S-1-2

Gut microbiota depletion does not affect the Intestinal Barrier Integrity Signaling Pathways in helminth infected individuals: Discussion from results of a randomized trial in Gabon

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Backround: The human gastrointestinal microbiota has co-evolved and shares its environment with parasitic worms. Several studies related to the concepts of the "hygiene hypothesis" and "immune distraction" demonstrate that interactions between the microbiota and helminths can influence both populations and host health. However, little is known about the context of endemic exposure to helminth infections. We hypothesized that antibiotic treatment-induced microbiota depletion may affect immune responses and gut homeostasis in people infected with helminths. We conducted a randomized trial to define the role of microbiota depletion on the immune response and pathophysiology of helminth-infected individuals in sub-Saharan regions.

Methods: A randomized, placebo-controlled, blinded study was conducted on 38 adults infected with *Trichuris trichiura* or hookworm, living in Lambaréné for 6 months. Participants received 2000 mg of neomycin or placebo 3 times daily for 10 days. After 90 days, they were treated with anthelmintic drugs. Clinical data, blood, and stool samples were collected at different time points. Parasite burden was assessed using the Kato-Katz technique. The abundance of the fecal microbiota was determined by 16S rRNA gene analysis, and microbial production was assessed by measuring total short-chain fatty acids (SCFAs) in plasma. Total RNA isolation and reverse transcription were performed from peripheral blood mononuclear cells (PBMCs). qRT-PCR was carried out using SYBR Green mix to assess the transcript levels of cytokines and genes involved in the G-coupled receptor signaling pathway (GPR41, GPR43, IFABP, IL-1β, IL-6, TNF-α, IL-10, IL-17, TLR2, TLR4) at days 0, 7, and 14. Additionally, markers of intestinal integrity and cytokines were quantified in plasma using human ELISA kits for Neutral ceramidase (Asah2), Neuregulin (Nrg1), IFN-γ, IL-1β, IL-6, TNF-α, IL-10, and IL-17.

Results: By day 7, there was a significant reduction in *Succinivibrio* and *Veillonella* populations, associated with a 2-fold reduction in hookworm egg counts. We next investigated whether microbiota depletion affected G-coupled receptor signaling pathways involved in peripheral immune responses and gut homeostasis. In contrast to the fecal microbiota results, total SCFA levels were similar in both groups, with a median of 10 ng/ml. We observed similar expression levels of GPCR-TLR genes in PBMCs and related cytokines in both groups. Additionally, circulating cytokine profiles (IL-1β, IFNγ, IL-6, TNFα, IL-10, IL-17) and intestinal permeability markers (NRG1, ASAH2) in plasma were also similar, independent of the treatment arm. No differences in adverse events were observed between participants treated with neomycin and those in the control group.

Conclusion: Our results demonstrate the resilience of human gut homeostasis and peripheral immune responses following gut microflora disruption in helminth infected individuals.

S-1-3

Within-host evolution of the gut microbiota enforces colonization resistance against enteric infection

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In the mammalian gastrointestinal tract, commensal organisms compete for a limited supply of nutrients, including carbohydrates and electron acceptors. Bacteria in these communities can quickly adapt to the constantly changing nutritional environment through various evolutionary mechanisms. While the evolution of specific bacterial species in the gut has been extensively researched, there still needs to be more understanding regarding how these evolutionary trajectories impact microbiome functions, such as colonization resistance against enteric pathogens. Here, we used experimental evolution as a tool to examine the adaptation of a defined microbial consortium, known as the Oligo-Mouse-Microbiota (OMM12), to the gut environment and to assess its functional relevance for colonization resistance against the enteric pathogen *Salmonella enterica* serovar Typhimurium (*S.* Tm). Through whole-genome analysis of 130 bacterial re-isolates derived from OMM12-colonized mice, we have identified numerous mutations that contribute to intra-species diversification. Specifically, we have observed a horizontal gene transfer event that influences the carbohydrate metabolism of *E. faecalis*. Employing a multi-omics approach, we demonstrate that *E. faecalis* evolves to more efficiently consume specific nutrient sources, increasing colonization resistance against *S.* Tm through competitive exclusion. We conclude that evolutionary trajectories influencing bacterial carbohydrate preferences in the gut microbiome contribute to colonization resistance against enteric pathogens. Unravelling the mechanisms underlying these evolutionary dynamics holds immense potential for engineering microbial communities that promote a healthier state in the host.

S-1-4

Commensal keystone species inhibit Salmonella infection through multiple microbiota-context-dependent mechanisms

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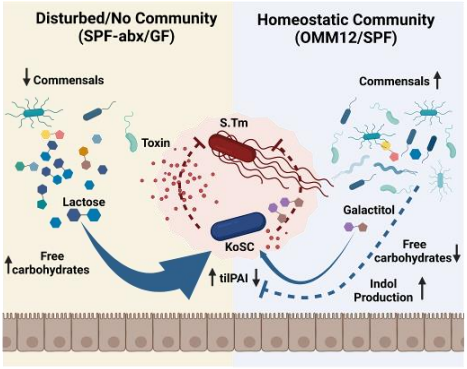
The *Klebsiella oxytoca* species complex (KoSC) is a component of the human microbiome, particularly abundant during infancy and childhood. Strains within the KoSC have a dual role in the literature. One the one hand, they have been identified as pathobionts, capable of producing enterotoxins such as tilimycin and tilivalline, which can lead to colitis under certain conditions. Conversely, various research groups have reported that KoSC strains can also provide colonization resistance (CR) against pathogens like *Klebsiella pneumoniae* and *Escherichia coli* and are associated with a reduced risk of sepsis in infants and transplant patients. The relationship between these seemingly contradictory roles is not well understood. Here, we investigated whether and how KoSC can protect against *Salmonella*-induced inflammation in under various microbiome conditions.

To better understand how the microbial context might influence the role of KoSC against the important and emerging enteropathogen *Salmonella Typhimurium*, we studied the competition between *K. oxytoca* and *S. Typhimurium* in vitro, as well as *S. Typhimurium*-induced enterocolitis in mouse models with both disturbed and undisturbed microbiota states. Many, but not all, KoSC strains inhibited *S. Typhimurium* in vitro. Suprisingly, genome analysis followed by gene targeting identified tilimycin production as a potent inhibitory factor in vitro. Notably, in mouse models, *K. oxytoca* provided CR against *S. Typhimurium* in different microbiota settings via distinct toxin-dependent and toxin-independent mechanisms. The availability of simple carbohydrates influenced both mechanisms: toxin production predominated in environments with high sugar availability, while microbial competition played a larger role when carbohydrates were scarce. This finding demonstrates that microbial competition is relevant not only to the interaction between *S. Typhimurium* and *K. oxytoca* but also to the broader microbial ecosystem surrounding *K. oxytoca*. Specific members of the homeostatic gut community appear capable of restricting available carbon sources to levels that prevent the production of detectable amounts of tilimycin, which would otherwise strongly inhibit these gut members. This study underlines the duality of specific members of the gut microbiota and their produced metabolites dependent on the gut environment and microbial context. While this is by itself an interesting biological observation, it might also be a relevant finding for future intervention strategies to prevent the negative effects of acute or chronic toxin production.

Fig. 1



S-1-5

Cervicovaginal bacterial communities in adult Malagasy women of reproductive age with human papillomavirus infection and female genital schistosomiasis

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The vaginal microbiome (VM) is described to have a role in the persistence of human papillomavirus (HPV) infection. Recent findings suggest that female genital schistosomiasis (FGS), caused by a chronic infection with *Schistosoma haematobium*, might exacerbate the risk of both HPV infection and cervical cancer. Typical symptoms of FGS are non-specific and diagnosis is done using colposcopy. Few studies have described the characteristics and variability of the VM in association with FGS, showing different bacterial communities or abundant *Trichomonas vaginalis*. The objective of our study was to characterize the VM of women of reproductive age in Madagascar, a country with high burden of both FGS and HPV.

A cross-sectional study was conducted at three Primary Health Care Centres in the district of Marovoay in the Boeny region of Madagascar. Data was collected from women aged 18-49 years between March and August 2021, including colposcopy results, cervicovaginal lavage (CVL) samples, socio-demographics, personal habits and clinical history. CVL samples were shipped to the Institute of Clinical Molecular Biology, Kiel, Germany, for 16S rDNA sequencing.

The sequencing of 16S rDNA of 414 women showed that *Gardnerella*, *Sneathia* and *Mycoplasma* were more abundant in HPV-positive women, but no differences were observed among women with different FGS and HPV/FGS statuses. Variability in the alpha and beta diversity was associated with urbanicity, profession, diet behavior, antibiotics usage and dyspareunia. Using VALENCIA, the five community state types (CST I - V) could be identified. The majority of VM were characterized as CST IV (highly diverse and without Lactobacillus dominance), followed by CST III (dominated by *L. iners*).

This first study of VM in Madagascar showed no Lactobacillus dominance in the Malagasy population. Further studies are needed to characterize the role of VM for high burden gynecological disorders, especially those affecting the most neglected populations.

S-1-6

Highly potent quinoxalindiones prevent tissue damage following *S. aureus* infection by inhibiting hemolysin alpha

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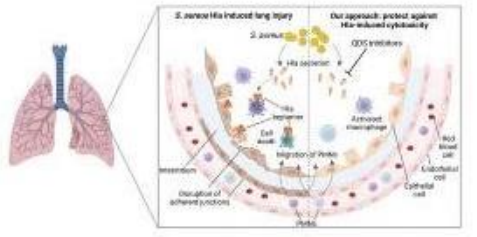
Hospital-acquired pneumonia caused by *Staphylococcus aureus* is associated with patient morbidity and mortality, in spite of adequate antibiotic therapy. It is considered a preventable condition as infection is typically preceded by airway colonization with the bacteria. Therefore, there is an opportunity for novel treatment concepts that go beyond antibiotics. The pore-forming heptameric toxin *α*-hemolysin (Hla) is a major pathogenicity factor of *S. aureus* and a clinically validated target. Using Hla-dependent phenotypic cellular assays, we discovered quinoxalindiones (QDS) as highly potent Hla inhibitors. QDS reverted the hallmarks of Hla pathogenicity by conferring protection against Hla-induced Ca2+ influx and cytotoxicity and by maintaining monolayer integrity of lung epithelial cells. The cellular effects were exerted in the nM range across all major Hla subtypes and shown in relevant cell types, including lung epithelial and endothelial cells, and primary human immune cells. QDS prevented the formation of functional Hla pores by interacting with the protein monomers. Structural data by NMR and chemoproteomics showed monomer binding near the phospholipid binding site of the protein, a functional site required for membrane integration. The analog H052 was active in mouse models of *S. aureus* lung infections in pre-emptive and therapeutic settings, as a monotherapy and in combination with subtherapeutic doses of linezolid. The QDS demonstrate that formation of large bacterial toxins complexes can be effectively inhibited by drug-like small molecules. The virulence-attenuating drug candidate may now be developed as a monotherapy in a pre-emptive intervention setting.

Fig. 1



Hallmarks of alpha-hemolysin (Hla)-induced lung injury following a *S. aureus* infection, and its prevention by QDS inhibitors.

S-2-2

Non-waning immunity and protection upon a single immunization with a self-boosting MCMV-vectored vaccine in hamsters

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Background: Cytomegaloviruses (CMV) elicit a lasting immune response with remarkably large populations of antigen-specific CD8+ T cells for life. Therefore, CMV has been proposed and investigated as a novel vaccine vector. Strict species specificity of CMV has compelled vaccine developers to use HCMV in clinical trials. We propose that non-human CMVs used in cross-species settings may overcome several limitations of HCMV vectors and be superior in terms of safety and immunogenicity. On one hand, such constructs are naturally unable to replicate in human cells, omitting the need to generate recombinant single-cycle CMV vectors grown on complementing cell lines. On the other, immune evasion genes are host-specific and do not affect responses in species discordant settings. A recombinant murine CMV (MCMV) vaccine vector expressing the spike protein of SARS-CoV-2 (MCMVS) showed a robust and long-lasting protection against distinct SARS-CoV-2 variants in the mouse model. We show in this study the immunogenicity and efficacy of our MCMV-based vector vaccine in the non-cognate hamster host.

Methods: We immunized hamsters with MCMVS and longitudinally quantified humoral and cellular immunogenicity following different immunization routes. At two, six, and ten months post immunization, immunized hamsters were challenged with SARS-CoV-2 including the Omicron BA.5 variant.

Results: Hamsters tolerated MCMVS well. They developed neutralizing antibodies and antigen-specific T cell responses, which expanded in size and breadth over time. Comparing intramuscular, subcutaneous, intranasal and intraperitoneal application routes, we found that robust humoral and cellular immune responses to spike antigens were elicited after all infection routes, but in particular after intramuscular injection. All immunized animals fully controlled productive SARS-CoV-2 infection upon challenge with the vaccine seed strain or with the antigenically distinct BA.5 variant up to ten months post-immunization.

Conclusion: Our data suggest that a single-dose vaccination with an MCMV-based vector vaccine elicits exceptionally long-lasting and broad protection in a non-cognate host species. Hence, this technology might be a promising platform for the development of broadly reactive and long-lasting immune responses and has the potential for use in clinical settings.

Funding/Support: This work was supported by the Helmholtz Association through the Impulse and Networking Fund grant PIE-008 to LCS and by the Federal Ministry of Education and Research through the GoBio Initial grant VIVA-VEK to HJ and LCS.

S-2-3

High *in vivo* expression of potent HIV-1 broadly neutralizing antibody mediated by AAVMYO vectors

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Broadly neutralizing antibodies (bNAbs) are a novel option for treatment and prevention of HIV-1 infection. While potent antiviral activity and favorable pharmacokinetic properties of bNAbs have been demonstrated in humans, the need for repeated administrations can limit antibody-mediated approaches against HIV-1. Recombinant adeno-associated viruses (rAAVs) are a promising tool for vector-mediated gene transfer to achieve sustained bNAb production. However, initial attempts for rAAV-mediated expression of HIV-1 bNAbs using AAV2- or AAV8-based vectors resulted in only low neutralization titers and the induction of anti-drug antibodies.

To develop novel vectors for HIV-1 bNAbs, we generated rAAVs based on the myotropic AAVMYO capsid protein that encode for the newly identified highly potent CD4 binding site bNAb A06. Functional bNAb expression from the single vector plasmid co-encoding antibody heavy and light chains was confirmed by HIV-1_{BG505} ELISA of transfected 293T cell supernatants. To determine the magnitude and durability of bNAb A06 expression under control of different promoters *in vivo*, we longitudinally analyzed serum antibody levels in NOD-*Rag1*^{null} *IL2rg*^{null} (NRG) mice. Consistent with high myocyte tropism of AAVMYO, the liver-specific albumin promoter resulted in low A06 IgG levels after application of 10^{12} rAAV genomes (geometric mean concentration of 1.5 µg/mL determined by total human IgG ELISA). In striking contrast, a single intravenous AAMVYO injection based on the CMV or muscle-specific SPc5-12 promoter led to high geometric mean A06 levels of 1000.0 and 721.7 µg/ml, respectively. IgG concentrations >100 µg/ml could be observed as early as one week after injection. Notably, A06 IgG levels of >500 µg/mL were maintained for at least 3 months after the single rAAV administration. TZM-bl cell pseudovirus assays using hard-to-neutralize tier-2 strains confirmed rAAVMYO-mediated functional A06 expression resulting in potent HIV-1 neutralizing activity in serum.

Subsequent experiments will evaluate the antiviral efficacy of A06-rAAVMYO constructs in treating and preventing HIV-1 infection *in vivo*. Based on the demonstrated high and persistent antibody expression following a single application, bNAb-encoding rAAVMYO vectors are a promising option for further development.

S-2-4
Multi-omics profiling of immunity to influenza vaccination reveals enhanced antibody responses in cancer patients

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Background/Question: Infectious diseases pose a significant threat to global health, causing more than 2 million deaths worldwide in 2019. Much of this burden is carried by immunocompromised individuals, such as cancer patients, who have a dysregulated immune system and die more often from infections than healthy individuals. Importantly, vaccines, which are a cornerstone of infectious disease prevention, are often less effective in this population. The molecular and cellular mechanisms underlying immune dysregulation and poor immunity are complex, variable among individual patients/conditions, and not well understood. An in-depth understanding of these mechanisms is crucial to identifying novel strategies to prevent infectious diseases and developing individualized therapies.

Method: To address this challenge and gain in-depth insights into the functioning of a dysregulated immune system, we have used a systems biology approach and analyzed blood samples from >130 healthy donors and cancer patients. We included two groups of cancer patients with an altered immune system: 1) patients with multiple myeloma, a hematological malignancy, receiving lenalidomide (an inhibitor of plasmablast [PB] development) and 2) patients with metastasized non-small cell lung cancer, a solid tumor, receiving chemotherapy and immune checkpoint inhibitors. Blood was collected at time points before and after immunization with an inactivated tetravalent influenza vaccine.

Results: In contrast to our initial hypothesis, our unpublished data demonstrate that both groups of cancer patients developed superior vaccine-induced binding and neutralizing antibody responses compared to matched healthy donors. In addition, single-cell RNA-seq analysis revealed that these cancer patients have a prolonged PB response that prevails for more than 30 days. Conversely, healthy donors in our cohort follow the "typical" pattern of a transient PB response, peaking at day 7 and returning to baseline at day 30. Transcriptional profiling indicates metabolic reprogramming in PBs from cancer patients, expressing higher levels of OXPHOS-associated genes – a phenotype previously linked to enhanced antibody production. Ongoing experiments using CyTOF, Olink, and BCR-seq are focused on gaining additional insights into the underlying mechanisms and determining how the administered oncology drugs contribute to the superior response.

Conclusion: Together, our results prove that potent vaccine responses can be induced in immunocompromised patients and raise the possibility that oncology drugs could boost vaccine responses. These insights serve as a starting point for developing improved vaccination approaches for vulnerable patients.

S-2-5
Therapeutic treatment of Hepatitis E virus infection in pigs with a neutralizing monoclonal antibody

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Question: Hepatitis E Virus (HEV) is a major public health concern and is recognized as the leading cause of acute viral hepatitis globally. Particularly in Europe, zoonotic infections with genotype 3 (HEV-3) can cause chronic hepatitis E in immunocompromised individuals and those with pre-existing liver disease. The prevalence of HEV-3 has been increasing in Europe, particularly in pigs and wild boars, which serve as main reservoir hosts. Chronic HEV infections can occur in immunocompromised patients or those with pre-existing liver damage, with approximately 15% of chronically infected patients failing to clear the virus due to factors like low treatment efficacy, adverse effects, or viral mutations Given the limitations of current treatments, there is an urgent need for alternative therapeutic options to manage chronic HEV infections and prevent HEV-related morbidity and mortality.

Methods: Monoclonal antibodies (mAb) have emerged as promising therapeutic agents for a variety of infectious

diseases over the past three decades, with regard to their specificity, low side-effect profiles, and ability to enhance immune responses. Given these attributes, mAb could offer a novel alternative for treating chronic HEV infections, especially in patients who do not respond to or cannot use current treatments . To explore this potential, we developed a set of monoclonal antibodies targeting the HEV capsid protein. These antibodies were tested in vitro to assess their neutralizing activity and the most effective antibody, mAb 5F6A1, was selected for further evaluation in an HEV-3-infected pig model. Following infection, mAb 5F6A1 was administered intravenously to pigs on days one and seven post-infection (Fig. 1).

Results: The in vivo study demonstrated that treatment with mAb 5F6A1 led to a significant reduction in both viremia and viral shedding in HEV-3-infected pigs compared to the untreated control group (Fig. 2). This reduction indicates the antibody's effectiveness in neutralizing the virus and limiting its replication within the host.

Conclusions: This study is the first to show a significant decrease in HEV replication using monoclonal antibody therapy in an animal model, highlighting a potential therapeutic option for patients with chronic HEV infection. The findings underscore the potential of monoclonal antibodies as an alternative or adjunctive treatment for chronic HEV cases, particularly when existing pharmaceutical options are ineffective or contraindicated.

Fig. 1 Experimental design and overview on pig groups and timeline in mab treatment experiment

Fig. 2 Detection of viral RNA in feces (A) and serum (B) of infected pigs

Fig. 1

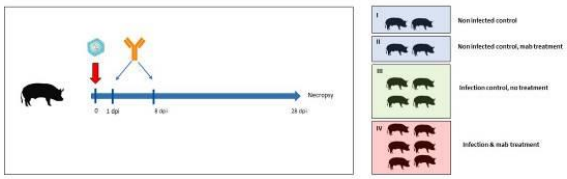

Figure 1

Fig. 2

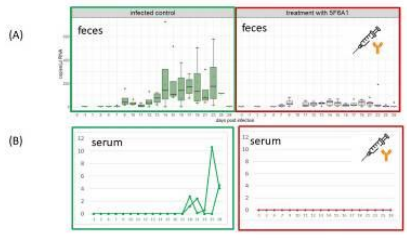

Figure 2

S-2-6

Mucosal IFNλ1 mRNA-based immunomodulation effectively reduces SARS-CoV-2 induced mortality in mice

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RNA vaccines are routinely used to induce protective immunity against SARS-CoV-2, but the potential of mRNA as an antiviral therapeutic remains largely unexplored. In this study, we examine the efficacy of lipidoid nanoparticle (LNP)-formulated mRNA encoding human IFNλ1 (ETH47), a key driver of antiviral responses at mucosal surfaces. Administration of IFNλ1 mRNA leads to dose-dependent protein translation, expression of interferon-stimulated genes but not inflammatory cytokines, and a significant reduction in SARS-CoV-2 replication in vitro. In mice, pulmonary delivery of IFNλ1 mRNA results in a strong decrease in viral load, mitigates virus-induced weight loss, and significantly improves survival rates. These findings highlight the potential of inhaled IFNλ1 mRNA as a prophylactic treatment for individuals exposed to SARS-CoV-2 or at high risk of developing COVID-19. Given the broad-spectrum antiviral properties of IFNλ1, this strategy could also apply to other respiratory viral infections, contributing to pandemic preparedness.

S-3-2

High burden of human papilloma virus infections and persistence in people living with HIV

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Question: In people living with HIV (PLH), coinfection with human papilloma virus (HPV) is a major source for comorbidity and mortality due to HPV-associated tumors. Despite highly efficient antiretroviral therapy (ART), HIV infection still increases the risk for HPV persistence, the cumulative lifetime risk of HPV disease progression and thus incidence of HPV-associated cervical, anal and oropharyngeal cancer (Perez-Gonzalez et al., 2022).

Methods: This study analyses the association of HIV-induced dysfunctional immune response and HPV persistence in an observational, prospective, longitudinal study (follow-up visits after 3, 6 and 12 months) conducted at the LMU University Hospital and Prinzmed practice in Munich, Germany. Anal and oral HPV status regarding 28 high- (HR), intermediate- (IR) and low-risk (LR) subtypes were evaluated. Concomitantly, myeloid-derived suppressor cell populations were defined by a flow cytometric approach and HPV-specific T cell responses targeting five HR-HPV types were monitored by ELISpot assays using freshly isolated peripheral blood mononuclear cells.

Results: 75 male and 3 female individuals with a median age of 54 (IQR 43-61) have been recruited. All 63 people living with HIV were on ART (viral load < 50cp/ml) with stable CD4 counts (median 603 (IQR 504-773) and 14/15 people living without HIV received pre-exposition prophylaxis. At baseline, HPV was anally and/or orally detectable in 89% and 33% of PLH or rather 93% and 40% in people living without HIV. While most (71% PLH, 80% no HIV) of the oral IR and HR subtypes were cleared in both groups, 71% (PLH) or rather 57% (no HIV) of anal IR and HR infections persisted up to 12 months. Only 28 out of 96 (29%; all timepoints merged) of PLH with HPV16, 18, 35, 45 or 58 showed an IFN-γ T cell response against the infecting subtype, which was associated with a more frequent remission of the respective subtype (p-value 0.0009; Figure 1). Individuals with a suspicious anal PAP smear (32%) had significantly more infecting high risk HPV subtypes and higher levels of polymorphonuclear myeloid-derived suppressor cells than those with no abnormalities detected (Figure 2).

Conclusion: In the presented study cohort, anal HPV infections are comparably abundant in people living with and without HIV. PLH lacking systemic HPV-specific IFN-γ responses or showing elevated levels of polymorphonuclear myeloid-derived suppressor cells are significantly more prone to HPV persistence or abnormal cytological findings in the PAP smear, respectively. Additional analysis of exhaustion marker and immune cell effector populations in peripheral blood as well as anal T cells by FACS and RNA-seq experiments will further characterize (dys)functionality of the immune system and provide insight on how the immune impairment associates with HPV persistence in PLH.

Fig. 1

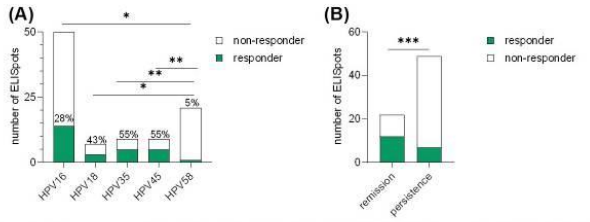

Figure 1: IFN-γ responses detected by ELISpot. Freshly isolated PBMC of PLH were stimulated for 20h with peptid pools from 5 HR subtypes. **(A)** Ratio between individuals with and without an IFN-γ response to either E6, E7 (HPV16, 18, 35, 45 and 58) or L1, E2 (HPV16, 18 and 45) is shown. Only individuals infected with the respective subtype are depicted. Fisher's exact test was applied. **(B)** PLH with remission or persistence of HPV16, 18, 35, 45 or 58 infection after 12 months were grouped into IFN-γ responders or non-responders to the corresponding subtype based on ELISpot data. Fisher's exact test was applied.

Fig. 2

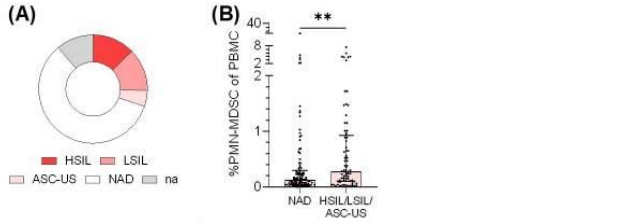

Figure 2: (A) Results of anal PAP smears of PLH collected as part of the routine clinical care during the study period. **(B)** Frequencies of PMN-MDSC (defined as CD15+CD14-CD33dimCD11b+) of total PBMC. Flow cytometric staining was performed within 2h after PBMC isolation. All four acquired timepoints are merged. Mann Whitney test was applied (p value 0.0036). (A-B) HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; ASC-US: atypical squamous cells of undetermined significance; NAD: no abnormalities detected; na: not available.

S-3-3

Chronic infection induces a broadly neutralizing B cell response to Elastase B of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PA) is a critical pathogen causing severe acute hospital-acquired infections, such as ventilator-associated pneumonia, and can significantly contribute to chronic infections in patients with underlying lung diseases. Among the various virulence factors related with pulmonary PA infections, the secreted bacterial elastase B (LasB) is particularly important, as it causes tissue damage and inflammation. Despite the long-recognized immunogenicity of LasB in humans, a detailed characterization of the humoral immune response against this enzyme remains elusive.

In our study, we investigated the humoral immune response to LasB in a cohort of individuals with cystic fibrosis (CF), who frequently suffer from chronic or recurrent PA infections. Notably, we observed protective effects of elevated anti-LasB antibody titers on lung function in chronically infected individuals with CF. Further single B cell analysis from six donors with elevated anti-LasB antibody titers revealed a diverse B cell receptor repertoire targeting LasB, which led to the production of monoclonal antibodies (mAbs) that effectively neutralize the proteolytic activity of LasB. In-depth analysis identified mAbs with broad-spectrum activity, unaffected by common mutational variations in LasB. Selected anti-LasB mAbs notably exhibited protective effects *in vivo*, reinforcing their potential as prophylactic or therapeutic agents.

To conclude, our findings indicate that chronic PA infection can elicit a protective, broadly neutralizing immune response against LasB, presenting a promising antivirulence strategy that could potentially be used against PA infections.

S-3-4

Host-derived lipid mediators as novel regulators of Treg cell development with distinct features in maintaining immune tolerance during human helminth infection

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Background and question: Distinct tissue-derived signals drive regulatory T cell (Treg) induction and shape their heterogeneity and functionality in chronic inflammation. In the inflammatory parasitic brain disease, neurocysticercosis (NCC), we have recently identified that the lipid mediator PGE2 secreted from parasitic glutamate dehydrogenase (GDH)-modulated monocyte and microglia is a central driver of Treg development, which is essential to control disease in asymptomatic, non-epileptic NCC patients. Here, we characterize the epigenetic and transcriptional determinants of GDH-PGE2-modulated Treg cell development and the clinical implications in brain inflammation and silencing and following anti-helminthic treatment.

Methods: Targeted lipidomics and extensive LC/MS/MS profiling of lipid mediators in controls, NCC asymptomatic and patients with epilepsy and neurological symptoms guided the identification of disease- and GDH-remodeling of eicosanoids, precursors and metabolites. In-depth immunophenotyping and pulsing of controls" and patients" peripheral cells with recombinantly expressed parasite GDH or PGE2 revealed the context-dependent Treg development with unique features in asymptomatic in contrast to symptomatic disease. Subsequent mechanistic pathways of lipid mediator regulation of Treg induction were elucidated by transcriptional profiling of *ex vivo* sorted monocytes. The epigenetic and transcriptional determinants of Treg development and landscapes in asymptomatic patients were furthermore assessed via ATACSeq and RNASeq as compared to *in vitro* induced Treg and *ex vivo* sorted Tconv from healthy individuals.

Results: Targeted lipidomics revealed a bias arachnoid acid metabolism in NCC patients with a pronounced COX2-PGE2 pathway in asymptomatic disease, which declined following anti-helminthic treatment. Importantly, the upregulation of the COX2-PGE2-Treg axis is associated with distinct features of enhanced CNS migration and endothelial cell adhesion potency ($CD69^{hi}$, $CCR7^{+}$, VLA-4^{hi}, LFA-1^{+}) of $ST2^{+}$Tregs, but absent in brain inflammatory symptomatic disease. The marked increase of PGE2 and precursor metabolites in patients" sera is positively correlated with pronounced EP2 and EP4 receptor expression on peripheral naïve $CD4^{+}CD25^{-}$ T cells. Moreover, integrative sequencing analyses interestingly revealed the non-canonical TNFR2-NF-kB and the JAK-STAT signaling pathways as important regulators controlling GDH-PGE2-driven Treg differentiation and thus a potential role in the regulation of inflammatory NCC.

Conclusions: This work highlights important insights into lipid mediators as novel regulators of Treg cell development with distinct features to maintain immune tolerance in NCC with relevance for other inflammatory brain diseases.

S-3-5

Dysregulated soluble immune checkpoints in plasma of Post-Covid syndrome patients correlate with altered immune phenotypes

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Post-Covid syndrome (PCS) occurs with an incidence of about 2% after infection with SARS-CoV-2. A wide spectrum of persistent symptoms such as fatigue or myalgia can be observed. The mechanisms contributing to the development of PCS are not yet fully understood. Long-term immune dysregulation has been discussed in other studies. The aim of our study is to investigate the cellular and humoral immune responses associated with PCS in order to identify new diagnostic and therapeutic approaches. Our study cohort consists of 120 PCS patients and 42 healthy controls who were thoroughly matched.

In an initial unbiased approach, we performed extensive analyses of cytokine and chemokine profiles in plasma from PCS patients, which revealed no significant changes in PCS patients compared with controls. However, when exposing human donor PBMCs to PCS and control plasma in a functional assay, we found a significant reduction of TNFa-release in the supernatant of cells treated with PCS plasma. This suggests that one or more soluble plasma factors directly mediate immune dysfunction in PCS patients. To correlate these findings with immune cell subsets and their activation state, we performed comprehensive flow cytometry analyses of PBMCs isolated from PCS patients and healthy controls. Here, monocytes showed significantly reduced expression of CD62L. This type I transmembrane glycoprotein and cell adhesion molecule mediates leukocyte-endothelial interactions. To determine if this phenotypic finding correlates with functional alterations, we performed migration assays revealing altered migratory behaviour of monocytes derived from PCS. Interestingly, a change in migration behaviour was also observed with the addition of PCS plasma to control PBMCs but not with plasma derived from non-PCS patients. To reveal the mechanism behind these PCS-plasma dependent effects we performed extensive plasma proteome analyses. Interestingly, we found 12 proteins which were differentially expressed in plasma of PCS patients. 4 of the upregulated proteins are known as soluble immune checkpoint factors (Serglycin, LILRB2, Siglec-9 and CXCL-7). Intriguingly, several of these proteins are known to alter TNF-signaling as well as migratory behaviour of immune cells. Thus, for the first time, we can provide a link between altered plasma factors and functional modifications of immune cells in blood of PCS patients.

Our findings can have future applications as biomarker-based diagnostic test. In addition, our data provide important insights into the pathogenesis of PCS that may lead to the development of urgently needed medicines since several of the identified checkpoint proteins are therapeutic targets in other disease backgrounds.

S-3-6

The LMP1-induced phosphoproteome is efficiently targeted by novel small molecule LMP1-TRAF2 interaction inhibitors in Epstein-Barr virus-transformed B cells

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Question: Latent membrane protein 1 (LMP1) is the primary oncogene of Epstein-Barr virus (EBV). It is expressed in most EBV-associated lymphomas including post-transplant lymphoproliferative disease (PTLD), EBV-positive Hodgkin (HL) and non-Hodgkin (NHL) lymphomas. In 2020, EBV was responsible for at least 137,900 cancer deaths worldwide, underscoring the urgent medical need for new EBV-specific therapeutics. LMP1 is essential for survival and proliferation

of EBV-transformed B cells and the development of most EBV-associated malignancies. Its subdomains CTAR1 and CTAR2 trigger cascades of phosphorylation events, which have not been studied at the proteome level. The direct interaction of TRAF2 with CTAR1 is critical for B cell transformation and serves as target structure for the development of inhibitors. Here we aimed at characterizing proteome-wide protein phosphorylation induced by LMP1 in LCLs to gain deeper insight into mechanisms of cell transformation by LMP1. We are currently in the lead optimization phase of our IP-protected novel small molecule inhibitors of LMP1-TRAF2 interaction. To demonstrate their potency and specificity in vivo, the effects of a highly active derivative on the LMP1-induced phosphoproteome was studied.

Methods: A comprehensive phosphoproteomic analysis of immediate LMP1 signaling events triggered by the activation of the inducible NGFR-LMP1 chimera in LCLs was performed in the absence or presence of the LMP1-TRAF2 inhibitor lead compound SBL316. The activity of SBL316 derivatives was measured in Alphascreen-based LMP1-TRAF2 interaction assays. The effects of inhibitor compound SBL316 was tested on LMP1-induced protein phosphorylation in LCLs at the proteome level. Highly interesting phosphotargets were confirmed by immunoblotting.

Results: More than 14,000 phosphosites were detected in LCLs. 537 phosphosites were induced by LMP1 ≥2.0-fold, whereas 81 sites were downregulated ≥2.0-fold. LMP1 activity causes the extensive phosphorylation of receptor-proximal signaling mediators involved in NF-kappaB and MAPK activation, among them many factors regulating ubiqitinylation processes including TRAF2 itself. Highly active derivatives with IC50 values in the nanomolar range kill LCLs and EBV-positive PTLDs. The derivative SBL316 showed broad activity on the LMP1-regulated phosphoproteome, whereas off-target effects were minor, demonstrating efficient inhibitor effects on LMP1 signaling and defining TRAF2-dependent LMP1 pathways in LCLs.

Conclusions: Phosphoproteomics revealed a complex LMP1 signaling network in LCLs. LMP1-TRAF2 interaction inhibitors show broad activity on LMP1 signaling, kill tumor cells, and will be further developed toward anti-EBV drugs.

S-4-2

Virologic auguring: Monitoring West Nile virus expansion in Germany through wild bird surveillance

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West Nile virus (WNV) is a well-known zoonotic arbovirus that has become a considerable health threat in Europe over the last decades. The enzootic infection cycles of this Orthoflavivirus and the closely related Usutu virus (USUV) include birds as reservoir hosts and mainly *Culex* mosquitoes as vectors resulting in a mostly seasonal transmission from June to November. Humans are dead-end hosts for these viruses but WNV infections in particular can result in severe neuroinvasive diseases with potentially lethal outcomes.

The wild bird-associated zoonoses network (WBA-Zoo) is a well-established monitoring structure for the surveillance of WNV and USUV in their reservoir hosts in Germany. Enabled by DZIF funding, members of the WBA-Zoo collect more than 1000 blood samples from live birds (sick or injured birds admitted to wildlife rescue centres or veterinary clinics) and organ samples from over 1500 dead birds per year, which are then submitted to serological and molecular biological analyses.

These analyses revealed increases in WNV and USUV circulation in 2022 compared to 2021, which mirrors the situation in other European countries. Comparable to preceding years, acute WNV infections were found preferentially in wild birds in central-eastern regions of Germany. In 2022, however, WNV specific RNA was also detected in birds outside this area indicating spatial expansion of the virus. Serological data from avian blood samples collected in the years 2021 to 2023 confirm enzootic circulation of WNV at additional locations in central-western and southern Germany, where no acute WNV cases in humans or animals have been identified, yet. Whole genome sequencing of more than 60 avian WNV cases (collected in 2021 and 2022) revealed the dominance of one previously identified subcluster of WNV lineage 2 (95%), while other clusters or subclusters of this lineage are underrepresented. The nationwide identification of USUV lineages Africa 3 and Europe 3 remained consistent in 2022, while lineage Europe 2 appears to have become more dominant and also seems to have expanded westward and southward from its formerly restricted area of circulation in the central-east.

The continuous identification of one subcluster implies that WNV circulation in Germany is driven by stable local transmission. New entries from outside have not yet become visible. At the same time, our serological data prove westward and southward expansion of the virus. In this context, the circulation dynamics of USUV can provide valuable information due to the high genetic and epidemiological similarity of the two viruses. We are very grateful to all cooperation partners, as their sampling efforts enable the overview of WNV and USUV circulation provided by the WBA-Zoo.

In conclusion, the results from this wild bird monitoring approach should be seen as an "early warning" as WNV circulation is expanding in Germany and the emergence of human infections at new locations may be imminent.

S-4-3

Mycophenolic acid leads to the emergence of novel SARS-CoV-2 variants

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The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 resulted in a serious threat to human lives, highlighting the vulnerability of specific populations with co-morbidities towards less favourable disease outcomes such as people who are immunosuppressed. The anti-proliferative drug mycophenolic

acid (MPA) is commonly utilized for immunosuppressant regimens following solid organ transplantation. Contradicting to the immunosuppressive features, MPA has been shown to successfully inhibit the replication of a variety of viruses, however under immunosuppressant concentrations viral suppression is suboptimal raising questions regarding viral adaptation and the emergence of novel viral variants.

In this study, we investigated the antiviral activity of the immunosuppressants, administered in effective concentrations, on SARS-CoV-2 infection *in vitro*. Solely MPA treatment resulted in a reduction of infectious virus. Moreover, MPA reduced coronavirus 229E, Hepatitis E virus, Respiratory syncytial virus, Influenza A virus and Mpox virus infection in a dose-dependent manner. Mechanistically, we identified that GTP depletion mediated the antiviral effect, which could be reversed by extracellular administration of guanosine. Despite the initial antiviral effect of MPA, SARS-CoV-2, but also 229E can overcome the selective pressure due to suboptimal viral suppression and adapt to MPA treatment when passaged *in vitro*. Deep sequencing revealed the presence of three commonly arising mutations during adaptation, present in the Spike protein, the accessory protein ORF3 and the Envelope protein. Interestingly, solely the combination of all three increased viral fitness in comparison to the WT virus. Notably, all three mutations have been described *in vivo*. We further observed dynamic alterations in the transcriptome of infected cells during the course of viral adaption *in vitro*. In early virus passage infected cells, a broad dysregulation of cellular genes, associated with enhanced protein modification processes as well as response to stress, NFκB signalling and metabolic processes was observed. In contrast, late passage virus-infected cells showed minimal transcriptional differences when compared to UTC. Additionally, the rapid loss of MPA associated inhibition, evidenced by the massive increase of viral replication observed with late passage virus, are indicative of the emergence of breakthrough mutations, conferring altered viral fitness restoring the ability of the virus to replicate efficiently in the context of cellular metabolic changes induced by MPA.

In conclusion, MPA impairs virus replication. However, when passaging the virus *in vitro* the virus can overcome the selective pressure imposed by MPA leading to the emergence of distinct variants with altered viral fitness. Consequently, this passaging approach holds significant potential as a valuable tool for assessing the development of viral variants under certain conditions.

S-4-4

Clinical and pathogenetic features of acute kidney injury in Lassa fever patients at the Irrua Specialist Teaching Hospital, Edo State, Nigeria 2022 - 2024

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Question: Lassa fever (LF) is an endemic viral hemorrhagic fever in West Africa, with Edo State in Nigeria being one of the main foci. The most important clinical complications of severe LF are acute kidney injury (AKI) and neurological sequelae. AKI is a common complication of LF and an important cause of mortality (1,2). The pathogenesis of AKI in LF is not well understood, both micro-bleeding and hyper-inflammation, as well as secondary causes have been discussed as potential mechanisms. Its management remains challenging, often requiring advanced management like hemodialysis.

Methods: We are conducting a longitudinal cohort study with the aim to explore the underlying pathophysiological mechanisms of AKI in LF and to ultimately improve patient management. Adult patients with RT-PCR confirmed LF hospitalized at the Irrua Specialist Teaching Hospital in Edo State, Nigeria, with laboratory diagnosis of AKI according to KDIGO criteria were enrolled and followed prospectively. Secondary causes of AKI such as pre- and postrenal failure, as well as pre-existing chronic renal disease and pre-disposing conditions (history of diabetes mellitus, hypertension, among others) were assessed. Study visits included clinical examination, recording of clinical parameters, and laboratory testing.

Results: We report the interim analysis consisting of clinical and laboratory findings of 107 LF patients with AKI (37 female, 67 male) and 250 LF patients without AKI (103 female, 140 male), recruited between January 2022 and May 2024.The median age of the LF-AKI patients was 42 years (18 – 92) vs. 32 years (18 – 80) in LF without AKI. Mortality was markedly higher in LF-AKI (41/107) compared to LF (6/250). LF-AKI patients are characterized by substantially higher systemic inflammation, more disease severity – by both, laboratory (liver function tests) and clinical parameters (NEWS2, qSOFA). Moderate hemorrhagic symptoms and coagulopathy was slightly more common in LF-AKI. Nephrotic-range proteinuria was common in LF-AKI patients and sonography revealed frequent presence of hyper-echoic kidneys and pleural effusions in a subset of patients. LF-AKI patients also tend to show predominantly respiratory complications, possible explaining the high mortality rather than hemodynamic shock. Recruitment ist ongoing until 03/2025 and interim data analysis is currently being conducted.

Conclusions: AKI poses a great challenge to the clinical management of LF. LF-AKI coinciding more strongly with hyperinflammation than coagulopathy suggests a primarily inflammatory pathogenesis. Whether this is intrinsically due to LF or secondary sepsis needs further investigation. Additionally, we noted severe features of VHF, such as pleural effusions during point-of-care ultrasound in LF-AKI patients.

References:

1 Duvignaud et al. Lancet Glob Health. 2021

2 Okokhere et al. Lancet Inf Dis. 2018

S-4-5

Experimental vaccination by single dose sporozoite injection of blood-stage attenuated malaria parasites

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Malaria remains a significant global health challenge with an estimated 250 million cases worldwide that resulted in 600.000 deaths in 2022, predominantly linked to *Plasmodium falciparum*. Children comprise approximately 60% of the victims.

Various strategies have been employed to develop an efficacious and safe vaccine against this deadly disease. These strategies are based on recombinant malarial proteins or genetically attenuated parasites. As the two currently approved vaccines - Mosquirix™ and R21 - present limited efficacy, the search for a sufficiently efficacious vaccine remains challenging. While subunit vaccines are popular for some viral diseases, only live attenuated vaccines offered a long-term sterile protection against malaria in controlled human malaria infections. Several malaria vaccination approaches using live attenuated *Plasmodium* parasites are currently explored. Attenuation is achieved either in sporozoites, the infectious form of the parasite in mosquitoes, or in the parasite´s blood-stage.

In our approach we screened gene-deletion mutants of the human parasite *P. falciparum* and the rodent parasite *P. berghei* for slow growth in the blood. Furthermore, we tested the *P. berghei* mutants for avirulence and resolution of blood-stage infections while still being able to progress through the entire life cycle. Out of numerous generated gene-deletion mutants, infections of mice with the two most growth-attenuated mutants, whether by blood stages or by sporozoites, resulted in full clearance by the immune response of mice. Immunizing mice with these mutants conferred protection from disease when later challenged with wild-type parasites.

In the human *P. falciparum*, a slow growth rate was detected in two out of seven generated gene-deletion mutants. Slow growing, avirulent *P. falciparum* mutants will enable further research on the induced immune responses and will contribute to developing new and ameliorating existing parasite vaccines.

S-4-6

Efficacy, safety and tolerability of a single day PYRAMAX® plus sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in adults and children in Gabon: Interim preliminary results of a randomized controlled clinical trial

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Background: There is an identified need for antimalarial medicines that are simpler and easier to take, such as a single-dose cure. Not only would such a medicine facilitate "directly observed therapy", which would help to prevent the emergence of drug resistance, it would also reduce the cost of treatment. The main objectives of the study are to assess the efficacy, safety and tolerability of single day AP plus SP particularly in children younger than 10 years

Methods: This is pragmatic comparative randomised non-inferiority study. All the patients from whom a diagnosis of uncomplicated malaria is suspected, undergo an RDT/microscopy. Positive patients with a signed informed consent and eligible are randomized in a 1:1 ratio to either the intervention group receiving a single day artesunate-pyronaridine (AP) plus sulfadoxine-pyrimethamine (SP) or the control group, receiving standard of care (SoC) 3-day artemether-lumefantrine (AL). Blood is taken for a blood spot for PCR analysis, for haematology and biochemistry. Follow-up visits are performed at home on Days 7, 28 and 42, with assessments including thick blood smear, dried blood spot and haematology. Based on the primary objective, at least 250 patients with a parasitaemia determined by microscopy at baseline have to be recruited (up to 1000 patients expected in total). The primary efficacy endpoint is Day 28 PCR-adjusted adequate clinical and parasitological response (ACPR) in intention to treat and per-protocol populations.

Results: Between May and August 2024, 68 patients have been screened and 65 enrolled, 36 (52.9%) in Tchibanga and 32 (47.1%) in Four-Place. Mean age is 15.2 (SD 16.3) years, with age ranging between 1 and 64 years. 33 (50.8%) were treated with AP+SP and 32 (49.2%) with AL. The interim preliminary Day 28 crude ACPR is 100% for AP+SP in available data. One moderate, transient adverse event, constipation, was reported in the AP+SP group. No serious adverse event nor adverse event of special interest has been reported.

Conclusion: The findings of this interim preliminary analysis show a high efficacy of a single day PYRAMAX® plus SP similar to SoC COARTEM. The single day multiple therapy is well tolerated and safe. If these findings are confirmed at the end of the study, this will be a revolutionary tool for the case management and elimination of malaria until the next generation antimalarial drugs are available.

S-5-2

Outer membrane vesicles as protective decoys: a novel mechanism of polymyxin resistance

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The continuous emergence of multidrug-resistant bacterial pathogens poses a major global healthcare challenge, with *Klebsiella pneumoniae* (*Kp*) being a prominent threat. We conducted a comprehensive study on *K. pneumoniae*"s antibiotic resistance mechanisms, focusing on outer membrane vesicles (OMVs) and polymyxin B (PB), a last-resort antibiotic.

Our research demonstrates that OMVs protect bacteria from polymyxins. OMVs derived from PB-stressed *K. pneumoniae* exhibited heightened protective efficacy due to increased vesiculation compared to OMVs from unstressed *Klebsiella*. Notably, these OMVs also shielded bacteria from other bacterial families. This protective effect was validated *ex vivo* and *in vivo* using precision-cut lung slices (PCLS) and *Galleria mellonella* models. In all models, OMVs protected *K. pneumoniae* from PB and reduced the associated stress response at the protein level.

Significant changes in the lipid composition of OMVs upon PB treatment were observed, affecting their binding capacity to PB. The altered binding capacity of OMVs from PB-stressed *K. pneumoniae* was linked to a reduction in lipid A content within the released vesicles. Despite the reduction in lipid A per vesicle, the overall increase in the number of vesicles resulted in enhanced protection, as the total number of PB binding sites increased. This discovery unravels the mechanism behind the altered PB protective efficacy of OMVs from PB-stressed *K. pneumoniae* compared to control OMVs. The lipid A-dependent protective effect against PB was further confirmed *in vitro* using artificial vesicles, which also successfully protected *Klebsiella* from PB *ex vivo* and *in vivo*.

Our findings indicate that OMVs act as protective shields for bacteria by binding to polymyxins, effectively serving as decoys and preventing antibiotic interaction with the bacterial cell surface. These insights into the mechanisms underlying antibiotic cross-protection offer potential avenues for the development of novel therapeutic interventions to address the escalating threat of multidrug-resistant bacterial infections.

S-5-3

Drug-repurposing screening identifies novel agents targeting persistent MRSA isolates

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Staphylococcal infectious diseases are associated with significant morbidity and mortality rates in the human population. Despite major advances in disease diagnostics and medical therapy, infections caused by antibiotic-resistant *Staphylococcus aureus* remain a leading cause of death worldwide, thereby urgently requiring novel therapeutic intervention strategies. Powered by a drug-repurposing screen, we here identified a set of alternative pharmacons that display antimicrobial activity against methicillin-resistant *S. aureus* (MRSA) and clinical derivatives thereof. Intriguingly, comparative analysis revealed that selected compounds including a potent anti-tumor agent exclusively eliminate persistent MRSA variants by restricting access to essential nucleic acid building blocks. In this manner, bacterial replication is hampered in dividing cells, thereby triggering rapidly occurring cell death, a phenomenon that can also be exploited in conventional staphylococci by applying advanced combination therapy. Together with resistance development testing and drug efficacy probing in progressive model systems of staphylococcal disease pathogenesis, these data indicate that targeting nucleic acid precursor metabolism may represent a powerful strategy to combat drug-resistant staphylococci, ultimately enhancing public health and infection control in hospital environments.

S-5-4

Small molecule antibiotic against *Acinatobacter baumannii* without cross-resistance and potential new mode-of-action

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The rise of drug-resistant pathogens is a ticking time bomb, threatening to set back decades of progress in global health. In the fight against these superbugs, carbapenem-resistant *Acinetobacter baumannii* emerges as one of the most critical bacteria, as classified by the World Health Organization.

In 2018, our collaboration partners identified the thiourea derivative SRI-12742 as an antibiotic against AB. The compound"s MIC is 4 µg/mL against the MDR AB isolate BAA-1605 and activity for clinical strains was assessed (MICs 4 µg/mL to >64 µg/mL). SRI-12742 exhibited concentration-dependent bactericidal activity (1.6 log10 CFU/mL reduction at 10×MIC in 24h), comparable with minocycline. In a murine neutropenic thigh infection model of AB infection, SRI-12742 reduced CFU counts by ca. 0.9 log10 CFU, comparable to polymyxin B. In addition, SRI-12742 synergised with all classes of antibiotics tested.

This hit was subsequently expanded with approx 150 synthetic derivatives. The structure-activity relationship (SAR) has been established. Highly active derivatives were identified with MICs down to 0.125-0.5 µg/mL against 25 clinical isolates. No cross-resistance has been observed. Activities for target identification revealed a novel target, explaining the observed absence of cross-resistance. The novel mode-of-action is currently under validation. Importantly, our compound exclusively hits A. baumannii, preserving the beneficial microbiome.

References:

Chopra et al, Int. J. Antimicrob. Agents (2018) 22–27

Czekanska, M.; Verma, S.; Chopra, S.; Titz, A. Thiourea-based derivatives as novel antimicrobials against A. baumannii, EP24180628.0, priority date Jun 6 2024.

S-5-5

Natural products chlorotonils exert a novel antibacterial mechanism and address multiple targets

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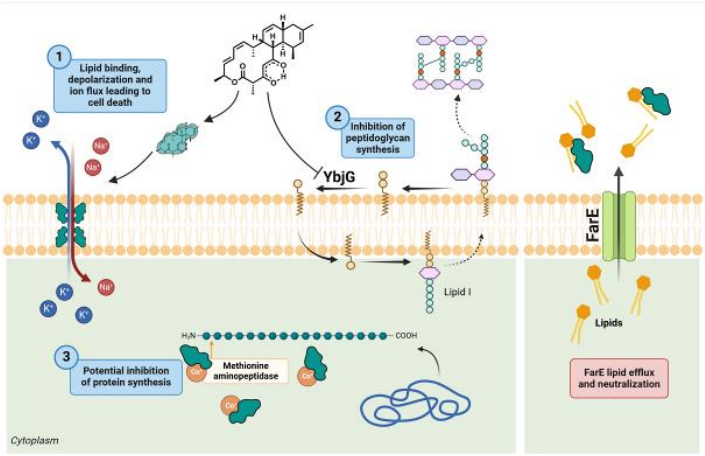
Antimicrobial resistance is a threat to human health rendering many current first-line antibiotics ineffective. New agents overcoming resistance mechanisms are urgently needed to guarantee successful treatment of human disease in the future. Chlorotonils, a natural product class with yet unknown mode of action, were shown to have broad-spectrum activity against multi-resistant Gram-positive bacteria as well as the malaria parasite *Plasmodium falciparum,* with promising activity and safety in murine infection models.

Here, we performed a series of microbiological, biochemical, and analytical data, including ICP-MS, microscale thermophoresis, proteomics, thermal proteome profiling, in vitro enzyme assays, resistance generation, and native lipid mass spectrometry to propose a complex activity profile of chlorotonils. We report that chlorotonils can target the cell membrane, cell wall, and protein biosynthesis. They can be characterised by a rapid onset of action via interference with ion homeostasis leading to membrane depolarisation, however, without inducing severe barrier failure or cellular lysis. Further characterisation confirmed binding of chlorotonils to bacterial membrane lipids eventually leading to uncontrolled potassium transport. Additionally, we identified functional inhibition of the peptidoglycan biosynthesis protein YbjG and the methionine aminopeptidase MetAP as secondary targets of chlorotonils. An initial toxicity model in mouse supports a safe in vivo profile of our lead molecule Dehalogenil.

Figure legend:

Complex mode of action (blue) of chlorotonils involves membrane depolarisation, as well as secondary effects targeting peptidoglycan and protein biosynthesis**.** Resistance (red) is mediated by lipid-efflux-detoxification of DHG at the outside of the cell via FarR/FarE. Created with BioRender

Fig. 1



S-5-6
Elucidating *Mycobacterium tuberculosis* resistance mechanisms using evolutionary principles
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Background and Objectives: The emergence of drug-resistant *Mycobacterium tuberculosis* complex (MTBC) strains poses a significant global public health threat, with varying resistance patterns observed across geographical regions. The intricate genetic composition of MTBC strains driven by the phylogeographical population structure, consisting of diverse lineages, plays a crucial role in influencing therapeutic outcomes and antibiotic response. To understand resistance evolution, our study aims to investigate the influence of the genetic background of clinical *M. tuberculosis* strains on *in vitro* antibiotic resistance development.

Methodology: An *in vitro* long-term evolution model was developed to enrich and select for high- and low-level resistant MTBC mutants following bedaquiline (BDQ) treatment. Single mutant colonies were isolated and characterized phenotypically (minimum inhibitory concentration) and genotypically (Next generation Sequencing). Transcriptomic analysis of selected mutants may further elucidate resistance mechanisms.

Results: Despite a noticeable reduction in bacterial growth after 5 weeks of treatment exposure, resistance in MTBC strains was observed at levels far below the MIC of the wild-type. This indicated a large mutant selection window that possessed varying resistance-associated variants even within the same gene. Characterization of mutants facilitated the detection of previously described and novel mutations. This enabled cataloguing of mutations, which may be used to advance treatment strategies. Preliminary results indicate a trend toward modern Lineage 2 strains more rapidly developing spontaneous BDQ resistance compared to Lineage 4 strains, and requires further characterization.

Conclusion: The application of our evolutionary model has elucidated key aspects of MTBC drug resistance evolution. This approach holds promise for identifying resistance mutations impacting bacterial fitness and contributing to cross-resistance with other antibiotics. Additionally, this model can be extended to assess the role of MTBC strain resistance development in response to both novel and existing TB drugs.

P-1-1
Differential rates of *Mycobacterium tuberculosis* transmission associate with host-pathogen sympatry
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Several human-adapted *Mycobacterium tuberculosis* complex (Mtbc) lineages exhibit a restricted geographical distribution globally. These lineages are hypothesised to transmit more effectively among sympatric hosts, i.e., those that share the same geographical area, though this is yet to be confirmed while controlling for exposure, social networks, and disease risk after exposure. Using pathogen genomic and contact tracing data from 2,279 tuberculosis cases linked to 12,749 contacts from three low incidence cities, we show that geographically restricted Mtbc lineages were less transmissible than lineages that demonstrate a widespread global distribution. Allopatric host-pathogen exposure, where the restricted pathogen and host are from non-overlapping areas, had a 38% decrease in the odds of infection among contacts compared to sympatric exposures. We measure 10-fold lower uptake of geographically restricted Lineage 6 strains compared to widespread Lineage 4 strains in allopatric macrophage infections. We conclude that Mtbc strain-human long-term co-existence has resulted in differential transmissibility of Mtbc lineages and that this differs by human population.

P-1-2

Trends in the availability and prices of quality-assured tuberculosis drugs: a systematic analysis of Global Drug Facility Product Catalogs from 2001 to 2024

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Background: The Global Drug Facility (GDF) of the Stop TB Partnership was launched in 2001 with the goal of increasing access to quality-assured tuberculosis (TB) drugs and products. We aimed to describe the TB drugs and prices available from the GDF over time and to assess trends.

Methods: We searched the internet, including an internet archive, for past and recent GDF Product Catalogs and extracted the listed TB drugs and prices. We calculated the lowest price for the most common drug formulations assuming drugs with similar active pharmaceutical ingredients (APIs) are substitutes for each other. We assessed time trends in the TB drugs and prices offered by the GDF in univariable regressions over the longest possible period.

Results: We identified 43 different GDF Product Catalogs published between November 2001 and May 2024. These product catalogs included 122 single medicines (31 APIs), 28 fixed-dose combinations (9 API combinations), and 8 patient kits (8 API regimens and other materials). The number of TB drugs listed in the GDF Product Catalog increased from 9 (8 APIs) to 55 (32 APIs). The price decreased for 17, increased for 19, and showed no trend for 12 APIs. The price of 15 (53.6%) of 28 APIs used against drug-resistant TB decreased, including the price of drugs used in new treatment regimens. The decreasing price trend was strongest for linezolid (-16.60 [95% CI: -26.35 to -6.85] percentage points [pp] per year), bedaquiline (-12.61 [95% CI: -18.00 to -7.22] pp per year), cycloserine (-11.20 [95% CI: -17.40 to -4.99] pp per year), pretomanid (-10.47 [95% CI: -15.06 to -5.89] pp per year), and rifapentine (-10.46 [95% CI: -12.86 to -8.06] pp per year). The prices of 16 (61.5%) of 23 APIs for standard drug-susceptible TB treatment increased, including rifampicin (23.70 [95% CI: 18.48 to 28.92] pp per year), isoniazid (20.95 [95% CI: 18.96 to 22.95] pp per year), ethambutol (9.85 [95% CI: 8.83 to 10.88] pp per year), and fixed-dose combinations thereof.

Conclusions: The number of TB drugs available from the GDF has substantially increased during its first 23 years of operation. The prices of most APIs for new TB treatments decreased or remained stable. The prices of most APIs for standard drug-sensitive TB treatment increased.

Fig. 1

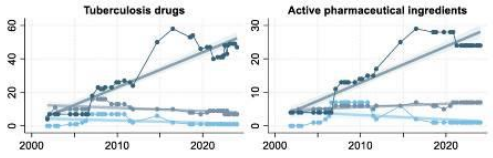

Figure 1: Tuberculosis drugs and active pharmaceutical ingredients listed in the Global Drug Facility Product Catalog, 2001–2024. ● = single medicines, ● = fixed-dose combinations, ● = patient kits. P<0.05 for all trends. N = 43.

Fig. 2

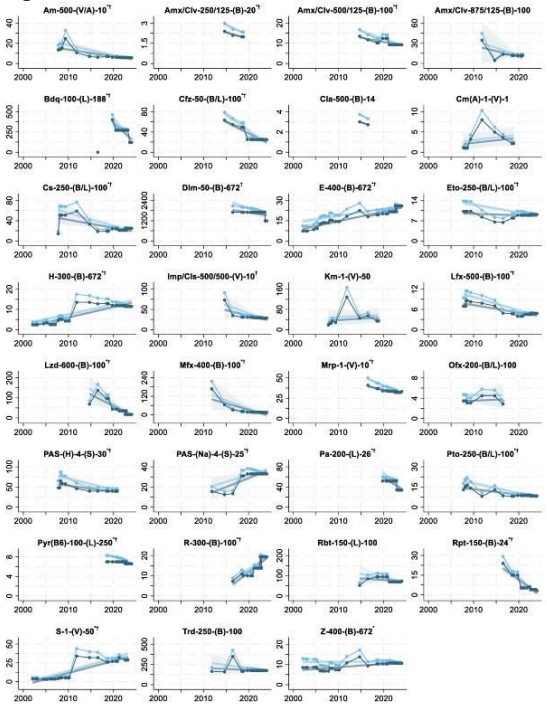

Figure 2: Prices of single medicines in the Global Drug Facility Product Catalog, 2001–2024. ● = price in $. *P<0.05 for price trend. ● = deflated price in $. †P<0.05 for deflated price trend. N = 9–40.

P-1-3

Body mass index trajectories and association with tuberculosis risk in a cohort of household contacts in southern Africa

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Question: Undernutrition and tuberculosis (TB) are syndemic, both being archetypal diseases of poverty. Undernutrition is both a cause and consequence of TB, but this complex relationship is poorly understood. We evaluated the baseline nutritional status of TB household contacts (HHCs), longitudinal trajectories, and association with incident TB.

Methods: ERASE-TB is a prospective longitudinal cohort study of TB HHCs aged ≥10 years in three countries in East and southern Africa (Zimbabwe, Tanzania, and Mozambique), with 6-monthly follow-up for up to 24 months. Undernutrition was defined as BMI<18.5 (adults) or BMI-for-age Z-score<-1 (adolescents). The household-level nutritional status was described including the prevalence of dual burden of malnutrition. The associations between baseline nutritional status and prevalent TB and incident TB

were investigated with logistic and Cox regressions, respectively, including the added value of measuring anaemia using haemoglobin. Growth mixture modelling (GMM) was used to model longitudinal latent trajectories and a Chi2 test or ANOVA was used to test associations between socio-demographic characteristics and latent groups.

Results: Of the 2,109 recruited HHCs (621 [29.5%] adolescents and 1,312 [62.2%] female), 517 (24.5%) were underweight. The households demonstrated a dual burden of malnutrition; while 353/822 (42.9%) had at least one member who was underweight, 257/822 (31.3%) had members who were overweight, and both conditions coexisted in 167/822 (20.3%) households. There were 24 prevalent and 43 incident TB cases, of which 5/24 (20.8%) and 14/43 (22.5%) were underweight. Baseline underweight was not associated with prevalent (aOR: 1.56, 95%CI: 0.46-4.36, p=0.429) but was with incident TB (aHR:2.41, 95%CI: 1.15-5.09, p=0.021). Including anaemia in the exposure strengthened the association (aOR: 4.15, 95%CI: 0.62-16.2, p=0.072 and aHR:5.15, 95%CI: 2.04-13.0, p<0.001) Three latent groups were defined in the GMM: increasing, decreasing, and stable nutritional status. Age, sex, baseline BMI, and TB-related outcomes were associated with latent class allocation.

Figure 1: Household-level dual burden of malnutrition exemplified by the overlap of underweight, overweight, and anaemia by site

Table 1: Univariable and multivariable associations between nutritional status and incident TB, evaluated based on baseline BMI, and combination of BMI and anaemia.

Conclusion: Low baseline BMI and decreasing BMI are strong predictors of TB risk among HHCs. Despite BMI being a crude assessment, prone to misclassification due to measurement error, it is easy to collect and should be part of routine TB assessments. Nutritional trajectories are especially important in determining risk of future TB and that longitudinal measurements of BMI should be considered in active case finding among TB-affected households.

Fig. 1

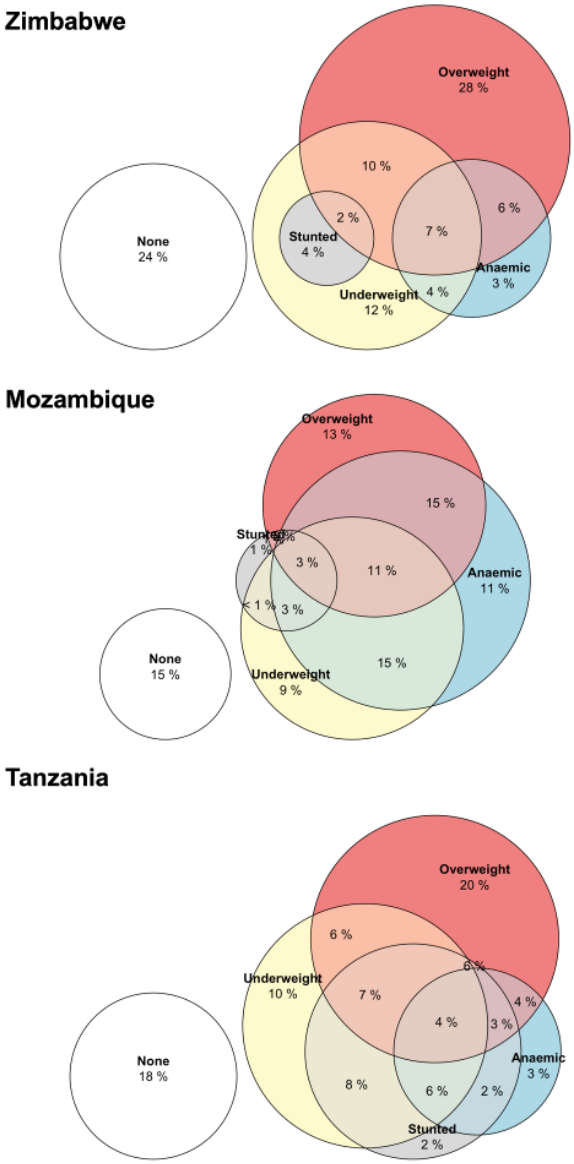

Fig. 2

			Crude		Adjusted[a]	
Prevalent TB						
Logistic regression	**N**	**n**	OR (95% CI)	*p**	OR (95% CI)	*p**
Baseline BMI						
Overweight	565	3	0.34 (0.08 – 1.01)	0.084	0.31 (0.07 – 0.99)	0.073
Normal	1021	16	1		1	
Underweight	520	5	0.61 (0.20 – 1.57)	0.346	1.55 (0.46 – 4.36)	0.429
Baseline BMI or anaemia						
Underweight or anaemic	809	9	1.15 (0.47 – 2.74)	0.737	2.07 (0.79 – 5.16)	0.121
Neither	1242	12	1		1	
Baseline BMI and anaemia						
Underweight and anaemic	92	2	2.09 (0.33 – 7.28)	0.324	4.15 (0.62 – 16.2)	0.072
Not underweight and anaemic	1997	21	1		1	
Incident TB						
Cox proportional hazards	**PY**	**n[1]**	HR (95% CI)	*p**	HR (95% CI)	*p**
Baseline BMI						
Overweight	817	6	0.39 (0.15 – 1.04)	0.060	0.38 (0.14 – 1.04)	0.059
Normal	1384	19	1		1	
Underweight	510	17	1.20 (0.62 – 2.34)	0.600	2.17 (1.02 – 4.63)	0.044
Baseline BMI or anaemia						
Underweight or anaemic	923	25	2.09 (1.11 – 3.91)	0.022	2.51 (1.28 – 4.94)	0.008
Neither	2615	14	1		1	
Baseline BMI and anaemia						
Underweight and anaemic	81	8	4.88 (2.16 – 11.0)	<0.001	6.20 (2.53 – 15.2)	<0.001
Not underweight and anaemic	1746	34	1		1	

P-1-4

Characterising the spectrum of tuberculosis by generation of reference standards and application of multi-state modelling: a proof of concept study

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Question: The conceptualisation of tuberculosis (TB) has undergone a paradigm shift from a binary state to a spectrum of disease. While discrete states of TB have been proposed, they were not accompanied by a well-defined and agreed-upon reference standard. Analytical approaches such as multi-state modelling may offer value in quantifying the progression and regression across the continuum.

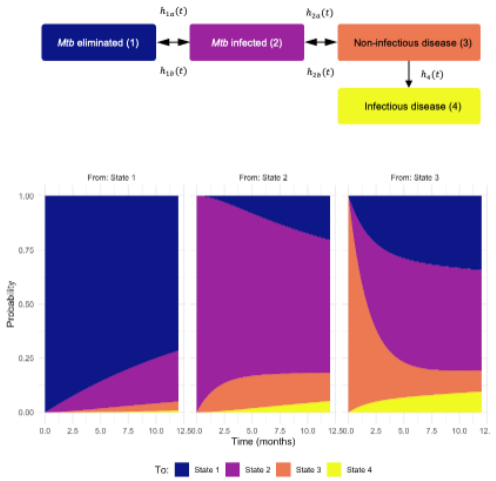
Methods: ERASE-TB is a prospective longitudinal cohort study in southern Africa (Zimbabwe, Tanzania, and Mozambique) aiming to evaluate diagnostics for the early TB states. Recruited household contacts are followed up 6-monthly for 18- 24 months with comprehensive TB investigations at each visit. We characterised the spectrum of TB using the new International Consensus on Early TB (ICE-TB) framework which uses information on symptomatology, pathology, and infectiousness to define the states. To model the use of different reference standards, we used different definitions for the three measures. A Markov multi-state model was applied with one initial state (*mycobacterium tuberculosis* [*Mtb]* elimination), two intermediate states (Mtb infection, non-infectious disease [subclinical and clinical]), and one absorbing state (infectious disease [subclinical and clinical]). Transition probabilities were predicted.

Results: Of the 2,109 recruited HHCs, 1,846 (87.5%) were included in this study (at least two time points with defined state classification and not diagnosed with prevalent TB). At enrolment, the majority did not have *Mtb* infection (990 [53.6%]), with 632 (34.2%) having *Mtb* infection, and 224 (12.1%) having non-infectious disease. The transition probability after one year from *Mtb* elimination to infection was 17.1%, from *Mtb* infection back to elimination 16.9%, from *Mtb* infection to non-infectious disease 9.3%, from non-infectious disease back to *Mtb* infection 42.8% and from non-infectious to infectious disease was 5.5% (Figure 1). Changes to definitions based on symptomatology accounted for more variation in the movement across states than changes to definitions for pathology and infectiousness.

Figure 1: Conceptual multi-state model for the progression pathways along the spectrum of TB based on the ICE-TB framework and predicted transition probabilities over 12 months

Conclusion: Despite having to make simplifying assumptions due the limited sample size of each state in the spectrum of TB, a multi-state approach proves useful in understanding progression and regression pathways. Application of this method to other observational cohort studies, especially individual person datasets, can provide valuable information in the absence of natural history studies. It is important to come up with well-defined and agreed upon reference standards of each state if we are to apply this new conceptual framework.

Fig. 1



P-1-5

Infectious diseases tissue biobanking and biodata management of the German Center for Infection Research – a key infrastructure for multiple research approaches

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Background & Objectives: The DZIF Tissue Bank, as part of the German Center for Infection Research (DZIF) Translational Infrastructure Bioresources, Biodata and Digital Health (TI BBD), supports multiple infectious disease-related research projects and various interdisciplinary studies with biosamples, biodata, latest technologies, and know-how.

Methods: As one of the first biobanks being accredited for the biobanking standard DIN EN ISO 20387, the DZIF Tissue Bank at the Heidelberg-site is the central national infectious disease tissue biobank, coordinating the infectious diseases biobanking of DZIF consisting of the DZIF Tissue Bank Heidelberg and the Liquid Biobank Munich. It supports numerous projects for infectious research leading to high-ranked publications and is part of the TI BBD infrastructure of DZIF.

Results: The services of the DZIF Tissue Bank (e.g. biosample-storage, IHC, IF, chemical stainings, Tissue-Micro-Array assembly, nucleotide extraction) follow Standard Operating Procedures, guaranteeing a maximum of quality, safety, and reproducibility. Located at the Institute of Pathology Heidelberg, the DZIF Tissue Bank has access to >800.000 biosamples including a broad range of infectious diseases samples, such as HBV/HCV/HEV, HPV, EBV, HIV etc. Due to the support of >100 research projects, it contributed to >40 high-ranked publications since 2013. Among achievements were the contribution and coordination of the unique COVID-19 autopsy registry in Baden-Württemberg, Germany, in which the DZIF Tissue Bank is storing over 12.000 COVID-19 biosamples (cryo and FFPE). Another major aspect of interest is the contribution to the Tx

cohort, in whose framework the significance of invasive fungal infection (IFI) as a severe complication in organ transplant patients was investigated.

Conclusion: Quality assured tissue biobanking as provided by the DZIF Tissue Bank is an important and efficient national research infrastructure and significantly contributes to infectious diseases research. Services and derivates provided by the DZIF Tissue Bank enhance the efficiency of biobanking for infectious diseases research and as part of the TI BBD, its biosamples and metadata management are of high relevance for good scientific practice and project management.

P-1-6

Digital services for DZIF researchers: Data & Tools-Hub, Metadata Repository and Central Biosample Register

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The DZIF infrastructure unit "Bioresources, Biodata and Digital Health" (TI-BBD) [1] provides several digital services to support research with Data/IT infrastructure supplying adequate tools. In this context the newly developed DZIF Web Portal offers common collaborative features like file sharing, code sharing and chat channels. The Data & Tools Hub (DZIF-DT-HUB), the Metadata Repository (DZIF-MDR) and the Central Biospecimen Registry (DZIF-ZBR) are associated services for which our group at Helmholtz Munich is responsible.

The DZIF-DT-HUB serves as a central repository to bundle the wide range of decentralized DZIF research resources. It collects and categorizes information on existing bioresources, biodata, studies, databases - including bioinformatic applications, instructions, templates, software tools & apps, protocols, etc.. Researchers can easily access a wealth of resources that improve collaboration and ensure quality assurance through standardized protocols and workflows. They are provided with established data and tools, which ultimately saves resources and promotes sustainability. The Data & Tools-Hub is publicly available at https://dt-hub.dzif.de.

The DZIF-MDR provides researchers with centralized and harmonized metadata that helps them create and manage data assets for research purposes more effectively. It stores structured information about the data collected in medical studies which is complemented by the DZIF Core Dataset. Thus, harmonization and standardization of collected data are fostered following the FAIR principles (Findable, Accessible, Interoperable, Re-usable) [2]. It is publicly available at https://mdr.dzif.de.

The DZIF-ZBR provides the DZIF scientists with an overview on collected biological materials and associated data. It merges and consolidates data on biosample collections from various DZIF sites and studies. Currently, information on more than 9,000 patients and over 350,000 biosamples is stored and scientists can run individual search queries. Thus, the DZIF-ZBR represents an effective instrument for project planning using biosamples across studies and sites. It can be accessed by registered users at https://zbr.dzif.de.

References:

[1] https://www.dzif.de/en/infrastructure/bioresources-biodata-and-digital-health

[2] https://www.go-fair.org/fair-principles

P-1-7

Oscillatory characteristics in infectious diseases

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Background: In 2019, Chowell and colleagues suggested that epidemics follow recurring characteristics that can be described by oscillatory properties which may allow accurate predictions about future waves of infection (1). We demonstrated that these oscillatory properties can be aptly described by a straightforward *duty cycle* (DC) model – in our case, the quotient of the duration of an epidemic wave and the duration between two peaks of two epidemic waves. Using parameters based of retrospective data derived from various historic pandemics that occurred during the last century, the DC model allowed accurate temporal predictions in COVID-19 (2, 3). Here we present further results of oscillatory characteristics in infectious diseases.

Methods: To support epidemiological research, we established a growing database comprising incidence data of en-, epi and pandemics of the last 130 years. Based on this database, we drew several random samples and ensured equal weighting of various epidemic events in the analysis. Based on this random sampling, we described the median and, if possible, the interquartile range of the DC, as well as characteristics regarding the height ratio and proportional frequencies of consecutive epidemic waves.

Results: For en-, epi- and pandemic waves, the DCs are relatively similar with values of 0.83 (IQR: 0.67/1.08), 0.87 (0.66/1.02), and 0.82 (0.68/1.08). Second and third waves are always higher than first waves. Only in pandemics, fourth and fifth waves are generally lower than first waves. Second waves occur least frequently in pandemics. Second waves occurred in 75% (50%/88%) of the events investigated, third waves in 40% (30%/52%), fourth waves in 10% (0%/25%) and fifth waves in 0% (0%/8%). Relevant deviations from these statistical regularities are: (I) in the DC domain: influenza B and parainfluenza; (II) in the height sequence: COVID-19; (III) in the area of subsequent wave frequencies: SARS from 2002, H1N1 from 1976/77, Mumps, Hepatitis-C-Virus and Enterovirus.

Conclusion: The description and measurement of en-, epi- and pandemics is of utmost public health relevance as it allows a specific management and prediction. Also, simulations and what-if-analyses can now be evaluated against the background of real-world evidence. Our results indicate relevant regularities in the height sequence and the

frequency of subsequent waves of infection. Whether a high DC indicates a particular virulence or the opposite should be investigated in the future.

Literature:

1. Chowell G, Tariq A, Hyman JM. A novel sub-epidemic modeling framework for short-term forecasting epidemic waves. BMC Medicine. 2019;17(1):164.
2. Standl F. Epidemiologische Charakteristika von Epi- und Pandemien 2023.
3. Standl F, Trilling M, Jansen P, Stich H, Stang A. Charakteristika von Epi- und Pandemien- eine epidemiologische Perspektive auf Basis historischer Daten. Medizin, Gesellschaft und Geschichte. [Article]. In press 2024.

P-1-8

A human cerebral organoid model to investigate the host-pathogen determinants of Ebola virus persistence in the central nervous system

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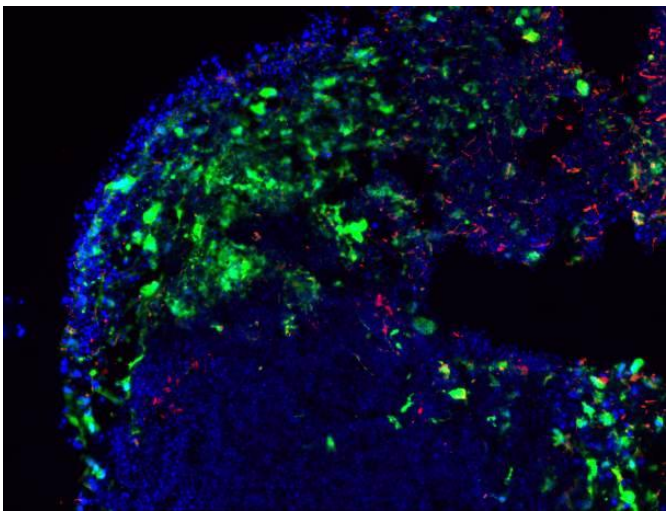
Endemic in equatorial Africa, Ebola virus (EBOV), a member of the *Filoviridae* family, causes Ebola virus disease (EVD), a severe, systemic illness with an average case-fatality ratio of 45%. The recent characterization of long-term EBOV persistence in immune-privileged sites in EVD survivors has implications both for public health (i.e., reigniting and prolonging outbreaks) and for individuals (i.e., recrudescent inflammatory syndromes in survivors). EBOV persistence in the central nervous system (CNS) associated with severe meningoencephalitis has been rarely documented in human and nonhuman primate survivors; however, the host-virus determinants of CNS persistence are unknown. The current absence of tractable animal and *in vitro* models to investigate EBOV persistence critically limits experimental investigation. To address this gap, we present a partially immunocompetent human cerebral organoid model to explore EBOV persistence in a broad range of CNS host cells, including glial and neuronal populations. In this model, we investigated putative mechanisms for establishing and maintaining EBOV persistence at tissue and cellular levels, revealing microglia accumulation in sites of infection and astrocytes as late targets of EBOV. In cerebral organoids, EBOV persistently replicated for at least 120 days, accompanied with the release of pro-inflammatory markers such as CCL-2 and IL-6. Over time, we observed the accumulation of defective viral genomes along with naturally occurring EBOV variants. In long-term 2D *in vitro* culture maintained for 20 to 91 days, we further gathered evidence for a differential antiviral response in persistently infected microglia and astrocytes and cell-to-cell EBOV transmission among microglia and astrocytes, suggesting a multitude of host and viral mechanisms collaboratively determining EBOV persistence in the CNS.

Fig. 1



P-1-9

Identification of a novel effector in HBV replication: PAXX

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The hepatitis B virus (HBV) is a significant global health concern, affecting millions of individuals, since complete eradication of the virus from the patient using available antiviral treatments is currently not possible. To develop new antiviral strategies, it is essential to gain a comprehensive understanding of the HBV replication cycle and the host factors involved.

To identify unknown host factors that play a role in HBV infection, a novel screening using pulsed stable isotope labeling with amino acids in cell culture (pSILAC) was conducted. In this setup, HepG2-NTCP cells were infected with either wild-type HBV or an X protein-deficient HBV strain. The infected cells were pulsed with a medium containing heavy isotopes at various time points ranging from the day of infection (day 0) to day 10 post-infection, each pulse lasting for 24 hours. Changes in the host proteome were subsequently analyzed via mass spectrometry (LC-MS/MS). After stringent filtering and manual verification, 104 out of approximately 5,400 quantified proteins were identified as having altered turnover rates in response to HBV infection. Among these, the proteome analysis identified PAXX, a protein known for its role in DNA repair, as significantly upregulated in response to HBV infection.

Initially, the upregulation of PAXX on mRNA and protein levels was confirmed in HBV-infected HepG2-NTCP cells. Viral protein complementation studies revealed that the upregulation of PAXX was attributed to the core and X protein, as well as potentially to NTCP signaling initiated by the interaction with HBV surface proteins in the extracellular environment.

By employing overexpression and knock-down models, the pro-viral role of PAXX was confirmed, with elevated PAXX levels being associated with enhanced HBV replication, while downregulation of PAXX impeded replication. Additionally, the simultaneous knock-down of PAXX and XLF revealed a higher decrease in HBV replication, implicating XLF as a potential functional substitute for PAXX. Furthermore, PAXX was observed to interact with viral nucleic acids, as evidenced in immunoprecipitation, suggesting its importance in cccDNA formation.

In summary, PAXX was identified as a novel pro-viral effector in HBV replication, which is upregulated through HBV directly after infection and potentially contributes to cccDNA formation. Further evaluating the function of PAXX as a factor in HBV replication may enhance our understanding of cccDNA formation and open the door for future antiviral therapeutic strategies.

P-1-10

Low humoral immunity against highly pathogenic avian influenza H5N1 Clade 2.3.4.4b

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The recent Influenza H5N1 outbreak in cattle and the subsequent spillover to humans raise new concerns about an H5N1 pandemic. Although H5 cross-reactive antibodies have been sporadically identified in humans, their prevalence and protective efficacy against clade 2.3.4.4b remain unclear. Here, we investigated serum neutralization and serum IgG binding of 66 individuals without known H5 exposure against H5N1 (A/Texas/37/2024), H1N1, and H3N2 hemagglutinins (HA) in a pseudo-typed lentivirus neutralization test, a cell-based binding assay, and an HA-ELISA. We then compared a subset of 20 sera for neutralization of an expanded HA pseudo-typed lentivirus panel consisting of 77 different HA variants (H1, H2, H3, H5, H7, and H9) previously isolated from humans. Additionally, we tested 9 previously isolated neutralizing monoclonal antibodies, some of which are under clinical evaluation. Our data reveal low cross-neutralizing titers against H5N1 clade 2.3.4.4b but show that clinically developed monoclonals retain their neutralization capacity against the current strain. These results may help estimate the risk of H5N1 clade 2.3.4.4b infection in humans and suggest potential therapeutic interventions with monoclonal antibodies.

P-1-11

Fever vs history of fever: Pyrogenic and cryogenic responses in children with acute febrile illnesses in Lambaréné, Gabon

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Question: Despites efforts put together to improve on the diagnosis of acute febrile illness (AFI) among children, it remains a challenge especially in low-income and middle-income countries (LMICs). Appropriate differential diagnostic tools are limited; hence, fever and history of fever represent in some circumstances the main criteria for guiding quick decision among clinicians. While this could be subjective, more immunological evidence is needed to understand the immune regulation of fever in children with AFI. Ancillary to the Find BFF-DX project[1], we investigated endogenous pyrogenic and cryogenic markers in children with AFI in Lambaréné, Gabon. We explored the hypothesis that children with fever and history of fever display similar immune regulatory profile in response to an exogenous pyrogen.

