would have benefits for global control of HCV disease burden. We screened a substance collection with licensed drugs for compounds with antiviral activity against HCV and identified one promising candidate: the migraine medicine flunarizine.

In the framework of the Helmholtz Alberta Initiative and the DZIF, and in collaboration with several German and international partners we screened a compound library that also included licensed drugs in order to identify possible new inhibitors of HCV replication. Repurposing a licensed medicine for a different indication is beneficial compared to a completely new developed substance, as the time-consuming, expensive and often unsuccessful studies to show safety of application in humans have already been passed. One of the promising candidates that we identified in this screening was flunarizine. This member of the chemical class of diphenylmethylpiperazines is a licensed prescription drug against migraine in Europe and Canada.



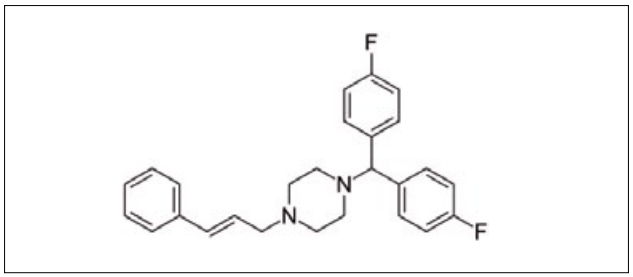


Figure 1: Structure of flunarizine.

Flunarizine is a calcium channel inhibitor. We hypothesized that it would act against the viral protein p7, which forms an ion channel and plays and important role for the secretion of newly synthesized virus particles from the infected cells. However, our experiments showed that flunarizine interferes with the membrane fusion of the virus which happens before the virus particles enter the cell. Interestingly, this effect was genotype-dependent and works primarily on HCV genotype 2 viruses. Using chimeric viruses consisting of portions from different viral genotypes and by selection for flunarizine resistance, we could show that flunarizine is targeting the functioning of the viral envelope proteins during membrane fusion. Thus, flunarizine emerged as a first in class HCV membrane fusion inhibitor. Finally, we could show that it also prevents HCV infection in an HCV animal model.

Together with partners from the Leibniz University in Hannover, we are currently conducting a larger structure activity relationship analysis of flunarizine and related molecules. Ultimately, repurposing of flunarizine or development of related compounds may enrich options for treating HCV patients.

Perin P.M., Haid S., Brown R.J., Doerrbecker J., Schulze K., Zeilinger C., von Schaewen M., Heller B., Vercauteren K., Luxenburger E., Baktash Y.M., Vondran F.W., Speerstra S., Awadh A., Mukhtarov F., Schang L.M., Kirschning A., Müller R., Guzmán C.A., Kaderali L., Randall G., Meuleman P., Ploss A., Pietschmann T. (2016) **Flunarizine prevents hepatitis C virus membrane fusion in a genotype-dependent manner by targeting the potential fusion peptide within E1** *Hepatology.* 63(1): 49-62

PARTNERS AND NETWORKS

ESTABLISHING A LINK BETWEEN BASIC RESEARCH AND CLINICAL MEDICINE

Ulrich Kalinke, Excecutive Director of TWINCORE

THE TWINCORE CENTRE FOR EXPERIMENTAL AND CLINICAL INFECTION RESEARCH

Founded in 2008 as a joint venture between HZI and MHH, the TWINCORE Centre for Experimental and Clinical Infection Research aims to enhance the interaction between basic researchers and clinician scientists. 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.

Research institutes at TWINCORE

Currently five W3 professorships are established at TWINCORE. The positions are held by the heads of the main divisions of TWINCORE: Ulrich Kalinke, Executive Director of TWINCORE and Director of the Institute for Experimental Infection Research, Thomas Pietschmann, Director of the Institute for Experimental Virology, Tim Sparwasser, Director of the Institute for Infection Immunology, Susanne Häußler, Director of the Institute for Molecular Bacteriology, and Gérard Krause, Director of the Institute for Infectious Disease Epidemiology.

The research group "Gene and Cell Therapy" at TWINCORE, headed by Michael Ott, has been delegated to TWINCORE by the Department of Gastroenterology, Hepatology and Endocrinology at MHH (headed by Michael P. Manns).

Translational infection research at TWINCORE

At TWINOCRE research projects are developed on the basis of clinical observations and clinical challenges. Projects typically follow combined approaches that are based on clinical and preclinical translational research. The projects are focused on the analysis of infections of the gastrointestinal tract, the liver, the lung, and the central nervous system. Par-

ticular attention is payed to the analysis of patients with enhanced vulnerability to infectious disease, such as newborns and the elderly. The clinical translational research is focused on innovative analytics, screening of natural compounds and formulations, the analysis of patient cohorts and observational clinical studies, and vaccine research; whereas the preclinical research addresses chronic viral infections, acute and chronic respiratory infections, organ-specific immunity, immunmodulation and metabolism.

Clinical cooperations

The programme "Junge Akademie" of the Hannover Medical School is supported by HZI and combines medicine and basic sciences in practice. This programme was established to allow young medical professionals combining their scientific work with high loads of clinical routine. The programme promotes "physician scientists" for a period of three years. During this time the physician scientists are supported by a medical and a basic research mentor. Such tandem projects turned out to be the basis for the sustained development of research cooperations between researchers at TWINCORE and clinicians at MHH.

The respiratory syncytial virus (RSV) can cause severe infections of the upper and lower respiratory tract. In particular, infants are at risk. The reason why RSV causes severe disease in some children but benign symptoms in others is not well understood. In collaboration with physicians of the clinics for Pediatric Pneumology, Allergology and Neonatology of MHH (Gesine Hansen), scientists of the Institute of Experimental Virology (Thomas Pietschmann) investigate genetic factors that determine the severity of RSV-infections. The ultimate objective is to use this information for the development of novel diagnostics and preventive measures.

In the years 2014-2016 two observational studies were carried out at the Clinical Research Center (CRC) Hannover under the supervision of Frank Pessler to address the efficacy of influenza vaccination in the elderly. To this end, more than 200 participants were recruited who were aged above 65. Currently sera drawn at different time points after vaccination are analyzed for biomarkers such as cytokines. First-line candidates have been identified that might inform on the immune responsiveness. The objective is to better understand why the immune responsiveness decreases with age. Together with the Department of Vaccinology at the HZI (Carlos Guzmán) currently it is being addressed whether in vaccination non-responders a therapy aiming at the replenishment of reduced cytokines would improve the vaccine responsiveness.

Patients with inflammatory autoimmune diseases such as rheumatoid arthritis (RA) not only suffer from autoimmunity but also from frequent and more severe infectious diseases. The higher risk for infections can either be caused by the immunosuppressive therapy or by an intrinsic defect of the immune system of RA Patients. Together with the Clinic for Immunology and Rheumatology of MHH (Torsten Witte) scientists of TWINCORE (Project Leader Annett Ziegler) at the Institute for Experimental Infection Research (Ulrich Kalinke) study the function of T helper cells in RA patients. These investigations revealed that T helper cells of RA patients show signs of functional exhaustion. One specific drug was identified that reverted T cell exhaustion to some extent. The hope is that one day treatment regimens can be improved in a manner that RA patients have T cells with an improved functionality.

Towards individualized infection medicine

The above translational research projects already point towards individualized approaches in infection medicine. The objective is that one day every patient with a severe infectious disease will be extensively analyzed and that based on the retrieved data treatment recommendations will be developed in order to assist the treating physician. To bring this vision into practice, next to TWINOCRE the Centre for Individualized Infection Medicine (CiiM) will be erected within the next few years. The close neighborhood of TWINOCRE and CiiM will catalyze the development of new qualities of translational research projects which will allow patients to profit from latest scientific breakthroughs more readily.

Perspectives

In the forthcoming years TWINCORE aims at recruiting also physician scientist group leaders who guide a research group and still are involved in patient care. The hope is that this way the cooperation between basic researcher and clinicians can be further enhanced.