Methods: Blood samples were collected from acute febrile children aged 1 to 15 years old. Endogenous pyrogens (IL-1beta, IL-6, TNF-α RANkL, PGE2) and cryogens (AVP, α-MSH, IL-10, IL-1RA, cytochrome P-450) levels were measured in the plasma by enzyme immunosorbent assay. Pyrogenic/cryogenic ratios representing the balance between each pyrogen and its antagonistic cryogen (IL-6/IL-10, TNF-α /IL-10, IL-1β /IL-10, IL-1β/IL-1RA, IL-6/α -MSH, TNF- α/α-MSH, PGE2/cytochrome P-450, RANkL/IL-10, PGE2/AVP, IL-6 + IL-1β /IL-10 + IL-RA) were then calculated for each sample. Data were analysed using non-parametric statistical tests.

Results: In total, 177 children were recruited, among which 125 had history of fever (median temperature = 36.7°C) and 52 had fever (median temperature = 38.3°C). The sex ratio was slightly in favour of female in the fever group and male in the history of fever group. Median IL-6, α-MSH and median IL-6/α -MSH and IL-6/IL-10 ratios were significantly higher ($p < 0.05$) in children with fever compared to children with history of fever. Similarly, median IL-6 and median IL-6/α-MSH, IL-6/IL-10, IL-6/α -MSH, TNF- α /IL-10 and IL-6 + IL-1β /IL-10 + IL-RA ratios were significantly higher in children with high grade fever as compared to moderate grade, who had slightly increase levels compare to low grade fever. Moreover, PGE2/AVP (Rho = 0.28, p = 0.04) and IL-1β /IL-10 (Rho = 0.026, p= 0.004) ratios correlated positively with fever and history of fever respectively.

Conclusion: These findings indicate that, the immune regulation of fever could differ according to the febrile status. This stress on the importance to further investigate these endogenous pyrogens and cryogens as potential tools to improve on the diagnosis and management of AFI.

Reference:

[1]Escadafal C, Geis S, Siqueira AM, Agnandji ST, Shimelis T, Tadesse BT, Massinga Loembé M, Harris V, Fernandez-Carballo BL, Macé A, Ongarello S, Rodriguez W, Dittrich S. Bacterial versus non-bacterial infections: a methodology to support use-case-driven product development of diagnostics. BMJ Glob Health. 2020 Oct;5(10):e003141.

Fig. 1

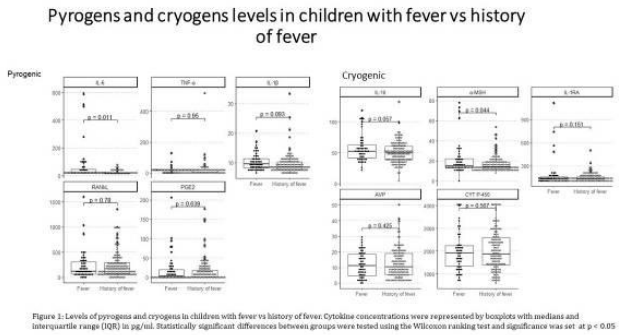

Figure 1: Levels of pyrogens and cryogens in children with fever vs history of fever. Cytokine concentrations were represented by boxplots with medians and interquartile range (IQR) in pg/ml. Statistically significant differences between groups were tested using the Wilcoxon ranking test and significance was set at $p < 0.05$

P-1-12

German clinical practice guideline for the management of Outpatient Parenteral Antimicrobial Therapy (OPAT)

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In 2024, the first German Clinical Practice S1-Guideline for the Management of Outpatient Parenteral Antimicrobial Therapy (OPAT) was published in the *Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften* e. V. (AWMF) guideline register.

OPAT is defined by the intravenous or intramuscular administration of antiinfective medication outside of the hospital. The indication for OPAT is made on an interdisciplinary basis by an infectious disease specialist and the specialists involved in the treatment. The outpatient therapy takes place in coordination with OPAT centres or in coordination with the treating family doctor.

Even though some few centers offer OPAT nowadays in Germany, so far, OPAT services are scarce and there is a lack of a structured and standardized OPAT service system in Germany. A first observational study on OPAT in Germany was recently published and the implementation of OPAT in Germany has been recommended as a result of the study.

OPAT largely depends on the presence of an infectious disease-led OPAT team and an intersectoral structured care system. As the number of complex infectious diseases and the number of comorbidities increases, the indications for antiinfective therapies with an indicated longer duration of therapy will also increase. In order to provide a broader range of OPAT as a form of therapy in Germany, nationwide standardized structures and formal financing models must be established in primary and secondary care.

The new guideline is intended to disseminate recommendations for action that can be used to put decisions in medical care on a more rational basis. This should lead to improvement of the structures, processes and quality of care in OPAT patients and strengthen the position of patients with complex infectious diseases.

It aims to provide insight for healthcare professionals who prescribe and oversee the provision of OPAT and considers various patient features, selection criteria of patients, infusion catheter issues, possible infectious diseases for OPAT, discussions around the anti-infective management regarding the selection of an anti-infective agent according to chemical-physical criteria, information on the legal regulation of the production, storage, transport and distribution of anti-infectives monitoring questions, and antimicrobial stewardship concerns. Comprehensive tables on important medication aspects are included. Also, economic aspects and future aspects are discussed. An extensive separat chapter on pediatric OPAT was developed in cooperation with the guideline development group and added to the guideline.

More than 20 questions are addressed in the guideline, and the guideline includes a check list for patient selection and flow charts.

The guideline was developed under the leadership of the *Deutsche Gesellschaft für Infektiologie* (DGI) and with the participation of six other medical associations and specialist societies.

P-1-13

Remote study participants recruitment, management and sampling: a swift combination of the PIA app and the dried blood spot (DBS) method

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Successfully launching a clinical trial or epidemiological study depends on accurately identifying eligible participants. Beyond clinical criteria, past infections often play a crucial role in eligibility. Infection history is typically assessed through immune responses in venous blood samples, but collecting these from large populations is costly and requires medical personnel[1,2]. Additionally, scheduling and travel logistics make gathering comprehensive data for longitudinal studies challenging.

To overcome these challenges, we developed a fully remote, digital recruitment, and self-sampling method. Participants are recruited via advertisements and directed via a link or QR code to the "Prospective Monitoring and Management App" (PIA), designed by the Helmholtz Centre for Infection Research in Braunschweig and customized in house (Fig a). After registration in PIA, participants can digitally provide consent (Fig b-c). Online questionnaires are administered afterwards. If inclusion criteria are met, a self-sampling kit is sent to collect a capillary blood sample using the dried blood spot (DBS) method on filter paper (Fig d-e). DBS collection is advantageous as cost-effective, minimally invasive, eliminating the need for medical personnel and allowing for stable long-term storage and transport, but requires development from the ground up. Although commercial antibody detection assays are well-established for serum, adapting these assays for use with DBS involves developing specific lab protocols for extracting and processing biological material.

Over the past four years, the remote DBS approach has been implemented in three longitudinal COVID-19 studies, enabling the quantification of SARS-CoV-2 anti-nucleocapsid and anti-spike SARS-CoV-2 antibodies, with over 80% retention rate and analyzing of over 200,000 samples[3]. The new combination of remote recruitment and DBS sampling is now established in a feasibility study involving up to 3,000 participants to determine the Epstein-Barr-Virus (EBV) sero-status by testing for responses to Capsid and Nuclear antigens. High acceptance and return rates for DBS samples and questionnaire data (>95%) have been observed in around 300 participants so far.

Our approach is cost-effective, suitable for large longitudinal cohorts, and highly transferable to other diseases and studies.

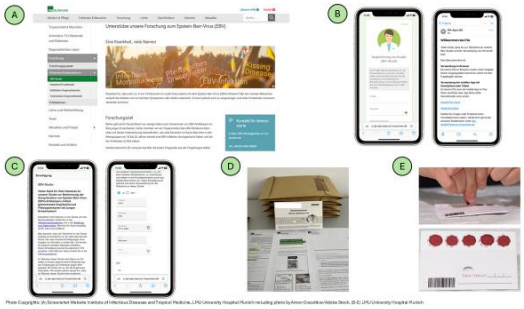
Fig: (A) recruitment via advertisement, (B) management via PIA for registration and questionnaire administration, (C) digital provision of inform consent via PIA, and (D-E) capillary blood sampling using DBS.

[1]Liu STH, et al. 2020. Convalescent plasma treatment of severe COVID-19: a propensity scorematched control study. Nat Med 26:1708–1713

[2]Arora RK, et al. 2021. SeroTracker: a global SARS-CoV-2 seroprevalence dashboard. Lancet Infect Dis 21:e75–e76

[3]Le Gleut, et al. 2023. The representative COVID-19 cohort Munich (KoCo19): from the beginning of the pandemic to the Delta virus variant Ronan Le Gleut. BMC Inf Dis 23:466

Fig. 1



P-1-14

Outer membrane vesicles of *Klebsiella pneumoniae* decrease bactericidal properties of alveolar macrophages

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Rationale: Alveolar Macrophages (AMs) are the sentinel cells in the lung, which clear bacteria and initiate inflammation. Gram-negative bacteria release outer membrane vesicles (OMVs) into the extracellular environment and antibiotics increase OMV production. OMVs of bacteria contain different types of cargo such as proteins, lipids, and nucleic acids. As colonization with *Klebsiella pneumoniae* (*K. pneumoniae*) constitutes a risk factor for infection, we hypothesized that OMVs of *K. pneumoniae* might alter bactericidal properties of AMs to facilitate pneumonia.

Methods: *pneumoniae* were cultured *in vitro*, treated with different subinhibitory concentrations of antibiotics, and the secreted OMVs were isolated. Murine AMs were harvested by bronchoalveolar lavage and treated with OMVs. Bactericidal properties were assessed in AMs infected with viable *K. pneumoniae* ex vivo. Reactive oxygen species (ROS) were quantified by flow cytometry. Cytokines were measured using a multiplex bead-based assay. Oxygen consumption rate (OCR) and glycolysis were measured using an extracellular flux analyzer.

Results: Preincubation with OMVs significantly decreased the killing capacity of AMs. In line, intratracheal instillation of OMVs facilitated bacterial outgrowth in a subsequent infection with *K. pneumoniae*. Whereas, OMVs did not alter cytosolic ROS production, they abrogated mitochondrial (mt) ROS release and decreased cellular respiration in AMs in response to *K. pneumoniae*. Specifically, OMVs isolated from *K. pneumoniae* treated with meropenem or piperacillin/tazobactam had the strongest effect. Human AMs were functionally similarly altered by OMVs. Inactivation of proteins and not DNA or RNA in permeabilized OMVs (perOMVs) abrogated inhibition of mtROS release upon bacterial encounter. Subsequently, the inactivation of proteins in perOMVs reverse the killing capacity of AMs. The proteomics data showed that OMVs of *K. pneumoniae* treated with meropenem revealed different protein composition compared to OMVs of non-treated bacteria. By using a BamA inhibitor to stop producing outer membrane proteins, we showed the effects of OMVs are reversed.

Conclusion: In summary, we found that OMVs of *K. pneumoniae* dampen the killing capacity of AMs. Therefore, we suggest that OMVs might facilitate the transition from bacterial colonization to infection in the lung by decreasing bactericidal properties of AMs.

P-1-15

Prevalence and associated factors of gametocyte carriage distribution in rural and urban areas

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Background: The presence of gametocytes plays a crucial role in malaria transmission. In order to better understand the dynamics of malaria transmission in endemic areas, we conducted a study to assess the prevalence and associated factors of gametocyte carriage in children and adults living in rural and urban areas of Gabon.

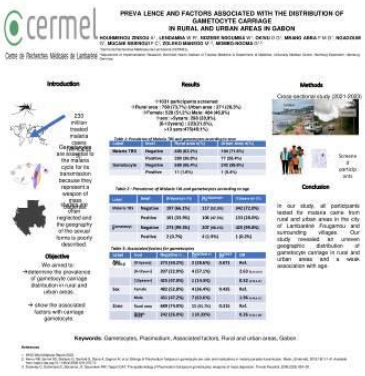
Material and method: This was a descriptive and analytical cross-sectional study conducted in Lambaréné and its surrounding villages between 2021 and 2023. Participants with a history of fever or fever, who had undergone malaria screening at CERMEL using thick film were included in this study. Gametocytes were detected by microscopy. Factors associated with gametocyte carriage were determined by univariate analysis.

Results: The prevalence of gametocyte carriage was 1.6% in rural areas and 0.4% in urban areas. Participants aged less than 5 years and male sex were important determinants associated with gametocyte carriage. Plasmodium falciparum was the most frequently observed parasite species with low parasitaemia in gametocyte carriers.

Conclusion: A good knowledge of the distribution of gametocyte carriage would enable early detection of transmission foci and better orientation of care policies directed towards rural areas, which are often less well supplied with primary health care.

Key words: Gametocytes, Plasmodium, Associated factors, Rural and urban areas, Gabon

Fig. 1



P-1-16

***M. tuberculosis* reroutes NETosis away from PAD4 towards Gasdermin D and its prevention results in bacterial control**

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The rise of multidrug-resistant tuberculosis highlight the importance of new treatment strategies besides conventional antibiotic therapy against *Mycobacterium tuberculosis* e.g., adjunct host-directed therapies. In previous studies, we showed that *M. tuberculosis* induced necrotic-like cell death in human neutrophils. This ensured the release of *M. tuberculosis* and subsequent proliferation within macrophages, establishing a vicious circle of host cell necrosis interspersed with bacterial replication, a scenario that likely takes place in lungs of tuberculosis patients causing tissue damage and transmission. Prevention of initial neutrophil necrosis resulted in subsequent growth control of *M. tuberculosis* by macrophages. To identify specific druggable targets, we aimed at essential check points for NETosis to deeper understand the neutrophilic death response towards *M. tuberculosis* infection. Myeloperoxidase inhibition as well as application of the antioxidant N-acetylcysteine prevented subsequent neutrophil necrotic-like cell death, which was independent of protein arginine deiminase 4 (PAD4). However, formation of NET-like structures was confirmed by microscopy and citrullinated histone H3 was detected. Inhibiting other essential key molecules for NETosis such as neutrophil elastase, histone deacetylases, and other PADs prevented *M. tuberculosis*-induced NETosis in a Gasdermin D-dependent manner and, thus, restricted bacterial growth. Our findings indicate the relevance of differential pathways leading to distinct types of NETosis. Identification of molecular mechanisms underlying host cell death of *M. tuberculosis*-infected neutrophils is essential to efficiently tailor specific host-directed therapies.

Fig. 1

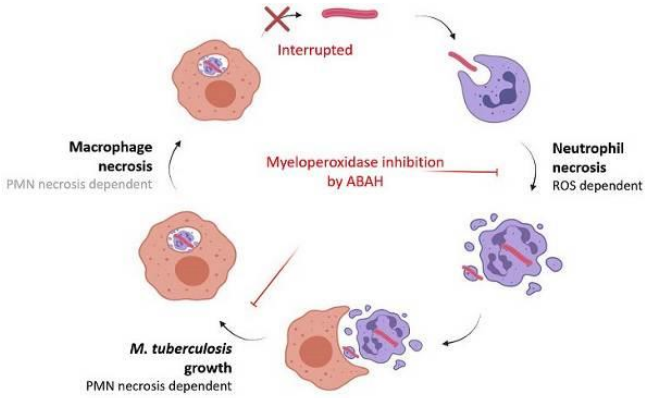
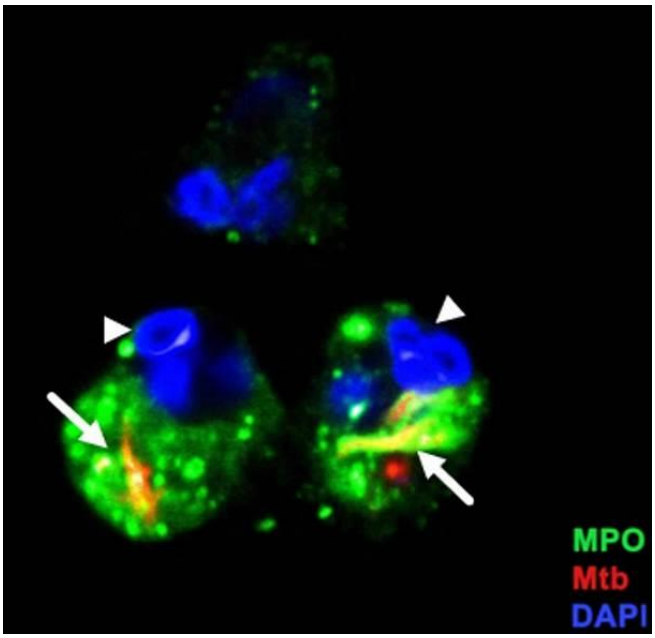


Fig. 2



P-1-17

Studies on the presence of neutrophil extracellular traps (NETs) in different types of urinary tract infections

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The formation of Extracellular traps (ETs) is a defense mechanism of several innate immune cell types, including polymorphonuclear neutrophils (PMN). Once activated, PMN undergoing NETosis release a DNA core into the extracellular space, concomitant with granular peptides and enzymes, which may capture and eventually kill pathogens. In case of urinary tract infections (UTI), leukocytes, especially PMN, migrate to infected tissues and are used as important clinical marker for inflammatory processes. Unfortunately, there is a lack of knowledge on the occurrence of NETs in human urine and their potential role in UTI. Therefore, the current project aims to analyze the presence of NETs in urine samples of patients with different types of UTI.

In total, x urine and y blood samples were collected from 3 different groups of UTI patients [(a) female n = 25, divided in cystitis n=11, pyelonephritis n=6 and asymptomatic

bacteriuria n= 8; (b) male n= 22 with asymptomatic bladder catheter associated UTI]. Samples of healthy individuals (group c, n=24) served as control group. Human PMN were isolated by negative selection (stem cell EasySep™). Immunofluorescence analyses of urine samples included the detection of neutrophil elastase (NE) and citrullinated histones to record and quantify NETs. To determine the presence of additional NET-typical markers, myeloperoxidase (MPO) and cathelicidin were detected by ELISAs, calprotectin via Western Blotting in addition to CD15, citrullinated histones and MPO via flow cytometry. Furthermore, the bacterial spectrum of each urine sample will be estimated via currently ongoing microbiomic analyses.

When overall comparing sexes, immunofluorescence analysis of urine samples revealed an average NET occurence of 23.29% (*SD +/- 16.89*) in group a (infected females), divided in their subgroups cystitis (27.72%, *SD +/- 17.88*), pyelonephritis (22.75%, SD +/- *12.91*) and asymptomatic bacteriuria (18.17%, *SD +/- 17.14*), and 30.63% (*SD +/- 17.88*) in group b (infected males). In both groups different phenotypes of NETs (spread, diffuse and aggregated NETs) were microscopically confirmed in urine of UTI patients. In all types of UTI, the NET markers MPO, cathelicidin, calprotectin and citrullinated histones were detected. There was no statistical significant difference in protein concentrations between groups a and b. As expected, the presence of immune cells and NETs (0.32%, *SD +/- 1.42*) in urines of healthy individuals was low and related markers could not be detected.

Of note, the abundance of NETs and the concentrations of related proteins varied considerbly between individuals.

In conclusion, the presence of PMN-derived NETs signifies a consistent finding in urine samples of UTI patients, but showing, high interindividual variations. Consequently, PMN-derived effector mechanisms should be studied in more detail in this patient group.

Acknowledgement: this study was supported by a DZIF MD stipend.

P-1-18

Testing of darobactin derivatives in pneumonia caused by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

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Rationale: *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are gram-negative bacteria that frequently cause nosocomial pneumonia and show both high pathogenicity and increasing resistance. The new antibiotic darobactin shows broad yet selective efficacy against gram-negative pathogens. Darobactin exhibits a unique mechanism of action, as it inhibits BamA, a vital component of the β-barrel assembly machinery (BAM) of the outer membrane of gram-negative bacteria. Here, we tested its in vivo efficacy in pneumonia.

Methods: Mice were infected with *P. aeruginosa* or *K. pneumoniae* intranasally. Darobactin B, darobactin B9 or NaCl 0,9% (=control) were administered intraperitoneally at three different time points or once intratracheally. At indicated time points mice were euthanized, and lung homogenates were plated on blood agar plates to determine bacterial outgrowth.

Results: We found that darobactin B and B9 were both locally and systemically effective in *P. aeruginosa* pneumonia. Darobactin B clearly reduced the bacterial loads in the lungs in *K. pneumoniae* pneumonia - for both local and systemic application. Interestingly, darobactin B9 was only effective locally, but not systemically in pneumonia caused by *K. pneumoniae*. Moreover, darobactin B was also effective against a multidrug-resistant *P. aeruginosa* when administered systemically.

Conclusion: In summary, we found that darobactin B has a better effect than darobactin B9, as it is effective against both tested bacteria regardless of the form of application. Since darobactin acts selectively on aerobic gram-negative bacteria, it is expected to have a minor impact on the gut microbiome compared to other antibiotics such as carbapenems. Therefore, we will next test its effect on microbiome composition.

References:

Imai et al., Nature, 2019

P-1-19

Galleria mellonella larvae as a model for studying implant-associated biofilm infections

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Question: Implant-associated biofilm infections, particularly those caused by *Staphylococcus aureus* and *Enterococcus faecalis*, pose significant clinical challenges. These infections often require complex treatments, including surgical removal of infected implants. The need to find effective treatment methods for these infections, while replicating clinically relevant conditions, drives the investigation into suitable *in vivo* models.

Model Rationale: *Galleria mellonella* larvae have emerged as a robust invertebrate model for studying biofilm-related infections at the implant-tissue interface. This model offers a cost-effective and ethically favorable alternative to vertebrate models, allowing for high-throughput studies under physiologically relevant conditions.

Methods: This study investigates biofilm formation and infection dynamics using *S. aureus* and *E. faecalis* on expanded polytetrafluoroethylene (ePTFE) implants, mimicking prosthetic valve endocarditis. Two biofilm formation methods were employed: *in vivo* biofilm development within the larvae, mimicking hematogenous spread, and pre-formed biofilm transplantation, mimicking contamination during surgery. Advanced scanning electron microscopy (SEM) was used to reveal intricate biofilm structures on implants, closely resembling clinical conditions.

Results: To assess treatment efficacy, the biofilm-infected larvae were treated with vancomycin, rifampicin, and their combination. The *in vivo* model demonstrated significant biofilm reduction, particularly in biofilms formed within the larvae, where complex host-pathogen interactions were

evident. Rifampicin alone and in combination with vancomycin showed the most significant biofilm eradication, leading to increased larval survival rates and reduced bacterial load on implants. Additionally, label-free multimodal imaging techniques, including Coherent Anti-Stokes Raman Scattering (CARS) and Fluorescence Lifetime Imaging (FLIM), provided insights into biofilm integrity and infection-induced tissue damage. These advanced imaging techniques highlighted the biofilm structure on implants and infection-induced morphological changes within the larvae.

Conclusions: The *Galleria mellonella* model, coupled with advance imaging, presents a powerful platform for studying implant-associated infections at interfaces. This model not only replicates key aspects of clinical biofilm infections but also facilitates the evaluation of antimicrobial treatments in a cost-effective, scalable, and ethically responsible manner. Its application offers significant potential for preclinical testing, providing valuable insights into biofilm-related infections and their treatment strategies.

P-1-20

The influence of comorbidity, age, sex, and comorbidities on COVID-19 mortality throughout the course of the SARS-CoV-2 pandemic in Europe: Data from the LEOSS study

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[2]Vivantes Humboldt Hospital, Department of Interdisciplinary Intensive Care, Berlin, Germany

[3]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I for Internal Medicine, Cologne, Germany

[4]Marien Hospital Herne Ruhr University Bochum, Department of Internal Medicine I, Herne, Germany

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[6]University Hospital Frankfurt Goethe University Frankfurt, Department of Internal Medicine, Infectious Diseases, Frankfurt a. M., Germany

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[8]St. Josef-Hospital Bochum, Ruhr University Bochum, Department of Neurology, Bochum, Germany

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Background: Population immunity against SARS-CoV-2 has increased, resulting in a decline of COVID-19 mortality. The aim of the study was to analyse the COVID-19-related mortality over the pandemic, stratified by a model of groups at risk of severe COVID-19.

Methods: COVID-19-related patients were included from January 2020 to November 2022 using the international multicentric cohort study Lean European Open Survey on SARS-CoV-2-Infected Patients (LEOSS). Predicted probability of COVID-19-related death was calculated using a multivariate logistic regression model adjusted to age, gender and vaccination status.

Results: In total, 12,096 patients were included. The overall mortality was 13% (n=1546), decreasing from 14% during the wildtype (wt) period (01/2020-12/2020), to 13%, 10%, and 6% in the alpha (α) (01/2021-06/2021), delta (δ) (07/2021-12/2021), and omicron (Ω) (01/2022-11/2022) periods, respectively. Patients aged 66-75, 76-85, and >85 years (y) had a 13.3-, 22.5-, and 40.4-fold higher odds of mortality compared to 26-35y old patients ($p<0.001$ in all listed comparisons). This increase in mortality between younger (age: 26-35y, mortality: wt 2%, Ω 1%) and older patients (age: >85y, mortality: wt 41%, Ω 23%) decreased with the shift from wt (increase of 39 percentage points) to Ω (increase of 22 percentage points). The overall mortality in males (m) (15%) was higher than in females (f) (10%), but this gender-specific difference leveled off with the shift from wt (m: 19%, f: 10%) to Ω (m: 9%, f: 9%). Referring to the difference in mortality between patients with zero and four comorbidities, the predicted increase in mortality during the periods wt, α, δ, and Ω was 14, 14, 1, and 0 percentage points, respectively. Concerning severely immunosuppressed patients, mortality significantly decreased throughout the pandemic (wt: 15%, Ω: 4%, $p=0.031$).

Conclusion: Overall mortality decreased during the pandemic. This change was observed even among severely immunosuppressed patients. Age, gender, and the number of comorbidities were identified as relevant mortality risk factors, with decreasing importance as the pandemic progressed.

P-1-21

Vaginal microbial dynamics are linked to functional shifts with implications on colonization resistance to STIs

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Chlamydia trachomatis is the most common bacterial sexually transmitted infection worldwide. The infection is frequently asymptomatic and, hence, left untreated in many infected individuals. These untreated infections can cause ascension of the pathogen, leading to severe sequels such as pelvic inflammatory disease, ectopic pregnancies, or infertility in women. Together with other sexually transmitted infections (STIs) such as *Neisseria gonorrhoeae*, genital tract infections are responsible for a high burden of childless couples in industrialized countries. With women younger than 25 years being of increased risk for exposure to the pathogens, recent data indicate that vaginal microbiota directly influence the susceptibility and course of STIs. However, no longitudinal data linking microbial dynamics with STIs exist and experimental approaches on microbiome-pathogen interactions are scarce. Little is, thus, known about the microbiome-induced colonization resistance to STIs and by what mechanisms the colonization resistance to STIs is perturbed by dysbiosic microbial communities.

We are targeting this question in a multidimensional approach consisting of observational studies in humans and a variety of experimental models to functionally uncover mechanisms of microbial interactions and their contribution to the course of the infection. We are currently disentangling microbial dynamics and co-occurrence networks in the vagina of healthy young women aged between 18 and 22 years via an app-based longitudinal study relying in self-sampling of cervical swabs of the participants. We have recently identified particular microbial structures consisting of a network of *Ureaplasma parvum* and several *Gardnerella* strains which provide functional shifts within the microbiome. In particular, over-representation of certain fermentation pathways in dysbiotic vaginal microbial communities suggests accumulation of acetat instead of lactic acid to be a

hallmark of reduced colonization resistance to STIs and fertility problems. Ongoing efforts are undertaken to identfy optimal candidate bacterial strains from well-defined, healthy women to enable development of validated life bio products for prevention and treatment of vaginal microbial dysbiosis and STIs.

P-1-22

Inhibition of homotypic fusion during co-incubation of *Chlamydia muridarum* and *Enterococcus faecalis* as a possible modulator of colonization resistance to chlamydial infection

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Chlamydia trachomatis is the most prevalent bacterial STI worldwide, frequently occurring asymptomatically. In females, the infection may potentially result in serious complications. Nevertheless, the complex interplay between infection, immune system, and the vaginal microbiota remains poorly understood. To investigate this relationship, we conducted *in vitro* as well as *in vivo* experiments to assess the impact of the vaginal microbiota on Chlamydia infections, using the murine strain *Chlamydia muridarum*. Our *in vivo* studies revealed, that *Enterococcus faecalis* is the most prevalent bacterial species in the vaginal microbiota of naturally cycling mice. We found that experimental modulation of the vaginal microbiome with *E. faecalis* provided partial protective effects against *C. muridarum* infection in mice, independent of other infection-modulating factors. *In vitro*, we observed that co-incubating *C. muridarum* with active *E. faecalis* and its supernatant, results in a delay in homotypic fusion, and a reduction in infection progeny. To further investigate the role of *E. faecalis*, we subsequently analyzed various knock-our mutants. We observed a decrease in the inclusion-to-cell ratio when using the epaB knock-out supernatant compared to the wild-type *E. faecalis* supernatant, suggesting that epaB influences homotypic fusion. EpaB, the enterococcal polysaccharide antigen, is located on the surface of the *Enterococci*. This species-specific effect suggests that microbial interactions affecting intracellular stages of the chlamydial lifecycle may play an important role in microbiota-mediated protection against chlamydial infection. This is further supported by observations that *C. trachomatis* strains defective in homotypic fusion cause milder symptoms and show a lower pathogen load in patients.

P-1-23

Easy to use application to monitor community-acquired infections (CAI-App)

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Longitudinal data are of uppermost importance in studying community-acquired infections. Therefore, we developed a browser-based application for the currently ongoing DZIF project PASTICCIO for longitudinal investigation of sexually transmitted infections (STI), which belong to the most frequent infections in a community setting. The browser-based application is designed to support the recruitment of study participants and validate the inclusion/exclusion criteria, obtain informed-consent and enable data and sample collection during the study period. In this project young females are enrolled on a voluntary basis in the presence or absence of clinical symptoms of BV or STI. Twice a year over five years, they obtain a self-sampling kit for vaginal swabbing and are asked to fill-out the online questionnaire about sexual behaviors, antibiotic treatments, potential contact partners, etc. Swabs are processed in the Institute of Medical Microbiology in Lübeck including molecular detection of the most frequent STI (C. trachomatis, Neisseria gonorrhoeae) and commensals of interest. Making use of an in-house pipeline for amplification, partial 16S sequencing for microbiota analysis from the sample´s isolated DNA is performed on a MiSeq sequencer (Illumina). Subsequently, raw data will be processed, quality filtered and aligned to a reference database as well as distance-based and taxonomic measures are used for microbiota analysis. Factors influencing microbiota composition and stability will be identified with the help of metadata from the participant´s questionnaire. With the CAI-App we also aim to establish a translational research platform for studies which can be further used for TTU-spanning research, e.g. for the longitudinal follow-up on MDRO patients. This platform will serve as a valuable resource to recruit patients for future interventional studies within the DZIF and allows broader community-based interventions. Acquiring app-based follow-up data at defined intervals should increase the efficiency of follow-up and minimize loss to follow-up. Further, the CAI-App can be used as a platform to collect decentralized and self-sampled biomaterials.

P-1-24

Advanced in vitro biofilm models for real-time analysis of *Pseudomonas aeruginosa* and *Staphylococcus aureus* responses to antimicrobials

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Biofilm-associated infections by *Pseudomonas aeruginosa* and *Staphylococcus aureus* pose substantial treatment challenges due to the robust resistance these pathogens exhibit within biofilms. Chronic and device-related infections from these bacteria highlight the need for advanced models that closely mimic in vivo conditions and enable in-depth investigation of biofilm dynamics and antimicrobial susceptibility. To address this need, we developed two advanced biofilm models: a dynamic microfluidic biofilm system coupled with confocal laser scanning microscopy (CLSM) and a light sheet fluorescence microscopy (LSFM) biofilm model, each optimized for detailed, time-resolved studies on *P. aeruginosa* and *S. aureus* biofilm formation and response to antimicrobial treatments such as antibiotics and bacteriophages.

The microfluidic biofilm system incorporates controlled flow dynamics to simulate nutrient exchange and shear forces, closely resembling in vivo environments. Using CLSM with targeted fluorophores, this model enables high-resolution visualization of biofilm structure, cellular organization, and adaptations, proving effective for examining spatial architecture and structural responses of *P. aeruginosa* and *S. aureus* biofilms to antimicrobials. It emphasizes the pivotal role of dynamic conditions in biofilm resilience.

The LSFM-based biofilm model allows non-invasive, real-time observation of biofilm development through time-lapse imaging, enabling us to track biofilm dynamics and monitor antimicrobial impact on live biofilms without compromising

sample integrity. This technique offers valuable insights into biofilm adaptability and resilience under therapeutic stress.

By integrating dynamic flow conditions with advanced imaging, our biofilm models provide a comprehensive platform to study the structure, development, and antimicrobial response of *P. aeruginosa* and *S. aureus* biofilms. These models significantly advance our understanding of biofilm biology and offer critical tools for developing targeted therapeutic strategies.

P-1-25

Prevalence study of the microbiome and development of antibiotic resistance in children with Crohn's disease

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Introduction/Question: As part of a prospective study, we examined the gut bacterial microbiome of a cohort of 39 pediatric Crohn"s disease (CD) patients at two time points (at baseline and five to 15 weeks later) and 27 pediatric controls from immunologically healthy children. The aim of this study is to provide improved therapeutic approaches for the treatment of the disease by analyzing the intestinal microbiome and its alteration under the influence of various therapeutic interventions.

Methods: Stool samples were collected and analyzed at the genus level by 16S rRNA sequencing of the V4 hypervariable region. Furthermore, specific samples underwent analysis through nanopore metagenome sequencing at the species level, with a focus on screening for antibiotic resistance genes.

Results and Discussion: To date, 66 subjects have been enrolled in the study (39 patients, 27 controls). The results show that various bacterial genera differ greatly in their frequency between the patients with CD and the patients in the healthy control group. The results also show that the frequency of various bacterial genera changed significantly under the therapy and that some, but not all, changed in the direction of the healthy control group. This affected, among others, the genera *Faecalibacterium*, *Escherichia-Shigella*, *Veillonella*, *Haemophilus* and *Fusobacterium*, whose relative abundance decreased during therapy.

Conclusion: Our preliminary results provide a comprehensive examination of the microbiome from stool samples of CD patients at the time of diagnosis. We were able to show that the microbiome of children with therapy-naive CD differs from that of healthy children and that it undergoes alterations with various therapies.

Acknowledgments: This work was supported by the German Center for Infection Research (TI07.003 /Karin Fetter). JdL was supported by FoKi-CED e.V., CEDATA and GPGE (Gesellschaft für pädiatrische Gastroenterologie und Ernährung e.V.).

P-1-26

Inhibitory effects of *Ruminococcus bromii* on *Clostridioides difficile* infections

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Clostridioides difficile is a significant gastrointestinal pathogen causing life-threatening infections especially in hospitals and old-age care centers. *Ruminococcus bromii* is a commensal bacterium ubiquitously found in mammals and a resistant starch degrader. Here, we assessed the capability of *R. bromii* to inhibit *C. difficile* infection in vitro, exploring its potential application in probiotic therapies for *C. difficile* infections. Utilizing cellular models, including HT29-MTX-E12 epithelial cells and human colon organoids, this study systematically examines the effects of *R. bromii* on *C. difficile* proliferation, toxin production, and gene expression under various experimental conditions. The methodology encompasses assessing the impact of different concentrations of *R. bromii* and *C. difficile*, variations in media composition, and the use of distinct *R. bromii* forms (live, heat-killed, supernatant). Our results demonstrate that *R. bromii* inhibits vegetative *C. difficile* and *C. difficile* sporulation in vitro and this inhibitory effect was even stronger in an infection model using HT29-MTX-E12 cells. The inhibitory action of *R. bromii* was dependent on growth media composition. However, *R. bromii* did not provide direct protection against *C. difficile* toxins at the tested concentrations. Our data contribute to the understanding of probiotic-pathogen interactions and highlight the potential of *R. bromii* as a probiotic candidate in management of *C. difficile* infections. However, our results also underscore the context-dependent nature of these interactions, suggesting the need for further in vivo studies to validate the efficacy of *R. bromii* in a more complex gut environment.

P-1-28

Characterisation of aerosolised SARS-CoV-2 spectral sensitivity to light from 185 to 528 nm

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Background: The COVID-19 pandemic has exerted unprecedented strain on global health and economic systems. Key factors facilitating the rapid dissemination of SARS-CoV-2 early in the onset of the pandemic were a lack of pandemic preparedness coupled with insufficient knowledge regarding the transmission and mitigation characteristics of this novel respiratory virus. Nowadays, the sensitivity of SARS-CoV-2 towards sunlight and germicidal irradiation, including exposure to the far-UV range, is widely accepted. However, our detailed understanding of the efficacy of UV-irradiation on aerosolized SARS-CoV-2 is still limited.

Methods: To examine the sensitivity of airborne SARS-CoV-2 to a comprehensive irradiation spectrum, we exposed aerosolized virus in a bioaerosol chamber to electromagnetic radiation of seven distinct wavelengths ranging from 185 to 528 nm under controlled temperature and humidity conditions. Following exposure, irradiated virus-laden aerosols were collected using a condensation growth tube sampler. The subsequent analysis involved a detailed evaluation of viral fitness using cell culture infectivity assays and genomic integrity by applying quantitative digital PCR. Additionally, two distinct suspension matrices were utilized to

mimic the potential protective effects of human saliva during transmission.

Results: Our study determines in detail the spectral sensitivity of aerosolized SARS-CoV-2 towards a wide spectrum of electromagnetic radiation including far-UV. We defined virus inactivation constants and dose-response curves across the above light spectrum and revealed a discrepancy between irradiation-mediated SARS-CoV-2 infectivity depletion and viral genome integrity and disruption after exposure of aerosolized virus.

Conclusions: Our analysis provides a comprehensive and detailed overview of the sensitivity of aerosolized SARS-CoV-2 to a wide spectrum of irradiation wavelengths. Our data further characterizes the sensitivity of aerosolized SARS-CoV-2 not only towards sunlight but also artificial germicidal irradiation sources. Our data set also enables us to now model viral inactivation based on individual wavelengths. Such modeling could further inform dose-level recommendations for UV-based germicidal applications in air filtering or circulation systems and, concerning far-UV wavelengths, for direct inactivation of aerosolized virus in occupied spaces.

P-1-29

Helicobacter pylori infection confers increased colorectal cancer risk depending on CagA by disrupting intestinal homeostasis

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Question: Infection with *Helicobacter pylori* CagA-positive strains is a significant risk factor for gastric cancer development and has also been implicated as a risk factor for colorectal cancer (CRC). Previous studies from our lab have demonstrated that *H. pylori* infection promotes colorectal tumorigenesis. However, whether these effects are dependent on the virulence factor CagA, and the mechanisms linking the extraintestinal infection to CRC, remain to be elucidated.

Methods: We infected Apc-mutant and wildtype mouse models with *H. pylori* CagA-proficient and CagA-mutant strains and conducted a comprehensive analysis of *H. pylori*-induced changes in intestinal T-cell responses using flow cytometry and scRNA/TCR-seq, as well as assessments of epithelial and microbial signatures. Immune responses were also evaluated in human colon biopsies, stratified according to CagA and eradication status via immunohistochemistry. Additionally, Apc-mutant mice were treated with antibiotics following long-term *H. pylori* infection to assess the therapeutic impact of eradication on colon tumorigenesis.

Results: *H. pylori* infection promoted tumor development in Apc-mutant mice and altered immune responses in the intestinal epithelium. Notably, the CagA-mutant strain was less effective in inducing the pro-carcinogenic and pro-inflammatory phenotype in Apc-mutant mice compared to the CagA-proficient strain. Furthermore, *H. pylori* CagA infection activated pSTAT3 signaling in the intestinal epithelium and shaped intestinal microbiome signatures, contributing further to tumor development. Importantly, antibiotic eradication of *H. pylori* reduced inflammation and tumor burden in Apc-mutant mice, though it induced persistent changes in the intestinal microbiota.

Conclusions: Together, our studies provide evidence that *H. pylori* infection is a potent promoter of intestinal inflammation and colorectal carcinogenesis, largely dependent on its virulence factor CagA. Consequently, the integration of *H. pylori* and CagA status into CRC prevention programs should be considered.

P-1-30

Quantification of bacterial sialidases and members of the _nan_ gene cluster in combination with CTACK in bile for the early detection of cholangiocellular carcinoma in patients with primary sclerosing cholangitis (PSC)

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Introduction and Aim: Primary sclerosing cholangitis (PSC) is a devastating disease of the biliary tract. Affected patients suffer from recurrent bacterial cholangitis due to the destruction of the biliary tract. Many patients develop cholangiocellular carcinoma (CCA) due to recurrent and/or chronic inflammation. However, the detection of CCA in these patients is difficult and in many cases not successful. In a former study we identified the degradation of cholangiocellular glycocalyx by bacterial sialidases as a new pathomechanism in PSC. In the present study we aimed to analyze the clinical use of detection and quantification of bacterial sialidases, lyases and kinases in combination with cytokines in bile of patients with PSC for the detection of CCA.

Methods: During routine endoscopic retrograde cholangiography (ERC) bile was collected in 112 patients with PSC with and without CCA during 2008-2020. Bile was aliquoted and stored immediately at -80°C. In order to quantify the amount of bacterial sialidases, lyases and kinases we developed an in-house qPCR. DNA was isolated after mechanic lysis with the EurX-Kit. In a sub-cohort of 88 patients with 137 samples 29 cytokines were measured.

Results: In the overall cohort, pool 1 (sialisases), pool 4 (lyases) and pool 5 (kinases and lyases) showed significantly higher DNA concentrations in patients with PSC and CCA compared to patients without CCA (pool 1: 28,005 vs. 709,800 copies/µl; $p<0.0001$) (pool 4: 13,127 vs. 722,228 copies/µl; $p<0.0001$) (pool 5: 2,912 vs. 16,662 copies/µl; $p<0.0001$). The AUC for the pools were 0.831 for pool 1, 0.793 for pool 4 and 0.754 for pool 5. Based on the Youden-Index we identified for each parameter a balanced cut-off with 164,628.77 copies/µl for pool 1, 316,334.54 copies/µl for pool 4 and 9,834.18 copies/ µl for pool 5.

In the sub-cohort, we identified 4 cytokines differentiating between patients with PSC with and without CCA. However, out of these CTACK (cutaneous T cell-attracting chemokine/ CCL27) showed the best performance with a specificity of 0.78, sensitivity of 0.90 and an area under the curve of (AUC) 0.872. Based on the Youden-Index we identified a balanced cut-off with 7,595.

In a next step we could increase the diagnostic value with a combination of our parameters. Patients with at least 2 out of

4 parameters being positive had a CCA with a specificity of 87.4% and a sensitivity of 84.6%.

Conclusions: Based on our new qPCR for bacterial sialidases, lyases and kinases in combination with CTACK we developed a new diagnostic tool for the early detection of CCA in patients with PSC. Early detection of CCA is crucial for the outcome of affected patients. Therefore, we see a huge diagnostic potential of our approach. However, further prospective studies in independent cohorts is needed to validate our system.

P-1-31

An anti-virulence strategy to treat Salmonella infections: Development of lead compounds targeting the transcriptional regulator HilD

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Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in Europe. While most non-typhoidal *Salmonella* strains cause self-limiting infections, several hypervirulent strains are associated with high hospitalization rates. Non-typhoidal *Salmonella* can cause systemic infections by invading the intestinal epithelium. The transcriptional regulator HilD is the central positive regulator of invasion-associated virulence genes. HilD is essential for intestinal colonization and systemic dissemination of *Salmonella* in mice and in chicken infection models.

Here we report advances in the discovery and optimization of synthetic small molecules targeting HilD. We provide evidence of a favorable spectrum of activity among clinical isolates of *S. enterica*, including multi-drug resistant strains. Following a structural characterization of the binding pocket using genetic and biophysical approaches, we undertook a structure-activity relationship analysis on more than 220 derivatives. We successfully identified compounds with activity at nM scale. Finally, we evaluated the antibacterial activity of our compounds against 20 representative gut microbiota species, aiming to demonstrate the potential of anti-virulence agents in mitigating dysbiosis-related disruptions, compared to the broader impact of conventional antibiotics.

HilD inhibitors could be used as standalone drugs or in combination with a standard-of-care antibiotic to reduce the risk of systemic *Salmonella* infections in human patients, and to shorten hospitalization rate and duration. HilD inhibitors could also be drug candidates to reduce the mortality rate among young broiler chicken.

P-1-32

Assessing the impact of anemia on infant cognitive development at Agogo, Asante Akyem North, Ghana

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Background: Anemia is a significant global health issue that affects approximately 1.62 billion people worldwide; particularly pregnant women and infants in low- and middle-income countries (WHO, 2021). Iron deficiency during infancy can lead to long-lasting consequences, including impaired cognitive abilities, behavioral issues, and delays in gross and fine motor skills. In Ghana, approximately 47% of children under five years are affected by anemia, with some regional variances (Ghana Health Service, 2020).

Objectives: This study examines the association between anemia and cognitive as well as developmental outcomes in the first 24 months of life.

Methodology: Data from the Malaria Birth Cohort (MBC, TTU 03.812) from Agogo, Ghana is used for this study, which assesses malaria infections and health related outcomes longitudinally. Cognitive development of 1,257 children was assessed at 12 and 24 months of age with the Developmental Milestone Checklist (DMC III) screening tool. The DMC assesses gross motor, fine motor and language competence of the children and used to estimate an individual cognitive development score. Simultaneously, the participants" hemoglobin levels were collected and type of anemia determined (i.e. microcytic hypochromic anemia, Macrocytic anemia, and normocytic normochromic anemia). These measurements will be used to define the level of anemia (mild, moderate, severe) and follow it over time. These data will be correlated with the calculated DMC-scores and with the respective individual score progression (i.e., score difference between measurements at 12 and 24 months).

Outcome: Insights into the impact of anemia on infant cognitive ability can inform targeted interventions to improve childhood development in malaria endemic areas. Addressing anemia in infants is critical for breaking the cycle of poverty and improving health outcomes for future generations.

P-1-33

Addressing host susceptibility in *mycobacterium tuberculosis* infection: A comparative analysis of lipid droplet accumulation in murine C3HeB/FeJ and C57BL/6 macrophages

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Mycobacterium tuberculosis (*Mtb*) is the intracellular bacterium responsible for tuberculosis (TB), infecting about one-fourth of the world's population, with 5-10% progressing to active disease. A hallmark of TB is granuloma formation in the lungs, which represents a complex structure of epithelial, innate, and adaptive immune cells with infected macrophages in its core. Macrophages are the preferred host cells of *Mtb* and often exhibit a foamy phenotype due to increased lipid droplet (LD) accumulation, composed of neutral lipids, cholesterol esters, and triacylglycerols. While lipids are a key carbon source for *Mtb*, LD biogenesis, dynamics, and their composition in primary macrophages are not well-studied.

No animal model fully replicates human TB disease and lung pathology because *Mtb* has co-evolved with humans and has adapted accordingly. However, animal models are crucial for studying TB. The commonly used C57BL/6 mouse model shows relative resistance to *Mtb*, but compared to humans differs in lung pathology. In contrast, the C3HeB/FeJ mouse strain is highly susceptible, rapidly developing active TB with centralized necrotic granulomas, hypoxia, and cavity formation.

The current study compares lipidomics data as well as LD formation in primary macrophages of C3HeB/FeJ and C57BL/6 mice infected with *Mtb* H37Rv. Bone-marrow-derived macrophages (BMDMs) from both strains were differentiated and infected with *Mtb* H37Rv at varying multiplicities of infection (MOI). By application of mass spectrometry, total lipid composition of BMDMs were qualitatively as well as quantitatively characterized at 24-hours post-infection. In addition, we used multi-mode quantitative microscopic analysis, employing Nile red to phenotypically address the presence of neutral lipids in murine macrophages. Our data suggest that macrophage activation appears to be an early key event in neutral lipid accumulation, which commences within the first 24-hours post-infection. Additionally, macrophages infected with a low MOI for a longer duration of time also showed an increased presence of lipid droplets, pointing to an infection-dependent lipid droplet formation during *Mtb* infection. While C3HeB/FeJ macrophages appear to have an inherently higher Nile red fluorescence compared to C57BL/6 macrophages, both cell types react with an increase in Nile red fluorescence upon *Mtb* infection, although it varies in its magnitude.

P-1-34

Giardia lamblia infection and its co-existence with malaria susceptibility in young children at Agogo, Asante Akyem North, Ghana: Implications for child health and nutrition

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Background: *Giardia lamblia* is a prevalent intestinal protozoan parasite responsible for significant morbidity and mortality in children, particularly in malaria-endemic areas where gut microbiota alterations may increase host susceptibility to other infections. This study examines the prevalence of *G. lamblia* infection and its co-infection with malaria susceptibility among children under five years in the Asante Akyem North District of Ghana, as part of a Malaria birth cohort (TTU 03.812).

Methods: Stool and blood samples were collected from children under five years old to assess parasitic infections. Stool samples were preserved in Sodium Acetate-Acetic Acid-Formalin (SAF) solution, processed by faecal sedimentation technique, and stained with iodine to aid in identifying *G. lamblia* parasites. Microscopic examination was conducted at X10 and high power. Blood samples were collected in EDTA tubes, and thick and thin blood films were prepared. Thin films were fixed in methanol, stained with 10% Giemsa solution, and examined under X100 magnification to identify malaria parasites.

Results: Stool samples were collected from 1,271 children born to 1,256 mothers who were recruited between April 2019 and June 2022 during their pregnancy. *G. lamblia* infections were detected in 46 (3.6%), 70 (5.5%), and 87 (6.8%) children at 12, 24, and 36 months, respectively. Concurrent *Plasmodium* infections were identified in 23 (2.3%), 86 (8.7%), and 143 (20.3%) among the cohort at the same intervals.

Discussion: We will assess and discuss the presence of *G. lamblia* and malaria co-infection at the cohort level highlighting potential implications of a complex interaction for child nutrition and growth. This duality may underscore the need for further research into these interactions in endemic regions.

P-1-35

Microbial profiling of urosepsis patients

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Background: Urosepsis is described as sepsis resulting from an infection in the genitourinary system. The causal role of e.g. *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus* spp., and *Pseudomonas aeruginosa* in urinary tract infections and subsequent urosepsis has been reported. Also, urosepsis caused by *Morganella morganii*, which is considered a rare pathogen in humans, has been revealed. Furthermore, there has been no investigation on the existence of analogous pathogen characterisation in the urine and blood samples of individuals with urosepsis. This study aimed to examine the microbiological profile of patients with urosepsis and identify uropathogens sequence types responsible for infection. Additionally, another purpose of this study is to ascertain whether the uropathogenic isolates of the same species present in the urine and blood samples of patients are identical.

Methods: From April 2024 to July 2024, 31 urosepsis patients were enrolled at the UKGM Clinic for Urology study site in Giessen. In this his study we employed 16S V4 rRNA Illumina sequencing of urine samples to examine the microbiome profile of patients with urosepsis. Additionally, Oxford Nanopore Technology (ONT) based long-read sequencing of the whole genome was conducted to assess the strain similarity of uropathogenic isolates identified in urine and blood samples from 9 out of 31 patients.

Results: The microbiome analysis indicated that *E. coli* as the predominant uropathogen was present in 12 of 31 urosepsis patients, accounting for 41.71 % of cases. Other prevalent species among patients comprised *Enterococcus* (10.49%), *Morganellaceae* (10.43%), *Enterobacteriaceae* (8.39%), and *Pseudomonas* (3.59%). Subsequent investigation of the isolates via ONT long-read sequencing demonstrated that in nine patients examined, the uropathogens present in both urine and blood samples were identical based on genome level and classified as *E. coli*. Furthermore, Multilocus Sequence Typing (MLST) analyses of these strains indicated that sequence types ST69 and ST131 were the predominant sequencing types detected.

Conclusion: This study underlines that uropathogenic *E. coli* (UPEC) are the predominant pathogenic species associated

with urosepsis. The second significant finding from ONT whole genome sequencing revealed that the predominant pathogen in the urine and blood samples of patients is *E. coli* which was identical in both sample types. Sequence types ST 69 and ST 131 were the most commonly identified types associated with the majority of *E. coli* caused urosepticemias.

P-1-36

Expression of the broad spectrum antiviral Griffithsin in human cells: looking for a selective window

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Griffithsin (GRFT) is a red algae-derived lectin of 121 amino acids, showing low in vitro and in vivo toxicity which has been shown to inhibit a broad spectrum of human viruses (1). In preliminary experiments we have determined, that GRFT both from red algae and expressed in E. coli is antivirally active against SARS CoV2. GRFT delivered into cells via lipid nanocarriers (NC) is more antivirally active and less toxic. In this project we aim to express the antiviral GRFT in eucaryotic/human cells to provide proof of concept that a mRNA in lipid nanovesicles would also exert an antiviral effect when administered to patients.

Material & Methods: GRFT was cloned into pLenti CMVtight eGFP Neo (Addgene w784-1) using 2-fragment Gibson assembly for expression in HEK 293T cells. In this plasmid the GRFT insert is under the control of a doxycycline inducible promoter (Tet-On system). HEK 293T were cotransfected with the GRFT containing plasmid and a Tet-On including plasmid (pLenti CMV rtTA3 Blast (Addgene w756-1)) using neomycin and blasticidin S for selection of stably transfected cells. GRFT is induced using doxycycline. Presence and transcription of GRFT is going to be tested by PCR /qrtPCR. Cells are then infected with a panel of cytolytic viruses, the effects on cell viability measured using a commercial viability assay (Promega CellTiter-Glo Luminescent Cell Viability Assay) and antiviral effect/ therapeutic window determined.

Results: Stably transfected cells were established and the inserts confirmed by sequence analysis. Currently induction and infection experiments are under way and will be discussed.

References:

1 Lusvarghi S, Bewley CA. Griffithsin: An Antiviral Lectin with Outstanding Therapeutic Potential. Viruses. 2016 Oct 24;8(10):296. doi: 10.3390/v8100296. PMID: 27783038; PMCID: PMC5086628.

P-1-37

Making health information accessible: The COVID-19 patient guideline

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Backround:

- evidence-based practice guidelines offer clinicians a **reliable source** of information based on the most recent **evidence**
- they are complex and difficult for laypersons to understand
- knowledge gap between clinicians and patients

Methods:

Creating the first version of the PatG (6 months)

- publish practice guideline
- prioritize topics for patients
- translate information to lay language
- feedback rounds for experts
- publish first version of PatG

Update: updating the PatG every 6 months (1 month)

- Publish updated practice guideline
- Revise information in PatG
- add new information
- feedback round with experts
- publish update of PatG

Main challenges and solutions:

Challenge 1: translating information without losing key information and the"duality of German language" (differences between technical terms andlay language are large)

Solution 1: we did not translate recommendations word for word, butrather added explaining sentences, kept the sentences as short aspossible, and added a dictionary for technical terms.

Challenge 2: including additonal information, while keeping thePatG short and accessible

Solution 2: we added graphics and flowcharts to shorten longparagraphs and explanations. Additionally, we linked additionalreliable resources rather than repeating already available information.

Conclusion and main messages:

- PatG provide reliable, evidence-based information
- PatG are a tool to bridge theknowledge gap and enable shared-decision making
- The COVID-19 PatG is updatedregularly and provides up-to-datescientific information in understandable language
- We constantly work on improving the accessibility and comprehensibility of COVID-19 PatG

P-1-39

The TTU-TB analytical platform

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We have established a number of mass spectrometry based workflows for preclinical studies in drug development, infection research and antimicrobial resistance. Here, we present our workflows for quantitation of pharmaceuticals to perform PK/PD measurements, determine lipid metabolic influence on viral infections and structural modification on the cell wall of bacterial.

Examples of recent studies are given concerning PK/PD of BDQ nanoparticles in a mouse model (1), cell lipid metabolic alterations during orthoflavivirus infection (2) and detection Lipid A modifications during development of collistin resistant in Enterobacter species (3).

The repertoire of bioanalytical methods is aimed to support translational studies and can be customized to specific needs in many areas of infection research.

(1) Intranasal Administration of Bedaquiline-Loaded Fucosylated Liposomes Provides Anti-Tubercular Activity while Reducing the Potential for Systemic Side Effects. Marwitz F, Hädrich G, Redinger N et al. ACS Infect Dis. 2024 Sep 13;10(9):3222. PMID: 39136125

(2) Glycerophospholipid remodeling is critical for orthoflavivirus infection.
Hehner J and Schneider L et al. Nat Commun. 2024 Oct 7;15(1):8683. PMID: 39375358

(3) Resolving colistin resistance and heteroresistance in Enterobacter species.
Doijad SP, Gisch N et. al. Nat Commun. 2023 Jan 10;14(1):140. PMID: 36627272

P-1-40

Comprehensive analysis of Tuberculostearic acid-containing lipids during host-pathogen interactions in *Mycobacterium tuberculosis* infection

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The *mycobacterium tuberculosis* (*Mtb*) cell wall contains the unique fatty acid (FA) 10-methylstearic acid, known as tuberculostearic acid (TSA), across various lipid species, but is absent in eukaryotic cells. Phosphatidylinositol (PI) 35:0 (16:0_19:0) has been identified as the major phosphoglycerolipid in multiple *Mtb*-complex lineages, and has been proposed as a marker for mycobacterial load in preclinical models and as a metabolic tracer [1,2]. Our studies aim to: (1) further explore PI 19:0_16:0 as a molecular marker of *Mtb* within host cells, and (2) investigate the metabolic pathways involving TSA within the host cell during infection. We used a cell culture system with bone marrow-derived murine macrophages (BMDMs) exposed to rifampicin-inactivated *Mtb* (H37Rv) over 24, 72, and 144 hours to examine PI 16:0_19:0 stability and its potential recycling into the host lipidome. Semi-targeted shotgun lipidomics, utilizing nano-ESI-MS/MS on the Q Exactive™ Plus platform (Thermo, Bremen, Germany) coupled with a Nanomate Triversa system (Advion, Ithaca, US), enabled us to identify phospholipid species containing TSA as potential macrophage metabolic products. Following incubation with the bacteria, we detected PI 19:0_14:0, PI 19:0_16:1, PI 19:0_16:0, PI 19:0_17:1, PI 19:0_15:0, PI 19:0_18:2, PI 19:0_18:1, and PI 19:0_20:4 in lipid extracts of the cells. Notably, the latter four species were absent in pure bacterial extracts, which suggests them to be metabolic products.

During these analyses and in a clinical study [1], we also observed FA 19:0 in control samples. In-depth structural elucidation is not possible with our commonly applied lipid analysis platform. To decipher which isomeric forms of FA 19:0 are present we used the capabilities of ion mobility array of the Cyclic MS (Waters, Manchester, UK) [3].

Additional experiments involved examining TSA-containing lipids in human monocyte-derived macrophages (HMDMs) infected with live *Mtb* (H37Rv) for 24 and 72 hours. We also infected HMDMs with fluorescently labeled *Mtb* H37Ra and used Fluorescence-Activated Cell Sorting (FACS; ARIAIII, BD) to specifically isolate macrophages harboring phagocytized bacteria.

This comprehensive analysis advances our understanding of TSA's role in host-pathogen interactions and highlights its potential as a biomarker and metabolic tracer in tuberculosis research

1. Brandenburg, J, Heyckendorf J, Marwitz F et al., *Tuberculostearic acid-containing phosphatidylinositols as markers of bacterial burden in tuberculosis.* ACS Infect Dis, 2022
2. Brandenburg, J. et al. *WNT6/ACC2-induced storage of triacylglycerols in macrophages is exploited by Mycobacterium tuberculosis.* J Clin Invest, 2021
3. Giles, K et a l. *A Cyclic Ion Mobility-Mass Spectrometry System*. Anal Chem, 2019

P-1-41

The CARV challenge: How do hematological malignancy patients struggle with diverse CARV pathogens

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Background: The increasing concern regarding the impact of community-acquired respiratory viruses (CARVs) on patients with hematological malignancies requires proactive measures to mitigate risks and enhance treatment outcomes. This study sought to gather and analyze epidemiological, management, and outcome data from hematological malignancy patients with CARV to inform tailored clinical management strategies.

Methods: Utilizing an online registry, data from of CARV infections in hematological malignancy patients were collected from January 2023 to January 2024, spanning 53 sites across 21 countries.

Results: Our survey encompassed 561 cases of CARV in patients with hematological malignancies. The majority of patients were from Italy (39%) and Spain (19%). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) accounted for 336 cases (63%), followed by influenza (n=77, 15%), respiratory syncytial virus (RSV) (N=63, 12%), and rhinovirus (n=50, 9%). The distribution of specific CARVs mirrored the overall prevalence across different malignancies, with 27% in acute leukemias and non-Hodgkin lymphoma each and 21% in multiple myeloma. Prior to CARV infection, 24% of patients had undergone stem cell transplantation or CAR-T therapy, particularly in cases of

metapneumovirus (67%), parainfluenza (60%), RSV, and rhinovirus (40% each). Chronic cardiopathies (41%) were the most common comorbidity across all CARV cases. Critical illness was more prevalent in patients infected with metapneumovirus (22%) or influenza (18%) compared to RSV (11%) or SARS-CoV-2 (8%). Invasive mechanical ventilation was more frequent in metapneumovirus (11%) and influenza cases (7%). Bacterial pathogens (17%) were the primary cause of secondary infections, followed by fungi and viruses (5% each), with consistent distribution across CARV types. Metapneumovirus and parainfluenza infections had the highest mortality rates (33% and 30%, respectively), surpassing those of influenza (16%) or SARS-CoV-2 (11%). SARS-CoV-2 (68%) and RSV (50%) had the highest associated mortality rates. Progression of baseline malignancy contributed to 50% of overall mortalities. Further details are provided in Table 1.

Conclusion: Our findings emphasize the profound impact of CARV on patients with hematological malignancies, notably with SARS-CoV-2 predominance. CARV distribution mirrored malignancy prevalence, with acute leukemias and non-Hodking lymphoma most affected. Additionally, our study highlights the increased severity and mortality rates linked to specific CARV pathogens like metapneumovirus and parainfluenza.

Fig. 1

	Overall		SARS-CoV-2		Influenza viruses		RSV		Rhinovirus		Parainfluenza virus		Meta-pneumovirus		Other viruses	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
	531	100.0	336	63.3	77	14.5	63	11.9	50	9.4	10	1.9	9	1.7	16	3.0
Baseline comorbidities																
Chronic cardiopathies	232	41.4	144	42.9	34	44.2	27	42.9	14	28.0	5	50.0	2	22.2	6	37.5
Diabetes mellitus	74	13.2	45	13.4	12	15.6	9	14.3	3	6.0	2	20.0	1	11.1	2	12.5
Chronic pulmonary disease	63	11.2	33	9.8	9	11.7	9	14.3	6	12.0	3	30.0	1	11.1	2	12.5
Smoking history	52	9.3	26	7.7	6	7.8	14	22.2	3	6.0	1	10.0	1	11.1	1	6.3
Renal impairment	35	6.2	19	5.7	3	3.9	8	12.7	2	4.0	2	20.0	0	0.0	1	6.3
Liver disease	23	4.1	11	3.3	5	6.5	3	4.8	2	4.0	0	0.0	1	11.1	1	6.3
Baseline malignancy																
Non-Hodgkin lymphoma	152	27.1	110	32.7	12	15.6	15	23.8	9	18.0	1	10.0	2	22.2	3	18.8
Multiple myeloma	120	21.4	68	20.2	19	24.7	15	23.8	12	24.0	4	40.0	1	11.1	1	6.3
Acute myeloid leukaemia	102	18.2	54	16.1	18	23.4	12	19.0	10	20.0	2	20.0	4	44.4	2	12.5
Acute lymphoblastic leukaemia	48	8.6	23	6.8	6	7.8	6	9.5	8	16.0	0	0.0	0	0.0	5	31.3
Chronic lymphocytic leukaemia	46	8.2	33	9.8	4	5.2	5	7.9	1	2.0	2	20.0	0	0.0	1	6.3
Myelodysplastic syndrome	31	5.5	15	4.5	7	9.1	3	4.8	4	8.0	0	0.0	0	0.0	2	12.5
Hodgkin lymphoma	17	3.0	7	2.1	5	6.5	2	3.2	1	2.0	0	0.0	1	11.1	1	6.3
Myelofibrosis	13	2.3	6	1.8	4	5.2	1	1.6	1	2.0	0	0.0	1	11.1	0	0.0
Chronic myeloid leukaemia	7	1.2	3	0.9	1	1.3	1	1.6	1	2.0	0	0.0	0	0.0	1	6.3
Hairy cell leukemia	7	1.2	4	1.2	1	1.3	1	1.6	1	2.0	0	0.0	0	0.0	0	0.0
Aplastic anaemia	6	1.1	5	1.5	0	0.0	1	1.6	0	0.0	0	0.0	0	0.0	0	0.0
T prolymphocytic leukemia	4	0.7	1	0.3	0	0.0	0	0.0	2	4.0	1	10.0	0	0.0	0	0.0
Amyloid light-chain amyloidosis	3	0.5	3	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Polycythemia vera	3	0.5	2	0.6	0	0.0	1	1.6	0	0.0	0	0.0	0	0.0	0	0.0
Essential thrombocythemia	1	0.2	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Systemic mastocytosis	1	0.2	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Transplantation history																
allogenetic HSCT	64	11.4	9	2.7	16	20.8	16	25.4	11	22.0	4	40.0	3	33.3	5	31.3
autologous HSCT	58	10.3	28	8.3	5	6.5	9	14.3	9	18.0	2	20.0	3	33.3	2	12.5
CAR-T	10	1.8	3	0.9	1	1.3	4	6.3	2	4.0	0	0.0	0	0.0	0	0.0
CARV severity																
ICU admission	54	9.6	26	7.7	14	18.2	7	11.1	3	6.0	0	0.0	2	22.2	2	12.5
Invasive mechanical ventilation	15	2.7	6	1.8	5	6.5	3	4.8	0	0.0	0	0.0	1	11.1	0	0.0
Secondary infections																
Bacterial	94	16.8	45	13.4	9	11.7	12	19.0	14	28.0	4	40.0	4	44.4	6	37.5
Fungal	28	5.0	10	3.0	5	6.5	4	6.3	4	8.0	1	10.0	2	22.2	2	12.5
Viral	28	5.0	10	3.0	3	3.9	3	4.8	6	12.0	2	20.0	2	22.2	2	12.5
Mortality	69	12.3	38	11.3	12	15.6	6	9.5	5	10.0	3	30.0	3	33.3	2	12.5
Reason for mortality *																
COVID-19	26	37.7	26	68.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	50.0
Influenza	5	7.2	0	0.0	5	41.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Respiratory syncytial virus	3	4.3	0	0.0	0	0.0	3	50.0	0	0.0	0	0.0	0	0.0	0	0.0
Rhinovirus	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Parainfluenza virus	1	1.4	0	0.0	0	0.0	0	0.0	0	0.0	1	33.3	0	0.0	0	0.0
Metapneumovirus	2	2.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	66.7	0	0.0
Hematological malignancy	35	50.7	17	44.7	8	66.7	2	33.3	3	60.0	1	33.3	2	66.7	2	100.0

* Mortality might be attributable not only to the viral infection. but also to the baseline hematological malignancy, or both

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P-1-42

Characterization of HIV-specific follicular memory CD8 T cells: Insights into transcriptional dynamics and functional attributes during acute and chronic infection

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Background: T helper cells in lymph node follicles are major reservoirs of HIV replication and continue to express viral gene products during treatment with ART. Follicular memory CD8 T cells expressing the homing receptor CXCR5 (mTfc) can access these sites and contribute to the clearance of HIV. This study aimed to characterize HIV-specific mTfc in people living with HIV (PLWH).

Methods: PLWH were recruited from Germany and Tanzania (total *n* = 30). HIV-specific and HIV-nonspecific memory CD8 T cells were identified in peripheral blood samples using peptide-HLA tetramers and sorted via flow cytometry. Low-input RNA sequencing and differential gene expression analyses were performed on sorted cell subsets stratified by the expression of CXCR5.

Results: Only a small fraction of circulating HIV-specific memory CD8 T cells expressed CXCR5 (median: 2.15% in the tanzanian cohort, 1.58% in the german cohort), which increases in treated PLWH. HIV-specific mTfcs exhibited a more distinct follicular transcriptomic profile in chronic than acute HIV infection. In HIV chronic infection, HIV-specific mTfcs showed a classical follicular, effector-memory phenotype with underexpression of genes associated with effector functionality, activation, and exhaustion and overexpression of genes encoding various chemokines and TLRs relative to HIV-specific memory CD8 T cells lacking expression of CXCR5. These transcriptional profiles were observed in both cohorts and were unaffected by ART. In addition, HIV-specific mTfc overexpressed genes associated with a type I interferon response relative to HIV-specific memory CD8 T cells lacking expression of CXCR5, even after prolonged treatment with ART.

Conclusions: These results provide insights into the transcriptional characteristics of HIV-specific mTfc at different stages of infection, highlighting functional pathways associated with immune control of viral replication in the absence or presence of ART.

P-1-43

Prospective cohort-study for the investigation of the vaccine-induced immune response after vaccination against RESPiratory viruses in patients with hematological and ONcological diseaSEs (RESPONSE)

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Respiratory virus infections, such as COVID19 and influenza, remain significant international public health concerns despite vaccines and anti-viral agents. While patients with cancer remain the most vulnerable group of patients with regard to morbidity and mortality, they show poorest vaccine response concurrently. Immunologic data in this population are limited mainly focusing on serologic parameters. However, cellular and especially T cell response seems often

to be induced more reliably in those patients than humoral response.

To gain further insights into vaccine-induced immune response and thereby improve protection from respiratory infection in hematological and oncological patients, we currently establish a structured prospective research program (RESPONSE). Apart from humoral and cellular immune response, we aim to investigate factors influencing the humoral and cellular vaccine-induced immune response in patients with hematological and oncological malignancies including state of disease, treatment, and demographic factors. As a pilot project, we investigated samples from patients with chronic lymphocytic leukemia (CLL; n=18) before and after influenza vaccination. Analyses of humoral and cellular vaccine-induced immunity are currently ongoing and results will be available by October 2024.

This study intends to lay a foundation for a structured translational research program of vaccination to aim for best protection from infection by different respiratory pathogens. Long-term objectives include to reach best protection from vaccine-preventable disease with a first focus on influenza infection (currently ongoing pilot project and planned study funded by DZIF Advanced Clinician Scientist Program with regard to optimized booster vaccination). In addition, we plan to investigate vaccine-immune response to the recently approved RSV vaccine within this platform and possibly further vaccines in future. Urging questions such as the influence of different targeted therapies on vaccine immune response will be part of these projects. Apart from immunological studies, we want to raise awareness for vaccination and increase vaccination rates among cancer patients.

P-1-44

Engineering CXCR5+ CD8 T Cells for targeted control of HIV Infection in Peripheral Blood and Secondary Lymphoid Tissues

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Background: Despite the effectiveness of antiretroviral treatment (ART), human immunodeficiency virus (HIV) remains a significant global challenge due to lifelong therapy costs, side effects, and persistent stigma. Viral rebound following ART cessation is attributed to integrated proviral DNA in latently infected long-lived memory cells, which constitute the viral reservoir. Targeting this reservoir is crucial for contemporary strategies seeking a functional HIV cure.

Follicular CD8 T cells, which express the lymphoid homing factor CXCR5, can enter B-cell follicles in secondary lymphoid tissue. These cells are of particular interest due to their access to these immune sanctuary sites that harbor a significant portion of the viral reservoir. Our group has recently performed an in-depth characterization of follicular CD8 T cells from peripheral blood, demonstrating a strong effector phenotype in individuals living with HIV who initiated ART early after infection (Rueger et al., manuscript in revision). Additionally, the antiviral activity of follicular CD8 T cells against HIV-1 has been shown in autologous viral outgrowth assays.

Methods: In this study, we aim to genetically modify primary CD8 T cells, sourced from peripheral blood and secondary lymphoid tissue, to constitutively express CXCR5 via lentiviral transduction and subsequently evaluate the functionality and antiviral capacity of the transduced cells. Primary CD8 T cells isolated from peripheral blood or tonsils will be transduced to express CXCR5 using lentiviral vectors constructed in collaboration with Prof. Boris Fehse and Dr. Kristoffer Riecken at the DZIF site Hamburg-Lübeck. The migration behaviour of the transduced cells will be evaluated using transwell migration assays and in vitro tonsil organoid models. Antiviral activity against HIV-1 infected cells from peripheral blood and secondary lymphoid tissue, in the form of HLAC cultures, will be assessed using a GFP-expressing HIV-1 reporter strain. Furthermore, remodelling the 3D structure of tonsils using a biomimetic tonsil organoid model will allow investigation of the killing potency of CXCR5+ CD8 T cells in a more secondary lymphoid-like environment.

Results: Preliminary results show transduction rates of approximately 5-10% of primary CD8 T cells isolated from peripheral blood. Downstream assays investigating migration behaviour and antiviral activity are yet to be performed. Results will be reported during the combined DZIF and DGI annual meeting.

Summary: This study aims to demonstrate proof of concept that CD8 T cells genetically engineered to constitutively express CXCR5 can mimic follicular CD8 T cells, localize to the germinal centers of B cell follicles, and exhibit antiviral activity against HIV-1. This research will lay the groundwork for future immunological cure strategies for HIV.

P-1-45

***In vivo* 4-1BB stimulation enhances the efficacy of therapeutic vaccination in high-titer HBV carrier mice**

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Nearly 4% of the world population is chronically infected with the hepatitis B virus (HBV) and at risk of developing liver cirrhosis and hepatocellular carcinoma. Numerous studies showed that HBV persistence correlates with a failure to develop efficient, virus-specific B- and T-cell responses. Hence, therapeutic vaccination represents a promising strategy to treat chronic hepatitis B. We developed *TherVacB*, a therapeutic hepatitis B vaccine based on a heterologous protein-prime/MVA boost vaccination scheme. In preclinical mouse models with low- to intermediate-levels of persistent HBV infection, *TherVacB* induced strong HBV-specific immunity and long-term immune control of HBV, whereas high levels of HBV replication negatively influenced vaccine-induced HBV-specific CD8 T cell response and antiviral efficacy.

We aimed to understand molecular mechanisms contributing to T-cell responsiveness by comparatively characterizing *TherVacB*-elicited liver-associated CD8 T cells in low- and high-titer HBV carrier mice to eventually find potential therapeutic targets to overcome T-cell dysfunctionality in high-titer HBV carriers.

Comparative transcriptome analysis revealed that, initially, *TherVacB* induced comparable numbers of effector CD8 T cells in low- and high-titer HBV carrier mice; however, their functionality and long-term survival were determined by hepatic HBV levels. Following the complete vaccination regimen, long-term effector, stem-like, and tissue-resident memory T-cell subsets were established in low-titer mice. In contrast, in high-titer mice, expression levels of exhaustion-related genes such as PD-1, Tim-3, Lag3, TOX, and TIGIT increased over time. In addition to those known factors contributing to T-cell dysfunctionality, vaccine-induced CD8 T cells from high-titer mice consistently expressed high levels of co-stimulatory molecule 4-1BB. Thus, we wondered whether combining *TherVacB* with 4-1BB targeting monoclonal antibodies (mAb) could improve the antiviral efficacy of therapeutic vaccination in high-level HBV infection. To address that, we immunized high-titer HBV-carrier mice with *TherVacB* with or without *in vivo* 4-1BB mAb treatment and compared HBV-specific immune responses and infection parameters.

Co-administration of 4-1BB mAbs resulted in elevated IFNγ, TNF, and GzmB production on vaccine-induced CD8 T-cells and decreased expression of exhaustion-related markers such as PD-1, Lag-3, and Tox compared to the only vaccinated mice. In addition, we found that combinatorial treatment with 4-1BB mAbs resulted in a significant reduction in serum HBeAg and HBsAg levels accompanied by mild, transient ALT flares.

In conclusion, combining *TherVacB* with 4-1BB monoclonal antibodies improved HBV-specific CD8 T-cell functionality and enhanced the antiviral efficacy of *TherVacB*. Our data suggest that activating 4-1BB signaling *in vivo* represents a promising strategy to overcome T-cell dysfunctionality in high-titer HBV carriers.

P-1-47

T cells expressing HBV-specific chimeric antigen receptors harboring a Fab fragment control HBV infection in mice

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Background and Aims: Chimeric-antigen-receptors (CARs) are synthetic receptors designed to drive antigen-specific activation of T cells upon binding to cognate antigen. CAR-T cells are used in cancer therapy but are also interesting for chronic viral infections. Our study aimed to generate novel CARs that target the hepatitis B virus envelope protein (HBVenv) on the membrane of HBV-infected cells using an antigen binding fragment (Fab) consisting of heavy and light chains instead of a variable single-chain fragment. The aim of this study was to overcome functional alterations of the new format, and to study the antiviral efficacy of FabCAR-engrafted T cells in vitro and in vivo.

Method: We constructed novel CARs containing the Fab fragment of HBVenv-specific monoclonal antibodies as binding domains and CD3 as well as CD28 intracellular signaling domains. We characterized the FabCAR T-cell function by T-cell activation upon HBsAg stimulation and elimination of HBVenv-transgenic hepatoma cells. The antiviral effect was accessed by coculturing FabCAR-T cells with HBV infected HepG2-NTCP cells. To study in vivo efficacy, CD45.1 murine T cells expressing FabCAR were transferred to CD45.2 AAV-HBV infected, HBV-carrier Rag1 knock-out mice.

Results: Multifunctional FabCAR-T cells could be induced via HBsAg stimulation. FabCAR-T cells specifically eliminated HBV envelop protein transgenic cell lines Huh7S and HepG2SML. Elimination of target cells was accompanied by secretion of interferon-gamma, tumor necrosis factor, and granzyme B. FabCAR-T cells showed antiviral activity by significantly decreasing the level of viral antigen, intracellular HBV DNA, and HBV cccDNA in HBV-infected HepG2-NTCP cells. In HBV-carrier mice, after adoptive transfer, FabCAR-T cells proliferated and localized to the liver, resulting in target cell killing indicated by ALT flare and an antiviral effect by HBsAg and HBeAg reduction.

Conclusion: T cells stably transduced with our FabCARs are polyfunctional and could efficiently eliminate HBVenv-positive cells in an antigen-dependent manner in cell culture and in a preclinical animal model. Thus, FabCAR-T cells are promising candidates for treating chronic hepatitis B and HBV-associated hepatocellular carcinoma.

P-1-48

Unveiling novel therapeutic targets for Human Cytomegalovirus (HCMV) Infection: Exploring the antibody response to the gH/UL116 complex

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The HCMV poses a significant public health challenge, especially for immunocompromised individuals and during congenital infections. Despite this, effective vaccines are lacking, and treatment options are limited. Antibody-based therapeutics have demonstrated effectiveness against viral infections; however, the lack of detailed mechanistic understanding of HCMV infection and neutralization impedes antibody application for HCMV prevention and therapy. In our study, we investigated the antibody response targeting the gH/UL116 HCMV-surface complex.

To this end, we selected individuals with high neutralizing capacities from a cohort of 9,000 donors. Among these, varying reactivity against UL116 was observed. Two donors with high and two with weak UL116 responses were chosen for B-cell analysis, showing a fraction of 0.072%-0.085% and 0.042%-0.048% gH/UL116-interacting, but gH/gL non-reactive memory B-cells, respectively. Of these we isolated 409 B-cells at single-cell level. Characterizing 107 representative antibodies, we identified a novel set of antibodies specific to gH and a set exclusively targeting UL116. Epitope mapping revealed four major antibody target sites on gH/UL116, further characterized by cryo-EM structural analysis.

Importantly, some antibodies showed potent neutralizing activity across various cell types, including potential HCMV reservoir cells like monocytes. Moreover, certain antibodies demonstrated superior neutralizing activity compared to

previously reported HCMV-specific antibodies, making them promising clinical candidates. Notably, a significant proportion of UL116-directed antibodies, along with a subset targeting gH, belonged to the IgG3 isotype, predisposing them to mediate alternative antibody effector functions. Initial experiments using a CD16a-reporting cell assay identified antibodies with strong CD16a-activating properties, pinpointing Fc-activating target sites on the gH/UL116 complex. Interestingly, gH/UL116 is strongly expressed on HCMV-infected cells, making it a promising target for ADCC-activating antibodies.

In summary, our findings unveil novel highly potent HCMV-antibodies as promising clinical candidates. Additionally, our data highlight gH/UL116 as an intriguing candidate for potential HCMV vaccine studies, stimulating diverse mechanisms of antibody responses against HCMV.

P-1-49

Cell-type-specific efferocytosis rewires mitochondrial metabolism in alveolar macrophages to prioritize resolution of inflammation over antibacterial responses

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Question: Resolution of lung injuries is vital to maintain gas exchange and restore homeostasis. Concurrently, there is an increased risk of secondary bacterial infections. Alveolar macrophages (AMs) are crucial to not only initiate inflammation and clear bacteria but also promote resolution. However, environmental cues that switch these seemingly opposing functional phenotypes of AMs remain elusive.

Results: Resolution of lung inflammation requires clearance of apoptotic cells (efferocytosis), mainly epithelial cells and neutrophils (PMNs). Here, we discovered an incapacity of AMs to mount an effective immune response to bacteria during resolution of inflammation. Efferocytosis of both cell types led to an anti-inflammatory phenotype in AMs. Intriguingly though, only efferocytosis of PMNs reprogrammed mitochondrial metabolism of AMs to restrict functional plasticity during resolution of inflammation. PMN-derived myeloperoxidase (MPO) fueled canonical glutaminolysis through uncoupling protein 2 (UCP2) resulting in decreased mtROS-dependent killing of bacteria and secretion of pro-inflammatory cytokines. Instead, MPO-mediated stabilization of UCP2 inhibited mitochondrial hyperpolarization and boosted efferocytosis irrespective of the presence of bacterial pathogens. In contrast, efferocytosis of alveolar epithelial cells resulted in a distinct anti-inflammatory phenotype of AMs maintaining phenotypic plasticity towards bacteria.

Conclusions: Overall, uptake of apoptotic PMNs switches AMs to prioritize resolution of inflammation over antibacterial responses and similarly affects murine macrophages at extra-pulmonary sites, and human AMs.

Fig. 1

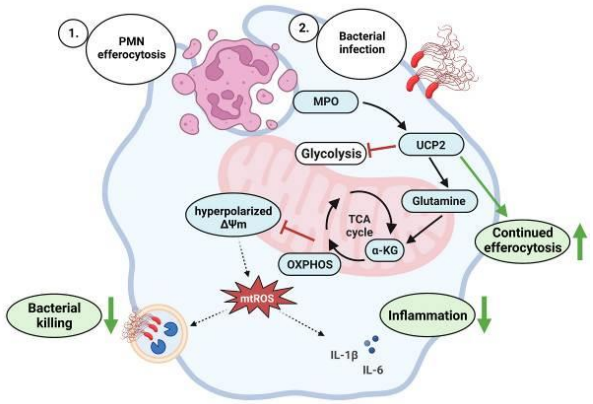
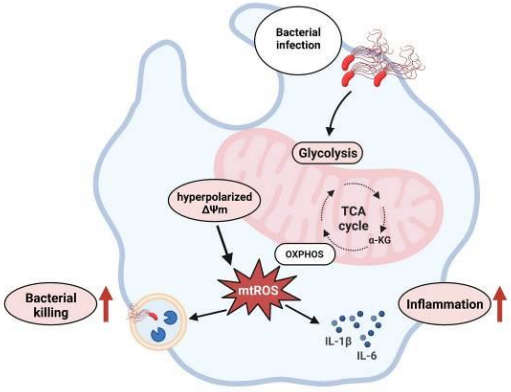


Fig. 2



P-1-50

A combination of direct acting and host-directed antivirals dramatically suppresses various Coronaviruses

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Cyclosporin A (CsA) is a cyclophilin A inhibitor used as an immunosuppressant to prevent transplant rejection. CsA can decrease the replication of various coronaviruses (CoVs), including SARS-CoV-2 and MERS-CoV, *in vitro* and *in vivo*. Alisporivir (ALV) is a non-immunosuppressive derivative of CsA that is also able to reduce the replication of several CoVs. The antiviral effect is partly due to CsA/ALV-induced IFN-λ, which mediates the expression of antiviral interferon-stimulated genes (ISGs). The coronavirus inhibitor 13b-K is directed against the viral main protease (MPro, also known as 3CLpro, encoded by nsp5), which is essential for processing and maturation of the coronavirus polyproteins. We could show previously that 13b-K is able to inhibit SARS-CoV-2 replication. Treatment with CsA or 13b-K alone reduced viral replication or egress by 2-3 log scales. In the present study, we investigated the effect of a combination treatment with cyclophilin and MPro inhibitor on the replication and release of different SARS-CoV-2 variants, MERS-CoV and HCoV-229E. Finally, we also investigated the effect of the combination of CsA or ALV and 13b-K against MERS-CoV *in vivo*.

First, we analysed CsA or ALV combined with 13b-K during infection of bronchial cells with HCoV-229E. We found an

almost complete block of viral replication (> 2-3 log scales) upon combination therapy in comparison with 1 log scale upon treatment with either CsA/ALV or 13b-K. We then examined the inhibitory effect on different SARS-CoV-2 variants of concern and MERS-CoV. The combination therapy resulted in a complete block of viral replication and release of all tested betacoronaviruses. There was also a significant reduction in virus-induced cytokines in inhibitor-treated and infected cells. These results suggest that the cytokine storm described in severe COVID-19, which can lead to tremendous lung damage, is also positively affected by the combination treatment.

The combination treatment described here is able to completely inhibit different CoVs in human-relevant cell culture models. By using two different substance classes, it is possible to counteract the viruses at different important propagation steps. 13b-K directly interferes with the virus replication while the cyclophilin inhibitor enhances cellular defense mechanisms due to an increased IFN-λ expression and ISG induction resulting in a potent inhibitory effect against several CoVs.

P-1-51

Therapeutic vaccination in combination with siRNA-mediated silencing of hepatitis B virus (HBV) and PD-L1 achieves immune control of persistent infection in high-titer HBV carrier mice

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Induction of hepatitis B virus (HBV)-specific immunity by therapeutic vaccination represents a promising treatment option of chronic hepatitis B. High levels of persistent HBV replication and antigen expression, however, prevent an effective therapeutic vaccination. Reducing HBV levels before vaccination by HBV-specific siRNAs (siHBV) enhanced the immunogenicity and antiviral efficacy of our clinical candidate protein-prime/MVA-boost therapeutic vaccine, TherVacB, in higher-titer HBV-carrier mice. We demonstrated that non-responsiveness to vaccination was associated with high PD-1 expression on vaccine-elicited hepatic CD8 T-cells. Consequently, silencing PD-1 ligand-1 by liver-targeted siRNA (siPD-L1) also improved the TherVacB-mediated therapeutic effects. We hypothesized that combining siHBV with siPD-L1 could further broaden the applicability of TherVacB in high-titer, persistent HBV infection settings.

We established high-titer persistent HBV infection in C57BL/6J mice using AAV-HBV, resulting in over 80% of HBV-positive hepatocytes and serum HBsAg levels of 5500 IU/ml. We pretreated five mice per group for eight weeks with siHBV before TherVacB and applied siPD-L1 during the two protein priming immunizations. We followed up with the mice for 7.5 months after the MVA boost.

Groups of mice receiving TherVacB and TherVacB+siPD-L1 demonstrated only a minor decrease in serum HBsAg and HBeAg levels shortly after treatment. Without vaccination, siHBV+siPD-L1 reduced HBsAg and HBeAg, as expected, but the antigen load eventually returned baseline values, and no induction of HBV-specific immunity was observed. Combining siHBV+TherVacB reduced HBsAg to undetectable levels for eight weeks, but a partial relapse finally resulted in only a 1-log10 decrease compared to the initial values. By contrast, mice receiving siHBV+TherVacB+siPD-L1 cleared HBsAg for 24 weeks. 3/5 mice remained negative for 7.5 months. Overall, the siHBV+TherVacB+siPD-L1 treatment resulted, on average, in a ≥3-log10 reduction in serum HBsAg, a 70% reduction in the numbers of HBV-positive hepatocytes and, a 90% reduction in intrahepatic HBV-DNA. A potent vaccine-elicited immunity accompanied this impressive antiviral effect.

Our data demonstrate that complementary siRNA-mediated silencing of HBV and the immune checkpoint PD-L1 helps to further enhance the efficacy of therapeutic vaccination in high-titer HBV carriers.

P-1-52

Implementing mRNA technology at DZIF: Benchmarking vaccines against three human pathogens of public health importance

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The advent of mRNA technology has marked a significant turning point in vaccine development, particularly highlighted by its pivotal role during the COVID-19 pandemic and the licensure of the first mRNA-based vaccine. This cutting-edge technology also has the potential to impact and enhance the research and translational capabilities of the members of the German Center for Infection Research (DZIF). Integrating mRNA technology into the DZIF framework requires a strategic approach. Sourcing the required lipid nanoparticle (LNP)-formulated mRNAs targeting specific pathogens from a Contract Research Organization (CRO) with a track record in GMP manufacturing seemed to be the most viable option.

In a project of the DZIF Bridging Topic Vaccines, three institutions from DZIF partner sites in Hannover, Marburg and Munich have joined forces to evaluate mRNA vaccine candidates against three different bacterial and viral human pathogens and benchmark them to other established DZIF technologies such as the viral vector Modified Vaccinia virus Ankara (MVA) and subunit approaches. In collaboration with the Product Development Unit of DZIF, a contract will be negotiated and agreed to establish a sustainable and long-term collaboration that will allow for the rapid provision of formulated mRNAs. We are targeting pathogens of public health importance, namely *Helicobacter pylori*, hepatitis C virus (HCV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Various vaccine candidates expressing either the MERS-CoV spike protein, *H. pylori* outer membrane proteins and virulence factors, or the HCV E2 protein will be tested using differently formulated LNPs in mice. The HCV vaccine candidate will be characterized for humoral immunogenicity, and the vaccine candidates for

MERS-CoV and *H. pylori* will be characterized for humoral and cellular immunogenicity. All vaccine candidates will be compared side-by-side with available vaccines against MERS-CoV, *H. pylori* and HCV with proven efficacy. If an mRNA vaccine candidate is at least similarly immunogenic or more immunogenic than the comparator vaccine, the efficacy of the vaccine candidate can be analyzed in the appropriate animal model, if available.

The described project will be carried out between May 2024 and April 2025 and the first results of the evaluation of the three subprojects will be presented at the joint meeting of the German Society for Infectious Diseases (DGI) and the DZIF in February 2025.

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P-1-53

Functional and phenotype analysis of SARS-CoV-2 specific T-cell responses before and after a breakthrough infection

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Since its emergence in 2019, the COVID-19 pandemic has presented a major global health challenge. Although widespread vaccination has significantly reduced the risk of severe illness, the precise role of T cells in protecting against breakthrough infections (BTI) remains unclear. This study presents a longitudinal evaluation of the functional and phenotypic characteristics of virus-specific T-cell responses in individuals who have experienced a SARS-CoV-2 BTI.

T-cell responses of 16 subjects were investigated longitudinally at two weeks, one, three, and six months after a SARS-CoV-2 BTI. Peripheral blood mononuclear cells (PBMC) were either stimulated for 24h/48h with overlapping SARS-CoV-2 peptide pools (Spike/NCAP) or labeled with peptide-specific MHC class I tetramers and specially designed panels, including markers for functional and phenotypical T-cell characterization. Subsequent analyses were conducted using a fully automated multiplex ELISA device or a flow cytometer.

We observed a time-dependent decline six weeks post-BTI in IFNγ-producing CD4 T cells reactive to Spike ($p=0.0003$) and NCAP ($p<0.0001$) viral antigens. Notably, two weeks after BTI, NCAP-specific CD4 T cells exhibited significantly elevated IFNγ production ($p=0.023$), as assessed by flow cytometry. Phenotypic analysis of CD8 T cells showed no significant changes in central memory, terminally differentiated, or effector memory subsets in a longitudinal progression post-BTI. The cytokine expression profile showed an increase of 8.6 % in NCAP-reactive trifunctional CD4 T cells (IL-2+, TNF+, IFNγ+) post-BTI. The number of Spike/NCAP-reactive IFNγ-producing monofunctional CD4 T cells both exhibited an increase of 76 % and 12 %, respectively, until one month after the BTI, followed by a longitudinal decrease of 23 % and 2 % six months post-BTI. Conversely, Spike/NCAP-reactive TNF-producing monofunctional CD4 T cells were both decreased by 56 % and 12 % , respectively, one month post-BTI, with an overall increase of 43 % and 2 %. The fully automated multiplex ELISA revealed a significant increase in the secretion of IL-1β, IL-2, IL-6, IL-10, IFNγ, TNF, and IL-4 by SARS-CoV-2 NCAP-specific PBMC following a BTI.

In conclusion, our longitudinal analysis revealed involvement of Th1, Th2, monocytes, macrophages, and T cells as a response to SARS-CoV-2 BTI, characterized by an increase in trifunctional NCAP-specific CD4 T cells and elevated cytokine secretion following a BTI. However, functional analysis indicated a decline in CD4 T-cell activity post-BTI over time, despite the phenotype of Spike/NCAP-specific CD8 T cells did not change over time. These results suggest that SARS-CoV-2-specific T cells do not provide long lasting protection against reinfection. Overall, these findings enhance our understanding of the T-cell response in breakthrough infections and provide valuable insights into the adaptive immune mechanisms involved in combating COVID-19.

P-1-54

Incidence and clearance of human Papilloma virus: Insights from a Madagascar population based female cohort

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Background: Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide, frequently self-cleared but still responsible for 660,000 cases of cervical cancer (CC) in 2022 due to infections with high-risk carcinogenic viral types. Vaccines preventing infections with HPV have been introduced since 2006, showing a high potential in reducing CC incidence following high vaccination coverage. The World Health Organization called out all member states to commit to increase HPV vaccination coverage to 90% of girls between 9-15 years old by 2030 to eliminate CC as a public health problem by 2120. Madagascar is among the countries with the most delayed HPV vaccination schedule. Further, there is very scarce data on both the frequency of HPV infection and circulating strains. This study aims to estimate the incidence and clearance of HPV to support and inform the implementation of vaccination programmes in the country.

Methods: A prospective cohort study was conducted between 2021 and 2022 on women aged 18-49 years old residing in the Boeny region of Madagascar. Participants underwent gynaecological examinations in three primary health care centres. Gynaecological investigations were performed and vaginal lavages were collected for HPV typification at baseline and 12-month follow-up visits. HPV genotypes were detected by a type specific E7 PCR bead-based multiplex genotyping assay and classified in high-risk carcinogenic and low-risk. Incidence and clearance per 1,000 person-months with 95% confidence intervals were estimated for any-, high- and low-risk genotypes using R software. Incidence was defined as a woman testing HPV positive at follow-up after testing negative at baseline, while clearance was defined as testing HPV positive at baseline but negative at follow-up for the respective group of HPV genotypes.

Results: A total of 110 women were included in the study. At baseline 49 (45.5%) tested positive and 61 (55.5%) negative for HPV. After 12 months, the incidence of new infections by any HPV, high-risk HPV, and low-risk HPV genotypes was 24.3 (CI 95%: 15.6-38.2), 15.6 (CI 95%: 9.3-26.4) and 7.8

(CI 95%: 4.1-15.0) per 1,000 person-months, respectively. After 12 months of follow-up, the clearance rates for any HPV, high-risk HPV, and low-risk HPV genotypes were 22.3 (CI 95%: 13.2-37.7), 25.5 (CI 95%: 14.8-43.9) and 39.3 (CI 95%: 21.1-73.0), respectively.

Conclusions: Our preliminary results show that the incidence of high-risk genotypes is higher while the clearance is lowered compared to the low risk, highlighting the importance for the implementation of vaccination programmes. Factors influencing incidence and clearance will be further evaluated in order to provide additional information to design prevention programmes for HPV infection and CC onset and progression in Madagascar.

P-1-55

HIV-1 infection of monocyte-derived microglia through cell-to-cell spread from CD4+ T cells

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HIV neuroinvasion occurs during initial viremia, with microglia representing the main reservoir in the central nervous system (CNS). Although the mechanisms of microglial HIV infection remain unclear, research on other myeloid cells suggests that interaction with HIV-infected CD4+ T cells is relevant for infection of myeloid cells. In HIV infection, the presence of infected CD4+ T cells and T-tropic virus in the CNS has been described. Here, we hypothesize that microglia become infected with HIV-1 through the phagocytosis of infected CD4+ T cells.

Monocyte-derived microglia (MDMi) were differentiated from human peripheral blood mononuclear cells (PBMCs) with IL-34 and GM-CSF for 14 days. Monocyte-derived macrophages (MDM) were differentiated from PBMCs with M-CSF for 7 days. CD4+ T cells were isolated from PBMCs, stimulated with anti-CD3/anti-CD28, and infected with HIV-1 strains 89.6 or CH077. Flow cytometry, immunofluorescence and Incucyte® live cell imaging were used to characterize the primary cells and quantify infection rates and cell uptake.

MDMi express the homeostatic microglia markers IBA1 and P2RY12, the phosphatidylserine receptors TREM2 and MerTK, the CD47 receptor CD172a as well as the HIV entry receptor CD4 and co-receptors CCR5 and CXCR4. HIV-1 89.6 infection of CD4+ T cells is associated with increased phosphatidylserine exposure on CD4+ T cells and with decreased CD47 expression on HIV-1 p24+CD4- cells. Live cell imaging showed phagocytosis of HIV-1 89.6-exposed CD4+ T cells by MDMi. Six days after 24-hour co-culture with 89.6-infected CD4+ T cells, the infection rate of MDMi was between 0.5-3.6 % as determined via flow cytometry. In contrast, MDMi that were separated from CD4+ T cells through a transwell insert during co-culture were not infected. 45-minute co-culture of MDM with Cell Tracer-labeled CH077-exposed CD4+ T cells resulted in higher uptake rates than observed after co-culture of MDM with labeled uninfected CD4+ T cells, as measured by the amount of Cell Tracer positive, CD3 negative MDM.

Cell-to-cell spread from infected CD4+ T cells is relevant for infection of MDMi with HIV-1 89.6. Further studies are needed to investigate the importance of phagocytosis in establishing the brain reservoir in HIV-1 infection.

P-1-56

Patient-derived monoclonal antibodies to treat Candida infections

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Candida spp. are the main pathogens causing life-threatening invasive fungal disease in the hospital setting.[1] Available treatment options are limited by drug interactions, toxicity and antifungal resistances. Morbidity and mortality of invasive candidiasis are high despite adequate treatment.[2] To overcome these limitations, there is an urgent need for alternative and adjunct therapeutic approaches.

Recently, a number of anti-infective human monoclonal antibodies (mAbs) for different infectious diseases have been successfully isolated from infected, convalescent or vaccinated individuals by antigen-specific single B cell sorting.[3,4,5] It is known that humans also mount an antibody response against fungal virulence factors during colonization and infection.[6] We aim to isolate human-derived mAbs that target virulence factors of *Candida* spp. as novel immunotherapeutic options.

In a cohort of patients with invasive *Candida* infections, we will perform serum ELISA to determine antibody titers against different secreted and cell surface-based fungal virulence factors of *Candida* spp. This will help us to identify individuals that mount a potentially protective antibody response against *Candida* spp. From selected individuals, we will perform an antigen-specific B cell sort with subsequent B cell receptor (BCR) amplification, BCR cloning, sequencing analysis and recombinant mAb production. This will allow us to isolate a set of diverse and novel human mAbs targeting *Candida* virulence factors. Afterwards, we will characterize these antibodies with functional assays to evaluate their anti-infective potential in vitro (Figure 1).

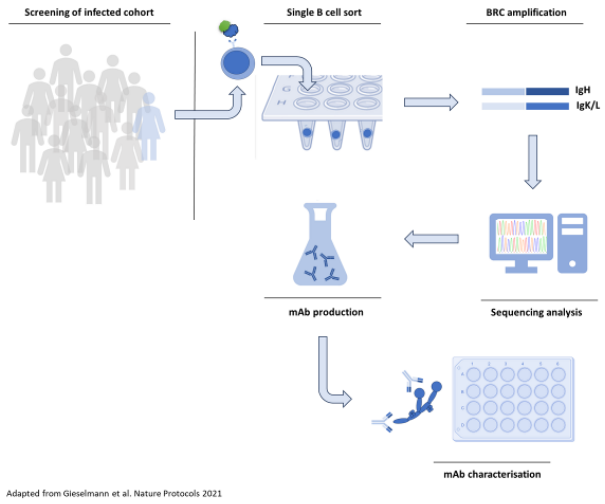
By targeting key virulence mechanisms of *Candida* spp. with human-derived antifungal mAbs, we address the urgent need for novel therapeutic options for these life-threatening fungal infections and pave the way for an antifungal immunotherapy.

1. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004.
2. Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis 2024.

3. Schommers P, Gruell H, Abernathy ME, et al. Restriction of HIV-1 Escape by a Highly Broad and Potent Neutralizing Antibody. Cell 2020.
4. Kreer C, Zehner M, Weber T, et al. Longitudinal Isolation of Potent Near-Germline SARS-CoV-2-Neutralizing Antibodies from COVID-19 Patients. Cell 2020.
5. Simonis A, Kreer C, Albus A, et al. Discovery of highly neutralizing human antibodies targeting Pseudomonas aeruginosa. Cell 2023.
6. Mochon AB, Jin Y, Kayala MA, et al. Serological profiling of a Candida albicans protein microarray reveals permanent host-pathogen interplay and stage-specific responses during candidemia. PLoS Pathog 2010.

Figure 1: Study work flow for the generation of human single B cell-derived monoclonal antibodies against *Candida* spp.

Fig. 1



P-1-57

NLRP3 inflammasomes as dual-hit host-directed targets in Tuberculosis – potent abrogation of necrosis and granuloma formation *in vitro* and *in vivo*

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Mycobacterium tuberculosis (Mtb) is the leading cause of morbidity and mortality worldwide among single infectious agents. The severity of an infection with Mtb depends on the nature of the host"s immune response. There is growing evidence that NLRP3 inflammasome associated cytokines and Type I Interferons (IFNs) are major drivers of TB pathogenesis. The expression of Type I IFNs depends largely on the activation of the cytosolic surveillance pathway cGAS-STING by dsDNA. However, there is little mechanistic insight in the exact signaling events leading to activation of these highly important innate immune pathways and their translational potential as host-directed therapies.

In our studies, we employed a combination of *in vitro*, *ex vivo* and *in vivo* methods to mechanistically investigate both inflammasome and type I IFN expression in several infection models including *ex vivo* granuloma-like structures (GLS) and the *Mtb*-infected C3HeB/FeJ mouse model which display human-like lung lesions. We also employed classical immunohistochemistry and CHIP-cytometry to show relevance of our findings in human lung and lymph node granulomas.

Using *Mtb*-infected THP-1 and primary macrophages, we were able to show that NLRP3 inflammasome inhibition leads to significantly improved cell survival and to abrogated secretion of pro-inflammatory cytokines, such as IL-1β. Surprisingly, we were able to link the expression of type I IFNs to NRLP3 mediated mitochondrial damage. Thus, for the first time, we provide evidence that inflammasome activation is linked to the expression of Type I IFNs and therefore represents a dual-hit target for host-directed therapies. Chemical inhibition of NLRP3 or cGAS-STING strongly impaired the formation of necrotic granuloma like structures (GLS) *ex vivo*. Most intriguingly, we found that treatment of *Mtb*-infected C3HeB/FeJ mice with a highly selective NLRP3 inhibitor strongly reduced the number and size of necrotic granuloma in the lungs. In-line, CHIP-cytometry revealed that both pathways are activated in human lung and lymph node granulomas of tuberculsis patients.

To conclude, we provide robust in *vitro*, as well as *in vivo* evidence that NLRP3 inflammasome inhibition represents a highly interesting dual hit target for host-directed therapies potently abrogating inflammation and tissue necrosis. Thus, treatment with these inhibitors may improve treatment outcomes and post-TB lung disease, a novel research area of the TTU-TB.

P-1-58

Small chemical compounds blocking herpes simplex virus assembly

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The WHO estimates that of the human population, two-thirds are infected with HSV-1 and 13% with HSV-2. Primary infections and reactivation from latency can lead to fatal encephalitis, blinding keratitis, disseminated disease, or eczema herpeticum, particularly in newborns or in immunocompromised patients. The nucleoside analog acyclovir and its derivatives inhibit the viral DNA polymerase, and a second drug candidate, pritelivir, inhibits the viral DNA helicase and is currently in phase III clinical trials. As both drugs target viral DNA replication, we designed phenotypic cell culture screens to identify novel antiviral compounds targeting other steps of the HSV-1 infection cycle.

Using HSV1(17+)Lox-GFP, we characterized 6 compounds of the DZIF PANH library that comply with the Lipinski rules, that inhibit productive infection of human epithelial and neuron-like cell lines as well as primary keratinocytes at high selectivity indices with IC_{50} ranging from 0.6 to 20 µM and CC_{50} higher than 150 µM. All inhibited the formation of HSV-1, HSV-2, and VZV plaques in Vero, HeLa, and HaCaT cells and of HSV-1 infection centers in murine skin explants. Like acyclovir and pritelivir, PANH_135, _173, and _174 inhibited

viral DNA replication and reduced nuclear C-capsid formation. In contrast, PANH_070, _128, and _184 did not impair DNA synthesis or capsid formation but perturbed later steps of the infection cycle. For example, PANH_070 led to higher amounts of nuclear and cytoplasmic capsids but reduced the number of enveloped cytoplasmic capsids.

We isolated several HSV1-GFP strains amplified at subinhibitory compound concentration and sequenced their genomes by NGS to identify potential resistance-conferring mutations. Candidate mutations were re-introduced into the parental HSV1-GFP by BAC mutagenesis, and their resistance to all PANH compounds was tested. Many of these BAC-derived HSV-1 strains were resistant to the given compound but remained sensitive to acyclovir and pritelivir, suggesting that each novel compound has a different mode of action. Furthermore, all compounds differ structurally from acyclovir and pritelivir. Also, resynthesized compounds and derivatives maintained their anti-HSV activities. Moreover, we have synthesized clickable and photoactivatable derivatives by adding alkyne and diazirine groups to identify their targets by cross-linking and protein mass spectrometry.

In summary, we have identified six novel small chemical compounds from the PANH library that inhibited HSV-1 and HSV-2 infection as potently as acyclovir in plaque assays, synchronous infection at high MOI, and in murine *ex vivo* skin infection. In ongoing work, we test potential resistance-conferring mutations by reintroducing them individually into the parental HSV1-GFP strain to characterize their molecular targets and mode of action.

P-1-59

Training versus tolerance – Previous adjuvant exposure impairs SARS-CoV-2 spike protein directed immune responses *in vitro* and *in vivo*

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In the past decade, training of innate immune cells for improved antigenic responses against pathogens and vaccines has become a highly important research focus. Both *in vitro* and *in vivo* experiments have shown that innate immune stimulation with adjuvants, pathogen-associated molecular patterns (PAMPs) or the BCG vaccine lead to altered immune responses towards non-related pathogens or vaccines (*Saeed et al., Zhang et al.*). However, translation of these findings into clinical studies failed for so far unknown reasons, possibly due to the induction of immune tolerance rather than immune training.

Using the SARS-CoV-2 spike protein and COVID-19 mRNA vaccine as models, we recently demonstrated that primary human macrophages from vaccinated individuals display a trained innate immune response towards a secondary stimulus *ex vivo* (*Theobald et al.*). In contrast, when transferring this experimental setup in an *in vitro* model, pre-stimulation of macrophages with the vaccine adjuvants (aluminum hydroxide, QS-21) or PAMPs (LPS, TDB, β-glucan) led to a significant decrease in secreted pro-inflammatory IL-1β, an NLRP3 inflammasome-dependent cytokine, when re-stimulated with the spike protein. Thus, sequential stimulation of innate immune cells with multiple adjuvants or ligands may either induce immune tolerance or immune training. To mechanistically address this phenomenon, we were able to rule out major alterations of immune signaling pathways in pre-stimulated macrophages, however, we were able to reveal that a first stimulatory hit induces significant alterations of metabolic activity which is known to regulate inflammasome activity. Thus, the immune cell metabolism of stimulated macrophages may result in dampening of the response following a second hit.

Inflammasome activation in innate immune cells is believed to be a key driver of potent adaptive immune responses. We used COVID-19 mRNA-vaccinated mice to assess whether our *in vitro* findings correlate with altered adaptive immune responses towards the SARS-CoV-2 spike protein. Interestingly, we were able to show that prior stimulation of mice with the adjuvant QS-21, a potent activator of the NLRP3 inflammasome, impaired the humoral immune response towards the spike protein. Most intriguingly, we found that T cells of mice that received QS-21 prior to two mRNA vaccinations secreted significantly less type II interferons upon peptide stimulation compared to mice that received mRNA vaccinations only.

Our data challenge the concept of trained innate immunity as a non-specific booster of both the innate and adaptive immune response towards non-related antigens. Our data also show that currently, our mechanistic understanding of training and induction of tolerance is insufficient to successfully translate these concepts into clinical application.

Saeed et al (2014) doi:10.1126/science.1251086
Zhang et al. (2022) doi:10.1172/JCI147719
Theobald et al. (2022) doi:10.15252/emmm.202215888

P-1-60

Comprehensive influenza a virus Hemagglutinin binding maps reveal antibody binding gaps and epitope dynamics

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Neutralizing antibodies are crucial for preventing and clearing Influenza A virus infections and are therefore being evaluated as novel therapeutics in the form of monoclonal antibodies (mAbs). However, influenza viruses rapidly evolve to escape the human immune response, which can quickly render mAbs ineffective. Assessing the limitations of mAb-mediated protection is critical for evaluating their therapeutic potential and understanding the epitope dynamics that significantly impact the success of antibody-based therapies. In this study, we developed a novel cell-based multiplex binding assay that rapidly determines antibody binding to hemagglutinin (HA), the primary target of influenza antibodies, using flow cytometry. By establishing a comprehensive influenza HA panel comprising historical and circulating H1N1, H2N2, and H3N2 strains, we generated a detailed binding map for 24 monoclonal antibodies, including several currently in clinical trials. Our data confirm that HA head-targeting antibodies are typically strain-specific, whereas HA stalk antibodies are more likely to cross-react with different HA subtypes. Additionally, we identified binding gaps in antibodies undergoing clinical trials, which could significantly affect their development and application. The presented assay is quickly adaptable to novel influenza strains, underscoring its value for continuously monitoring potential immune evasion by influenza viruses.

P-1-61

Helminth infection modulates the immune responses to SARS-CoV-2 vaccination

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Introduction: Helminth parasites infect over a quarter of the global population and can potentially influence host immune responses to various vaccines. The SARS-CoV-2 pandemic led to the development of various vaccine strategies, including the first licensed mRNA-based vaccines and classical protein-based vaccines. However, the efficacy of these vaccines among individuals with existing parasitic infections is uncertain.

Objectives: Here, we aimed to explore how the SARS-CoV-2 vaccination was influenced by an underlying helminth infection using an experimental *Schistosoma mansoni*-infection mouse model.

Materials & methods: Naïve mice and mice chronically infected with *S. mansoni* were immunized intramuscularly with mRNA-based Comirnaty vaccine or Aluminum adjuvanted spike protein, and boosted after four weeks. Vaccine-induced humoral and cellular immune responses were evaluated one week after boost immunization by quantifying and subtyping spike-specific IgG responses, assessing SARS-CoV-2 virus-neutralizing activity of sera, and performing spike-specific tetramer staining and intracellular cytokine staining on murine splenocytes.

Results: Immunization with the mRNA-based Comirnaty vaccine induced significantly higher titers of spike-specific IgG antibodies compared to spike protein immunization. Following both types of immunizations, however, the IgG antibody titers and SARS-CoV-2 infection-neutralization capacity were similar in naïve and helminth-infected mice. Nevertheless, despite the comparable magnitude of IgG responses, spike-specific IgG subclasses revealed distinct patterns in these two mouse models. Immunization of naïve mice primarily induced the IgG_{2c} subclass associated with a Th1 response, while in helminth-infected mice, a predominance of the IgG_1 subclass was observed, indicative of a Th2-prone response. Unlike the weak T-cell responses observed in the spike protein immunization group, Comirnaty immunization induced robust spike-specific CD4 and CD8 T-cell responses. In helminth-infected mice, Comirnaty immunization led to strong, dose-dependent spike-specific IFNγ+ CD4 T-cell responses, similar to those in naïve mice. However, spike-specific IFNγ+ CD8 T-cell responses were significantly lower in helminth-infected mice compared to their naïve counterparts. Furthermore, helminth infection notably altered the polyfunctionality of Comirnaty vaccine-induced CD8 T cells. In helminth-infected mice, there was a significant reduction in spike-specific IFNγ+ TNFα+ IL-2+ CD8 T cells, accompanied by a notable increase in spike-specific PD-1+ Lag3+ CD8 T cells, indicating functional suppression of CD8 T cells.

Conclusion: Chronic infection with a helminth that elicits mainly liver and intestinal inflammation surprisingly altered the characteristics of antibody responses and substantially decreased the magnitude and functionality of CD8 T-cell responses elicited by SARS-CoV-2 vaccination.

P-1-62

Targeting *Enterococcus faecalis* adhesin Ace with patient-derived monoclonal antibodies

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The Gram-positive bacterium *Enterococcus faecalis* (EF) is a significant nosocomial pathogen, especially affecting patients with disrupted intestinal microbiota due to previous antibiotic treatment. Moreover, EF causes 10–20% of all cases of infective endocarditis (IE). The pathogenesis of EF is driven by various virulence factors, including the adhesin Ace, which mediates adhesion to host tissues such as collagen.

In this study, we characterized the human antibody response to Ace across different patient cohorts, including individuals diagnosed with IE as well as those with cystic fibrosis (CF), a population frequently affected by polymicrobial infections and repetitive antibiotic treatment. Notably, we observed elevated titers against Ace in both IE and CF patients compared to healthy individuals as well as immunocompromised patients following EF infections. To further investigate the humoral immune response to Ace, we isolated Ace-specific B cells from individuals with elevated Ace titers and conducted single B cell analysis of the variable immunoglobulin regions. The resulting antibody repertoire analysis revealed a diverse B cell receptor repertoire, facilitating the generation of patient-derived monoclonal antibodies (mAbs) showing high affinity to Ace in an enzyme-linked immunosorbent assay.

By producing the first patient-derived mAbs against the enterococcal adhesin Ace, we aim to further characterize their functionality, such as their ability to inhibit the adhesion of EF to collagen. This will elucidate the potential of fully human anti-Ace mAbs as the basis for an anti-virulence therapy. Potential clinical applications might include treatment of acute EF infections and passive immunization strategies for individuals at high risk of invasive enterococcal infections.

P-1-63

Deciphering heterologous-induced immunity to tropical infectious diseases

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Question: Encountering several antigens, could modify subsequent immune response to unrelated antigen leading to non-specific adaptive immune response, so called heterologous immunity, involving cross reacting memory T cells, neighboring T cells activated by innate cytokines and memory B cells producing cross-reacting antibodies. Characterizing cellular and cytokines immune responses with reproducible *in vitro* stimulations of human peripheral blood mononuclear cells (PBMCs) and reduce volume of whole blood is crucial to select potential biomarkers of heterologous immunity which could be translated *ex-vivo*.

Methods: To establish a baseline of immune markers for heterologous immunity to tropical diseases, we first characterized T cells, B cells and monocytes response to positive controls, malaria antigen and tropical viral peptides in PBMCs and whole blood from non-exposed individuals to tropical infectious diseases. Frequencies of cells responses were compared to positive controls using Kruskal-Wallis test. Also, a comparison was done between frequencies of cells responses in PBMCs and whole blood using a chi-square test. A p value below, was considered statistically significant.

Results: Overall, positive controls showed good stimulation on cells and non-exposed individuals did not have strong reaction against malaria and tropical viral peptides. Also, there was no significant differences in frequencies of cells between PBMCs and whole blood stimulation.

Conclusion: Memory T cells, B cells and cytokines immune responses could be reliable immune biomarkers to translate *ex vivo* for the assessment of heterologous immunity status in individuals with whole blood. Further cytokines network and MCH tetramers assessments are needed to confirm bystander activation of T cells, T cells receptor and B cell receptor cross-reactivity respectively.

P-1-64
rVSVΔG-ZEBOV-GP vaccine bystander effect on EPI vaccines responses

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Background: The understanding of immune mechanisms underlying the Non-specific vaccine-related effects (NSEs), known as heterologous immunity, has strengthened its importance in vaccination and epidemiology. Bystander activation, a third mechanism underlying NSEs, implies the activation of neighboring non-specific T cells activated via cytokines, against vaccine components (antigen, adjuvant, or vector) and induces responses of unrelated plasma cells. We hypothesized that vaccination with the vector-based rVSVΔG-ZEBOV-GP Ebola vaccine contributes to the serological memory maintenance of other vaccines received by children through the expanded program on immunization (EPI).

Methods: Immunoglobulin G titers of Yellow Fever, Measles, *Haemophilus Influenzae* b, Tetanus Toxin/Toxoid, Diphtheria, Hepatitis B, *Bordetella pertussis* and Poliomyelitis Viruses vaccines were determined from serum samples of children randomly assigned to receive the rVSVΔG-ZEBOV-GP Ebola vaccine or the varicella-zoster virus vaccine in a phase 2 clinical trial (PACTR202005733552021). We defined serological maintenance as non-inferior titers of each EPI antibody by individual participant across timepoints and between vaccine groups.

Results: Immunoglobulin G titers against Yellow Fever, Measles, *Haemophilus Influenzae* b, Tetanus Toxin/Toxoid, Diphtheria, Hepatitis B, *Bordetella pertussis* and Poliomyelitis Viruses are being evaluated before vaccination and within a serological window of 28 after vaccination. Difference in seroprotection and seropositivity are being calculated for non-inferiority analysis between rVSVΔG-ZEBOV-GP and varicella-zoster virus vaccines groups.

Conclusion: rVSVΔG-ZEBOV-GP Ebola vaccine would not interfere with other vaccines received by children through EPI and could help in their serological immune response maintenance.

P-1-65
Deciphering the human B cell response to exotoxin A of Pseudomonas aeruginosa

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Pseudomonas aeruginosa (PA) presents a significant therapeutic challenge as a nosocomial pathogen, causing severe infections, particularly in immunocompromised patients. A major virulence factor of PA is exotoxin A (ETA), a highly potent toxin that disrupts cellular protein synthesis and induces cell death. The internalization of ETA is mediated by the interaction of ETA domain-1A with the alpha-2-macroglobulin receptor on eukaryotic cells followed by clathrin-dependent endocytosis. Here, we investigate the human B cell response to ETA and the impact of human antibodies on ETA function and uptake as potential antivirulence therapy.

By screening a study cohort of 102 individuals with cystic fibrosis (CF) mainly with chronic or intermittent PA colonization status, we detected elevated aniti-ETA IgG titers within the CF group. These antibodies demonstrated protective effects in *in vitro* cytotoxicity assays, indicating a protective immune response upon exposure to ETA. Further single B cell analyses of individuals with increased titers revealed a diverse B cell receptor repertoire directed against ETA and enabled the production of ETA-specific monoclonal antibodies (mAbs).

Our study provides the first in-depth analysis of the human B cell response to ETA of PA. ETA-specific mAbs will be further evaluated for their mode of action in ETA neutralization and their potential as an antivirulence therapy for severe PA infections. Therefore, we will assess the virulence-inhibiting activity of the ETA-specific mAbs *in vitro* and *in vivo*.

P-1-66

Differential gene expression in patients with pyrazinamide-associated hepatoxicity before the start of drug-susceptible tuberculosis treatment

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Background: Pyrazinamide (PZA) is an important component of the treatment of drug-susceptible tuberculosis (DS-TB). However, PZA has the potential to cause hepatotoxicity. The underlying mechanisms of PZA-associated hepatotoxicity are not yet fully understood. We aimed to identify genes that are differentially expressed in patients with PZA-associated hepatotoxicity before the start of DS-TB treatment.

Methods: Data from prospectively enrolled adult patients initiating PZA-containing DS-TB treatment from three independent cohorts in Germany were used. Whole blood RNA before treatment initiation was used for the transcriptomic analysis. Clinical data were collected throughout treatment. The outcome was defined as clinically relevant increased liver enzymes leading to discontinuation of PZA. The severity of the increased liver enzymes was retrospectively quantified using the Common Terminology Criteria for Adverse Events (CTCAE) grading scale. Genes were classified as significant differentially expressed when Benjamini-Hocherg adjusted p-value was below 0.05.

Results: Out of the three cohorts, 59 patients on PZA-containing DS-TB treatment were included in the analysis. Six patients (10.2%) had to discontinue PZA due to hepatotoxicity. According to the CTCAE grading scale, the hepatopathy was classified as grade 2 in two cases (33.3%), grade 3 in three cases (50.0%) and grade 4 in one case (16.7%). In total, 926 genes were statistically significantly differentially expressed before the start of treatment when comparing patients with PZA-associated hepatotoxicity to patients without PZA-associated hepatotoxicity. Using hierarchical clustering on the *z* score normalized 926 genes, two clusters were identified (Figure 1).

Conclusion: We identified genes and a cluster that were associated with developing PZA-associated hepatotoxicity in DS-TB treatment prior to treatment initiation.

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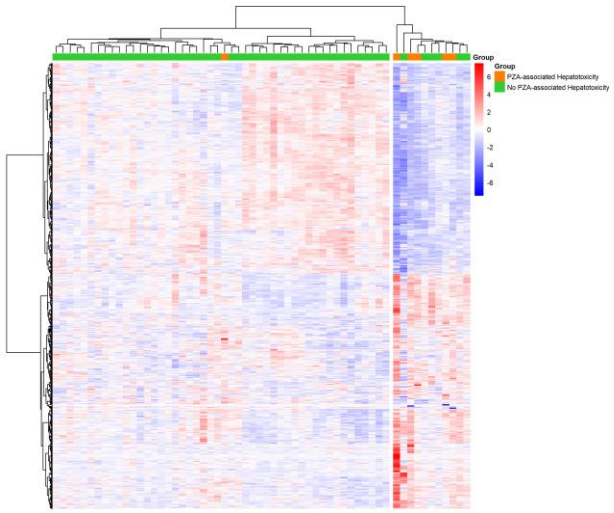
Fig. 1



P-1-67

Optimal pneumococcal vaccination strategy in older adults: a randomized trial comparing sequential, simultaneous, and single vaccination with (13-valent) conjugate vaccine and (23-valent) polysaccharide vaccine

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Background: Concerns about vaccine-driven depletion of antigen-specific memory B cells after vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPSV23) have influenced vaccination strategies. We hypothesized that sequential vaccination (with 13-valent conjugate vaccine (PCV13) followed by PPSV23 after six months) might reduce memory B cells, while simultaneous vaccination could preserve them.

Methods: In this randomized controlled trial, 123 vaccine-naïve adults aged 60 to 78 years were assigned to three groups: (1) simultaneous PCV13 and PPSV23, (2) sequential vaccination or (3) single PPSV23 vaccination. The primary endpoint was the change in circulating memory B cells to four vaccine-serotypes (STs; ST3, ST14, ST19A and ST23F) at 27-28 weeks. Secondary outcomes included antibody levels, memory B cell responses within 24 months, and safety.

Results: 118 persons (74 females and 44 males) reached the primary endpoint. At 27-28 weeks, there were no relevant differences in memory B cells response between the simultaneous and sequential groups. Sequential vaccination showed a significant increase in memory B cells for ST3 compared to single vaccination (median change +0.005% versus 0% of B cells, p=0.009). Simultaneous vaccination induced the largest early rise in plasma cells (+14.5%, p=0.004 versus sequential), but this did not translate into long-term benefits. Sequential vaccination demonstrated higher antibody levels at 24 months against ST3 than simultaneous vaccination and against ST3, ST4, and ST9V than single vaccination (p<0.05 for all). No serious adverse events were recorded.

Conclusion: Sequential vaccination provides the most durable immune response for ST3. While safe, simultaneous vaccination does not offer superior long-term benefits.

Figure 1: Study design and interventions with schedule of vaccinations and blood sampling.

Figure 2: Kinetics of GMFR of IgG against pneumococcal vaccine serotypes with at least one significant difference between study groups. The biggest differences were detected for serotype 3 and serotype 4. Arrows indicate the timing of vaccination (vaccinations at baseline and 2nd sequential vaccination after 6 months), and the asterisks denote statistically significant differences (p-value <0.05) among the three study groups (simultaneous versus

sequential vaccination, simultaneous versus single vaccination, and sequential versus single vaccination) as determined by the two-sided Wilcoxon-Mann-Whitney test.

Fig. 1

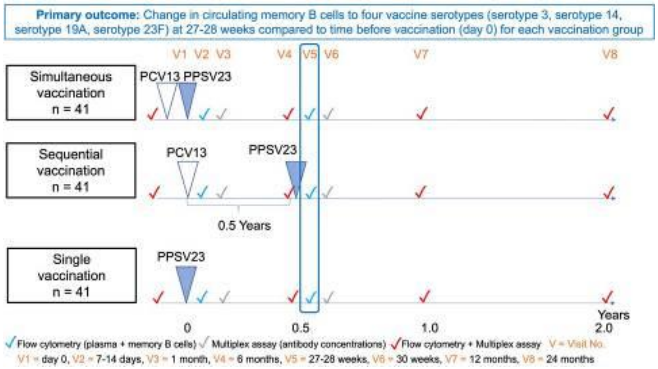
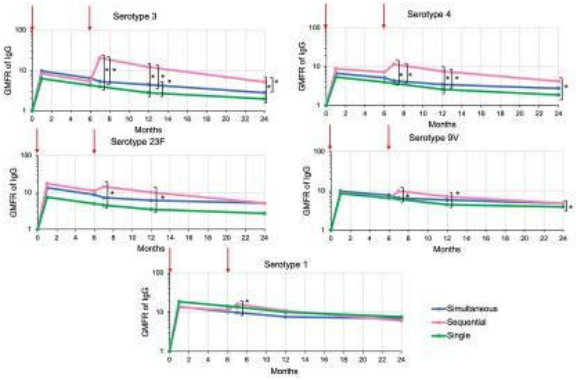


Fig. 2



P-1-68

Age- and gender-specific changes in immune cell populations

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It is already known that the function of our immune system becames impaired during aging process. Besides the age, also sex plays a significant role on the incidence and severity of infectious diseases, but barely is known about the extent of age-associated changes between men and women.

In this study, we investigated the age and sex interaction changes in the immune cells composition of 243 (120 females, 123 males) inhabitants between the age of 19 and 93 from Neustadt am Rennsteig, Thuringia as part of the CoNAN-Study. Peripheral blood was processed using the MAXPAR Deep Immune Profiling Assay, measured on a Helios instrument, and analyzed with Pathsetter software v3 (Standard BioTools, South San Francisco, CA, USA). Unlike the other published studies, we further determined changes of either frequency or absolute counts of major leukocyte subsets by using smoothing spline regression and therefore have circumvented to group the cohort into age ranges.

Our data confirm previous findings such as the decrease in the number of naïve lymphocytes, including naïve T cells and naïve B cells during aging. However, this decline was particularly observed in men, while women retain their naïve B cells one decade longer than men. Since naïve lymphocytes are important for mounting an immune response to new antigens, an age-associated decline might significantly impact on the cellular and humoral immunity in context of new infections. Additionally, an age-associated increase in effector memory T cells (particularly central memory CD8+ T cells) was observed in men, which was aligned with a decreasing trend in dendritic cell counts in both men and women. In the context of innate immune cells, an increase of transitional monocytes occurred specific to men, while other innate immune cells such as granulocytes (Neus, Baso, Eos) did not show any compositional changes over the lifespan.

Using multi-dimensional mass spectrometry combined with the spline regression approach uncovers new insights into age-sex-interaction-specific changes in immune cell composition that occur with aging. These findings may help to understand the immune status prevalent among various age cohorts. Nevertheless, it is necessary to extend the analysis of other cohorts for validation of our findings since our study was restricted to one village in rural Thuringia only.

P-1-69

When biofilms signal danger: PNAG-induced Dectin-1 pathway modulates immunity through CXCL10

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Introduction: *Staphylococcus aureus (S. aureus),* a human pathogen, is notably virulent due to its capacity to form recalcitrant biofilms (1). A major component of the staphylococcal biofilm matrix is poly-*N*-acetylglucosamine (PNAG) (2). This polysaccharide plays a vital role in conferring adhesion. Thus, we aimed to analyze the effect of *S. aureus* biofilm-associated carbohydrate, PNAG, on the immune chemotaxis process of peripheral blood mononuclear cells (PBMC).

Methods: For all experiments, the laboratory standard strain *S. aureus ATCC 43300* was used. Isolated PBMC or monocytes from four healthy volunteers with or without antibody blockade of Dectin-1 were exposed to planktonic bacteria, biofilms or to their respective products i.e. *S. aureus* protein A (SpA), or PNAG. After 24 h incubation, immune cells were analyzed using FACS and chemotaxis assay, and bacteria were assessed by viable cell counting using confocal microscopy. The supernatant of PBMC or monocytes stimulated by biofilm, planktonic bacteria, SpA or PNAG was analyzed using LC-MS/MS and cytometric bead array.

Results: Proteomics identified 143 differentially expressed proteins (DEPs) in PBMC when exposed to biofilm (Fig 1). We could show that PNAG, in contrast to SpA, significantly elevates the secretion of CXCL10 in PBMC (Fig 2), which is known to stimulate the motility of other immune cells via the CXCL10/CXCR3 pathway (3). We show that PNAG treatment increases Dectin-1 and CARD9 as well as CXCL10 and NF-κB transcription (Fig 3). Further, blocking Dectin-1 in PBMC decreases CXCL10 secretion upon exposure to PNAG (Fig 4). In our observations on monocytes, we identified 40 expressed targets upon treatment with PNAG. Further, we noted absolute count of CD14+ monocytes expressing CXCL10+ did not change after stimulation by SpA, while CXCL10+ monocytes increased upon stimulation by PNAG (Fig 5 A-C).

Conclusion: Our findings show that PNAG, as a component of S. aureus biofilms, possesses immune chemotactic properties, e.g., CXCL10 secretion by triggering Dectin-1-Syk-CARD9 signaling in PBMC. This process might be linked to the recruitment of immune cells to the site of infection,

influencing the eventual clearance or persistence of the pathogen.

Acknowledgment: This project has received funding from the European Union"s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 861323.

Fig. 1

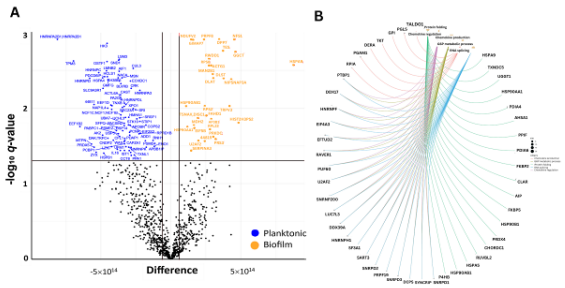
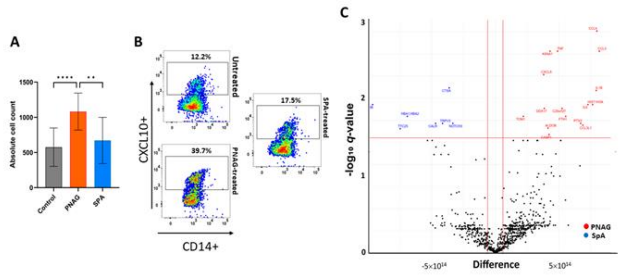


Fig. 2



P-1-70

Generating off-the-shelf TCR libraries for treatment of viral infections by CRISPR/Cas9-mediated TCR engineering

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Reactivation of latent viral infections poses a serious threat to immunocompromised individuals, such as transplant recipients. Traditional antiviral treatments are often inadequate due to limited efficacy and drug resistance. Adoptive T-cell transfer, addressing viral reactivation while promising, faces logistical challenges in isolating virus-specific T cells for widespread use.

To address this, we are developing "off-the-shelf" libraries of virus-specific TCRs that serve virtually any patient. We developed a pipeline enabling identification and selection of candidate TCRs for therapy. We identified the minimum number (11) of Human Leukocyte Antigens (HLAs) covering 95% of the European population, followed by searching for immunodominant viral epitopes, starting with CMV. We screened over 180 healthy donors by stimulating PBMCs and assessing the activation markers IFNγ and CD107a. Using single-cell sequencing (scRNAseq) on activated cells, we uncovered donors with varying diversity of TCR repertoires and levels of cytokine expression, independent of clonal expansion. By re-expressing selected TCRs and performing retrospective transcriptomic analysis, we linked their reactivity to defined gene signatures, with which we can now predict the reactivity of TCRs in new scRNAseq data sets reliably.

Using this approach we re-expressed over 30 pre-selected CMV-specific TCRs in Jurkat reporter cell lines assessing peptide sensitivity, adding to our diverse array of TCRs spanning 8/11 HLAs of interest, with further candidates in the pipeline.

Furthermore, we are preparing a phase I clinical trial using CMV-specific TCR-engineered T cells using orthotopic TCR replacement (OTR) to generate physiological T cells for stem cell transplant recipients at high risk for CMV infection.

P-1-71

Fluoxetine and sertraline potently neutralize the replication of distinct SARS-CoV-2 variants

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The pandemic caused by SARS-CoV-2 is still a major health problem. Newly emerging variants and long-COVID-19 represent a challenge for the global health system. In particular, individuals in developing countries with insufficient health care need easily accessible, affordable and effective treatments of COVID-19. Previous studies have demonstrated the efficacy of functional inhibitors of acid sphingomyelinase against infections with various viruses, including early variants of SARS-CoV-2. This work investigated whether the acid sphingomyelinase inhibitors fluoxetine and sertraline, usually used as antidepressant molecules in clinical practice, can inhibit the replication of the former and recently emerged SARS-CoV-2 variants in vitro. Fluoxetine and sertraline potently inhibited the infection with pseudotyped virus-like particles and SARS-CoV-2 variants D614G, alpha, delta, omicron BA.1 and omicron BA.5. These results highlight fluoxetine and sertraline as priority candidates for large-scale phase 3 clinical trials at different stages of SARS-CoV-2 infections, either alone or in combination with other medications.

P-1-72

Amitripytiline potently neutralizes distinct SARS-CoV-2 variants

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COVID-19, driven by the SARS-CoV-2 virus, remains a significant global health challenge, particularly with the emergence of new variants and the persistence of long COVID symptoms. This situation is exacerbated in developing countries, where access to affordable and effective treatments is severely limited. Functional inhibitors of acid sphingomyelinase have been identified as potential therapeutic agents against various viral infections, including early SARS-CoV-2 variants. In this study, we evaluated the antiviral potential of amitriptyline, a commonly used antidepressant and known functional acid sphingomyelinase inhibitor, against both early and newly emerged SARS-CoV-2 variants in vitro. Our findings demonstrate that amitriptyline effectively inhibits cell entry by pseudotyped virus-like particles with various mutations in the receptor binding domain of the SARS-CoV-2 spike protein. Furthermore, we examined the antiviral effect of amitriptyline against clinical SARS-CoV-2 isolates D614G, Omicron BA.5, and Omicron XBB.1. Amitriptyline significantly reduced the viral load of all three tested variants in a dose-dependent manner at subtoxic concentrations. The results are promising and provide a basis for the further development of amitriptyline for clinical use in the treatment of SARS-CoV-2 infections. These results suggest that amitriptyline holds promise as a candidate for further investigation in large-scale phase 3 clinical trials, either as a monotherapy or in combination with other therapeutic agents, across various stages of SARS-CoV-2 infection.

P-1-73

***In vivo* effects of the 3rd generation peptidic LasB inhibitor (R)-30 on disease progression in an experimental mouse model of Pseudomonas keratitis**

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Bacterial keratitis is a severe infection of the cornea that may lead to loss of vision. The Gram-negative bacterium *Pseudomonas aeruginosa* is an important cause of this disease, especially in contact lens wearers. The bacterium secretes a number of virulence factors into the host tissue to support its spread and propagation, including the zinc-dependent metalloprotease elastase B (LasB), which degrades host extracellular matrix components such as collagen and mucins, and interferes with various components of the host immune system. Immunization with elastase was shown to confer protection against *Pseudomonas* keratitis in both rabbits and mice, and recent studies demonstrated that metal-chelating elastase inhibitors reduce the risk of corneal melting in an experimental rabbit model of *Pseudomonas* keratitis.

In this study, we tested the capability of the 3rd generation peptidic LasB inhibitor (*R*)-30 to affect disease progression in an experimental mouse model of *Pseudomonas* keratitis, either as stand-alone treatment or in combination with the carbapenem-antibiotic meropenem.

While treatment of mechanically harmed and *P. aeruginosa* PA54-infected corneas with 5 µl doses of (*R*)-30 (1 mg/ml) at 8-hour intervals over a period of 72 hours clearly impacted the immune response in the infected eye tissue, it neither reduced the symptoms of the disease nor decreased the bacterial load in the eye at 3 days post infection when compared to sham treatment. However, when used in combination with meropenem at a dose of 0.5 mg/ml, a different picture emerged. Treatment of the infected eyes at 8-hour intervals with (*R*)-30 and meropenem yielded in a 4-log_{10} reduction of the bacterial load at the infection site and significantly reduced the opacity of the cornea and the infiltration of neutrophils into the eye tissue at 3 days post infection when compared to sham-treatment. Importantly, treatment with the combination was also more effective than treatment with the antibiotic alone, which only allowed for a 2-log_{10} reduction with the treatment scheme used and was less effective in reducing the opacity of the cornea and neutrophils infiltration than the combination therapy.

Our findings strongly suggest that a combination therapy of (*R*)-30 with an antibiotic that is active against the *P. aeruginosa* isolate to be treated is likely to reduce the infection symptoms and to clear a *Pseudomonas* keratitis faster than the standard therapy with the antibiotic alone.

P-1-74

Generation of aspergillus-specific CAR-NK Cells for treatment of invasive fungal infections

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Introduction: Invasive aspergillosis is a significant cause of morbidity and mortality in immunosuppressed patients, particularly in hematopoietic stem cell transplant recipients [1]. High levels of immunosuppresion in these patients and antifungal resistance can lead to the failure of conventional antifungal therapy [2]. We aim to develop a novel, off-the-shelf therapeutic approach using Aspergillus-specific chimeric antigen receptor (CAR) natural killer (NK) cell therapy. CAR-NK cell therapy is an innovative and promising immunotherapeutic strategy for treating various malignancies as well as non-malignant diseases. By generating Aspergillus-specific CARs, we aim to enhance NK cell-mediated immune defense and thus develop an effective immunotherapy.

Methods: Through an antibody discovery campaign, Aspergillus fumigatus-specific single-chain variable fragments (scFvs) were identified from a proprietary naïve phage display library with an estimated diversity of 1.07 x 10^6 unique scFv sequences. Selected scFvs were analyzed by high-throughput sequencing, with immunoglobulin domains

annotated and compared to the IMGT database. Using In-fusion cloning, scFvs of interest were then inserted into the third-generation CAR backbone. Subsequently, Aspergillus-specific CAR-NK cells were generated via retroviral transduction. Functional assays will evaluate CAR-NK cell activity in vitro, with high-potential candidates identified for further in vivo testing.

Results: Our library was previously screened for cancer antigens, leading to the discovery of 30 unique scFvs targeting 14 different antigens. For this project, we identified 10 unique scFvs directed against three Aspergillus fumigatus antigens. These scFvs were successfully cloned into a third-generation CAR backbone, with accuracy confirmed by Sanger sequencing, and demonstrated expression levels of 20-25% in a model cell line. These CAR constructs are now ready for transduction into NK cells for subsequent functional testing.

Conclusion: Phage display libraries are effective tools for antibody discovery, enabling the development of target-specific cell therapies. The incorporation of Aspergillus-specific CAR-NK cells alongside conventional antifungal therapy has the potential of improving clinical outcomes of patients with invasive aspergillosis and can serve as a model for developing further cell-based anti-infective therapeutic strategies.

Refrences:

1) Puerta-Alcalde, P., & Garcia-Vidal, C. (2021). Changing epidemiology of invasive fungal disease in allogeneic hematopoietic stem cell transplantation. *Journal of Fungi*, *7*(10), Article 848. https://doi.org/10.3390/jof7100848

2) Verweij, P. E., Chowdhary, A., Melchers, W. J. G., & Meis, J. F. (2016). Azole resistance in *Aspergillus fumigatus*: Can we retain the clinical use of mold-active antifungal azoles? *Clinical Infectious Diseases, 62*(3), 362–368. https://doi.org/10.1093/cid/civ885

P-1-77

Induction of mucosal anti-HPV16 T cell responses in an HPV16 E6/E7-dependent orthotopic tumor model in MHC-humanized mice

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Persistent infection with high-risk types of human papillomavirus (HPV), such as HPV16, can cause cancer in both, women and men, and accounts for approximately 5% of cancer cases worldwide. Many therapeutic vaccines targeting HPV16-associated malignancies have shown to be highly effective in preclinical studies but lacked effectiveness when tested in human patients. To overcome this discrepancy, our group developed model systems that resemble the human situation more closely. We established two orthotopic HPV16-dependent tumor models in MHC-humanized mice, which are located in the mucosa of the female genital tract and the base of the tongue, respectively. They allow testing of vaccine platforms containing clinically relevant HLA-A2-restricted epitopes against tumors at sites of natural infection. To evaluate different vaccine platforms, mice were vaccinated, and splenocytes and lymphocytes of the female genital tract were analyzed via flow cytometry. In first vaccination studies, we identified two platforms either consisting of amphiphilic constructs or silica nanoparticles (SiNP), which, when combined with the CD8+ T cell epitope HPV16 E7/11-19, both successfully induced systemic CD8+ T cell responses. SiNP were found to produce more reproducible results due to their robust synthesis procedure, which is why we focused on those in subsequent experiments. Addition of the CD4 T cell epitope PADRE (pan-DR epitope) in the SiNP vaccine formulation significantly enhanced the immune response. By comparing different administration routes and prime-pull approaches, we determined the vaccination strategy that not only resulted in a strong systemic immune response but also induced a local immune response in the mucosa of the female genital tract. The most promising vaccination strategies are currently being evaluated in terms of their effects on orthotopically located HPV16 E6/E7-positive tumors. Taken together, these experiments will be a crucial step in the preclinical assessment of new therapeutic HPV vaccine formulations. The obtained results will provide important insights for the design of clinical trials for therapeutic HPV vaccination.

P-1-78

Development and characterization of novel hepatitis C virus vaccine candidates

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Spontaneous clearance of hepatitis C (HCV) infection is linked to a rapid and broad HCV-specific adaptive immune response. However, immunity gained either by spontaneous resolution or after direct acting antiviral (DAA) therapy of chronic infections, is not sufficient to prevent HCV reinfection. Thus, the need for a prophylactic HCV vaccine is evident. We propose a novel vaccine candidate - the subgenomic replicon (SGR) of HCV. The SGR is non-infectious, but self-sufficient for replication without introducing viral vectors. Furthermore, it is self-limiting and all cells harboring the replicon will express high antigen levels until they are cleared by the induced immune response. We aim to use the SGR as a booster vaccine in a heterologous vaccination system that targets T-cells activation, combined with a priming step utilizing standard mRNA constructs encoding consensus sequences of non-structural proteins that entail major T-cell target epitopes (NS3-4A and NS5B).

To evaluate the efficacy of our vaccine candidates, we established a simplified coculture system of HCV-specific CD8+ T-cells and Huh7-Lunet cells with ectopic HLA-A2 expression. Huh7-Lunet cells are transfected with *in vitro* transcribed RNA of our vaccine candidates. This system enables the measurement of T-cell activation directly via activation marker staining and for SGRs it offers an indirect measurement of T-cell activation as well, by quantifying SGR replication levels through expression of luciferase, a reporter protein in the SGR. For the priming step, we set out to compare two different mRNA configurations – one encoding NS3-4A and NS5B resulting in membrane-bound proteins and one encoding NS3 and NS5B lacking its anchoring domain and resulting in a soluble polyprotein.

We have demonstrated that the HCV SGR induces CD8+ T-cell activation *in vitro* and that activated T-cells in turn reduce its replication levels. Moreover, we have examined the mRNA vaccine candidates" capacity to induce CD8+ T-cell

activation in a coculture system where we achieved stronger induction levels with the membrane-bound variant. In the next steps, we want to showcase the ability of our mRNA vaccine constructs in activating CD8+ T-cells in an *in vivo* setting, by injecting C57BL/6 mice with LNP-mRNA particles. Additionally, we intend to further explore the replication competence of our SGR vaccine candidate *in vivo* in Alb-uPA mice.

This project explores the possibility of a novel HCV vaccination approach that has the potential of eliciting a robust CD8+ T-cell response.

P-1-79

Broad and potent NTD and RBD antibodies for SARS-CoV-2 combination therapy

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Despite the availability of vaccines and antiviral drugs, SARS-CoV-2 infection remains a substantial risk for immunocompromised and elderly individuals. Viral evolution challenges immunity to SARS-CoV-2 and has rendered most clinically used monoclonal antibodies ineffective. Antibody-based strategies against COVID-19 therefore require new candidates with broad and potent activity as well as a high barrier for viral escape.

Using an extended panel of 83 pseudoviruses comprised of 59 B.1 mutants with single NTD, RBD, or S2 amino acid substitutions and 24 SARS-CoV-2 variants, we reveal high neutralizing activity of antibodies isolated from convalescent individuals. In addition to RBD-targeting antibodies (TV1t4p2_A5 and TV1t4p3_E8), these included the NTD-reactive antibody R568-2E7. Compared with the RBD against which all clinically used antibodies are directed, phylogenetic SARS-CoV-2 sequence analysis demonstrated consistently lower rates of mutation in the NTD, making it an attractive target for antibody-based strategies. Differing from the typical pattern of SARS-CoV-2 antibodies, the NTD antibody R568-2E7 showed limited activity against pre-Omicron viruses but had high potency against recently emerged Omicron variants including XBB.1.5, JN.1, and KP.3 (IC_{50}s of 0.007, 0.019, and 0.26 µg/ml, respectively). Cryo-EM structural analysis using the Wu01 Hexapro spike protein revealed that R568-2E7 targets the membrane proximal side of the NTD, contacts NTD and SD2 domains, and binds the spike trimer in the RBD down configuration. Deep mutational scanning (DMS) using XBB.1.5-based pseudovirus libraries indicated limited pathways of potential escape from the NTD-targeting antibody R568-2E7 and residues identified by DMS were highly conserved amongst circulating viruses. For example, <0.1% of GISAID-deposited viral sequences between April and October 2024 showed amino acid substitutions at the top 10 residues of potential R568-2E7 escape. Escape and neutralization profiles of R568-2E7 and the RBD-targeting antibodies TV1t4p2_A5 and TV1t4p3_E8 were highly complementary, suggesting a benefit of combination immunotherapy. Indicating their potential for clinical application, all three antibodies demonstrated favorable *in vivo* pharmacokinetic characteristics in mice.

Our analyses reveal the potent activity of monoclonal antibodies against the most recently emerged SARS-CoV-2 Omicron variants with limited options for viral escape. Notably, in contrast to antibodies previously developed for clinical use, R568-2E7 targets the NTD domain of the SARS-CoV-2 spike protein and ranks amongst the most potent NTD antibodies identified to-date. Thus, R568-2E7 and antibodies against the non-overlapping RBD epitope (e.g., TV1t4p2_A5 or TV1t4p3_E8) are promising options for the development of novel SARS-CoV-2 immunotherapies.

P-1-80

Identifying AML-cell specific AAV variants through parallel library screenings

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Introduction: Due to its nonpathogenic nature, adeno-associated viruses (AAV) are a promising tool for gene therapy delivery in humans. Advancements in this field such as display libraries and directed evolution make it possible to now design variants with a higher specificity and efficiency for desired cell types, as has been shown in several studies [1, 2].

Acute myeloid leukemia (AML) is an aggressive disease that is characterized by high heterology and high mortality rates. Given the growing variety of therapeutical targets in AML, it is important to develop an accordingly wide array of therapy delivery tools.

In this study we aim for the development and selection of AAV variants with enhanced efficiency and specificity in human AML cells.

Methods: To this end, we have designed a barcoded natural benchmark AAV library as well as seven different engineered peptide and nanobody display AAV libraries from which the most specific and efficient variants will be selected.

The engineered libraries use the AAV6 and AAV9 capsids as scaffolds. This decision is based on the expectation that AAV6 will be the best variant out of the natural benchmark AAVs and that AAV9 is a naturally good transducer with great potential when enhanced by peptide or nanobody display. The experiments will be carried out in AML cell lines, AML primary cells, or both, according to results of preliminary screenings.

For the specificity testing, we are planning to use cell lines derived from different blood cell types for negative selection of the efficient candidates.

Results: While the peptide and nanobody display libraries are still undergoing the last stages of development, the barcoded library has shown a good variant distribution in NGS and is ready to be used for screening in the target cells. DNA and RNA isolation has already been successfully performed in three different AML cell lines, which provides future reference for expectations regarding transcription rates and DNA/RNA amounts.

Conclusion: The multitude of clinical trials in the field of AAV have shown its potential as a gene delivery tool as well as its disadvantages, consisting mainly of its wide tissue tropism. Through this approach we aim to identify suitable AAV variants with an enhanced specificity for future gene therapies targeting AML.

References:

1: https://doi.org/10.1038/s41467-020-19230-w

2: https://doi.org/10.1016/j.omtm.2019.09.003

P-1-81

Engineering multi-specific HIV antibodies to eliminate the HIV reservoir and antibody resistance

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Background: HIV/AIDS remains a major global health challenge despite remarkable advances in antiretroviral therapy. The persistence of latent HIV reservoirs and the emergence of antibody resistance pose significant challenges to the development of effective future HIV treatments. Multi-specific antibodies are promising new options to prevent HIV antibody resistance and eradicate the latent HIV-1 reservoir.

Methods: We have established a high-throughput cassette cloning platform that allows us to rapidly design, clone, produce, and test novel multi-specific antibodies in 2-3 weeks. The different binding antibody fragment antigen-binding region (Fabs) are expressed on one antibody with up to four different specificities. To prevent production of single chains or mispairing we included a knob-hole system into the Fc-part of the construct. Using this platform, we have already tested over 50 different constructs, the vast majority of which retained their intended binding and neutralizing specificity. Moreover, we have cloned antibodies that bind to the HIV-1 envelope and T-cell activation markers such as CD28 and CD3 in order to activate and clear latently infected CD4 T cells.

Results: SDS-page gels of our multispecific neutralizing antibodies showed single proteins of about 175 or 200 kDa of our tri- or quattro-specific antibodies proofing their correct production. Correct binding of each specific Fab specificity was validated via ELISA. We further constructed a set of multispecific antibodies to test whether each Fab arm retained its neutralizing activity. By selecting viruses resistant to all but one Fab in the combination, we confirmed that each Fab in a tri-specific antibody maintained its activity. Via including several highly potent anti-HIV-1 neutralizing Fabs that target different epitopes (CD4 binding site: 1-18 and 3BNC117, V3 loop: PGT128) on one antibody we were able to develop a highly neutralizing antibody that was able to neutralize 12/12 viruses of the global panel with a mean neutralizing activity of 0,022 µg/ml. Regarding the design of our constructs, including neutralizing antibodies and T-cell engagers (TCE), we showed potent CD8 T cell activation via FACS. The TCE comprising of CD3 and co-stimulatory CD28 showed superior induction of CD25, CD69, and Cytokines such as IFN-γ and TNF-α compared to CD3-only TCE and controls. We further performed a bridging assay via FACS and our results show that our multi-specific antibodies mediate T cell redirection towards HIV env trimers.

Conclusion: We have successfully established a design, production, and validation pipeline for multispecific antibodies targeting HIV-1. Next, we aim to further characterize these antibodies *in vitro* and *in vivo* and employ this pipeline to develop multispecific antibodies that not only show a combined effect of the added Fab arms but show synergistic effects, leading to a significantly higher breath and potency compared to their parental monovalent binding bNAbs.

P-1-82

Determining the interplay between HIV-1 antigen exposure and serum neutralization

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Background: Understanding the factors that lead to potent HIV neutralization is critical to developing new future options for the prevention and treatment of HIV-1 infection. In addition to the possibility of identifying novel highly potent broadly neutralizing antibodies (bNAbs), the study of large cohorts of people living with HIV (PLWH) is critical to identifying factors that lead to potent serum HIV neutralization. In addition, understanding the impact of potent HIV-1 neutralization on the "viral reservoir" is important for future HIV-1 cure approaches.

Methods: We performed blood draws and collected clinical data from PLWH at the University Hospitals of Cologne and Bonn, including sex, age, origin, ethnicity, mode of transmission, antiretroviral therapy (ART) regimen, viral load, and CD4 T-cell count. To investigate the viral reservoir in the next step of the project, we collected 100 ml of whole blood from all participants. We also isolated immunoglobulin G (IgG) from all patients and tested its HIV-1 binding and neutralizing activity against the well-established 12-strain global panel, which is representative of the strains circulating worldwide.

Results: 294 patients were enrolled. The median age was 53 years (range 24-80 years) and 16% were female. 288 (99%) of the participants were receiving ART, with 22% of the patients having a viral blip in the previous 12 months. The median CD4 count was 630/µl (range 67 - 1890/µl). After PBMC isolation, we were able to isolate a median of 99 million PBMCs per patient.

All patients showed binding to the B BG505SOSIP.664 protein. Neutralization of the cohort showed an overall lower neutralization compared to previous neutralization screenings. Of all patients tested (n=288), we found the vast majority (92%) without or with weak IgG neutralization and only a small subset of patients with cross (7.6%) or broad

(0.3%) neutralization. None of the patients tested showed elite neutralizing activity. The neutralization profile against the 12-strain global panel was comparable to previous screenings such as Schommers et al, Nat Med 2023, with the most strongly neutralized strains being 398F1 (clade A), 246F3 (clade AC) and CNE55 (clade AE).

Conclusion: The results suggest that effective antiretroviral therapy (ART) influences neutralizing antibody responses. The weaker neutralization in our cohort could be partly explained by the high rates of effectively ART-treated patients in our cohort. Thus, our data are consistent with previous reports showing that neutralizing activity of patients declines over time with lower antigen exposure. Future experiments will show if and how reservoir size correlates with neutralizing activity in this cohort.

P-1-83

IGHV1-69 dominated memory B cell response upon MVA-MERS-S vaccination targets multiple neutralizing and non-neutralizing epitopes

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Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a WHO-listed priority pathogen with pandemic potential and a high case fatality rate. As a result, each MERS-CoV outbreak or related emerging Merbecovirus variant poses a significant risk to public health, underscoring the urgent need for a protective vaccine. Previously, a Modified Vaccinia Ankara (MVA) vector vaccine candidate showed promising results in an open-label phase I trial with the generation of virus-neutralizing antibodies and MERS-CoV spike-specific T cells.

To investigate the long-term memory B cell response to this vaccine candidate, we isolated and sequenced 1.195 MERS-S protein-reactive single memory B cells from five vaccinated study participants at two time points, 21–24 and 29–32 months after immunization, and produced 186 monoclonal antibodies for in vitro characterization. Our results show a strong VH1-69-biased antibody response targeting the S1 subunit of the MERS-CoV spike protein, with highly potent neutralizing antibodies identified in each individual at both time points. Detailed functional epitope mapping revealed multiple target sites on the surface of the MERS-S protein, including novel binding areas that have not been targeted by previously reported antibodies. Notably, cryo-EM structures show at least four different binding modes for VH1-69-based antibodies, highlighting the role and diversity of this particular V gene in the MERS-S antibody response.

Our findings suggest that the MVA-based vaccine candidate induces long-lasting memory B cells that persist for at least 32 months and produce potent neutralizing antibodies. These antibodies might provide cross-protection against closely related zoonotic Merbecoviruses, indicating the vaccine's potential to combat emerging viral threats.

P-1-84

Screening pipeline for predicting broadly neutralizing antibody sensitivity in people living with HIV

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Background: HIV-1 reactive broadly neutralizing antibodies (bNAbs) that bind directly to the HIV-1 envelope protein (HIV-1env) represent a promising new approach to the treatment and prevention of HIV-1 infection. However, the extensive genetic diversity of the HIV-1env remains a major challenge for the future clinical use of bNAbs. Therefore, pre-screening of people living with HIV (PLWH) for antibody resistance to broadly neutralizing antibodies (bNAbs) will be critical. However, assays that can be used for rapid pre-screening of patients are lacking. Therefore, we aimed to develop a pipeline for the rapid detection and characterization of HIV-1env in PLWH. We here used this pipeline to analyze the impact of serum neutralization on proviral reservoir in a unique cohort of matched neutralizing and non-neutralizing patients.

Methods and Results: We established a cohort of matched neutralizing and non-neutralizing patients (mean neutralizing activity against the 12-strain global panel of elite neutralizers (EN): 75.1%; non-neutralizers (NN): 15.0%, $p<0.0001$). Patients were well balanced with respect to gender (elite neutralizers (EN): 40% female; non-neutralizers (NN): 39% female), time since HIV-1 infection (EN: median 5.5 years; NN: median 8.6 years, $p=0.06$) and mean time off ART (EN: 8.3 years; NN: 5.9 years, $p=0.36$). However, they slightly differed in mean CD4 cell count (EN: 431/µl; NN: 572/µl, $p=0.04$) and origin (EN: 44% Tanzania, 56% Germany; NN 100% Germany). We used PBMCs of all patients to isolate individual HIV-1env genomes from these patients. A phylogenetic approach using PhytClust was then applied. PhytClust clusters the sequences from the phylogenetic tree into different subclusters representing closely related sequences. Once the most representative strains have been identified, we can rapidly clone them into envelope expression vectors that can be used to produce pseudoviruses with patient-derived HIV-1 envelopes. Using this pipeline, we have already isolated a total of 630 envelope sequences (EN: 326, NN: 304) with a median of 9 sequences per patient, of which the vast majority (86%, n=540) have already been sequenced. Sequencing revealed that the majority of the identified HIV-1 envs were fully

functional without ambiguities in NGS, ensuring their single viral origin (67%, n=360). The next step will be to analyze the sensitivity of these strains. Given our two different cohorts, we will then be able to determine the impact of IgG neutralization on the viral reservoir.

Conclusion and Outlook: We have developed a pipeline for rapid single-genome amplification of proviral HIV-1 env sequences in PLWH, allowing direct testing of the sensitivity of these sequences to bNAbs. This approach will generate a large dataset of paired neutralization and sequence information, which we intend to use in the future with informatics tools to predict individual antibody susceptibility based solely on HIV-1 env genetic data.

P-1-85

Next generation broad-spectrum human papillomavirus vaccine based on a recombinant measles virus vector

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Question: High-risk types of human papillomavirus (hrHPV) have carcinogenic potential. Approximately 5% of all cancers, in particular all cases of cervical cancer can be attributed to persistent hrHPV infection, which can be prevented by prophylactic vaccination. Current HPV vaccines consist of adjuvanted recombinant HPV major capsid protein L1, which self-assembles into immunogenic virus-like particles (VLP). However, protection is largely serotype-specific due to variation of L1. Next-generation HPV vaccines aim to provide a broader protection. Among promising broad-spectrum HPV vaccine candidates are RG1-VLPs, which assemble from a fusion protein of the HPV16 L1 protein including an additional, conserved epitope for cross-type neutralizing antibodies, "RG1," provided by the minor capsid protein L2. It has been demonstrated that rec. measles virus (MeV) can be used as cheap and effective platform technology for L1-VLP vaccination. The potential for induction of cross-protection by presenting L1-RG1 fusion protein by MeV is analyzed, in this study.

Methods: The antigen ORF encoding the L1-RG1 fusion protein was cloned into an additional transcription unit in either of two positions of the MeV Moraten vaccine strain genome. Multi-step growth kinetics of the respective recombinant vaccine viruses were analyzed and antigen expression in infected Vero cells was demonstrated via immunoblot. MeV-susceptible IFNAR$^{-/-}$ mice and New Zealand White rabbits were vaccinated twice in a 4 weeks interval. Binding and neutralizing antibodies were determined by ELISA and titration, respectively. IFN-γ ELISpot and intracellular cytokine staining for IFN-γ, TNF-α and IL-2 described T cell responses. Rabbit sera were transferred into BALB/c mice, which were subsequently challenged with different hrHPV pseudoviruses to show protection.

Results: The recombinant vaccine viruses revealed robust replication, while antigen expression in infected Vero cells was demonstrated in all cases. Sera of vaccinated mice included both target antigen- as well as vector-specific binding and functional nAb. Moreover, IFN-γ ELISpot showed high HPV antigen-specific cellular immune responses for mice immunized with the next generation vaccine candidate. Vaccinated NZW rabbits also presented with MeV-specific bAbs and nAbs as well as RG1-specific bAb and HPV16 nAb. Transfer of rabbit sera into BALB/c mice and subsequent challenge with HPV pseudoviruses of HPV serotype 39 (PsV39) showed significant protection by sera of rabbits immunized with the MeV-RG1 vaccine candidate, while mice challenged with PsV45 or PsV59 still revealed a trend for protection by the transferred sera.

Conclusion: The MeV-derived HPV vaccine candidate induced robust humoral and cellular immune responses in animals and also showed cross-protection in a pseudovirus challenge model. Therefore, our data reveal the potential for broad-spectrum protection against HPV by next-generation MeV-based HPV-vaccines.

P-1-86

Clinical translation of CRISPR-Cas9-mediated TCR engineering for the treatment of viral infections

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The transfer of naturally occurring, virus-specific T cells has proven effective in treating a potentially fatal reactivation of latent viruses such as Cytomegalovirus (CMV) in immunocompromised patients (e.g., after stem cell transplantation). However, finding suitable seropositive donors is often challenging. Moreover, this approach is not an option in the case of seronegative donors. In contrast, antigen-specific TCR-engineered T cells allow for the development of adaptable 'living therapeutics", also for the high-risk setting of CMV+ recipient/CMV- donor. We recently devised a CRISPR-Cas9-mediated T-cell receptor (TCR) delivery technique termed 'orthotopic TCR replacement' (OTR), which facilitates the generation of near-to-physiological engineered T cells with highly predictable functional properties.

We isolated several TCRs specific for an immunodominant HLA-A*02:01-restricted epitope derived from the CMV pp65 antigen via pMHC multimer staining using CMV seropositive individuals. TCRs were engineered into both primary T cells and Jurkat-based reporter cell lines to characterize the peptide sensitivity and cytotoxic capacity of OTR-T cells. Cytotoxicity was evaluated in an advanced model for endogenous epitope presentation. Based on these data, a lead candidate for clinical translation has been selected. In parallel, we translated the OTR approach into a GMP-compliant T-cell manufacturing process by designing and optimizing a 5-day process.

Currently, we are devising and compiling all necessary steps to start a first-in-human phase I clinical trial, which will encompass the prophylactic infusion of CMV-specific OTR-engineered T cells for stem cell transplant recipients with seronegative donors and high-risk of CMV infections.

P-1-87

Isolation and characterization of a novel V3 glycan site HIV-1 bNAb with a unique binding mode

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Broadly neutralizing antibodies (bNAbs) targeting highly conserved epitopes on the HIV-1 envelope trimer (Env) have become promising tools in HIV-1 prevention and therapeutic strategies. However, their clinical application is challenged by broad viral sequence diversity and emergence of escape. Combination therapies comprised of bNAbs with complementary neutralization profiles can potentially help overcome such challenges and are currently being investigated in clinical trials. Thus, identifying novel bNAbs with high antiviral activity, unique binding modes and distinct viral escape profiles is essential to advance clinical applicability of bNAbs.

To identify novel bNAb candidates, we set up a multinational cohort of 2,354 HIV-1 infected individuals. From the top elite neutralizer of this cohort, we were able to isolate the novel bNAb 007 targeting the V3 glycan site on the HIV-1 Env. Evaluation against the global and large multiclade HIV-1 pseudovirus panels showed that 007 ranks among the most potent and broadest V3 glycan site bNAbs neutralizing 100% and 66% of pseudoviruses with IC_{50}s of 0.008mg/ml and 0.01mg/ml, respectively. Furthermore, functional analyses indicated a distinct neutralization and binding profile compared to previously described V3 glycan site bNAbs. Cryo-EM analyses revealed that 007 contacts the ^{324}GDIR^{327} amino acid motif at the base of the V3 loop and interacts with glycans at positions N156 and N301. Unlike most other bNAb members of the V3 glycan site family 007 is not dependent on glycan N332, rendering it unaffected by common V3 escape mutations at this site. In addition, the cryo-EM data revealed that 007 Fab displays substoichiometric binding to the Env trimer. Subsequent comparisons of neutralization potencies between the 007 Fab and the IgG showed that the IgG is much more potent than the Fab. EM experiments with the 007 IgG are ongoing and have revealed a previously unseen trimer-dimer structure, crosslinked by three IgG molecules, which may explain the enhanced potency of the IgG. Finally, 007 achieves transient decline of viremia and is able to overcome classical V3 escape mutations in HIV-1_{ADA}-infected humanized mice.

Our studies have led to the identification of a novel V3 glycan site bNAb with high breadth and potency. 007 displays a unique binding mode and is capable of suppressing viremia *in vivo*, making it a valuable addition to the existing HIV-1 bNAb repertoire.

P-1-88

Modified vaccinia virus Ankara-based vaccine candidates provide cross-protection against lethal MARV challenge in a single-dose vaccination regimen

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Marburg virus (MARV) is a member of the *Filoviridae* family causing severe hemorrhagic fever with high mortality rates in humans. Despite its significant pathogenicity and the recurring nature of MARV outbreaks, most recently observed in Rwanda with over 60 confirmed cases, no licensed vaccines or antiviral therapies are currently available, underscoring the critical need for effective countermeasures. Here, we report on the development and preclinical evaluation of a Modified Vaccinia virus Ankara (MVA)-based vaccine candidate expressing the glycoprotein (MVA-MARV-GP) or the nucleoprotein (MVA-MARV-NP) of the MARV Guinea isolate (2022).

While recombinantly generated MARV based on the sequence of an isolate from the Guinean outbreak (2022) did not elicit clinical symptoms in the IFNAR (interferon receptor alpha/beta) knockout mouse model, infection with a natural MARV Leiden isolate (2008) resulted in fulminant disease progression and was uniformly lethal. Both MVA-MARV-NP and MVA-MARV-GP were analyzed for protective efficacy against MARV Leiden in the IFNAR knockout mouse model. Therefore, mice were immunized in a prime-boost or prime-only vaccination regimen and infected with 1000 PFU of MARV Leiden isolate. Blood samples were taken at distinct time points for antibody and T cell analysis as well as for determination of viral titers. At the predetermined end of the experiment or when mice reached the humane end point, organ samples were collected to determine viral load and to perform histopathological analyses.

In contrast to mice immunized with MVA, all mice vaccinated in the prime/boost or prime only groups with either MVA-MARV-GP or MVA-MARV-NP survived the infection with no to minimal clinical disease. The infection was successfully cleared, as evidenced by the absence of infectious virus in both serum and organs.

Our findings demonstrate that a single immunization with the MARV Guinea-based MVA-MARV-GP or MVA-MARV-NP vaccine is sufficient to confer protection against a lethal challenge with the heterologous Leiden isolate. This cross-protective efficacy observed in the IFNAR mouse model highlights the potential of these vaccine candidates as a robust prophylactic option to protect against MARV,

especially in in outbreak scenarios where rapid protection is critical. These results provide a promising basis for further development of the MVA-MARV vaccine candidate to provide a viable solution to address the unmet medical need for MARV outbreaks.

P-1-89

Preclinical development of patient-derived anti-PcrV monoclonal antibodies targeting *Pseudomonas aeruginosa*

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Antimicrobial resistance (AMR) represents a critical global health threat, outpacing the development of new antibiotics. To mitigate the shortage of novel anti-infectives, a paradigm shift in antimicrobial therapy is imperative, similar to innovations in cancer therapy that have transitioned from non-specific chemotherapies to targeted treatments. In this context, we have recently focused on the development of patient-derived antibodies neutralizing the type III secretion system (T3SS) of *Pseudomonas aeruginosa* (PA), which is associated with increased morbidity and mortality rates in acute PA infections.

By conducting a screening in a cohort of patients with chronic PA infections (n = 51), we identified several individuals with T3SS-neutralizing antibodies that abrogate PA-mediated cytotoxicity *in vitro*. Further characterization of human anti-PcrV mAbs revealed a series of antibodies with highly neutralizing qualities against a broad range of clinical PA isolates and an antibiotic-like effect *in vivo*. Importantly, patient-derived mAbs showed significantly improved potency compared to mouse-derived anti-PcrV mAbs such as MEDI3902 and 1F3 (COT-143) *in vitro* and *in vivo*.

Based on our data, we will further explore the therapeutic potential of human anti-PcrV mAbs by extensive preclinical pharmacokinetic and efficacy studies leading to the identification of a lead candidate. This candidate will undergo cell line development and master cell banking under Good Manufacturing Practice (GMP) to provide the basis for further GMP-grade antibody production. Successful implementation of our project will pave the way for the translation of patient-derived anti-PcrV antibodies into clinical trials and integration into patient healthcare, thereby addressing critical challenges posed by AMR.

P-1-90

Detection of a MERS-CoV-reactive B cell response in individuals immunized against SARS-CoV-2

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Over the course of the COVID-19 pandemic, SARS-CoV-2 variants emerged that escaped vaccine-induced humoral immunity. In addition, there are numerous coronaviruses including Middle East respiratory syndrome–related coronavirus (MERS-CoV) circulating in the animal reservoir and future zoonotic spillovers are likely. Therefore, there is a compelling need for pan-coronavirus vaccines and therapeutics. Notably, since the COVID-19 pandemic, there has been a decline in confirmed MERS cases and potential cross-protection against MERS-CoV infections due to SARS-CoV-2 immunization has been hypothesized.

In order to investigate a potential MERS-CoV cross-reactive immune response upon SARS-CoV-2 vaccination, we study the B cell and antibody response in individuals immunized against SARS-CoV-2 and the effect of immune imprinting in a study population vaccinated against MERS-CoV and SARS-CoV-2. We evaluated plasma antibody reactivity by ELISA and isolated and sequenced 1,108 MERS/SARS-CoV-2-S protein-reactive single memory B cells from 10 SARS-CoV-2 immunized donors and two MERS-CoV vaccinated study participants.