IN SEARCH OF NOVEL ANTI-INFECTIVES

Rolf Müller, Managing Director of HIPS

HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL RESEARCH SAARLAND (HIPS)

The Helmholtz Institute for Pharmaceutical Research (HIPS) was founded in 2009 by HZI and Saarland University (UdS). Since 2015 the new research building hosts three departments and three junior research groups, currently employing more than 130 people. HIPS is the first public research institute in Germany that is explicitly devoted to the pharmaceutical sciences, and also the first institute representing Germany's largest research organization, the Helmholtz Association, in the Saarland. 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.

The Department **Microbial Natural Products** (MINS, head: Rolf Müller) focuses on the identification and development of natural compounds, primarily from myxobacteria and actinomycetes. Intensive studies on myxobacterial biodiversity in 2016/2017 resulted in the discovery that the taxonomic distance between bacterial species is correlated with the production of structurally distinct secondary metabolites, which led to new strategies in identifying so far unknown, chemically novel drug molecules. Further achievements were made in the pharmacological profiling of Amidochelocardin and Cystobactamids, which are current antibiotic lead candidates with broad-spectrum activities. The heterologous production of Corallopyronin A, an anti-parasitic agent for treatment of filariasis, has made superb progress in a joint project with HZI and Bonn University, and attracted further funding from the German Center for Infection Research (DZIF). Chlorotonil and Telomycin are further anti-infective candidates that are currently in the lead development phase in order to treat malarial and *S. aureus* infections, respectively. In addition to its S2 safety laboratory to test *in vitro* bioactivities of novel compounds against a broad spectrum of relevant pathogens, MINS recently established a Zebrafish facility at HIPS for toxicity and efficacy studies of novel lead compounds in simplified *in vivo* infection model systems. The junior research group "Structural Biology of Biosynthetic Enzymes" uses a combination of X-ray crystal-

lography, biochemistry and chemical biology to elucidate the biosynthesis of natural products enabling compound engineering and understanding their mode of action as demonstrated for Carolacton. The Department **Drug Design and Optimization** (DDOP) headed by Rolf Hartmann and Anna Hirsch (since 05/2017) is specialized in medicinal and pharmaceutical chemistry.

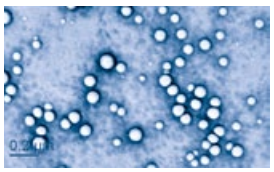
The DDOP team follows a target-based rational design strategy focusing on biologically relevant enzymes, transporters and regulators within bacterial pathogens. The portfolio of drug targets can be grouped into those that either impair bacterial viability and neutralize them effectively (*e.g.*, DXS and RNAP) or "patho-blockers" that interfere with pathogenicity and virulence (*e.g.*, PqsR and ColH). Various novel compound classes were identified as highly active inhibitors of these targets, also by using innovative techniques such as dynamic combinatorial chemistry. The researchers are performing multiparameter optimisation of these novel anti-infectives, taking advantage of their biochemical, microbiological and biophysical as well as *in silico* investigation. In order to bridge the translational gap, the evaluation of the PK/PD profile of anti-microbial lead compounds and their testing in preclinical *in vivo* models was initiated. The junior research group "Chemical Biology of Carbohydrates" has developed novel hybrid-type glycomimetics with high affinities towards the *P. aeruginosa* virulence factor LecB, which are now ready for *in vivo* testing. Furthermore, a first pathogen-specific probe was developed and employed in biofilm imaging.

HIPS HELMHOLTZ Institute for Pharmaceutical Research Saarland

Improving the delivery of drugs to their site of action and facilitating their transport across relevant biological barriers is the focus of the department **Drug Delivery** (DDEL, head: Claus-Michael Lehr). A significant part of this research is on human cell and tissue models as an alternative to animal testing. Once established, such models may significantly fasten the translation of new drugs into the clinic. In this context, the first human alveolar epithelial Typ1-like cell line with tight intercellular junctions (hAELVi) has been made commercially available. Co-culture models of epithelial and immune cells are being currently expanded towards bacterial infections for studying host-pathogen interactions and the effects of aerosolized anti-infectives. Innovative polymeric nanocarriers shall enable intracellular delivery of macromolecular biopharmaceuticals, like *e.g.* mRNA, for minimal invasive vaccination strategies and contemporary gene therapy approaches. In collaboration with various clinically-oriented partners, encouraging results have been obtained in relevant animal models for severe diseases like colitis, surfactant protein deficiency, cystic fibrosis and diabetes.

Discovery & production of microbial antibiotics | **Screening, profiling, mode of action** | **Hit-to-lead optimisation** | **Formulation & delivery**



LEARNING THE LANGUAGE OF RNA TO COMBAT INFECTION

Jörg Vogel, Managing Director of HIRI

Photo: HIRI/Mario Schmitt

HELMHOLTZ INSTITUTE FOR RNA-BASED INFECTION RESEARCH (HIRI)

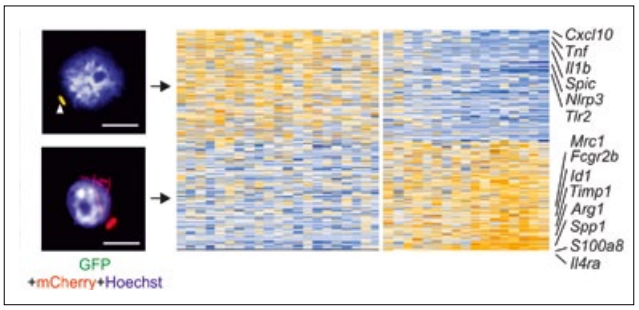
As a partnership between the Helmholtz Centre for Infection Research (HZI) and the Julius-Maximilian-University of Würzburg (JMU), the Helmholtz Institute for RNA-based Infection Research (HIRI) was established in May 2017. As the first research institution worldwide bridging the fields of ribonucleic acid (RNA) biology and infectious disease, HIRI will pioneer an integrative approach to exploit the vast potential of RNA as a diagnostic, drug and target for new strategies to combat infectious diseases. Within the first year after its foundation, HIRI has already grown to seven research groups and a total of 35 people.

Rising antimicrobial resistance, chronic infections, and (re-) emerging pathogens are among the major challenges facing humanity. RNA is increasingly understood to contribute to key regulatory and sensory processes in the cell, but the role of RNA in infection biology remains understudied. HIRI will combine interdisciplinary expertise with cutting-edge research infrastructure to exploit the vast potential of RNA as a diagnostic molecule, target, and drug to combat infectious diseases.

HIRI HELMHOLTZ Institute for RNA-based Infection Research

Research at HIRI focuses on four areas: bacterial infections, viral infections, host response, and RNA delivery, complemented by the strategic pursuit of emerging topics in RNA research. Research in the first three areas will provide a comprehensive analysis of the role RNA plays in infections and utilise RNA delivery for diagnostic and therapeutic purposes. The first HIRI department "RNA biology of bacterial infections" was established in June 2017 (head: Jörg Vogel). The aim of this department is to develop novel procedures to understand the RNA world of bacterial pathogens and use RNA-centric approaches to target pathogens and manipulate the microbiota. The second HIRI research group "Single cell analysis" was set up by Antoine-Emmanuel Saliba. His primary goal is to use and advance single-cell RNA-seq approaches to study heterogeneity in host responses to infections and its impact on disease outcome on the single cell level.

On January 1st, Chase Beisel joined the HIRI from the North Carolina State University, (USA). His group "RNA Synthetic Biology" will investigate and harness the functional diversity of CRISPR-Cas immune systems for the development of new foundational technologies. They aim to develop a new generation of CRISPR technologies that can be employed to better understand, diagnose, and combat human infections. Neva Caliskan established her new group "Recoding mechanisms in infection processes" to identify and characterise the mechanisms and regulatory implications of translational recoding in RNA viruses and pathogenic bacteria. The third junior PI to join HIRI in 2018 was Lars Barquist. With his group "Integrative Informatics in Infection Biology" he will develop systems approaches to RNA and infection, using modern visualization, data science, and machine learning technologies to integrate large-scale functional genomics data.



Differential transcriptomics signature associated with macrophages with non-growing and growing bacteria. (adapted from Saliba et al. 2016 Nat Microbiol).