We found a significant increase in antibody binding to MERS-CoV spike protein comparing post-pandemic plasma samples (n=62) and pre-pandemic donors (n=57; $p<0.0001$). The antibody response was mainly focused on the MERS-CoV spike protein S2 subunit. Using flow cytometry, we detected in SARS-CoV-2-immunized individuals that 0.015% (±0.014) IgG+ B cells were double-positive for the MERS and SARS-CoV-2 S ectodomains. The memory B cell response was polyclonal and biased towards the usage of κ light chains (94.5%) and the expression of VH1-69 and VH3-33 antibody gene segments.

Taken together, our preliminary results indicate that SARS-CoV-2 immunization may induce a cross-reactive antibody response against MERS-CoV, which could potentially contribute to a more rapidly immune response to MERS-CoV in SARS-CoV-2 immunized individuals.

P-1-91

Towards targeted immune interventions to treat invasive fungal infections

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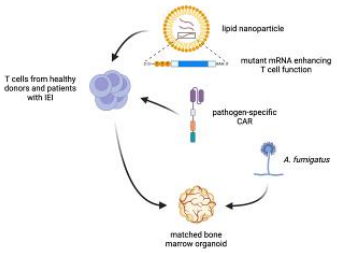
Pathogen-specific chimeric antigen receptor (CAR) T cells offer a novel, targeted approach to treating invasive fungal infections, especially in cases complicated by drug resistance, immunocompromised status, or prolonged antifungal exposure. Recent proof-of-concept studies have demonstrated the efficacy of *A. fumigatus*-specific CAR T cells in a mouse model of invasive pulmonary aspergillosis, highlighting their promise as a targeted therapy against resistant fungal pathogens. To advance this approach toward clinical application, we propose developing a human model system based on genetically engineered immune cells and

our recently developed bone marrow organoid (BMO). Specifically, we aim to leverage molecular insights from the study of inborn errors of immunity to rationally design T cell products with augmented anti-microbial potency and test their efficacy in the physiologically relevant microenvironment of the BMO.

Our group recently identified mutations that enhance TCR signaling and tumor cell killing, and we have demonstrated that these effects can be reproduced through transient delivery of mutant mRNA using electroporation or lipid nanoparticles (LNP). To evaluate the potential of this approach to treat fungal infections, we will generate induced pluripotent stem cells (iPSC) from healthy donors and patients with inborn errors of immunity and differentiate them into bone marrow organoids. T cells from the same individuals will be engineered to transiently express both a pathogen-specific CAR and a dominant mutant protein that boosts T cell function. We will assess the functional properties of these engineered T cells, including their persistence, expansion, cytokine production profiles, and ability to clear pathogens within the BMO. Furthermore, we will compare the anti-fungal activity of the engineered CAR T cells against conventional antifungal therapies to establish a benchmark for efficacy. In addition, we will attempt to generate pathogen-specific CAR T cells in situ by leveraging recently developed enveloped delivery vehicles (EDV) or LNPs decorated with T cell-targeting single chain variable fragments.

Thus, by combining insights from inborn errors of immunity with genetic engineering, our study aims to pave the way for targeted immune interventions to treat invasive fungal infections, particularly in immunocompromised patients.

Fig. 1



P-1-92
The DZIF Transplant Cohort
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The DZIF Transplantation Cohort e.V. is a large multicenter prospective observational cohort of transplant recipients and donors, enabling extensive collection of medical data as well biological samples. Infections in transplant recipients have a major impact on the overall therapy success and clinical outcome. However, many issues regarding long-term consequences of infections are not well understood. How severe is the impact of defined infections on graft survival/function and graft- versus-host disease (GvHD)? How to assess the individual susceptibility to bacterial, viral and fungal colonization under immune suppression? What is the long- term impact of anti-infective therapy on graft and patient survival or changes in the physiological microbiome that may be significant for colonization with pathogenic and antibiotic-resistant microbes? These are just a few examples of important research questions. Worldwide there exist only few and relatively small prospective cohort studies on transplant patients with a focus on infectious disease. Therefore, the DZIF Tx Cohort will contribute significantly to a more careful epidemiological and experimental analysis of the impact of infections on transplant function and survival by using standardized protocols for the collection of multiple biosamples and patient data at defined time points before and after transplantation.

Collection of data and samples takes place in university hospitals and clinics in Hannover, Heidelberg, München and Tübingen at time of transplantation, after 3, 6, 9 and 12 months and then yearly thereafter; as well as in case of infection. The cohort enables studies to investigate correlations between infections and immune alterations with the development of transplant complications in a prospective manner. Biosamples are preserved in a quality conform to state-of-the art genomic and epigenomic technologies for future analyses. The distribution of data and samples to researchers is linked to a detailed review process by the cohort internal scientific steering committee and a pool of external international reviewers.

In collaboration with the TI BBD, distinct quality controls of biosamples, audits and local trainings are performed to ensure the quality of collecting biosamples, procession regarding SOPs and storage. Control of quality and quantity of documented data is performed in collaboration with epidemiology experts.

Up to now, there are about 2.700 patients included in the data base and more than 50.665 biosamples collected, including PBMCs, RNA-stabilized blood, serum, urine, feces.

P-1-94
HIF-2α Pathway Inhibition as a therapeutic strategy for Endothelial Dysfunction and Hypoxia in Long COVID
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Questions: Long COVID is still an ongoing challenge for our healthcare systems and economic recovery, with unfortunately still an absence of consensus on the cause of and possible treatments for Long COVID across the medical community. In our previous publication, we were able to show that persistent endothelial dysfunction could be a hallmark of ongoing symptoms in Long COVID, and others have summarized that persistent microvascular endotheliopathy might be a suitable therapeutic target. However, the underlying biological mechanisms remain unclear.

Methods: In this prospective observational study, we characterized 41 LC patients and 24 healthy controls (HC) and investigated the effect of SARS-CoV-2 Spike protein 1 (S1) and plasma from LC patients on human retinal endothelial cells (HREC).

Results: Plasma samples from LC patients exhibited significantly elevated VEGF and MCP-1 alongside decreased IL-6 levels compared to HC. VEGF levels were positively correlated with Anti-S1 IgG levels in patients and upregulated in HREC exposed to S1. Additionally, S1 exposure increased VEGF mRNA, promoted ROS production, reduced NO synthesis, and transiently activated HIF-1α in HREC. Persistent activation of HIF-2α by S1 led to disrupted endothelial integrity. Treatment with Belzutifan, a HIF-2α inhibitor, restored barrier integrity in HREC exposed to S1. Plasma from Long COVID patients induced similar endothelial dysfunction in HREC, increasing ROS production and reducing NO synthesis, and Belzutifan was able to restore barrier integrity in these cells.

Conclusions: Our findings underscore the importance of addressing HIF-2α-mediated endothelial dysfunction in Long COVID and suggest for the first time that targeting HIF-2α with Belzutifan could offer a novel therapeutic approach.

1) doi.org/10.1007/s10456-023-09885-6

2) doi.org/10.1172/JCI161167

P-1-95

Characterization of COX6C as a proviral factor in HBV infection and its role in oxidative phosphorylation

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Hepatitis B virus (HBV) is a hepatotropic virus that depends on various host factors for its replication. Metabolic reprogramming is a common strategy viruses use to ensure sufficient energy production for replication and virion production.
To identify pro- or antiviral factors of HBV involved in host energy metabolism, we developed a novel pulsed stable isotope labeling with amino acids in cell culture (SILAC) screening. We infected HepG2-NTCP cells with either wildtype HBV or HBV in which the X protein was knocked out and then pulsed the infected cells at different time points from day 0 to day 10 post infections for 24 hours with SILAC medium. Following each pulse, changes in host protein levels were analyzed using mass spectrometry (LC-MS/MS). The proteome analysis identified cytochrome C oxidase subunit 6C (COX6C), a regulatory factor in oxidative phosphorylation, to be stabilized upon HBV infection. We confirmed by qPCR analysis that HBV led to an increase in COX6C levels in infected HepG2-NTCP cells. To further validate COX6C as a proviral factor in HBV infection, we generated cell lines with a COX6C knock-out and knock-down. Both models showed a decrease in HBV infection parameters compared to controls. In contrast, upregulation of COX6C expression enhanced HBV infection efficiency. Using a non-toxic concentration of a COX inhibitor, we confirmed that oxidative phosphorylation is essential for productive HBV infection. In summary, we have identified the mitochondrial protein COX6C as a novel proviral factor in HBV infection. This finding has significant implications for the development of novel therapies targeting energy metabolism in viral infections. We aim to further characterize the mitochondrial function in HBV infection in COX6C knock-out and control cells to identify regulatory pathways that could be targeted for such therapies.

P-1-96

Improving T cell control of HCMV with a mAb targeting the immunosuppressive glycoprotein pUL11

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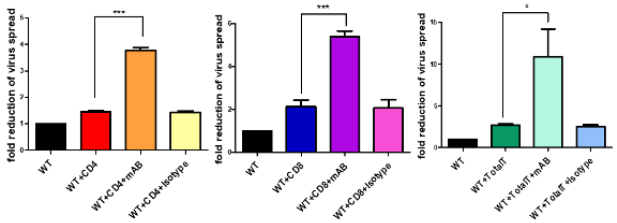
Human Cytomegalovirus (HCMV) modulates the host´s immune system in many ways. Infected cells are manipulated to avoid immune surveillance and, by less well studied mechanisms, uninfected immune cells are suppressed by the virus. We have identified an HCMV glycoprotein, pUL11, which is expressed on the surface of infected cells and binds to the leukocyte surface protein CD45. The presence of pUL11, as a purified protein or in the context of HCMV infection, results in decreased CD45 phosphatase activity in CD4+ and CD8+ T cells expressing CD45. pUL11 impairs T cell functions via this inhibition of CD45, thereby reducing T cell control of HCMV spread. Mechanistically, pUL11 induces IL-10 production in CD4+ central memory T cells, resulting in the suppression of CD4+ T cell-mediated control. CD8+ T cell function is suppressed through IL-10-independent mechanisms. We have shown that the enhanced control of viral spread in the absence of pUL11 is due to both increased T cell cytotoxicity and increased anti-viral cytokine production. A monoclonal antibody targeting pUL11 disrupts its interaction with CD45 and, as a result, improves T cell control of HCMV. Both CD4 and CD8 T cell responses are markedly enhanced, leading to greater clearance of HCMV infected cells in coculture assays. While HCMV infections are generally not problematic for healthy individuals, immunocompromised people, such as hematopoietic stem cell transplant (HSCT) recipients, can experience severe disease upon reactivation of HCMV. For these patients, functional T cells are critical for controlling HCMV. We hypothesize that blocking pUL11 with a therapeutic mAb could enhance control of active HCMV infections by the patients" own or adoptively transferred T cells and thus be beneficial for this patient group.

Fig. 1: Blocking pUL11 improves the T cell control of HCMV.**R**etinal pigment epithelial (RPE) cells were infected with HCMV Merlin GFP. The cells were treated with an anti-pUL11 monoclonal antibody (mAb) or an isotype control. Enriched CD4+, CD8+ or total T cells from healthy blood donors were stimulated and added to the co-culture for 7 days. GFP+ cells were quantified by flow cytometry and the fold reduction of the virus spread in the presence of T cells and antibody is shown.

Fig. 1



P-1-97

High-risk human papillomavirus persistence and incidence among women living with HIV in the African Cohort Study

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Background: Women living with HIV (WLWH) have increased risk of high-risk (HR) human papillomavirus (HPV) infection and developing cervical cancer (CC) as compared to women without HIV. Many African countries aim to start HPV molecular screening as recommended by the World Health Organization. However, the optimal interval to re-screen HR-HPV negative and positive WLWH is unclear and could be influenced by the HPV type. We evaluated HR-HPV persistence and incidence in previously HR-HPV positive and negative WLWH in four African countries.

Methods: Participants were enrolled into the African Cohort Study (AFRICOS) from 12 clinics in Tanzania, Kenya, Uganda, and Nigeria. From 2015 onwards, adult WLWH within AFRICOS were offered HR-HPV testing annually, where cervical cytobrush specimens from women were genotyped for 14 HR-HPV types using the multiplex Seegene Anyplex real-time PCR.

Results: From 2015 to 2022, 383 WLWH underwent serial screening for HR-HPV. Their median age was 43.2 years (interquartile range 36.3-49.1 years) and 370 (96.6%) of them were on ART. A positive first HPV test was observed in 247 participants (64.5%), of whom 167 (67.6%) had persistent HPV one year later. We also observed a 29%-61% type-specific HPV persistence with HPV45 being the most persistent (61.5% (8 of 13) persistent infections), followed by HPV52, HPV33, HPV16 and HPV35 (55.8% (24/43), 54.5% (18/33), 51.4% (37/72) and 51.1% (23/45) respectively). HPV18 infections were persistent in 33.3% (10/30) of cases. The frequency of persistent HR-HPV in WLWH with CD4 T-cells of <200, 200-500 or >500 cell/mm3 at their first HPV test was 80.9%, 72.2% and 61.5% respectively (p=0.095). Among 136 participants with a negative first HPV test, 37 (27.2%) had incident HR-HPV infections one year later, of whom 12 (32.4%) had HPV16/18/45 and 8 (21.6%) had HPV35.

Conclusion: HR-HPV type-specific short-term persistence and incidence is high even in ART-treated WLWH with high CD4 T-cell counts from four African countries. These preliminary data support yearly HPV re-screening of WLWH.

P-1-98

Dissecting the impact of HIV on HPV-specific immunity, viral persistence and molecular signatures of cervical dysplasia

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Introduction: To better understand the underlying patho-mechanisms contributing to the multi-fold increased cervical cancer risk in Women Living with HIV (WLWH), we investigated HPV16-specific T-cell responses, HPV16 viral load and oncogene expression, molecular signatures of dysplasia and HR HPV persistence in relation to HIV status in the Tanzanian 2H study cohort.

Methods: 2H study volunteers were studied after stratification by both cytology and histology diagnosis. HPV typing was determined by the Roche Linear Array assay. HPV16 viral load and oncogenic signatures were assessed by RT-PCR and the Luminex-based QuantigGene assay, respectively. HPV16 E6/E7-oncoprotein-specific T-cell frequencies were determined after in vitro restimulation of peripheral blood mononuclear cells with peptide pools by intracellular cytokine staining.

Results: WLWH were frequently characterized by HR HPV-specific immune dysfunction and molecular signatures of dysplasia, even in women without visible cervical lesions. Specifically, in women with HPV infection and without lesions, HIV infection was associated with depletion of autologous HPV16-oncoprotein-specific CD4+IFNg+ T-cells (median: 0.02% HIV- vs 0.00% HIV+, p = 0.020), with a multifold increased HPV16 viral load quantified as copies per cell (median: 0.000 HIV- vs 0.001 HIV+ ,p = 0.041) and E6 mRNA expression (mean: 0.00 rMFI HIV- vs 0.14 rMFI HIV+ ,p = 0.008). Considering any HR HPV infection, we observed higher expression of cellular transformation-associated biomarkers, including p16 and STMN1, MCM2 (all p<0.05) compared to HIV- controls. Studying all women with

longitudinal HPV data, who did not receive cryotherapy or other ablative treatment, HR HPV annual persistence was 3.2-fold higher in HIV+ versus HIV- women (42% v 13%, p<0.001, n=324).

Conclusions: Consequently, HIV-induced immune suppression contributes to reduced HR HPV viral clearance allowing persistence, accumulation of mutations leading to malignant transformation, and in turn enhanced oncogene expression accelerating cervical cancer.

P-1-99

Human intestinal organoids to study the stem cell niche as a potential reservoir for hepatitis E virus infections

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Background/Aim: Hepatitis E and A viruses (HEV/HAV) are transmitted by the faecal-oral route. While certain HEV genotypes can persist, HAV infections are always self-limiting. There is evidence that both viruses actively infect the intestinal epithelium to gain access to hepatocytes and HEV antigen was detected in the intestinal crypt of a chronically HEV-infected patient. Here, we aimed to use human pluripotent stem cell (hPSC)-derived intestinal organoids (hIOs) to identify the specific cell types targeted by each virus and to investigate whether the stem cells in the intestinal crypt can be infected by HEV.

Methods: The hPSC line iPSC.C3A was differentiated into hIOs, which were infected with the HEV GT3 Kernow-C1 p6 and an HAV GT IIIA stool isolate by either mechanical dissociation or microinjection. Viral infection was assessed by RT-qPCR or staining using SPIM or confocal microscopy. Sections of a paraffin-embedded intestinal tissue sample from a chronic HEV patient were analysed by RNA-FISH.

Results: hIOs reflect the cellular diversity of the intestinal epithelium with their apical membranes facing the organoid lumen. Infection of hIOs with HEV but not HAV was maintained over a 40-day observation period, suggesting that HEV but not HAV infection can persist in hIOs. By isolating LGR5+ cells from infected hIOs, we found that HEV appears to target the stem cell niche for infection. Consistent with this, we detected HEV RNA in LRG5+/OLMF4+ stem cells in the intestinal crypt of a chronic HEV patient. Since stem cells are resistant to viral infection by intrinsically expressing a subset of interferon-stimulated genes (ISGs), we are currently investigating the expression of potential proviral ISGs in the intestinal stem cell niche that could support HEV infection.

Discussion: We have succeeded in infecting hIOSs with HEV and HAV allowing us to study cell-specific determinants. Our results suggest that the intestinal stem cell niche acts as a reservoir in chronic HEV patients, with potential repercussions on infection and treatment outcomes.

P-1-100

Predictors of postviral symptoms following Epstein-Barr-Virus-Associated infectious Mononucleosis in young people – data from the IMMUC Study

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Introduction: Epstein-Barr virus-associated Infectious Mononucleosis (EBV-IM) is a frequent illness following primary EBV infection in young individuals. Occasionally, EBV-IM does not resolve. Instead, symptoms such as fatigue may be protracted for months or even progress to myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS). This large prospective observational study identifies risk factors associated with protraction and fatigue ≥ 6 months post EBV-IM symptom onset (T_{onset}).

Material & Methods: Two hundred young individuals (aged 1 - 33 years) with acute primary EBV infection were recruited. Data on patients" history, as well as clinical and routine laboratory parameters, were collected at a baseline visit (≤4 weeks) and two follow-up visits, one and 6 months after T_{onset}. Multivariate logistic regression models identified risk factors for protraction and late fatigue.

Results: Protraction was observed in 55/183 (30.1%) and fatigue in 34/181 (18.8%) patients at ≥ 6 months after T_{onset}. Susceptibility to infectious diseases, indicated by ≥1 positive ELVIS criteria, was significantly associated with protraction [OR: 2.31; P=0.011] and fatigue [OR: 2.98; P=0.006]. We

further identified the severity of individual early clinical symptoms, e.g., gastrointestinal symptoms, as significant risk factors for protraction [OR: 3.42; P=0.027] and fatigue [OR: 3.54; P=0.034]. Within the first four weeks after T_{onset}, more than 12 features (clinical and laboratory) best-predicted protraction (OR 2.47, 95%-CI: [1.26, 4.87]) and best-predicted fatigue at ≥ 6 months after T_{onset} (OR 2.43, 95%-CI: [1.07, 5.49]).

Discussion: A clinical history of immune dysregulation alongside pronounced symptoms of severe infectious mononucleosis may indicate prolonged postviral illness, aiding in recognizing young individuals vulnerable to such conditions.

P-1-101

Longitudinal metabolomic profiling in long COVID

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Question: Long COVID is a multisystem condition occurring after SARS-CoV-2 infection. Several hypotheses for its pathogenesis have been suggested, including persisting reservoirs of SARS-CoV-2 in tissues. Long COVID has been shown to be associated with obesity and other preexisting comorbidities like diabetes mellitus, cardiovascular disease and rheumatoid arthritis. To date, there is only limited understanding of the intricate metabolic interactions between long COVID and coexisting diseases and of isolated effects of each comorbidity on long COVID. Despite the valuable insights provided by prior metabolomic studies on long COVID, the limitations of small sample sizes - typically no more than 40 long COVID samples per study - have hindered the generalizability of their findings. While previous studies have identified significant alterations in metabolic concentrations when comparing long COVID patients to either healthy or COVID-recovered controls, the variability in the specific metabolites identified across different studies shows the need for a more robust investigation.

Our study addresses this gap through analysis of the large and well-defined EPILOC cohort to decipher the metabolic profiles associated with long COVID in the context of comorbidities.

Methods: The Baden-Württemberg EPILOC study is a prospective, longitudinal, population-based nested case-control study with comprehensive medical evaluation and comparison of persistent cases with a control group of age- and sex-matched stably convalescent controls. Due to its cohort size and the possibility of comparing long COVID cases with controls and the long-term course, the EPILOC cohort is ideal for validly assessing persistent metabolomic effects in the longitudinal course in long COVID patients.

Results: Targeted metabolomic profiling of blood plasma samples of ~1,100 EPILOC study participants (700 long COVID, 400 COVID-recovered controls) ~ 18 months after acute COVID-19 and of a longitudinal second timepoint ~3 years after COVID-19 (~ 600 participants) is nearly complete, with results from the first timepoint already under bioinformatical analysis. Although these analyses are still in progress, preliminary findings have revealed a significantly reduced glutamine-to-glutamate ratio in persistent cases compared to controls. This parameter was not significantly different between cases with and cases without post-exertional malaise (PEM).

Conclusions: In conclusion, by integrating metabolomic features with comprehensive clinical data, including covariates such as BMI and PEM, our study is uniquely capable of exploring the complex dynamics of long COVID. The complete results are currently being finalized and will be presented at the meeting scheduled for February 2025.

P-1-102

Targeting immune checkpoint receptor ligands to restore T cell activity and eliminate persisting hepatitis B virus infection

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Background/Aims: Chronic hepatitis B virus (HBV) infection is a major global health problem, with the potential to cause cirrhosis, liver failure, and hepatocellular carcinoma. Antivirals control the HBV replication, but virus clearance is seldom achieved. Since a strong T-cell response indicates successful viral clearance during acute infection, restoring T-cell functionality through targeted therapies is considered a promising approach. The aim of this study is to suppress ligands for the PD-1 and TIM-3 exhaustion markers, namely PD-L1, PD-L2, and Galectin-9 (Gal-9), which are mainly expressed in non-parenchymal liver cells, in order to reconstitute T cell functionality and cure chronic hepatitis B infection.

Methods: Short hairpin RNAs (shRNAs) were designed and screened for PD-L1, PD-L2, and Gal-9. For cell-type specific delivery of the shRNAs we chose adeno-associated viruses (AAVs). Specific AAV capsids were selected to target non-parenchymal liver cells and liver macrophages. Selected shRNAs repressing immune checkpoint receptor ligands were cloned in a single self-complementary AAV genome (AAV-ICI). To establish persistent HBV replication, an AAV carrying a 1.2-fold overlength genome of HBV was used.

Results: Neither AAV-ICI nor AAV-Ctrl showed a toxic effect in vitro in primary mouse hepatocytes. In vivo, however, high alanine transaminase (ALT) levels indicating liver damage were detected, and a strong innate immune response was induced in AAV-ICI and AAV-Ctrl groups. In addition, no persistent HBV replication could be established when mice were co-infected with AAV-HBV and AAV-ICI or AAV-Ctrl.

Conclusions: Targeting liver macrophages with a self-complementary AAV led to significant liver damage with both AAV-ICI and AAV-Ctrl treatments. Thus, suppression of

immune checkpoint ligands has to be tackled using small interfering RNAs with diverse modifications to specifically target non-parenchymal cells.

P-1-103
Investigation of the HBV infection cycle of rabbit hepatocytes

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Hepatitis B Virus (HBV) is a global health burden, with over 256 million chronically infected patients and approximately 1.1 million annual deaths, attributed to the infection or its consequences. Chronically infected patients are at high risk of developing hepatocellular carcinoma and liver cirrhosis. So far, there is no reliable treatment option, and there is a critical need for the development of novel therapies and validation in suitable animal models. For HBV, the only naturally infectable non-human species is the chimpanzee, whose use is highly restricted. Therefore, there is an urgent need for the establishment of new HBV animal models.

The bona fide entry receptor for HBV, the sodium taurocholate co-transporting polypeptide (NTCP), is a key factor for HBV infection and is considered a species barrier due to naturally occurring cross-species sequence variations. We have shown that pig and macaque hepatocytes, which are not susceptible to HBV infection, can be rendered fully permissive upon expression of a human or humanized chimeric NTCP. However, respective animal models rely on a vector-mediated transgene expression or the generation of transgenic animals.

In this project, our goal is to identify de novo permissive species. We tested several species-specific NTCPs on their capability to bind HBV and support HBV infection in vitro and identified ferret, horse, and rabbit NTCPs as functional HBV entry receptors. To validate this result, we isolated primary hepatocytes from rabbits. We tested whether they support HBV binding using the receptor binding domain of the HBV surface protein, Myrcludex B (MyrB). We confirmed the binding of a fluorescently labeled MyrB to the cell surface of the cells. We next infected the hepatocytes with a recombinant HBV, leading to Luciferase expression upon infection, and wildtype HBV and could see no markers for infection. We then transduced the cells with an adenovirus harboring a 1.3-fold HBV genome and detected the expression of HBV-specific proteins such as HBeAg and HBsAg, indicating the block for HBV in the early steps of the replication cycles. To determine whether HBV enters the cell, we established an in-situ hybridization assay (RNAscope) to detect and localize single viral particles intracellularly. This assay revealed that HBV was not able to enter the rabbit hepatocytes after binding, indicating that a yet unknown factor or co-factor essential for HBV cell entry is absent or altered. In summary, our results indicate that further investigations on the HBV cellular entry are necessary to establish rabbits as an hbv animal model.

P-1-104
TIGIT-expression on NK cell subsets is an early indicator of alleviating liver inflammation following BLV treatment in chronic hepatitis D

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Question: Bulevirtide (BLV) is a novel and the only approved treatment option for patients with chronic hepatitis D (CHD). BLV alleviates liver inflammation already early during treatment when only minor HDV RNA changes are observed. However, some patients are not responding by week 48 after therapy (TW 48). Previous studies showed that natural killer (NK) cells were functionally compromised in patients with CHD. Thus, the study aims to analyze NK cell immunotypes during BLV treatment.

Methods: BLV-treated chronic hepatitis D patients (n=20) from a single-center cohort were longitudinally analyzed for clinical, molecular, and virological parameters. PBMC were studied before treatment start (baseline (BL)), at therapy weeks 3 (TW3) and 48 (TW48) by spectral flow cytometry investigating 20 immune markers followed by high dimensional data processing. Chronic HCV-infected patients treated with direct-acting antivirals were used as controls.

Results: Overall NK cell frequencies remained stable during BLV-treatment. However, biochemical responders (BR) showed distinct NK cell immunophenotypic features before and during therapy. TIGIT-expression increased on CD56dim and CD56bright NK cells during the course of BLV-treatment and inversely correlated with alanine aminotransferase levels in HDV but not HCV infection. High frequencies of TIGIT-CD57+ CD56dim NK cells at BL and low levels during therapy were indicative of a biochemical response.

Conclusions: We here suggest that the lack of expression of the immune checkpoint inhibitor TIGIT on NK cell subtypes may be a hallmark of liver inflammation in HDV infection. BLV-therapy is associated with a reappearance of TIGIT on these cells, which may be one mechanism of why liver enzymes rapidly improve during therapy.

Figure 1: Schematic representation of the study design.

Figure 2: Longitudinal FlowSOM in BR and NBR with four populations for the markers TIGIT and CD57.

Fig. 1

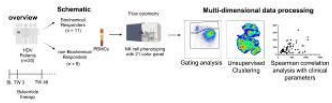
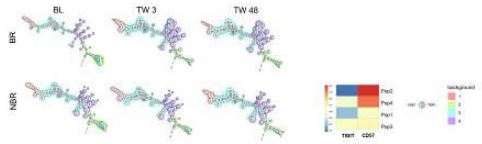


Fig. 2



P-1-105

Purification of HBV core antigen particles using a one-step anion exchange chromatography process

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Background: The Hepatitis B Virus (HBV) poses a major challenge to global health. During its replication cycle, the viral capsid plays an essential role, making the HBV core (HBc) protein an important target for new therapeutic strategies. The 183 aa HBc protein forms dimers and 90 or 120 dimers self-assemble into virus-like particles (VLPs) or – in presence of the HBV pregenome - into the HBV capsid. Recombinant VLPs bear great potential for translational research. Consequently, optimization of their production represents a key area of investigation, as it helps to minimize costs and enhance yields. This study aimed to optimize VLP composition and establish a rapid purification process using a one-step anion exchange chromatography (AEX) resulting in high purity.

Methods: We expressed three HBV genotype D capsid particles composed of HBc proteins of different lengths with different nucleic acid packaging properties in *E. coli*: D (full-length, 183 aa), ΔD163 (partially truncated C-terminus, 163 aa) and ΔD149 (maximally truncated C-terminus, 149 aa). After benzonase treatment of E. coli lysates, polyethyleneimine (PEI) precipitation of HBV capsid particles further reduced excess nucleic acids, followed by two stages of ammonium sulfate (AS) precipitation. For a final purification, AEX was performed.

Results: Purified HBV capsid particles were analyzed for purity and stability by SDS-PAGE, native agarose gel electrophoresis, ELISA, dynamic light scattering (DLS), electron microscopy and host cell protein and DNA and endotoxin assays. Irrespective of the HBc protein length, 22 nm VLPs were formed, demonstrated by corresponding peaks in the DLS measurement. The chromatograms revealed elution peaks with UV254 nm signal specific for the respective nucleic acid-packaging capabilities of the VLPs indicating successful one-step AEX purification after PEI and AS precipitation. The gradual increase in purity during the purification process could be visualized by SDS-PAGE, with only minimal impurities evident in the final product (>95% purity). Structural analysis subsequently proved the elution of capsid particles in their native form with a final yield of 5.2 mg for D, 25.9 mg for ΔD163 and 27.8 mg for ΔD149 per liter expression culture. The resulting VLPs showed low host cell protein and DNA residues and endotoxin levels. HBV capsid particles retained their integrity over one month of storage at 4 °C. Minimal aggregation of larger structures was observed.

Conclusion: We successfully designed a one-step chromatography purification technique for different HBV capsid-like VLPs. Our data revealed that all three VLPs retained their integrity, exhibited minimal aggregation of the VLPs, and preparations showed low host cell residues. The findings of this study provide the basis to increase efficiency of larger scale productions.

P-1-106

Spectrum and prevalence of psychiatric disorders depending on latent toxoplasmosis at initial HIV diagnosis in 2019 and 2020 – HIV/PSYCH

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Question: The relationship between HIV and psychiatric disorders is suspected to be bidirectional. HIV infection increases the risk of psychiatric disorders and vice versa [1]. A higher seroprevalence of toxoplasmosis was found in people with certain psychiatric disorders, compared to healthy control groups [2]. The aim of this study is to map the prevalence of mental illness in PLWH and to answer the question of whether this prevalence is associated with latent toxoplasmosis infection.

Methods: Cross-sectional analysis of 800 PLWH treated in participating study centers who were first diagnosed with HIV in 2019 or 2020. Patient characteristics, information on the HIV testing, clinical data including psychiatric comorbidity and laboratory parameters including toxoplasmosis serology were documented retrospectively.

Results: A total of 800 individuals with HIV-first diagnosis were analyzed in this project. 22.4% presented with at least one AIDS defining illness, most frequently pneumocystis-pneumonia (32.7%), candidiasis (28.5%), and wasting (24%). Cerebral toxoplasmosis was found in 6.7%. 13% presented with one or more psychiatric disorder at time of initial HIV diagnosis. Most frequent were affective disorders (60.6%), neurotic disorders (36.6%) and sleep disorders (7.7%). Among these recurrent depressive (51%) and adjustment disorders (22.1%) were the most commonly observed. Substance abuse was documented in 31%, the most frequent being nicotine abuse (79.8%). Serology testing for toxoplasmosis was performed in 71.3% and 33.7% tested positive. In PLWH with latent toxoplasmosis, psychiatric disorders and substance abuse were not found more often compared to those who tested negative (12% vs. 14.3% and 32,3% vs. 32,3%; $p > 0.05$). However, AIDS- defining conditions were observed significantly more often in those with latent toxoplasmosis compared to those without (32.3% vs. 22.8%; $p < 0.05$), although cerebral toxoplasmosis was rather rare in the study population.

Conclusions: This preliminary evaluation of HIV/PSYCH shows that mental illnesses at 13% in PLWH are not more common than in the general population. However, this result could be influenced by inadequate documentation of mental illnesses in the patient files. The suspected influence of latent toxoplasmosis on the prevalence of psychiatric comorbidities could not be confirmed. However, latent toxoplasmosis has been found to significantly increase the risk of AIDS which could possibly indicate changed risk behavior due to the latent parasitic infection.

References:

[1] Nedelcovych et al. The Psychiatric Impact of HIV. ACS Chem Neurosci. 2017 Jul 19; 8(7): 1432–1434. DOI: 10.1021/acschemneuro.7b00169

[2] Fernandes et al. Association between exposure to toxoplasmosis and major psychiatric disorders: a systematic review. Braz J Psychiatry. 2021 Jul-Aug;43(4): 438-445. DOI: 10.1590/1516-4446-2020-0904

P-1-107

Bulevirtide in combination with pegylated interferon alfa-2a shows a sustained response to treatment in the liver

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Background and Aims: Bulevirtide (BLV) is a first-in-class entry inhibitor approved in the EU for the treatment of chronic hepatitis D (CHD). In a multicentre, open-label, randomized phase 2b study (MYR204; NCT03852433), safety and efficacy of BLV (2 and 10mg/day) with or without pegylated interferon alfa-2a (PegIFNα) were evaluated in patients with CHD and compensated liver disease. This sub-study aimed to evaluate intrahepatic virologic changes 24 weeks after end of treatment (EOT) compared to baseline. To further understand the effect of treatment, the relationship between intrahepatic outcomes and peripheral parameters, including intrahepatic innate and inflammatory gene expression profiles, was evaluated

Method: 174 CHD patients were randomized (1:2:2:2) to receive (A) PegIFNα for 48 weeks (48w); (B) BLV 2mg + PegIFNα or (C) BLV 10mg + PegIFNα for 48w, both followed by 48w of monotherapy with BLV 2mg or 10mg, respectively; or (D) BLV 10mg for 96w. Paired liver biopsies were available at baseline and 24w after EOT in in a subset of patients (n=42 for molecular and 44 for histological analysis). HDV parameters and infection-related host genes were assessed by qPCR and HDAg immunohistochemistry.

Results: At follow-up (FU), 24w after EOT, median intrahepatic HDV RNA declines from baseline were 1.9Log10 in arm A (n=5), 2.0Log10 in arm B (n=14), 3.2Log10 in arm C (n=11), and 0.8Log10 in arm D (n=12). Intrahepatic HDV RNA levels strongly correlated with the number of HDAg positive cells ($r=0.85$; $p<0.0001$), which declined by 1.9Log10 in arm A (n=6), 1.0Log10 in arm B (n=14), 1.9Log10 in arm C (n=11), and 0.8Log10 in arm D (n=13). In the two combination arms B and C, 8/14 (57%) and 8/11 (73%) patients, respectively, were negative for HDV RNA at FU, while in monotherapy arms A and D, 3/5 (60%) and 1/12 (8%) patients had undetectable intrahepatic HDV RNA levels, respectively. Intrahepatic changes of HDV RNA mirrored HDV RNA changes determined in the serum ($r=0.82$; $p<0.0001$) while intrahepatic HBV parameters did not change significantly apart from a modest median reduction of total HBV RNA in both combination arms. Transcriptional levels of inflammatory chemokines and infection-related genes were decreased at FU in combination treatment arms. Notably, decreases from BL to FU in chemokines, such as CXCL10, strongly correlated with the reduction of intrahepatic HDV RNA ($r=0.67$; $p<0.0001$) and with changes of peripheral ALT levels ($r=0.75$; $p<0.0001$), suggesting an amelioration of liver inflammation.

Conclusion: Intrahepatic analysis in paired BL and post-treatment biopsies demonstrated a strong correlation between intrahepatic and serum HDV RNA reductions. Concomitant with the decrease of HDV parameters, expression levels of innate immune genes declined. The highest rate of the off-treatment virologic response in the liver was observed in the arm that received combination of BLV 10mg + PegIFNα.

P-1-108

Assessing determinants of optimal replication fitness for a subgenomic replicon vaccine against hepatitis C virus

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The hepatitis C virus (HCV) is divided into 8 genotypes (gt) with 93 subtypes. There are now effective therapies against HCV, but the lack of a protective vaccine is still a major obstacle to eradicating the virus. One potential vaccine approach would be self-replicating HCV mRNAs, so called subgenomic replicons (SGR). For this approach, a good understanding of the viral determinants of efficient HCV RNA replication is essential. For HCV gt1b, key determinants of high replication fitness were identified in the non-structural protein 5A (NS5A) in a region we called the Replication Enhancing Domain (ReED). This allowed for selection of potential vaccine candidates for gt1b. This project aims for

getting a similar understanding of determinants of replication fitness for the clinically relevant gt1a and gt3a to subsequently identify suitable vaccine candidates. For an informed vaccine design, it is also necessary to understand the interplay between HCV isolates of varying replication fitness and the immune system. To this end, we assessed a patient cohort of acute HCV patients comparing the frequency of sequence patterns of high replication fitness in patients who developed a chronic infection vs. patients who cleared the virus.

From the "HCV Research UK" cohort, viral sequences with ReED mutations (18 for gt1a and 10 for gt3a) were selected from liver transplant patients. These ReED sequences were cloned into prototype subgenomic reporter replicons (H77 for gt1a and S52 for gt3a) and then tested in cultured hepatoma cells. The most common mutations found in constructs with high replication fitness were then further characterized as individual point mutations in SGRs. Regarding the acute HCV infections, the research group of Naglaa Shoukry provided us with 32 patient sera with either chronic or resolved HCV infections. Viral RNA was extracted from the serum, reverse transcribed, amplified and sequenced via Sanger sequencing.

Through our experiments, the mutation at the P2209L site was identified for both genotypes as the mutation that influences replication the most. This mutation has been described for gt1b, although the effect seems to be milder for gt1a and gt3a. Nevertheless, this insight shows that sequence determinants can increase replication fitness across different genotypes. In addition, our work shows that patients who cleared an HCV infection have the tendency to contain more mutations associated with high replication fitness than chronic HCV patients do. This argues for HCV with high replication fitness being less capable in inducing a chronic infection. The most likely explanation would be that high replicators are too immunogenic to progress to a chronic infection which would be a crucial information for vaccine design.

P-1-109

Cytotoxic CD16$^+$ γδ T cells ere associated with virus control in chronic Hepatitis B virus (HBV) infection by mediating antibody-dependent cellular cytotoxicity (ADCC)

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The role of the immune system in the pathogenesis of HBV infection and the correlates of functional cure are not fully understood. Antibody-dependent cellular cytotoxicity (ADCC) is a possible important mechanism for controlling viral replication. However, its role in CHB, especially the involvement of NK and γδ T cells, which are abundant in the liver, is still unclear.

This study analyzed peripheral NK and γδ T cells in individuals with acute (n=13) and chronic HBV (n=57) using spectral flow cytometry and single-cell RNA sequencing, alongside HBV viral markers. To assess NK and γδ T cell-mediated ADCC, isolated NK or γδ T cells from HBV patients and cord blood were stimulated with HBsAg and intravenous immunoglobulin (IVIG).

We showed that CD16$^+$ γδ T cells but not CD16$^+$ NK cells negatively correlate with HBcrAg, a marker of intrahepatic HBV replication in CHB. These cells expressed higher levels of cytotoxic markers (granzyme B, perforin, NKG2D), and their stimulation with HBsAg and IVIG led to increased IFN-γ, TNF-α, and CD107a expression. *Ex vivo* staining of CD16$^+$ γδ T cells positively correlated with ADCC in individuals with CHB and acute HBV, while γδ T cells from cord blood, with low CD16 expression, lacked ADCC function.

In conclusion, our results emphasize the role of CD16$^+$ γδ T cells and ADCC in the control of HBV during chronic infection. The absence or low levels of CD16$^+$ γδ T cell-associated ADCC in cord blood may explain the high rate of CHB in the context of vertical transmission.

P-1-110

Intactness and function of Nef proteins from an elite-neutralizer patient cohort

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While people living with HIV (PLWH) generally mount robust antibody responses against the virus, only rare patients develop high affinity, broadly neutralizing antibodies (bNAbs). Difficulties in generating bNAbs include that the conformation of the viral glycoprotein shields neutralization epitopes from antibody recognition and that the bNAb generation requires particularly complex genetic rearrangements. In addition, HIV infection leads to a profound and generalized B-cell dysfunction, including B cell exhaustion and hypergammaglobulinemia. Using experimental model systems, we previously identified the HIV pathogenesis factor Nef as necessary and sufficient to impair the helper function of HIV-1 infected CD4 T helper cells to B cells and demonstrated that Nef impairs the induction and maturation of high affinity, antigen-specific antibodies by disrupting immunological synapses between CD4 T- and B-cells (Kaw et al., 2020, Embo J.).

If Nef drives evasion from humoral immunity, one reason why PLWH with efficient bNAb responses (elite neutralizer) may be infected with HIV variants whose Nef proteins are defective in this activity. To test this hypothesis, we set out to analyze *nef* sequence and function in recently described patient cohorts without bNAb response (non-neutralizers) and elite neutralizers (Schommers et al., 2023 Nat. Med.). The 3' half of proviral genomes was amplified from patient peripheral blood mononuclear cells and subjected to oxford nanopore sequencing. We developed an analysis workflow that corrects noisy long reads, reconstructs major and minor pro-viral haplotypes *de novo*, and enables detailed annotation, characterization, and visualization of associated viral variants. Our results indicate that high neutralizing ability is associated with a decrease in intact *nef* sequences. In particular, proviruses from elite neutralizers exhibit a

higher frequency of APOBEC-mediated hypermutation. Most of these hypermutations reflect G-to-A mutation at the *nef* start codon, which is embedded in a previously unrecognized APOBEC3G consensus sequence. Intact *nef* variants code for all previously identified protein interaction motifs that mediate classical Nef activities. However, the molecular determinants of Nef-mediated humoral immunity disruption are unclear and we are therefore currently cloning patient-derived *nef* sequences into expression vectors. The initial functional characterization of these Nef variants will be presented.

P-1-111

Persistent changes in the NK cell and UTC immune signatures after cure of hepatitis C

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Despite the effectiveness of direct-acting antivirals (DAAs) in treating hepatitis C, it remains a prominent topic of investigation due to its widespread prevalence and significant morbidity and mortality. Hepatitis C leads to immune system dysfunction, characterized by alterations in the phenotype and function of immune cells. This impairment has been shown to persist even after successful treatment with DAAs. The role of hepatitis C associated long-term alterations in NK cell- and UTC immune signatures is unclear.

Thus, a comprehensive analysis of UTC and NK cell immune signatures of patients with chronic hepatitis C prior to treatment and 4-6 years post HCV eradication, was conducted by utilizing multi-color flow cytometry.

We identified a persistent reduction in the frequency of MAIT cells in patients with chronic hepatitis C, even after the elimination of HCV. Additionally, patients with chronic hepatitis C exhibited significant phenotypic alterations indicative of activation and exhaustion in UTCs and NK cells, with the most notable changes occurring in NK cells, succeeded by MAIT cells, γδ T cells, and DN T cells. Following DAA therapy, NK cells displayed the most pronounced recovery, succeeded by γδ T cells, MAIT cells, and DN T cells, with the SDI of NK cells achieving full normalization, in contrast to earlier studies with a shorter follow-up duration. Importantly, although certain markers showed partial or complete normalization following DAA therapy, hepatitis C-associated phenotypic changes in UTCs and NK cells were still present for several years after cure of chronic hepatitis C. Moreover, post-DAA therapy, NK cells and UTCs exhibited novel phenotypic modifications that were absent prior to DAA treatment.

Consequently, these findings indicate that the recovery process of the immune signatures persists for several years post DAA treatment while concurrent new phenotypic transitions occur, necessitating additional long-term follow-up studies over extended periods and epigenetic investigations.

Fig. 1

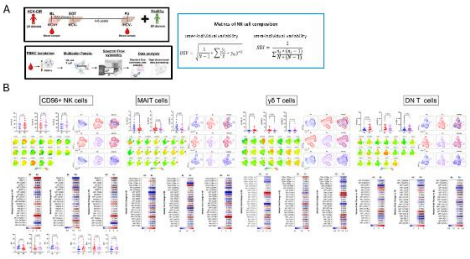
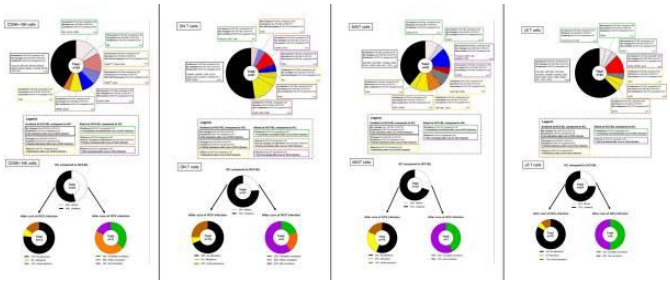


Fig. 2



P-1-112

Improved targeting of human CD4+ T cells by single domain antibody coupled lipid nanoparticles

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Background: Antiretroviral therapeutic regimens do not specifically address the integrated proviral DNA (i.e., the provirus). PROVIREX is pioneering novel curative strategies utilizing the *in vitro*-engineered Brec1 recombinase, which excises the provirus from a majority of HIV-1 strains and subtypes with notable specificity. The prevailing method involves the lentiviral transduction of hematopoietic stem cells followed by the autologous transplantation of these genetically modified cells, a process that is intricate and resource-intensive. The application of single domain antibody (sd-Ab)-conjugated lipid nanoparticles (LNP) has the potential to streamline the administration of the HIV-targeted recombinase, rendering this personalized medical approach accessible to low-income countries.

Methods: LNPs conjugated with CD4-specific sd-Ab were evaluated on a T cell line, primary CD4+ T cells, as well as peripheral blood mononuclear cells (PBMC), regarding transfection efficiency, specificity, and antiviral efficacy. Both cell lines and primary cells underwent transfection using LNPs, followed by analysis through flow cytometry, digital droplet PCR (ddPCR), and endpoint PCR.

Results: The transfection assays utilizing LNPs coupled with sd-Ab demonstrated remarkable transfection efficiencies exceeding 90% in T-cell lines. Notably, high transfection

rates were observed in primary CD4+ T cells, achieving up to 30% with minimal LNP concentrations. In PBMC, CD4-dependent transfection rates reaching 50% were recorded in CD4+ T cells, accompanied by low background in other cell populations.

Conclusions: LNPs conjugated with CD4-specific sd-Ab effectively facilitate the transfection of cell lines, isolated primary CD4+ T cells, and CD4+ T cells within mixed blood cell populations (PBMC). *In vivo* targeting of infected CD4+ T cells may contribute to the reduction of viral reservoirs, promoting immune system reconstitution, which enables people living with HIV (PLWH) to sustain stable viral loads without ongoing medication ("functional cure"). This strategy could also lead to extended periods without antiretroviral therapy (ART), thereby diminishing the risks associated with drug resistance and long-term toxicity.

P-1-113

Inflammation metabolism in tuberculosis: Impact of ROS and RedOx Metabolites on active TB pathology and PTLD development

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More than 1/3 of patients with pulmonary tuberculosis suffer from exacerbations leading to severe long-term lung impairment and post TB lung disease (PTLD). In TB sequel I, we showed association of neutrophil activity with severity of immunopathology by correlating concentrations in blood and sputum of neutrophil marker proteins such as MMP8 / 9, MPO, Lipocalin2 and calprotectin with lung impairment. Infection of neutrophils with *Mycobacterium tuberculosis* (Mtb) leads to oxidative burst followed by necrotic cell death. Different intrinsic RedOx Buffer systems, such es glutathione (GSH), thioredoxin, or taurine can reduce reactive oxygen species (ROS) and protect proteins and other molecules from oxidative damage. However, these protection mechanisms seem to fail in long-lasting inflammatory diseases such as TB. In the upcoming TB Sequel II study, we will focus on the metabolism of inflammation by investigating ROS induction, their impact on disease exacerbation and outcome and influence of N-Acetyl-Cystein (NAC) supplementation to recover GSH as RedOx-Buffersystem.

To analyses the capacity of ROS and necrosis induction, as well as mycobacterial growth, we established a 96 well plate based whole blood infection assay. First data showed significant reduction of ROS and necrosis by in vitro NAC and GSH treatment after Mtb infection. We will further comprehensively characterize neutrophils from whole blood and isolated from sputum by flow cytometry to elucidate the impact of pro and anti-inflammatory or low / high density neutrophil phenotypes.

To assess oxidative stress in the lungs of active TB patients, we measure ROS as well as protein carbonyls directly from sputum. RedOx metabolites i.p. from cysteine pathways, as well as energy molecules will be analyzed using 1H NMR and compared in whole blood and sputum. We established a metabolite extraction protocol preserving the redox state of cysteine residues to compare reduced and oxidized GSH and protein glutathionylation. *In vitro* metabolomics of neutrophils already revealed significant metabolic shifts upon activation, including increased glucose, NADP, and acetate, indicating glycolysis activation, while decreases in NAD, tryptophan, taurine, and total GSH suggest oxidative stress responses, with further investigation needed for the fate of taurine and its derivatives hypotaurine and taurine chloramine.

The generated data will show new insights into neutrophil phenotypes, their interaction with human inflammation metabolism and its impact on TB exacerbations.

P-1-114

Early Neutrophil associated markers correlating with postTB lung impairment in MDR TB

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Background: Tuberculosis (TB) is the #1 bacterial killer worldwide. Despite successful antibiotic treatment, patients with exacerbated lung inflammation do not regain sufficient lung capacity and develop postTB lung disease (PTLD). We hypothesized that 1) neutrophils play an important role during the acute phase of TB leading to disease exacerbation and PTLD and 2) associated markers can be used for early prediction of PTLD and as targets for personalized host-directed therapy.

Methods: In two cohorts, patients with confirmed MDR and DS TB were recruited at the East European Study Site in Bucharest. Sputum samples were taken over 6 months (MDR) or 2 months (DS) during antibiotic treatment. Sputum derived leucocytes were counted, and neutrophil associated proteins such as Interleukin 8, Calprotectin, lipocalin 2 (NGAL), myeloperoxidase (MPO), neutrophil elastase (ELA2), matrix metalloproteinase (MMP) 8/9 and regulators were analyzed by ELISA. In addition, we performed comprehensive proteomic analysis of plasma samples using O-Link multiplex technique. Mycobacterial burden was assessed by smear microscopy, solid and liquid culture. TB severity was assessed at baseline and month 6 using spirometry (lung function) and x-ray (lung pathology) and patients were categorized in mild and severe diseased.

Results: Patients with MDR-TB showed higher concentration with neutrophil proteins with comparable lung impairment. 16 patients (64 %) from MDR cohort had severe impairment in lung function after treatment initiation and month 6, but 44 % showed at least an improvement of 10 % either in FEV1 or FVC. In contrast, x-ray pathology was improving in 10 patients (40 %) and remained stable severe in 10 patients (40 %). Ralph scores were significantly higher in patients with impaired lung function. Leucocyte concentrations and neutrophil associated markers significantly declined under antibiotic treatment. Patients with stable severe impairment showed significantly increased MMP8/9 sputum concentrations at baseline and increased concentrations of Calprotectin, MPO, ELA2 and NGAL at week 2. In addition, Calprotectin, MMP8 and NGAL

concentrations were increased in males at month 4, while no sex differences in x-ray pathology was observed. No correlation of baseline mycobacterial burden with lung impairment or concentration of neutrophil associated proteins were observed.

Conclusion and Outlook: Early postTB lung impairment was associated with neutrophil proteins in the acute phase of TB and demonstrates the impact of neutrophils on disease progression and immune pathology. Transcriptome and multiplex protein analysis of corresponding blood samples will further elucidate systemic inflammatory properties leading to PTLD. Neutrophils associated proteins will be further analyzed as targets for host-directed therapies to reduce oxidative stress (MPO), tissue degradation (proteases), as well as immune modulators (calprotectin) to prevent PTLD.

P-1-115

Dissecting host immune responses in hepatitis E virus infection: NanoString analysis across diverse disease courses

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Hepatitis E virus (HEV) infection can clinically range from asymptomatic and acute symptomatic cases to chronic infections, particularly in immunocompromised individuals. This study employs systems biology, utilizing NanoString's nCounter® Host Response Panel to dissect the expression of 785 genes across 50+ host-response-related pathways, assessed tissue-specific damage, developed host-response signatures, identified biomarkers for disease severity, and quantified the relative abundance of 14 immune cell types in patients with different HEV disease courses. Our cohort comprised peripheral blood mononuclear cells (PBMCs) from 35 HEV-infected patients and uninfected controls. Subgroups included acute HEV patients without significant comorbidities (n=10), chronic HEV patients post-solid organ transplantation (n=4), asymptomatic HEV PCR positive blood donors (n=7), and acute patients with rheumatologic diseases or lymphomas (n=3). We analyzed gene expression patterns relevant to host susceptibility, interferon response, innate and adaptive immune activation, and immune homeostasis. Notably, patients with rheumatologic conditions or lymphomas displayed a significant downregulation of HLA-DQB1 (immune modulation) and upregulation of OASL (antiviral RNA degradation) compared to asymptomatic individuals. Chronic HEV patients post-transplantation showed upregulation of IL1RN (inflammatory regulation), IL1B (pro-inflammatory cytokine), and MARCKS (mucin and inflammatory response regulation), along with PTGS2, SOD2, and IL6—key markers of chronic inflammation and liver damage. Our results found no significant expression differences between asymptomatic and acute patients without relevant underlying conditions or between acute patients with and without rheumatologic or lymphoproliferative conditions. Flow cytometry is underway to validate these findings. This study highlights distinct immunological profiles associated with HEV in various clinical groups, emphasizing key pathways such as IL-1 signaling, leukotriene and prostaglandin regulation, oxidative stress, MHC class II antigen presentation, and virus-host interactions. These insights contribute to understanding HEV pathogenesis and may guide therapeutic strategies, particularly for vulnerable patient populations. Further investigation is essential to clarify mechanisms underlying HEV"s clinical heterogeneity.

P-1-116

Analysis of the CXCL9-11/CXCR3 axis in hepatitis D

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Question: In this study, we characterized the induction pattern of inflammatory chemokines in HDV-infected primary human hepatocytes (PHHs) and CXCR3-mediated chemotaxis of T cells in chronic hepatitis D (CHD).

Methods: We performed qPCR, RNA in situ hybridization (ISH) and FACS analysis in liver biopsies and blood samples from patients with chronic HBV infection (CHB) and CHD. Chemokine expression was investigated in cultured HBV/HDV-infected PHHs and in livers of HBV/HDV-infected humanized mice, in the presence or absence of adoptively transferred human T cells.

Results: HDV infection highlighted CXCL9-11 as the most strongly induced chemokines. Interferon lambda-1 (IFNL1) was also strongly induced by HDV and blocking of the IFNL receptor before HDV infection resulted in reduced CXCL9-11 induction in cultured PHHs. ISH analysis of HDV-infected livers from patients and chimeric mouse revealed that PHHs substantially contribute to chemokine expression in vivo. Moreover, the corresponding chemokine receptor CXCR3 was enhanced on CD4 T cells in the periphery of CHD patients. CXCR3-upregulation was unspecific and was not detected on HDAg- or HBsAg-specific CD4 T cells by AIM assay. Adoptive transfer of human T cells in humanized mice led to the recruitment of non-HBV/HDV-specific CD4+ T cells only in the setting of HBV/HDV co-infection, but not in HBV-mono-infected mice.

Conclusions: HDV infection enhanced the expression of CXCL9-11 in hepatocytes, and such induction was augmented by IFNL1 production. The CXCL9-11 increase correlated with the accumulation of bulk CXCR3+T cells in HDV-infected liver. This pathway may contribute to the aggravated liver inflammation in CHD patients.

P-1-117

Targeting the methyl-D-erythritol phosphate (MEP) pathway in *M. tuberculosis*: CRISPRi-based hypomorphs and novel reporter strains in antitubercular compound discovery

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As part of global efforts to accelerate and advance the development of novel and effective antitubercular therapies, our group has recently expanded its research interests into early-stage anti-Tb drug discovery. With a strong collaborative network across Germany and internationally, we have access to a diverse array of novel chemical scaffolds and have now established a robust *in vitro* workflow for antitubercular compound testing, mammalian cytotoxicity assessment, and mechanism of action elucidation. In this poster we present an overview of the methods and technologies that we currently use in our drug discovery work, as well as several examples of chemical series that have been assessed in this way. In particular, we focus on our efforts to identify novel compounds targeting enzymes of the methyl-D-erythritol phosphate (MEP) pathway, involved

in isoprenoid biosynthesis. We have so far generated CRISPRi-based hypomorph strains of the first two enzymes of this pathway, Dxs1 and Dxr, and through them validated Dxr as the target of one novel antitubercular compound. CRISPRi-mediated transcriptional repression is a powerful tool for whole cell-based target validation of test compounds, and we are currently expanding this technology to cover further mycobacterial genes of high relevance and importance in antimycobacterial drug discovery (e.g. MmpL3, DprE1, QcrB). Elsewhere, we are developing methods for rapid compound mechanism of action identification, including the use of fluorescent reporter strains (fluorescence induction upon exposure to various stresses, such as cell wall damage, iron limitation, or DNA damage). We are constantly seeking to update and improve our drug discovery platform, and therefore conclude with a roadmap of future goals and aims that should help us to strengthen our competencies in this field.

P-1-118

Ten-year follow-up after 96 weeks treatment with peginterferon plus tenofovir in hepatitis D (HIDIT-II)

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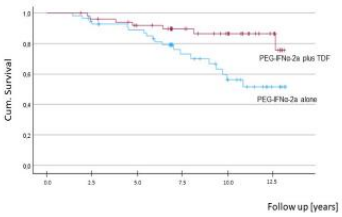
Background and Aims: Chronic delta hepatitis represents a major health burden. Until recently, pegylated interferon-alfa-2a (PEG-IFNa) therapy was the only treatment option for patients infected with hepatitis D virus (HDV). The aim of this study was to evaluate long-term clinical and virological outcomes after 96 weeks of treatment with PEG-IFNa with or without tenofovir disoproxil fumarate (TDF).

Methods: We performed a retrospective follow-up study of the Hep-Net-International-Delta-Hepatitis-Intervention-Study 2 (HIDIT-2 trial). Patients had received 96 weeks of treatment with either PEG-IFNα-2a plus TDF or PEG-IFNα-2a alone. Patients were included if they had completed the 96-week treatment period and had at least one follow-up visit (PEG-IFNα-2a plus TDF; n=51, PEG-IFNα-2a alone; n=56). Liver-related complications were defined as liver-related death, liver transplantation, hepatocellular carcinoma (HCC) and hepatic decompensation defined as ascites, variceal bleeding and/or hepatic encephalopathy.

Results: Patients who received PEG-IFNα-2a plus TDF were younger (37 vs 42 years), no significant differences were observed in other baseline characteristics between the two treatment groups. The mean follow-up period was 8.4 years (with a range of 1.8 to 13.1 years). A total of 26 patients (24%) developed one or more liver-related endpoints after a mean time of 6.0 (1.4-12.6) years. Twent-two patients were re-treated with IFN and 41 with Nucs, whereas 4 patients were treated with bulevirtide. Histological cirrhosis was detected in 42 patients (39%) at baseline and another 29 patients progressed to cirrhosis after a mean time of 1.8 (0.9-6.8) years. Following the conclusion of the therapeutic regimen, 48 patients had undetectable HDV RNA, of whom 59% patients experienced a relapse during the mean follow-up of 8.5 years. Fifteen patients lost HBsAg; none of them developed an event (p=0.01). No significant differences were observed between the two treatment arms with regard to undetectable HDV RNA, HDV RNA relapse, HBsAg loss, re-treatment administration, or progression to cirrhosis. It is of note that patients who received PEG-IFNα-2a alone demonstrated a significantly greater incidence of clinical complications (p=0.01). The development of clinical complications was further found to be associated with non-response to therapy (HDV RNA and HBsAg), age and baseline cirrhosis, as well as baseline values of platelets, AST, GGT, bilirubin and albumin using a Cox regression model. In multivariate analysis HDV RNA ever negative, treatment without TDF, age, platelets, GGT and albumin were significantly associated with endpoints.

Conclusions: The 10-year follow-up of a large randomised clinical trial demonstrates that HDV RNA response to PEG-IFNα-2a treatment and loss of HBsAg are associated with an improved clinical long-term outcome. Furthermore, concomitant therapy with TDF seems to be independently associated with a favourable clinical course.

Fig. 1



P-1-119

Post covid predictors in the cross-sectional cohort platform (SUEP) of the national pandemic cohort network (NAPKON) in Germany

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Previously established risk factors for the development of Post-COVID syndrome (PCS) are hospitalization, length of hospital stay, intensive care unit admission, COVID-19 symptom severity, obesity, smoking, age, sex, comorbidities, COVID-19 vaccinations, and COVID-19 drug treatments.1-3 In the prospective and multi-center Cross-Sectoral Platform (SUEP) of the German National Pandemic Cohort Network (NAPKON), we aimed to confirm previously found predictors and identify new ones. PCS scores were calculated using a symptom-based Post-COVID score at the 12-month follow-up.4 New predictors were identified using an XGBoost algorithm, selecting the top 10% most important variables for PCS classification. Odds ratios (ORs) for PCS predictors were assessed via both uni- and multivariable analyses. Missing 12-month follow-up data were imputed using the missing indicator method (NA category) and multiple chained equations with random forest. Among 1,088 patients, the risk of PCS was increased if, during the acute infection, the patient was older (ref: 18-45 years, 45-64 years: OR 1.65 [95%CI: 1.24;2.21]; >64 years: OR 1.99 [95%CI: 1.46;2.73]), had more symptoms (ref: 0-2; 3-5: OR 1.42 [95%CI: 1.06; 1.92]; 6-8: OR 1.62 [95%CI: 1.04;2.58]; >9: OR 2.38 [95%CI: 1.26;4.74]), had a higher disease severity (including hospitalization and oxygenation), had a immunological disease history (e.g. diabetes, cancer, rheumatism) (OR 2.70 [95%CI: 1.05;8.36]) or had an infection with the alpha variant of the virus compared to omicron (OR 1.67 [95%CI:1.16;2.40]). In addition, we found a lower risk of PCS among vaccinated patients (OR 0.57 [95%CI: 0.38;0.85]). In conclusion, this study enhances our understanding of key predictors associated with PCS, contributing to refined risk assessment and tailored preventive strategies, with the notable finding that vaccinated patients have a reduced the risk of PCS by 43%.

References:

1. Tsampasian V, Elghazaly H, Chattopadhyay R, et al. Risk Factors Associated With Post-COVID-19 Condition: A Systematic Review and Meta-analysis. *JAMA Intern Med*. 2023;183(6):566-580. doi:10.1001/jamainternmed.2023.0750
2. Boscolo-Rizzo P, Guida F, Polesel J, et al. Sequelae in adults at 12 months after mild-to-moderate coronavirus disease 2019 (COVID-19). *Int Forum Allergy Rhinol*. 2021;11(12):1685-1688. doi:10.1002/alr.22832
3. Bramante CT, Buse JB, Liebovitz DM, et al. Outpatient treatment of COVID-19 and incidence of post-COVID-19 condition over 10 months (COVID-OUT): a multicentre, randomised, quadruple-blind, parallel-group, phase 3 trial. *Lancet Infect Dis*. 2023;23(10):1119-1129. doi:10.1016/S1473-3099(23)00299-2
4. Appel KS, Nürnberger C, Bahmer T, et al. Definition of the Post-COVID syndrome using a symptom-based Post-COVID score in a prospective, multi-center, cross-sectoral cohort of the German National Pandemic Cohort Network (NAPKON). *Infection*. 2024;52(5):1813-1829. doi:10.1007/s15010-024-02226-9

P-1-120

Genome-wide transcriptome analysis reveals unique transcriptional control of effector functions in tissue-resident CD8 T cells during persistent viral infection

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Introduction: Chronic hepatitis B virus infection, 300 million patients worldwide, and virus-specific CD8 T cells loss of function of these cells are not well understood. An efficient CD8 T cell response is vital to preventing viral infection. Thus, we aimed to understand the transcription regulation of weak T cell response, which characterizes dysfunctional CD8 T cells in persistent viral liver infection.

Methods: C57BL/6 mice were infected with recombinant adenoviruses encoding the model antigen ovalbumin either under the ubiquitous CMV promoter (Ad-CMV-GOL) or the hepatocyte-specific TTR promoter (Ad-TTR-GOL), allowing for the induction of a resolved or persistent liver infection, respectively. Antigen-specific CD8 T cells were isolated and analyzed by flow cytometry and subjected to transcriptome profiling. To study the regulation of gene expression in hepatic cells after acute-resolving and chronic viral infection, we generated the genome-scale transcriptome profile using RNA sequencing. Differentially expressed genes (DEGs) between resolved and persistent liver were identified using the DSeq2 algorithm.

Results: Antigen-specific liver resident cells identified by expression of CXCR6 and CD69 developed after acute self-limited Ad-CMV-GOL and persistent Ad-TTR-GOL infection. In contrast to acute self-limited infection, no CX3CR1-expressing effector memory T cells were detected in the liver during persistent Ad-TTR-GOL infection. The transcriptome analysis enabled us to capture the core TRM signature by comparing tissue-resident populations from different tissues and infection models. Furthermore, we constructed transcription factor target networks using a computational approach and predicted the hierarchy and dynamics of those networks. This analysis identified *Crem* transcription factor, which regulates T cells. We also observed the *Crem* activity in circulated HBVcore-specific CD8 Tcells from HBV-infected patients.

Conclusion: We describe liver-resident CD8 T cells during persistent viral infection. Regulation of these T cells is governed by one single transcription factor whose activity is induced by the regional environment in the liver in preclinical models and HBV patients.

P-1-121

Anti-HDV prevalence and virological characteristics of samples in routine diagnostics at a major German university hospital (2008–2024)

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Background and Aim: Hepatitis D virus (HDV) coinfection is the most severe form of viral hepatitis, yet data on its prevalence and patient characteristics in Western Europe remain limited. This study aimed to assess the prevalence of anti-HDV antibodies and examine the virological characteristics of anti-HDV positive patients in a large German Center.

Methods: We retrospectively analyzed testing records from our laboratory information system, including HBsAg (Centaur Siemens, since 2018 Alinity i), quantitative HBsAg (Architect, since 2018 Alinity i; Abbott), anti-HDV antibodies (Diasorin, with the Liaison XL system since 2018), HBV DNA quantification (Cobas Roche, since 2016), and HDV RNA (LDT qPCR assay since 2022, normalized to IU/ml). HDV genotype was determined by Sanger sequencing (R0 or R1 region) or NGS using the iSeq100 system for HDV RNA-positive samples.

Results: From 2008 to 2024, over 6000 individuals tested positive for HBsAg, with a median of 10 [range 4-28] new anti-HDV positive cases identified per year. The anti-HDV screening rate was approximately 60% across the period. However, reflecting increased clinical awareness and new treatment options, screening rates rose, from <100 individuals in 2008 (4 anti-HDV positive) to >300 in 2023 (26 anti-HDV positive). The median anti-HDV prevalence was 6% [range 3-9%/year], with anti-HDV positivity rates remaining stable despite increased screening. Among 214 anti-HDV positive patients, 63% were male, with a median age of 42 years [range 19-76]. HBsAg was reactive in 94%, with a median quantitative HBsAg level of 4365 IU/ml [n=177; range 0.05 - 1x10^5 IU/ml]. HBV DNA was detectable in 70% of cases, with a median viral load of 570 IU/ml [range 2 - 9x10^8 IU/ml], and HBeAg positivity was observed in only 9%. HDV RNA testing was performed in 72% (156/217) of patients; of these, 43% had detectable HDV RNA, with a median HDV RNA titer of 15000 copies/ml (n=52; range 20 - 2x10^8) and 165,070 IU/ml (n=16; range 86 - 1x10^7). HDV genotyping in 60/68 HDV RNA-positive patients showed 88% genotype 1 (n=53), 10% genotype 5 (n=6), and 1 patient with genotype 8.

Conclusion: This large single-center cohort study confirms an anti-HDV prevalence of 5-10% among HBsAg-positive patients in Germany. Higher screening compliance for newly diagnosed HBsAg-positive patients did not reduce the anti-HDV antibody positivity rate but did decrease the overall HDV RNA positivity rate. In addition, genotyping unexpectedly revealed that 10% of HDV RNA-positive patients in our cohort were infected with non-genotype 1 HDV strains.

P-1-122

Pediatric tuberculosis testing: A systematic review and meta-analysis of individual participant data comparing the diagnostic yield of urine and respiratory tests for pulmonary tuberculosis in children

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Background: Tuberculosis causes high mortality in children, yet diagnosis is often delayed or missed due to limited access to rapid tests and difficulties in obtaining sputum samples. Urine-based lipoarabinomannan (LAM) tests, offer a promising alternative to conventional sputum testing. Comparing novel tests on more accessible sample types only on accuracy, overlooks important practical aspects, such as sample availability. To better capture clinical utility, we estimated the diagnostic yields, considering both sample availability and accuracy, in all tested (DYT) and all diagnosed (DYD), for urine LAM point-of-care and respiratory WHO-recommended molecular tests.

Methods: We systematically searched six databases up to December 20, 2023, for randomized controlled trials, cross-sectional studies, and cohort studies. Using a two-stage individual participant data meta-analysis, we predicted diagnostic yields (DYD and DYT) for each test per study and pooled DYD across studies. We also calculated sample availability and accuracy for each test, diagnostic yields for test combinations and subgroup analyses including clinical setting, HIV status, age and mortality. DYD"s denominator included confirmed and unconfirmed cases. Bayesian latent

class analysis (LCA) addressed the absence of a perfect reference standard. This study is registered with PROSPERO (CRD42021230337).

Preliminary findings: From 537 records, we included six datasets with 2,276 participants. All studies assessed sputum Xpert MTB/RIF or Ultra (Cepheid) along with urine AlereLAM (Abbott). In a preliminary analysis of 746 children, 85.5% provided urine and 56.5% provided sputum (spontaneously or induced). Sensitivity with LCA was 58% [95% credible interval (CrI): 52-67] for AlereLAM and 58% [95%CrI: 51-68] for Xpert on sputum samples. DYD was 42% [95%CrI: 39-45] for AlereLAM, and 36% [95%CrI: 33-39] for Xpert on sputum samples.

Preliminary interpretation: AlereLAM demonstrated a notable diagnostic yield, exceeding Xpert"s yield on sputum samples, suggesting practical value for LAM tests, especially in setting with limited facilities for respiratory sampling. Data from three additional studies will further clarify these trends.

Funding: German Center for Infection Research (DZIF).

P-1-123

Costs for global guideline-based diagnosis of mucormycosis

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Question: Mucormycosis is a rare invasive fungal infection which is characterized by prolonged antifungal therapy, high morbidity and mortality rates, as well as increased treatment costs. Appropriate diagnosis of mucormycosis is a fundamental component of successful treatment, however, evidence about health economic expenses does not exist.

Methods: Based on an international guideline approach by the European Confederation of Medical Mycology (ECMM) and the Mycoses Study Group Education and Research Consortium (MSGERC) for diagnosis of mucormycosis, we calculated costs for imaging-based and laboratory procedures as well as susceptibility testing from the German payer perspective. All costs were calculated by using flat rates of the so-called Uniform Valuation Standard ["Einheitlicher Bewertungsmaßstab" (EBM)], representing costs for medical services of the statutory health insurance. We therefore analyzed the diagnostic recommendations for patients at increased progression risk, i.e., neutropenia, previous solid organ transplantation or haematopoietic stem cell transplantation.

Results: By using a micro-costing approach, we calculated diagnostic costs of €499.40 per mucormycosis case. The most important cost drivers (approximately 85% of overall costs) were imaging procedures such as CT scans of sinuses, chest, abdomen, and pelvis as well as CT-guided biopsy. Costs for microbiological detection were relatively low compared to other cost factors. The overall diagnostic costs for 193 confirmed mucormycosis cases in 2022, which corresponds to an incidence rate of 0.232 cases per 100,000 people per year, were €96,384.

Conclusions: From the health economic point of view, our analysis underlines the relevance of appropriate guideline-based diagnosis of mucormycosis. The overall costs are relatively low compared to other components in the management of mucormycosis, such as cost-intensive treatment with antifungal agents. Nevertheless, it is important to bear in mind that the level of diagnostic accuracy in line with the global guidelines by the ECMM/MSGERC requires substantial resources, which may not be available in all countries or centers, especially in those with low income.

P-1-124

Immunogenicity of COVID-19 vaccination in immunocompromised patients (Auto-COVID-VACC): Protocol for a multicenter prospective non-interventional study

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Introduction: Despite availability of vaccines, immunocompromised patients are still at high risk for severe COVID-19. While vaccination schedules for the general population have been defined, achieving immunogenicity in immunocompromised patients remains a challenge. The primary objective is to analyze anti-spike-IgG titers after repeated mRNA vaccinations in immunocompromised patients. Further objectives are to analyze data on humoral immune responses and to evaluate data on cellular immune response.

Methods: This multicenter, prospective, non-interventional study aims to determine immunogenicity and reactogenicity of an implemented standard-of-care COVID-19 vaccination strategy in immunocompromised patients. A total of 100 patients will be recruited at three study sites. Additional blood samples will be drawn at each scheduled outpatient visit. Study-related blood samples will be used for evaluation of T and B cell response to COVID-19 vaccinations. For this study, no additional visits or invasive procedures will be performed in addition to standard care.

Discussion: Results will be used to optimize vaccination and booster schedules for immunocompromised patients and to increase rates of protection against severe SARS-CoV-2 infections. Further, results may identify risk and treatment factors, which lead to low immune responses in patients vaccinated against COVID-19, as well as the impact of repeated vaccination on B and T cell responses.

P-1-125

Seroprevalence of maternal TORCH infections and their association with adverse birth outcomes in a multicentre cohort study of pregnant migrant women in Germany

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Background: Congenitally transmitted infections, known as TORCH, have been linked to increased risks of adverse birth outcomes. Pregnant migrant women are particularly vulnerable and lack sufficient data on seroprevalence.

Methods: We examined IgG seroprevalence among 85 pregnant migrants from five medical centers, assessing toxoplasmosis, measles, varicella zoster virus (VZV), mumps, parvovirus B19, rubella, and cytomegalovirus. At birth, we measured newborn parameters and calculated risks for prematurity, low birth weight (LBW), and being small for gestational age (SGA) based on maternal serostatus.

Results: Median age of participants was 27 years (range 18-45) and the majority (51.2%) came from middle eastern countries followed by sub-Saharan Africa (41.5%). Seroprevalence was highest for Rubella and VZV (each 81.5%), followed by measles (44.4%) and lowest for PB19 (28.4%). An age- dependent increase of seropositivity was observed for VZV, whereas the opposite was the case for PB19. Seroprevalence didn"t differ among regions of origin. Prematurity and decreased birthweight were significantly associated with seronegativity of mothers against Rubella (OR 0.12 [0.02-0.66] p = 0.045 and 2954g vs 3346g p=0.017, respectively). LBW and SGA were not significantly associated with the serostatus of any TORCH infection, the same as head circumference and newborn length.

Discussion: Protective immunity against frequent and vaccine-preventable diseases such as measles was alarmingly low. Although congenital infections with rubella have become a rarity, seronegative status correlated with prematurity and LBW.

Conclusion: Completing the vaccination status of migrant women of reproductive age at entry requires special attention to ensure herd immunity and prevent subsequent congenital infections.

P-1-126

MALDI imaging of the clinical-stage anti-tuberculosis drug BTZ-043 in mouse tissue

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains a global health threat due to the emergence of drug-resistant strains and the formation of necrotic granulomas in the lung that are difficult for antibiotics to penetrate. The development of new drugs that can effectively target these lesions is crucial for improving TB treatment outcomes. BTZ-043 is a novel antibiotic that has shown promising bactericidal activity against Mtb in a phase IIa clinical trial. In this presentation we focus on the MALDI imaging methodology, which is the only technique capable of providing detailed information regarding the penetration process and accumulation behavior of drugs within affected tissue.

A number of measures were taken in order to insure the reliable and accurate detection of the drug. Prior to analysis, lung tissue sections were treated with γ-irradiation to inactivate the pathogen. A mimetic tissue model spiked with BTZ-043 as a model system. In addition to MALDI imaging, these samples were analyzed by HPLC-MS/MS measurements in order to exclude potential irradiation effects. Ion suppression effects, i.e. the influence of tissue composition on ionization efficiency was evaluated by applying a deuterated standard of BTZ-043. No significant effects were detected in these measurements. In conclusion, our workflow allowed investigating the distribution and efficacy of BTZ-043 in Mtb-infected IL-13tg mouse lung tissue with high reliability.

P-1-128

IKARUS research consortium "Infections in the cardiovascular system – pathophysiology, therapy and diagnostics"

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The clinical research consortium IKARUS (*Infektionen im Kardiovaskulären System: Pathophysiologie, Therapie und Diagnostik*/ Infections in the Cardiovascular System: Pathophysiology, Diagnostics and Therapy) aims to develop new diagnostic and therapeutic approaches to combat bacterial infections in the cardiovascular system. These infections commonly affect both natural and artificial heart valves or pacemakers, and their incidence is rising due to an aging population and advancements in medical technology.

Treating such infections poses significant challenges as bacteria can colonize implant surfaces, forming biofilms that shield them from antibiotics and the immune system. To address these issues, the IKARUS consortium integrates expertise from infectious medicine, cardiac surgery, cardiology, and microbiology, fostering innovation in both diagnostics and therapies. By focusing on the pathophysiology of bacterial infections, the consortium investigates novel diagnostic and therapeutic approaches while promoting young clinicians in the Clinician Scientist Program, which bridges clinical practice and research.

The consortium''s primary research areas include the analysis of microbial cell-free DNA (mcfDNA) as a biomarker for infective endocarditis, retrospective imaging evaluations to assess diagnostic sensitivity, development of risk scores for left ventricular assist device (LVAD) infections, metabolome analyses in heart failure, testing of antibiotic combinations, and resistance prophylaxis for *Staphylococcus aureus*. Additionally, the consortium is conducting studies on risk factors for post-interventional infections to optimize prophylactic treatments.

Although the research is in its early phase and results are not yet available, significant outcomes are expected following the implementation of new diagnostic and therapeutic procedures and clinical studies are planned, following any results derived from the basic projects in this consortium. Despite advancements in clinical and basic scientific research, current recommendations for the prevention and treatment of cardiovascular infections still rely heavily on outdated data and the experience of individual clinicians. IKARUS aims to change this by acquiring comprehensive and extensive data, leading to improved treatment strategies for patients. The creation of risk profiles and the development of scoring systems will enable the application of targeted prophylactic therapies. The next step will involve enhancing microbial diagnostics, a field desperate for innovation, as current diagnostic gold standards are outdated and fail to align with the technological progress of the 21st century.

Once infections are properly diagnosed and pathogens identified, the challenge will be to initiate optimized and individualized treatment regimens that are tailored to each patient''s specific needs. Interested researchers and clinicians alike are warmly welcome to participate in this future-oriented project.

P-1-129

Home care strategies for childhood illnesses before medical consultation in Ghana

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Caregivers in Sub-Saharan Africa often initially attempt to manage childhood illnesses at home, which can delay or complicate later diagnosis and treatment at a health facility. Understanding home treatment practices among children could help to characterize treatment history when information is unavailable or unreliable. We investigated these practices among children seeking care at three levels of a healthcare system in Ghana.

Children <15 years of age and their caregivers were recruited at Community Health Services and Planning (CHPS) clinics, outpatient departments (OPD) and inpatient departments IPD) in Agogo and Assin Foso, Ghana. Demographic, clinical, socioeconomic, and home treatment information were collected via interviews. Urine samples from children were cultured to test for antibiotic use. Hierarchical log-binomial models were fitted in R.

Caregivers of 1,503 children were interviewed. Forty-six percent (n = 689) reported any home treatment prior to the visit: 37% (n = 560) reported antipyretic use, 11% (n = 167) antimalarial, and 7% (n = 103) antibiotic use. Home medication was lower in the CHPS clinics (30%, n/N = 148/500) compared to the OPD (61%, n/N = 308/509) and IPD (47%, n/N = 233/494). Children treated at home had longer delays in seeking treatment (median 3 days, IQR: 1,3) compared to those not (median 2 days, IQR: 1, 3). In regression models, illness severity and specific symptoms were associated with antimalarial use but not antibiotic use. No prior antibiotic use was reported among most (*n*/*N* = 33/46) samples where antibiotic inhibition was indicated.

Home treatment practices in this region were common, although highly localized in prevalence, and home antibiotic use was not well-targeted. The discrepancy between self-reported antibiotic use and antibiotic inhibition suggests lack of awareness about medication identification and appropriate use. This also presents a challenge for clinicians in obtaining an accurate treatment history, which is highly relevant to the timely diagnosis and treatment of the illness in the facility.

P-1-130

Molecular epidemiology of respiratory syncytial virus (rsv) in children from Munich, Germany during the Covid-19 pandemic

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Question: Respiratory syncytial virus (RSV) is the major pathogen of lower respiratory tract infection (LRTI) in infants and young children, causing significant morbidity and mortality. In moderate climate zones, RSV infections usually occur seasonally with the highest incidence during the winter months from November to March. RSV strains can be divided into two different subtypes RSV A and RSV B and into further genotypes. ON1 and BAIX have been the predominant pre-pandemic genotypes of RSV A and B, respectively. The COVID-19 pandemic disrupted RSV and other respiratory viral infection patterns worldwide. In Germany, the expected winter season of 2020/2021 was absent, and unusually high rates of hospitalizations with RSV infections were reported during the summer months of 2021. This study aims to investigate the molecular epidemiology of RSV in Munich, Germany, during the COVID-19 pandemic.

Methods: Out of 6154 tested children, nasopharyngeal swabs from 328 children tested positive for RSV infection during the year 2021 at the Dr. von Hauner Children''s Hospital LMU University Hospital. RSV RT-PCR and sequence analysis of the second hypervariable region of the G gene coding for the attachment G glycoprotein was performed for RSV-positive tested samples. Phylogenetic analysis was conducted to identify the diversity of circulating RSV genotypes. Corresponding clinical data was retrospectively retrieved from medical records.

Results: A total of 328 children with a median age of 15.4 months (IQR 3.4 – 28.9) tested RSV-positive and were included in the analysis. More than half of RSV-positive

children were male (n=178/328; 54,3%) and had symptoms of a lower respiratory tract infection (n=258/326; 78,7%). The incidence of RSV infections started to rise in August and peaked in October and November. Sequence analysis of the second hypervariable region of the G gene was successfully conducted for 69 samples. The predominant RSV subtype was RSV A (n=52; 75,4%). All RSV-A strains belonged to the ON1 genotype containing a 72-nucleotide duplication. All RSV-B strains (n=17; 24,6%) could be attributed to the BAIX genotype containing a 60-nucleotide duplication. Identified ON1 and BAIX strains could be subdivided into four clusters in the phylogenetic tree corresponding to four defined ON1 and BAIX lineages, respectively.

Conclusion: We present phylogenetic and corresponding demographic and clinical data of RSV strains circulating during the COVID-19 pandemic in Munich, Germany. Mapping the spread of known RSV genotypes can contribute to a better understanding of unusual seasonal patterns. Our results suggest that rather non-viral influences on RSV transmission may have contributed to the surge of RSV infections during the summer of 2021.

P-1-131

Knowledge and perception of tick exposure and Lyme borreliosis in the general population in Germany

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Introduction: Lyme borreliosis (LB), a bacterial infection caused by various genospecies of *B. burgdorferi s.l.* complex, is transmitted to humans through the bite of infected *Ixodes spp* ticks and is the most common tick-borne disease in Europe. In Germany, data about the general population"s knowledge and risk perception towards LB are limited. In 2022, we surveyed adults in 20 European countries to investigate awareness about ticks and LB and level of concern about contracting LB. This abstract presents the results of the survey for respondents in Germany.

Methods: We used an existing survey panel to conduct an online survey of adults aged 18-65 years old, with recruitment quotas on age, gender, and region. The survey included questions about LB knowledge, perception of tick exposure, and outdoor activities. We conducted descriptive analyses with weighting to adjust for the complex survey design.

Results: Of 2733 respondents, 96% were aware of ticks and 85% were aware of LB. Among respondents that were aware of ticks and LB, 79% considered LB a severe disease, 38% were concerned about contracting LB, and 33% perceived themselves at risk of contracting LB. Overall, 58% of respondents reported a tick bite at least once in their lifetime. Always or often conducting tick checks was reported by 48% of respondents. Respondents reported spending an average of 10 hours outdoors at home and an average of 11 hours outdoors away from home each week.

Conclusion: In Germany, there is a broad knowledge of ticks and LB in the general population. Because people spend considerable time outdoors and do not regularly use prevention measures, new interventions are needed to address the risk for contracting LB and to mitigate this important public health concern.