On March 1st, Alexander Westermann was admitted to the ranks of a Junior professor at HIRI to start his group on "Host-pathogen-microbiota interactions". His research centers on the identification and functional characterization of noncoding RNA molecules in pathogens, microbiota and the host.

Two months later, on May 1st, Junior professor Redmond Smyth followed with his Helmholtz junior research group "Genome architecture and evolution of RNA viruses" to investigate how RNA virus genome architecture can be exploited in drug discovery and vaccine design.

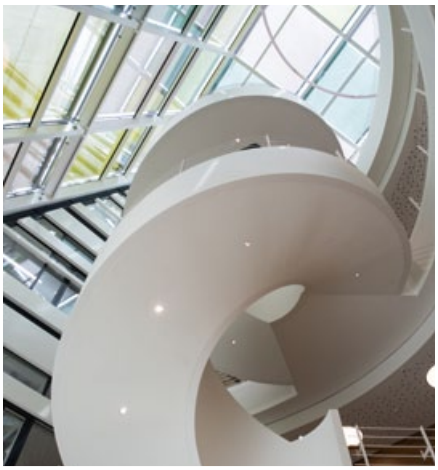
July 1st 2017 saw the inception of the "HIRI Seed Grant", a HIRI-based funding initiative to foster synergies between HZI, the University of Würzburg (JMU), and HIRI and to generate publishable results shortly after the foundation of HIRI. In all, 22 projects, amounting to a total funding volume of 1.9 million Euro, have been selected. At halfway stage of the funding period, in January 2018, three trans-institutional articles have already been published. Another four manuscripts are submitted, under revision or already accepted (one of them in *Nature Communications*). Eight manuscripts are currently being finalised.

Until HIRI can move to its own building, 1500 qm of space are being provided within the premises of JMU's Institute for Molecular Infection Biology. The limited available office and laboratory space, however, is already becoming challenging with respect to the further growth of the HIRI, particularly when it comes to recruitment of new department leaders on W3 level. Thus, the establishment of the new HIRI building is being pursued strenuously. The architecture competition for the new HIRI building was launched in March 2018. Submissions will first be exhibited and awarded at HZI in Braunschweig and then at HIRI in Würzburg. The much-anticipated jury meeting will take place in October 2018.

With its integrated scientific concept, tight interconnections between research areas and cutting-edge infrastructure, HIRI will provide a vibrant research environment for both established and young scientists. The natural synergy between this new research institute and the infection research and translational competences of its parent institutions will create unique opportunities to effectively convert knowledge into applications. This setting will also stimulate collaborations with industry in the development of novel therapeutic and diagnostic strategies for the treatment of infectious diseases.

POWERFUL LIGHT SOURCES FOR INFECTION RESEARCH

Michael Kolbe, Head of the department "Structural Infection Biology"



THE CENTRE FOR STRUCTURAL SYSTEMS BIOLOGY (CSSB)

The Centre for Structural Systems Biology (CSSB) in Hamburg opened its doors on June 29, 2017. This brand new Centre is the result of the joint effort of ten partner institutions in North Germany. It aims to employ cutting-edge light sources 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 specialized secretion systems that facilitate the invasion of enteric pathogens, more specifically of bacteria that cause diarrhea, 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). 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. These high-resolution studies will definitely enable the development of new therapeutics to block Gram-negative infections.

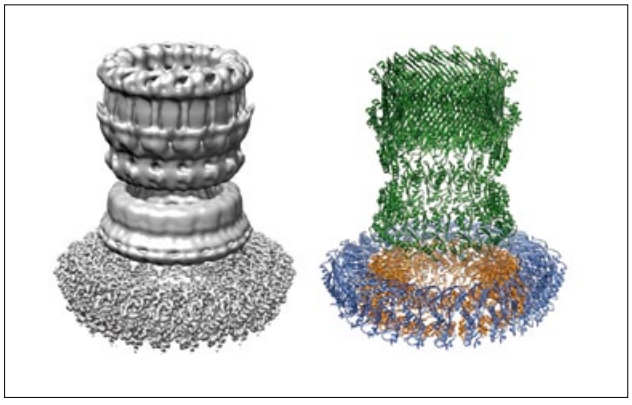
The group moved to the CSSB building in June 2017. Together with this group, the CSSB hosted the laboratories of two group leaders with expertise in structural biochemistry of membrane protein complexes. This exquisite combination of expert partners will provide the group with many opportunities to improve 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. By the end of summer 2018, the protein characterization and crystallization will be opened. Additionally, HZI and the group will benefit greatly from access to the S2 laboratories equipped with last generation cryo-electron microscopes and high – end fluorescence microscopes. Future cryo-electron microscopy analysis studying the fine 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 near-atomic resolution.

In fall 2017, the CSSB underwent its first scientific evaluation 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" from 13-15 November, 2017 organised by HZI in cooperation with Kai Grünewald (HPI) and 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.

CSSB
Centre for Structural Systems Biology

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



The needle complex of the type III secretion system (T3SS) of *Shigella flexneri*. Left: Cryo-electron microscopy, right: atomic model.

CSSB Partners:
- Bernhard Nocht Institute for Tropical Medicine (BNITM)
- Deutsches Elektronen-Synchrotron (DESY)
- European Molecular Biology Laboratory (EMBL)
- Forschungszentrum Jülich (FZJ)
- Hannover Medical School (MHH)
- Heinrich Pette Institute, Leibniz Institute for Experimental Virology (HPI)
- Helmholtz Centre for Infection Research (HZI)
- Research Center Borstel (FZB)
- Universität Hamburg (UHH)
- University Medical Center Hamburg-Eppendorf (UKE)

TRANSLATION: THE DZIF AND ITS MISSION

Timo Jäger, Managing Director of the DZIF

Combating epidemics worldwide: The DZIF meets global challenges posed by infectious diseases.

THE GERMAN CENTER FOR INFECTION RESEARCH (DZIF)

Infectious disease pathogens are ubiquitous. They spread rapidly, develop further and are becoming increasingly resistant to conventional drugs. Diseases caused by bacteria, viruses, parasites and fungi constitute one of the major health threats of our time. The German Center for Infection Research (DZIF) faces this challenge with a mission to translate biomedical research results more rapidly into clinical applications to help prevent, treat and cure infectious diseases. As one of 35 member institutions HZI has played an important role in the development of DZIF, especially at the partner site Hannover-Braunschweig.

DZIF bridges the gap between basic research and clinical application from test tube to patient, from testing to treatment. In order to fulfill this mission, more than 500 scientists and clinicians collaborate beyond boundaries of the institutions and professions and ensure successful multidirectional information flow and workflow. DZIF has organised and thematically structured itself into a decentralized virtual centre across different institutions: scientists and doctors research infectious diseases in nine research fields (*see text box*).

In 2016 and 2017 the research field "Emerging Infections" gained particular attention. DZIF researchers were involved in testing an Ebola vaccine, called rVSV-ZEBOV. As members of a WHO team of experts, they tested the potential vaccine in humans for the first time. For another dreaded virus, Zika, DZIF scientists analyzed and optimised diagnostic tools. Reliable diagnostics are very important, for example to confirm suspected Zika infections in pregnant women. The virus can cause severe malformations of the fetal brain. The DZIF development of a vaccine against

DZIF is an affiliation of 35 research institutes, located at seven sites distributed throughout Germany. Thirteen additional sites are associated partners: Charité -Universitätsmedizin Berlin, German Liver Foundation, Goethe University Frankfurt, University of Würzburg, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Max-Planck Institute for Informatics, Faculty of medicine – Martin Luther University Halle-Wittenberg, Otto von Guericke University Magdeburg, University Bayreuth, University Erfurt, Essen University Hospital, Medical Center – University of Freiburg, University of Münster.

the dangerous MERS-coronavirus is supported by CEPI, the newly founded international vaccine initiative Coalition for Epidemic Preparedness Innovations. The first clinical study has just been started.