P-1-132

Intracellular signaling cascades and cell death mechanisms of dendritic cells induced by *Mycobacterium tuberculosis* infection

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Tuberculosis remains a highly relevant infectious disease with more than 1.5 million deaths per year and a fast-evolving incidence of multi-drug resistant cases. To combat this, understanding how *Mycobacterium tuberculosis* (Mtb) interacts with human immune cells is critical for developing new host-directed therapies and efficient vaccines. As most *Mtb* related research focuses on Macrophages, the mechanisms of host cell infection, intracellular trafficking, and cell-death in other cell types, particularly Dendritic cells (DCs), are not yet well understood.

In our study, we dived deep into the response of DCs to *Mtb* infection, revealing striking contrasts between DCs and macrophages. Through global RNA analysis, we uncovered distinct transcriptomic profiles, with DCs exhibiting increased TNF-α and interferon signalling, and macrophages inducing a pro-inflammatory innate immune response with upregulation of antibacterial defence genes. This was further validated by multiplex cytokine analysis, where DCs and Macrophages showed increased levels of TNF-α and type I/II interferons, or pro-inflammatory cytokines, respectively.

Additionally, we were able to demonstrate increased resistance of DCs towards *Mtb*-induced cell death, with DCs showing significantly higher viability in flow cytometric analysis than macrophages up to 72 hours post-infection. Remarkably, AnnexinV/PI staining revealed that while infected Macrophages succumbed to a lytic cell death, DCs leaned towards apoptosis. This was further supported through a caspase 3/7 activity assay, which showed more caspase activity in infected DCs than macrophages. This finding is interesting concerning the pro-inflammatory response in *Mtb* infection and the spread of *Mtb* within its host. Protein expression analysis revealed further differences, such as a strong upregulation of autophagy and IFN-signalling related proteins in infected DCs, as well as less pronounced mitochondrial damage compared to macrophages.

Overall, these findings suggest a distinct phenotypic *Mtb*-response in DCs compared to macrophages, which could enhance our understanding of the human immune-response against *Mtb* and shed light on new possible targeted therapies.

P-1-133

Collateral damage of antibiotic eradication of nasal *Staphylococcus* aureus requires alternative decolonization strategies

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Question: *Staphylococcus aureus* is a major human pathogen which causes a wide-variety of infections. S. aureus colonizes approximately the nares of 20% of the human population. Decolonization treatments prior to invasive medical inventions lead to a reduced risk in subsequent infections. The gold standard method for decolonization of *S. aureus* is a treatment with the antibiotic

mupirocin, which causes faces several hurdles on the long run. Firstly *S. aureus* is usually recolonization after the treatment has stopped. Secondly antibiotic resistance to mupirocin in *S. aureus* is emerging. S*. aureus* is part of a complex microbiome in the human nares. Other bacterial species have promoting or suppressive effects on *S. aureus* nasal colonization. The effect of mupirocin treatment on the nasal microbiome is poorly understood on the species and strain level. In this study we determine the global effect of nasal decolonization strategies on the nasal microbiome. In addition, we propose potential alternative methods with increased precision and selectivity.

Methods: We assembled a comprehensive collection of nasal commensals from several human volunteers. Subsequently we used a combination of *in vitro* techniques and *in silico* prediction to determine antibiotic susceptibility and colonization dynamics upon mupirocin treatment. Furthermore, we validated our results with *in vivo* colonization data.

Results: Resistance to mupirocin is highly correlated with presence of genetic determinants. We show that presence of certain alleles of the mupirocin target explains antibiotic resistance across a wide variety of nasal commensals from very diverse phylogenetic clades. Presence of identified resistance determinants governs growth in a competitive *in vitro* system. Observed microbiome disturbances caused by mupirocin treatment correlate well with *in vivo* colonization data.

Conclusion: We show that mupirocin treatment has notable collateral side effects on nasal commensals perturbing the composition of nasal microbiomes. Side effects are especially pronounced on other- often harmless - commensals staphylococci, which are frequently antagonizing *S. aureus* colonization. We propose alternative antimicrobial approaches targeting molecular structures which are unique to pathogenic commensals. A promising alternative could be bacteriophages, microbial viruses. In contrast to frequently used antibiotics bacteriophages target distinct surface structures that are unique to *S. aureus* allowing potentially selective and controlled eradication of this pathogens from the human nose.

P-1-134

PhageSurf - Improving effectiveness of phage medicinal product manufacture and therapeutic administration by investigating phage-surface interactions in medically relevant devices

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Background: Antimicrobial resistance (AMR) is a major global health threat, rendering many conventional antibiotics ineffective against bacterial infections. Bacteriophages (phages), selectively target and lyse bacterial cells, offering an innovative and targeted approach to combat infections caused by resistant bacteria.

Problem statement: Despite the potential of phage therapy, its clinical application faces several challenges, particularly in the production, stability, and delivery of phage therapeutic medicinal products (PTMPs). A significant hurdle is phage adsorption (PA) to surfaces of bioreactors and medical devices, which leads to substantial loss of phage activity during manufacture and therapeutic administration. This loss impacts both the production process and clinical outcomes, but the extent and implications of PA on medically relevant devices are underexplored.

Objective: The project, PhageSurf, aims to systematically assess PA on surfaces of medically relevant devices, such as bioreactors, infusion and catheter systems, and its impact on PTMP production, delivery, and administration for urogenital and bloodstream infections (UTIs and BSIs), with a focus on the clinically relevant pathogens *Acinetobacter baumannii* and *Escherichia coli*.

Methods: Data will be collected from various experimental setups and testing approaches focused on assessing phage concentration before and after contact with testing material. This study, conducted across two laboratories in Cologne and Frankfurt, is designed to ensure reproducibility and broad applicability of results.

Conclusion: By improving our understanding of phage-surface interactions and optimizing phage manufacturing, concepts of phage therapy can be improved, providing a powerful tool in the treatment of bacterial infections that no longer respond to traditional antibiotics. This collaborative study will not only enhance phage production processes but also provide foundational guidelines for future personalized phage therapy studies in Germany.

P-1-135

Detecting *S. mansoni* and *S. haematobium* infections in endemic countries: A diagnostic accuracy study in an adult population in rural Madagascar

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Background and Objectives: *S. haematobium* and *S. mansoni* are endemic in Madagascar, but reliable diagnostic tools are often lacking, contributing to exacerbate transmission and morbidity. This study evaluated the diagnostic accuracy of three tests for schistosome infection

in Malagasy adults from areas of medium to high endemicity, assessing sensitivity (Se) and specificity (Sp).

Methods: This cross-sectional study enrolled adults from three primary health care centres in Madagascar between March 2020 and January 2021. Urine and blood samples were tested for schistosome infection using polymerase chain reaction (PCR), up-converting phosphor lateral flow test for the circulating anodic antigen (UCP-LF CAA), and point-of-care circulating cathodic antigen (POC-CCA) tests. Bayesian latent class models were used to assess diagnostic accuracies and disease prevalence.

Results: A total of 1,339 participants were analysed: 461 from a *S. haematobium* and 878 from an *S. mansoni* endemic area. In the *S. haematobium* area, positivity rates were 52% (POC-CCA), 60% (UCP-LF CAA), and 66% (PCR). In the *S. mansoni* area, rates were 54% (POC-CCA), 55% (UCP-LF-CAA), and 59% (PCR). POC-CCA had low Se and Sp in the *S. haematobium* area. UCP-LF CAA and PCR showed high Se but imperfect Sp. For *S. mansoni*, POC-CCA and PCR provided high Se and Sp, whereas UCP-LF CAA had high Se but imperfect Sp.

Discussion: At the population level, diagnostic tests showed similar prevalence in both endemic areas, but individual-level agreement was only low to moderate. Variability in Se and Sp across tests highlights uncertainty in true infection status. Continued development of accurate diagnostics suitable for highly endemic settings is essential to achieve the WHO 2030 target of eliminating schistosomiasis as a public health problem.

P-1-136

Integration of screening for soil transmitted helminths, filariasis, and schistosomiasis into outreach services for cervical cancer and HIV

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Introduction: In southwestern Tanzania, mobile health services have been implemented by PEPFAR to provide mobile HIV Testing and Counseling service (mHTC) to key vulnerable populations and conduct screening for sexual transmitted diseases and cervical cancer. The geographical areas covered by the mHTC are rural areas, that in addition are also known for high prevalence of soil transmitted helminths and schistosomiasis, and previously filariasis. Surveillance data of these areas showed a prevalence of *S. haematobium* of ~ 15%, *S. mansoni* of 11%, and hookworm of 50%. One are was endemic for *Wuchereria bancrofti* before govermental treatment activities reduced the prevalence.

Schistosomiasis, lymphatic filariasis and soil-transmitted helminths belong to the group of neglected tropical diseases affecting people mainly living in rural areas with limited access to clean water and proper sanitary. Schistosomiasis can lead to liver fibrosis, bladder cancer etc. Genital schistosomiasis is defined by egg deposition in the mucosa, leading to disruption of the barrier function and with that, an increased likelihood of transmission of sexual transmitted infections, HIV and human papilloma virus (HPV). For infections with *Wuchereria bancrofti* an increased HIV incidence was described earlier by our group, for hookworm infections an increase in HPV viral load was found recently.

Method: Cervical cancer screening is implemented in Tanzania using visual inspection after applying acidic acid. During the mHTC services cervical swabs are collected for PCR testing, targeting HR HPV. Stored samples will be used to test for *S. haematobium, and S. mansoni* in the future. Urine and stool samples will be collected in adition to test for intestinal helminths. HIV testing is offered and done according to the national guidelines using finger prick blood; circulating filarial antigen measurement can be added using this method. We plan to train and introduce self-collection of vaginal samples, reducing the barrier to accessing health personnel. In addition, local staff who already perform visual inspections for cervical cancer as part of a national cervical cancer screening program will be trained in the diagnosis of FGS in collaboration with local gynecologists. To improve outcome measurement, an open data kit (ODK)-based digital questionnaire was developed to collect socioeconomic and behavioral information.

Outcome: Integrating screening of NTDs into mobile health services for HIV and cervical cancer will be beneficial for more than only the targeted disease with no additional inconvenience for the particicants. Using existing structures and adding diagnostic measurements will be cost-effective. Preventing or treating one of the diseases may affect the outcome of the others in a positive way, as an impact of helminth diseases on the susceptibility towards HIV and HPV has already been shown.

P-1-137

Gram-negative bloodstream infections: Are we missing opportunities for better treatment? A retrospective cohort study

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Background: Gram-negative bloodstream infections (GN-BSI) are a major cause of hospitalizations and are associated with high morbidity and mortality rates. Although current guidelines recommend de-escalation, oralization, and appropriate treatment durations for GN-BSI, it remains uncertain if these practices are consistently implemented in clinical settings. This study investigates the management of GN-BSI in a tertiary care hospital, comparing uncomplicated and complicated cases and identifying risk factors for suboptimal therapy.

Methods: A retrospective study was conducted at University Medical Center Hamburg-Eppendorf from 02/2022 to 02/2023. Adult patients (≥18 years) with positive blood cultures for Enterobacterales or *Pseudomonas aeruginosa* were included, excluding those with concurrent gram-positive bacteremia. Optimal therapy, defined as using the narrowest-spectrum antibiotic within 24 hours of antibiogram

availability, was the primary outcome. Logistic regression analysis was used to identify factors associated with suboptimal therapy. Secondary outcomes included comparing actual versus expected therapy duration and evaluating missed opportunities for oralization.

Results: The study included 194 GN-BSI patients, with 93 having complicated and 101 having uncomplicated infections. Patients with complicated GN-BSI were younger (mean 58 years, SD 14.8), had higher rates of AmpC producers (22.6%) and *P. aeruginosa* (8.6%), and more frequent use of vascular devices (32.3%). Infectious disease (ID) consultation was obtained in only 12.9% of cases. Antibiotic duration was longer in complicated GN-BSI (median 12 days, range 5–105) compared to uncomplicated cases (median 10 days, range 4–32, $p < 0.01$), with a median deviation from the expected duration of 2 days. Optimal therapy rates were similar for both groups (31.2% in complicated vs. 30.7% in uncomplicated), but time to optimal therapy was longer for complicated cases (median 4 days vs. 3 days, p=0.02). Uncomplicated GN-BSI showed higher missed opportunities for oralization (83.9% vs. 65.5%, $p < 0.01$). Logistic regression analysis indicated a higher likelihood of suboptimal therapy in cases of *Klebsiella* infection (OR 3.8), immunosuppression (OR 8.5), elevated CRP, and a Charlson comorbidity index >3. Clinical improvement at 72 hours (OR 0.15) and source control (OR 0.4) were associated with better management. Complicated GN-BSI was not significantly associated with suboptimal treatment (OR 0.11).

Conclusion: Despite evidence supporting optimal GN-BSI management, gaps remain in achieving appropriate treatment in both complicated and uncomplicated cases. Antibiotic stewardship programs should address these gaps by encouraging oralization and de-escalation strategies, especially in settings with limited ID consultation resources

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P-1-138

Citrobacter freundii ST396 has emerged as a prevalent and high-risk pathogen of bloodstream infections in Germany

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Question: Common bacterial species responsible for bloodstream infections (BSI) include *E. coli*, *Enterococcus*, *Staphylococcus*, *Klebsiella*, and *Pseudomonas*. In recent years, bloodstream infections with *Citrobacter* have been reported with increasing frequency. Concurrently, the emergence of multidrug resistance (MDR) in *Citrobacter* is well reported in prevalence studies. No extensive comparative data exists regarding the association of BSI-causing emerging MDR *Citrobacter* species and the prevalence of virulence factors that may contribute to adverse clinical outcomes in these organisms. This study aimed to examine the genomic landscape of BSI *Citrobacter* (CITROBSI) from four German university hospitals.

Methods: From 2016 to 2023, 257 *Citrobacter* isolates were obtained from study sites in Cologne, Giessen, Lübeck, and Tübingen. 102 isolates were sequenced utilizing long-read sequencing by Oxford Nanopore Technology, prioritizing specific strains of *C. freundii* (CF) and *C. braakii* (CB). Genomic characteristics, including antibiotic resistance genes (ARGs), virulence factor genes (VFGs), and phylogenetic relationships, were assessed. *In vivo* infection experiments involving the invertebrate *Galleria mellonella* were conducted to assess virulence capabilities of *Citrobacter* isolates.

Results: The genomes of the isolates were taxonomically assigned to various species: 57 CF, 15 CB, 8 *C. portucalensis*, 7 *C. europaeus*, 7 *C. koseri*, 4 *C. youngae*, 3 *C. werkmannii*, and 1 *C. meridianamericanus*. Strains of the same species were classified into distinct MLST types and exhibited significant genetic diversity. The most prevalent MLST types within the CFs were ST396, ST18, ST19, and ST98. The predominant plasmid types identified were pKPC-CAV1321, IncFII(SARC14), and IncFIB(pHCM2). The beta-lactamase gene *bla*CMY and the quinolone resistance gene *qnrB* were commonly detected on chromosomal loci. A total of 108 VFGs encompassing the categories of adherence, invasion, effector transport system, motility, exotoxin, immunomodulation, biofilm, nutritional/metabolic factors, regulation, and antimicrobial activity/competitive advantage were identified in the sequenced isolates. CF encompassed approximately 21 VFGs per genome related to adherence (curli), invasion, immunomodulation, nutritional/metabolic factors, and regulatory mechanisms. Certain CFs also possessed T6SS-VFGs and exhibited genes for biofilm production, which correlated with various phylogenetic clades. In *Galleria mellonella* infection assays, the T6SS-positive ST396 strain exhibited the highest level of pathogenicity.

Conclusions: There is an increase in Citrobacter bloodstream infections in the university hospitals examined. The CITROBSI isolates exhibited significant genetic diversity and originated from various sources of infection. CF ST396, ST18, ST19, and ST98 were the predominant strains. Their pathogenicity varied among the genetic clades, with ST396 emerging as a potential novel high-risk clone.

P-1-139

Immune regulatory role of myeloid-rerived suppressor cells in Epstein-Barr virus-associated infectious mononucleosis – result from the IMMUC studies

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Background: Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of regulatory immune cells from the myeloid lineage with immunosuppressive activities. Previous studies reported an increase in MDSC frequencies in patients with EBV-associated nasopharyngeal carcinoma and chronic active EBV infection disease. However, little is

known about the role of MDSC during infectious mononucleosis (IM). Our study provides important insight into the frequencies of polymorphonuclear (PMN-) (CD33+HLA-DR-negative/lowCD14-CD15+), monocytic (M-) (CD33+HLA-DR-negative/lowCD14+CD15-), and early (e) (CD33+ HLA-DR-negative CD14-CD15-) -MDSC in pediatric patients during the early and late phases of IM. In addition, we determined the correlation of frequencies of MDSC subpopulations with the complexity and severity of laboratory and clinical, as well as virological IM features.

Material and Methods: Our study, conducted as part of the comprehensive IMMUC study, included 37 patients with IM following confirmed primary EBV infection. All patients were investigated at three study visits (V): V1 as early as possible after symptom onset (Tonset), V2 four to six weeks after Tonset, and V3 six to 12 months after Tonset. At each visit, we performed flow cytometry-based analyses of PMN-, M-, and e-MDSC using freshly isolated PBMC and assessed the severity and complexity of a wide range of clinical and laboratory IM features, as well as various virological parameters (EBV DNA load, EBV-specific antibodies), ensuring a comprehensive understanding of the dynamics of MDSC in IM.

Results: We observed decreased relative frequencies of PMN-MDSC from V1 to V2 and V1 to V3 (p=0.039 and 0.015) with simultaneously increased frequencies of M-MDSC from V1 to V3 (p=0.005) and V2 to V3 (p=0.015), and increased frequencies of e-MDSC from V1 to V2 and V1 to V3 (p=0.005 and <0.001), and from V2 to V3 (p=0.039). Dynamics of MDSC frequencies over time were significantly correlated with IM complexity (p=0.035), IM severity (p=0.023), presence of neurological symptoms (p=0.025) or hepatitis (p=0.007) as well as ferritin level (p=0.025) and positivity for EBV EBNA-1 IgG (p=0.032) at the same visit. Specifically, the proportion of PMN-MDSC increased with the complexity and severity of pathological laboratory features, as well as with ferritin levels.

Conclusion: Our data provide important insights into the temporal dynamics of MDSC subpopulations during the course of IM and their correlation with clinical, laboratory, and virological features. Changes in the frequencies of MDSC subpopulations related to IM complexity and IM severity may contribute to the understanding of early or late complications of IM. They thus might serve as biomarkers, especially for protracted disease. Further studies in a validation cohort will be conducted to support these findings.

P-1-140

Effects of Wuchereria bancrofti infection on CD4 T cell responses to specific and non-specific antigens

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Backround: Lymphatic filariasis, primarily caused by *Wuchereria bancrofti,* is a mosquito-transmitted disease that affects people living in tropical regions. Infection with *W. bancrofti* is associated with chronic inflammations that may cause lymphedema and hydroceles. The adult worm of *W. bancrofti* lives for many years in the human host and even without disfiguring consequences leads to modulation of the adaptive immune response. The aim of our study was to determine whether chronic *W. bancrofti* infection affects CD4 T cell responses to specific and non-specific antigens.

Methodology: Blood samples were collected from 140 participants living in two *W. bancrofti* endemic regions in Tanzania: Mbeya (Kyela district) and Lindi region. Samples were stimulated with the whole lysate of *Mycobacterium tuberculosis* (Mtb), Staphylococcus Enterotoxin B (SEB) or PBS (control) for 16 hours. The frequency of CD4 T cells responding to stimulation by secreting interferon gamma (IFN-γ) or interleukin 2 (IL-2) cytokines was measured by flow cytometry. The frequencies of antigen-specific CD4 T cells were compared between the FTS+ and FTS- groups after background subtraction.

Results: *W.bancrofti* infected individuals had significantly low Mtb specific IL-2 producing CD4 T cells compared to uninfected individuals (p =0.0091). In addition, *W.bancrofti* infection reduced the IL-2 responses in individuals aged 25 to <45 years. Interestingly, *W. bancrofti* infection showed significantly reduced frequencies of both IFN-γ(p<0.0001) and IL-2 (p<0.0001) CD4 T cell responses upon stimulation with SEB compared to uninfected individuals. This reduced immune response upon SEB stimulation was noted in the 14-<25 and 25-<45 age groups.

Conclusion: Our findings show diminished CD4 T cell responses to SEB in W. bancrofti-infected individuals. On the other hand, only Mtb-specific, IL-2 (and not IFNγ) releasing CD4 T cells were reduced in FTS+ individuals. FTS+ individuals in the age range between 14 to <25 and 25 to <45 showed significantly low IL-2 and IFNγ responses upon non-specific antigen stimulation, while reduced IL-2 responses were only in the 25 to <45 group upon specific antigen stimulation. Our results indicate chronic infection with *W. bancrofti* suppresses CD4 T cell responses, most likely as part of the immune evasion strategy of the parasite. These reduced immune responses, which are age-dependent, might have a deleterious impact on the ability of the host to fight other infections.

P-1-141

Epithelial precision: Targeting Coronaviruses in lung Epithelium with antiviral siRNA

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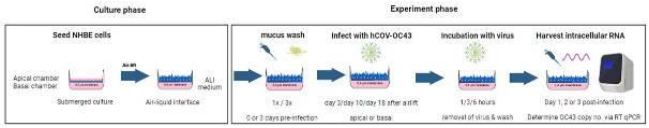
Short-interfering (si)RNA are an emerging class of therapeutics that, due to their sequence-specific mechanism of action, hold the potential to treat a myriad of diseases. In recent years, six siRNA therapies have been approved for clinical use, underscoring their clinical potential. siRNAs can be engineered to target viral RNAs and are thus a promising

tool to combat emerging viral infections. Our group has previously developed highly potent siRNAs with broad silencing efficiency against the severe respiratory coronaviruses SARS-CoV-2, SARS-CoV-1, MERS, as well as other endemic human coronaviruses such as OC43. To successfully bring these siRNAs to the clinic, however, an efficient delivery to the human respiratory tract is prerequisite. We established a coronavirus infection model of the human lung, using primary human bronchial epithelial cells cultured in an air-liquid interface. Using a design of experiment (DOE) algorithm, we investigated variables such as day of infection after airlift, timepoint and number of mucus washing and apical versus basal infection on the replication of the endemic coronavirus hCOV-OC43. Interestingly, we discovered that infecting primary NHBE cells from the basal rather than the apical side of the polarized epithelium enhances viral replication significantly. This hints at the protective role of mucus, which is deposited on the apical side of differentiated, air-exposed lung cells, towards foreign particles including viruses,. The optimized *in vitro* model of human coronavirus infection will be used to investigate the delivery and silencing efficiency of our novel siRNA conjugate. This construct comprises an anti-CoV siRNA covalently linked to an αvβ6-ligand. The αvβ6 receptor is exclusively expressed in epithelial cells, enabling selective delivery into lung epithelium where coronaviruses replicate. This research will enhance our understanding of siRNA delivery to the lung and has implication for pandemic preparedness.

Fig. 1



P-1-142

Evaluating phage-resistant *Pseudomonas aeruginosa* variants against clinically relevant phages in Germany: Insights into resistance trade-offs and therapy potential

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Background: Bacteriophages (phages) present a promising approach for treating infections caused by antibiotic resistant or recalcitrant bacterial strains. However, the evolution of phage resistance of *Pseudomonas aeruginosa* poses significant challenges to the effective use of phage therapy. In some cases, evolutionary changes in bacteria conferring phage resistance incur fitness costs, such as reduced motility or lowered toxin production. These trade-offs could potentially be leveraged to improve the efficacy and safety of personalized phage therapies, yet they are still not well understood.

Objectives: This study aims to advance phage therapy research by investigating the adaptive traits of phage-resistant bacterial breakout variants relevant to clinically significant phages in Germany.

Methods and Project Outline: Phage-resistant breakout variants of well-characterized laboratory strains and clinical isolates of *Pseudomonas aeruginosa* were developed via phage-driven selection. The phage-sensitive ancestral strains, PA01, PA14, and CH3549, were exposed to the phages JG005, JG024, and Bhz 17, respectively. Our analysis pipeline begins with purifying colonies and confirming phage resistance. These breakout variants are then comprehensively compared to their ancestral strains in terms of growth rate, motility, antibiotic susceptibility, biofilm formation and stability, toxin production, inflammatory response induction, and genomic changes.

Growth rates are measured through kinetic assays in liquid media. Antibiotic susceptibility is evaluated using Minimum Inhibitory Concentration (MIC) tests per EUCAST guidelines. Motility is assessed by twitching, swimming, and swarming assays. Biofilm formation and stability are analyzed via static and flow-through assays. Toxin production focuses on characteristic Pseudomonas products such as pyocyanin and elastase, while inflammation studies assess interleukin and LDH release in human cell cultures stimulated by bacterial variants or ancestor strains. Genomic changes are identified through in-house Nanopore sequencing and comparative analyses.

Conclusion: This pipeline for clinically relevant phages in Germany aims to elucidate the evolutionary trade-offs linked to phage resistance and to inform strategies for phage therapy. By examining these characteristics, this work supports the development of safer and more effective personalized phage-based therapies for combating antibiotic-resistant and persistent bacterial infections.

P-1-143

Transcriptomic changes associated with lung function impairment after successful tuberculosis treatment

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Approximately half of individuals cured from tuberculosis (TB) experience post-treatment lung function (LF) impairment, yet the mechanisms underlying this process remain unclear. Studying these pathways is challenging due to the intense immune activation that occurs during TB infection.

As part of the TB Sequel cohort study, we performed RNA sequencing on samples from 108 patients without HIV to investigate transcriptomic changes associated with LF outcomes post-treatment. Participants with mycobacteriologically confirmed pulmonary TB were stratified into two groups based on post-treatment

measurements of forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). We analyzed differential gene expression and pathway enrichment from baseline (start of treatment), month 2, and month 6 (end of treatment), using multiple gene set and pathway databases to identify the difference between patients with either severe or no impairment after treatment.

Our findings show transcriptomic differences across all time points, with the largest divergence occurring at month 2, when a high proportion of genes exhibited significantly lower expression in individuals with poor LF outcomes. At baseline, individuals with LF impairment post-treatment showed increased activation of immune-related genes and pathways, including Toll-like receptors, NOD-like receptors, and TNF signaling. By month 2, higher immune-related activation persisted, with a shift toward adaptive immune and cytokine response as well as pathways involving pattern recognition. Interestingly, gene sets associated with morphogenesis and differentiation were upregulated in individuals with favorable LF outcomes, along with gene sets related to cell-cell adhesion and junctions.Further, in individuals with post-treatment LF impairment, also gene set clusters not related to immune or regenerative pathways such as cardiac function and neuronal morphogenesis and GABA receptor signaling were downregulated at month 2. Additionally, at month 2 olfactory receptor signaling was downregulated in individuals with poor LF outcomes.

At month 6 overall transcriptomic differences between the groups decreased. Several gene sets associated with B cell and immunoglobulin mediated immunity as well as interferon type I signaling remain elevated in the group with poor LF outcomes. A highly significant cluster of gene sets from multiple databases associated with olfactory receptor signaling, downregulated at month 2, is then found to be upregulated in the group with poor LF outcomes.

This study provides insights into mechanisms that may contribute to more severe lung impairment in certain individuals and underscores the dynamic nature of this process. Our findings suggest that the pathways leading to LF impairment extend beyond immune response alone, pointing to a multifactorial nature of this pathology.

P-1-144

Autopsy-based COVID-19 tissue biobanking – a successful example for integration of external biobanking projects into DZIF infrastructure

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Introduction: Since the beginning of the SARS-CoV-2 pandemic, structured and quality assured autopsy-based biobanking has been performed in the context of the COVID-19 Autopsy and Biosample Registry Baden-Württemberg (BW), encompassing the five university pathologies (Heidelberg, Freiburg, Mannheim, Tübingen, Ulm). The consortium, coordinated by Heidelberg site, was funded by the Ministry of Science, Research and Arts of Baden-Württemberg, Germany, from 2020 until the end of 2024 and during this time closely cooperates with the German Center for Infectious Diseases (DZIF). As part of the sustainability of the project, the later integration into the DZIF Tissue Bank Heidelberg was initially planned.

Structure: The main part is a structured biobanking and registry function to support latest research and to facilitate and increase autopsy frequency. Tissue samples (FFPE, cryopreserved) of all relevant organs are collected in a standardized approach from SARS-CoV-2 infected and/or anti-SARS-CoV-2 vaccinated deceased patients. Samples are stored in a harmonized decentral manner. Sample data including relevant patient datasets, autopsy data, histopathological/radiological characteristics, immunization status, as well as clinical and virologic data are recorded in a web-based platform.

Results: From 2020 to 2024 around 12,500 tissue samples were collected, resulting in over 40 peer-reviewed publications. The established registry of all collected cases as well as the biomaterial collection at the Heidelberg site are transferred and integrated into the DZIF Tissue Bank in the end of 2024. The biomaterial collective of the Heidelberg site will thereby be maintained, and research projects supported by the quality assured tissue biobanking experts of the DZIF Tissue Bank. The overall web-based autopsy registry will continuously be hosted by the DZIF Tissue Bank and integrated data sets are accessible for every DZIF scientist via the Central Biosample Registry (ZBR) at the Helmholtz Center in Munich.

Conclusion: With ending of the funded project period, the transfer of the SARS-CoV-2 autopsy registry as well as the biomaterial collection at the Heidelberg site into the DZIF Tissue Bank is conducted. This integration assures the accessibility and sustainability of the data and biomaterial collectives remain available for future research projects, especially within DZIF. This is an excellent example of how the DZIF Tissue Bank as an DZIF infrastructure improves and expands research resources and how the whole DZIF community can benefit from externally funded and structured infectious diseases research projects which include biobanking.

P-1-145

Microbiome based clinical pathogen detection in bronchoalveolar lavage fluid using next-generation sequencing

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Question: Lower respiratory tract infections (LRTI) are a leading contributor for morbidity and mortality worldwide, demanding accurate and streamlined pathogen identification for effective patient treatment. While culture-based methods remain the gold standard in routine diagnostics, they fail to detect pathogens in nearly half of the cases. Next-generation sequencing (NGS) embodies a prospective, unbiased method for microbial detection. Does it have the potential to improve or expand clinical diagnostics?

Methods: This study investigates the bacterial microbiome profiles of 144 bronchoalveolar lavage fluid samples using Illumina V4 16S rRNA gene sequencing and comparing the results with traditional culture methods. To achieve higher taxonomic resolution, 21 samples were further analyzed with Nanopore long-read sequencing. Microbiome profiles were categorized as mono-, poly- and multi-bacterial, enhancing understanding of microbial diversity in clinical samples and facilitating the comparison with culture results.

Results: The most abundant bacterial genera identified were *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Prevotella*, *Veillonella* and *Rothia*. Illumina sequencing accurately traced 91.5% of cultured bacteria, though these were not always among the most dominant genera in the microbiome profiles. Nanopore sequencing matched the cultured species in 76.5% of the cases and detected potential pathogens (*H. influenzae* and *S. pneumoniae*) that were missed by culture in two instances. Samples with potential pathogens in culture showed higher DNA yield, but did not exhibit significantly different α-diversity indices than samples with a negative culture or a cultured commensal.

Conclusion: NGS results demonstrated partial overlap with culture as the current diagnostic gold standard in LRTIs. Its strengths lie in the ability to capture a vast range of bacteria. Additionally, NGS provides insights into the complex microbial ecosystem of the lungs, revealing features that traditional culture methods may overlook.

P-1-146

Engineering of myotropic AAV vectors for high in vivo expression of broadly neutralizing anti-HIV-1 antibodies

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Broadly neutralizing antibody (bNAb)-based therapeutics have the potential to act as effective tools for the prevention or control of HIV-1 infection. Recombinant adeno-associated viruses (rAAVs)-mediated gene transfer is considered safe and could provide long-term bNAb expression, but intramuscular injections of AAV1 or AAV8 vectors resulted in low bNAb titers in humans. Thus, improved vectors will be critical to advance bNAb-mediated strategies to target HIV-1 using AAVs. In addition to optimized bNAb expression cassette design, AAV capsid protein selection can strongly influence transgene expression by modulating vector tropism.

Here, we describe the development of novel rAAV vectors, based on the myotropic AAVMYO capsid protein, for the expression of a newly identified highly potent anti HIV-1 bNAb. To achieve high bNAb expression in vivo, the AAV vector genome was further optimized as a bi-cistronic system encoding a heavy and a light chain of a full-length human IgG, which are separated by a self-cleaving 2A peptide. Additionally, several other pivotal modifications were incorporated in our transgene cassette: (i) a Kozak consensus sequence upstream of the first cistron; (ii) a furin cleavage site (RRKR) as well as a GSG-linker at the N-terminus of the 2A peptide; and (iii) a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE3) fused to a SV40 polyadenylation signal. Transcription levels were assessed by RT-qPCR in transfected HEK293T cells, resulting in a ~2-fold higher expression in comparison to our previous tri-cistronic approach (containing an additional eYFP marker). Functional bNAb expression was confirmed by HIV-1BG505 ELISA of transfected HEK293T and Huh7 cell supernatants, showing an up to ~8-fold increase of bNAb concentration. Under the control of different promoters, high and durable rAAVMYO-mediated bNAb expression *in vivo* was demonstrated in NOD-Rag1null IL2rgnull (NRG) mice.

In imminent studies, we will evaluate the capacity of the new bNAb-rAAVMYO vectors to treat or prevent HIV-1 infection *in vivo*. The encouraging results obtained thus far warrant further investigation and highlight the great potential of bNAb-encoding rAAVMYO constructs as a novel, clinically relevant modality for people living with HIV or at risk of infection.

P-1-147

Unravelling the pathogenesis of post-tuberculosis lung function impairment through serum proteomics

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While a substantial percentage of TB survivors retain lung impairments, the pathology underlying post-tuberculosis lung disease (PTLD) remains incompletely understood, limiting advancements in targeted interventions. Tuberculosis (TB) inflicts substantial disruption on the immune system, masking the mechanisms that contribute to subsequent lung impairment. Furthermore, immune responses initially engaged to combat TB may contribute to chronic lung damage. This study aimed to characterize serum protein expression changes from TB diagnosis to treatment completion, disentangling these mechanisms in participants of the TB Sequel cohort.

We conducted targeted proteomic analyses using the Olink Target 96 Inflammation and Organ Damage protein panels, analyzing serum samples from 70 individuals from the TB Sequel site in Tanzania at TB diagnosis and at treatment completion. Lung function impairment was classified using spirometry, specifically FEV1, FVC, and FEV1/FVC measures.

Our analysis identified 26 out of 184 measured proteins as significantly differentially expressed (fold change >1, $p<0.05$) over the treatment course. These were mainly inflammatory proteins, involving pathways mediating leucocyte migration through cytokine and interleukin signalling, as well as signal transduction (via G-protein coupled receptor (GPCR), MAP kinase, and receptor tyrosine kinases). Unsupervised computational signature identification using Bayesian and Random Forest classification algorithms identified 3 or 22 proteins capable of distinguishing TB disease from cure, achieving sensitivities of 92% and specificities of 94% and 100%,, respectively.

Although differential protein expression among individuals with post-treatment lung function impairment was subtle, a few significant differences were observed at baseline, differentially regulated proteins included those involved in immune signaling. Proteins associated in chemokine and GPCR signalling remained elevated throughout the TB treatment phase; however, when analyzed cross-sectionally at treatment completion, no significant differences were observed. No classifiers for lung function impairment could be identified.

In conclusion, these findings offer insights into the complex immune response dynamics in PTLD. Further investigation may identify biomarkers and therapeutic targets for mitigating long-term lung impairment following TB.

P-1-148

Heterocellular spheroids as a new model for antivirals testing at the blood-brain-barrier (BBB)

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At the Bundeswehr Institute of Microbiology (IMB) we aim to create heterocellular neurovascular spheroids to study viral infection in this model of the BBB and the effect of antivirals in this process.

Material & Methods: Infection rates of endothelial (HBMEC NY), pericytes (HBMVPC) and glioblastoma (U138) cell lines with EGFP based reporter viruses are monitored in single or multiple-cell cultures and in 2D or 3D growth environments. The detection of pericytes, endothelia cells and their tight junction proteins at the border of the glial cells is used as an indicator of a functional BBB. For this we use the antibodies NG2+ (Pericytes), ZO1 and CD31 (Endothelia cells). The progression of viral infection with measles virus (IC323-GFP) in the presence and absence of Molnupiravir is monitored by detection of reporter activity in live and inactivated spheroid cells.

Results: Preliminary results show that 3D grown mixtures of endothelial and glioblastoma cell lines form heterocellular spheroids and are viable for at least 9 days. Heterocellular neurovascular spheroids can be infected with EGFP reporter viruses and virus propagation starts in endothelial cells.

Conclusion: We show for the first time infection of heterocellular neurovascular spheroids with reporterviruses and their suitability to assess the effect of antivirals in this process.

P-1-149

Telemedicine care for patients with an OPAT – concept presentation and interim evaluation of a prospective, exploratory, and multicenter trial APAT DTB proto

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Question: Patients requiring long-term intravenous (IV) antimicrobial therapy often remain in the hospital despite being clinically stable. Discharge would be possible through Outpatient Parenteral Antimicrobial Therapy (OPAT), which enables IV treatment at home and is considered safe for younger, fitter patients. However, OPAT is often denied for older, multimorbid patients or those residing abroad due to safety concerns. This study aims to evaluate the feasibility of a telemedicine-supported OPAT for all patients.

Methods: After providing written consent, patients receive a free app that allows them to submit vital signs, report side effects of the IV therapy, use a chat feature, and receive therapy reminders through alerts. A telemedicine team monitors incoming data via a web-based platform and responds as needed. Support services include phone calls, video consultations, or outpatient care appointments. All patients continue to receive standard care. The program emphasizes strong communication between hospital physicians (standard care), the telemedicine team (primary contact for questions and monitoring), the pharmacy (to announce any OPAT changes early), OPAT-trained nursing experts (support at home if possible), and the patients. End points are the feasibility, life quality (PROMIS-29), treatment quality (ZAP, ZUF), adherence (A14), usability (SUS), OPAT safety and clinical outcome.

Inclusion criteria: Eligible patients must be >18 years old, have a valid OPAT indication, and possess a smartphone. Exclusion criteria include language barriers, IV drug addiction, and any inability to use a smartphone.

Recruitment will take place at Charité – Universitätsmedizin Berlin, Vivantes Auguste-Viktoria-Klinikum Berlin, and Marienhospital Stuttgart from January 2024 to March 2025, with additional centers expected to join. The app is available on both iPhone and Android platforms, with a recruitment goal of 100 patients. The trial adheres to ethical standards for medical research and GCP guidelines for clinical trials, including EU Regulation 2017/745, and is registered at DRKS (00030938) with ethical approval (100_EK_20240411084431_22-834).

Results: Of the patients enrolled, 23 have received telemedicine support so far. Further data will be presented in the poster (if approved!). Initial findings suggest that less fit patients are supported effectively. However, most patients over 60 declined participation, often due to concerns such as mental stress or lack of familiarity with smartphones. Technical issues were common exclusion factors for middle-aged patients. Younger patients declined if they were heavily committed to work and family. More males than females enrolled. Currently, improvements on the telemedicine dashboard are ongoing. The app itself has been well-received, with minimal need for patient training.

Preliminary Conclusion: Middle-aged and older patients may require an adapted approach to encourage participation.

P-1-150

Respiratory disease outbreaks in wild Bonobos: Noninvasive pathogen identification & conservation implications in the context of the Covid-19 crisis

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Question: Bonobos are classified as endangered according to the IUCN Red List, with infectious diseases being one of their primary threats. Respiratory disease outbreaks in wild, human-habituated great ape populations have been associated with the spillover of common human respiratory viruses on multiple occasions. This study investigated potential infectious agents associated with respiratory disease outbreaks among wild bonobos in the Kokolopori Bonobo Reserve, Democratic Republic of the Congo, from 2020 to 2023.

Methods: Fecal samples were collected from 3 groups of wild bonobos habituated to human presence for behavior research as part of the health monitoring program in the Kokolopori Bonobo Reserve. Total nucleic acids were extracted from 65 fecal samples collected from symptomatic bonobos between 2020 and 2023. PCR screening targeted major human respiratory viruses, including SARS-CoV-2, human metapneumovirus (HMPV), human respiratory syncytial virus (HRSV), Influenza A virus, parainfluenza virus, rhinoviruses, and other coronaviruses. Positive samples were sequenced via Sanger sequencing.

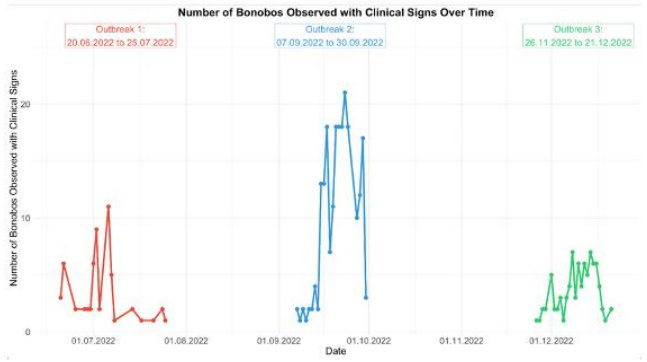
Results: Signs of respiratory disease, such as coughing and sneezing in the three bonobo groups were associated with three distinct human pathogens identified in closely timed outbreaks: Human respiratory syncytial virus A (HRSV A) (13/21) in July 2022, human metapneumovirus (HMPV) (5/21) in September 2022, and Rhinovirus C (RV C) (2/14) in December 2022 (Fig. 1).

Discussion: Our study identified three distinct human viruses as the causative agent of respiratory disease outbreaks in wild bonobos, highlighting the high risk of pathogen transmission at this interface and the importance of prevention measures. To the best of our knowledge, this study marks the first identification of HMPV and Rhinovirus C in wild bonobos. The rapid increase in case frequency over brief periods and the absence of re-infections or co-infections suggests point-source outbreaks rather than prolonged propagation within bonobo populations (Fig. 2). Since HRSV, HMPV, and Rhinovirus C have no known reservoirs other than humans and are common human pathogens, we suspect three separate introductions of these pathogens from humans into the bonobo population within six months. The findings underscore the urgent need for vigilant surveillance in animals and humans, and preventative measures to protect bonobos from population declines, caused by human pathogens. Studying disease epidemiology in animals and humans is crucial to better understand the disease epidemiology in these areas of high habitat overlap.

Fig. 1

Outbreak	1	2	3
Date	20th June 2022 – 25th July 2022	7th September 2022 – 30th September 2022	26th November 2022 – 21st December 2022
Sample Size	21	21	14
Positive Samples	13	5	2
Pathogen	HRSV A	HMPV	RVC

Fig. 2



P-1-151

The clinical-stage drug BTZ-043 accumulates in murine tuberculosis lesions and efficiently acts against Mycobacterium tuberculosis

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The hallmark of human tuberculosis (TB) is the formation of granulomas with central necrosis that harbor *Mycobacterium tuberculosis* (Mtb). Anti-TB drugs must effectively penetrate the cellular as well as the necrotic, non-vascularized regions of these lesions and reach sufficient concentrations in order to eliminate Mtb. BTZ-043 is a novel antibiotic with potent bactericidal activity in humans in a phase IIa trial.

In this study we comprehensively evaluated BTZ-043 in two pre-clinical TB mouse models, the standard mouse model and the advanced IL-13-overexpressing model which reflects granuloma necrosis observed in humans. Using the later model we determined pulmonary BTZ-043 concentrations and its concentration in necrotic lesions by laser capture microdissection and subsequent LC-MS/MS. The lesional activity of BTZ-043 was analyzed by quantitative Mtb 16S rRNA expression. The spatial and temporal distribution of BTZ-043 within centrally necrotizing granulomas was assessed by high resolution MALDI imaging.

We demonstrate that BTZ-043 reaches concentrations in centrally necrotizing granulomas several times above its minimal-inhibitory concentration and that it also exerts its antimycobacterial activity in these lesions. MALDI imaging revealed an accumulation of BTZ-043 in the cellular compartment of granulomas early after administration and at later time points a full penetration of the necrotic center

which is the most problematic region in terms of drug penetration.

Based on the observed penetration, local concentration and lesional activity of BTZ-043 in centrally necrotizing granulomas we conclude that BTZ-043 has the potential to complement existing antibiotics and could contribute to an improved treatment of TB patients.

P-1-152

The Data Hub: Enhancing infectious disease research and global health action with reproducible data harmonization

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Challenge: In an increasingly interconnected world, with global shifts in demographics, society, and climate, the complexity of Global Health challenges is escalating. Infectious diseases, in particular, are embedded within complex socio-ecological systems that shape their emergence, spread, control, and prevention. Addressing this requires collaborative intelligence that synthesizes evidence from multiple health disciplines, complemented by contextual insights (1). However, data often remain dispersed across disciplines and siloed within domain-specific repositories and inaccessible sharepoints, which hinders efficient data collaboration.

Methods: Harnessing computational sciences, we developed a flexible, self-hostable open-source software framework - the Data Hub -, supporting reproducible data harmonization and exploratory visualization for Global Health research and action. The development considered data practices and needs of potential users in research and global public health, as well as data standards relevant to the community. The software was developed using Python, PostGIS data architecture and the Django web framework, published under MIT license.

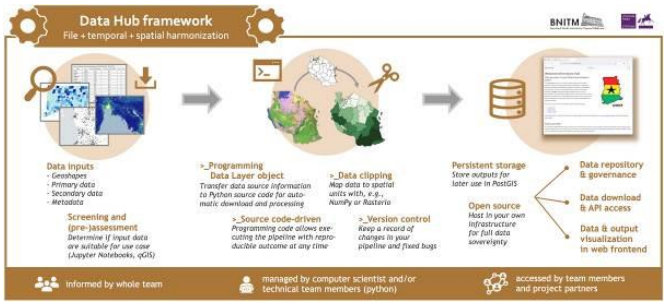
Results: The Data Hub framework integrates a data fusion engine and an interactive dashboard for data collaboration (2). It further supports metadata management in compliance with FAIR data principles (3), enhancing data accessibility, interoperability, and reusability. With each stage of data processing and output generation encoded, the framework ensures transparency, reproducibility and verifiable workflow. A demonstration Data Hub was created using Ghana as a case study, incorporating more than 40 open data layers across 6 categories, covering diverse spatial and temporal dimensions (2). Identified areas of application include planning and conducting complex and interdisciplinary studies (e.g., cluster sampling), integrated risk assessment frameworks and analytical models (e.g., agent-based models), and data-driven simulation exercises to strengthen collaboration (e.g., epidemic investigation).

Conclusion: The Data Hub represents a step toward fostering collaborative intelligence to confront the current and future multifaceted challenges in infectious disease and Global Health research. The core framework is openly accessible to interested users worldwide and undergoing testing to minimize user impact and improve usability, currently in its beta phase. Future objectives include simplifying the framework's setup process, evaluating its effectiveness in low-resource settings, and enhancing features for visual data comparison, dataset creation, and tool administration (e.g., user management, language settings).

(1) Morgan OW et al. Nat Med. 2022; 28. doi:10.1038/s41591-022-01900-5.
(2) Data Snack. Data Hub framework overview. 2024. Available online: https://datasnack.org/.
(3) Wilkinson MD et al. Sci Data. 2016; 3. doi:10.1038/sdata.2016.18.

Figure 1: The Data Hub framework

Fig. 1



P-1-153

Treatment of Hepatitis E virus infected humanized mice with the nucleoside analog NITD008

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Background/Aim: Hepatitis E virus (HEV) infection has an emerging clinical relevance for patients worldwide, but there is currently no approved antiviral therapy. For immunosuppressed people with chronic hepatitis E, off-label treatment with ribavirin is used in daily clinical practice, but ribavirin can cause serious side effects and resistance does emerge. Therefore, there is an urgent need for new and effective antiviral drugs against HEV infection. The adenosine nucleoside analog inhibitor NIDT008 is one such candidate that has been shown to be effective in vitro against a broad range of RNA viruses and in vivo in animal models against Dengue, Zika or Hepatitis C virus. The aim of this study was to evaluate the efficacy of NITD008 in vivo in human liver chimeric mice stably infected with HEV.

Methods: Humanized immunodeficient mice, repopulated with human hepatocytes, were infected with hepatitis E virus (genotype 1) via the co-housing fecal-oral route. After stable infection was achieved (6-8 weeks), median viremia level was 1,5*107, mice received 20µg/g of the polymerase inhibitor NITD008 by oral gavage daily for up to 2 weeks (n=5), while a control group (n=4) received mock treatment. Changes in HEV RNA titers were determined by qPCR in faeces and blood. Mice (n=3) were sacrificed after 1 or 2 weeks of treatment, while a subgroup was observed for a 3-week rebound phase (n=2). Intrahepatic analysis including immunohistochemistry, multiplex RNA in situ hybridization (RNA-ISH) and qPCR was performed in all mice at sacrifice.

Results: NITD008 effectively reduced fecal HEV loads by up to 4log already in the first week of treatment, with some mice showing no detectable virus shedding. All mice had undetectable serum HEV titers (LLoQ) after 7 days, while vehicle-only controls showed no evidence of a decrease in fecal and serum viral titers (median 7,7*106 IU/mL and 1*106 copies/ml, respectively). Intrahepatic analysis showed a 1.5 log reduction in HEV RNA/human housekeeper levels in treated mice compared to controls after 1 week of treatment and a 2.5 log reduction after 14 days of treatment.

Consistent with the qPCR data, RNA-ISH analysis confirmed the presence of only a few HEV-RNA-positive human hepatocytes in treated mice, whereas approximately 95% of human liver cells were positive in control animals. Two weeks of treatment with NITD008 did not induce ALT elevation or significant weight loss in treated mice. Despite the strong reduction in HEV infection, HEV rebound was observed after 3 weeks of treatment cessation in this immunodeficient system.

Conclusion: This study demonstrates the efficacy and non-toxicity of the adenosine nucleoside analog NITD008 towards human hepatocytes after 2 weeks of treatment in HEV infected human-chimeric mice. Further studies, such as combination regimens with ribavirin, are needed to further optimize results and achieve complete viral clearance even in the absence of a humoral immune response.

P-1-154

The mechanism of action of semisynthetic guanidino lipoglycopeptides with potent antibacterial activity

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Increasing numbers of infections with antibiotic-resistant pathogens, like vancomycin-resistant enterococci or staphylococci, are a major global health problem. To overcome resistance, semisynthetic glycopeptide derivatives like the clinically used telavancin have been developed. These lipoglycopeptides exhibit enhanced antibacterial potency but additionally raised toxicity concerns. The group of Nathaniel I. Martin, Leiden University, The Netherlands, recently developed semisynthetic lipoglycopeptide antibiotics, which contain a positively charged guanidino moiety and a variable lipid group. These guanidino lipoglycopeptides exhibit a highly increased activity against a variety of Gram-positive bacteria, including vancomycin-resistant strains. Moreover, they exhibited minimal to mild toxicity towards eukaryotic cells suggesting an improved therapeutic safety profile compared to vancomycin. In-depth analysis of the modes of action of the guanidino lipoglycopeptides revealed that they bind the peptidoglycan precursor lipid II-D-Ala-D-Ala with a higher affinity than vancomycin. Additionally, in contrast to vancomycin, they showed a high affinity interaction with lipid II-D-Ala-D-Lac found in resistant strains, providing a rational for the enhanced activity against vancomycin-resistant isolates. According to these findings, the guanidino lipoglycopeptides represent promising candidates for further development of antibiotics against clinically relevant multidrug-resistant Gram-positive infections.

van Groesen, E., Mons, E., Kotsogianni, I., Arts, M., Tehrani, Kamaleddin H. M. E., Wade, N., *et al.* (2024) Semisynthetic guanidino lipoglycopeptides with potent *in vitro* and *in vivo* antibacterial activity. *Sci Transl Med* **16** (759).

P-1-155

Data linkage in the MuDTX project: Integrating TX-Cohort study and routine clinical data for enhanced infection prediction

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The MuDTX project aims to develop predictive models for opportunistic infections in post-transplant patients by integrating data from the DZIF Transplant Cohort (TX-Cohort) with clinical data routinely collected at each transplant site. This integration requires linking patient data on key clinical variables, such as diagnoses, heart rate, temperature, and procedure codes (OPS), from both the TX-Cohort and site-specific Data Integration Centers (DICs). One of MuDTX"s main objectives is to evaluate the use case for linking study-specific and routine clinical datasets, which would not only increase the amount of data we have but also adds data not captured in the TX-Cohort. To achieve this, we submitted formal data requests to obtain pseudonymized records, in line with study and privacy requirements. In this use case, we used two main software components: the bridgehead, for securely storing the data, and TransFAIR, for converting and linking the datasets. TransFAIR links the different data sources by matching pseudonyms to IDATs (Identification and Demographic Attributes) when the data is managed in the TX-Cohort"s Electronic Data Capture (EDC) system. The linkage process involves several organized steps. First, a data manager from each site downloads the relevant data into their bridgehead. The TransFAIR then connects to the FHIR server provided by the DIC, the FHIR server of the bridgehead and connects to the Trusted Third Party (TTP) at the site. Through TransFAIR"s workflow, data from both sources are matched and merged into the bridgehead. After successful linkage, the combined dataset is made available for download by the site and then transmitted to the MuDTX team. We then combine all datasets and work on developing predictive models for infection risk.

P-2-1

Mortality attributable to candidemia: Results from the european confederation of medical Mycology multinational observational cohort study Candida III

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Background: Despite advances in antifungals, Candida infections still have a high mortality rate of up to 40%. The ECMM Candida III study in Europe investigated the changing epidemiology and outcomes, highlighting the need to understand and manage these infections.

Methods: In this observational cohort study, participating hospitals enrolled the first ten consecutive adults with confirmed candidemia. Data collected included patient demographics, risk factors, hospital stay length (with a 90-day follow-up), diagnostic procedures, Candida species, treatment details, and outcome. Controls were matched in a 1:1 ratio from the same hospitals, ensuring similarity in age, underlying illness, ICU versus normal ward stay, and recent major surgery. The study described overall and attributable mortality and assessed survival probability for both cases and controls.

Findings: The study included 171 pairs consisting of patients with candidemia and matched controls from 28 institutions. In those with candidemia, overall mortality was 40.4%. The attributable mortality was 18.1% overall but differed among the causative Candida species (7.7% for Candida albicans, 23.7% for Candida glabrata, 7.7% for Candida parapsilosis, and 63.6% for Candida tropicalis). Regarding risk factors, the presence of central venous catheter, total parenteral nutrition, and acute or chronic kidney disease were significantly more common in cases versus controls. Length of hospitalization and ICU stay were significantly longer in candidemia cases (20 days (IQR 10-33) vs. 15 days (IQR 7-28); p=0.004).

Interpretation: Although overall mortality remains high in this matched case/control analysis, attributable mortality has decreased compared to historical cohorts. This may be due to a better prognosis for candidemia caused by Candida albicans, which has an attributable mortality of 7.7%, while candidemia cases caused by non-albicans Candida exhibit higher attributable mortality.

P-2-2

Reactive nitrogen species in insecticide resistance and mosquito immunity against malaria parasites

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Massive deployment of insecticide-based control strategies substantially curtailed malaria incidence from 2000 to 2015; however, the escalating threat of insecticide resistance (IR) has drastically dampened hopes for swift malaria eradication. New strategies and countermeasures are imperative to bypass this rising challenge. Pyrethroids are the most important class of insecticides, utilized on all insecticide treated bednets (ITNs), but their efficacy is threatened by IR. Due to their importance, it is critical to understand changes to mosquito biology caused by IR and exposure which could impact vector competence. Reactive nitrogen species (RNS) have a well-known effect on the mosquito immune system during invasion of *Plasmodium* parasites, the causative species complex of malaria disease. In contrast, their role in insecticide resistance remains unexplored. Furthermore, the effect of perturbation of such reactive molecules to manipulate the whole redox system of mosquitoes has not been investigated in terms of infection with malaria, most importantly with human pathogenic *Plasmodium falciparum* parasites. New knowledge about the interplay between RNS, IR, insecticides and mosquito immunity against this parasite could have huge implications for future decisions in integrated vector control management. Here, we utilize confocal imaging, RNAseq, qPCR, phenotyping and infection experiments to determine the impact of pyrethroid exposure on the mosquito. In this study we show that pyrethroids increase oxidative stress levels, especially RNS, in various tissues, including those relevant for parasite transmission and circulating phagocytic immune cells. Interestingly, targeted perturbation of RNS in mosquitoes of the *Anopheles gambiae* complex, the main African vector species, results in changes to both vector competence to *Plasmodium falciparum* and insecticide resistance. Accompanying RNA sequencing data shows that boosting RNS levels drastically alters mosquito metabolism in a manner akin to insecticide exposure and changes the expression of multiple IR and immunity genes and pathways. Our study unveils valuable new insights into a potential novel target to reduce malaria transmission.

P-2-3

Gamma-pyrone synthetic compounds display broad-spectrum antiviral activity against RNA viruses by targeting mitochondrial metabolism

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The emergence and re-emergence of epidemic and pandemic RNA viruses urgently requires the development of novel direct-targeting antivirals (DTAs) and host-targeting antivirals (HTAs). Our previous research identified a class of gamma-pyrone inspired compounds, which displayed low nanomolar activity against HIV-1 infection. In this study, we investigated the antiviral activity of gamma-pyrone inspired compounds against SARS-CoV-2 and DENV-2, as representatives of emerging and reoccurring RNA viruses. We found that compound #7 displayed antiviral activity at low nanomolar concentrations against SARS-CoV-2 and DENV-2. Furthermore, we designed a version of compound #7, to be used for photo-affinity labeling (PAL) and mass spectrometry, aiming to identify the cellular target(s) responsible for its antiviral activity. We identified MCAD as the main target, as well as additional candidates which localize to mitochondria and are involved in the fatty acid oxidation and in the oxidative phosphorylation. In conclusion, our work showed that gamma-pyrone synthetic compounds are a class of broad-spectrum antivirals targeting the host metabolism and mitochondrial energy production, providing a novel HTA strategy to target RNA viruses.

P-2-4

Recombinant receptor binding proteins of bacteriophages as versatile tools for pathogen detection

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[3]*Bundeswehr Institute of Microbiology, Munich, Germany*

For highly pathogenic bacteria, such as *Bacillus anthracis*, *Yersina pestis*, *Burkholderia pseudomallei* or *Brucella* spp., rapid and unambiguous detection is crucial for timely antibiotic therapy of infected patients. While polymerase chain reaction (PCR) is the gold standard for diagnostics of most infectious diseases, antibody-based assays that detect specific antigens of the pathogen are commonly used as confirmatory methods. However, these antibodies often feature insufficient specificity due to the high degree of relatedness of these pathogens to their non- or less pathogenic relatives. Receptor binding proteins (RBPs) of bacteriophages, which mediate recognition and binding to host bacteria, represent a promising alternative to antibodies. Here, we identified RBPs of a variety of phages specific for *Bacillus anthracis*, *Yersina pestis*, *Burkholderia pseudomallei* and *Brucella* spp. and utilized them to develop a set of novel tools for detection of these notorious pathogens. For this, recombinant RBPs were produced as fusions with different reporter proteins, such as fluorescent proteins or enzymes. In addition, RBPs were coupled to magnetic beads to serve as highly specific capture molecules for bacterial pathogen enrichment or isolation approaches.

P-2-5

Uridine diphosphate (UDP)- glycosyltransferases (UGTs) are associated with insecticide resistance in the major *malaria vectors Anopheles gambiae s.l* and *Anopheles funestus*

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Malaria remains one of the highest causes of morbidity and mortality, with 249 million cases and over 608,000 deaths in 2022. Insecticides, which target the *Anopheles* mosquito vector, are the primary method to control malaria. The widespread nature of resistance to the most important insecticide class, the pyrethroids, threatens the control of this disease. To reverse the stall in malaria control there is urgent need for new vector control tools, which necessitates understanding the molecular basis of pyrethroid resistance. In this study we utilised multi-omics data to identify uridine-diphosphate (UDP)- glycosyltransferases (UGTs) potentially involved in resistance across multiple *Anopheles* species. Phylogenetic analysis identifies sequence similarities between Anopheline UGTs and those involved in agricultural pesticide resistance to pyrethroids, pyrroles and spinosyns. Expression of five UGTs was characterised in *An. gambiae* and *An. coluzzii* to determine constitutive over- expression, induction, and tissue specificity. Furthermore, a UGT inhibitor, sulfinpyrazone, restored susceptibility to pyrethroids and DDT in *An. gambiae*, *An. coluzzii*, *An. arabiensis* and *An. funestus,* the major African malaria vectors. Taken together, this provides clear association of UGTs with pyrethroid resistance as well as highlighting the potential use of sulfinpyrazone as a novel synergist for vector control. Characterisation of these Anopheline UGTs using a bacterial expression system has also been adopted for *in vitro* enzyme assays to investigate their relationship with key public health insecticides, as well as the UGT inhibitor.

P-2-6

Prevalence and mortality of patients with clinically diagnosed tuberculosis in high burden countries: a systematic review and meta-analysis

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Background: Clinical diagnosis of tuberculosis (TB), referring to TB without bacteriological confirmation, is common and may undermine the impact of new diagnostic tests. We aimed to describe the proportion of people with presumed TB who were diagnosed clinically, and their outcomes compared to those with bacteriologically confirmed TB.

Methods: We systematically searched Medline, Web of Science, Embase and Cochrane Library from January 2010 to May 2024, using terms for "TB", "diagnostics" and "high TB burden". We excluded studies with participants aged <15 years, not reporting clinical and bacteriologically confirmed TB, those restricted to sub-populations, and studies that were not trials, cohort or cross-sectional in design. Data was extracted and risk of bias assessed using a custom-designed tool based on validated tools. Forest plots and summary estimates were calculated overall and by pre-specified subgroups including HIV status, diagnostics available and healthcare level. Risk ratio (RR) for mortality of clinical compared to bacteriological diagnosis was evaluated by random effects meta-analysis. The review is registered on PROSPERO (CRD42023404419).

Results: Searches identified 5,365 records, 51 studies were included in our analysis. 12 studies (23.5%) were rated as low risk of bias. Overall, the median proportion of TB diagnosed clinically (n=80,249 patients) was 39.3% (95% CI [32.3%, 45.7%], ranging from 8.1% to 76.3%), with high heterogeneity across studies (I2=99.3%). The proportion diagnosed clinically was higher in people living with HIV (45.6%, 95% CI [37.5%, 54.0%] vs. HIV negative: 36.6%, 95% CI [24.9%, 55.1%]), extrapulmonary TB (71.0%, 95% CI [50.4%, 90.5%] vs. pulmonary TB: 37.7%, 95% CI [31.1%, 46.6%]), and in secondary level healthcare facilities (48.8%, 95% CI [39.9%, 59.5%], vs. primary healthcare: 35.4%, 95% CI [28.5%, 44.3%]). Studies using only sputum smear microscopy compared to studies using Xpert MTB/RIF (PCR) had similar proportions diagnosed clinically (32.1%, 95% CI [21.6%, 40.9%], and 37.7%, 95% CI [11.0%, 71.8%]). Proportions did not differ by study year. The pooled RR for mortality (n= 20,523 patients, 10 studies) was 1.52 (95% CI [1.05%, 2.19%], I2 = 78.7%) indicating higher mortality for patients diagnosed clinically.

Discussion: The proportion of patients diagnosed clinically remains high and has not declined despite introduction of more accurate molecular diagnostics such as Xpert MTB/RIF. Mortality risk is higher in clinically diagnosed patients compared to bacteriologically diagnosed, suggesting some of these patients have conditions other than TB that are not being adequately treated. High heterogeneity across studies is a limitation. Clinical diagnosis may undermine the impact of new diagnostic strategies with improved accuracy in TB, and further research is needed to understand drivers and impact of clinical diagnosis and associated poorer outcomes.

Fig. 1

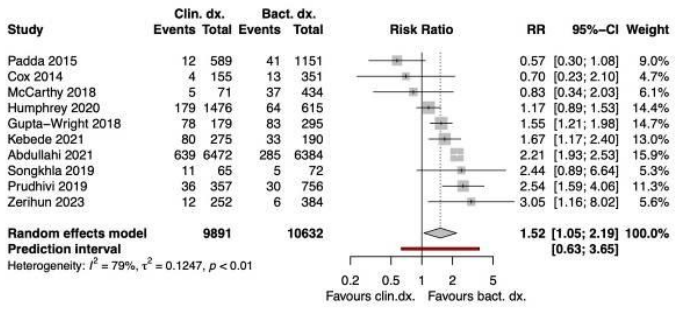
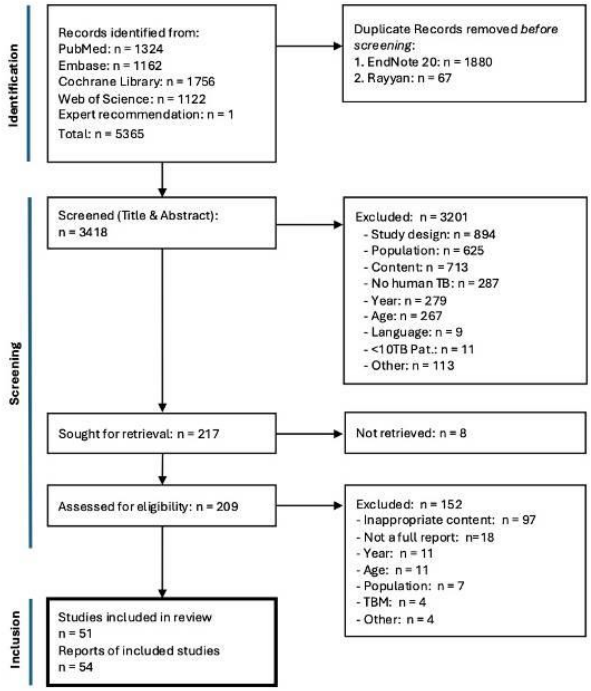


Fig. 2



P-2-7

The impact of chronic schistosomiasis on co-infections with dengue virus in Madagascar

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Chronic schistosomiasis is highly prevalent in the tropics and can cause high morbidity. Research on the impact of this disease on morbidity caused by viruses circulating in the same areas, such as dengue virus (DENV), is scarce. Madagascar is a country highly endemic for schistosomiasis with only sporadic outbreaks of DENV. Our hypothesis is that chronic schistosomiasis might confer protection against DENV infections or dengue fever progression.

Serum samples, collected through a cross sectional study in schistosomiasis and DENV endemic regions of Madagascar, were used in a plaque reduction neutralization test (PRNT) after assessment of Schistosome infection and DENV/*Flaviviridae* seroprevalence through an in-house PCR and pan-DENV IgG ELISA, respectively.

A total of 990 serum samples have been collected and analyzed for Schistosome infection (59,5 %) and DENV/*Flaviviridae* seroprevalence (3,3 %/16,9 %) and 822 samples were submitted to the PRNT. Samples with pre-existing DENV/*Flaviviridae* antibodies were excluded from further analysis. A significant reduction of the median plaque number in Schistosome-infected participants was observed and this effect remained significant when adjusting for other biological variables such as age and sex.

In our Malagasy study population, we observed a low seroprevalence of DENV in a highly endemic schistosomiasis area. Our preliminary results corroborate our initial hypothesis of schistosomiasis interfering with DENV infections with the underlying molecular mechanisms remaining to be further investigated.

P-2-8

Emergence of *Mycoplasma pneumoniae* before and after COVID-19 pandemic in Germany

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Purpose: *Mycoplasma (M.) pneumoniae* is a common pathogen of community-acquired pneumonia (CAP). Epidemics occur every 3-7 years especially in pediatric patients. We collected data from a large laboratory network in Germany to define the epidemiological dynamics in the pre- and post-COVID-19 pandemic period.

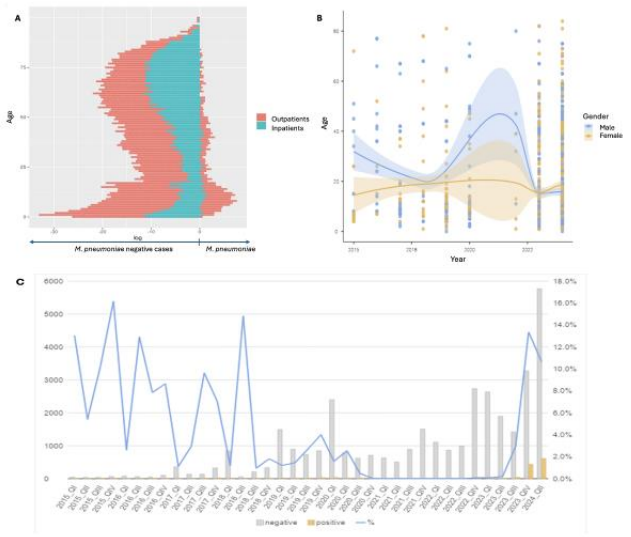
Methods: In this retrospective cohort study we included all patients that obtained targeted or multiplex PCR for *M. pneumoniae* from nasopharyngeal swabs, sputum or bronchoalveolar fluids from 2015 - 2024. Demographic data (age, sex, place of residence, in- or outpatient status) were compared between *M. pneumoniae* positive and negative patients and co-infections with bacterial or viral pathogens analyzed.

Results: We screened 38.204 patients for *M. pneumoniae*. 1448 cases (3.8 %) of *M. pneumoniae* were identified (48.8% females). Pediatric patients ≤18 years represented 75.7% of *M. pneumoniae* patients and 2.3% were ≥60 years. 95.0% of *M. pneumoniae* positive cases were taken from outpatients compared to 78.4% in *M. pneumoniae* negative cases (p<0.001, OR 0.19 95%-confidence interval (CI): 0.15 – 0.24)(figure 1A). We observed differences in age (p = 0.006) and sex (p = 0.04) in *M. pneumoniae* cases from 2015 –

2024 (figure 1B). Incidence of *M. pneumoniae* increased in fourth quartile 2015 (16.2%), second quartile 2018 (14.8%) and fourth quartile 2023 (13.4%). No cases were detected during COVID-19 pandemic 2021 (figure 1C). Young age, outpatient status and year of testing were predictors of *M. pneumoniae* detection in multivariate analysis (p<0.001). Most common co-infections were influenza A/B, rhinovirus, metapneumovirus and respiratory syncytial virus.

Conclusions: Our study highlights the epidemic spread of *M. pneumoniae* during the winter season 2015 and spring / summer 2018 in Germany. *M. pneumoniae* re-emergence in 2023/2024 in Germany is comparable to the pre-pandemic number of cases. Empirical treatment of CAP patients often does not include coverage of *M. pneumoniae*. A more thorough implementation of available surveillance data into clinical routine, respective therapies could be adapted more quickly during epidemic outbreaks of *M. pneumoniae* infections. As hospitalization is increased in adult patients and severe courses of disease have been frequently reported, physicians should be aware and test for *M. pneumoniae* in CAP.

Fig. 1



P-2-9

Preclinical development of the antibiotic Corallopyronin A for the treatment of filarial infections

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Question: The goals of the WHO Roadmap 2021-2030 to eliminate the debilitating Neglected Tropical Diseases onchocerciasis and lymphatic filariasis are hampered by the lack of a safe, short-term macrofilaricidal – adult worm killing – drug. Here, we present the development of Corallopyronin A (CorA), a bacterial RNA polymerase inhibitor, which kills the essential Gram-negative *Wolbachia* endosymbiont present in filariae. Depleting the *Wolbachia* endosymbionts prevents filarial development, causes worm sterility and slowly kills the adult filariae, thus it is a promising macrofilaricidal candidate for human filarial infections.

Methods: Efficacy of CorA against filarial nematodes was tested in surrogate animal models (*Litomosoides sigmodontis, Onchocerca ochengi*, a species closely related to the nematode that infects humans). Furthermore, PK studies in mice, rats and dogs were performed and the relationship of CorA exposure levels and drug efficacy was assessed. Standard toxicity studies were performed *in vitro* and *in vivo*, including non-GLP 7-day repeated dose studies in rats and dogs. Using amorphous solid dispersion, two solid formulations were developed, which are suitable for clinical trials.

Results: Using an oral solid formulation in the *L. sigmodontis* gerbil model, CorA depleted >99% of *Wolbachia* and was macrofilaricidal with a 2-week monotherapy (30 mg/kg TID; 60 mg/kg BID) or ten-day co-administration with albendazole (CorA 60 mg/kg TID plus albendazole 10 mg/kg BID for 7 days). The *Wolbachia*-depleting and macrofilaricidal activity of a 2-week CorA treatment was confirmed in mice implanted with *O. ochengi*. *In vitro* toxicity tests demonstrated CorA as safe and 7-day repeated dose studies in rats and dogs demonstrated no prohibitive safety issues [No Observed Effect Level (dog) = 150 mg/kg; lowest observed adverse effect level (rat) = 250 mg/kg]. From the PK/PD data, a Physiologically Based Biopharmaceutics Model (PBBM) was developed and predicted a safety margin for the predicted human efficacious dose that supports clinical trial. PBBM modeling, including intravenous PK results from gerbils, is ongoing and suggests an even greater therapeutic window. The results of GLP toxicology studies conducted in Q3/2024 will be presented. The developed solid formulations had a stability of >3 months at 30 °C and >6 months at 25 °C. CorA production was successfully established at a GMP-certified contract manufacturing organization to produce the CorA for phase 1 clinical studies in 2026.

Conclusions: CorA is one of only three novel macrofilaricidal candidates in late preclinical/clinical development and the only *Wolbachia*-targeting candidate, a mode of action previously demonstrated to be safe and well tolerated. In comparison to doxycycline, the gold standard for individual macrofilaricidal treatment, we predict that the treatment can be reduced from 4-6 weeks to 10-14 days.

P-2-11

Improving prediction of RSV infection dynamics and disease burden based on population-based assessments with novel multiplex tools

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Background: The lack of well-parametrized mathematical models, gaps in understanding transmission, and insufficient reinfection data challenge the accuracy of respiratory

syncytial virus (RSV) forecast models. In fact, only a few European projections were able to predict the high RSV hospitalizations in 2021 and 2022, raising the need for a comprehensive strategy incorporating population-based surveillance data and state-of-the-art mathematical models.

Methods: We used an RSV multiplex serological assay in an established population-based panel (n=1,572 in 2022 for the seasons 20-22 and n=836 in 2023 for the season 22/23) in conjunction with sentinel and notification data to parametrize ordinary differential equation models, able to trace dynamics in Germany in 2021 and 2022, and inform and asses projections for 2023/24.

Results: Estimates of RSV reinfection based on a 20% post-F and a 45% N antibody increase in the assay were 5.7% (95%CI: 4.7-6.9) from 2020 to 2022 and 12.7% (95%CI: 10.5-15.2) from 2022 to 2023. Age-specific dynamics of reinfection varied between the waves. In 2021, the 30-39 age group had a higher risk of reinfection, while in 2022 all age groups except the 30-39 age group had a higher risk of reinfection. Projections for 2023/2024 suggested a season similar to 2022, with a later peak. This aligned with the actual RSV season in Germany, which peaked even later than predicted.

Conclusion: Population-based assessments revealed unexpected age-specific reinfection patterns in Germany's 2021 and 2022 RSV seasons, which are not evident in public surveillance data but are crucial for parameterizing dynamic models. Rapid age-specific reinfection assessments, and models that incorporate these data will be critical for understanding and predicting RSV dynamics, especially with changing post-pandemic patterns and new prevention strategies as newly introduced vaccines and monoclonal antibodies.

Helmholtz Association, EU Horizon 2020 research and innovation program, Federal Ministry of Education and Research, and German Research Foundation supported this work.

P-2-12

Identification of genetic variants associated with post-TB lung disease

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Patients recovering from Tuberculosis (TB) may suffer from post-TB lung disease (PTLD) characterised by spirometric and radiological abnormalities, as well as respiratory impairments. Although the literature on PTLD is expanding, its genetics remains largely unexplored. This study, as part of the TB-Sequel study (https://www.tbsequel.org/), utilised genome-wide association studies (GWAS) to identify genetic variants associated with lung function in TB patients for the first time.

Lung function was assessed via spirometry, specifically, forced expired volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio. We performed GWAS based on lung function measures at the end of TB treatment (cross-sectional GWAS) and the change in lung function over the first year from the start of the TB treatment (longitudinal GWAS) in patients diagnosed with TB from four African countries (Gambia, Mozambique, Tanzania, and South Africa). 765 individuals were included in the cross-sectional GWAS and 976 individuals in the longitudinal GWAS. Genome-wide significant single nucleotide polymorphisms (SNPs) (p-value < 5E-8) were assigned to putative causal genes using in silico methods. The prioritised genes were further investigated using publicly available single-cell RNA-sequencing data from lung tissue.

As the first GWAS examining post-TB lung function, we identified three SNPs associated with lower FEV1, higher FVC at the treatment end and decreasing FVC over time, respectively. The putative genes prioritised are involved in lung remodelling, repression of Wnt/-catenin signalling (activated in alveolar type 2 (AT2) cells during lung injury), and inflammatory signalling, respectively. The expression of these genes in relevant cell types within TB-affected lung tissue can be confirmed using a publicly available single-cell dataset.

The SNPs identified may affect post-TB lung function by impacting lung tissue repair or the extent of immune response to Mycobacterium tuberculosis.

P-2-15

The interaction of schistosomes with the maternal microbiota and effects on the offspring immune system

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Question: Schistosomes manipulate the host immune system to ensure their prolonged survival, with bystander effects on the infected host and their progeny including reduced allergic sensitivities and impaired vaccine responses. We propose that these immune alterations in the offspring stem from specific maternal signals that are modified during chronic infection. Given that 40 million women of child-bearing age are infected in Sub-Saharan Africa, it is imperative to delve deeper into these signals. One possible mechanistic angle behind these changes is the modification of the maternal microbiota, which plays a large role in shaping the offspring immune system and has been investigated during schistosome infection, but not in a fetomaternal setting.

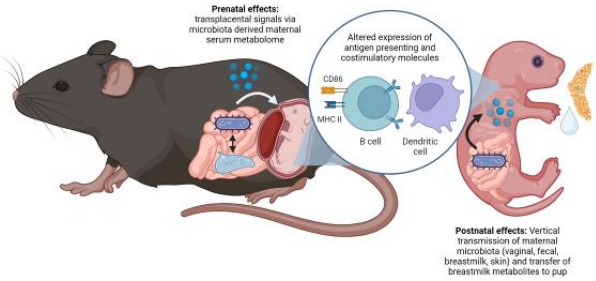
Methods: To disentangle pre- vs postnatal effects, we carry out a cross-foster experiment in a mouse model and investigate the expression of antigen-presenting and costimulatory molecules on B cells and dendritic cells in the spleen, mesenteric lymph node and bone marrow as well as stem cell frequencies in the bone marrow using FACS. We also analyse the maternal and offspring stool microbiota from

regulatory phase of infection by 16s rRNA sequencing in mice and complement this with metabolomic analysis of stool and serum.

Results: We show that the expression of antigen-presenting and costimulatory molecules is consistently increased in offspring suckled by an infected mother, compared to those gestated by one. We identify changes in the maternal microbiota and bile acid levels in the stool and serum due to schistosomiasis, however these changes in the microbiota are not inherited by the offspring.

Outlook: We will complement our metabolomic analysis by investigating whether changes can also be seen in the offspring of infected mice, as well as in serum samples from our human mother-child cohort (Helmvit). We will also carry out metabolomic and proteomic analysis of mouse breastmilk.

Fig. 1



P-2-16

Self-amplifying RNA vaccine protects mice against lethal homologous and heterologous CCHFV infection

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Infection with Crimean-Congo hemorrhagic fever virus (CCHFV) can cause hemorrhagic fever in humans, which is fatal in several hundred cases per year. To date, there are no approved antiviral therapeutics or vaccines, and CCHFV was recently listed by the WHO as a priority pathogen for research, with high potential for Public Health Emergencies of International Concern (PHEIC).

Due to the high genetic diversity of the different CCHFV lineages, preclinical efficacy studies should be performed with distantly related CCHFV isolates to demonstrate cross-protection. To analyze the broad efficacy of antiviral drugs or vaccines against CCHFV, we established two mouse models with the phylogenetically distantly related CCHFV strains Afg09-2990 and Kosovo Hoti. Interferon alpha/beta receptor knockout (IFNAR-/-) mice infected with 100 TCID50 of either virus showed severe weight loss, as well as impaired general condition and spontaneous behavior before reaching humane endpoint criteria. It should be emphasized that the clinical score was significantly higher in Afg09-2990-infected animals versus Kosovo Hoti-infected animals.

Both models were used to evaluate the efficacy of vaccination with self-amplifying RNAs (saRNAs) encoding the CCHFV Afg09-2990 major viral glycoprotein (Gc) or the nucleoprotein (NP) co-formulated in lipid nanoparticles (saRNA Gc+NP). In contrast to the vehicle-immunized control mice, all prime/boost-immunized animals developed NP- and Gc-specific antibodies and survived both homologous and heterologous infection. Moreover, even a single dose immunization with saRNA Gc+NP induced an immune response capable of conferring complete protection against lethal Afg09-2990 challenge. The protected mice showed no clinical symptoms, apart from slight weight loss or transient ruffled fur in individual animals. A comprehensive post-mortem analysis of the serum and various organ samples showed that CCHFV-specific RNA and infectious CCHFV were not detectable in the vaccinated animals.

In summary, we have established two lethal CCHFV mouse models and used them to test vaccine-mediated cross-protection. We successfully demonstrated the efficacy of a saRNA-based vaccine administered as a single dose and as a prime/boost vaccination. The use of saRNA Gc+NP resulted in complete protection of mice against lethal CCHFV challenge with different isolates.

P-2-17

First Controlled Human Hookworm (Gabonese Necator americanus) Infection in Africa: A single-center participant-blinded placebo-controlled trial

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Question: Efforts are underway to develop vaccines against Hookworm. Expanding scientific capacities of research institutions working in disease endemic regions is essential in accelerating the development of new drugs and vaccines. Controlled human hookworm infection (CHHI) studies in the global north in unexposed individuals, like natural infection, have not contributed to developing sterilizing immunity, the end-goal of vaccine development, initiating efforts centralized in disease-endemic areas and populations. This CHHI study was conducted to assess safety and patency of locally derived larvae of Gabonese *Necator americanus* (G-*Na*) in a hookworm-endemic region.

Methods: We enrolled 15 male participants with limited exposure to hookworm living in Lambaréné, Gabon, including 2 Caucasians, and allocated them, in a participant-blinded manner, into intervention or placebo groups in a 2:1 ratio. Ten healthy individuals were infected with 50 G-*Na* larvae and followed up weekly for 24 weeks, with abrogation of infection with three-day dosing of 400mg of albendazole at week 16. The primary endpoints of the study included the frequency and intensity of adverse events, and the detection of hookworm in infected participants 9-16 weeks after challenge. Stools were collected and tested for G-*Na*. Collected G-*Na*, blood and urine samples were stored for immunologic assays.

Results: The mean age and BMI of participants was 34 years and 23 kg/m2, respectively. One participant was unable to complete the study due to moving out of the study area. Participants in the intervention vs placebo groups reported a greater proportion of solicited AEs, including diarrhea (50% vs 0%), Itching at inoculation site (90% vs 20%), and nausea/vomiting (70% vs 20%). There was one serious AE, occurring in the placebo group and deemed unrelated to the study by investigators. Stool culture data showed that infection was established between weeks 6-8 after challenge, in all participants. Immunologic analyses are ongoing.

Conclusion: This is the first time a CHHI model has been established in Africa. The model is safe and patent, paving the way for accelerated hookworm drug and vaccine development and assessment using locally derived G-*Na* in populations living in Hookworm-endemic Gabon.

P-2-18

Association of sonographic findings with severity of dengue fever: A systematic review and meta-analysis

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Question: evaluate the association between sonographic findings and severity of dengue fever

Methods: This systematic review and meta-analysis was performed following the PRISMA Guidelines. Data were gathered from PubMed, Embase, LILACS, and Cochrane databases from inception until June 2024. We included original studies of adults with serologically confirmed dengue fever without concurrent diagnosis who underwent sonographic imaging assessment. Outcomes included overall mortality and severity of dengue fever, as per WHO standard definitions of 1997 and 2009. Plasma leakage was defined as sonographic evidence of ascites and/or pleural effusion. After deduplication and initial triage of 1795 studies, 94 were selected for full-text screening, and 21 were included in the final analysis. Data were plotted onto Excel spreadsheets and analyzed using R version 4.4.1. A random-effects model was used due to anticipated heterogeneity between studies in clinical practice. Heterogeneity was evaluated with the Cochrane Q test and I2 statistics; p values inferior to 0.10 and I2 > 25% were considered significant.

Results: A total of 21 studies of 3,214 individuals met inclusion criteria. Most studies were observational cohorts of hospitalized patients. The most frequently reported sonographic findings associated with dengue fever were gallbladder wall thickening (GBWT), ascites, and pleural effusion. Overall analysis showed a pooled sensitivity of sonographically identified GBWT for the outcome of severe dengue of 0.83 (95%CI 0.70-0.91) with specificity of 0.70 (95%CI 0.53-0.83), with an elevated heterogeneity (87%), which could be partially explained by threshold effect. Pooled sensitivity and specificity of sonographically evidenced ascites were respectively 0.60 (95%CI 0.42-0.76) and 0.89 (95%CI 0.77-0.95) for severe dengue. Evidence of pleural effusion showed a pooled sensitivity and specificity of respectively 0.47 (95%CI 0.24-0.72) and 0.93 (95%CI 0.76-0.98) for the outcome of severe dengue, with high heterogeneity 86-90%. The AUC of GBWT, ascites, and pleural effusion in this context were respectively 0.80 (95%CI 0.68-0.87), 0.78 (95%CI 0.66-0.84), and 0.74(95%CI 0.55-0.88). Sonographic evidence of plasma leakage showed a pooled sensitivity of 0.67 (95%CI 0.46-0.84) and specificity of 0.58 (95%CI 0.22-0.64) for the prediction of mortality in dengue fever.