Regarding the broad spectrum of themes dealt with in DZIF, we just pick a second problem area with outstanding results in the last two years. DZIF scientists meet the global challenge of increasing antimicrobial resistance in different fields. Coordinated by the HIPS/HZI, scientists from the research field “Novel Antibiotics” have generated several promising new active agents. Parallel to this development, scientists and clinicians are working on the important strategy of using antibiotics more targeted and intelligently in hospitals. This approach is also termed “Antibiotic Stewardship” – a strategy where European clinics are working together

with the aim of managing the problem of multidrug-resistant pathogens. For detailed information about our results in all nine research fields see www.dzif.de

Whether it is antimicrobial resistance, AIDS, malaria, Zika or hepatitis – viruses and bacteria know no borders and demand global action. In 2016 and 2017 the DZIF duly upgraded and strengthened the international cooperations – with its four African partner institutions as well as with new international coalitions as CEPI or CARA (Conscience of Antimicrobial Resistance Accountability).

Last but not least: Promoting the next generation of infection researchers is one of the important tasks in the DZIF. Our integrative approach is attractive for upcoming generations of scientists. PhD stipends and special workshops, as well as other funding possibilities at the DZIF Academy enable doctors to better combine clinical work and research. Laboratory rotations permit young scientists to gain insight into other research fields and methods. Funding programmes for young mothers support combining family with a scientific career. Funding for students, young scientists and clinicians is available on an individual basis at the different DZIF partner sites.

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

Research fields:

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

infrastructures:

- African Partner Institutions
- Novel Antivirals (since 2018)
- Biobanking
- Bioinfomatics
- Clinical Trial Unit
- DZIF Academy
- Epidemiology
- Pathogen Repository
- Product Development Unit

COMBINING EXPERTISE AND INFRASTRUCTURE TO ENHANCE COLLABORATION

Ulrich Kalinke, Coordinator of TRAIN

The first graduates of The TRAIN Academy.

TRANSLATIONAL ALLIANCE IN LOWER SAXONY (TRAIN)

The biomedical Translational Alliance in Lower Saxony (TRAIN) was founded in December 2008 with the objective to jointly establish critical research infrastructures and to enhance collaboration in the Braunschweig/Hannover region in order to develop new approaches in the diagnosis, therapy and prevention of diseases. The intention of the involved partners is to bundle infrastructure and expertise in order to foster the entire value chain from the idea to the application in clinical practice. The Alliance has made good progress combining the competences of various university and non-university research establishments in Lower Saxony.

Bundled competence in health science

The initiative was started when it was recognised that in the Hannover-Braunschweig region outstanding clinical and research expertise in the field of infectious diseases can be found. However, it was spread across different locations and institutions. In the initial phase, TRAIN assisted in the planning and construction of new research buildings and the creation of infrastructure to provide a basis for new translational research and development projects. Various centres have been built and have begun their activities. Furthermore, two new partners have been acquired: the DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, and the NIFE (Niedersächsisches Zentrum für Biomedizintechnik, Implantatforschung und Entwicklung – Lower Saxony Centre for Biomedical Engineering, Implant Research and Development), Hannover.

At the beginning of 2016, the TRAIN Partners decided to develop a platform strategy for key requirements in health – especially infection – research:

- TRAIN Academy
- TRAIN Omics
- TRAIN Projects

These first three platforms address the most important future requirements in the field of translational research. The TRAIN Academy ensures a steady supply of up-and-coming talented scientists and fosters the close network within the translational community. TRAIN Omics bundles infrastructure and expertise in the rapidly developing field of omics technologies and TRAIN Projects allows to take advantage of the synergies the TRAIN network can provide to outstanding translational projects in developing new drugs or medical products.

The TRAIN Academy

A two-year curriculum for extra-occupational training has been developed for physicians and scientists in the field of translational research and medicine. The programme was initiated in October 2015, and in October 2017 final certificates were awarded to the first eight graduates of the first course. Furthermore, in October 2018 the fourth year TRAIN Academy will be initiated.

In parallel to the development of the curriculum, a six-member steering group and a Scientific Advisory Board with three experienced and prominent members from biotechnology, pharmaceutical industry and regulatory authorities have been established for the TRAIN Academy. In addition, the TRAIN Academy is supported by approximately 70 lecturers, most of them from the TRAIN institutions. Importantly, the training series is certified by the Central Evaluation and Accreditation Agency Hannover (ZEvA) for the years 2016-2021.

Currently additional training formats are being developed in order to offer optimal conditions for professional development of young scientists in the area.

TRAIN Omics

At the beginning of 2016, the TRAIN partners decided to develop a common omics strategy: TRAIN Omics. The aim of TRAIN Omics is to develop a viable concept as well as the successive expansion of omics technologies within the region. Coordinated collaboration aims to increase the availability and efficiency of omics technologies in the region by making cooperative and complementary use of already available (or yet to be created) resources and further developing them in the most efficient way in future. In order to generate manageable packages, subgroups in the areas of genomics & transcriptomics, proteomics & metabolomics, cost and accounting models, education and teaching, bioinformatics, glycomics and content aspects have been established.

Within 2018/2019, a call for tenders is expected from the Federal Ministry of Education and Research (BMBF) in which approximately five leading Next Generation Sequencing sites in Germany will be determined in a competitive selection process. TRAIN Omics is intensively preparing for this call with its activities in Braunschweig and Hannover. More recently also the Georg-August Universität Göttingen got interested in joining the TRAIN Omics initiative.

COMMUNICATION OF METABOLISMS – METABOLIC CROSSTALK UNDERLYING INFECTION

Dieter Jahn, Vice President Research of the Technische Universität Braunschweig and Speaker of the Braunschweig Integrated Centre of Systems Biology (BRICS)

The BRICS building: On four floors, laboratories, offices and meeting rooms are used for joint experimental and theoretical work. The building also provides a training lab, a computer pool and large meeting rooms for student education.

THE BRAUNSCHWEIG INTEGRATED CENTRE OF SYSTEMS BIOLOGY (BRICS)

BRICS
Braunschweig Integrated Centre of Systems Biology

BRICS is an interdisciplinary research centre of Technische Universität Braunschweig (TU Braunschweig), HZI and Leibniz Institute German Collection of Microorganisms and Cell Cultures (DSMZ) with systems biology as the central joint scientific approach. 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 2017, BRICS comprised 24 principal investigators (PIs) from TU, HZI, DSMZ and TWINCORE. The common aim of BRICS is to exploit the full potential of the integration of state-of-the-art experimental approaches with strong bioinformatics based modelling in infection research. Thus, the holistic understanding needed for the development of directed intervention strategies is generated.

Communication of metabolisms

Currently, our major scientific focus is on the metabolism of the organisms of interest as one of the major readouts of cellular adaptation. In order to understand the regulatory and structural basis of metabolism underlying gene regulatory and protein networks are determined.

For infection processes we follow the concept of biochemically interacting cells. In this context, the fast developing techniques of metabolomics and fluxomics allow for the dynamic elucidation of the metabolic changes in an infectious bacterium and an infected host cell. Clearly, they influence strongly each other. During gut infections we encounter an even more complex situation. The infectious bacterium is part of the microbiome of hundreds of different microorganisms. The host is represented by the epithelium, cells of the immune system and the mucus. Moreover, the composition of the whole consortium and its metabolic turnover is dependent on the nutrition. Multiple levels of metabolic interactions of many different organisms can be expected. These processes we refer to as "communication of metabolisms". However, the number of partners influencing each other prevents solid molecular research using current experimental approaches. In order to understand the critical metabolic processes involved in bacterial infection we follow a reductionist approach, re-building the gut situation step by step from simple to complex in a defined manner as outlined in figure 1.

Key project: Host-pathogen metabolism

Infections have in common that the agents causing disease require energy. The energy resources are taken from the host organism and weaken the immune response. Host-pathogen-interaction can be seen as a competition for energy resources, and this competition turns out to be critical for disease dynamics and pathogen clearance. A frequent strategy of pathogens is the generation of an environment which is hostile to immune effector cells and inhibits energy consumption by immune cells. Switching between different metabolic strategies appears to be a crucial factor for disease control. At BRICS we investigate the metabolism of host-pathogen interactions.

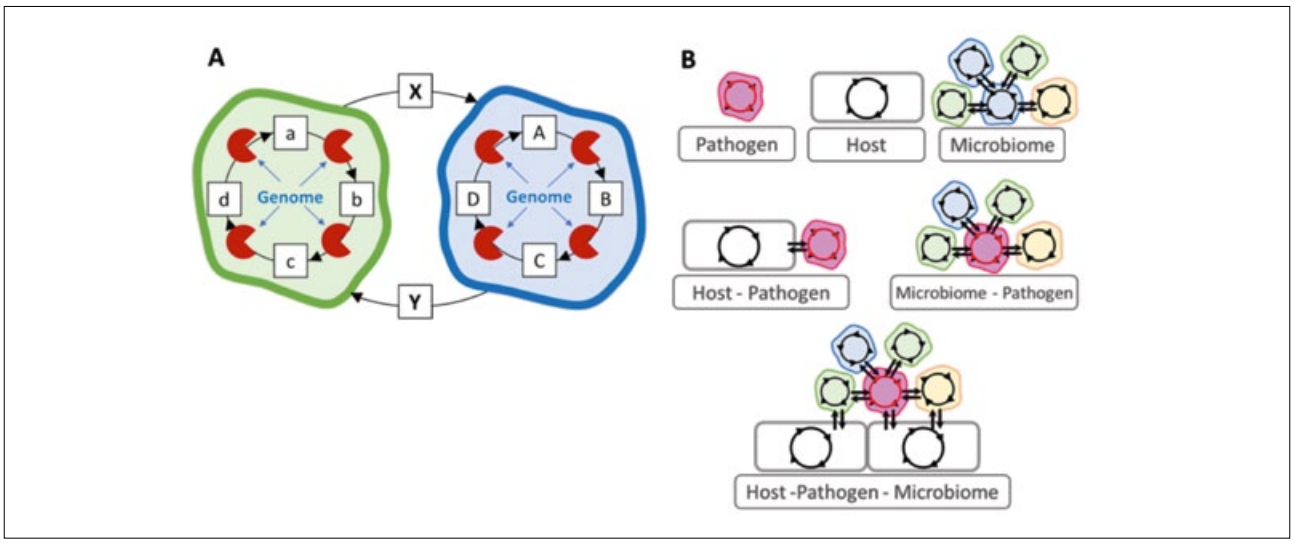


Figure 1: Communication of metabolisms. A: In two organisms the metabolites a-b-c-d and A-B-C-D are interconverted by enzymes (red) that are encoded by the corresponding genome; both interact by the exchange of metabolites (X)/(Y). B: We analyse the major players during a gut infection on increasing levels of complexity. In particular, the following interactions are analysed: host - pathogen, microbiome - pathogen, host - pathogen- microbiome.

Key project: *Clostridium 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 "CDiff" research consortium assembles an interdisciplinary team of researchers which explores the systems biology, the toxins and the interactions of the pathogen with the host and with the microbiome. The goal is a basic molecular understanding of the success of this organism in its environment. In this project, the communication of metabolisms is an essential element.

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 lung is the formation of biofilms in an anaerobic environment. In the DFG funded Research Training Group GRK2223 "Protein Complex Assembly (PROCOMPAS)" a team of scientists from TU 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.

Technologies and Resources at BRICS

Genome Analysis	HZI Genome Analytics platform (Geffers) PacBio DNA Sequencing (Overmann, DSMZ)
Proteome Analysis	Microbial Proteomics (Engelmann, HZI/TU) Cellular Proteomics (Jänsch)
Metabolome Analysis	Immune metabolism (Hiller, HZI/TU)
Databases	BRENDA - The Comprehensive Enzyme Information System (Schomburg, TU) BacDive – The Bacterial Diversity Metadatabase (Overmann, DSMZ) PRODORIC2 – Gene Regulation in Prokaryotes (Jahn, TU)
Biotechnology	Human Antibody Engineering and Phage Display (Dübel, TU)
Bioengineering	Systems Biotechnology and Fermentation (Spieß/Krull, TU)
Nanomicroscopy	Superresolution Microscopy and Single Molecule Flurescence Spectroscopy (Tinnefeld, TU)

Collaboration Projects at BRICS

- AMPro: Aging and Metabolic Programming. Helmholtz Association.
- CDiff: Epidemiology and systems biology of the bacterial pathogen *Clostridium difficile*. 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.

ORGANISATION CHART

WK
Scientific Commitee
Prof. Dr. Wolf-Dietrich Hardt, Chair

AR – Supervisory Board
MinDir'in Bärbel Brumme-Bothe (BMBF), Chair
MinDirig Rüdiger Eichel (MWK Niedersachen), Vice Chair

KD
Clinical Director
Prof. Dr. med. Michael Manns

LA
Steering Committee
Prof. Dr. Dirk Heinz, Chair

WISKO
Council of Scientists
Prof. Dr. Michael Meyer-Hermann, Chair

GFW – Scientific Management ***Prof. Dr. Dirk Heinz***

GFA – Administrative Management ***Silke Tannapfel***

TOPIC 1: Bacterial and Viral Pathogens

Dep. **BIFO** (BRICS)
Computational Biology of Infection Research
Prof. Dr. Alice McHardy

Dep. **EPID**
Epidemiology
Prof. Dr. Gérard Krause

Dep. **EVIR** (TC)
Experimental Virology
Prof. Dr. Thomas Pietschmann

RG **AIVE** (TC)
Innate Immunity and Viral Evasion
Prof. Dr. Christine Goffinet

RG **IMMI**
Innate Immunity and Infection
Prof. Dr. Andrea Kröger

RG **VIRT** (TC)
Virus Transmission
PD Dr. Eike Steinmann

RG **INFG**
Infection Genetics
Prof. Dr. Klaus Schughart

RG **TEE**
Experimental Animal Unit
Dr. Bastian Pasche

Dep. **MIBI**
Molecular Infection Biology
Prof. Dr. Petra Dersch

JRG **IBIS**
Infection Research of Salmonella
Dr. Marc Erhardt

RG MINP
Microbial Interactions and Processes
Prof. Dr. Dietmar Pieper

RG **KOM**
Microbial Communication
Prof. Dr. Irene Wagner-Döbler

RG **ZEIM**
Central Facility for Microscopy
Prof. Dr. Manfred Rohde

Dep. **MOBA**
Molecular Biology
Prof. Dr. Susanne Häußler

RG **GMAK**
Genome Analytics
Dr. Robert Geffers

Dep. **RABI** (HIRI)
RNA-Biology of Bacterial Infections
Prof. Dr. Jörg Vogel

RG **SIGA** (HIRI)
Single Cell Analysis
Dr. A.-Emmanuel Saliba

Dep. SFPR
Structure and Function of Proteins
Prof. Dr. Wulf Blankenfeldt

RG **CPRO**
Cellular Proteome Research
Prof. Dr. Lothar Jänsch

RG **MPRO**
Microbial Proteomics
Prof. Dr. Susanne Engelmann

RG **NBSC**
Structural Chemistry
Prof. Dr. Teresa Carlomagno

RG **RPEX**
Recombinant Protein Expression
Dr. Joop van den Heuvel

Dep. **STIB** (CSSB)
Structural Infection Biology
Prof. Dr. Michael Kolbe

Dep. **ZBIO**
Cell Biology
Prof. Dr. Theresia Stradal

RG **MZBI**
Molecular Cell Biology
Prof. Dr. Klemens Rottner

JRG **VIMM**
Virale Immune Modulation
Prof. Dr. Melanie Brinkmann

TOPIC 2: Immune Response and Interventions

Dep. **EXIM**
Experimental Immunology
Prof. Dr. Jochen Hühn

Dep. **EXPI** (TC)
Experimental Infection Research
Prof. Dr. Ulrich Kalinke

Dep. **BIOM** (TC)
Biomarkers in Infection and Immunity
PD Dr. Frank Pessler

Dep. **IMMK**
Immune Control
Prof. Dr. Burkhart Schraven

RG **INMI**
Intravital Microscopy in Infection and Immunity
Prof. Dr. Andreas Müller