Conclusions: Our findings suggest that ultrasound screening may be a valuable tool to identify individuals who may potentially develop severe dengue fever, particularly in resource-limited settings. The most frequently associated sonographic findings were thickening of the gallbladder wall, ascites, and pleural effusion.

P-2-19

Genomic sequencing based hybrid characterisation of Schistosoma worms from clinical cohorts - "GENOSCHIS"

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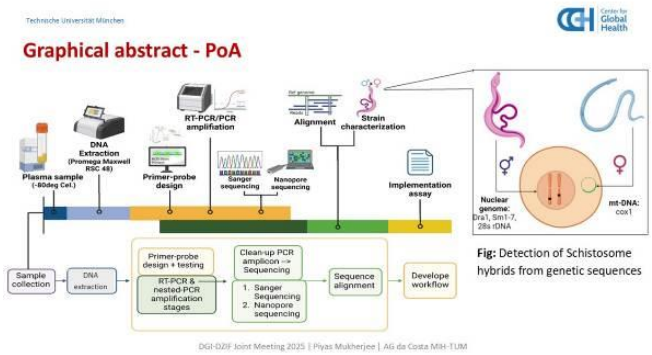
The Neglected Tropical Disease (NTDs) Schistosomiasis, caused by blood-flukes of genus Schistosoma sp., is a disease of great medical and veterinary significance in tropical and subtropical regions. In the backdrop of (re-)emergence and spread of (zoonotic) hybrids in the endemic zones; that disrupts existing diagnosis, treatment & control measure, and the epidemiological burden as well, there is an impetus for novel technologies and diagnostic tools. ("The Roadmap to Elimination of NTDs (2021-2030), the WHO, 2020). This opportunity is provided by molecular genetic tools to target DNA/genetic materials of the worm in patient samples known as cell-free DNA (cf-DNA). The circulating cf-DNA in the patient blood samples (plasma or serum), provides nuclear & mitochondrial gene markers, for detection via qPCR & PCR techniques (Wichmann et al., 2009). The significance of utilizing a nuclear & mitochondrial (mt-DNA) marker lies with the identification of hybrid strains (Cnops et al. 2021), as mt-DNA is inherited only via the female worm in parental generation. The dra1 (Hamburger et al., 2006), and 28S rDNA (Sondoval et al., 2006) were selected as nuclear gene targets. And the cox1 (cytochrome c oxidase subunit-1) was considered for its well described characteristics as mt-DNA target (Littlewood et al., 1997). To that end, we aimed to investigate the application of NGS-based assay for detection and characterization of hybrid infection from patient samples in a clinical cohort. In addition to that, we also targeted at validation of the described genetic markers for their viability in specifically elucidating the nature of the hybrid strains. Finally, we wanted to explore and comment on the optimization of Oxford Nanopore sequencing of target amplicons, and downstream analysis workflow for a potential on-field application. We performed a serial qPCR/PCR-assay with Dra1-PCR, followed by positive samples processed via 28S-genus qPCR, and finally nested-PCR approach for the cox1 gene. For the experiment, we processed a total of n= 183 patient blood plasma & serum samples (PBMC) in a clinical cohort, as a part of existing study at our lab, from Gabon, Africa. Following, the qPCR/PCR assays, the amplicons of positive test samples were validated via gel electrophoresis or melt-curve analyses. Finally, the purified extracted amplicons were sequences via Sanger sequencing & Oxford Nanopore NGS-based assay. The data could be further processed for strain characterization with reference sequences from databases, and strain characterization of the hybrids infection. The qPCR/PCR tests provided conclusive indications, particularly for serum & plasma samples. It is a high-throughput, efficient solution for diagnosis & strain identification with higher congruency. and more reliable.

Hence, the NGS-based & molecular genetic tools provide novel technological application in development of diagnostic tools for detection, control & treatment of Schistosomiasis.

Fig. 1



P-2-20

Assessment of the performance of a newly developed PCR test, compared to CAA and urine filtration for the detection of *Schistosoma hematobium* in Lambarene, Gabon

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Schistosomiasis is a neglected tropical disease caused by the parasitic flatworms of the genus *Schistosoma*. It is the second most impactful parasitic disease after malaria, with over 250 million people infected, with substantial associated morbidity and mortality. The WHO roadmap for NTDs to 2030 targets schistosomiasis for elimination as a public health concern in all countries, key to achieving this target is developing novel diagnostics. Current diagnostic approaches are inadequate for mapping, monitoring treatment and post-treatment surveillance, with the goal being to develop sensitive point-of-care tests that aid in decision-making and mapping, as well as provide additional information regarding drug resistance, environmental and xeno-monitoring and testing for the under-served genital manifestation of schistosomiasis. To this end we are developing a novel, field-applicable PCR test for *Schistosoma mansoni* and *hematobium* infection. We have designed new assays and protocols to bring high-throughput, high-sensitivity diagnostics to the field. As part of this development, we have compared the performance of our assay to established egg microscopy and antigen testing methods, through the testing of over plasma 300 samples collected as part of the DFG HelmVit study in Lambarene, Gabon. Our test uses crude DNA extraction methods, that are applicable to low-resource settings, and which only needs 20µl of sample input. Despite this low sample input, it performs comparatively to existing PCR methods using up to 1ml of sample input. Future directions include further optimising the DNA extraction protocols, through active engagement with industry; and similarly taking advantage of new technologies to bring even greater sensitivity and througput to the field; with the latter consisting of the adaptation of the PCR to a platform from HP Health Solutions Germany (formerly GNA Biosolutions), as a part of a long-standing collaboration.

P-2-21

Identifying novel antiviral small molecules and their targets in coronavirus-infected cells

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Coronavirus disease 2019 (COVID-19) has been a disastrous pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A panel of small molecules targeting the viral polymerase and main protease has been approved for clinical use. New drugs with broad activity against different members of the *Coronaviridae* family and which inhibit new viral targets are needed for future zoonotic outbreaks. Hence, we aimed to find novel inhibitors against infections with human coronaviruses (HCoVs) and identify their druggable targets.

After screening 54,528 small molecules against a HCoV-229E reporter virus, we selected the 300 compounds with the strongest antiviral effect. Next, we confirmed the antiviral effect of 73 compounds in a plaque assay. Ultimately, we selected the best 5 compounds for the target identification phase: 3 compounds (C1, D1, E2) were chosen due to their excellent selectivity indices against HCoV-229E, and 2 because of their cross-reactivity against SARS-CoV-2 (D2, E1). By serial passage in the presence of high compound concentrations, we generated resistant HCoV-229E mutants against C1 and D1. For the viral clones generated in the presence of C1, next-generation sequencing (NGS) revealed a double mutation in nsp4 and nsp6 with additional mutations in nsp15. C1 inhibits late in the replication cycle and one of the drug-resistant isolates is also cross-resistant towards K22. Our finding that the double nsp4/6 mutation confers resistance towards C1 was confirmed by reintroducing these mutations in a HCoV-229E wildtype genome. As nsp4 and nsp6 are essential for double-membrane vesicle (DMV) formation and K22 is known to affect DMV formation, we hypothesize that C1 might represent a novel class of DMV formation inhibitors. For the drug-resistant virus generated in the presence of D1, we found a point mutation in the spike open reading frame. Studying viral attachment/entry using vesicular stomatitis virus particles pseudotyped with the HCoV-229E spike protein, we confirmed the spike protein as the viral target of D1.

In summary, we identified 5 lead novel antiviral small molecules with potent activity against HCoV-229E, of which 2 also inhibit SARS-CoV-2. A double mutation in nsp4 and nsp6 (involved in DMV formation) confers drug resistance towards C1, and the spike protein is the viral target of D1. By identifying novel lead antivirals against HCoVs and extending our knowledge on their druggable targets, we are laying a crucial foundation for future drug development against HCoVs.

P-2-22

Impact of helminth infections during pregnancy on maternal and newborn Vitamin D and on birth outcomes

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Question: Poor birth outcomes in low- and middle income countries are associated with maternal vitamin D deficiency and chronic helminth infections. Here, we investigated whether maternal *Schistosoma haematobium* affects maternal or cord vitamin D status as well as birth outcomes.

Methods: In a prospective cross-sectional study of pregnant women conducted in Lambaréné, Gabon, we diagnosed maternal parasitic infections in blood, urine and stool. At delivery we measured vitamin D in maternal and cord blood.

Result: *S.haematobium*, soil-transmitted helminths, and microfilariae were found at prevalences of 30.2%, 13.0%, and 8.8%, respectively. Insufficient vitamin D and calcium levels were found in 28% and 15% of mothers, and in 11.5% and 1.5% of newborns. Mothers with adequate vitamin D had lower risk of low birthweight babies (aOR=0.11, 95%CI: 0.02 - 0.52, *p*=0.01), whilst offspring of primipars had low cord vitamin D levels, and low vitamin D levels increased the risk of maternal inflammation. Maternal filariasis was associated with low calcium levels, but other helminth infections affected neither vitamin D nor calcium levels in either mothers or newborns.

Conclusion: Healthy birth outcomes require maintenance of adequate vitamin D and calcium levels. Chronic maternal helminth infections do not disrupt those levels in a semi-rural setting in sub-Saharan Africa.

P-2-23

Comparison of POC-CCA and UCP-LF-CAA for the detection of Schistosoma mansoni infection in pregnant women and pre-school-aged children from endemic area

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Background: Accurate and affordable diagnostics for the detection of schistosome infection are pivotal for clinical care, disease surveillance and control programmes towards the elimination of schistosomiasis as a public health problem. Prior research has shown that the point-of-care (POC-CCA) circulating cathodic antigen urine test is well suited for the detection of *S. mansoni* infections in endemic areas. However, data on the performance of this assay in vulnerable populations at risk of infection such as pre-school-aged children and pregnant women, for which WHO recommends treatment based on diagnosis, is limited. To address this research gap, this study aimed to compare the diagnostic performance of POC-CCA and UCP-LF-CAA (Up-Converting reporter Particle Lateral Flow Circulating Anodic Antigen) assays in pregnant women and infants in an endemic area of Madagascar.

Methods: As part of the cluster randomized controlled trial "Fast and reliable easy-to-use-diagnostics for eliminating bilharzia in young children and mothers" (Pan-African Clinical Trial Register PACTR201905784271304) conducted in 2020-2022 in 42 primary health care centers in the Itasy, Bongolava and Amoron"i Mania regions of Madagascar urine samples were collected from 4615 women at 17-36 weeks of pregnancy and from 2008 infants of 6-12 months of age.

The UCP-LF-CAA and POC-CCA were performed at the *Centre d"Infectiologie Charles Mérieux* in Antananarivo. The threshold of 2 pg/ml was used to define UCP-LF-CAA positivity. For POC-CCA the results were scored as negative, trace or positive. The proportion of agreement between the assays was estimated under two scenarios: considering POC-CCA trace results as positive, and as negative.

Results: In pregnant women, 6.5% (n=299), 12.6% (n=579) and 80.9% (n=3737) of the samples were classified as negative, trace, and positive, based on POC-CCA and 56.0% (n=2,585) were positive with the UCP-LF-CAA. The proportion of agreement between the two assays was 55.1% and 55.7% considering trace POC-CCA results as negative and as positive, respectively.

In infants, 29.2% (n=586) were POC-CCA negative, 29.6% (n=594) had trace result and 41.2% (n=828) were positive. Based on UCP-LF-CAA, 93.8% (n=1884) were negative and 6.2% (n=124) were positive for schistosome infection. The proportion of concordant results on both assays ranged from 57.9% when trace POC-CCA results were assumed negative to 30.7% when those were considered positive.

Conclusions: Our preliminary results show considerable differences in positivity rates for *S. mansoni* infections comparing the POC-CCA and UCP-LF-CAA in a large sample of pregnant women and children from endemic areas. This raises concerns regarding the use of the POC-CCA in these populations highlighting the need for alternative rapid diagnostics to detect schistosome infection.

P-2-24

Helminthic larval stage mediates cellular apoptosis in neurocysticercosis through alteration of caspase pathways

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Introduction: In human neurocysticercosis (NCC), the cellular and molecular mechanisms of host-parasite interactions triggering brain inflammation and acquired epilepsy, especially in children in Sub-Saharan Africa, are poorly understood. Emerging evidence indicates that the sustained brain inflammation leading to symptom development and disease severity is determined by the viability of the cyst of the pork tapeworm *Taenia solium*. Whereas viable cysts in the brain potentiate immune regulation associated with asymptomatic disease, we recently showed that decaying cysts, such as following treatment, cause brain microglia inflammation and peripheral immune cell apoptosis by yet unidentified mechanism.

Material and methods: We investigated the cellular interactions and molecular mechanisms governing decaying cyst-driven cell apoptosis and necrosis in healthy human peripheral blood immune cells, purified monocytes as well as murine brain microglia via FACS. Guided by inhibitor-based green and red fluorescent probe detection of active caspases, we explored apoptotic signaling pathways and related parasitic molecules by differential mass spectrometry analysis of cyst components. Additionally, sera from NCC-patients during and after anti-helminthic treatment were screened for known apoptosis mediators such as FasL, TNFα, ROS and caspases and cyst-cell specific apoptotic interactions were confirmed by immunohistochemistry staining in naturally-infected pig brain slices.

Results: We report here that vesicular fluid derived from intact cysts barely induced necrosis but rather strong levels of apoptosis in a dose-dependent manner *in vitro*, preferentially in CD16+ monocytes, microglia, CD3+ T cells and CD3- lymphocyte populations, which infiltrated infected pig brain near the cyst. This process was insensitive to heat treatment and Proteinase K digestion and was furthermore primarily induced by small molecules (<30kDa) involving dynamic changes in caspases, especially caspase 9 signaling in microglia. Furthermore, this effect correlated with an upregulation of caspase 9 but a decrease in Bid, Bcl2 and Ripk3 transcription, suggesting a more dominant involvement of the intrinsic rather than the extrinsic pathway mechanisms of apoptosis. Accordingly, anti-helminthic treatment of confirmed NCC patients resulted in a significant drop in previously elevated FasL levels, an important apoptosis mediator of the extrinsic pathway.

Conclusion: Our study reveals that helminth-induced cellular apoptosis via caspases is a central mechanism that may contribute to brain inflammation and immunopathogenesis in neurocysticercosis and could become important to guide future therapeutic strategies including monitoring of treatment responses.

P-2-25

Diagnostic accuracy of colposcopy screening for female genital schis-tosomiasis implemented at primary level of care: A cross- sectional study

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Question: Diagnosis of neglected tropical diseases poses a major challenge due to the lack of tools that are adapted to endemic contexts. Among others, Female Genital Schistosomiasis (FGS), caused by persistent infection with *Schistosoma haematobium,* is an illustrative example of how the diagnosis of these diseases often represents an unmet medical need in endemic areas. Left untreated, it can lead to complex gynecological syndromes with consequences such as pelvic pain or infertility. The standard screening for FGS is colposcopy, a complex clinical examination that often cannot be performed in resource-limited contexts due to lack of expertise or insufficiently equipped infrastructures. This study addresses the question of what is the diagnostic accuracy of colposcopy in detecting FGS by trained midwives at primary care level and what factors influence this.

Method: The study was implemented in the rural region of Boeny, Madagascar, where a prevalence of FGS above 60% is reported. Colposcopy images were collected and participants screened for signs of FGS by trained midwives at three primary health care centers. Images were re-evaluated by two gynecologists through a blinded reconciliation process. Reference diagnosis was defined as agreement of both gynecologists on the FGS diagnosis; images with a conflicting interpretation were excluded from the analysis. Statistical analysis using R included descriptive statistics, measures of diagnostic accuracy and binary Poisson regression with robust standard errors.

Results: Among 660 women screened for eligibility, 521 participants colposcopy images were analyzed. A final diagnosis from a gynecologist was available in 495 cases. The colposcopy-based detection of FGS signs by midwifes in comparison to the gynecologists showed a sensitivity of 96.4 % (95%CI 93.7-98.0) and specificity of 28.7 % (95%CI 21.8-36.5) with an overall agreement in the diagnostic of 75.0% (95%CI 70.9- 78.7). Multivariate regression showed a positive influence on diagnostic agreement of increasing colposcopy routine in comparison to the start of the study. One study centre presents a negative influence on the agreement of diagnosis.

Conclusion: This study shows the potential of introducing colposcopy at the primary care level as a screening tool for FGS due to its high sensitivity. This could enable a more targeted use of Praziquantel in adults and close the access gap to advanced screening methods at the primary care level in rural areas.

P-2-26

High prevalence of plasmodium infections and low *P. malariae* msp-1 diversity in beninese population: Implications for Malaria surveillance and vaccine development

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Question: *Plasmodium malariae,* one of the five parasite species causing malaria in humans, though less widespread than *P. falciparum*, can result in prolonged and persistent infections. This research aims to monitor asymptomatic *Plasmodium* infections and characterize genetic diversity in the merozoite surface protein (msp-1) of *P. malariae* in Beninese population.

Methods: Blood samples collected from 484 asymptomatic participants in Southern Benin underwent analysis for all human *Plasmodium* species utilizing rapid diagnostic tests (RDT - PfHRP2/LDH), microscopy, nested PCR, and nested real-time qPCR assay for the quantification. Mono- or mixed infections of *P. malariae* underwent *msp-1* genotyping. The *P. malariae msp-1* gene were amplified by a nested PCR, followed by sanger sequencing.

Results: Microscopy and RDTs identified infection rates of *Plasmodium* spp. at 17% and 20%, respectively, whereas nested PCR significantly elevated rates of 38% (P<0.0001) (Table 1). Furthermore, the utilisation of nested qPCR revealed a 12% increase in *Plasmodium* spp. prevalence in

individuals who were uninfected using nested PCR. Molecular methods notably enhanced the detection of *P. malariae* compared to microscopy, with prevalence rates of 7% and 12% using nested PCR and qPCR, respectively (P<0.05). Genetic analysis of *msp-1* revealed a high nucleotide sequence similarity (95-100%) (Figure 1). The mean pairwise nucleotide divergence (π) of *P. malariae* was 0.00602. Gender-based analysis indicated a marginal risk of *P. malariae* infection in females compared to males (RR: 1.3, 95% CI: 0.9 - 8; P = 0.04).

Conclusions: The present study uncovered a high prevalence of *Plasmodium* infections, including sub-microscopic *P. malariae*. Furthermore, our findings demonstrated a low sequence diversity of *P. malariae msp-1* compared to other *Plasmodium* species. This suggests that msp-1 holds promise as a vaccine candidate against *P. malariae* infection, given its low genetic diversity and highly conserved amino acids.

P-2-27

Bringing bioactive secondary metabolites to the clinic – The story of Corallopyronin A production

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The natural product Corallopyronin A, originating from *Corallococcus coralloides*, inhibits bacterial DNA-dependent RNA polymerase [1]. It is currently developed to threat filariases as primary indications and multiresistant *Staphylococcus* infections as secondary indication. To overcome process inhomogeneities, the drug candidate is produced with the recombinant producer strain *Myxococcus xanthus* [2]. Within the last six years, the Helmholtz Centre for Infection Research (HZI) developed a robust bioprocess, optimized and scaled up to technical scale yielding on average 100 mg/L CorA. Since 2016 the HZI has produced more than 500 g of high-quality research grade material (HQ-RGM; >90% purity) for formulation development, stability studies, and preclinical trials.

In 2021, the Belgium company Bio Base Europe Pilot Plant (BBEPP, Ghent, Belgium) was contracted for further scale up (first bioprocess with recombinant myxobacterium in such scale) to industrial scale. In March 2022 USP and DSP were successfully scaled up to 15 m3 scale with a titer of 90 mg/L CorA and purified with an overall yield of 60 %.

In 2023 the German/Canadian company Phyton Biotech GmbH was contracted to evolve the process towards GMP production. Due to increased documentation efforts and high requirements for GMP production, preparation of the first feasibility run took one year. This included validation of existing methods, process adjustments to alternative equipment, and certification of incoming media components and excipients. In addition, experimental data for biological safety assessment, cleaning verification, and wastewater treatment were generated. Challenges of bringing scientific work towards the highly regulated GMP environment were identified and successfully addressed to produce Corallopyronin A for drug product development, produce clinical trial material, and conduct a phase 1 clinical trial scheduled in 2025/2026.

[1] Krome *et al.* 2022: Corallopyronin A: antimicrobial discovery to preclinical development. DOI: 10.1039/D2NP00012A

[2] Pogorevc *et al.* 2019: Production optimization and biosynthesis revision of corallopyronin A, a potent anti-filarial antibiotic. DOI: 10.1016/j.ymben.2019.07.010

P-2-28

Awareness and knowledge about female genital schistosomiasis among European healthcare workers

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Background: Female Genital Schistosomiasis (FGS) is a gynaecological manifestation of persistent infection with *Schistosoma haematobium,* which can lead to severe consequences, such as miscarriage and infertility. It is estimated to affect 56 million women globally, mostly in sub-Saharan Africa (SSA). Most migrant people in Europe are female, often from SSA and therefore at risk of FGS, hence healthcare workers (HCWs) knowledge of FGS is essential for the provision of adequate care. This study aims to assess awareness and knowledge of FGS among European HCWs to inform strategies to improve the management of migrant health.

Methods: In June 2023 - January 2024 we conducted a cross-sectional online survey targeting medical doctors (MDs), nurses and midwives (NMs) working in fields of infectiology, gynaecology, urology, family, travel, internal or occupational medicine. The prevalence of FGS awareness and knowledge was estimated, Poisson regression was used to identify factors associated with MDs' awareness of FGS.

Results: Among 922 surveyed HCWs, 43.7% (CI95%: 39.6-47.9) of MDs and 12.0% (CI95%: 8.8-16.0) NMs have heard about FGS. FGS awareness among MDs was associated with work in clinics for migrants (adjusted prevalence ratio (aPR)=1.33, CI95%: 1.10-1.59) and specialization, being lower for gynaecology (aPR=0.67; CI95%:0.51-0.88), and family medicine (aPR=0.42, CI95%:0.30-0.59). Among MDs, 7.1% (CI95%: 5.1- 9.5) had medium knowledge, while 25.3% (CI95%: 21.8-29.0) had low, and 67.6% (CI95%: 63.7-71.4) no knowledge of symptoms, complications or diagnostic tools for FGS. FGS knowledge was mostly acquired through academic curricula (34.7%), scientific literature (28.4%) and conferences (25.6%).

Conclusions: The study shows limited awareness of FGS among European MDs and NMs, and highlights that European HCWs may not be adequately prepared to deal with diseases that are gaining relevance in the European continent due to the global connectivity and the dynamic nature of our societies.

P-2-29

Factors influencing the uptake of mass drug administration for schistosomiasis among preschool-aged children: a cross-sectional study from Madagascar

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Background: The World Health Organization (WHO) has recently recommended mass drug administration (MDA) with praziquantel (PZQ), the main strategy to control schistosomiasis, for preschool-aged children (PSAC). Additionally, a paediatric formulation of PZQ has been recently approved by the European Medicines Agency.

The availability of the drug alone is not enough to guarantee a successful uptake and experiences from MDAs for other diseases highlight that acceptance of treatment for PSAC may be influenced by several factors, such as fear of adverse events (AE).

This study aims at exploring the factors influencing the uptake of PZQ through MDAs in children aged 9-24 months (mo) in the regions of Boeny and Haute Matsiatra of Madagascar.

Methods: The cross-sectional study was performed from February to December 2023 to enrol 5000 children. A PZQ treatment was offered to the caregivers of children in medical and non-medical settings. Quantitative data were collected to assess factors influencing the uptake, including socio-demographic characteristics, individual awareness, previous experience with PZQ and knowledge of schistosomiasis.

Results: A preliminary analysis of the data of 1880 children (925, 51.4% females and 875, 48.6% males) showed that the most of them (614, 34.1%) were in the age group 18-24 mo. Among the caregivers, 1649 (91.5%) were the mother, 542 (30.1%) were accompanied by a relative, and most of the interviewed had secondary education (833, 46.2%), while 126 (7.6%) had no education. Many of them (945, 52.4%) had heard of PZQ, 740 (41.1%) had previous experience with PZQ treatment for other children, 1002 (55.7%) had no concerns about AE. The treatment uptake was 84.7% (95%IC 82.9-86.3). Having heard of PZQ, previous experience with PZQ and being accompanied were positively associated with the uptake, the fear of AE and the level of education showed a negative association. For children, the older age and having siblings presented a positive association.

Conclusions: Our preliminary results show an uptake higher than the treatment coverage suggested by WHO, encouraging the promotion of this intervention in Madagascar. This study will contribute to shape the global strategy for the implementation of PZQ treatment among PSAC that is being rolled out in the next years.

P-2-30

Factors influencing the resolution of female genital schistosomiasis: a longitudinal study in rural Madagascar

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Background: Female genital schistosomiasis (FGS) is the chronic manifestation of Schistosoma haematobium infection. Complications include infertility, ectopic pregnancy, and increased risk of HIV acquisition, while the association with HPV infection is still unclear.

Schistosomiasis is highly endemic in Madagascar and cervical cancer is the most frequent cancer among Malagasy women. This study aims to assess the rate of resolution and the factors associated with lesion regression following treatment with praziquantel (PZQ) in women of reproductive age.

Methods: The study is implemented at three Primary Health Care Centers (PHCCs) in the rural district of Marovoay, Boeny region. Enrolment of women in this longitudinal study started in 2021 and is currently ongoing with a scheduled 4-year follow-up.

Women were invited to participate in FGS screening by colposcopy (CLP) through community outreach activities. Every year, enrolled women are contacted for follow-up visits at 12-month intervals (12 +/- 3 months).

FGS is diagnosed using CLP. Each woman screened positive for FGS is offered 40mg/kg PZQ treatment. FGS diagnosis is confirmed through a blind assessment of CLP images by two specialists. Cervical vaginal lavages (CVLs) are collected to assess the role of sexually transmitted infections, such as HPV.

The data collected at the time of recruitment were analyzed to estimate the baseline prevalence of the disease. The follow-up will be completed in 2024.

Results: By February 2024, 1,073 women underwent CLP and CVLs were collected at least once. Specifically, 551 women underwent CLP once, 429 had one baseline and one follow-up visit, and 93 had two follow-up visits. Among 500 women enrolled in 2021, 302 had a final FGS diagnosis: FGS prevalence was 62.6% (189, 95% CI: 56.9-68.1), and 26.5% (80, 95% CI: 21.6-31.8) of women with FGS were also infected with HPV.

Conclusions: Our preliminary data show that Madagascar has a high prevalence of FGS among women of reproductive health. The cohort established in this study will contribute to clarifying the role of PZQ treatment in this complicated form of schistosomiasis, informing the clinical management of FGS, and the development of targeted public health interventions.

P-2-31

Generation and characterization of two recombinant MVA candidate vaccines expressing Lassa virus glycoprotein or nucleoprotein

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Lassa virus (LASV), causative agent of the severe infectious disease Lassa fever (LF), is endemic in several countries in West Africa. Up to 300,000 cases are reported every year and an expansion of the virus to non-endemic regions within the last years highlights the importance of developing effective vaccines and treatments. Although several DNA and RNA-based vaccines are tested in preclinical and clinical research, no LASV-specific vaccines are licensed for prevention of LF by now.

Modified Vaccinia virus Ankara (MVA) is a well characterized orthopoxvirus and vaccine strain, which is unable to productively replicate in cells of human origin. Due to an established record of clinical safety and its capacity to efficiently deliver various recombinant proteins as antigens, MVA is a promising tool to create candidate vector vaccines against emerging infections.

Here, we describe the generation and characterization of two recombinant MVA candidate vaccines expressing either LASV glycoprotein (MVA-LASV-GP) or LASV nucleoprotein (MVA-LASV-NP) using our MVA vector technology platform compliant with requirements for clinical research. The two candidate vaccines were generated by inserting the modified target genes into the MVA genome via homologous recombination and were clonally isolated by consecutive rounds of plaque purification. Afterwards, MVA-MARV-GP and MVA-MARV-NP were *in vitro* characterized according to our standardized quality control procedures. Furthermore, tolerability and immunogenicity were evaluated in wild type C57BL/6 mice and *HLA-A2.1-/HLA-DR1*-transgenic *H-2 class I-/class II*-knockout mice. We confirmed that vaccination with MVA-LASV-GP induced strong humoral and cellular immune responses. By restimulating isolated splenocytes from C57BL/6 mice with putative GP-specific peptide epitopes specific for the mouse alleles H2-Db and H2-Kb, we detected LASV-GP- specific IFN-γ /TNF-α producing CD8 T cells. Furthermore, we confirmed high LASV-GP-specific serum IgG binding antibodies after a single immunization, which increased after the booster immunization.

MVA-LASV-NP-vaccination induced strong cellular immune responses. By restimulating isolated splenocytes from C57BL/6 mice with overlapping peptides covering the entire LASV-NP protein, we identified one H2-Db-restricted 8mer peptide epitope with CD8 T cell antigenicity. In addition, by restimulating isolated splenocytes from *HLA-A2.1-/HLA-DR1*-transgenic *H-2 class I-/class II*-knockout mice with putative NP-specific peptide epitopes specific for the alleles HLA-A*02:01 and HLA-DR1, we detected LASV-NP specific IFN-γ /TNF-α producing CD8 T cells. In the future we aim to evaluate the efficacy of our two candidate vaccines in a suitable animal challenge model.

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Strong and early activation of mpox virus-specific immunity associated with mild disease after intradermal clade IIb-infection in CAST/Eij-mice

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Mpox virus (MPXV) is a poxvirus long endemic in West and Central Africa. MPXV zoonoses can be followed by limited human-to-human transmission that is self-limiting, at least until recently. Outbreaks, first the global spread of clade II outside Africa in 2022, and since 2023 the accelerating spread of clade I in central Africa and beyond point to MPXV adaptations that pose the risk of MPXV becoming a more transmissible human pathogen with pandemic potential. Animal models that mimic the clinical disease outcome in humans are important to better understand MPXV pathogenesis, host tropism, and the contribution of new genetic mutations. Here, we demonstrate that MPXV infection via tail-scarification in CAST/Eij mice is an appropriate animal model to mimic human mpox disease. In our study disease outcome was milder in MPXV clade IIb than clade IIa infected mice, which was associated with enhanced titers of neutralizing antibodies early during infection. This suggests that MPXV clade IIb more efficiently activates the host immune responses, highlighting how this animal model could facilitate studying the pathogenesis, virulence and immunity of new MPXV clade variants to help us develop efficient antivirals and preventive measures.

P-2-33

Infektiopod – 5 Jahre Podcasten über Infektionsmedizin

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Einleitung: Der *Infektiopod* ist der älteste deutschsprachige Podcast zum Thema Infektionsmedizin. Seit Januar 2019 werden im Podcast Infektionskrankheiten vorgestellt und aktuelle Forschungsergebnisse diskutiert, bislang wurden 85 Folgen produziert deren Themen von Typhus über Ebola und Covid bis zu Endokarditis reichen. Um ein besseres Verständnis für die Zuhörerschaft zu gewinnen, wurde eine Onlineumfrage durchgeführt.

Methoden: Mit dem Online-Datenerfassungstool REDCap (Research Electronic Data Capture) wurde ein strukturierter Fragebogen entwickelt. Dieser enthielt Fragen zu Demographischen Daten der Teilnehmenden, inhaltlichen Aspekten des Podcasts und Wünsche für die Weiterentwicklung. Der Fragebogen wurde auf der Seite "infektiopod.de" verlinkt und die Hörer:innen in den Folgen des Infektiopod auf die Verlinkung aufmerksam gemacht.

Ergebnisse: Die Umfrage wurde vom 25. Oktober 2023 bis zum 28. Januar 2024 durchgeführt und verzeichnete die Teilnahme von 104 Personen. Die meisten Teilnehmenden befanden sich im Altersbereich von 30 bis 49 Jahren. 60 % von ihnen waren Ärzt:innen, während die restlichen 40 % aus anderen Berufsgruppen kamen. Die ärztlichen Teilnehmer:innen arbeiteten überwiegend in nicht-universitären Krankenhäusern mit einer Bettenkapazität von über 800 Betten. Etwa ein Drittel der Befragten war wissenschaftlich tätig, jedoch engagieren sich nur wenige

aktiv in der Wissenschaftskommunikation. Die meisten Teilnehmenden wurden über die Plattform X (ehemals Twitter) oder durch persönliche Empfehlungen auf den Podcast aufmerksam.

Schlussfolgerung: Die Umfrageergebnisse liefern wertvolle Einblicke in die demografischen Merkmale, Interessen und Vorlieben der Hörenden des Infektiopod. Mit einer überwiegend ärztlichen Zuhörerschaft, die in großen, nicht-universitären Krankenhäusern tätig ist, zeigt sich ein klares Interesse an praxisrelevanten Inhalten wie Infektionsmedizin, Diagnostik und Therapie. Die bevorzugte Episodenlänge von 60 Minuten und der Wunsch nach Fallbesprechungen sowie tiefgehenden Diskussionen über wissenschaftliche Publikationen unterstreichen den Bedarf an fundierten, ausführlichen Formaten. Diese Erkenntnisse bieten wertvolle Anhaltspunkte, um den Podcast gezielt weiterzuentwickeln und noch besser an die Bedürfnisse des Publikums anzupassen.

P-2-34

Malaria and hypertension in volunteers attending peripheral health services in Lambaréné, Gabon

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Introduction: The last few decades have been marked by an increase in the incidence of non-communicable diseases, including cardiovascular diseases, particularly arterial hypertension. This growing phenomenon, although evolving in parallel with the constant state of malaria endemicity in sub-Saharan Africa, remains worrying because of the alarming number of deaths. Although the potential link between these two entities, hypertension and malaria, has been described in the literature, there are few studies on African populations where malaria is endemic. Our study will aim to explore the association between malaria parasitaemia and hypertension in patients and volunteers screened for malaria in Gabon.

Methods: This study took place at the Lambaréné Medical Research Centre. Data were collected retrospectively during screening activities in Lambaréné clinics from 2017 to 2020 and then prospectively on consenting adult subjects seen in routine consultation in the clinical operations department of the said centre from 2020 to 2023. The populations recruited were screened for malaria and had their blood pressure measured. Blood pressure was diagnosed based on systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg. A rapid diagnostic test determined malaria parasitaemia.

Results: A total of 913 participants were included in this study, with a male-female sex ratio of 0.86. Most participants lived in urban areas (70.1%, 640/913). In this study, the prevalence of malaria was estimated at 69% (630/913). Cases of arterial hypertension were encountered with an estimated prevalence of 47.5% (n=434). 10.7% (n=68) of participants with hypertension tested positive for malaria. Our study revealed a significant association between malaria and arterial hypertension (p-value=0.030).

Conclusion: Although the risk of developing non-communicable diseases, in particular cardiovascular diseases, is linked to lifestyle habits, if it is attributable in part to malaria infection in the literature by some authors arterial hypertension is very weakly related to malaria in our study.

Keywords: malaria, hypertension, Gabon.

P-2-35

A randomized controlled phase II clinical trial to evaluate the safety and tolerability of adjunct dexamethasone for the treatment of Lassa fever

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Lassa fever (LF) is a severe and often fatal systemic disease in humans. It is caused by the Lassa virus (LASV) which belongs to the segmented negative stranded ribonucleic acid (RNA) viruses of the Arenaviridae family. After spill-over from the animal reservoir, the virus can be transmitted from human to human. LASV is endemic to several West African countries with the highest disease burden in Nigeria. Vaccines are not available yet and treatment options are limited to supportive care and ribavirin.

Dexamethasone is a corticosteroid which can modulate inflammatory-mediated tissue damage associated with a variety of infectious diseases. It has been successfully used in COVID-19 and selected other infectious disease. Based on the understanding of a hyperinflammatory response to Lassa virus infection and its preliminary and promising safety data, dexamethasone is proposed as adjunct treatment candidate for LF. To systematically assess the potential benefit of adjunct dexamethasone, a randomized controlled phase II clinical trial is being conducted.

The aim of this exploratory study is to assess the safety and tolerability of dexamethasone as an adjunct treatment in symptomatic LF cases as well as to explore immunological characteristics. Following provision of written informed consent, participants are randomized to a standard of care arm or a standard of care + dexamethasone arm and are being followed up for ten days. Clinical data and blood samples for assessment of hemogram, clinical chemistry, molecular diagnostics and immunologic analyses are collected throughout this period.

Safety and tolerability data will be presented to provide first insights into prospects of this novel treatment candidate for LF.

P-2-36

Exploring adaptive immunity in response to Coronavirus infection and vaccine treatment via (lymphatic) immune organoids

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Introduction: Vaccines are among the most effective public health measures, preventing millions of infections and deaths each year. Viral vector vaccines (VVVs), in particular, have gained attention during recent epidemics and pandemics, such as the Ebola virus outbreak and the recent Coronavirus disease 2019 (COVID-19) pandemic, due to their ability to induce strong immune responses. However, there remains a limited understanding of the detailed human immune response mechanisms triggered by the wide array of viral pathogens and developed VVVs. Currently, research in this regard is primarily confined to preclinical animal models and *ex vivo* analyses of human peripheral blood. Especially peripheral blood does, however, not sufficiently reflect the reactions and interactions of immune cells within lymphatic tissue, where a significant portion of the adaptive immune response occurs. This gap can be bridged by employing human immune organoid technologies. Organoids, in general, offer the potential to complement *in vivo* experiments using animal models, while providing a more accurate prediction of the human immune responses to infections and vaccinations. Human tonsils, as part of the lymphatic tissue, serve as one of the body's first lines of defense when it comes to respiratory infections. Thus, examining the effects of infection and vaccination in organoids derived from tonsil tissue is especially relevant and will enhance our understanding of immunity in humans.

Methods and Results: To this end, we have successfully established a human tonsil organoid model. Following organoid culture induction, we identified distinct immune cell compostions that differ clearly from the typically oberserved distribution in PBMCs, showing elevated B cell, but reduced T cell numbers. In addition, the immune cells accumulated in germinal center-like structures, which do not arise during PBMC culture.

Stimulation of the organoid model using MVA-based vaccines against the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) resulted in the development of plasmablasts, whereby major T cell populations remained unchanged when compared to unstimulated organoids.

Moreover, we established a co-culture setup of the tonsil organoid model with a lung cell line expressing ACE2, TMPRSS2, and DPP4 to compare the response of vaccination to natural infection with SARS-CoV-2. Upon infection, the lung cells produced infectious virus and the together with the eventually secreted cytokines lead to superior stimulation of plasmablast development compared to the tested vaccine candidates.

Conclusion: Overall, our preliminary data suggest that stimulation with infectious virus results in elevated plasmablast counts when compared to vaccination. Moreover, we expect that the different vaccine platforms available induce distinct patterns of immune cell activation and stimulation.

P-2-37

Immunogenicity of pre-fusion stabilized, and wild-type MERS-CoV spike proteins delivered by modified vaccinia virus Ankara in mice

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Introduction & Aims: The Middle East respiratory syndrome coronavirus (MERS-CoV) is a member of the *Betacoronavirus* genus and first emerged in 2012. Evidence suggests that dromedary camels are the natural reservoir of the pathogen. Humans can become infected with MERS-CoV via direct physical contact with camels or, to a limited extend, via direct human-to-human transmission, leading to the Middle East respiratory syndrome (MERS). Human MERS infections range from mild upper respiratory symptoms to severe pneumonia and multi-organ failure. The case fatality rate is 35%. We have already generated and characterized a MERS candidate vaccine based on the Modified Vaccinia virus Ankara (MVA) platform, MVA-MERS-S, which targets the immunogenic spike protein (S). We aimed to determine if vaccine immunogenicity can be improved by the administration of an MVA candidate vaccine delivering a pre-fusion stabilized form of MERS-S (MVA-MERS-ST).

Methods: We generated a recombinant MVA virus expressing MERS-ST by cloning the modified target gene into a MVA vector plasmid and introducing it into the MVA genome by homologous recombination. The recombinant virus was generated by serial plaque passaging and tested by standardized quality control procedures. To test immunogenicity, BALB/c were immunized twice with 10^7 PFU of MVA-MERS-S or MVA-MERS-ST over a 21-day interval. Fourteen days after the booster immunization, mice were sacrificed and splenocytes were isolated and restimulated with MERS-S-specific peptides. T cell immunity was measured by IFN-γ ELISPOT assay and intracellular cytokine staining plus FACS analysis. Additionally, serum MERS-S IgG binding antibodies were measured by ELISA.

Results: After prime-boost immunization with MVA-MERS-S and MVA-MERS-ST, we observed comparable IFN-γ and dual cytokine (IFN-γ+TNF-α+) production by MERS-S-specific CD8 T cells. By testing the serum samples by ELISA, we found that MVA-MERS-S induced higher MERS-S2 IgG binding titres after prime immunization, whereas MVA-MERS-ST induced higher MERS-S1 IgG binding titres. However, the titres were comparable for both MERS-S subunits after the booster immunisation.

Conclusions: Overall, we found that MVA-MERS-ST induced comparable T cell immunogenicity to MVA-MERS-S. Although some differences in humoral immunity were observed after prime immunization in mice, after the booster the responses were similar. In a next step, the immunogenicity and efficacy of the MVA-MERS-S and MVA-MERS-ST vaccine candidate will be comparatively characterized in a mouse challenge study against lethal doses of MERS-CoV.

P-2-38

Identification of a novel widespread Mycobacterium intracellulare subsp. chimaera clone in drinking water dispensers and patient isolates across Germany

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Question: Non-tuberculous mycobacteria (NTM) are increasingly recognized as emerging opportunistic pathogens causing pulmonary and other diseases. In 2022 and 2023, an unusually high number of lower respiratory tract samples from 63 patients attending the same hospital in Germany tested positive for mycobacteria that could not be differentiated at the species level using routine diagnostic tests. This raised the question whether a hospital outbreak, colonization, or contamination was occurring. We aimed to investigate the species, source, extent, and potential spread of this peculiar *Mycobacterium* strain.

Methods: To investigate the source of the potential (pseudo-)outbreak, extensive environmental sampling was conducted within the hospital. Water samples and swabs were collected from bronchoscopes, disinfection equipment, drinking water dispensers, showers, and sinks. Whole genome sequencing (WGS) was performed on all patient and environmental isolates, followed by core genome multi-locus sequence typing (cgMLST) based on 3719 genes to determine genetic relatedness. Isolates were also compared to an in-house global database of over 600 *Mycobacterium intracellulare subsp. chimaera* strains to assess potential connections beyond the affected hospital.

Results: WGS identified all patient isolates as *Mycobacterium intracellulare* subsp. *chimaera*. Two clusters of closely related strains (less than 10 alleles differences) were identified. Cluster 1, the larger of the two, included 44 isolates: 38 isolates from 35 patients and 6 isolates from different drinking water dispensers within the hospital. Cluster 2 consisted of 1 patient isolate and 4 isolates from two additional water dispensers. Comparing these isolates to the global database revealed that 7 patient isolates from 4 other German hospitals also clustered within Cluster 1, the latest case from 2024, suggesting an ongoing and wider geographical spread. The most closely related non-German isolates were collected from patients in the UK and the Netherlands before 2016 and showed 16-17 allele differences. After implementing prevention measures, no additional patient isolates were found positive with the cluster 1 clone in the hospital where the clone was first identified.

Conclusions: Altogether, this suggests the existence of a new *M. chimaera* clone that is widespread at least in Germany. However, whether the patients are truly infected, colonized temporarily or samples are merely contaminated is still under investigation. Hospitals and other institutions across Germany are advised to assess their water systems regularly and submit samples to the National Reference Laboratory for Mycobacteria in Borstel to determine the spread of this clone.

P-2-39

Immune cell characterization of Lassa fever patients durig acute disease in glovebox-based immunology laboratory in Nigeria

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The Lassa fever (LF) outbreak caused by Lassa virus (LASV, *Arenaviridae* family), results in up to 900,000 infections yearly. With no approved vaccine and lacking therapeutic treatments LF has a high in West Africa. Still, the causal mechanisms leading to an unfavorable disease outcome remain poorly understood. One potential reason for the dearth of knowledge regarding the disease pathophysiology is the inherent difficulty of conducting immunological research in endemic countries under high-containment biosafety level conditions.

Although non-human primate infection experiments showed that the early activation of the adaptive immune response is associated with survival, the immune response in human LF patients has not been the subject of much detailed investigation to date.

To address this knowledge gap, we aim to gain insight into the underlying immunological mechanisms influencing disease outcome by analyzing antigen-presenting cells (APCs) and T cells during LASV infection in human patients.

In Nigeria, we established a glovebox-based immunology laboratory to process blood samples from patients with LF in collaboration with our partner at the Irrua Specialist Teaching Hospital (ISTH). This enabled the characterization of immune cell population phenotypes through flow cytometry staining as part of an observational clinical study. In 2022, 86 patients were enrolled and about 400 samples were processed of at consecutive time points (day 1, 2, 4, 6, 8 and 10 after enrollment) to differentiate and characterize different immune cell subsets. We observed variances in dendritic cell subtypes and altered frequencies of myeloid cells in LF patients. Maturation markers on the cell surface of APCs are deregulated over time and can be used as indicators of acute disease. Moreover, effector T cells are highly activated. A comparison of LF survivors and fatal cases revealed an increase in T cell exhaustion marker expression, accompanied by a reduction in activation markers, resulting in the dysfunction of T cells, which may be a pivotal factor in severe disease progression.

Further analysis is underway to gain a better understanding of the immune response-driven pathology following LASV infection.

P-2-40

Multi-omics characterization of the monkeypox virus infection

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Multiple omics analyzes have defined molecular characteristics of poxvirus infections biology. However, relatively little is known about the monkeypox (mpox) virus (MPXV), which, despite its high sequence similarity to VACV, has a different disease manifestation. We performed

an in-depth multi-omics analysis of the transcriptome, proteome, and phosphoproteome signatures of MPXV-infected primary human fibroblasts to gain insights into the virus-host interplay. In addition to expected perturbations of immune-related pathways, we uncover regulation of the HIPPO and TGF-β pathways. We identify dynamic phosphorylation of both host and viral proteins, which suggests that MAPKs are key regulators of differential phosphorylation in MPXV-infected cells. Among the viral proteins, we find dynamic phosphorylation of H5 that influenced the binding of H5 to dsDNA. Our extensive dataset highlights signaling events and hotspots perturbed by MPXV, extending the current knowledge on poxviruses. We use integrated pathway analysis and drug-target prediction approaches to identify potential drug targets that affect virus growth. Functionally, we exemplify the utility of this approach by identifying inhibitors of MTOR, CHUK/IKBKB, and splicing factor kinases with potent antiviral efficacy against MPXV and VACV.

P-2-41

Establishment of high throughput screening assays for antiviral compounds against hemorrhagic fever viruses

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Introduction: Viral hemorrhagic fever like Crimean Congo Hemorrhagic fever, Ebola virus disease and Lassa fever pose serious public health threats and are on the WHO R&D blueprint of high priority pathogens. Since no vaccines or treatment, besides supportive care, are available for most of these pathogens, the research for antivirals is crucial. However, research on these viruses can only be performed under BSL-4 conditions, which require special facilities and expertise that only a small fraction of laboratories possess. To drive the research for antivirals forward even though no BSL-4 conditions are available, closely related, non-pathogenic surrogate viruses are an option for antiviral research.

Objective: Our project aims to establish a simple and inexpensive high throughput screening assay based on fluorescence microscopy using surrogate viruses for hemorrhagic fever viruses. The assay is suitable for different viruses and various staining methods.

Methods: The surrogate viruses used in our project are Mopeia Virus (MOPV), Taccaribe Virus (TACV) and Hazara Virus (HAZV). They are surrogates for old world Arenaviridae such as Lassa Virus, new world Arenaviridae such as Junín Virus and Crimean Congo Hemorrhagic fever virus, respectively.

We explored different staining methods for these viruses: MOPV wildtype and TACV are stained with classical immunofluorescence staining using pathogen-specific antibodies. Additionally to the classic approach, we used a trisegmented recombinant MOPV, which expresses eGFP as a reporter gene during replication. Therefore, the antibody staining can be neglected.

In the case of HAZV, no commercially antibodies are available, and no reverse genetic system is established. Therefore, we use fluorescence in situ hybridization (FISH) as a different approach. Here, we tag the RNA expressing the NP of HAZV with DNA probes which all have the same FLAP sequence at the end. To this FLAP sequence, a complementary sequence coupled to a fluorophore is added.

Summary: We established a simple and inexpensive high-throughput screening assay suitable for different staining methods which are translatable to a broad variety of viruses, even if no virus-specific antibodies are available. The subsequent step is the screening of different compound libraries for potential antiviral candidates.

P-2-42

Long-term immunogenicity of the MVA-MERS-S vaccine candidate in humans

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MVA-MERS-S, a Modified Vaccinia virus Ankara (MVA) viral vector vaccine candidate against the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), was recently tested in a two-center, randomized, placebo-controlled phase 1b trial (NCT04119440) to investigate its safety, immunogenicity, and optimal dosing in healthy adults. Participants of four treatment arms were randomized to receive two MVA-MERS-S doses of 1x107plaque-forming units (PFU, high dose (HD)) or 1x108 PFU (low dose (LD)) either on days 0 and 28 (short interval (SI)) or on days 0 and 56 (long interval (LI)), followed by a third dose on day 224. A placebo arm was included for comparison (Fig. 1).

Fig. 1

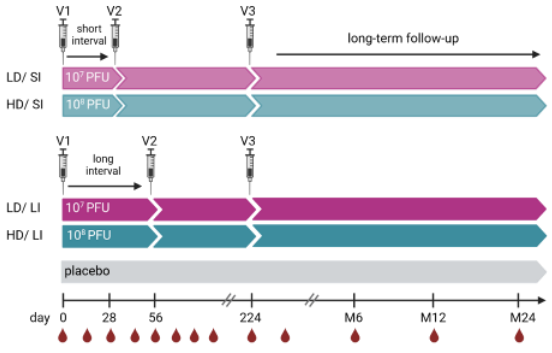

Figure 1: MVA-MERS-S phase 1b study design with long-term follow-up. LD = low dose (1x107 PFU), HD = high dose (1x108 PFU), SI = short interval (28 days), LI = long interval (56 days).

Following the second vaccination, MERS-CoV-neutralizing antibody responses were highest in the HD/LI cohort with geometric mean titers of 238 IU/ml (95% CI 128·9-438·4). A third vaccination boosted neutralizing responses in all treatment arms, with 100%, 89%, 71%, and 86%

seroconversion observed in the HD/LI, HD/SI, LD/LI, and LD/SI arms, respectively (Raadsen et. al, 2024, accepted at Lancet Infect Dis). Contrary to the antibody response, the strongest MERS-CoV spike-specific T cell response (measured by ELISpot) was observed in the LD/LI arm, with 75% and 69% of assay responders after the second and third vaccinations, respectively.

As one of the goals of vaccination is to provide long-term protection, we conducted a long-term follow-up of the MVA-MERS-S phase 1b trial to investigate the persistence of MERS-CoV-specific immune responses over time. Peripheral blood samples were collected from 54 study participants 6, 12, and 24 months after the third vaccination. Neutralizing antibody titers decayed more slowly after the third than the second dose and remained detectable for up to 2 years following vaccination in all treatment arms. To measure spike-specific T cell responses, we established an interferon-gamma release assay (IGRA) in which whole blood was stimulated with a peptide pool covering the MERS-CoV spike sequence. MERS-specific T cell responses were still detectable 2 years after vaccination with significantly higher IFNγ levels in MVA-MERS-S-vaccinees (median = 433 mIU/ml) compared to healthy controls (median = 66 mIU/ml; p<0.0001).

In conclusion, these data show, that MVA-MERS-S is immunogenic, inducing robust MERS-specific antibody and T cell responses that persist for at least two years following vaccination. While the LD was beneficial for T cell immunogenicity, the HD induced more robust neutralizing antibody responses. Immunogenicity was enhanced by prolonging the interval between the first and second vaccine doses to 56 days.

P-2-43

Systems vaccinology reveals enhanced innate and T follicular helper cell responses after a late third MVA-MERS-S vaccination in humans

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The Middle East respiratory syndrome (MERS) is a WHO priority disease caused by the MERS coronavirus (MERS-CoV), warranting research and development of countermeasures. The Modified Vaccinia virus Ankara (MVA)-vectored vaccine candidate MVA-MERS-S was evaluated in a phase 1a clinical trial at the University Medical Center Hamburg-Eppendorf. Study participants received two vaccinations 28 days apart, and a late booster vaccination was administered approximately one year after prime. MVA-MERS-S revealed to be safe and immunogenic, with the late boost enhancing titers, functionality and persistence of antibodies.

In the present study, we applied a systems vaccinology approach in the context of three vaccinations with MVA-MERS-S to explore associations between innate and adaptive immune responses. Various factors can influence innate immune responses, which may in turn shape adaptive immunity. Based on peripheral blood samples collected from ten study participants at baseline as well as 1, 3, 7 and 14 days after vaccination, multiple techniques were applied to comprehensively characterize the longitudinal dynamics of gene expression, cytokine/ chemokine secretion, and activation of innate and T follicular helper (TFH) cells.

An upregulation of transcriptional pathways related to interferon signaling and pathogen recognition was observed following the first and third vaccination, whereas responses to the second vaccination were less pronounced. Compared to the primary vaccination series, a late third vaccination with MVA-MERS-S induced more rapid responses of intermediate monocytes, CD16+ dendritic cells and TFH1 cells, as well as a stronger increase in the plasma levels of TNFα, IL-6, CCL3 and CCL4. Several of these parameters correlated positively with the adaptive immune response against both MERS-CoV and the MVA vector, induced by the late booster vaccination.

Overall, our findings suggest an impact of the number and interval of vaccinations on the innate immune response and indicate that both innate immune responses and an enhanced interplay with TFH cells may contribute to the recall responses of memory B cells, resulting in improved humoral immune responses after the late boost. Supported by systems vaccinology, a better understanding of MVA-mediated immune mechanisms can contribute to the optimization of vaccination strategies for both recombinant vaccines based on the MVA platform and non-recombinant MVA administered against mpox.

P-2-44

Malaria infection, parasite density and Abo-Rhesus blood groups in febrile children under five years of age at Cottage Hospital, Tiko, Cameroon

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Introduction: Malaria is a serious and fatal mosquito-borne disease caused by protozoan parasites of the genus

Plasmodium. Children aged 5 and below are the most vulnerable to this deadly disease. ABO blood group antigens are inherited among individuals and populations. Differences in blood group antigens can increase or decrease host susceptibility to many infections. Previous Research has claimed that there is a relationship between malaria and ABO Rhesus blood groups.

Methods: This study was carried out for a period of 3 months, from February to April 2023. A total of 160 participants subscribed to the study. Venous blood was collected from each participant using syringes, transferred into EDTA tubes and analyzed within 30 minutes. HB measurements were taken using the URIT 12 HB meter. The malaria thin and thick films were prepared and stained using Giemsa for microscopy. The blood group was determined using the Cell or Forward grouping.

Results: Out of all 160 participants, 133(84.2%) tested positive for malaria. The mean of Malaria Parasite (MP) density was 55169.8 T/ul and most had high parasitemia (5000-99,999 T/ul) 49.4%. T/ul. The most prevalent blood group was O (48.8%) and most were Rhesus positive (89.4%). The mean malaria parasitemia was high in all the different blood groups. Malaria parasite density decreased with increasing hemoglobin and increased with temperature (P 0.05). Rhesus-positive blood group A had an increased risk of developing severe malaria (29.35%).

Coclusion: Malaria parasitemia is high in the different blood groups, with O+ having the least parasitemia. Blood group does not play a role in determining the parasitemia of malaria in febrile children aged 5 and below.

Keywords: *Plasmodium falciparum*, ABO, Rhesus, Blood groups, febrile, Cameroon

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P-2-45

Human Placental Schistosomiasis – A systematic review of the literature

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Question: Schistosomiasis in pregnant women has been a neglected issue for many years. Egg deposition may affect the placenta of infected mothers and cause Placental Schistosomiasis (PS). Placental inflammation is one discussed mechanism how Maternal Schistosomiasis may contribute to adverse birth outcomes. Meanwhile, diagnosis of PS remains difficult as standard histopathological examination of placental tissue is an inadequate detection method due to low sensitivity. One alternative approach is to use maceration of placental tissue. However, there are still only few detailed case reports on PS. We aim to review and summarize the existing cases and discuss potential links between PS and adverse birth outcomes.

Methods: A systematic literature search on PubMed, EMBASE and Medline was conducted. All publications that reported microscopically confirmed cases of PS were eligible, without limitations regarding study design or language. Publications identified in the citations of the primarily included publications were also screened for eligibility. This systematic review follows the recommendations of "Preferred Reporting Items for Systematic reviews and Meta-Analyses" (PRISMA).

Results: After removal of duplicates, 113 abstracts were screened, and 4 were found to be eligible. In the secondary literature another 4 abstracts were identified, giving a total of 8 publications reporting a total of 92 cases of PS. Described cases included egg deposition of dead and/or viable eggs and worms of S. haematobium and S. mansoni in placental tissue. 7 publications were case reports, and one a cross-sectional study investigating the prevalence of PS and its association to adverse birth outcomes. The latter found 22% of placentas to be infested using a maceration technique but only < 1% using histologic examination. An increased risk for deleterious pregnancy outcomes in mothers with PS could not be shown.

Conclusions: Placental Schistosomiasis is an unattended and underdiagnosed condition in endemic populations, due to a lack of awareness as well as low sensitivity of histopathological examinations. However, PS may play an important role in mediating or reinforcing adverse birth outcomes (ABO) such as fetal growth restriction (FGR) in maternal schistosomiasis. Possible mediators are the transfer of soluble egg antigens or proinflammatory cytokines across the placenta and placental inflammation. Treatment during pregnancy may influence pregnancy outcomes, and is now formally recommended by WHO.

References:

[1] Gerstenberg, J.; Mishra, S.; Holtfreter, M.; Richter, J.; Davi, S.D.; Okwu, D.G.; Ramharter, M.; Mischlinger, J.; Schleenvoigt, B.T., on behalf of the TropNet. Human Placental Schistosomiasis—A Systematic Review of the Literature. Pathogens 2024, 13, 470. https:// doi.org/10.3390/pathogens13060470

P-2-46

Generation and characterization of MVA-based vaccines targeting Nipah virus soluble Glycoprotein, Fusion and Matrix protein

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Introduction: Nipah virus (NiV), causative agent of the severe Nipah disease, belongs to the *Paramyxoviridae,* and is prevalent in Asia, where it circulates in *Pteropus* bats. Transmission of the virus to humans mainly occurs via direct contact with *Pteropus* bats or via intermediate hosts. Infections in humans are associated with severe respiratory symptoms and encephalitis. The case fatality rate is high and so far, only therapeutic measures, using monoclonal

antibodies, exist, highlighting the need for a preventive approach such as effective vaccines against NiV.

We previously developed two candidate vaccines targeting the glycoprotein (G) of NiV using our Modified Vaccinia virus Ankara (MVA) viral vector platform. We aimed to develop and characterize two additional candidate vaccines that target the fusion protein (F) and the matrix protein (M) of NiV for future testing in mouse immunization trials.

Modified Vaccinia virus Ankara (MVA), a well characterized vaccinia virus strain, is a promising viral vector platform for vaccine development against emerging infections, due to its capacity to successfully deliver multiple recombinant antigens and its established clinical safety.

Materials & Methods: Recombinant MVA-NiV candidate vaccines (MVA-NiV-F, MVA-NiV-M) were generated by initial cloning of the modified target sequences into MVA vector plasmids allowing for an insertion into the MVA genome by homologous recombination. Purification of recombinant MVA-NiV-F and MVA-NiV-M was done by serial rounds of plaque purification on chicken embryonic fibroblast (CEF) cells, and the vaccines were further characterized by standardized quality control procedures.

Results: PCR-analysis of recombinant MVA-NiV-F and MVA-NiV-M candidate vaccines confirmed stability and identity of the inserted sequences. Unimpaired expression of the target proteins could be demonstrated by Western blot analysis. Furthermore, recombinant MVA-NiV vaccines were able to replicate to high titers on CEF cells, but not in cells of human origin.

Conclusion: Nipah disease remains a challenge in respect of its mortality and health care burden, highlighting the need of safe and effective vaccines against NiV. We successfully generated and characterized MVA-NiV candidate vaccines targeting the F and M proteins, leading the path for evaluation of preclinical immunogenicity, preclinical efficacy, and future clinical development.

P-2-47

Complications and risk factors for postoperative bile leakage and recurrence in patients with hepatic cystic echinococcosis

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Background: Cystic echinococcosis (CE) is a zoonotic neglected tropical disease with global relevance. The liver and lung are the most frequently affected organs, compression of surrounding tissues and vessels and cysto-biliary / cysto-bronchial fistulas the most common complications. Open cystectomy is the most frequent treatment for complicated cysts.

Objective: This study describes a large series of patients with hepatic CE surgically treated by open cystectomy in a national reference center.

Patients and methods: Retrospective case series of patients with hepatic CE treated by open cystectomy at the University Hospital Heidelberg and postoperative follow-up between January 2000 and January 2023. The data was analysed with descriptive analysis and multiple logistic regression.

Results: 105 patients were included in the analysis. 44 % (46/105) of patients presented with CE-associated complications prior to surgery, most frequently cysto-biliary fistulas. Postoperative complications occurred in 48 % (50/105) of patients. The most frequent postoperative complications were bile leakage (19 %) and bilioma (18 %) followed by postoperative abscess/infection of the residual cavity (10 %). 8,6 % of patients had local cyst-recurrence. The mean time to diagnosis of recurrence was 29 (± 10) months. There was no death related to surgery. Mean length of hospital stay was 13 (± 16) days. Mean long-term follow-up was 55 (± 48) months (min.: < 1, max.: 219). In a multiple logistic regression pre-operative cysto-biliary fistulas were associated with a substantial risk for postoperative bile associated complications (leakage, bilioma) ($p < 0,01$). Complex cysts (WHO stage CE2 and CE3b) seem to be more likely to show cyst recurrence compared to unilocular cysts ($p < 0,1$). Omentoplasty of the residual cavity may reduce the risk of post-operative biliary leakage ($p < 0,1$).

Conclusion: Our analysis shows that postoperative morbidity is predominantly associated with biliary complications. Patients with preoperative cysto-biliary fistulas are most at risk. Biliomas are observed in 18 % of patients in our cohort. Early postoperative ultrasound three months after surgery is recommended to distinguish bilioma and seroma from CE cyst recurrence which manifest later. In our analysis CE2 and CE3b (complex cysts) seem to bear a higher risk of recurrence. These cysts contain multiple daughter cysts and it may be more difficult to evacuate the cyst content. Patients with biliary complications prior to surgery and complex cyst stages should be red flagged for surgeons. The former to put particular emphasis on biliary fistula detection and closure, the latter to take particular care evacuating cyst content and flushing the residual cavity. In our cohort local recurrence occurred within four years of follow-up. A total of 5 years of unremarkable follow-up after surgery seems to be sufficient.

P-2-48

Safety, tolerability and efficacy of moxidectin 4 mg in subjects with microfilaremic Loa loa infection – preliminary data from a phase 2a randomized, ascending dose, placebo-controlled, assessor-blind trial

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Background: More than 20 million people are chronically infected with *Loa loa*. Recent studies have revealed that the burden of loiasis is comparable to the disease burden of other major neglected tropical diseases (NTDs), along with an associated increased mortality. Despite this, treatment options continue to be scarce.

Moxidectin has shown potential to help eliminate onchocerciasis due to its favorable pharmacokinetic profile, warranting its evaluation for treating loiasis. Preliminary data from a clinical trial assessing the safety and efficacy of moxidectin in patients with loiasis will be presented.

Methods: During the first phase of a randomized, assessor-blind, placebo-controlled phase 2a trial 35 individuals with

microfilaremic loiasis (microfilarial density 1-2,000mf/ml) were randomly assigned to one of the three groups: moxidectin 4 mg, ivermectin (150 µg/kg) or placebo.

The primary endpoint was the safety and tolerability assessed by the number of adverse events occurring within 28 days post-treatment administration compared among treatment groups. Efficacy was assessed by comparing the reduction of microfilaremia and patient-reported signs and symptoms.

Results: The preliminary analysis of the first cohort suggests a similar safety profile for moxidectin and ivermectin. Most adverse events were mild, including symptoms such as headache, rhinitis, and pruritus, occurring within the first 28 days post-treatment. Notably, no grade 3 or serious adverse events (SAE) were recorded following treatment administration. Efficacy analysis suggests that moxidectin 4 mg suppresses only slightly microfilaremia within the first days following treatment.

Conclusions: If confirmed in the final analysis, moxidectin may prove as a promising new treatment option for microfilaremic loiasis. The complete results of this phase 2a trial are highly anticipated.

P-2-49

Rapid development of Modified Vaccinia virus Ankara (MVA)-based vaccine candidates against Marburg virus suitable for clinical use in humans

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Marburg virus (MARV) is the etiological agent of Marburg Virus Disease (MVD), a rare, but severe hemorrhagic fever disease with high case fatality rates in humans. Smaller outbreaks were frequently reported from countries in Africa within the last years, and confirmed human cases outside Africa are, so far, exclusively imported by returning travelers. Over the last years, MARV has also spread to non-endemic African countries, demonstrating its potential to cause epidemics, and although MARV-specific vaccines are evaluated in preclinical and clinical research, none received approval for human use to date. Modified Vaccinia virus Ankara (MVA), a well-established and characterized vaccine strain against emerging infections, has the capability to deliver multiple antigens and has a remarkable record of clinical safety. Here, we have used an optimized methodology to generate and characterize recombinant MVA candidate vaccines that deliver either the MARV glycoprotein (MVA-MARV-GP) or the MARV nucleoprotein (MVA-MARV-NP) and meet the requirements to proceed to human clinical trials. Infections of human cell cultures with recombinant MVA-MARV-GP and MVA-MARV-NP confirmed efficient synthesis of MARV-GP and MARV-NP proteins in mammalian cells, which are non-permissive for MVA replication. Prime-boost immunizations in C57BL/6J mice readily induced circulating serum antibodies binding to recombinant MARV-GP and MARV-NP proteins. Moreover, the MVA-MARV-candidate vaccines elicited MARV-specific T cell responses in C57BL/6J mice. Thus, further studies are warranted to characterize the protective efficacy of these recombinant MVA-MARV vaccines in other preclinical models and to evaluate them as vaccine candidates in humans

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P-2-50

Clinical course and influences on anaemia during and after TB treatment: a multinational cohort analysis

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Pulmonary tuberculosis (TB) and iron deficiency anemia are widely prevalent in sub-Saharan Africa, where their endemic regions overlap considerably.[1] We analyze site-specific factors influencing Hb in TB-patients in a multinational Sub-Saharan African cohort.

Data stems from the TB sequel cohort [2] where microbiologically positive TB patients were recruited 2017-19. Study sites were Fajara (The Gambia), Johannesburg (South Africa), Maputo (Mozambique) and Mbeya (Tanzania). Anemia was defined as Hb <13 g/dl (male) and <12 g/dl (female). Long-term outcomes from month 24 to 36 after diagnosis were pooled. Patients receiving oral iron substitution (Fe) at TB diagnosis were retrospectively matched with untreated controls. Significance level was set at $p<0.05$.

1008/1356 eligible patients (74.3%) were anemic at TB-diagnoses. 446/1356 (32.9%) had microcytic-hypochromic anemia and 335/1356 (24.7%) had at least moderate anemia <10 g/dl. At month 6, anemia prevalence receded to 318/1052 (30.2%) and to 190/780 (24.4%) at month 24. Likewise, prevalence of moderate-severe anemia receded with study time, Fig 1. 49 Patients received Fe for at least 14 days. Oral Fe did not show a significant influence on Hb at month 6 nor did it change prevalence of adverse TB-outcomes. HIV+ status and low CD4+ count were associated with lower Hb, as was higher Ralph score. BMI >18.5, male sex and elevation of dwelling above sea level were associated with higher Hb ($p<0.05$). Adjusting Hb for elevation [3] temporarily inverted a positive correlation at TB-diagnosis ($p<0.05$), which could not be observed 2 years after. Low Hb at TB-diagnosis correlated significantly with all cause hospitalization and poor spirometry outcomes 2 years after ($p<0.05$).

Anemia was a prevalent TB-comorbidity within our cohort, but a wide majority of cases resolved spontaneously with TB-treatment. Early oral Fe did not affect anemia resolution at month 6 nor other patient outcomes. Site-specific differences in mean Hb are explained by differences in HIV-status, sex, Ralph score and elevation of dwelling.

1. Safiri, S., et al., Burden of anemia and its underlying causes in 204 countries and territories, 1990-2019: results from the Global Burden of Disease Study 2019. J Hematol Oncol, 2021. 14(1): p. 185.
2. Rachow, A., et al., TB sequel: incidence, pathogenesis and risk factors of long-term medical and social sequelae of pulmonary TB - a study protocol. BMC Pulm Med, 2019. 19(1): p. 4.

3. WHO Guidelines Approved by the Guidelines Review Committee, in Guideline on haemoglobin cutoffs to define anaemia in individuals and populations. 2024, World Health Organization

Fig. 1

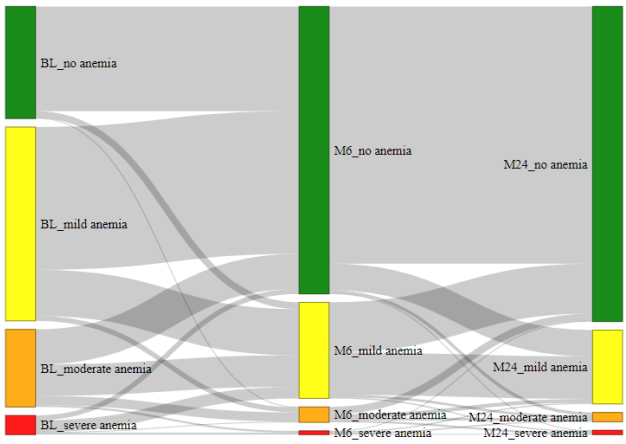

Figure 1. Course of anemia severity from Baseline (BL) to month 6 (M6) and month 24 (M24). Mild anemia: 12-10 g/dl (female) and 13-10 g/dl (male), moderate: <10 g/dl, severe: <8.5 g/dl.

P-2-51
Kidney involvement in Schistosomiasis

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Schistosomiasis (Bilharziasis) is a Neglected Tropical Disease (NTD), which is caused by trematodes (flukes). One of the main species causing urogenital Schistosomiasis in humans is *Schistosoma haematobium*, which is highly endemic in sub-Saharan Africa.

S. haematobium has a complex life cycle. Humans are the final hosts and freshwater snails are the intermediate hosts. After a prepatent period, paired adult worms produce large amounts of eggs in the venous plexus of the bladder, which are shed from infected humans. However, some eggs trap in the tissue, leading to granulomatous formations and inflammation in urinary tract.

Here, we aim to investigate immune cells and Tubular Epithelial Cells (TECs) in urine as well as inflammatory and immune markers such as cytokines and antibodies against *S. haematobium* in blood samples. The hypothesis is: The more advanced the infection, the more immune cells, TECs and inflammatory markers are present in urine and blood.

Prelimary findings: *S. haematobium* negative participants (n=4) show a diverser distribution of Immune and TECs, whereas the median of Immune cells are higher in *S. haematobium* positive participants (n=4). This is not shown for TECs.

P-2-52
Developing broad-spectrum antivirals against emerging viral threats: A systematic approach using the antiviral compound testing platform

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For most emerging viral infections, no vaccines or specific antiviral drugs are available, with symptomatic treatment as the only option. Recent examples of major epidemics and pandemics caused by Ebola, Zika, Chikungunya, and SARS-CoV-2, highlight the need for better preparedness. Given the inevitability of future outbreaks and the unpredictability of their causes, developing broad-spectrum antiviral drugs is one of a strategic approach to mitigate these threats. In this effort, the TTU Emerging Infections (EI) of the German Center for Infection Research (DZIF) focuses on developing broad-spectrum antivirals. By uniting experts from different fields of virology and combining their expertise and infrastructures, the Antiviral Compound Testing Platform (ACTP) has been established to enable rapid screening of potential antiviral compounds against a wide range of high-risk viruses. In this regard, the Nucleoside Booster Flex Fund project was initiated to systematically explore a diverse chemistry set of nucleoside analogues (NAs) and identify promising broad-spectrum antiviral drug candidates. Nucleoside analogs (NAs) are a promising class for broad-spectrum antivirals due to their mechanism of action and high resistance barrier. In close collaboration with the NGO Drugs for Neglected Diseases Initiative (DNDi), a pre-selected library of NAs was screened within the ACTP against a number of high-risk pandemic viruses. The screening comprised a total of 129 NAs that were probed against 10 distinct viruses and identified 21 hit candidates. These candidates underwent further confirmation through dose-response analyses and were evaluated for antiviral activity across 17 different assays and 10 different viruses, with cytotoxicity assessed in 9 cell lines. Based on their antiviral and cytotoxicity profiles, 5 NAs were identified as promising candidates for broad-spectrum antiviral activity and are currently undergoing additional validation.

At the Heidelberg site, the NA screening project has focused on orthoflavivirus members, notably Dengue and Zika virus. Selected NAs were confirmed via dose-response analysis, and hit candidates with inhibitory effects against both viruses are now being evaluated for broad-spectrum efficacy against other orthoflaviviruses, including West Nile and Yellow Fever virus. The NA candidates are also being tested in primary cell culture systems to better reflect target organs and viral pathogenesis, advancing their potential as pan-flavivirus antiviral candidates. This initiative demonstrates that the ACTP serves as a robust platform technology for comprehensive testing of antiviral molecules, providing a

strategic approach to rapidly respond to future viral outbreaks with effective therapeutic options.

P-2-53

RCHY1 targets and is utilized by SARS-CoV-2 Nsp13 to modulate Lys63-linked polyubiquitination of Nsp13 and inhibit NF-κB signaling

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RCHY1, a RING finger and CHY zinc finger domain-containing protein, acts as an E3 ubiquitin ligase, promoting the ubiquitination and subsequent proteasomal degradation of substrates like p53. In the context of SARS-CoV-2, non-structural protein 13 (Nsp13), a helicase crucial for viral replication, is a key target. Nsp13 unwinds double-stranded DNA and RNA in a 5′ to 3′ direction, and its high sequence conservation makes it an attractive candidate for antiviral strategies.

Our study reveals that RCHY1 interacts with Nsp13 via residues 95-144, as demonstrated by co-immunoprecipitation (Co-IP). Nsp13 can be divided into two main fragments: an N-terminal fragment (amino acids 1-259), containing a Zinc Binding Domain (ZBD) responsible for coordinating structural zinc ions, and a C-terminal fragment (amino acids 260-601), which houses two "RecA-like" helicase subdomains involved in nucleotide binding and hydrolysis. Both fragments were found to bind RCHY1, leading to increased polyubiquitination of Nsp13. Deleting RCHY1's RING domain, which is essential for its E3 ligase activity, partially reduced Nsp13 ubiquitination. Furthermore, inhibition of Nsp13 helicase activity also significantly diminished RCHY1-mediated Nsp13 ubiquitination.

Ubiquitination can involve different linkages, with Lys63-linked and Lys48-linked chains being well-characterized. To investigate this further, we performed an in vivo ubiquitination assay using wild-type ubiquitin and ubiquitin variants with substitutions at Lys63 or Lys48 (myc-Ub-K63R, myc-Ub-K48R). A notable reduction in K63-linked ubiquitin chains on Nsp13 was observed in cells expressing myc-Ub-K63R, but not myc-Ub-K48R. This suggests that RCHY1 specifically promotes Lys63-linked ubiquitination of Nsp13.

Moreover, co-expression of RCHY1 and Nsp13 inhibited NF-κB signaling, a key pathway in the host immune response. Interestingly, mutations in three known ubiquitination sites on Nsp13 did not abolish the suppression of NF-κB activity, indicating that RCHY1's repression of NF-κB is independent of Nsp13 ubiquitination. However, this post-translational modification may influence other aspects of Nsp13 function, such as its interaction with other viral proteins or its role in viral replication.

In summary, our findings highlight that RCHY1 targets Nsp13 for Lys63-linked polyubiquitination. This ubiquitination, while not directly linked to NF-κB suppression, may play a role in other physiological processes critical for SARS-CoV-2 replication and immune evasion.

P-2-54

SARS-CoV-2 orf3a disrupts the mRNA export machinery to inhibit host gene expression

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Viruses employ various strategies to suppress host gene expression and antiviral responses to enhance their replication. One such process targeted by many viruses is the nuclear export of mRNA. SARS-CoV-2, the virus responsible for severe acute respiratory syndrome, similarly inhibits mRNA export from the host nucleus. Identifying viral proteins that interfere with host cell translation is crucial for developing antiviral therapies.

In this study, we identified the viral accessory protein orf3a as a key factor in disrupting mRNA export and repressing protein translation. Orf3a induces the accumulation of poly(A)+ RNA in the nucleus, hindering mRNA from reaching the cytoplasm for translation. While orf3a is predominantly localized in the cytosol, including the endoplasmic reticulum and Golgi apparatus, it also partially resides in the nucleus. There, it colocalizes with eukaryotic translation initiation factor 4A1 (eIF4A1), which is essential for mRNA binding to the ribosome.

Orf3a was found to interact with mRNA export factors UAP56 and ALY/REF, disrupting their functional interaction. Specifically, the N-terminal region of orf3a (amino acids 1-132) binds to ALY/REF and nucleoporin 62 (Nup62), key components of the mRNA export machinery. Although orf3a does not affect the nuclear localization of ALY/REF and Nup62, it significantly decreases their expression level, thereby inhibiting mRNA export. Orf3a also suppresses the host antiviral response, with the aa1-132 region playing a critical role in this suppression. Knockdown of orf3a reduces SARS-CoV-2 replication in Vero E6 and BEAS-2B cells.

These findings suggest that targeting orf3a's inhibition of mRNA export and antiviral pathways could offer a therapeutic approach to restoring proper antiviral host gene expression during SARS-CoV-2 infection.

Keywords: SARS-CoV-2 orf3a, mRNA exporter, nucleoporin, antiviral response, replication

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P-2-55

Investigating sex differences following vaccination with an MVA-based vaccine against the Middle East Respiratory Syndrome (MERS)

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Sex differences have been observed for various vaccines, with women generally reporting more adverse events and developing stronger antibody responses. However, the impact of biological sex on responses to vector vaccines has not been well elucidated. We here elucidate sex differences in the reactogenicity and immunogenicity profiles of participants in a double-blind, placebo-controlled clinical Phase 1b trial of MVA-MERS-S, a Modified Vaccinia virus Ankara (MVA)-based vaccine candidate against the Middle East respiratory syndrome-related coronavirus (MERS-CoV), a WHO priority pathogen with epidemic potential spread.

Healthy adults were randomized into five arms and received four injections (3x vaccine, 1x placebo, or 4x placebo). All participants except the placebo arm received low (10^7 plaque-forming units, PFU) or high dose (10^8 PFU) of MVA-MERS-S on day 0, days 28 (short interval) or 56 (long interval), and day 224. Multiple blood samples were collected longitudinally from baseline up to 252 days after prime vaccination.

Following MVA-MERS-S vaccination, females reported a higher number of systemic adverse events. This was observed also in the placebo study arm, highlighting gender rather than sex differences. Our data showed no sex differences in neutralising antibody titres. Interestingly, 28 days after booster vaccination, males had higher geometric mean titres of MERS-CoV S1 specific antibodies compared to females, as also observed for MVA-based vaccines in previous studies. Additionally, we investigated sex-specific differences in whole-blood gene expression profiles and the correlation between sex hormones and antibody levels.

A better understanding of newer vaccine platforms and the mechanisms involved in sex differences in response to vector-based vaccines will facilitate future strategic vaccine development.

P-2-56
Recurrent events in clinical cohorts: Modeling the occurrence of malaria infections

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There is limited methodological information about the occurrence of recurrent events in longitudinal studies. However, in diseases like malaria, recurrent events are common and they are the subject of analyses in many clinical studies. A proper understanding of disease occurrence within a cohort provides a basis for study planning and sample size estimation. This study mathematically describes the occurrence of recurrent malaria infections in a naïve cohort and highlights the necessary assumptions to inform study planning.

A common estimator of the frequency of disease in a population at risk is the cumulative incidence, which defines the individual risk of experiencing a single disease episode. In contrast, the risk to experience recurrent events is given by the event rate (ER), which represents the observed number of events in a population at risk over time. Mathematical models parameterized with an ER of 400% (i.e., each individual experiences on average 4 malaria infections per year) were established to show the proportion of study individuals moving through different infection-related states over a follow-up period of 2 years. The transition between these states is described by a compartment model characterized by a system of ordinary differential equations.

At 12 months, 33% and at 24 months, 55% of the study population experienced at least one malaria infection. Over time, individuals who experienced their first infection could become reinfected, and as the number of recurrent infections increased, the rate of single infections decreased. At 24 months, 14% experienced two, 4% experienced three, and 1% experienced four or more infections. The number of patients who experienced recurrent infections was 19% at 12 months and 35% at 24 months. At month 12, an average of 4 and at month 24, an average of 8 infections per individual were observed, representing the ER used to calculate the model.

Recurrent infections in longitudinal studies cannot be directly estimated from disease frequency data. However, this study provides a simple set of equations to calculate the number of expected recurrent events. The presented formulas represent simple transmission dynamics and ignore factors such as heterogeneous infection risk or immunity development over time. However, the model can easily be adapted to represent additional transmission and infection dynamics. Malaria serves, as an example; but the model can also be used for other recurrent diseases like influenza.

P-2-57
Strategy to identify and characterize pan-filovirus reactive antibodies

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Filoviruses such as ebolaviruses Ebola virus (EBOV), Sudan virus (SUDV) and Bundibugyo virus (BDBV) and marburgviruses (Marburg virus and Ravn virus) are highly contagious and can cause severe diseases with significant case fatality rates in humans. Treatment options for filovirus infections are limited and mainly based on supportive care. In addition, for EBOV disease few monoclonal antibodies (mAbs) or cocktails as well as three vaccines licensed by the European Medicines Agency are available. However, cross-reactivity of vaccines as well as isolated mAbs with other filovirus strains is restricted or not known. Moreover, other strain specific vaccines are still in development and evaluated in (pre-)clinical trials. Therefore, a more comprehensive understanding of filovirus infection pathways, the resulting B cell response as well as the different neutralization mechanisms plays a central role in the identification of potent neutralizing antibodies. Of note, identification and targeting of highly conserved and sensitive

antibody target sites will be important to be prepared against spillovers of novel filoviruses in the future.

To gain better insights into the development, duration and cross-reactivity of a filovirus-specific humoral immune response, we analysed longitudinal dynamics of EBOV-specific antibodies induced by vaccination. Therefore, we collected samples of participants of a clinical phase I study vaccinated with the rVSV-ZEBOV vaccine in 2015. Of these the primary response has been studied 2019 by Erhardt et al. This enabled us to get information about the development of a B cell response seven years after vaccination. Of all collected samples we performed pseudotyped neutralization assays and tested the serum for their neutralizing activity. In addition, we performed B cell isolation and single cell sequencing. Our preliminary data suggests the presence of a long-lasting B cell response seven years after vaccination. Moreover, using surface proteins of SUDV and BDBV we were able to identify potentially cross-reactive B cells in rVSV-ZEBOV vaccinated individuals.

These findings provide important insights into the duration and development of a filovirus-specific immune response and the efficacy of the rVSV-ZEBOV vaccine. Further analysis will include the selection of representative B cells, the in vitro production of corresponding antibodies and binding as well as neutralization studies with various filoviruses. Based on this we will aim to identify promising candidates with high neutralization potency as well as broad filovirus reactivity for the next upcoming therapeutic approaches.

P-2-58

The impact of insecticide resistance and oxidative stress on the development of Plasmodium falciparum in Anopheles coluzzii

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Malaria remains a major public health challenge, with 249 million cases and 608,000 deaths reported in 2022. Insecticide-treated nets, which all contain pyrethroids, target the *Anopheles* mosquito vector and have proved to be the most effective tools in the prevention of Malaria over the last decades. However, the efficacy of this class of insecticides is threatened by the rapid spread of insecticide resistance (IR) across Africa. Pyrethroids induce oxidative stress (OS), and IR mosquitoes also exhibit higher respiration and endogenous production of reactive oxygen species (ROS), a marker of OS. ROS play a key role in the mosquito's immune response to pathogens and so elevated levels in IR *Anopheles* could enhance immunity towards *Plasmodium falciparum*. To explore this, increasing OS by feeding *An. coluzzii* hydrogen peroxide (H2O2) and RNAi-induced silencing of Catalase was used to investigate immune responses to *P. falciparum* by measuring parasite prevalence and intensity. Surprisingly, H2O2 feeding increased infection possibly due to microbiome disturbance. Through the comparison of IR and susceptible *An. coluzzii* we can further understand other resistance mechanisms and their impact on *P. falciparum* development and its extrinsic incubation period. Together, these results shed new light on the interplay of IR and immunity and contribute to the much needed knowledge for the design of future vector control strategies.