RG **IREG**
Immune Regulation
Prof. Dr. Dunja Bruder

RG **SIME**
System-oriented Immunology and Inflammation Research
Prof. Dr. Ingo Schmitz

RG **NIND**
Neuroinflammation and Neurodegeneration
Prof. Dr. Martin Korte

Dep. SIMM (BRICS)
Systems Immunology
Prof. Dr. Micheal Meyer-Hermann

Dep. **VAC**
Vaccinology and Applied Microbiology
Prof. Dr. Carlos A. Guzmán *(Deputy Director)*

RG **IMCI**
Immune Aging and Chronic Infections
Prof. Dr. Luca Cicin-Sain

RG **MIDI**
Microbial Diagnostics
Prof. Dr. Manfred Höfle

JRG **MIKI**
Microbial Immune Regulation
Dr. Till Strowig

RG **MSYS**
Model Systems for Infection and Immunity
Prof. Dr. Dagmar Wirth

Helmholtz Centre for Infection Research GmbH
Inhoffenstraße 7
38124 Braunschweig

Other Locations:

BRICS	BRICS, Braunschweig Integrated Centre of Systems Biology, Rebenring 56, 38106 Braunschweig
CSSB	CSSB, Centre for Structural Systems Biology, Notkestraße 85, 22607 Hamburg
HIPS	HIPS, Helmholtz Institute for Pharmaceutical Research Saarland, Universitätscampus E8.1, 66123 Saarbrücken
HIRI	HIRI, Helmholtz Institute for RNA-based Infection Research, Josef-Schneider-Straße 2, 97080 Würzburg
TC	TWINCORE, Centre for Experimental and Clinical Infection Research GmbH, Feodor-Lynen-Str. 7, 300625 Hannover

HZI HELMHOLTZ Centre for Infection Research

BR – Staff Council
John Aubert, Chair

BEM – Occupational Re-entry Management
Angela Walter

GB – Equal Opportunity Commissioner
Katja Flaig

VPS – Represent. Body for Disabled Employees
Carolin Heidler

IT-Safety Officer
Christoph Barlag *(temp.)*

Animal Welfare Officer
Dr. Marina Pils

TOPIC 3: Anti-Infectives

Dep. **CBIO**
Chemical Biology
Prof. Dr. Mark Brönstrup

RG **COPS**
Compound Profiling and Screening
Prof. Dr. Ursula Bilitewski

Dep. **DDEL** — HIPS
Drug Delivery
Prof. Dr. Claus-Michael Lehr

JRG **BION** — HIPS
Biogenic Nanotherapeutics
Dr. Gregor Fuhrmann

Dep. **DDOP** — HIPS
Drug Design and Optimization
Prof. Dr. Rolf Hartmann
Prof. Dr. Anna Hirsch

JRG **CBCH** — HIPS
Chemical Biology of Carbohydrates
Dr. Alexander Titz

Dep. **MCH**
Medical Chemistry
Prof. Dr. Markus Kalesse

Dep. **MINS** — HIPS
Microbial Natural Products
Prof. Dr. Rolf Müller

RG AMEG — HIPS
Actinobacteria Metabolic Engineering Group
Prof. Dr. Andriy Luzhetskyy

RG **INI**
Infection Immunology and Processes
PD Dr. Eva Medina

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

JRG **SBBE** — HIPS
Structural Biology of Biosynthetic Enzymes
Dr. Jesko Köhnke

Dep. **MWIS**
Microbial Drugs
Prof. Dr. Marc Stadler

Administrative Management

Departments

EM – Purchasing Department
Anja Anfang

FA – Finance Department
Dirk-Michael Reinhardt

JUR – Legal Affairs and Licences
Dr. Christiane Kuegler-Walkemeyer
(Technology Transfer Commissioner)

ORG – Organisation and Administration-IT
Harald Ohrdorf
(Data Protection Manager)

PA – Human Resources
Jörg Schinkel

PS – Patents
Dagmar Meseke

RZ – Computer Centre
Dr. Joachim Metge

SU – Safety and Environmental Affairs
Dr. Erwin Grund

TB – Technical Services
Olaf Rabe

Staff Units

BIB – Library
Axel Plähn

CO – Controlling
BCO ***Elisabeth Gerndt***
DMC ***Dr. Michael Strätz*** *(Deputy Director)*
PWC ***Dr. Rolf-Joachim Müller***

FASI – Occupational Safety Spezialist
Carsten Strömpl

GS – HZI International Graduate School for Infection Research
Dr. Sabine Kirchhoff

IR – Internal Auditing
Richard Lomberg
(Anti-Corruption Commissioner)

PuK – Press and Communications
Susanne Thiele

SKO – Strategic Communication
Manfred Braun

WST – Scientific Strategy
Dr. Corinna Richthammer

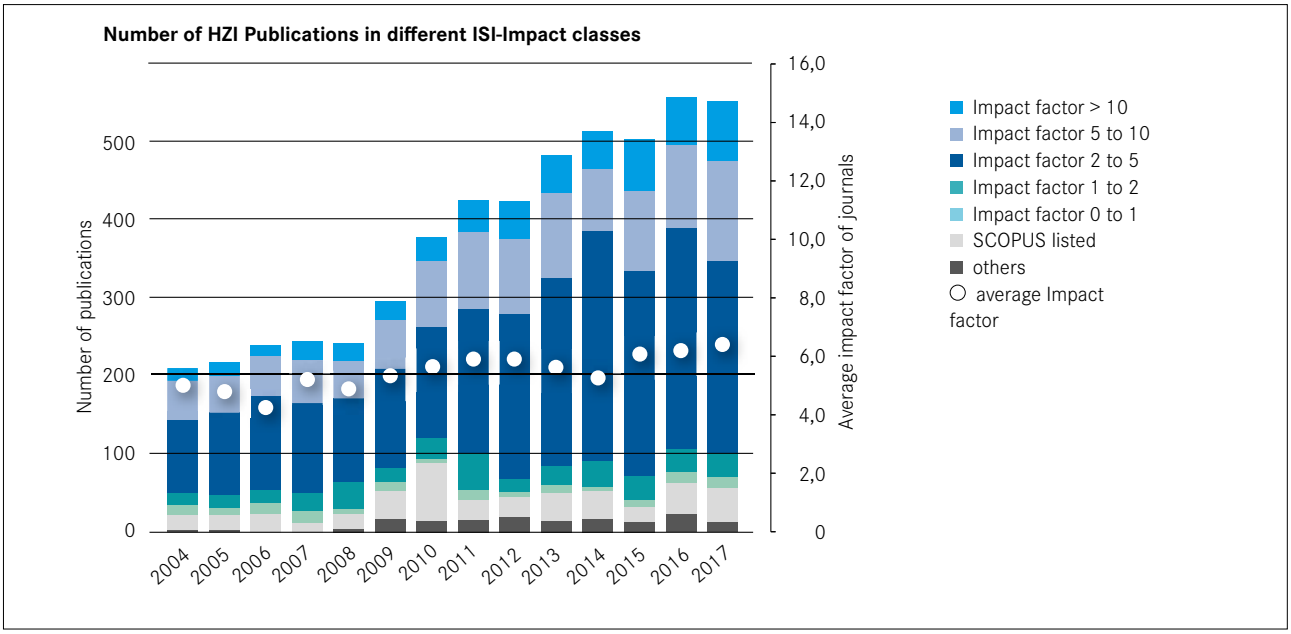
Dep.: Department
RG: Research Group
JRG: Junior Research Group

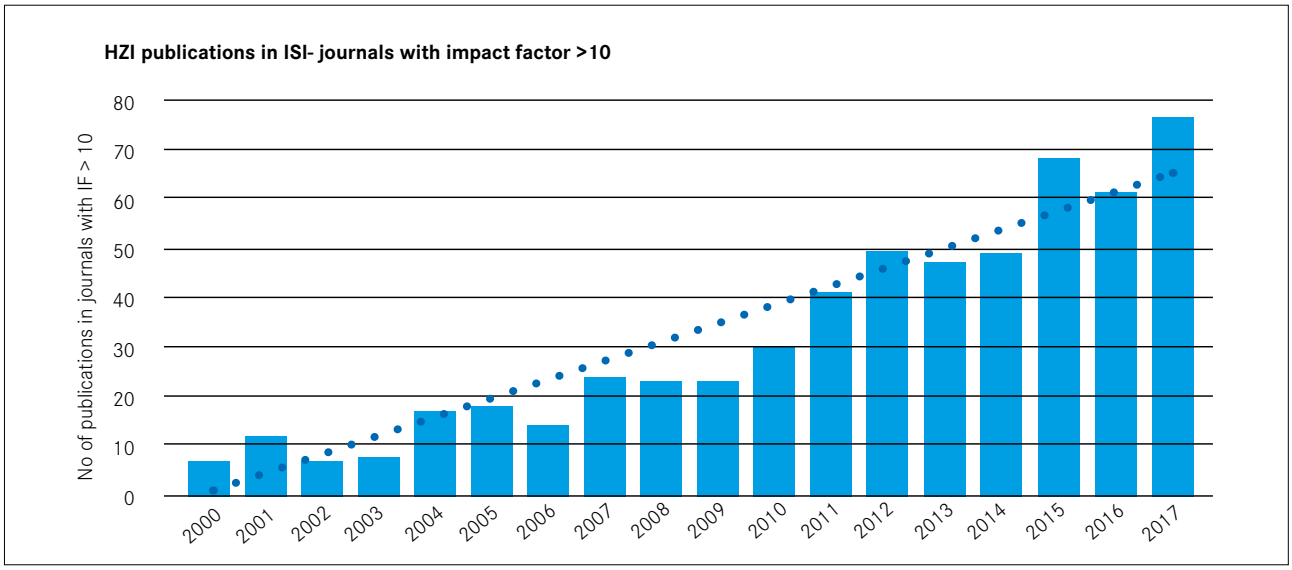
BCO: Financial Controlling
DMC: External Funding Controlling
PWC: Planning Programme and Scientific Controlling

Version: 31. December 2017

PUBLICATIONS

In 2016 and 2017, more than 1100 scientific articles were published by HZI scientists. Over the recent years, the number of publications in general as well as the number of articles in high impact journals have been increasing.





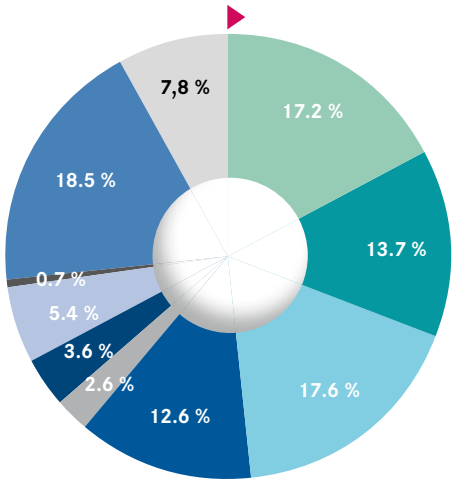
FINANCING

In 2017, the complete budget of HZI amounted to 80.1 Mio € including 19.0 Mio. € of Third Party Funding.

More than 60 per cent of the external funding came from national programmes, about 20 per cent were from EU programmes and industry.

Third party funding of Research in 2017 (in 1000 €)

Source	Full cost
BMBF	3,278
DFG	2,611
EU	3,346
Helmholtz Association	2,396
Industry	0,505
Länder	0,686
NAKO (LKII)	1,030
Construction/Investments	0,141
DZIF	3,530
Others	1,499
Total Sum	**19,022**



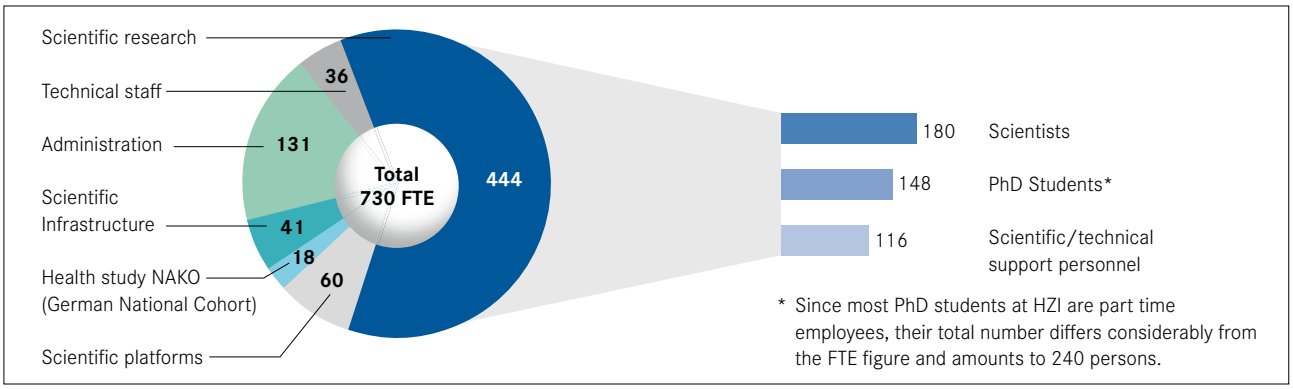
PARTICIPATION IN RELEVANT RESEARCH NETWORKS

In 2017, HZI and TWINCORE participated in 17 DFG Programmes (including Clusters of Excellence and Collaborative Research Centres), 32 EU projects (including ERC Starting, Consolidator and Proof-of-Concept Grants) and 29 BMBF / BMG / BMWi projects.

Patents, property rights and licenses

	2016	2017
Priority based applications	2	10
Total number of held property rights	515	438
Licence agreements	26	24
Licence proceeds (thousand €)	889	1,833

PERSONNEL



At the end of 2017 the HZI staff comprised 822 full time and part time employees, amounting to 730 full time equivalents (FTE). Scientific personnel constitutes the majority of HZI staff (444 FTE). Additionally, 255 guests worked in various projects, receiving their payment from third parties.

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: January 2018)

Function	Name, Titel	Organisation	Location
SB	Prof. Dr. Christopher Baum	MHH	Hannover
SB	Prof. Dr. Jan Buer	University Clinic	Essen
Chair SB	MinDir Bärbel Brumme-Bothe	BMBF	Berlin
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Frank Bierstedt – page: 37
Uwe Bellhäuser – pages: 11, 115
CSSB/Tina Mavric – pages: 1, 12, 26, 118
CRC collection – page: 12
DESY – page: 12
DFG/David Ausserhofer – page: 15
DZIF collection – pages: 120, 122
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- 14, 110 (Alexander Rats)
- 57 (Sebastian Kaulitzki)

Johannes Huisman – pages: 12, 125
HZI collection – pages:
- cover (Till Strowig), 20, 21 (Heinrich Lünsdorf), 23, 27, 28 (Verena Meier), 36, 44, 45, 46, 56, 88, 89,106, 108, 111, 115 (Manfred Rohde), 72 (Department CPRO/Marco van Ham)
1, 6, 7, 15, 20, 22, 23, 26, 29, 33, 34, 36, 37, 38, 41, 46, 54, 62, 70, 78, 86, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 115, 118, 123,
HIRI/Mario Schmitt – pages: 1, 30, 32, 116
Jenko Sternberg Design – pages: inside cover “The HZI at a Glance”
JMU Würzburg – page: 11
MHH collection – pages: 112, 123 (Karin Kaiser)
Pixabay – pages: 64, 73, 107
UdS/Jörg Pütz – pages: 114
Stefan Rampfel – page: 9
ScienceRELATIONS – page: 122
Thomas Steuer – page: 8
TU Braunschweig – page: 27 (Stephan Nachtigall)
TWINCORE collection – pages: 1, 11, 112, 123