P-2-59

Co-administration of the novel antibiotic Corallopyronin A with benzimidazoles improves efficacy in the Litomosoides sigmodontis rodent model

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Current treatment options for human filarial diseases are largely limited to microfilaricidal drugs, i.e., drugs that predominantly target the first larval stages. As such, therapies based on these drugs can only interrupt transmission and are unable to cure the infected patients. The development of a safe, macrofilaricidal (=adult worm killing) drug could improve the well-being of millions of people that are still infected with a variety of filariae across the tropics. Depleting the essential *Wolbachia* endosymbionts of filarial nematodes prevents development, causes worm sterility and slowly kills the adult filariae. Corallopyronin A (CorA), a bacterial RNA polymerase inhibitor, kills *Wolbachia* endosymbionts present in filariae and is such a potential new macrofilaricide. Based on a strong synergistic effect of anti-wolbachials with benzimidazoles that has been observed previously for other compounds, we investigated the potential of a co-administrative therapy of anti-wolbachial compounds (CorA, doxycycline) with two different benzimidazoles, albendazole (ALB) and oxfendazole (OXF).

The efficacy of anti-wolbachial compounds was assessed in the *Litomosoides sigmodontis* model. Gerbils (CorA) or mice (doxycycline) naturally infected with *L. sigmodontis* were treated either with the anti-wolbachial alone or together with ALB or OXF. Rodents were analyzed for the number of microfilariae in the peripheral blood throughout the infection. All animals were assessed for changes in the worm number, length, worm fertility and number of *Wolbachia* endosymbionts.

The current minimal efficacious regimen of oral CorA monotherapy is a 14-day treatment of 30 mg/kg TID or 60 mg/kg BID in the *L. sigmodontis* model. Co-administration with ALB reduced the dose required to deplete *Wolbachia* >99% to 45 mg/kg CorA BID for 14 days. Since OXF is generally more effective against filariae than ALB, we compared the efficacy of lower doses (30 and 20 mg/kg CorA BID for 14 days ± 3 days of either OXF or ALB). As expected, the co-administration with OXF led to a stronger median reduction of *Wolbachia* than the CorA monotherapy (97.8% vs. 33.3%). In the case of doxycycline, co-administration with OXF did not improve the depletion of *Wolbachia*, however, the co-administrative therapy led to a strong macrofilaricidal effect surpassing the worm clearance of the OXF monotherapy.

Novel macrofilaricidal treatments for human filarial infections are urgently needed. CorA is the only *Wolbachia*-targeting drug that is likely to be tested in clinical trials within the next 2-3 years. In this project, we report on potential co-administrative therapies with drugs that are either already

approved for human use (ALB) or in phase 2 clinical trials (OXF). We demonstrate that a co-administrative therapy with either benzimidazole has the potential to reduce the required daily dose and/or treatment duration of CorA for a successful treatment in the *L. sigmodontis* model.

P-2-60

Correlates of protection against MERS-CoV in mice immunized with an S protein-encoding measles-derived vaccine candidate

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Question: Protective efficacy of vaccine candidates against infectious diseases must be verified for each targeted pathogen, especially when using vaccine platform technologies such as mRNA vaccines or viral vectors. For that purpose, knowledge of protective immune responses against a pathogen, so-called correlates of protection (CoPs), is crucial for success. Despite its emergence in 2012, CoPs against the Middle East Respiratory Syndrome coronavirus (MERS-CoV) are still unclear. A recombinant MeV-derived vaccine candidate expressing the spike (S) protein of MERS-CoV, MeV-MERS, was shown to induce both S-specific antibodies and T cell responses that protected vaccinated mice during lethal challenge. Here, we strive to identify CoPs for MERS-CoV using this MeV-derived vaccine that efficiently stimulates both arms of the adaptive immune system.

Methods: To identify CoPs against MERS, depletion of $CD4^+$ and/or $CD8^+$ T cells, B cells, or NK cells by i.p. administration of monoclonal antibodies was established and monitored by FACS analysis. Established protocols were then used for B cell depletion before or depletion of T and NK cells after vaccination in MeV-MERS-vaccinated, MERS-CoV-susceptible $IFNAR^{-/-}$-hDPP4 mice, which were subsequently challenged with >10 LD_{100} of MERS-CoV. In addition, naïve mice, vaccinated mice, or naïve mice after transfer of serum or T cell pools isolated from vaccinated mice were infected.

Results: Depletion of T cell subsets or NK cells in peripheral blood and spleens was successful after administration of 0.5 µg (GK1.5, αCD4), 0.1 µg (GK2.43, αCD8) or 0.3 µg (PK136, αNK1.1) mAb 8 and 6 days (only αCD4) as well as 3, 2 and 1 days (all) before infection. B cells were depleted after administration of 0.1 µg mAb (MB20-11, αCD20) twice in a 3 days interval. Mice vaccinated with MeV-MERS were fully protected after infection, in contrast to naïve control mice, which uniformly succumbed to challenge. Challenge of vaccinated mice after immune cell depletion revealed survival independent of the presence of $CD4^+$ T cells. However, depletion of $CD8^+$ T cells or NK cells rendered 2/7 or 1/7 animals susceptible for disease, respectively. Also B-cell depletion before vaccination rendered 1/7 vaccinated animals susceptible for disease. Neither transfer of serum nor adoptive transfer of T cells from vaccinated donors into naïve mice did rescue the acceptor animals from disease.

Conclusion: In our MERS vaccine and challenge model, an important role for NK, B and $CD8^+$ T cell responses for protection became evident. Our data moreover suggest an interplay of several factors that control infection, since depletion of single immune cells subsets was not sufficient to fully ablate protection nor did serum or T cell transfer alone rescue the receiving animals.

P-2-61

Estimated incidence of respiratory syncytial virus (RSV)-related hospitalizations for acute respiratory infections (ARIs) and associated risk for cardiovascular events in adults in Germany

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Background: RSV is a leading cause of ARI, including CAP, in older adults, but available data often substantially underestimate incidence. We estimated RSV-related ARI hospitalization incidence from a prospective CAP study and adjusted for undiagnosed RSV infections due to use of nasopharyngeal/nasal swab testing only.

Methods: We conducted active, population-based surveillance of adult CAP hospitalizations in Thuringia (Germany) between 2021–2023. Participant nasopharyngeal/nasal swabs were RSV-tested by multiplex nucleic acid amplification testing. To estimate RSV-related CAP incidence, age-group specific proportions of RSV positivity among tested patients were applied to all-cause CAP incidence. To adjust for underdiagnosis due to nasopharyngeal/nasal swab sampling only and the percentage of ARI with pneumonia diagnoses, we used data from a large, prospective, multispecimen study assessing impact of collecting multiple specimens (nasopharyngeal/nasal swab, saliva, paired serology, and sputum) among 3,669 adults hospitalized for ARI.

Results: Among 1,040 enrolled adults (≥18 years) with radiologically confirmed CAP, 38 tested RSV-positive via nasopharyngeal/nasal swab (3.7%). The percentage positive increased to 7.8% after adjusting for higher RSV detection with multiple specimens compared to nasopharyngeal/nasal swab only. Adjusted RSV-related CAP hospitalization rates were 4.7 (95%CI 1.5–11.2) and 109.1 (95%CI 89.6–131.6) per 100,000 adults aged 18–59 and ≥60 years, respectively. Adjusted incidences of RSV-related ARI were 18.4 (95%CI 11.0–28.9) and 377.6 (95%CI 340.5–417.7) per 100,000 adults aged 18–59 and ≥60 years, respectively. Among RSV-positive CAP hospitalizations, 12.1% of patients aged ≥65 years died within 30 days, with no deaths in those aged 18–64 years. Cardiovascular events occurred in 11.1% of patients aged 18–64 and 36.4% of those aged ≥65 years.

Conclusions: Older adults in Germany face a high burden of RSV-related ARI hospitalizations, including CAP, underscoring RSV vaccination"s potential utility for this population.

Fig. 1

Table 1. Characteristics of adults hospitalized at Jena University Hospital with radiologically-confirmed CAP patients by RSV testing status

	NP/Nasal Swab Tested		NP/Nasal Swab Untested***		
	N	%	N	%	P-Value
Overall	293	100	741	100	
Age group					
18-59 years	38	13	73	9.9	0.145
≥60 years	255	87	668	90.1	0.145
18-64 years	66	22.5	117	15.8	0.010
≥65 years	227	77.5	624	84.2	0.010
Sex					
Female	125	42.7	309	41.7	0.778
Male	168	57.3	432	58.3	
Study period*					
Year 1	135	46.1	341	46	0.987
Year 2	158	53.9	400	54	0.987
Overall study period	293	100	741	100	
Risk status**					
Low-risk	18	6.1	62	8.4	0.228
At-risk	104	35.5	391	52.8	<0.001
High-risk	171	58.4	287	38.7	<0.001

Abbreviations: CAP=community-acquired pneumonia, CDC = centers for disease, NP=nasal swab=nasopharyngeal (NP)/nasal swab
* Year 1: 01Jul2021 – 30Jun2022; Year 2: 01Jul2022 – 30Jun2023
** The risk status was classified using CDC definitions for pneumococcal disease [23]
*** Include enrolled and non-enrolled patients

Fig. 2

Table 2 Unadjusted and adjusted incidence rates of RSV-related CAP and ARI hospitalizations in Thuringia/Germany 2021–2023

Age group (Years)	CAP incidence rate (per 100, 000) [1]	RSV positive proportion based on NP/nasal swab [1]	Percentage increase in RSV detection [2]	RSV-related CAP incidence rate per 100,000 (95% confidence interval) [3]		Proportion of CAP in RSV-related ARI hospitalization [5]	RSV-related ARI incidence rate per 100,000 (95% confidence interval) [6]	
				Unadjusted	Adjusted [4]		Unadjusted	Adjusted
Year 1: 01Jul2021 – 30Jun2022								
≥18	451.0 (410.0, 494.6)	2.3%	112%	10.4 (5.1,18.9)	22.0 (13.8, 33.3)	28.0%	37.0 (26.1, 51.0)	78.5 (62.1, 97.9)
18-59	87.7 (70.6, 108.4)	2.3%	148%	2.0 (0.2,7.2)	5.0 (1.6, 11.7)	25.4%	7.9 (3.4, 15.6)	19.7 (12.0, 30.5)
≥60	1189.0 (1121.8, 1257.1)	2.3%	101%	27.3 (18.0,39.6)	54.9 (41.3, 71.5)	28.9%	94.5 (76.4, 115.6)	190.0 (163.9, 219.0)
18-64	119.4 (98.8, 142.4)	1.7%	141%	2.0 (0.2, 7.2)	4.9 (1.6, 11.5)	22.6%	9.0 (4.1, 17.1)	21.6 (13.5, 32.8)
≥65	1384.5 (1312.5, 1458.3)	2.7%	95%	37.4 (26.4, 51.5)	72.9 (57.1, 91.7)	31.8%	117.6 (97.3, 140.9)	229.2 (200.5, 260.9)
Year 2: 01Jul2022 – 30Jun2023								
≥18	528.7 (485.0, 575.5)	5.5%	112%	29.1 (19.5, 41.8)	61.6 (47.2, 79.0)	28.0%	103.9 (84.9, 125.8)	220.2 (192.1, 251.3)
18-59	69.3 (53.7, 87.3)	2.6%	148%	1.8 (0.2, 6.9)	4.5 (1.4, 11.0)	25.4%	7.1 (2.9, 14.6)	17.6 (10.4, 28.0)
≥60	1460.6 (1387.5, 1537.3)	6.1%	101%	89.1 (71.6, 109.6)	179.1 (153.6, 207.3)	28.9%	308.3 (274.8, 344.7)	619.7 (571.9, 670.5)
18-64	116.6 (65.0, 136.1)	4.2%	141%	4.9 (1.6, 11.5)	11.7 (6.0, 20.6)	22.6%	21.5 (13.4, 32.7)	51.8 (38.7, 68.0)
≥65	1691.7 (1613.0, 1773.6)	6.0%	95%	101.6 (82.7, 123.3)	197.9 (171.3, 227.5)	31.8%	319.2 (285.1, 356.2)	622.4 (574.5, 673.3)
Overall study period: 01Jul2021 – 30Jun2023								
≥18	489.8 (460.4, 520.6)	3.7%	112%	18.1 (10.7, 28.6)	38.4 (27.2, 52.6)	28.0%	64.7 (49.9, 82.5)	137.2 (115.2, 162.2)
18-59	78.5 (64.6, 94.5)	2.4%	148%	1.9 (0.2, 7.1)	4.7 (1.5, 11.2)	25.4%	7.4 (3.1, 15.0)	18.4 (11.0, 28.9)
≥60	1324.3 (1241.3, 1411.3)	4.1%	101%	54.3 (40.8, 70.8)	109.1 (89.6, 131.6)	28.9%	187.9 (162.0, 216.8)	377.9 (340.5, 417.7)
18-64	117.5 (101.1, 135.8)	2.5%	141%	2.9 (0.6, 8.6)	7.1 (2.9, 14.6)	22.6%	13.0 (6.9, 22.2)	31.3 (21.3, 44.4)
≥65	1538.1 (1437.9, 1643.2)	4.2%	95%	64.6 (49.8, 82.4)	126.0 (105.0, 150.0)	31.8%	203.1 (176.1, 233.0)	396.1 (356.0, 437.5)

Abbreviations: CAP=community-acquired pneumonia; ARI= acute respiratory infection; RSV=respiratory syncytial virus. NP/nasal swab=nasopharyngeal (NP)/nasal swab1. Obtained from the active, population-based surveillance study. CAP incidence estimates are taking into account Jena patients only; however, all three sites contributed to the RSV positive proportion

2. Percentage increase with additional specimen type results beyond NP/nasal swab, obtained from the multi-specimen study*

3. Assumes the RSV positive proportion is the same among CAP patients tested and untested

4. Adjusted for the underascertainment of RSV detection using the percentage increase with additional specimen type results beyond NP/nasal swab, obtained from the multi-specimen study*

5. Proportions of CAP among RSV-related ARI hospitalizations were obtained from the multi-specimen study*

6. Calculated by dividing RSV-related CAP incidence by the proportion of CAP in RSV-related ARI hospitalizations

* Alhadi N. et al Detection of Respiratory Syncytial Virus (RSV) using nasopharyngeal specimens (NPS) alone significantly underestimates disease incidence in adults hospitalized with acute respiratory infections (ARI): an updated, pooled analysis from North America. 8th Global RSV-NET Conference. Mumbai, India 2024.

P-2-62

Five years of molecular epidemiology of Lassa virus in Nigeria

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Lassa fever (LF) is a viral hemorrhagic disease endemic in West Africa with a case fatality rate up to 20%. Lassa virus (LASV), the etiologic agent of LF, is estimated to infect hundreds of thousands of individuals per year and is mainly transmitted to humans from infected rodents, the primary LASV reservoir. While human-to-human transmission of LASV has been reported in healthcare-associated settings, genomic surveillance efforts from previous outbreaks suggest independent rodent-to-human spillovers as predominant route of infection. Nevertheless, current sequencing approaches are heavily biased towards samples with high viral loads, associated with increased case fatality rates, and frequently fail to generate consensus genomes at low viral titers. Consequently, we currently lack a detailed picture of LASV genetic diversity in patients with medium to low viral loads, which blurs our understanding of virus transmission and genomic signatures possibly associated with LF severity and outcome.

To address this gap, we use targeted whole-genome sequencing of diagnostic samples from LASV-confirmed patients at the Irrua Specialist Teaching Hospital (ISTH) in Nigeria between 2018 and 2022, and combine the phylogenetic LASV analysis with the related clinical data. We first collated and cleaned a pseudonymized database of all LASV diagnostic samples and associated metadata obtained at ISTH from 2018 to 2022 and consequently selected approximately 100 random LASV-positive samples per study year for sequencing. A target enrichment approach is being developed to enable the generation of consensus genomes for samples with low viral loads whereas samples with high viral loads are already being sequenced using a metagenomic approach. Obtained LASV genome sequences will be continuously included in our phylogenetic analysis to finally provide a comprehensive snapshot of the molecular epidemiology of LASV in Nigeria over five years. Our findings will provide novel insights into LASV evolution dynamics and stimulate novel and innovative approaches in LASV diagnostics and therapy.

P-2-63

Management of malaria in neonates: A systematic review

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Background: Neonatal malaria is a potentially life-threatening condition that is often underdiagnosed due to its symptom overlap with conditions like sepsis. Management strategies for neonatal malaria vary both within and across endemic and non-endemic regions, reflecting the lack of specific guidelines for this age group. This review aims to identify approaches for managing malaria in newborns within their first month of life.

Methods: Pubmed and Web of Science search from January 2003 to December 2023 for full-text original publications, using key terms around "management", "neonatal", and "malaria". Inclusion criteria were age 0-28 days, parasitological confirmation of malaria and availability of antimalarial treatment data, including case reports. Data was extracted and risk of bias assessed using Critical Appraisal Tools for Case Reports in JBI Systematic Reviews. The review is registered on PROSPERO (CRD42023414278).

Results: Searches identified 3736 records, of these 25 studies were included; all studies were rated as low risk of bias. Overall, the 25 studies comprised data on management of malaria in n=37 neonates; studies were from endemic regions with India (10/25, 40%) and Colombia (3/25, 12%) contributing most cases. Maternal history of malaria in 14/19 (74%) of cases, and 5/7 (71%) of those mothers had received preventive treatment during pregnancy. Most (24/25, 96%) cases were diagnosed by microscopy;*Plasmodium vivax* was the most identified species (17/25, 68%), followed by *Plasmodium falciparum* (6/25, 24%). Most cases (18/21, 86%) were reported as congenital malaria. Fever was the most frequent symptom in *P. vivax* (14/17, 82%) and *P. falciparum* (5/6, 83%) malaria; anaemia was more common in *P. vivax* than *P. falciparum* malaria (14/17, 82% vs. 1/6, 17%). Initial misdiagnosis was common, with 68% (17/25) initially suspected to have sepsis. Treatment involved chloroquine for *P. vivax* (in 10/17, 59%) and for *P. falciparum* artemisinin-based (2/6, 33%) and non-artemisinin-based treatments (4/6,66%). Antimalarial treatment was administered either intravenously or orally. Oral administration was syrup or tablet-based, with 4/25 (16%) studies reporting crushed tablets suspended in water. Species-independent average parasite clearance time was 4.5 days.

Discussion: This review highlights the challenges in diagnosing and managing malaria in neonates. Fever and anemia emerge as the most common symptoms, yet diagnosis remains challenging due to the overlap with other neonatal illnesses. Treatment approaches vary significantly based on the malaria species and regional practices. Findings point to a pressing need for management guidelines specific to neonatal malaria, focusing on improved diagnostic tools and consistent treatment strategies.

Reference: Danwang C, Bigna JJ, Nzalie RNT, Robert A. Epidemiology of clinical congenital and neonatal malaria in endemic settings: A systematic review and meta-analysis. Malar J [Internet]. 2020;19(1):1–8.

P-2-64

Therapeutics for pandemic preparedness in Germany

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The Center for Pandemic Vaccines and Therapeutics (ZEPAI) was established during the COVID-19 pandemic to execute fast vaccine distribution and to prepare for future pandemics using vaccines and therapeutics. We focus on preparing concepts to protect the German public in case of new health emergency situations. Therefore, partnerships with academia and industry are built and strengthened to implement innovative techniques in the field of vaccines and therapeutics into our strategies for pandemic preparedness. Government funding or contractual partnerships will be necessary to achieve these goals and to support the development of therapeutics as well as to reserve production capacities for an immediate response to a health crisis.

As seen in the COVID-19 pandemic, therapeutics like (broad-spectrum) antivirals and monoclonal antibodies (mAB) play an important role in combination with vaccine strategies to prevent disease spread in vulnerable and high-risk groups. Antivirals are important in the early phase of a pandemic as vaccine production needs several months once the virus is identified. During a pandemic, therapeutics stay important, as they might reduce severe illness in patients and are options for subgroups that cannot be vaccinated for certain reasons. Limitations, as decreased efficacy against rising variants, should be overcome with technological advancements and a well-prepared pharmaceutical infrastructure. Therefore, the ZEPAI focusses on the preparation of therapeutic concepts for mAbs, immune modulators and (broad-spectrum) antivirals connecting research institutes and industrial stakeholders in a pandemic preparedness partnership.

Monoclonal antibodies are of particular interest for our preparedness strategy. The development time of mAB products during the COVID-19 outbreak was comparable to that of employed vaccines. Our goal is to use technological advances made during the pandemic to create a mAb production platform infrastructure in Germany/Europe. The ZEPAI plans to partner with biotech companies and academia partners that were either active in the field of mAb development during the COVID-19 pandemic or have extensive experience in mAb development and manufacturing. We aim to develop prototype-mAbs against a set of known pathogens with high endemic/pandemic potential up to phase I clinical trials or even beyond. The combination of prototype-mAbs and an established manufacturing infrastructure, including preferred manufacturing agreements, will give us the opportunity to develop mAbs at the same speed as pandemic (mRNA) vaccines in cased of a new public health crisis. Thus, mAbs can be used as a first line of defense to prevent infection in high-risk groups, such as hospitalized patients and vulnerable groups, including elderly and immunocompromised persons but also health personnel in a dual defense strategy together with vaccines.

P-2-65

Placental health in malaria-endemic settings: Insights from a birth cohort study

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Question: Placental health is increasingly recognized as a critical determinant of early-life outcomes, particularly for mothers exposed to malaria and other infectious diseases. To explore this, a detailed histopathological analysis of placental health was conducted as part of the longitudinal Malaria Birth Cohort that was established in 2019 in Agogo, Ghana. This analysis aims to determine whether adverse placental conditions affect birth outcomes and assess whether children with measurable health disadvantages at birth (such as being small-for-gestational-age or born prematurely) show signs of recovery within their first year of life.

Methods: Pregnant women were recruited between April 2019 and 2022 and the placentas along with the umbilical cord collected at birth. They were stored in 50 ml of 10% neutral buffered formalin until processed for histopathological examination.

Results: Out of 1,010 recorded births, 987 placentas were collected, with histopathological results available for 860 specimens. Among these, 627 of 835 (75%) showed infarction, 74 of 837 (9%) had microinfarcts, and 664 of 834 (80%) exhibited fibrosis. Acute and chronic microinflammation were noted in 6 (0.85%) and 7 (0.9%) of the 792 samples, respectively. Malaria pigment was present in 7 of 835 (0.8%) placentas, and no viral inclusions were detected. Pathological fibrosis and infarction covering more than 20% of tissue were seen in 243 of 664 (37%) and 337 of 627 (54%) samples, respectively. Altogether 47% (461 of 987) of the women exhibited placental tissue above the defined pathological threshold.

Conclusion: Insights leading to a broader understanding of how maternal health is impacted and impacts child developmental outcomes in malaria-endemic settings may improve early-life health disparities in LMICs. The histopathological placenta analyses will be evaluated for an association with birth outcome (i.e. birthweight, duration of pregnancy), cognitive development, anthropometric measurements (e.g. height, weight, MUAC, head circumference), and other health indicators (e.g. frequency of hospitalization) of the child at 12 months.

P-2-66

Makorin ring finger protein 2 dysregulated by SARS-CoV-2 infection is a substrate for SARS-CoV-2 nonstructural protein 3

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Makorin Ring Finger Protein 2 (MKRN2) is a RING domain containing E3 ubiquitin ligase targeting tumor suppressor p53 and the NF-κB subunit p65 for polyubiquitination and proteasomal degradation. In A549_ACE2 cells, infection with SARS-CoV-2 results in increased protein level of endogenous MKRN2. SARS-CoV-2 nonstructural protein 3

(Nsp3) interacts with MKRN2 via SARS Unique Domain (SUD). The papain-like protease (PLpro) domain in Nsp3 deubiquitinates as well as deISGylates MKRN2. Deubiquitination of MKRN2 by PLpro in Nsp3 disturbs MKRN2 proteasomal degradation and hence causes MKRN2 protein accumulation. Subsequently, the enhanced MKRN2 boosts degradation of antiviral factor p53 and NF-κB subunit p65.

P-2-67

Mobile laboratory for malaria: Field evaluation of a simplified PCR-free detection assay

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Background: Accurate diagnosis is essential for effective case management of malaria infection. Microscopy and antigen-detecting rapid diagnostic tests (RDTs) are the most widely used malaria diagnostic tools for clinical management and surveillance; however, these diagnostic tests have limited sensitivity in detecting low-density infections. Accurate identification of submicroscopic parasitemia is particularly important for malaria elimination campaigns and research, such as controlled human malaria infection studies. Ultra-sensitive nucleic acid amplification technology (NAAT) assays, such as reverse transcription quantitative PCR (RT-qPCR), achieve detection levels of 6 parasites/mL (0.06 parasites per µL); however, the complexity of PCR workflows and reliance on sophisticated laboratory equipment limit their use to centralized facilities and result in long diagnostic turnaround times. To address these limitations, our DZIF-funded malaria diagnostics project (TTU 03.811, MALDx) aims to simplify NAAT-based malaria diagnostics for rapid field use.

Methods: First, we simplified nucleic acid extraction by replacing traditional silica spin column purification with a magnetic bead suspension method that eliminates the need for high-speed centrifugation. Next, we developed a novel isothermal amplification technique based on reverse transcription-recombinase-polymerase-aided amplification (RT-RAA) as an alternative to PCR. We then streamlined workflows to enable molecular diagnosis of malaria directly in the field using a mobile suitcase laboratory powered by a solar panel battery. Finally, we evaluated the performance of the RT-RAA assay compared to microscopy, RDT, and ultrasensitive RT-qPCR in a prospective field trial in Lambaréné and surrounding villages in Gabon. The sensitivity of RT-RAA was assessed using two nucleic acid extraction methods.

Results: Between April and June 2024, we evaluated 165 individuals. The sensitivity of RT-RAA reached 64.4% (extraction method 1) and 98.7% (extraction method 2) compared to ultrasensitive RT-qPCR and was superior to the sensitivities of microscopy and RDT. Cohen's kappa agreement between RT-RAA and reference was good for method 1 (κ = 0.61) and excellent for method 2 (κ = 0.98); in contrast, agreement was poor for microscopy (κ = 0.27) and fair for RDTs (κ = 0.41).

Conclusion: This innovative and simplified molecular assay shows significant potential as a point-of-need screening or diagnostic test, offering high sensitivity comparable to ultrasensitive RT-qPCR. It can be used in settings where such diagnostic accuracy is critical, including drug/vaccine trial screening in endemic areas, mass drug administration test-and-treat campaigns, and intermittent screening and treatment (ISTp) for pregnant women.

P-2-68

Lack of evidence for HEV infection in Baltic Sea mussels (Mytilidae): A comprehensive analysis

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Background: Similar to hepatitis A virus infections, cases of hepatitis E virus infections associated with the consumption of mussels have been described and HEV has been detected in commercial mussels in various studies. While mussels from tropical regions, the Mediterranean Sea, and the North Sea have already been tested for HEV, there have been no reliable statements on the risk posed by common mussels from the Baltic Sea (Mytilidae). Furthermore, no one has attempted to infect mussels from the Baltic Sea with HEV experimentally. Methods:392 hospital employees have been asked about mussel consumption and were tested for anti-HEV IgG (Wantai test). Commercial Mytilidae from the Baltic Sea obtained from a discounter have been tested for HEV by PCR. 50 living Mytilidae have been experimentally exposed to HEV followed by dissection and separate PCR testing of the gastrointestinal tract, gonad, and muscle tissue.

Results::The likelihood of anti-HEV IgG positivity differed not significantly in people eating mussels, compared to those not. None of the 40 commercial mussels out of 2 packs tested positive for HEV. HEV RNA could be detected in the gastrointestinal tract of experimentally exposed Mytilidae but not in the gonad or muscle tissue. HEV-RNA can persist for more than 16 but less than 24 days in the digestive tract.

Conclusion: In contrast to other reports on other mussels from various regions, Mytilidae from the Baltic Sea do not pose a relevant risk for HEV transmission to consumers and can be consumed safely.

P-2-69

HELMSYS: The impact of helminth infections on vaccine response in humans: A systematic literature review

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Background: Vaccination holds the promise of eradicating infectious diseases, but parasitic helminth infections can immunomodulate the response of vaccines, hindering optimal protection; especially in endemic settings of low-and-lower-middle-income countries (LMIC).

Objectives: We conducted a systematic review of literature to examine the impact of helminth infections on vaccine immunogenicity, efficacy and effectiveness, in humans.

Methods
This review was PROSPERO-registered, and follows PRISMA guidelines. PubMed, Scopus, Web of Science, and clinical trial registries were searched; using search terms related to helminths and vaccine responses; targeting studies from January 1970 to June 2024 (in progress). Reference lists of articles and websites were hand-searched;and expert recommendations sought. The title and abstract and full-texts of the retrieved records were screened by two independent reviewers; studies with undetermined helminth-infection or natural immunity were excluded. Study setting, sample size, diagnostics, anti-helminthic treatment, and vaccine response parameters were extracted; means and SDs were calculated or converted for comparability. Quality was assessed in an independent, blinded manner by RoB 2.0 for RCTs and Newcastle-Ottawa Scale for observational studies. Analysis was stratified by direct helminth exposure or anti-helminthic treatment, with only qualitative analysis for studies with non-quantifiable data.

Results: We included 38 studies on 13 different vaccines from 15 LMIC-populations of adults, children or both. All studies reported immunogenicity. Of the 29 observational studies analyzed for impact of direct helminth exposure: on BCG vaccination: raised IFN-γ and IL-10; on tetanus: reduced IL-13 and IFN-γ but protective antibody levels. Quantitatively, on HBV: lowered antibodies and heightened IL-5; on polio: reduced IgG and heightened cytokines; on HPV: reduced HPV18 IgG; in helminth-infected populations. Qualitatively, lowered antibodies in helminth-infected groups in *S. typhi*, Ebola, and malaria vaccines. Of the 9 RCTs analyzed for impact of antihelminthic treatment; quantitatively, on BCG: raised IFN-γ, IL-12 but lowered TGF-β post-treatment; on cholera: only a modest boost in humoral responses; in helminth-infected groups. Qualitatively, on measles: no effect in adults but raised IgG in children; on influenza: antibodies boosted initially but declined, but memory B-cells remained stable; meningococcal and *N. meningitidis* vaccine showed no response to deworming on immunized, infected groups. RCTs showed moderate to high risk of bias, while observational studies showed low to high bias.

Conclusion (in progress): Cumulatively, the findings suggest that treatment of direct helminth infection before vaccination may help improve responses, though the evidence is inconsistent. However, more robust RCTs are needed to ascertain whether deworming before vaccination may improve responses.

P-2-70
Breadth and specificity of CD4⁺ T-cell responses to MPXV Proteins H3L, A35R, and B6R at the single-peptide level in recovered mpox patients vs. MVA-BN-vaccinated individuals

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Introduction: Mpox is a zoonotic disease caused by the monkeypox virus (MPXV), part of the Orthopoxviridae genus that also includes the vaccinia virus (VACV). To prevent MPXV infection, high-risk individuals are vaccinated with two doses of MVA-BN vaccine. Also, poxviruses show high homology, with the H3L protein sharing 93.5% sequence similarity with its VACV counterpart, and A35R and B6R proteins having 95.0% and 96.5% similarity respectively. However, little is known about T-cell responses and epitope recognition in both vaccinated and mpox-recovered individuals.

Methods: We examined MPXV-specific T-cell responses to glycoproteins H3L, A35R, and B6R. Peripheral blood mononuclear cells (PBMCs) were collected from mpox-recovered patients (n = 10) and MVA-BN double-vaccinated individuals (n = 8). Clinical data, including HIV status, were recorded; 3 of 10 mpox-recovered patients and 2 of 8 vaccinated subjects were HIV-positive. PBMCs were cultured for 10 days with peptide pools corresponding to 81 20-mer peptides covering the H3L, A35R, and B6R proteins. T-cell responses were evaluated using IFN-γ ELISpot assays and intracellular cytokine staining (ICS).

Results: We identified 267 peptide-specific CD4+ T-cell responses across the 18 study participants. In the mpox-recovered group, all participants responded to at least one H3L- and A35R-derived peptide, and 70% responded to B6R-derived peptides. Among vaccinated participants, all responded to H3L, while only 37.5% and 12.5% showed responses to A35R and B6R, respectively. H3L elicited the highest number of IFN-γ responses per participant (median = 5.5), with no significant difference between vaccinated (median = 6.5, range 2–12) and recovered individuals (median = 5, range 1–10). The number of IFN-γ responses towards B6R was significantly higher in recovered individuals (median = 2, range 0–5) than in vaccinated individuals (median = 0, range 0–4). We observed similar trends for A35R. No significant differences in IFN-γ response magnitude were observed between recovered (median H3L: 0.09, A35R: 0.11, B6R: 0.11) and vaccinated individuals (median H3L: 0.08, A35R: 0.12, B6R: 0.06). Overall, we identified 14 H3L and 1 A35R peptide with a response frequency above 30% across both groups. In the recovered group, H3Laa251-270 was the most frequently recognized peptide (70%), while H3Laa221-240 was most recognized in the vaccinated group (50%). No differences in IFN-γ response breadth or magnitude were observed between HIV-positive and HIV-negative individuals.

Conclusion: This study characterizes the T-cell response to MPXV proteins H3L, A35R, and B6R, showing that MVA-BN vaccination induces comparable H3L responses to natural infection. These findings aid in evaluating T-cell immunity and assessing long-term protection from MPXV provided by infection and vaccination.

P-2-71
Corallopyronin A as an example for rational drug product development guided by physiologically based biopharmaceutics modeling in pre-clinical phase using mouse PK/PD relationships

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An early understanding of the relationship between pharmacokinetics (PK) and pharmacodynamics (PD) in drug development helps adapt formulations for an optimized outcome in clinical trials. It is crucial to determine whether a single high dose or multiple small doses per day are more effective for treatment. PD-optimized PK profiles of prototypes have been tested in Phase I clinical trials despite their focus being primarily drug safety. Nonetheless, this information will significantly increase the likelihood of success in proof of concept (POC) trials during Phase II, especially in cases in which the budget allows only one POC study. Animal studies guide dose and formulation selection, but non-mechanistic approaches like conversion tables, based on body surface and weight, create uncertainty due to physiological differences between species. A physiologically based biopharmaceutical model (PBBM) offers a mechanistic understanding, helps selecting formulation and dosage regimen without additional trials.

A mouse PBBM was developed using the software GastroPlus® v9.8.3. Distribution and elimination behavior were determined from *in vivo* i.v. experiments. Further, physicochemical properties were measured *in vitro* and predicted *in silico.* A product particle size distribution related dissolution model for the Corallopyronin A (CorA)-Povidone formulation was implemented using a mechanistic model based on the effect of surface pH on the dissolution rate. Validation of the model was achieved using PK data obtained from *in vivo* oral administration. Simulated plasma concentration-time profiles were generated for all repeated p.o. doses over the entire treatment period of 14 days. Key PK parameters such as area under the curve, time spent above certain concentrations, maximum and trough concentration were extracted.

The relationship between *in vivo* efficacy data for CorA in a rodent mouse model of filarial infection (*Litomosoides sigmodontis*) and the simulated PK parameters were investigated. A repeated single daily dose was shown to increase effectiveness with increasing dose (9, 18, 24, 36 mg/kg), though dividing the dose into twice daily was more effective. Therefore, the maximum concentration was not the decisive parameter. Administering the dose as once (36 or 24 mg/kg), twice (18 or 12 mg/kg) or thrice daily (12 or 8 mg/kg) showed that maintaining a consistent plasma concentration, either via trough concentration or time above a certain threshold, was advantageous for efficacy in the mouse model.

These results emphasize the importance of including a sustained release formulation already in Phase I in order to combine once daily application with more constant plasma concentrations. Additionally, PK and PD data in larger species like rat and dog will enhance the precision of the PBBM for more accurate dose estimates in humans.

P-2-72
Organotypic brain slices as a model to study the neurotropism of the highly pathogenic Nipah and Ebola viruses

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Ebola (EBOV) and Nipah virus (NiV) are highly pathogenic viruses with high case fatality rates. While the brain is known to be one of the main target organs of NiV infection, often resulting in encephalitis, neurological complications during EBOV infection were not frequently observed before the West African outbreak in 2013-2016, where acute or late-onset neurological dysfunctions were noted. Due to the high pathogenicity, limited information is available regarding the infection route, target cells, and immune responses within the CNS.

An *ex vivo* organotypic brain slice model (BS) was established based on either wildtype (wt) or type I interferon knockout ($IFNAR^{-/-}$) adult mice to analyze virus infection and spread in a setting that maintains the neuronal network. The suitability of the model for infection with neurotropic viruses was first assessed under BSL-2 conditions with the Vesicular stomatitis virus (VSV). Next, BS were infected with NiV or EBOV under BSL-4 conditions. Infection was evaluated at different time points by immunofluorescence and *in-situ* hybridization to assess infected cell types and brain areas, as well as by RT-qPCR and $TCID_{50}$ assay to evaluate replication and release of infectious particles. Brain-specific responses to infection were analyzed via multiplex ELISA.

Both NiV and EBOV demonstrated the capacity to infect BS from adult wt as well as $IFNAR^{-/-}$ mice and targeted various cell types. NiV was observed to replicate in BS derived from both mouse strains, yet no release of infectious particles was detected. In contrast, EBOV replication was limited in both BS models. The release of several pro-inflammatory cytokines and chemokines, including eotaxin, IFN-γ, IL-1α, IL-9, IL-17a and KC, was observed in both virus-infected models, suggesting a potential role of the inflammatory response in NiV or EBOV-induced neuropathology. It is noteworthy that the choroid plexus was identified as a highly susceptible target for EBOV and NiV infection, suggesting that the blood-cerebrospinal fluid barrier may serve as a potential entry point for these viruses.

The established models may thus promote the investigation of CNS target cells and entry sites during EBOV and NiV infection as well as of brain specific inflammatory responses. The BS model represents a promising and urgently needed tool to test the efficacy of antiviral compounds in neuronal tissues.

P-2-73
Human iPSC-Derived lung organoid model for respiratory virus research

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Emerging viral diseases represent a global challenge, with the capacity to cause considerable morbidity, mortality, and socio-economic disruption. Developing novel, physiologically

relevant laboratory models for studying emerging viruses is essential for understanding virus-host interactions, disease mechanisms, and fast therapeutic development. Here, we have established a novel human lung organoid system to study Coronavirus and Influenza A virus (IAV) infection.
Human induced pluripotent stem cells (iPSC) were used to derive lung "bud tip" organoids (LO) which at a later developmental stage exhibit branched bronchial and alveolar morphology with proximal-distal cellular patterning of polarized epithelium, congaing goblet, club, and alveolar epithelial type II cells. To increase the complexity of the LOs, we established a LO-microinjection protocol using iPSC-derived macrophages (iMacs) and developed endothelialized LOs incorporating iPSC-derived endothelial cells (iECs). We demonstrated that iMacs engrafted into the alveolar compartments of LOs, adopting an alveolar macrophage phenotype within 7 days. Co-culturing iECs with LOs resulted in the formation of vascularized organoids, with endothelial cells integration into the LOs after 3-4 weeks. The iMac-LO and iEC-LO present platforms for study virus-induced lung epithelial pathology and for assessing the antiviral effects of therapeutics in the context of immune and endothelial cell interactions, which are often overlooked in epithelial mono-cultures.
To evaluate the susceptibility of LOs to viral infection, we tested different conditions of infection. The spread of IAV (H1N1, H5N1, H7N7) in LOs was observed only after injection of the viral inoculum into the organoid lumen, whereas apical virus application led to infection of only the outer cell monolayer and did not result in virus spread. SARS-CoV2 (CoV2) exposure at the apical surface of LOs enabled productive replication, with sgRNA accumulation observed at 6 hpi and viral particles release detected at 12 hpi. Conversely, despite high CD13 mRNA expression - hCoV-229E virus receptor, virus replication was not observed, likely due to low CD13 protein expression on the outer layer of the LOs.

Importantly, our LO platform facilitates the evaluation of antiviral agents. We previously showed the IFNλ-dependent antiviral activity of Cyclosporin A (CsA) against MERS and CoV2 in organotypic human respiratory models and mice. Treatment of CoV2-infected LOs with CsA or its non-immunosuppressive analog, alisporivir, resulted in a significant reduction of viral replication and an elevation in IFNλ1 expression at 24hpi, with IFNλ-dependent response observed at 48 hpi.

Thus, we have developed and characterized a novel, complex human lung organoid infection model, that not only facilitates the study of virus-host interactions, but also provides a standardized system for testing various therapeutic strategies aimed at reducing virus-induced lung injury.

P-2-74
Towards unraveling antimicrobial resistance dynamics: A longitudinal exploration of rectal swab metagenomes
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The increasing prevalence of antimicrobial resistance (AMR) poses significant challenges in clinical settings. In particular, early screening and detection of colonization by multidrug-resistant organisms (MDROs) in patients at admission is crucial. In this context, the clinical use of metagenomics (mNGS) holds promise for fast and untargeted diagnostic methods. Here, we aimed to evaluate the long-term stability of the rectal microbiome and the diagnostic accuracy of mNGS in comparison to culture and whole-genome sequencing (WGS) of MDROs.

We analyzed rectal swabs from 26 patients with two consecutive admissions over a four-year period. The detected antimicrobial resistance genes and assembled metagenomes were compared to those obtained via classical culture-based antimicrobial susceptibility testing and WGS of isolated MDROs.

Our results showed that the rectal microbiome is variable during the two timepoints, with a β-diversity greater in magnitude than what is currently known for the gut microbiome, highlighting the variability in the niche. Nevertheless, we also observed strong co-occurrence of taxa, suggesting that the rectal swab microbiome is also a regulated niche with cooperative biotic interactions. In total, we isolated and sequenced 6 MDROs from 6 patients at individual timepoints. Almost all AMR genes from the genomes of the isolates (median: 100%, range: 84.6-100%) could be detected by mNGS of the rectal swabs. Thus, in patients with positive cultures, we could not detect the isolated MDRO species or associated AMR genes at all screening visits. In addition, we detected AMR genes and pathogenic species in patients with negative cultures.

In conclusion, our study showed that, in principle, mNGS of rectal swabs can detect clinically relevant AMR profiles. However, the cooccurrence of AMR genes and pathogenic species does not always correlate with culture-based diagnostic results but rather indicates a potential risk of horizontal AMR gene transfer. However, it is unclear whether the observed discrepancies are due to transient or locally confined colonization of MDROs, limits of detection, or variability of the sampling method and specimens.

P-2-75
Gut decolonization of multidrug-resistant Escherichia coli clinical isolates via cooperative niche exclusion
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The fight against multi-drug resistant Enterobacterales (MDR-E) has been declared a high priority by the WHO. Colonization of the human gut with MDR-E, including MDR *E. coli,* is associated with an increased risk of infection and dissemination within the community. Several experimental interventions have been explored to promote decolonization of the gut of MDR-E, for example, treatment with antibiotics or fecal microbiota transplantation (FMT). However, both potentially exhibit adverse effects on the gut microbiota (for example diarrhea and loss of colonization resistance). In contrast, the potential of probiotics to selectively decolonize the microbiota of carriers from MDR strains is a promising alternative, specifically, if they achieve their aim without affecting health-promoting commensals. Previous studies showed that closely related commensal Enterobacterales can compete against each other in the murine gut resulting in the displacement of the losing species from the ecosystem. We hypothesize that the human gut is a great resource for such probiotics, which show the potential to selectively decolonize MDR-E.

As a novel resource for identifying potentially probiotic bacteria, a strain collection of Enterobacterales was generated from 630 donors from four cohorts comprising individuals from different age groups and nationalities. As it is of great interest to screen as many strains as possible due

to the high genetic diversity of bacterial isolates, we established an *ex vivo* assay to identify strains with protective properties. Here, we assessed the strain-specific potential of 430 commensal *Escherichia coli* isolates to inhibit the growth of an MDR *E. coli* strain. Comparative analyses in *in vitro*, *ex vivo*, and mouse models revealed that only a subset of commensal strains could promote gut decolonization. Bioinformatic and experimental analysis of the antagonism for representative strains demonstrated the contribution of direct and indirect carbohydrate competition to niche exclusion between *E. coli* strains. Lastly, the combination of a protective *E. coli* strain with a *Klebsiella oxytoca* strain expanded the gut decolonization potential to metabolically diverse MDR *E. coli* strains and additional MDR-E species, demonstrating that rational metabolically complementary design is crucial to developing next-generation probiotics with broad-spectrum activity. Currently, we are assessing the safety profile of the strains that have demonstrated significant decolonization potential using bioinformatics, and phenotypic and functional analyses.

P-2-76

Identification of potentially new anti-infectives against *H. pylori* by repurposing FDA-approved drugs

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Helicobacter pylori shows high resistance rates to the most commonly used antibiotics, metronidazole (25-60%), clarithromycin (15-30%), and levofloxacin (20%) (Bujanda et al. 2021). These high resistance rates, in combination with the role of *H. pylori* in carcinogenesis, represent an adverse constellation that underlines the need for alternative treatment approaches to overcome antibiotic resistance. Our strategy is drug repurposing, which aims to screen drugs already approved by the US Food and Drug Administration (FDA) for new therapeutic applications. Screening these drugs for their ability to hinder the growth of *H. pylori* is a faster and more cost-effective option than conventional drug discovery, as it only takes approximately six years for approval, lowering the costs to nearly one quarter (Nosengo 2016). This project aims to perform drug screening for *H. pylori* bactericidal and bacteriostatic compounds.

We have developed a robust screening platform to identify compounds effective against *H. pylori*. We are using the Prestwick Chemical Library, a collection of 1500 FDA-approved drugs for a first screening. Our initial results have yielded active candidates that are now being further validated. We plan to investigate these active *in vitro* and *in vivo* to uncover a lead candidate that could potentially translate into clinical trials.

Overall, the outcomes of this drug screening and validation process hold the potential to identify new *H. pylori* treatment strategies to mitigate the rising rates of antibiotic resistance.

P-2-77

Rapid Detection and Real-Time Antibiotic Susceptibility Testing of *Klebsiella pneumoniae* and *Yersinia pestis* using Recombinant Reporter Phages

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Infections caused by the highly pathogenic bacterium *Yersinia pestis* are rare, however, the pathogen still pose a major biosecurity risk due to the potential misuse for biological warfare or bioterrorism. In contrast, the massive emergence of multi-drug-resistant (MDR) bacteria, such as *Klebsiella pneumoniae*, constitutes an enormous threat to global health as MDR-associated treatment failure causes high mortality rates in nosocomial infections. In both cases, rapid pathogen detection and antibiotic resistance screening are crucial for successful therapy and thus patient survival. Reporter phage-based diagnostics offer an avenue to expedite pathogen identification and resistance testing. Reporter phages feature integrated reporter genes that enable real-time detection of living target bacteria upon infection. Here, we developed and engineered highly specific reporter phages which produce nanoluciferase (nLuc) as a reporter enzyme upon host infection that enable rapid detection of *K. pneumoniae* or *Y. pestis* cells in clinical matrices within a few hours. At the same time, these reporter phage assays can be utilized in real-time antibiotic susceptibility testing to provide rapid identification of suitable antibiotic treatment options.

P-2-78

PMT-Uro: Using phages in combination with fecal microbiota transfer to access different reservoirs of uropathogens: a DZIF stiped project for women after maternity leave

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This research focuses on the potential of bacteriophages in combination with fecal microbiota transfer (FMT) as a treatment option for recurrent urinary tract infections (rUTI) caused by uropathogenic *E. coli* (UPEC). Urinary tract infections belong to the most common bacterial infections affecting about 150 million people every year worldwide with a high frequency of recurrences and chronicity. Bacteria causing recurrent UTI are able to invade and replicate in epithelial cells of the bladder lumen forming a persisting reservoir undetected by immune surveillance mechanisms and protected from antibiotics. This generates the need to develop new approaches for the treatment and prevention of UTIs, among which phage therapy may represent a promising option (1). In addition of overcoming antibiotic resistance, phages bear the advantage of being effective in biofilm reduction and being able to persist in the mucosa, which partly protects them from washout during urine voiding (2). The gastrointestinal tract is considered the major reservoir of uropathogens in the body, suggesting that decolonization strategies like FMT might prove useful in the prevention of further episodes of UTI. The proposed translational project is destined for studying growth kinetics and antimicrobial activity of *E. coli*phages alone or in combination with antibiotics and/or fecal microbiota transfer (FMT). In the first project phase the most efficient among 6 previously isolated *E. coli* phages will be identified. This will be accomplished by phage stability and activity testing in pooled human urine, antibiotic synergy testing and host range determination. Additionally, the decolonization potential of FMT and phages will be assessed in a human intestinal model. In a third step, treatment strategies and the ability of phages to reach bacteria residing in bladder epithelial cells will be evaluated in a vitro UTI infection model.

Preliminary results indicate that phage activity in urine is phage dependent and generally severely reduced, which underlines the importance of phage effectivity testing in the designated clinical medium. Interestingly, adjusting the urine

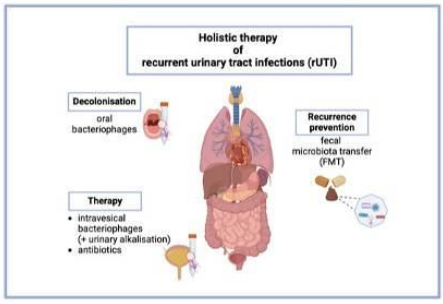
pH restores phage activity, suggesting adjunctive urine alcalisation may be necessary to ensure treatment success.

The findings of this projects can eventually contribute to the development of improved clinical strategies for a holistic therapy of rUTI.

References:

1. Loubet P, Ranfaing J, Dinh A, Dunyach-Remy C, Bernard L, Bruyère F, et al. Alternative Therapeutic Options to Antibiotics for the Treatment of Urinary Tract Infections. Front Microbiol. 2020;11:1509.
2. Blanco C, Chen IA. Phage therapy administered noninvasively could be effective in thin tubes subject to episodic flow despite washout: a simulation study. Phys Biol. 2019;16(5):054001.

Fig. 1



P-2-79

Impact of loss of exclusivity and generic entry on antibiotic consumption rates in German hospitals and out-patient sector

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Introduction: Infections caused by multidrug-resistant pathogens pose a global challenge. The correlation between intensive antibiotic use and the spread of resistance is well-established[1]. Therefore, an appropriate use of antibiotics is mandated by medical guidelines, German infection control laws, and the DART 2030 strategy. However, the financial constraints of the DRG system in hospitals can discourage the use of more expensive, innovative antibiotics in favor of cheaper generics. Data is lacking to investigate a potential impact of antibiotic's loss of exclusivity and subsequent generic entry - usually accompanied with a decrease in price - on consumption rates. For this study, the development of in-patient and out-patient consumption rates were investigated for eleven marketed antibiotic substances with generic entry taking place in Germany between Jan-2010 and Apr-2021. The prescription trends post-patent expiry will be compared with those in six other European countries.

Methods: Epidemiological data analysis of University Hospital Jena included annual retail and hospital consumption data for moxifloxacin, levofloxacin, linezolid, tigecycline, daptomycin, meropenem, imipenem, cefepime, azithromycin, teicoplanin and amikacin from Jan-2005 to Dec-2021. Data was retrieved from IQVIA PharmaScope® und IQVIA DKM®, Retail and Hospital, 12/2022– and analysed by University Hospital Jena as days of treatment per 1,000 overall treatment days (Hospital) respectively per 100,000 inhabitants (Retail). Differences in consumption rate at time of generic entry (GE) and during the time following GE were analysed applying segmented linear regression modelling with a significance level of 0.05.

Results: For hospital consumption data, a significant increase in consumption rates at time of and/or following GE could be determined for daptomycin and azithromycin while consumption rates for imipenem, moxifloxacin i.v., levofloxacin, teicoplanin and amikacin decreased significantly. For linezolid and meropenem, we could determine a significant increase of consumption during the observation period but without observed evidence for a correlation to GE. Further results will be presented.

Conclusions: Based on the data evaluated, there was no observable overall trend in development of antibiotic consumption data following generic entry but the results differed between antibiotic substances. Further analyses are necessary to evaluate a potential impact of Antibiotic Stewardship strategies as well as a potential correlation between increased consumption rates and bacterial resistance development against the respective antibiotics, amongst others.

Based on internal analysis by University Hospital Jena using data from the following source: IQVIA PharmaScope® und IQVIA DKM®, Retail and Hospital, 12/2022–for the period 2005 - 2022 reflecting estimates of real-world activity. Copyright IQVIA. All rights reserved.

[1]Kern WV Dtsch Med Wochenschr. 2018 May;143(9):643-650

P-2-80

Structure- and activity-guided engineering and profiling of anti-Gram-negative darobactins underpin their potential for antibiotic development

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The darobactins are a recently discovered class of antibiotic candidates showing antibacterial activity against a broad range of Gram-negative pathogens. We showcase the establishment of a versatile heterologous expression platform to overproduce these ribosomally produced and post-translationally modified peptides to investigate this pharmaceutically promising compound class and to make darobactins available for further development. The biotechnological approach combined with elucidation of the molecule"s binding mode with the target protein faciliated a structure-activity relationship, enabling comprehensive understanding of the influence of different substitutions at positions 2, 4, 5, 6 and 7 of the heptapeptide for bioactivity. Moreover, diverse *in vitro* and *in vivo* models were utilized to profile darobactins and pave the way for potential clinical studies.

1.

11882. Groß1, F. Panter1, D. Pogorevc1, **C. E. Seyfert**1, S. Deckarm, C. D. Bader, J. Herrmann and R. Müller*: Improved broad-spectrum antibiotics against Gram-negative pathogens via darobactin

biosynthetic pathway engineering **Chem. Sci.** 2021, 12, 11882.

2.

C. E. Seyfert1, C. Porten1, B. Yuan1, S. Deckarm1, F. Panter, C. Bader, J. Coetzee, K. H. M. E. Tehrani, P. G. Higgins, H. Seifert, T. C. Marlovits*, J. Herrmann* and R. Müller*: Darobactins exhibiting superior antibiotic activity by cryo-EM guided biosynthetic engineering **Angew. Chem. Int. Ed.** 2023, 62, e202214094.

3.

16330. **C. E. Seyfert**, A. V. Müller, D. J. Walsh, J. Birkelbach, A. M. Kany, C. Porten, B. Yuan, D. Krug, J. Herrmann, T. C. Marlovits, A. K. H. Hirsch, R. Müller*: New genetically engineered derivatives of antibacterial darobactins underpin their potential for antibiotic development **J. Med. Chem.** 2023, 66, 23, 16330.

4.

C. E. Seyfert, C. Porten and R. Müller*: Darobactin – ein neues Antibiotikum in Entwicklung **BIOspektrum** 2023, 05, 539

P-2-81

Long-term evolution of β-lactam resistance in *Haemophilus influenzae*

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Introduction: *Haemophilus influenzae* is an opportunistic bacterial pathogen that can cause pneumonia, meningitis, and bacteremia. The increased prevalence of ampicillin resistant strains led to the endorsement of β-lactamase inhibitors and third-generation cephalosporins. However, evolutionary pathways towards cross-resistance against these new treatment options are only poorly understood.

Materials/Methods: We established long-term evolution experiments with the reference strain *H. influenzae* Rd KW20 under stepwise increasing concentrations of ampicillin, cefotaxime, and ceftriaxone. For more than 300 clones we performed whole genome sequencing, phylogenomic analysis and determination of minimum inhibitory concentrations (MICs). For selected isolates we further assessed the bacterial fitness and collateral effects against other antibiotics.

Results: Exposure to ampicillin and cefotaxime led to step wise evolution of mutations in *ftsI* (encoding the main target of β-lactam antibiotics) which were associated with increased MICs to all three β-lactams. Evolution under ceftriaxone, however, reproducibly selected different mutations in *ompP2,* coding for an outer membrane protein. *OmpP2* mediated resistance was associated with unstable hetero-resistance. Interestingly, we observed for some resistant clones a reduced MIC to other antibiotics (collateral susceptibility) and variable fitness effects.

Discussion: Our data provide new insights into the evolutionary pathways of β-lactam resistance of *H. influenzae*. We highlight the role of *ompP2* as resistance determinant, and possible mechanisms to induce an unstable hetero-resistant phenotype.

P-2-82

Corallopyronin A: A potential antibiotic against difficult to treat *Staphylococcus spp.* infections

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Multidrug-resistant *Staphylococcus* spp. infections are listed on the WHO Priority Pathogen List for R&D as in the high category of infections that need new antibiotics. Treatment-recalcitrant *Staphylococcus* spp. infections are a special burden not only due to the combination of wide-spread antimicrobial resistant strains, but also due to issues with biofilms, site of infection and device-related infections (e.g., bone or prosthetics/foreign bodies). Corallopyronin A (CorA) is a novel inhibitor of the bacterial DNA-dependent RNA polymerase currently under development to treat filarial nematode infections based on the established targeting of *Wolbachia* symbionts. CorA is also effective against Gram-positive bacteria like *S. aureus* and we showed that the *S. aureus* mutation frequency leading to resistance was lower than previously reported.

Because preliminary experiments showed good bone penetration and activity in biofilms, we proposed in-depth experiments to generate new data needed to extend CorA development to infections caused by biofilms. Thus, we evaluated CorA against laboratory and clinical strains of *S. aureus* and *S. epidermidis* with biofilm-building and antimicrobial resistance phenotypes using complementary *in vitro* models established at the University of Bonn and Helmholtz Centre for Infection Research. Biofilm parameters, including minimal biofilm inhibitory concentration, minimal biofilm-eradication concentration, and spatiotemporal killing kinetics were determined for CorA alone and in combination, and compared to clinically applied antibiotics, e.g., rifampicin and dalbavancin.

The MIC_{90} for 103 strains was 0.5 mg/L for *S. aureus* and 1 mg/L for coagulase negative strains. Interestingly, bactericidal assays (70 strains) showed that CorA was more bacteriostatic against 66% of the *S. aureus* strains tested, while it was bactericidal against 71% of the coagulase negative strains. Microtiter plate-based biofilm assays (crystal violet and MBEC plate) for 14 strains showed that CorA both efficiently inhibits (0.06-4 µg/ml) and eradicates (0.125-2 µg/ml) biofilms; performing equal to- or better than dalbavancin and rifampicin. We will present our recent data on CorA pharmacokinetics and pharmacodynamics in the neutropenic mouse lung and thigh infection models as well as data on *in vivo* biofilm/foreign-body infection model. The results of the project will guide us in selecting the best clinical indication(s) for using CorA as an antibiotic to address the huge health problem of multidrug-resistant staphylococci in biofilms.

P-2-83

Nationwide survey on penicillinallergy delabeling among german healthcare professionals

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Background: Penicillinallergy (PA) is reported by 8-12% of hospital inpatients but on testing more than 90% of these patients will not have a true allergy to penicillin. Use of second-line antibiotics in these patients is associated with increased rates of multidrug-resistant organisms, prolonged and more frequent hospitalisation, worse clinical outcomes as well as increased costs. Multidisciplinary PA-delabeling interventions can remove the majority of these labels and are therefore recommended in national guidelines. We aimed to evaluate the current landscape of PA-delabeling in Germany.

Methods: A nationwide web-based anonymous survey was distributed via professional organizational emails to physicians and hospital pharmacists. Interview questions were designed by a team of infectious diseases specialists consisting of physicians and antimicrobial pharmacists from different teaching hospitals in Germany. The survey consisted of four main parts: demographics, antimicrobial stewardship team, allergy assessment, PA-delabeling.

Results: A total of 611 participated in the survey and 504 responses (49.4% physicians, 50.6% pharmacists) were analysed further. 67% of respondents had an antimicrobial stewardship (AMS) team but access to allergy specialists was just available for 31% on site. 46% of AMS teams were active in PA-delabeling, but only 32% of respondents had access to local guidelines for PA assessment. Whilst a total of 75% reported routine documentation of drug allergy, 44% of physicians and 21% of pharmacists reported an extended assessment of drug allergy history and 56% of physicians and 31% of pharmacists reported following standardized procedures for allergy assessment. 42% of physicians and 26% of pharmacists reported experience with PA-delabeling. Reasons for not performing PA-delabeling were lack of time and availability of clear algorithms as well as lack of experience with the procedures. 80% of respondents would perform PA-delabeling if they had access to a clear algorithm.

Conclusion: Assessment and PA-delabeling is carried out insufficiently by German health care professionals. Clear national guidelines and algorithms are needed to support non-allergy specialists to delabel incorrect PA. These guidelines should take into account distinct requirements for physicians and hospital pharmacists. This data will support our efforts to study and implement these guidelines for Germany.

P-2-84

Development of BamA inhibitors to kill gram-negative pathogens

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Innovative treatment options against the AMR strains of highest concern call for novel drugs that address novel targets. The latter should be specific, essential and easily accessible for the drug. The natural product darobactin was the first small molecule detected that targets and inhibits the outer membrane protein BamA. It arrests the function of the beta-barrel assembly machinery, a protein complex that is required for viability and pathogenesis of specifically Gram-negative bacteria.

We optimized the molecule to increase potency and efficacy. Therefore, a heterologous expression platform was implemented that enables generation of (libraries of) derivatives and the fast activity screening of new variants. Frontrunners with improved antibiotic activity were selected and profiled against clinical isolates of concern. Furthermore, *in vitro* ADME and toxicity tests were done, and *in vivo* mouse models for different indications performed.

The current status in lead optimization and development of a first-in-class antibiotic will be presented.

P-2-85

Why do routine genome-oriented surveillance for multidrug-resistant *Pseudomonas aeruginosa*?

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Question: The German Infection Control Act legally requires hospitals to perform surveillance of multidrug resistant (MDR) microorganisms. However, current diagnostic tools like species identification and antimicrobial resistance testing do not discriminate with sufficient precision between different strains of the same species. We thus evaluated how classical surveillance procedures of MDR *Pseudomonas aeruginosa* (PA) between 2019-2023 were impacted by genome-oriented analysis.

Methods: At the University Hospital of Regensburg, proactive molecular surveillance consisting of whole genome sequencing (WGS) of one isolate of MDR PA per year and patient using a NextSeq device (Illumina) was introduced in 2022. For the years 2019-2021, WGS was done retrospectively. MDR PA is defined in accordance with the German definition of 4-MRGN (resistance to piperacillin, ceftazidim, cefepim, meropenem, imipenem and ciprofloxacin).

Quality control was done using the AQUAMIS pipeline, data analysis with SeqSphere+ (Ridom GmbH, version 9.0.1 2023-04) -including the herein implemented definitions for multilocus sequence typing (MLST) and core-genome (cg) MLST - and tree annotation with iTOL.

Results: Between 2019-2023, 159 predominantly male patients (73.7%) aged an average of 62 years (range: 26-88 years) were diagnosed with MDR PA.

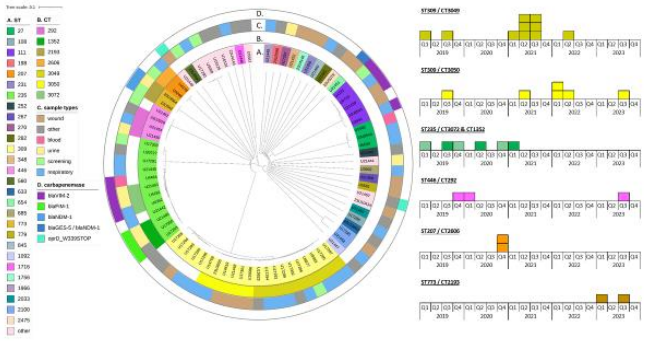
By August 2024, 81 MDR PA mainly cultivated from respiratory materials (n=28) and wounds (n=17), but also from urine (n=9), rectal screening swabs (n=9), blood (n=2) or other sample types (n=16) were sequenced, and passed quality control. Most isolates (n=41, 53.9%) were collected during hospitalization in an intensive care unit (ICU). Three isolates were included for one patient due to morphologic and genetic differences. Further isolates are currently under investigation.

The genetic diversity proved high (42 sequence types [ST]), but the high-risk clones ST309 (n=21), ST235 (n=12) and ST111 (n=5) predominated. The VIM-2 carbapenemase was found in 16 isolates. Using cgMLST, isolates were sub-divided in clusters of 2-10 isolates per complex type [CT]. The largest clusters were assigned to ST309/CT3049 (Cluster 1) and ST309/CT3050 (Cluster 2) (Figure 1).

In Cluster 1 (n=10), isolates differed by up to 14 alleles in pairwise comparison per CT. They were collected between 2019-2021 from four different departments including two ICUs. Isolates of Cluster 2 (n=6), however, differed by <9 alleles, and only spread within one department. Environmental investigations identified the same strain in sinks at this department. Due to missing carbapenemase and long periods between isolates, the outbreak could only be detected due to genome-oriented surveillance. Outbreak management is still ongoing in August 2024.

Conclusions: The genome-oriented surveillance of MDR PA revealed several outbreaks of MDR PA, which were overlooked during classic surveillance due to insufficient discriminatory power of routine microbiological diagnostics.

Fig. 1



P-2-86

Multi-omics techniques deliver highly accurate diagnostic gene expression signatures for precision medicine in extrapulmonary tuberculosis

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Objectives: With an estimated 10 million new infections worldwide in 2023, tuberculosis (TB) remains a leading cause of mortality and morbidity among infectious agents. Globally, approximately 20 % of diagnosed cases are extrapulmonary TB (EPTB), with an increasing incidence in several European countries. While there is a plethora of research data on pulmonary TB (PTB), knowledge on EPTB remains scarce. Diagnosis and assessment of therapeutic success are particularly challenging. Blood-based diagnostics are urgently needed with whole-blood gene expression signatures providing an elegant approach for clinical use.

Methods: Within the Cologne DZIF EX-TB cohort we collected baseline and longitudinal data including information on the diagnostic process and clinical parameters. A broad range of state-of-the-art OMICS methods (bulk and single cell RNAseq, multicolor flow cytometry and cytokine profiling) were employed in 29 patients to deeply characterize immunotypes of EPTB. Bioinformatic tools were applied to generate highly accurate diagnostic gene expression signature.

Results: Whole blood bulk and scRNA sequencing as well as multicolor flow cytometry (40 color) lead to the detection of three immunotypes (mild, intermediate and severe) of EPTB each characterized by a distinct immunological program. Here, type I and II interferon and IL-1β signaling represent the main contributing pathways for EPTB pathogenicity. Using an advanced bioinformatic approach we developed a novel EPTB signature (15 gene core-EPTB signature) for diagnostic purposes with significantly advanced performance compared to previously published TB signatures. Moreover, the core-EPTB signature outperformed competitor signatures in differentiating a large range of other diseases from TB and lastly, showed significantly better performance in detecting all forms of TB (PTB and EPTB).

Conclusion/Outlook: EPTB presents with highly heterogeneous disease manifestations. Whole blood RNAseq revealed distinct immunotypes of EPTB disease. Exploiting these highly heterogenous disease manifestations allowed us to generate the most accurate whole blood based diagnostic gene expression signature described so far. Next, we are aiming to perform longitudinal analysis within the novel DZIF-funded multicentric EX-TB cohort to characterize EPTB treatment responses and comprehensively analyze the underlying immune responses. Patent applications have been submitted for exploitation of our findings in future clinical applications.

P-2-87

A highly selective inhibitor of the 1-deoxy-D-xylulose 5-phosphate synthase with good oral bioavailability and activity against MDR tuberculosis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading infectious disease, claiming 1.3 million lives and resulting in 10 million new active cases each year. Unfortunately, progress to eradicate TB has stalled partly due to the emergence of Multidrug-Resistant (MDR-TB) and Extensively Drug-Resistant (XDR-TB) tuberculosis, which do not respond to the standard treatment regimen.

At the DZIF TTU-TB high-throughput screening unit in Cologne we lately screened a library of 10,000 synthetic small molecules and identified a promising hit candidate named MJ-8. The compound demonstrated strong inhibitory activity against laboratory strains with an MIC90 of 2.52±0.73 µg/mL as well as MDR-TB clinical isolates, with a MIC90 as low as 0.25 µg/mL. Panel testing against other mycobacteria, Gram-positive and Gram-negative bacteria, indicated that MJ-8 is specific for mycobacteria. Interestingly, MJ-8 showed activity against *M. abscessus,* a clinically relevant multi-drug resistant non-tuberculous mycobacterium. Cytotoxicity in HepG2 cell lines revealed a selectivity index (SI) of 12.07±1.95, no cytotoxicity was observed in THP-1 cell line. In subsequent intracellular activity studies using human-derived CD14+ PBMCs, MJ-8 was able to limit growth of intracellular MTB. Target identification studies, by generating spontaneous resistant mutants, revealed that resistance was associated with the 1-deoxy-D-xylulose 5-phosphate synthase (DXPS), the first enzyme in the MEP pathway. The pathway, which is absent in human cells, produces several essential downstream products such as vitamin B1 or

terpene precursors. Resistant mutations were either in the promoter of the *dxs1* (rv2682c) gene, leading to a significant upregulation, or within a conserved mycobacterial motif in the binding pocket of the cofactor thiamine diphosphate (ThDP). Targeted recombineering of the conserved pattern region confirmed that MJ-8 targets *dxs1*. The mutation in this highly conserved motif may explain the compound"s selectivity against mycobacteria. Given its unique mode of action and potent intracellular activity, oral bioavailability becomes a critical factor, especially for anti-TB drugs which need to be applied for several months. Poor bioavailability can result in bloodstream concentrations below therapeutic levels and therefore hinder the progression of the drug candidate. In *in vivo* pharmacokinetics studies in mice, after oral administration of 10 mg/kg MJ-8, bloodstream concentrations exceeded the MIC for the duration of 4h, with a Cmax of 19.65 µg/mL. Taking all the data into account, MJ-8 represents a potential clinical candidate for which we are now planning to conduct efficacy studies in mice.

P-2-88

Alarming rates of bedaquiline resistance in drug-resistant tuberculosis samples from Mozambique

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Introduction: Bedaquiline (BDQ) is a key component of the World Health Organization endorsed 6-month BPaLM regimen (BDQ, pretomanid, linezolid and moxifloxacin) for treatment of patients with rifampicin resistant (RR) or multi-drug-resistant (MDR, isoniazid and rifampicin resistance [INHr, RR]) tuberculosis (TB). Recent data from Mozambique indicated an increase of BDQ resistance (BDQr) from 3% to 14% from 2016-2021, suggesting that current RR/MDR-TB treatment regimens are not able to prevent BDQr development at population level (1).

Methods: To study current resistance levels, we performed targeted Next-Generation-Sequencing using Deeplex®-Myc-TB from GenoLyse® DNAs extracted from 255 INHr and/or RR (classified by Bruker-Hain GenoTypeMTBDR*plus*) clinical and cultured samples submitted to the National Tuberculosis Reference Laboratory in Maputo between January/2021 and June/2024.

Results: 222 samples were classified RR, 194 at least MDR/RR, 60 fluoroquinolone resistant (24%, FQr), 16 pre-XDR (6%, MDR+FQr), and 34 XDR, (13%, MDR+FQr+BDQr). 72 were BDQr (28%): two INHr, one FQr+BDQr, one INHr+FQr+BDQr, one RR+BDQr, four RR+FQr+BDQr, 29 MDR+BDQr, and 34 XDR. Ten samples (4%) had the *rpoB* I491F mutation as sole RR marker (two MDR, five MDR+BDQ, one preXDR, two XDR). Alarmingly, these samples were classified rifampicin susceptible by GenoTypeMTBDR*plus.* **Conclusions:** Increasing BDQr and FQr rates found are alarming and potentially jeopardize DR-TB control in the country. BDQr is observed in a broad spectrum of resistance combinations. "Diagnostic escape" *Mycobacterium tuberculosis* strains with *rpoB* I491F RR mutation, not detected by commercial molecular drug resistance assays such as Xpert® MTB/RIF and GenoTypeMTBDR*plus*, cause an additional challenge for the current DR TB test algorithms, and underline the urgent need for implementation of rapid comprehensive resistance testing in country.

1. Barilar I, Fernando T, Utpatel C, Abujate C, Madeira CM, José B, et al. Emergence of bedaquiline-resistant tuberculosis and of multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis strains with rpoB Ile491Phe mutation not detected by Xpert MTB/RIF in Mozambique: a retrospective observational study. Lancet Infect Dis. 2024 Mar;24(3):297–307.

P-2-89

Antiinfektivakurs Nord – ein interdisziplinärer jährlicher multizentrischer Online-Kurs vermittelt zentrale Inhalte zu Antibiotic Stewardship und erhöhte das Zutrauen von Teilnehmenden, Antibiotika rational einzusetzen

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Die Anwendung von Antibiotika ist eine zentrale Kompetenz für medizinisches Personal wie Ärzt:innen und Apotheker:innen. Die rationale Anwendung von Antibiotika, wie sie in Antibiotic Stewardship Programmen gelehrt wird, ist von zentraler Bedeutung, um Antibiotikaresistenz zu reduzieren und die Behandlung der individuellen Patient:innen zu verbessern. Viele Kolleg:innen haben jedoch wenig Zutrauen in Ihre Fähigkeiten, Antibiotika rational einzusetzen. Wir beschreiben hier die Effekte eines interdisziplinären, jährlich stattfindenden multizentrischen Online-Kurses, der zentrale Inhalte zu Antibiotic Stewardship vermittelt. Der Kursus war in acht thematische Einheiten unterteilt und fand in den Jahren 2023 und 2024 statt. Teilnehmende wurden um eine Selbsteinschätzung gebeten, ihr Wissen zur rationalen Antibiotikaverwendung vor und nach dem Kurs zu beurteilen. Die durchschnittlich 95 Teilnehmenden aus vier verschiedenen Krankenhäusern berichteten eine deutliche Zunahme im Zutrauen, Antibiotika rational einzusetzen.

P-2-90

Introducing Murein endopeptidases as novel targets to fight multidrug-resistant *Pseudomonas aeruginosa* – function and inhibition

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Nosocomial infections with multidrug-resistant (MDR) Gram-negative bacteria are an increasing threat today. Novel approaches to fight Gram-negative pathogens are highly needed. However, original compounds against novel innovative targets remain scarce. We recently identified in a Transposon-insertion directed sequencing approach [1] that

four Mep genes encoding, namely *mepM1*, *mepM2*, *mepM3*, and *mepH2* are candidates contributing to b-lactam resistance in a MDR *Pa* bloodstream isolate. Gram-negative bacteria possess an outer membrane (OM) and inner membrane (IM), and separated by the periplasm, which contains the peptidoglycan (murein) sacculus. The sacculus determines cell shape and stability and consists of short interconnected glycan chains arranged perpendicular along the long axis of the cells. The glycan building blocks are disaccharide units with muropeptide side chains which are crosslinked to each other. Assembly of novel glycan building blocks into the sacculus requires Murein endopeptidases (Mep) which cleave the crosslinks. It was shown in *E. coli* (*Ec*) that three such hydrolases (*Ec*MepM (YebA), *Ec*MepH and *Ec*MepS) are important for the assembly of murein building blocks into the cell wall and consequently also for growth of bacteria. The three putative MepM proteins identified in our screen, namely *Pa*MepM1, *Pa*MepM2, *Pa*MepM3, are phylogenetically highly related to *Ec*MepM and we could validate that *Pa*MepMs in fact do contribute to b-lactam resistance. Also, we found a reduction of biofilm formation and swarming motility, a substantial change in cell morphology and reduced membrane integrity and an increased sensitivity against high salt concentrations. Hence, murein endopeptidases are promising targets to develop novel drugs as antivirulence agents and to sensitize MDR pathogens to b-lactam treatment. As the active sites of *Pa*MepM1, *Pa*MepM2 and to lesser extent *Pa*MepM3 are highly homologous, we performed a virtual screen to identify inhibitors targeting both *Pa*MepM1 and *Pa*MepM2. For inhibitor testing, we developed an *in vitro* MepM activity assay. This assay uses a quenched fluorescence minimal endopeptidase substrate, resembling the muropeptide crosslinks. With this assay, we validated MepM inhibitors identified *in silico* and could show that it is indeed possible to simultaneously target both *Pa*MepM1 and *Pa*MepM2. These promising results open up a novel route to combat MDR *P. aeruginosa*.

1. Sonnabend, M.S., et al., *Identification of Drug Resistance Determinants in a Clinical Isolate of Pseudomonas aeruginosa by High-Density Transposon Mutagenesis.* Antimicrob Agents Chemother, 2020. **64**(3).

P-2-91

Pathogen and host determinants of extrapulmonary tuberculosis among 1035 patients in Frankfurt am Main, Germany, 2008-2023

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Background: Extrapulmonary tuberculosis (EPTB) presents with nonspecific symptoms which can pose a significant diagnostic challenge. Various factors, including age, sex, and HIV status, have been associated with an increased risk of developing EPTB. However, the influence of the lineage of the infecting *Mycobacterium tuberculosis* complex (Mtbc) strain remains controversial.

Methods: Between 2008 and 2023, comprehensive clinical data from 1035 cases, along with whole genome sequencing (WGS) data of the respective Mtbcstrains have been collected. To examine the association between Mtbc lineage, sex, region of birth, HIV infection status, diabetes and EPTB, we calculated crude and adjusted odds ratios (OR) using logistic regression.

Results: Of the 1035 patients, 272 had exclusively extrapulmonary disease and 138 had both pulmonary and extrapulmonary disease. Patients infected with a lineage 1 strain had the highest odds of developing EPTB in the univariate analysis (OR: 3.30, 95% CI: 1.97-5.49). However, Mtbc lineage was not a significant predictor in the multivariable model, while the odds of developing extrapulmonary disease were higher among patients born in the South-East Asian region (adjusted OR: 6.48, 95% CI: 3.72-11.43) and the Eastern Mediterranean Region (adjusted OR: 5.54, 95% CI: 3.41-9.11) compared to those born in the European region.

Conclusion: While infection with a strain of lineage 1 was associated with higher rates of EPTB, our results demonstrate that host factors, such as geographic origin and sex are stronger predictors than infection with a Mtbc strain of a particular lineage alone. Further investigation of this host-pathogen interaction is needed.

Fig. 1

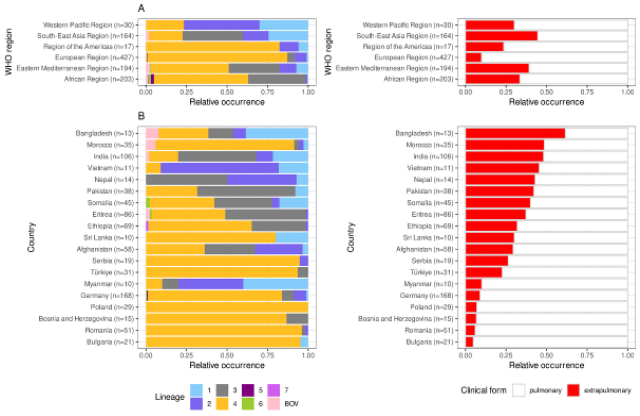
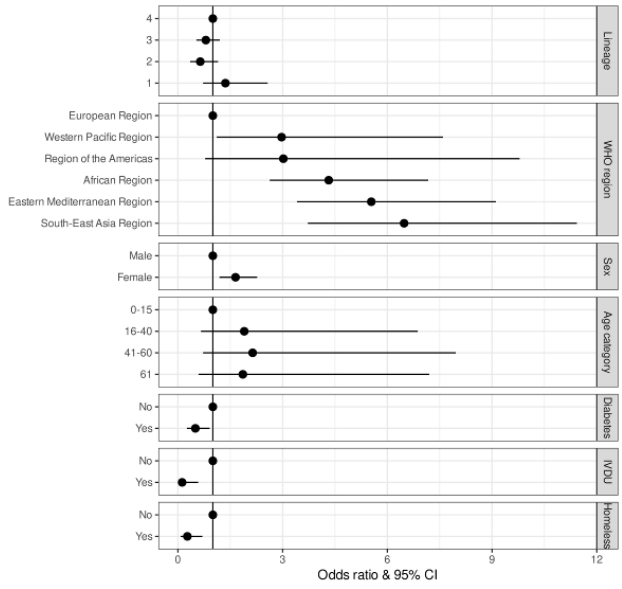


Fig. 2



P-2-92

Comprehensive evaluation of genomic antibiotic resistance prediction in Tuberculosis using whole genome sequencing for routine diagnostics in Germany

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Introduction: Phenotypic drug susceptibility testing (pDST) remains the gold standard for evaluating drug resistance in Germany, despite limitations. Since 2020, the National Reference Center (NRC) for Mycobacteria has integrated whole genome sequencing (WGS) for Mycobacterium tuberculosis complex (Mtbc) strains within the Robert Koch Institute (RKI) coordinated IMS-TB project. However, the potential for WGS-based resistance prediction to replace pDST in low-incidence settings remains uncertain.

Goals This study evaluates the efficacy of WGS-based resistance prediction using an advanced interpretation database, within routine diagnostics in a low burden setting.

Materials & Method: Between 2020 and 2023, over 4,400 isolates underwent pDST at the NRC using the proportion method in the MGIT system. As part of the IMS-TB project, each Mtbc strain was analyzed using next-generation sequencing (NGS). Resistance-causing variants were identified using a comprehensive inhouse database to predict resistance to first-line and Group A second-line drugs (fluoroquinolones (FQ), bedaquiline (BDQ), and linezolid (LZD)). The results were compared with pDST findings.

Results: For 4,401 isolates, a complete phenotypic and genotypic first-line resistance profile was generated. Among these, 3,205 isolates (72.8%) were fully susceptible to first-line drugs. NGS predictions demonstrated sensitivities above 95% for each drug except pyrazinamide, with a sensitivity of 87.9% (CI 83.7–91.4%). Specificities exceeded 97% for all four first-line drugs, with the negative predictive value above 99%. Group A drug analysis included 481 full datasets, comprising 109 resistant isolates for FQ, 13 for BDQ, and 7 for LZD. High sensitivity and specificity were observed for FQ resistance detection (moxifloxacin 93.6% [CI 87.2–97.4%], levofloxacin 96.0% [CI 90.1–98.9%]) and BDQ (84.6% [CI 54.6% –98.1%]), although BDQ and LZD results require individual evaluation due to limited cases.

Summary: NGS-based genomic DST reliably predicts first-line susceptibility. Standard sequencing of all culture-positive isolates may replace phenotypic testing if technical and quality management standards are met.

P-2-93

Glycemic status and therapy with Metformin impact long-term pulmonary outcome of tuberculosis patients

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The interplay of diabetes mellitus (DM) and tuberculosis (TB) is complex and increasingly important to understand as anti-DM drugs are being evaluated as host-directed TB therapies. However, the influence of glycemic profile, DM and DM-treatment status on pulmonary recovery and long-term outcomes is currently unknown.

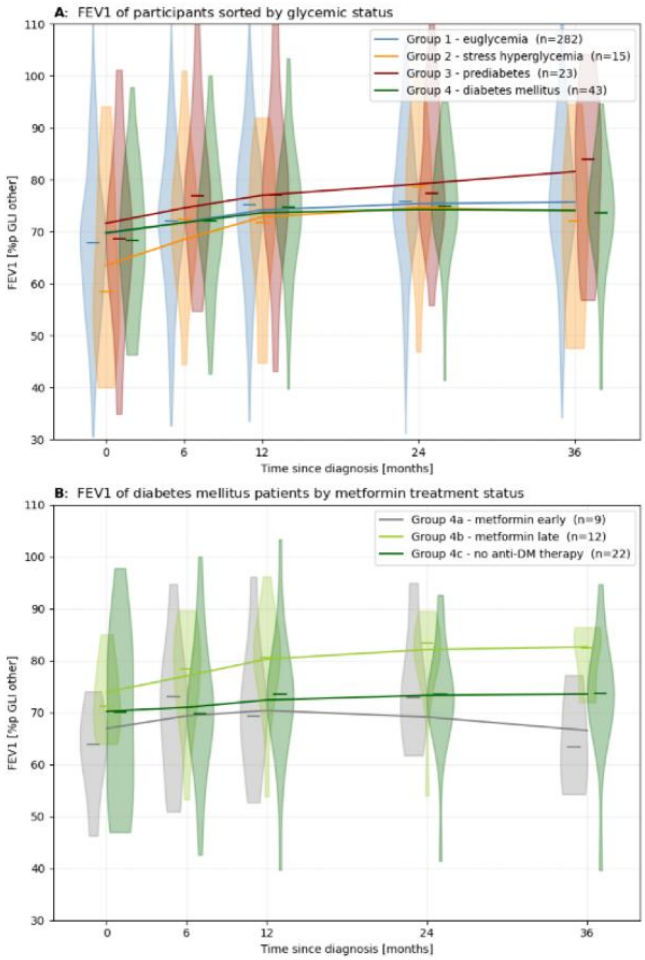
Microbiologically confirmed, drug-susceptible pulmonary TB patients were included upon diagnosis as part of a TB Sequel cohort sub-study. A maximal follow-up of 36 months included glycemic profile monitoring (fasting blood glucose (FBG), HbA1c) and spirometry (results expressed as means of %predicted FEV1±SD/FVC±SD according to GLI 2012 "other" category and as mean absolute change in ml/month).