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Inhoffenstraße 7 | D-38124 Braunschweig, Germany
Telephone +49 531 6181-0 | Fax +49 531 6181-2655
info@helmholtz-hzi.de | www.helmholtz-hzi.de
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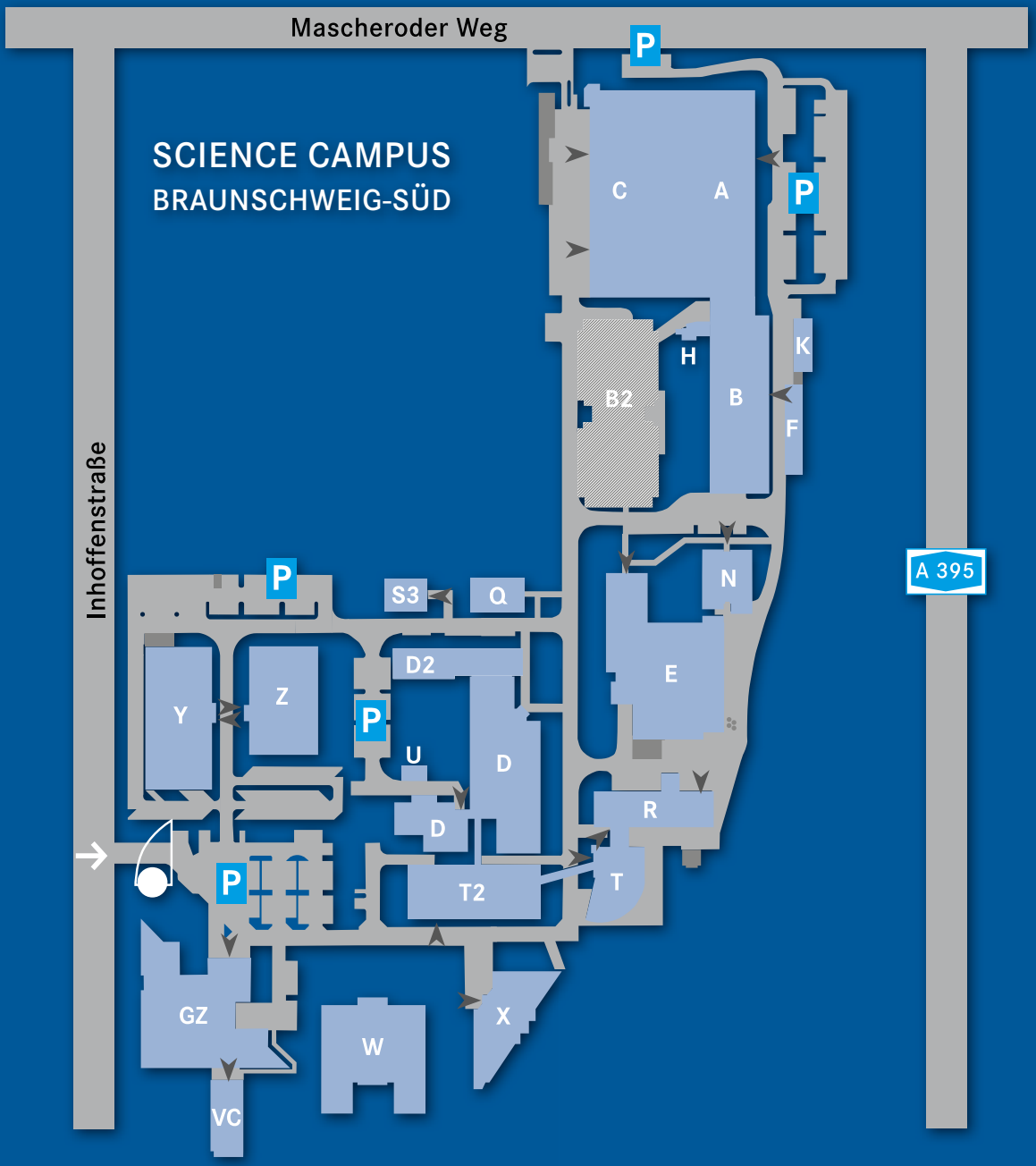

The HZI Campus

A, B, C, D, GZ	Research Labs
A	YUMAB GmbH
B2	Drug research & Functional genomics (under construction)
D2	Offices
E, F, H, K, Q, R, U	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

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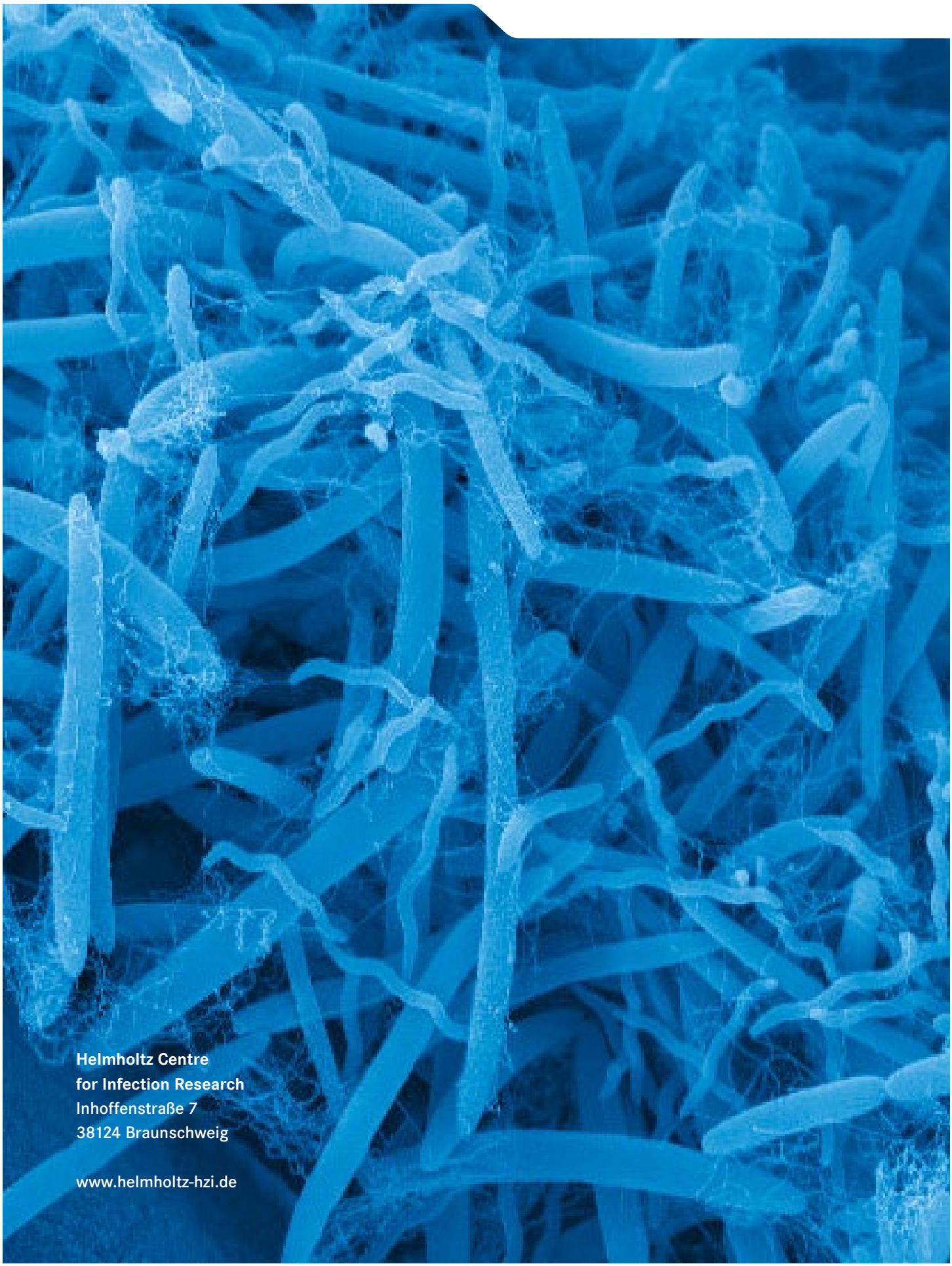

Helmholtz Centre
for Infection Research
Inhoffenstraße 7
38124 Braunschweig

www.helmholtz-hzi.de