363 patients met eligibility and data completeness criteria for the following groups: *euglycemia* (HbA1c <5.6%, FBG <5.7mmol/l, n=282), *stress hyperglycemia* (baseline FBG ≥7mmol/l, later normalizing, n=15), *prediabetes* (HbA1c 5.60-6.49%, n=23) and *diabetes* (n=43, subdivided into: *early metformin,* established before study entry (n=9); *late metformin,* established until month 6 (n=12); *no anti-DM therapy* with FBG ≥7mmol/l, HbA1c ≥6.5% (n=22)). Overall spirometry at TB diagnosis was most impaired within *stress hyperglycemia* (FEV1: 63.35±16.96), figure A. *Prediabetes* and *diabetes+late metformin* exhibited the least spirometry impairment at TB diagnosis and long-term (month 36-FEV1: 81.03±15.14 and 80.41±6.5). *Stress hyperglycemia* improved the most within 12 months (+29ml FEV1/month) but did not improve to the level of any other group. *Diabetes+early metformin* was associated with more spirometry impairment compared to other diabetes subgroups and was the only subgroup to show FEV1-decline after month 24 (-17ml FEV1/month), figure B.

Manifest DM did not negatively affect overall spirometry results in TB patients. Prediabetes was associated with generally improved spirometry results. Lung function in TB patients with DM only benefitted from metformin when started with/after TB therapy. An established metformin therapy prior to initation of TB therpy was associated with baseline spirometry impairment and FEV1 deterioration after month 24.

Figure 1. FEV1% change over time, starting from initiation of TB therapy. **A** - Main groups: *euglycemia* (n=282), *stress hyperglycemia* (n=15), *prediabetes* (n=23) and *diabetes* (n=43). **B** - Subgroups: *early metformin* (n=9), *late metformin* (n=12), *no anti-DM therapy* with FBG ≥7mmol/l, HbA1c ≥6.5% (n=22). Violins show distribution within group. (**-**) show group means.

Fig. 1



P-2-94

DZIF EX-TB study: Challenges during treatment of extrapulmonary tuberculosis and treatment response assessment

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Background: Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide with 1.3 million deaths in 2022. Pulmonary involvement is more common; however, extrapulmonary TB (EPTB) accounts for approximately 20% of TB cases worldwide. The diagnosis of EPTB is complicated by the wide variability in symptoms based on the organ affected.

Methods: Within a prospective cohort study (Cologne EX-TB study), we examined 44 EPTB patients treated at the University Hospital of Cologne, Germany, before and throughout treatment from 2018 to 2023.

Results: The Cologne EX-TB cohort comprised 44 patients with a median age of 35 years, originating from 21 countries. 19/44 (43%) patients were classified as having disseminated disease. Paradoxical reactions (PR) occurred in 31% of the patients who were followed up (13/42). Treatment duration in patients with a PR was significantly longer (median 15 months) compared to patients without PR (median 9 months).

A previously published clinical scoring system assessing EPTB treatment responses showed a favorable treatment outcome in 68% of patients only (21/31).

Conclusion and outlook: EPTB exhibits highly variable disease severity. Management of therapy is further complicated by the frequent occurrence of PR, which can lead to treatment durations exceeding standard recommendations. Further understanding of risk factors for PR is needed to anticipate worsening of symptoms. Clinical scores developed in high-incidence settings may not be reliably applicable for patients in low-incidence and high-resource countries. This highlights the need for alternative biomarkers in EPTB to potentially shorten treatment regimens with a high burden of side effects. Further data are expected from the multicentre mEX-TB study located at 6 DZIF sites, initiated in 2023.

References:

1. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.

P-2-95

Fine-tuning preclinical animal models for providing proof-of-concept for targeted click-to-release activated antibacterials

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The use of colistin as a last-resort antibacterial in case of antimicrobial resistance has become inevitable. Often, high doses are necessary to confer an effect. This results in potential kidney toxicity as adverse side effect.[1,2] We aimed to explore local chemically-triggered activation of colistin derivatives by using targeted click-to-release reactions bearing in mind that local activation of colistin might reduce systemic toxicity. For antibacterial drug development, typically classic PK-PD models such as the neutropenic thigh infection model are used. However, these models cannot demonstrate an effect for these novel chemical entities. Thus, novel methods are needed to advance systemically administered compounds that are specifically activated at the infection site in preclinical development.

For the neutropenic thigh infection model, it is known that bacteria can disseminate from the primary infection site, i.e. thigh, toward e.g. lung tissue.[3] To demonstrate that the

concept of targeted click-to-release also has potential for clinical applications, we decided to make use of the secondary infection site and use this classical model with small adjustments as a surrogate. Therefore, we administered a tetrazine derivative, D-Ubi-Tz [4,5], intended for triggering release by click chemistry, locally via nebulization into the lung. By contrast, the colistin-conjugate, Col-$(TCO\text{-}Asp)_1$ was administered intravenously, i.e. directly into the system. Colistin itself served as a control. Bacterial burden was then determined in thigh and in lung tissue.

Our results show that colistin reduced bacterial burden back to baseline in thigh and in lung tissue. Col-$(TCO\text{-}Asp)_1$ and D-Ubi-Tz alone did neither impact bacterial burden in thigh or lung tissue. This demonstrated that both did not have any residual activity. However, combination of nebulized D-Ubi-Tz with systemically administered Col-$(TCO\text{-}Asp)_1$ showed a reduction of bacterial burden in the lung, in a similar range as colistin, whereas no effect was seen on bacterial burden in thigh tissue. This demonstrated that Col-$(TCO\text{-}Asp)_1$ was locally activated with D-Ubi-Tz.[5]

In summary, our study provides the first in vivo proof-of-concept of locally activated targeted click-to-release antibacterials. Future studies need to investigate in detail the dimension of reduced toxicity of local activation. Finally, our study shows that smaller adjustments of a classical PK-PD model serve as a good surrogate model for non-classical, novel antibacterials.

[1] Poirel et al., Clin Microbiol Rev. 2017

[2] Chien et al., J Antimicrob Agents 2020

[3] Rox, Sci Rep 2022

[4] Tegge et al., Angew Chem Int Ed 2021

[5] Charoenpattarapreeda et al., Angew Chem Int Ed 2024

P-2-96

Use of hepatoprotective drugs as an adjunct to anti-tuberculosis treatment in Europe – a TBnet survey

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Backround: Several drugs used to treat tuberculosis can be associated with hepatotoxicity. In this context, several hepatoprotective agents have been proposed to mitigate liver damage and thus represent potentially promising substances to minimise the adverse effects of anti-tuberculosis drugs and improve overall treatment success. While the benefits of hepatoprotective drugs are controversial, there is little information on whether and how often they are currently used in Europe.

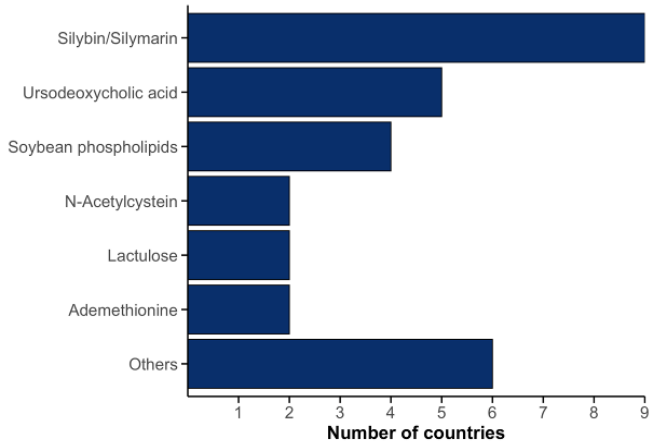
Methods: Between November 2023 and May 2024, we conducted a standardised questionnaire survey on the use of hepatoprotective drugs in patients receiving antimicrobial treatment for tuberculosis among representatives of the Tuberculosis Network European Trials Group (TBnet) in 41 of the 53 countries in the World Health Organization (WHO) European Region. Andorra, Azerbaijan, Bosnia-Herzegovina, Israel, Kazakhstan, Kyrgyzstan, Monaco, Montenegro, San Marino, Tajikistan, Turkmenistan and Uzbekistan were excluded because of their small populations or lack of contacts.

Results: Responses were received from representatives of 37 of the 41 countries contacted (90.2%). Of these, only Slovenia was able to provide national data on the incidence of hepatotoxicity in patients receiving anti-tuberculosis treatment in 2020, reporting an incidence of 8%. Among the countries without national data that estimated the incidence of hepatotoxicity for their country, the median estimated incidence was 8.0% (IQR: 8.0 - 15.5%; n=22 respondents). A total of 15 countries (40.5%) reported the regular use of hepatoprotective drugs in patients receiving standard anti-tuberculosis therapy (Figure 1). Almost half of these countries (n=7) belong to the former Soviet Union. In five countries, recommendations to use hepatoprotective drugs as an adjunct to anti-tuberculosis treatment were included in national guidelines. The most commonly used hepatoprotective drugs were silibin/silymarin (n=9), ursodeoxycholic acid (n=5) and soy phospholipids (n=4) (Figure 2). The regular duration of treatment with hepatoprotective drugs ranged from less than one month (56.25%, n=9), one to three months (18.7%, n=3) and four to six months (18.7%, n=3). Eleven countries reported financial coverage for these drugs, but in all but one country only for inpatients. The median average monthly cost of outpatient treatment in the eight countries providing this information was 10€ (IQR: 9-15€).

Conclusion: Hepatoprotective drugs are widely used in the WHO European Region, particularly in the countries of the former Soviet Union, as an adjunct to anti-tuberculosis therapy, and some countries have included them in their national policies and guidelines. However, there is a considerable need for further research into the potential role of various hepatoprotective drugs as an adjunct to anti-tuberculosis treatment in reducing liver toxicity and improving treatment outcomes.

Fig. 1

Fig. 2



P-2-97

UNMASK-TB: Face mask sampling for the detection of *Mycobacterium tuberculosis* in children and adults

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Background: Diagnosing tuberculosis (TB) remains a major challenge because diagnostic methods are often invasive, time-consuming and inaccessible in many regions. To overcome these limitations, there is an urgent need for non-invasive, rapid and cost-effective diagnostic tools. This study evaluates face mask sampling as a simple non-invasive method for detecting *Mycobacterium tuberculosis* DNA in adults and children. The research is being conducted in Chisinau, Moldova, where rates of rifampicin-resistant and multidrug-resistant TB (RR/MDR-TB) are particularly high, to determine whether this approach can also detect rifampicin resistance - a critical area where no data exist. We report preliminary data from this ongoing study.

Methods: In the current study, adult patients with suspected pulmonary TB are recruited from the Chiril Draganiuc Institute of Phthisiopneumology in Chisinau, Moldova, while children with suspected pulmonary TB are recruited from the paediatric ward of the Chisinau Municipal TB Hospital. Each participant is provided with three face masks, each containing four polyvinyl alcohol (PVA) strips, to wear for 30 minutes, with a 30-minute interval between mask applications (Figure 1). The face masks are then analysed at the National Reference Laboratory for TB in Chisinau, Moldova. First, PVA strips removed from the face masks and dissolved in sterile water using a sample rotator. Then, 2ml of the PVA solution are transferred directly to a GeneXpert MTB/RIF Ultra cartridge (Cepheid, USA).

Results: To date, 44 adult patients with suspected pulmonary TB have been enrolled in the study. Of these, 38 were confirmed to have TB disease by positive sputum smear microscopy and positive GeneXpert MTB/RIF (Figure 2). Of these confirmed cases, 16 (42%) had at least one face mask that tested positive for *M. tuberculosis* using GeneXpert MTB/RIF. In addition, of the six patients identified with rifampicin resistance detected in their sputum samples, all four patients with a positive GeneXpert MTB/RIF result in their face mask samples also had rifampicin resistance detected in their face mask. Six children with suspected pulmonary tuberculosis were also included in the study. All of them tested negative for *M. tuberculosis* in both sputum samples and face mask samples using GeneXpert MTB/RIF.

Conclusions: Preliminary results suggest that detection of M. tuberculosis DNA using GeneXpert MTB/RIF on exhaled breath collected by face mask sampling is possible in almost half of adult patients. Rifampicin resistance may also be reliably detected in face masks. However, diagnosis of TB in children remains a challenge and further research is needed to determine the utility of face mask samples in a larger paediatric cohort.

Fig. 1

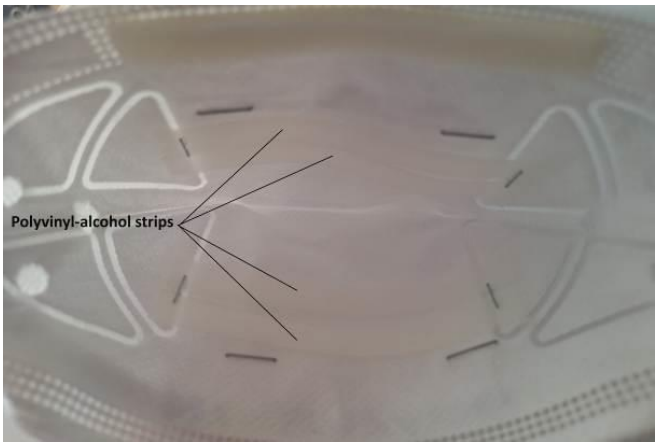
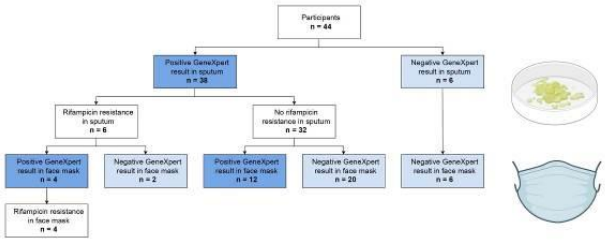


Fig. 2



P-2-98

Multiparameter optimization of pathoblockers targeting elastase (LasB) for the treatment of Pseudomonas aeruginosa lung infections

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Infections caused by *Pseudomonas aeruginosa* are becoming increasingly difficult to treat due to the rise of antimicrobial resistance. This poses a particular threat to

patients suffering from *e.g.* hospital-acquired or ventilator-associated pneumonia (HAP/VAP), cystic fibrosis (CF) or non-cystic fibrosis bronchiectasis (NCFB). To develop novel, non-traditional treatments targeting *P. aeruginosa* virulence, the secreted protease elastase (LasB) represents a prime target due to its key role in bacterial virulence and its extracellular localization. [1,2]

We have recently discovered a potent and selective phosphonate-based scaffold of LasB inhibitors, which we are optimizing towards novel treatment options for lung infections. [3,4] In the frame of this medicinal chemistry optimization campaign, we employ an in house screening cascade for *in vitro* ADMET properties, such as metabolic stability, Calu-3 permeability, plasma protein binding and cytotoxicity. Applying this multiparameter approach together with *in vivo* pharmacokinetic profiling, we rationalized compound properties that lead to favorable bioavailability in the lung after both inhalative and systemic administration. These findings could be translated into *in vivo* efficacy in combination with standard-of-care antibiotics in murine *in vivo* infection models.

[1] Luyt, C.-E., et al. (2018) Curr. Opin. Crit. Care. 24(5), 332-338. [2] Everett, M. J., Davies, D. T. (2021) Drug Discov. Today. 26(9), 2108-2123. [3] Konstantinovic et al. (2023) ACS Cent. Sci. 9(12):2205-2215. [4] WO 2022/043322 A1.

P-2-99

Therapeutic drug monitoring of long acting Rilpivirine and Cabotegravir for treatment of HIV-1 infection – A case series of five patients with one virologic failure after development of two-class resistance

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Question: Phase III trials have shown good virological efficacy of Long-acting (LA) rilpivirine/cabotegravir, with incidence rates of virological failure (VF) of 1.2% (n=19/1651) and 1.4% (n=23/1651) after 48 and 152 weeks, respectively. Data from real-world cohorts have confirmed these findings with similar risk of VF (1.8%, 2.0% and 1.9%, respectively). There remains an ongoing debate if and when to implement routine Therapeutic drug monitoring (TDM) in at-risk-patients to prevent VF. As TDM data of patients with VF in real-world settings are scarce we aim to contribute to this knowledge gap with the presentation of regular TDM data from a case series of 5 patients on LA RPV/CAB including one experiencing VF.

Methods: Plasma samples from viral load monitoring from the five patients since their switch to LA RPV/CAB were stored at -20°C. All available samples which were collected directly before the injection of LA RPV/CAB, were used to retrospectively investigate drug trough-levels using a validated high-performance liquid chromatography (HPLC)-based method. Drug trough-levels were interpreted using different thresholds, that were recently discussed by Thoueille (2024), including protein adjusted concentration for 90% viral replication (PAIC90) and two and four times this respective value.

Results: Two patients (P4 and P5) showed a rebound of viral load at week 30 post-treatment switch coinciding with an acute SARS-CoV 2 infection. Retrospective TDM at week 30 showed worrisome low drug levels for one of them (CAB: 361 ng/ml and RPV: 16 ng/ml). Unfortunately, patient 4's week 30 plasma sample volume did not allow for retrospective TDM. When patient 4 developed confirmed VF at week 64, retrospective TDM showed values above the 4 x PAIC90 threshold for both RPV and CAB, while other cohort patients showed trough levels below 4 x PAIC90 4 x PAIC90 for both RPV (P1) and CAB (P5) without any signs of VF.

Conclusions: Even if the frequency of VF during ART using LA RPV/CAB appears to be low, the risk of emerging 2-class resistance should not be underestimated, as treatment options are severely limited thereafter. Careful selection of patients to switch to LA ART is therefore of absolute clinical importance. Currently known predictive risk factors for VF help to identify patients at risk. However, more research on LAART is needed to better understand the value of TDM as well as mechanisms leading to VF in patients previously unidentified by known risk factors.

References:

[1] Gerstenberg et al. Therapeutic Drug Monitoring of Long Acting Rilpivirine and Cabotegravir for Treatment of HIV-1 Infection – A Case Series of Five Patients with One Virologic Failure after Development of Tow-Class Resistance. Open Forum Infect Dis. DOI: 10.1093/ofid/ofae480.

P-2-100

Fluoroquinolone-resistant and extended-spectrum β-lactamase (esbl)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with sepsis and urinary tract infections at Asante-Akyem Agogo Presbyterian Hospital, Ghana

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Fluoroquinolone-resistant and ESBL-producing strains of *E. coli* and *K. pneumoniae* have become more prevalent in recent years, presenting significant challenges for infection control and antibiotic treatment. Research suggests that ESBL-producing *Enterobacterales* may also carry genes conferring resistance to fluoroquinolones, limiting treatment options and potentially necessitating more expensive therapies. Therefore, this study aimed to identify and describe fluoroquinolone-resistant and ESBL-producing *E. coli* and *K. pneumoniae* strains associated with cases of sepsis and urinary tract infections (UTI) in patients at Asante-Akyem Agogo Presbyterian Hospital (APH).

Blood and urine samples were collected from patients with sepsis and UTI symptoms, respectively. Gram-negative bacteria were identified using API 20E, an antimicrobial susceptibility test was performed, and fluoroquinolone-resistant and ESBL genes were detected using PCR. Samples of blood (56.7%, 785/1383) and urine (43.3%, 598/1383) were collected from 1,334 patients, with 49 providing both types of samples.

The study reports *E. coli* and *K. pneumoniae* as the most commonly isolated Gram-negative bacteria, with respective prevalence of 1.7% (14/785) and 1.0% (7/60) in sepsis, and 15.0% (90/598) and 4.6% (28/598) in UTI cases. *K. pneumoniae* isolates displayed the highest resistance to ampicillin (97.1%) and cefuroxime (91.4%) but the lowest resistance to meropenem (5.7%). Also, *E.* coli isolates exhibited the lowest resistance to meropenem (4.8%), but the highest resistance to ampicillin (92.3%) and tetracycline (87.5%). Overall, ESBL genes were detected in 85.6% (119/139) of the *E. coli* and *K. pneumoniae* isolates, while fluoroquinolone-resistant genes were found in 82.7% (115/139) of the *E. coli* and *K. pneumoniae* isolates. The *bla*CTX-M gene was the most commonly detected ESBL gene, present in 77.7% of the isolates and the *bla*SHV was the least detected present in 25.1% of the isolates. Regarding fluoroquinolone resistance, the *gyrA* gene mutation was most frequently encountered (81.3%), while the *parC* gene mutation was least detected (27.3%). The majority (72.0%, 100/139) of isolates carried both ESBL and fluoroquinolone-resistant genes, with no observed association between the carriage of these genes in *E. coli* and *K. pneumoniae* isolates.

In summary, this study identified prevalent fluoroquinolone-resistant and ESBL-producing *E. coli* and *K. pneumoniae* strains associated with sepsis and UTI in patients at Asante-Akyem Agogo Presbyterian Hospital, highlighting the urgent need for targeted antimicrobial stewardship efforts and infection control measures. Continuous surveillance is vital for tracking trends in antibiotic resistance, detecting emerging patterns, and conducting additional research to comprehend the underlying mechanisms behind resistance.

Fig. 1

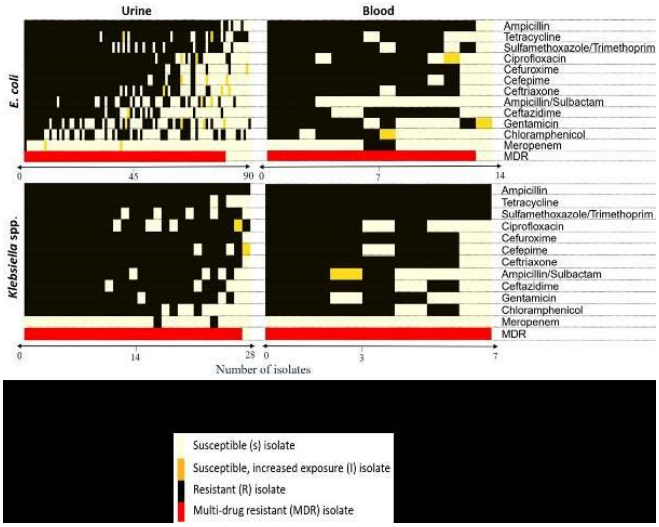
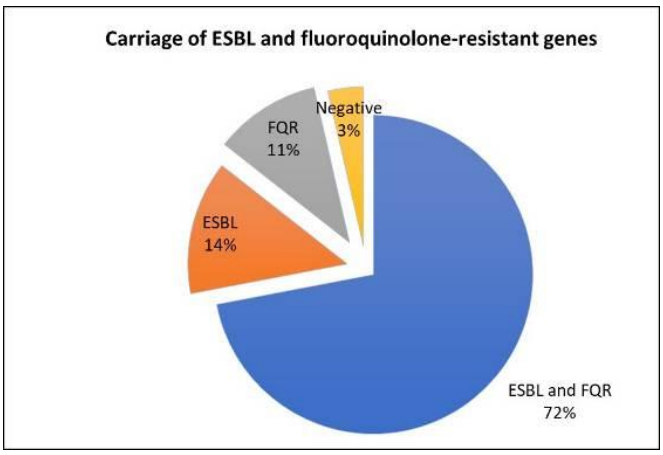


Fig. 2



P-2-101

Extended spectrum β-lactamase (ESBL)-producing E. coli and *K. pneumoniae* isolated from raw meat and poultry in Ouagadougou and Nouna, Burkina Faso

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Introduction: Livestock contributes 40% of the global value of agricultural output and supports the livelihoods and food security of almost a billion people worldwide. Intensification of livestock production has led to increased use of veterinary drugs, including antibiotics. Studies have indicated that antimicrobial resistance of livestock origin is rising in low- and middle-income countries. In Burkina Faso, self-medication is practiced by 75% of poultry farmers, and growth promoters are used in 94% of poultry farms. The poultry production-food-consumer chain was chosen for assessing baseline data of ESBL-producing *E. coli* and *K. pneumoniae*.

Method: This descriptive and analytical study covered the period from 2022 to 2024. It was carried out at two sites (backyards and farms for poultry, markets, and abattoirs for meat) in Nouna and poultry farms in Ouagadougou. Stool and raw meat samples were enriched in Brain-heart infusion broth and plated on CHROMagar ESBL to isolate *E. coli* and *K. pneumoniae.* Strains were identified based on their morphological characterization on CHROMagar and by biochemical methods. The isolates were further confirmed using the VITEK2 compact system and confirmation of ESBL was done using a combined disk method. Antimicrobial susceptibility testing was performed on all confirmed ESBL isolates using the Kirby-Bauer Method and interpreted using the EUCAST guidelines.

Results: In total 409 samples were collected. This includes 149 samples from poultry stool collected in Ouagadougou and 260 samples from Nouna, including 177 poultry stool and 83 meat samples. In all samples, ESBL-positive *E. coli* was more frequent (18%, n/N = 75/409) than ESBL-positive *K. pneumoniae* (6%, n/N = 24/409). Based on sample type, from all poultry stool samples taken in Ouagadougou, 21% (n/N = 32/149) were ESBL positive for *E. coli* and 13% (n/N = 19/149) were positive *for K. pneumoniae*. Similarly, 24% (n/N = 43/177) of all poultry stool samples collected in Nouna were ESBL-positive for *E. coli*, and 3% (n/N = 5/177) were positive for *K. pneumoniae*. Of all the meat samples tested

from Nouna, 25% (n/N = 21/83) were ESBL positive for *E. coli* and 2% (n/N = 2/83) were positive for *K. pneumoniae*. There was no statistically significant difference in the distribution of ESBL-positive *E. coli* between Ouagadougou and Nouna; x*2* (1, N = 149) = 0.51, p = 0.47. However, the distribution of ESBL is Positive for *K. pneumoniae.* Significantly higher in Ouagadougou compared to Nouna: *x2* (1, N = 149) = 17.9, p < 0.01. All bacteria were sensitive to meropenem; however, *K. pneumoniae* was resistant to tetracycline and cotrimoxazole.

Conclusion: Our study found a high prevalence of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* in the poultry in Burkina Faso. These findings suggest that antimicrobial resistance is a significant issue in urban areas and is also a serious threat in rural.

P-2-102

MraY and WbpL from *Pseudomonas aeruginosa* are inhibited by uridyl peptide antibiotics

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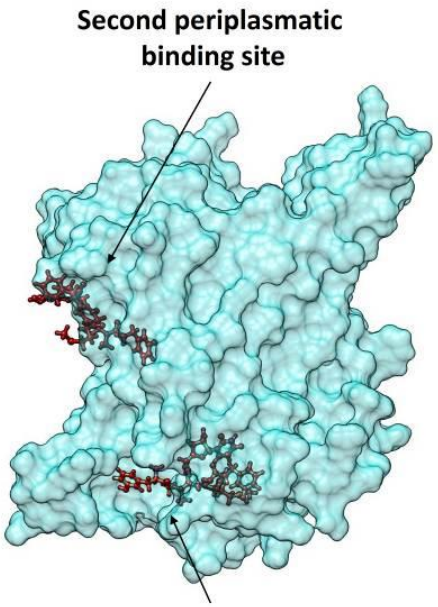
Antibiotic resistance is one of the most serious health threats and the therapeutic options to treat infections caused by multidrug-resistant strains are seriously compromised. Especially for infections caused by *Pseudomonas aeruginosa* isolates which have emerged resistance towards all carbapenems, aminoglycosides, and fluoroquinolones, novel antibiotics with new targets or unprecedented mechanisms of action are urgently needed. Integral component of the drug development process is the analysis of the mechanism of action of an antibiotic, as well as identification of the molecular target. Without this detailed knowledge, rational drug design is strongly hampered.

Uridyl-peptide antibiotics (UPA) are a promising group of nucleoside natural products, which show potent activity against *P. aeruginosa*. An important molecular target of this compound class is the phospho-N-acetylmuramoyl-pentapeptide-transferase (MraY), which catalyses the transfer of phospho-MurNAc pentapeptide to the membrane-standing lipid carrier undecaprenyl phosphate (C_{55}-P) resulting in the formation of Lipid I. Using a fluorometric assay with the heterologously expressed MraY from *P. aeruginosa*, eight UPA"s were characterized, and all showed IC_{50} values in the nanomolar range. Further, a second periplasmatic binding site was identified and confirmed by using site-directed mutagenesis resulting in significant elevation of the IC_{50} compared to the wildtype MraY, making UPA"s the first antibiotics with a dual binding motifs against the MraY of *P. aeruginosa*. To corroborate the data, a bioinformatical approach was used, by calculating the ΔG of the wildtype MraY and the mutants with AutoDock Vina (Trott & Olson, 2010). The theoretical binding showed very similar results to the biological data. Additionally, we could identify LPS synthesis of *Pseudomonas* as a new target of the UPA"s, by developing an in vitro assay for the first membrane standing protein of LPS biosynthesis (WbpL)

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461.

Fig. 1



(A) Schemtic representation of the cytoplasmatic and periplasmatic binding site at MraY

P-2-103

Targeting the replication of mycobacterial DNA: corramycin as an antituberculous agent

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Despite significant advancements in drug development and discovery, tuberculosis (TB) remains one of the world's deadliest infectious diseases, claiming an estimated 1.3 million lives in 2022. The emergence and spread of multidrug-resistant (MDR-)TB highlight the urgent need for novel antibiotics. Screening of our DZIF natural product library against *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB, led to the discovery of corramycin (COR), an antibiotic from *Corallococcus coralloides* with promising activity against Gram-negative pathogens that was recently developed for the treatment of urinary tract infections. Here we describe the previously neglected antitubercular activity of corramycin, report on the potential import mechanism into *Mtb*, and provide initial insights into its mode of action along with the identification of the DNA gyrase as its target.

P-2-105

Mode of action of the defensin-like compound actifensin

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Bacteriocins are ribosomally produced antimicrobial peptides that have been suggested as an alternative to conventional antibiotics due to their efficacy at low concentrations and their potential to be genetically modified. *Actinomyces ruminicola* produces a potent bacteriocin, actifensin (AfnA),

with activity against a broad spectrum of Gram-positive bacteria, including VRE and MRSA. Actifensin was purified and found to have a mass of 4,091 Da and a sequence containing three disulfide bridges. Actifensin shows high similarity to a number of related eukaryotic defensins, such as plectasin (Sugrue et al., 2020). Plectasin is a fungal defensin that targets the cell wall biosynthetic pathway by binding to the cell wall precursor lipid II (Jekhmane et al., 2024; Schneider et al., 2010). Here we investigated the mode of action of actifensin, specifically focusing on the effects on the cell wall biosynthetic pathway.

Jekhmane, S., et al. (2024). Host defence peptide plectasin targets bacterial cell wall precursor lipid II by a calcium-sensitive supramolecular mechanism. *Nat Microbiol*. doi:10.1038/s41564-024-01696-9

Schneider, T., et al. (2010). Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. *Science, 328*(5982), 1168-1172. doi:10.1126/science.1185723

Sugrue, I., et al. (2020). Actinomyces Produces Defensin-Like Bacteriocins (Actifensins) with a Highly Degenerate Structure and Broad Antimicrobial Activity. *J Bacteriol, 202*(4). doi:10.1128/jb.00529-19

P-2-106

Elucidating the effects of bituminosulfonates on the bacterial cell envelope

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The emergence of antibiotic resistance is a major health threat. Recently, there has been a growing interest in a new category of medicinal products, known as nonbiological complex drugs (NBCDs), due to their highly complex pharmacological profiles and low potential for resistance development (Schellekens et al., 2014). Bituminosulfonates, a multi-component mixture which have been tentatively designated as NBCDs, have been the subject of extensive pharmacological research and demonstrated a low potential for resistance development (Schwalb et al., 2023). The use and efficacy of sodium bituminosulfonates (SBS) and ammonium bituminosulfonates (ABS) as therapeutic agents have been documented since 1882 (Tiemann et al., 2024). Here, we investigate the mode of action of SBS and ABS, provided by Ichthyol®, mainly focusing on the effect on the cell envelope in Gram-positive bacteria.

Schellekens, H., et al. (2014). How to regulate nonbiological complex drugs (NBCD) and their follow-on versions: points to consider. *Aaps j, 16*(1), 15-21. doi:10.1208/s12248-013-9533-z

Schwalb, L., et al. (2023). Analysis of complex drugs by comprehensive two-dimensional gas chromatography and high-resolution mass spectrometry: detailed chemical description of the active pharmaceutical ingredient sodium bituminosulfonate and its process intermediates. *Anal Bioanal Chem, 415*(13), 2471-2481. doi:10.1007/s00216-022-04393-w

Tiemann, O., et al. (2024). Rock-to-Pharma: Characterization of Shale Oil-Based Nonbiological Complex Drugs along the Production Process by High-Resolution Mass Spectrometry. *Anal Chem, 96*(32), 13050-13060. doi:10.1021/acs.analchem.4c01288

P-2-107

Linking genomic and metabolic diversity of the myxobacterium Sorangium spp

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Next generation genome sequencing in combination with genome mining approaches has led to the prediction of a multitude of hypothetical biosynthetic gene clusters (BGCs), suggesting an enormous potential for the biosynthesis of diverse natural products. Especially the myxobacteria *Sorangium* spp., with their large genomes, harbor many such BGCs. For the vast majority of these BGCs however, associated natural products are currently unknown. On the other hand, for many natural products the underlying BGCs are not known. We aim to close this gap by linking genomic and metabolic diversity using a novel metabologenomics approach.

To this end we have integrated genome sequences and experimental data on natural products from 72 *Sorangium* spp. strains to identify statistical associations between BGCs and the production of potentially biotechnologically useful metabolites. We predicted 2,029 BGCs in the 72 *Sorangium* spp. genomes, which clustered into 265 gene cluster families (GCFs) based on their sequence similarities. Combining high-resolution mass spectrometry with our in-house database on natural products from myxobacteria, these strains were analyzed for their metabolite production under controlled conditions. 880 distinct molecular features from natural products could be differentiated, that further classified into 99 metabolite families (MFs). For the statistical association of GCFs and MFs, we applied a maximum-likelihood approach, considering the genome-based phylogeny of *Sorangium* spp. As a result, 43 MF/GCF pairs were statistically associated, including correct links for the majority of published pairs of BGCs and natural products present and predictable in the 72 *Sorangium* spp. strains.

Our results demonstrate that statistical association analysis is a powerful tool for accelerating the identification of links between BGCs and natural products, which is a prerequisite for understanding bacterial biosynthetic potential, for investigating its genetic regulation, and using synthetic biology approaches for rational engineering of molecules.

P-2-108

From antimotilin antivirulence compounds to intrabacterial targets

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Bacterial motility and flagellar assembly are complex traits which require the multifactorial regulation and hierarchical expression of contributing factors. We develop novel antibacterial strategies targeting bacterial motility. We have explored the potential as a new antibacterial therapeutical approach to overcome high antibiotic resistance in the human pathogenic *Helicobacter pylori* which causes severe stomach diseases including cancer. Thereby, we have recently discovered and patented a new compound class which is able to inhibit flagellin FlaA expression and motility of *H. pylori* in vitro and in vivo in a mouse model [1]. The next step of our research was now to identify molecular target(s)of the activities of such compounds to make them more amenable to lead optimization and further translational efforts.

In the present work, in addition to numerous phenotypic and regulation experiments to assess influences on bacterial traits such as motility, we have performed transcriptome and epigenome analyses of *H. pylori* under the influence of various active compounds including the identified antimotilins. Transcriptome analyses suggested a pleitropic action of the motility-inhibiting compounds, with a likely target in epigenetics. Using EM-Seq [2] and several control conditions to quantitatively assess modifications in bacterial epigenetics, we determined that selected compounds acted on local quantitative epigenetic DNA modifications (cytosine methylation). Our results show that specific epigenetic modifications, as previously suggested [3], are an additional layer to structure gene regulation, including flagellar genes. We have also synthesized a family of related compounds which show differential effects in certain assays, suggesting a variability in molecular targets. Molecular modelling is currently attempted as a complementary methodology to refine the assessment of molecular target interaction of selected compounds. We also assessed molecular effects on the microbiota.

Taken together, we have established novel methods and uncovered novel compound targets for a novel family of motility-inhibiting compounds that influence, among others, bacterial motility regulation in *H. pylori* through epigenetics. These results will help refine our antibacterial strategy.

[1] Suerbaum S. et al., mBio 2022;13(2):e0375521. doi: 10.1128/mbio.03755-21.

[2] Patel, L. et al., BMC Biol. 2024;22:125. doi: 10.1186/s12915-024-01921-1.

[3] Estibariz, I. et al., Nucleic Acids Res. 2019;47(5):2336-2348. doi: 10.1093/nar/gky1307.

P-2-109

Evaluation of microbiome enrichment and host DNA depletion in human vaginal samples using Oxford Nanopore"s adaptive sequencing & Nanopore-based enrichment of antimicrobial resistance genes – a case-based study

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Adaptive sampling on Oxford Nanopore sequencing platforms emerges as a powerful real-time method for selective DNA enrichment or depletion. The method enables direct, on-device rejection or acceptance of DNA molecules during sequencing, offering significant advantages for clinical microbial research without requiring specialized sample preparation. We conducted two studies to evaluate adaptive sampling in a clinical setting: i) examining human host DNA depletion in vaginal microbiome samples from patients with Premature Rupture of Membranes (PROM) to increase microbial sequencing depth, and ii) assessing targeted enrichment of antimicrobial resistance genes in clinical bacterial isolates using a miniature flow cell (Flongle).In our vaginal microbiome study, human host depletion achieved a 1.70-fold increase in microbial sequencing depth compared to control experiments. The enhanced sequencing depth directly translated to improved taxonomic profiling sensitivity, enabling more detailed characterization of the vaginal microbiome while maintaining microbial abundance. However, the complete removal of all human host sequences is not yet possible and should be considered as an ethical approval statement might still be necessary.

When adaptive sampling was applied to antimicrobial resistance surveillance, the technique successfully enabled real-time on-device target enrichment of 1,147 resistance genes on a miniature flow cell to create an ultra-high multiplex assay. Multiple carbapenem-resistant strains were identified in a rectal swab from a patient, and their genomes were reconstructed and supplemented with metagenomic data from the swab. 57.9% (22/38) of the resistance genes from the fully reconstructed genome of the R. ornithinolytica isolate were detected, including all three plasmid-encoded carbapenemases (NDM, KPC, VIM), even when using miniature Flongle flow cells. Computational modeling of the experimental data revealed that enrichment efficiency was primarily influenced by two key factors: nucleotide identity (with higher identity yielding better results) and read length (where shorter reads performed better).

These findings establish Oxford Nanopore"s adaptive sampling as a valuable tool for clinical surveillance and medical research, offering particular utility in scenarios requiring host DNA depletion or targeted pathogen detection. The implications of this technology extend beyond immediate clinical applications, suggesting potential use in rapid outbreak surveillance, routine microbiome monitoring, and antimicrobial resistance tracking in healthcare settings. The ability to perform targeted sequencing in real-time particularly benefits time-sensitive clinical decisions, potentially reducing the turnaround time for pathogen identification and resistance profiling from days to hours.

Fig. 1

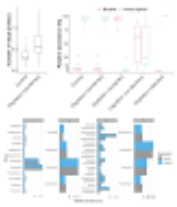
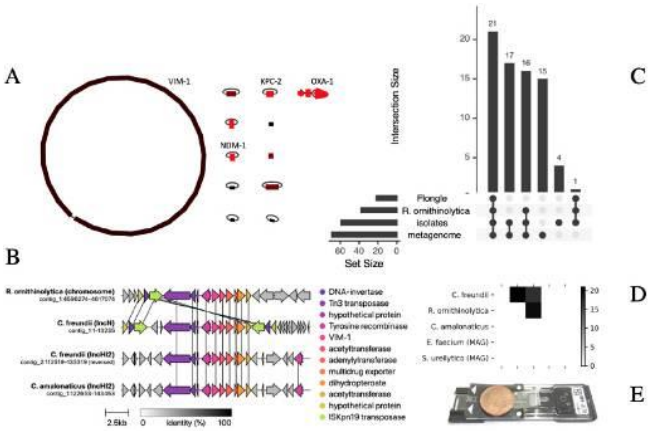


Fig. 2



P-2-110

Data-driven identification of possible transmission sites for carbapenem-resistant gram-negative bacteria

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Question: Since carbapenems are last-resort antibiotics to treat bacterial infections, it is crucial to control the spread of carbapenem-resistant bacteria. Often, the resistance is caused by carbapenemase enzymes, which occur as multiple different types and subtypes. In 2011, the state of Hesse, Germany, has established a notification system where cases of carbapenem non-susceptibility are reported. We use the notification data from 2012 to 2024 to develop and evaluate an automated computational approach that not only detects putative outbreaks of carbapenem-resistant bacteria but also their most likely sources.

Methods: The approach has been designed to deal with varying levels of specification for the resistance type in the notification data and allows for user-defined groupings of resistance types for the analysis. Furthermore, the user has the option to restrict the analysis to specific groups of bacterial species and to either focus on single-species outbreaks or allow for multi-species outbreak predictions. A graphical user interface makes the tool accessible to non-programmers. The main input is a time frame of interest, given by an end date and the number of weeks beforehand that should be considered. The output is a table of putative outbreak events that connect subsets of patients that were reported within that time frame.

Results: Our study shows that it is beneficial for the outbreak prediction to take patients' prior hospital stays into account. Comparing four alternative machine learning pipelines for the prediction task, we see clear performance differences in recovering manually labeled outbreaks. Each pipeline is queried with the same test time frames and has obtained the same annotated data of a preceding time frame for hyperparameter selection. In addition to fine-tuning the final prediction pipeline, we investigate appropriate statistical measures to assess anomaly of case frequencies and prioritize actions among predicted outbreaks if needed. To make potential users aware of the limitations and risks of such an automated system, we carefully analyze ethical implications and provide guidelines on how to interpret its outputs.

P-2-111

Development of Vancomycin-resistant-enterococci rapid detection kit for routine clinical care

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Integrating rapid diagnostic tests into clinical management has great potential to improve, therapeutic processes, and patient outcomes. For this, we develop an antibody-based lateral flow assay (LFA) for the detection of vancomycin resistant Enterococcus faecium (VREfm). Resistance to vancomycin is disseminating rapidly also in other more virulent bacterial species. Hospitalized patients with gastrointestinal carriage of VREfm appear to be the major reservoir of the pathogen. Two major vancomycin resistance determinants (ligases VanA and VanB) have been described in enterococci. Expression of the ligases, VanA or VanB, results in the synthesis of altered peptidoglycan precursors to which vancomycin binds with markedly lower affinity loosing its bactericidal activity. VanA mediated resistance is characterized by high-level resistance to vancomycin, and was up to recently the most prevalent type in Germany. New epidemiological studies revealed an increasing prevalence of vanB in VREfm and particularly in sequence type ST117 during the last five years in German hospitals. VanB expressing isolates show inducible resistance to more modest levels of vancomycin rendering to false susceptible results in routine phenotypic tests. We focused primarily on the development of an LFA to detect VanA and VanB resistance determinants with the option to expand it towards a biomarker for VREfm sequence type ST117. We cloned, expressed and purified representative VanA / VanB ligases in E. coli for immunization of mice. Generated monoclonal antibodies (mAbs) by hybridoma technology were screened by ELISA and Westernblot for their binding properties. We identified 15 mAbs that specifically react with different variants of endogenous VanA or VanB, or both of clinical isolates. In contrast to VanA, VanB expression needs to be induced by vancomycin, which will be considered in the VRE-LFA workflow. The aim is to rapidly detect VREfm in cultivated isolates from patient samples in order to simplify and expedite diagnosis and treatment options. The VRE-LFA will be integrated into a pilot study to analyze its potential impact on clinical management.

P-2-112

LL-37 derived antimicrobial peptides against uropathogenic Escherichia coli

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Question: Urinary tract infections (UTIs) impact more than 150 million people annually. Uropathogenic *Escherichia coli* (*E.coli*) (UPEC) are identified in up to 80% of cases of UTI [1]. Antimicrobial peptides (AMPs) are a promising alternative therapeutic approach against drug-resistant pathogens and have been shown to have broad-spectrum antimicrobial activity against pathogenic microorganisms [2].

Methods: We tested three AMPs, the human cathelicidin LL-37 [3], and the N-terminal fragments of LL-37 106 (aa 106-140) and 110 (aa 110-140) [4]. The AMPs were tested for the inhibitory effect against seven *E. coli* strains. The *E. coli* strains CFT073, 536, S115, CDF6 and ATCC BAA-2469

were isolated from clinical patients' urine, and the strain NRZ14408 from wound swab, ATCC25922 was used as control strain. We evaluated the AMPs' effectiveness with the minimal inhibitory concentration (MIC) in the broth microdilution test. The MIC assays were performed in the media M9 minimal medium (pH 7.0), and Müller-Hinton-Broth (MHB, pH 7.2).

Results: For LL-37, the MICs in MHB were 3.3 µM for CFT073 and 6.6 µM for S115 and 536. For 106 the MIC was 6.6 µM for CFT073, 536, ATCC25922 and ATCC BAA-2469. For 110 MICs were 2.8 µM for ATCC BAA-2469 and 5.6 µM for CFT073, 536 and ATCC25922. No bacterial inhibition was observed for the tested peptides in M9 medium. The measurement of pH in the medium MHB for LL-37, after incubation for 24 h, 37°C, revealed a change in the pH, to pH 8 of the well with the AMP and the *E. coli* strains and a pH value pH 9 in the positive control.

Conclusion: We determined the MIC for the peptides LL-37 and the terminal fragments of LL-37, 106, and 110 for all seven *E. coli* strains in MHB. The results show that in MHB (pH 7.2), the tested AMPs are more effective against the tested *E. coli* strains, than in the M9 medium (pH 7.0). The MHB medium's pH changes to a more basic pH value during the 24-hour incubation for LL-37.

References: [1] Schwartz L, Bochter MS, Simoni A, Bender K, Dios Ruiz Rosado J de, Cotzomi-Ortega I et al. Repurposing HDAC inhibitors to enhance ribonuclease 4 and 7 expression and reduce urinary tract infection. Proc Natl Acad Sci U S A 2023;120(4):e2213363120. https://doi.org/10.1073/pnas.2213363120.[2] Mba IE, Nweze EI. Antimicrobial Peptides Therapy: An Emerging Alternative for Treating Drug-Resistant Bacteria. Yale J Biol Med 2022;95(4):445–63.[3] Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001;97(12):3951–9. https://doi.org/10.1182/blood.v97.12.3951.[4] Ciornei CD, Sigurdardóttir T, Schmidtchen A, Bodelsson M. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. Antimicrob Agents Chemother 2005;49(7):2845–50. https://doi.org/10.1128/AAC.49.7.2845-2850.2005.

P-2-113

EnteroBase: Global genomic surveillance of bacterial pathogens

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EnteroBase is an analysis platform for the genomic surveillance of select bacterial pathogens. It provides databases with currently >1.2 million quality-controlled genome assemblies from microbial strains, together with pre-calculated genotyping data for each genome, associatedepidemiological metadata, and powerful graphical tools for molecular epidemiological analyses.

EnteroBase greatly facilitates the detection of transmission chains, outbreaks, and epidemics. Importantly, it also provides for large-scale international contextualisation of pathogen genome data. Recently we have implemented the genome-based detection of antimicrobial resistance (AMR) determinants. EnteroBase can now be used to compare the genetic determinants of AMR among tens of thousands of genomes, to track the emergence and spread of AMR across space and time.

EnteroBase is being developed and operated collaboratively at the University of Warwick (https://enterobase.warwick.ac.uk/) and the Leibniz Institute DSMZ (https://enterobase.dsmz.de/). This international collaboration accelerates the development of the platform and ensures the longevity of the resources built. In the DZIF TI Bioresources, Biodata and Digital Health, we are expanding EnteroBase with novel databases and analysis tools for pathogens that are particularly relevant for DZIF. In collaboration with the TTU Tuberculosis we currently focus on Mycobacterium tuberculosis epidemiology and AMR.

P-2-114

Integration of whole genome sequencing data for cluster analysis as part of the tuberculosis surveillance in Germany - results from three cities

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Question: Tuberculosis (TB), particularly the emergence of drug resistant *Mycobacterium tuberculosis* complex (MTBC) strains, remains a major public health challenge. Whole genome sequencing (WGS) of MTBC isolates accompanying classical epidemiology augments comprehensive TB surveillance, including monitoring of resistance levels and impact of migration on transmission. Local public health authorities (LHAs) in Hannover, Hamburg, and Frankfurt/Main, the Robert Koch Institute and the National Reference Center for Mycobacteria delivered and analysed a comprehensive set of integrated molecular and epidemiological data to enhance TB surveillance in Germany.

Methods: Key epidemiological parameters of the patients notified within the TB notification system were linked with WGS results, providing information on molecular clusters as indication of a possible recent transmission. We present descriptive analysis of the data from 2020-2023.

Results: 1,056 culture-positive TB cases were notified from the study sites; WGS data was linked to 615 (58%) of them. 219/615 (36%) of these cases were part of 118 molecular clusters, which also involved cases notified by other LHAs (n=75 cases in 42/118 (36%) of the clusters), and cases without linked epidemiological data (n=277 cases in 93/118 (79%) of the clusters; Table 1).

102/118 clusters (86%) included cases born outside of Germany (n=211), four of them with children below the age of 15 (n=5). Further four clusters involved children born in Germany (n=6). Thirty-five clusters (30%) contained cases with resistances against at least one anti-TB drug (n=122), among them one with pre-XDR-TB cases (n=7; Table 2).

Conclusions: Clusters overarching multiple regions emphasize the importance of the integration of WGS within the nationwide TB surveillance. Integration of epidemiological and molecular information is crucial to identify events requiring immediate further investigation and targeted interventions such as clusters involving children or resistant-TB. Collected data will contribute to improving (trans-) regional TB control and developing interventions for the detected transmission events.

Table legends:

Table 1: Number of cases in the detected molecular clusters by year and region; n (%).

Table 2: Molecular resistance prediction for cases in the detected molecular clusters per year; n (%).

Fig. 1

District/Year	2020	2021	2022	2023	2024	NA	Total
Region Hannover	6 (10.5%)	19 (57.6%)	9 (15.5%)	12 (12.1%)	5 (10.6%)	0 (0.0%)	51 (8.9%)
Frankfurt am Main	16 (28.1%)	14 (42.4%)	22 (37.9%)	9 (9.1%)	0 (0.0%)	0 (0.0%)	61 (10.7%)
Hamburg	35 (61.4%)	0 (0.0%)	11 (19.0%)	39 (39.4%)	22 (46.8%)	0 (0.0%)	107 (18.7%)
Other counties	0 (0.0%)	0 (0.0%)	16 (27.6%)	39 (39.4%)	20 (42.6%)	0 (0.0%)	75 (13.1%)
Unlinked cluster cases	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	277 (100.0%)	277 (100.0%)
Total	57 (100.0%)	33 (100.0%)	58 (100.0%)	99 (100.0%)	47 (100.0%)	277 (100.0%)	571 (100.0%)

Fig. 2

Year/ Resistance prediction	Susceptible	non-MDR*	MDR	pre-XDR	XDR	NA	Total
2020	51 (89.5%)	6 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	57 (100.0%)
2021	25 (75.8%)	8 (24.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	33 (100.0%)
2022	47 (81.0%)	11 (19.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	58 (100.0%)
2023	79 (79.8%)	18 (18.2%)	0 (0.0%)	2 (2.0%)	0 (0.0%)	0 (0.0%)	99 (100.0%)
2024	35 (74.5%)	4 (8.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (17.0%)	47 (100.0%)
Unlinked cluster cases	195 (70.4%)	68 (24.5%)	0 (0.0%)	5 (1.8%)	0 (0.0%)	9 (3.2%)	277 (100.0%)
Total	432 (75.7%)	115 (20.2%)	0 (0.0%)	7 (1.2%)	0 (0.0%)	17 (3.0%)	571 (100.0%)

*Non-MDR: TB cases with molecularly predicted resistance against at least one anti-TB medication, but not MDR-TB, pre-XDR TB or XDR-TB.

P-2-115

Exploring the antibiotic stress transcriptome: Response patterns in *Enterobacter cloacae* strains with varying meropenem susceptibility

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Background: RNA expression profiles provide a novel approach for determining antibiotic resistance phenotypes. We characterized the antibiotic resistance response patterns in *Enterobacter cloacae*, analyzing both sensitive and resistant strains. Our aim was to discover transcriptome profile variations unrelated to known antibiotic resistance genes. Thus enhancing our understanding of the *Enterobacter cloacae* response to antibiotic stress, while offering a novel way of antibiotic resistance prediction, and identifying potential targets to combat it.

Methods: To investigate carbapenem resistance in *Enterobacter cloacae*, we selected both a resistant and a sensitive clinical strain. Strains were genotyped, and the resistant strain was screened for the clinically significant VIM resistance gene. We cultivated and collected samples for transcriptomic analysis during different time point of the logarithmic growth phase. Subsequently, total RNA was extracted, rRNA was depleted and reverse transcription was performed. The resulting cDNA were sequenced using an Illumina platform. The sequencing data was analyzed via RNAflow pipeline while differential expression analysis was performed using DESeq2, and over-representation analysis was conducted using ClusterProfiler, with annotations from Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Clusters of Orthologous Groups (COG).

Results: We observe significant differences in expression patterns in *Enterobacter cloacae* as early as 2 hours after culturing. Identifying 3,230 significantly differentially expressed genes in the sensitive strain and 3,437 in the resistant strain, with 194 genes being highly upregulated in the sensitive strain and 103 in the resistant strain. Functional analysis showed increased expression of genes involved in oxidative stress, metabolism, and energy production in the sensitive strain, while the resistant strain exhibited upregulation in gene expression regulation and transporter-related genes. We also observed inverse regulation in several stress response genes (downregulated in sensitive strain and upregulated in resistant strain) such as general stress response and cellular transport functional groups, highlighting distinct patterns characteristic of each strain type.

Conclusion: Our research identified key differences in gene expression between antibiotic-sensitive and resistant strains of *Enterobacter cloacae*, revealing biological pathways involved in antibiotic response. These findings offer insights into bacterial adaptation mechanisms, suggesting potential avenues for developing novel therapeutic and diagnostic strategies.

P-2-116

Intravenous antibiotics in patients with bloodstream infection: Switch to oral therapy remains an unpopular choice

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Background: Switch from intravenous (i.v.) to oral antibiotics when feasible has proven to be safe in patients with bloodstream infection (BSI) and may further reduce length of hospital stay [1]. We present the development and application of an algorithm to evaluate i.v. to oral switch in a cohort with microbiologically-proven BSI.

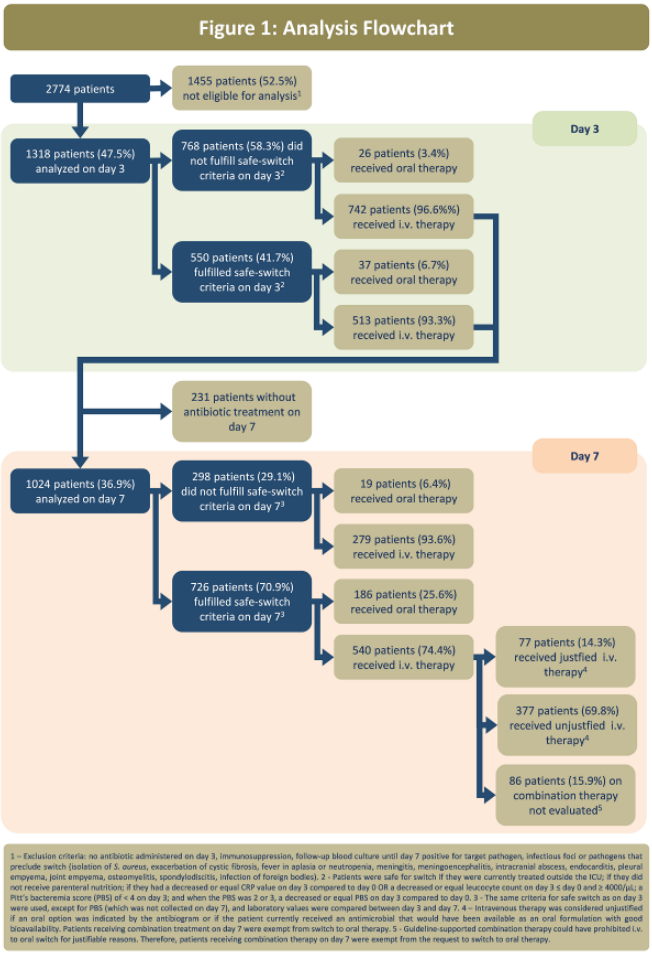
Methods: Among patients eligible for safe switch, route of administration was evaluated both on day 3 and 7 after positive blood culture (algorithm in figure 1). Switch by day 3 was considered early switch, by day 7 it was considered late but appropriate switch. In patients not receiving oral therapy by day 7, justification for i.v. administration was investigated. The algorithm was used for a secondary analysis of the observational BLOOMY and PREDICT cohorts including hospitalized adult patients with BSI due to ESKAPE pathogens. Data was prospectively collected in six German university hospitals between 2017 and 2021 [2]. There was no uniform policy for i.v. to oral step-down in place during the study period.

Results: Out of 2774 patients included in the study cohort, 1318 and 1024 were evaluated for switch to oral therapy on day 3 and day 7, respectively. 1001 patients were fully evaluable for the quality indicator, out of which 580 (57.9%) received antibiotics through the route appropriate for their clinical condition. 223 patients were appropriately switched to oral therapy by day 7, with 37 (16.6%) of them being considered early switch. 540 patients received i.v. antibiotics on day 7 in spite of fulfilling safe switch criteria. For 376 (69.6%) of them, an oral alternative was indicated by the antibiogram. Allergies prevented switch to the oral option in only 2 patients (0.4%). Restricted analysis revealed that in summary, 37.2% of all patients eligible for switch received oral therapy. When comparing patients remaining on i.v. therapy to patients who were switched, those switched showed a lower burden of comorbidity, less severe disease, lower in-hospital mortality (0% vs. 11.2%, p<0.001) and shorter LOS after positive blood culture (7 vs. 12 days, p<0.001). In univariate analysis, patients with respiratory focus (6/29 (20.7%)) and nosocomial infection (42/153 (27.5%)) were among the groups least likely to be switched, while patients with urogenital focus received oral therapy frequently (100/229 (43.7%)).

Conclusion: Evaluation of i.v. to oral switch using the developed algorithm showed that even in a very selected cohort of patients, opportunities for oral administration of antibiotics were rarely utilized. Obstacles to switch may have included combination therapies and concerns about the safety of oral antibiotics in sicker patients. Hence, i.v. to oral switch should be promoted further as an opportunity in ABS-programs.

References: [1] N. Wald-Dickler *et al.*, *The American Journal of Medicine*. **135**, 369-379.e1 (2021)., [2] E. Tacconelli *et al.*, *The Lancet Infectious Diseases*. **22**, 731–741 (2022).

Fig. 1



P-2-117

A practice-based approach to teaching antimicrobial therapy using artificial intelligence and gamified learning

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Question: Scalable teaching through apps and artificial intelligence (AI) is of rising interest in academic practice. We focused on how medical students could benefit from this trend in learning antibiotic stewardship (ABS). Our study evaluated the impact of gamified learning on factual knowledge and uncertainty in antibiotic prescription. We also

assessed an opportunity for AI-empowered evaluation of freeform answers.

Methods: We offered four short courses focusing on ABS, with 46 participating medical students who self-selected themselves into the elective course. Course size was limited by the faculty. At the start of the course, students were given a questionnaire about microbiology, infectious diseases, pharmacy and qualitative questions regarding their proficiency of selecting antibiotics for therapy. Students were followed up with the same questionnaire for up to 12 months. We selected popular game mechanics with commonly known rules for teaching and an AI for evaluating freeform questions.

Results: The number of correctly answered questions improved significantly for three topics asked in the introductory examination, as did the self-assessed safety of prescribing antibiotics. The AI-based review of freeform answers was found to be capable of revealing students" learning gaps and identifying topics in which students needed further teaching.

Conclusions: We showed how an interdisciplinary short course on ABS featuring gamified learning and AI could substantially improve learning. Even though large language models are a relatively new technology that sometimes fails to produce the anticipated results, they are a possible first step in scaling a tutor-based teaching approach in ABS. As a result, application-oriented learning is expected to be enabled for professional training. This can potentially lead to both - more comprehensive knowledge of ABS and thus a more rational use of anti-infectives.

Fig. 1

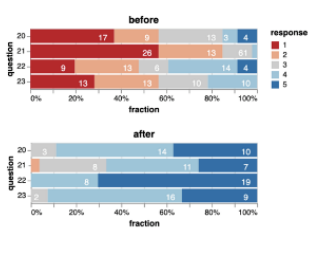

Figure 2: Subjective confidence rating of 46 students regarding antibiotic therapy initiation before (upper panel) and after (lower panel) the course. Rating was performed on a Likert scale from 1 ("no agreement") to 5 ("full agreement"). A substantial improvement can be observed across all questionnaire items in this block (blue is better).

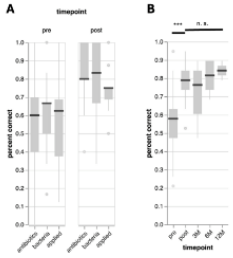

Figure 3: Knowledge increases over the course and persists for up to one year. **(A)** A comparison of answers to 18 factual questions related to three topics (antibiotics, bacteria, and "applied" infectious diseases) before ("pre") and after ("post") the course revealed a significant improvement in all topics. **(B)** Over the long term, this knowledge persists for up to one year, with no significant (n. s.) decline in correctly answered questions when comparing the "post" assessment to all subsequent time points at three, six, and twelve months.

P-2-118

Preclinical and clinical development of HY-133, a recombinant bacteriophage endolysin for rapid nasal eradication of *Staphylococcus aureus*

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Current strategies to eradicate multidrug-resistant organisms in high-risk patients are limited. Existing approaches, such as the use of the antibiotic mupirocin for *Staphylococcus aureus* decolonization from nasal habitats, carry significant risks and drawbacks including the development of resistance, impairment of the microbiome, and lengthy and often frustrating application protocols. This highlights the need for highly selective, efficient, and fast-acting eradication strategies.

In this context, bacteriolytic proteins derived from bacteriophages present a promising alternative. These proteins exhibit rapid bactericidal activity, are stable under various conditions, and are highly specific to their bacterial targets. In addition, an intelligent design could overcome the disadvantages of using complete phages, such as strain-specific specificity and drug regulatory restrictions. The cysteine- and histidine-dependent aminopeptidase/hydrolase (CHAP) domain from the endolysin of phage K as enzymatic active domain was combined with the bacteriocin lysostaphin to enable a species-specific cell wall binding domain. The resulting recombinant chimeric agents, the pilot candidate PRF-119 and the optimized lead candidate HY-133, showed strong efficacy against *S. aureus*, including methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus*, while sparing coagulase-negative staphylococci. Time-kill curves revealed a rapid bactericidal effect of HY-133 within the first two hours.

HY-133 has been designed to meet critical medical needs by potentially offering (i) a stable, nasally applicable agent with rapid bactericidal action and being effective directly before or at the hospital admission, (ii) activity against mupirocin-resistant strains with low potential for resistance development, and (iii) high specificity for MSSA and MRSA without effects on the co-colonizing microbiota thereby reducing the risk of rapid re-colonization. Antimicrobial activity of HY-133 has been thoroughly characterized in several studies challenging it with a large collection of MSSA and MRSA strains covering more than 100 *spa* types. Also phenotypic variants of *S. aureus* (small-colony variants) were successfully tested. In-vivo studies have demonstrated safety and efficacy of HY-133 in animals.

HY-133 is manufactured in GMP quality as a stable, application-ready formulation. Currently, a randomized double-blinded placebo-controlled first-in-man single dose and multiple dose study evaluates the safety, tolerability and efficacy of HY-133. An extended phase will evaluate additionally effects of the nasal microbiome. Selective *S. aureus* eradication via specific lytic phage protein may offer a powerful approach for combating nosocomial infections. Clinical study results remain essential to fully assess the efficacy of this approach.

P-2-119

Group B streptococcus in Franceville, Gabon: Vaginal carriage and phenotypic resistance profiles

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Background: Group B Streptococcus (GBS), known to colonize the vaginal and rectal tracts, is a significant cause of neonatal mortality and morbidity. This study aims to determine the phenotypic antibiotic resistance profiles of GBS and to evaluate the prevalence and associated risk factors of colonization in outpatient pregnant women at the medical lab analysis of the Interdisciplinary Center for Medical Research of Franceville (CIRMF, Gabon).

Method: This retrospective and prospective study examined GBS vaginal carriage and resistance phenotypes among pregnant women (ages 14-45) living in Franceville over six months. Vaginal swabs were cultured on GRANADA selective medium. Antibiotic susceptibility tests were performed on the Vitek 2 Compact 15 system. Data gathered in Franceville were redesigned at the Centre de recherche de Lambaréné (Cermel, Gabon) and statistically analyzed using R studio version 4.3.3 to examine the relationships between exposure factors and GBS carriage, using the Chi-square test, Pearson correlation, and Odds ratio, with a risk α set at 5%.

Results: Vaginal secretions from 100 women were analyzed. The prevalence of GBS colonization was 13%, with parity being the only associated risk factor. GBS carriage is higher among women with bacterial vaginosis (8%) and its odds ratio (OR) of 0.8 suggests a weak association. Antibiotic susceptibility tests revealed high resistance to clindamycin (77%), tetracycline (54%), oxacillin (31%), and penicillin (23%). GBS strains were frequently resistant to tetracycline and clindamycin, with 46% being multi-drug resistant.

Conclusion: These findings highlight a significant concern in Franceville, with potentially severe implications such as spontaneous abortions, premature deliveries, and neonatal infections, potentially increasing neonatal mortality rates. It is crucial to use antibiotic susceptibility tests to guide the management and treatment of GBS colonization.

Fig. 1

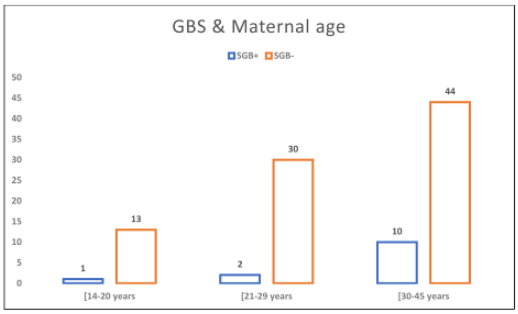

Figure 1. Distribution of GBS by maternal age

Fig. 2

Table 1. Gynaecological History and GBS Carriage

Antécédents gynécologiques		SGB+	SGB-	Spearson Cor (t, df)	p
Pregnancy age (weeks)	[1-12	7 (7%)	40 (40%)	0.786 (t=1.27 ; df=1)	0.425
	[16-28	3 (3%)	31 (31%)		
	[30-34	3 (3%)	16 (16%)		
Parity	Nulliparous	2 (2%)	22 (22%)	0.998 (t=20.29 ; df=1)	**0.031**
	Primiparous	1 (1%)	17 (17%)		
	Multiparous	10 (10%)	48 (48%)		
Abortion history	Yes	7 (7%)	45 (45%)	-	0.886
	No	6 (6%)	42 (42%)		
Preterm birth	Preterm+	0 (0%)	5 (5%)	-	0.375
	Preterm-	13 (13%)	82 (82%)		

P-2-120

Treatment failure or delayed microbial clearance despite usage of *in-vitro* active antibiotic agents in invasive bacterial infections has heterogeneous causes: Preliminary results from a deep assessment study

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We report preliminary results from the DZIF-Project FABULOUS – *Treatment failures in the primary absence of phenotypic resistance: a deep assessment for underlying causes to improve diagnostic and therapeutic approaches*.

The FABULOUS project encompasses isolates and data from TTU HAI (Healthcare-Associated Infections) cohort studies like TIARA and R-Net, with a focus on *Enterobacterales*. It aims at identifying potential (intrinsic or adaptive) resistance mechanisms which are undetectable by routine antimicrobial sensitivity testing (AST). 120 clinical isolates from invasive bacterial infections (e.g. Bloodstream Infections or wound infections) with repetitive isolation >48 h after first description in a clinical sample were selected from the different projects and analyzed. Additionally, we selected 15 control isolates with single isolation from the TIARA cohort. After assessment of the antibiotic therapy, antibiotic sensitivity testing was repeated for the administered antibiotic agents of interest. Whole-genome sequencing (WGS) to genome closure was performed on all isolates and the genomes were analyzed for potential causes of heteroresistance or tolerance. All isolates were also subjected to a tolerance disc (TD) test to reveal possible heteroresistance or tolerance.

For three cases, ongoing therapy was tested resistant following AST results, which was adapted after the results were available, and no further positive samples were found afterwards. For other isolates a persistent infection focus led to prolonged detection of the same bacterium. Wherever plasma samples were available from the time of the antibiotic treatment (some samples within TIARA), therapeutic drug management (TDM) measurements were performed. Although some plasma samples showed sub therapeutic concentrations of the administered antibiotic agent, mostly the concentration was within the therapeutic range.

Overall, in most cases the patient received the correct antibiotic therapy according to the resistance pattern of the isolates, thus further analyses regarding heteroresistance and antimicrobial tolerance were performed. A large portion of the tested isolates displayed in-vitro tolerance in a first screening (TD test). Long-read WGS detected a known

genetic cause for heteroresistance only in a few cases, e.g. amplification of an *ampC* beta-lactamase under antibiotic pressure. The isolates are currently under in-depth assessment by multiple time-point sequencing under antibiotic pressure, growth curve analysis, population analysis tests and dynamic transcriptome sequencing. However, the current results suggest that only a distinct subpopulation displays in-vitro causes for potential treatment failure or delayed microbial clearance.

P-2-121

Therapeutic drug monitoring-guided treatment of XDR tuberculosis with an RpoB I491F mutation in a ukrainian patient in Germany, 2023

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Background: In 2022, tuberculosis (TB) caused an estimated 10.6 million active infections and 1.3 million deaths worldwide[1]. The emergence of drug-resistant strains, such as multidrug-resistant TB (MDR-TB), which is resistant to at least first-line drugs isoniazid (INH) and rifampicin (RIF), is concerning. Extensively drug-resistant TB (XDR-TB) involves resistance to any fluoroquinolone (FLQ) and at least one other World Health Organization (WHO) Group A drug, such as bedaquiline (BDQ) or linezolid (LZD). Individualized treatment regimens with a minimum duration of 18 months are recommended by the WHO for XDR-TB [2].

Clinical case: A Ukrainian woman who fled to Germany in 2023 presented with symptoms of pulmonary TB. The Xpert® MTB/RIF Ultra (Cepheid) test in sputum samples yielded positive results for *Mycobacterium tuberculosis* (Mtb) detection but negative results for RIF resistance. However, phenotypic resistance testing revealed borderline resistance, as well as resistance to isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB). Further genomic and phenotypic testing in the national reference center for mycobacteria revealed resistance to FLQ, BDQ, LZD and clofazimine (CFZ). Targeted sequencing identified the I491F mutation in rpoB. Subsequent whole genome sequencing classified the isolate as a lineage 2/Beijing strain.

A regimen containing terizidone (TZD), amikacin (AMK), meropenem with clavulanic acid, pretomanid (PTM) and rifabutin (RFB) was initiated. Therapeutic drug monitoring (TDM) of PTM revealed decreased PTM levels upon co-administration with RFB. The treatment was further complicated by AMK-induced ototoxicity and increased Minimal Inhibitory Concentration (MIC) of PTM.

Conclusion: The presence of the rpoB I491F mutation, formerly predominantly found in lineage 4 strains of the sub-Saharan African region, in the Beijing/lineage 2 strain is concerning [3]. Low-level RIF resistance due to this mutation evades detection by the GeneXpert test. Tailored regimens for XDR-TB carry a high risk of side effects and drug interactions. Rifamycins reduce PTM serum concentrations, making TDM essential. The emergence of an increased MIC of PTM underscores the importance of regular phenotypic susceptibility testing, preferably with determining MICs.

References:

1. Global Tuberculosis Report 2023. Geneva: World Health Organization; 2023. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023. Date last accessed: August 2 2024.
2. World Health Organization. WHO operational handbook on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment, 2022 update. https://www.who.int/publications/i/item/9789240065116. Date last accessed: September 4 2024.
3. Friesen I, Dreyer V, Klingmuller A, et al. First detection of a Mycobacterium tuberculosis XDR clinical isolate harbouring an RpoB I491F mutation in a Ukrainian patient treated in Germany, October 2023. Euro Surveill. 2024;29(28).

P-2-122

A widespread SCCmec-located gene cluster protects MRSA against toxic polysulfides

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Methicillin-resistant *Staphylococcus (S.) aureus* (MRSA) is the most common antibiotic-resistant nosocomial pathogen, responsible for millions of life-threatening infections worldwide[1]. The bacterium frequently colonizes mucosal surfaces of the human body, exposing it to a variety of environmental stress factors. One of these stressors is hydrogen sulfide (H_2S). In low concentrations, it acts as a physiological signaling molecule in the host. At higher concentrations, H_2S is infamous for its toxicity, stemming from its ability to inhibit respiratory chains. High concentrations of H_2S are primarily the result of bacterial metabolization of sulfur compounds. Especially on mucosal surfaces, e.g. in the nose and the gut, the degradation of sulfated sugar moieties and l-cysteine residues[2] from mucin leads to H_2S concentrations of up to 0.4 mM[3] and 0.3 – 3.4 mM[4,5], respectively. Under oxic conditions, which are also present on mucosal surfaces[6], H_2S oxidizes to highly reactive polysulfides, thus creating another toxic stressor. It is known that *S. aureus* protects itself from H_2S with its *cst* gene cluster, consisting of *tauE, cstR, cstA, cstB,* and *sqr*[7]. In addition, we could show that it also uses an *sqr*-independent variant of the *cst* pathway to detoxify polysulfides. Furthermore, we found that *cst* is heterogeneously distributed in the genomes of staphylococci and that multiple clinically relevant *SCCmec* types introduce an additional *cst* (*cst2*; without *sqr*) into the genomes of MRSA strains. Phenotypic analyses of a comprehensive set of clinical and laboratory-derived strains revealed that *cst2* confers high

polysulfide tolerance to MRSA. Moreover, we could demonstrate that *cst2* in MRSA leads to a remarkable fitness benefit in polysulfide-rich environments, resulting in the displacement of MSSA strains in direct intraspecies competition**.**

1 **Murray *et al.* (2022):** *in: The Lancet 399, 629–655.*

2 **Stümmer *et al.* (2023):** *in: Antioxidants (Basel, Switzerland) 12.*

3 **Ikeda *et al.* (2019):** *in: Molecules (Basel, Switzerland) 24.*

4 **Braccia *et al.* (2021):** *in: Frontiers in microbiology* 12: 705583.

5 **Dordević *et al.* (2021):** *in: Journal of advanced research* 27: 55–69.

6 **Espey (2013):** *in: Free radical biology & medicine* 55: 130–140.

7 **Shen, *et al*. (2015):** *in: Biochemistry* 54 29, 4542–4554.

P-2-123

Isolation of hospital-associated VRE clones from German rivers; results of the DZIF GAP-project

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Background: Previous DZIF-funded studies (i.e. R-NET/Control) have revealed a high clonality of clinical vancomycin resistant *Enterococcus faecium* (VRE) isolated from different DZIF-affiliated hospitals, with ST117/CT71 in particular showing a remarkable clonality between sites. The GAP project was initiated to investigate VRE isolated from German rivers and wastewater treatment plants in areas close to Hamburg, Lübeck, Freiburg, and Cologne/Bonn, and compare them to previously sequenced clinical isolates.

Methods: Environmental isolates (n=203, 73 from rivers isolated 2016-2024, and 130 from wastewater influent/effluent isolated 2016-2018), were plated onto appropriate media. Confirmed VRE were sequenced (MiSeq) and compared by cgMLST (SeqSphere) to all DZIF VRE sequenced to date. The rivers were not connected to wastewater treatment plants that received hospital wastewater.

Results: The vast majority of environmental isolates were *vanB*-positive (n=168) and the remaining were *vanA*-positive (n=35). MLST analysis revealed 124 ST117, and 60 ST80. Nine isolates were ST1299, a ST recently described as dominant in Bavaria, and all were isolated 2023-2024. Five isolates were ST323, two were ST2176, and one each ST192, ST375, and ST1478. cgMLST analysis using a threshold of 20 alleles (Fig 1) reveals that both river and wastewater isolates clustered together with some isolates identical, and some clusters contain isolates from multiple sites. Coincidentally, 117 of the ST117 were the epidemic CT71.

Using a threshold of ≤20 alleles, 1300 clinical *E. faecium* cluster with the environmental isolates. With a lower threshold of ≤3 alleles, 154 clinical VRE clustered with the environmental VRE. Interestingly some clinical isolates were identical to environmental isolates despite them being isolated in different regions (Fig 2).

Conclusion: These data show that clinically relevant VRE strains can be isolated not only from wastewater, but also from river water which were not connected to a hospital upstream of the collection point, suggesting that the isolates are present in the community. Furthermore, the VRE appear to persist over the 8-year period that samples were collected, particularly the epidemic CT71 strain. Therefore, VRE can be considered a One-Health problem with a reach beyond the clinics. Further work is necessary to understand how the isolates are cycled back into the community and how to break this cycle.

Fig. 1

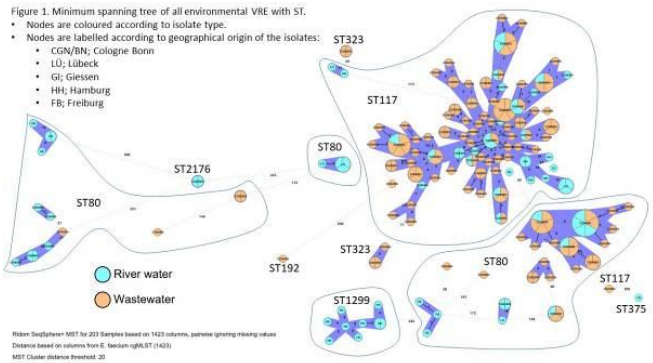
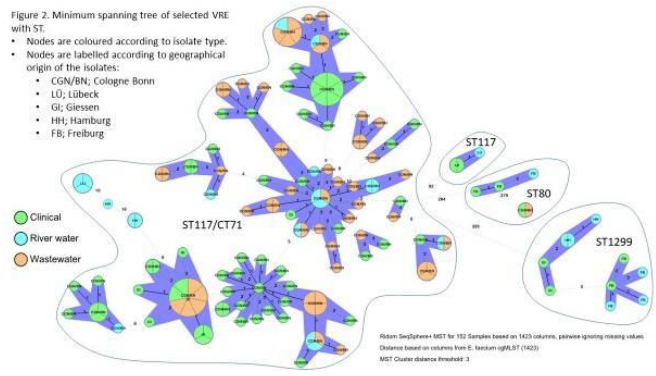


Fig. 2



P-2-124

Preclinical development of vancomycin polycationic peptide conjugate (V_N-R_6C) with high antimicrobial activity in vitro and in vivo

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Multidrug-resistant bacteria are considered as one of the most imminent threats to modern medicine worldwide. While there are numerous drugs available for standard therapy, there are only a few compounds capable of serving as a last resort treatment for severe infections. Especially, infections caused by the ESKAPE pathogens are associated with numerous types of resistances. Therefore, approaches to treat infections with multidrug-resistant bacteria must be implemented.

Here, a strategy of reactivating the established glycopeptide antibiotic vancomycin by structural modification with a hexa-arginine polycationic peptide was investigated (Figure 1). The conjugate synthesis provided yields of over 65% in each of the two reaction steps required. The lead conjugate V_N-R_6C showed high antimicrobial potential on over 50 clinical isolates of linezolid- and/or vancomycin-resistant enterococci (VRE, LVRE; Figure 2). The higher antimicrobial activity was also demonstrated by improved killing kinetics against selected strains. Radiolabeling with ^{125}I enabled the *in vivo* determination of the pharmacokinetics in SWISS mice by molecular imaging and biodistribution studies. In comparison to unmodified vancomycin, an altered biodistribution profile was observed. While vancomycin is rapidly excreted by the kidneys, the polycationic-conjugate shows a hepatobiliary excretion profile. *In vitro* biocompatibility studies on liver (Hep-G2 and primary human heptocytes), kidney (HEK-293) and human red blood cells as well as a murine toxicity study showed no relevant toxicity. Further ADME screening, including serum, plasma and S9 liver microsome stability and metabolite profiling, CYP-inhibition and hERG-channel blocking emphasized drug-like properties. The *in vivo* efficacy of the conjugate was confirmed by VRE infection models in *G. mellonella*. Additionally, a systemic vancomycin-susceptible *S. aureus* murine infection model resulted in a significant reduction of CFU in the liver. The transfer to murine VRE infection models is still ongoing. In conclusion, these results highlight the drug-like properties of the lead conjugate V_N-R_6C. The combination of low toxicity and high *in vivo* efficacy of the hexa-arginine vancomycin conjugate makes it well suitable for further preclinical and potential clinical development as new antibiotic against multidrug-resistant bacteria.

Figure 1. Structure of V_N-R_6C. First, the vancomycin-linker precursor is synthesized using the hetero-bifunctional linker SMCC. Second, the polycationic hexa-arginine is coupled to the maleimide via a thioether bond.

Figure 2. Minimal inhibitory concentration (MIC) of vancomycin and the polycationic-peptide conjugate V_N-R_6C on over 50 VRE clinical isolates, including *vanA*- and *vanB*-type resistant *E. faecalis* and *E. faecium*. The dotted lines indicate the concentration (4 mg/L) at which bacterial strains are considered to be resistant towards vancomycin.

Fig. 1

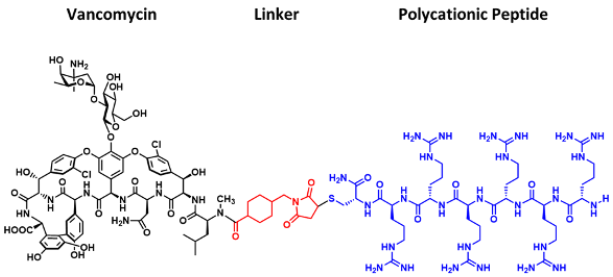
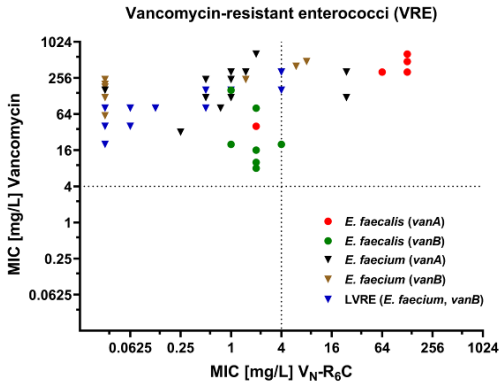


Fig. 2



P-2-125

Nasal application of staphylococcus lugdunensis for eradication of Staphylococcus aureus – a first-in-man microbiome intervention study

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Antibiotic-resistant bacterial pathogens (ARBPs), like methicillin-resistant *Staphylococcus aureus* (MRSA), cause numerous severe and often deadly infections, particularly in hospitalized and immunocompromised patients, with an estimated 1.27 million deaths annually worldwide. Treatment options for ARBPs are limited, with available antibiotics often only bacteriostatic, damaging the microbiome, and increasing resistance development. Infections commonly stem from ARBP clones already present in the patient's microbiome. Thus, the application of beneficial human commensal bacteria producing antimicrobial compounds could become an effective tool for the targeted decolonization of specific ARBPs but also their antibiotic-sensitive strains within a microbiome.

Our promising novel approach utilizes the nasal commensal *Staphylococcus lugdunensis*, which produces an antimicrobial called lugdunin, to specifically target *Staphylococcus aureus*. Research has demonstrated that *S. lugdunensis* effectively eradicates *S. aureus* in both in vitro co-cultures and in vivo nasal colonization models in cotton rats. Furthermore, two independent studies have shown that humans naturally colonized by *S. lugdunensis* exhibit an about six-fold reduced risk of being also colonized by *S. aureus*, underscoring the great potential of lugdunin-producing *S. lugdunensis* for preventive and decolonizing applications to avoid subsequent systemic *S. aureus* infection of the host.

A master cell bank (MCB) of *S. lugdunensis* IVK28, a fully characterized strain isolated from a healthy individual, has been established under GMP conditions. From this MCB the

strain was manufactured under GMP to an applicable product, which will be applied within a forthcoming proof-of-concept, first-in-human clinical trial at the Institute for Topical Medicine (ITM) within the Clinical Trial Platform (CTP) of the Cluster of Excellence "Controlling Microbes to Fight Infections" (CMFI). This monocentric trial will use a conservative 3+3 dose escalation model, prioritizing safety and gathering reliable data on nasal colonization and the reduction of *S. aureus*. Increasing doses of *S. lugdunensis* will be applied to the nasal cavities of healthy volunteers, aiming to identify a safe, well-tolerated dose while monitoring colonization impact on the nasal microbiome.

Following a successful phase I trial, phase II and III trials are anticipated. The ultimate goal is to develop a widely available and easily applicable treatment for at-risk patients worldwide, offering a novel approach to combating bacterial infections.

P-2-126

Diversity of the vanB transposon in CT71 vancomycin-resistant *Enterococcus faecium* isolates from Germany

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Introduction: The increasing prevalence of vancomycin-resistant *Enterococcus faecium* (VREfm) is concerning in Germany. We demonstrated the inter-regional clonal expansion of the CT71/*vanB* subpopulation of ST117 VREfm in patients upon hospital admission.[1] Furthermore, previous studies showed that clonal VREfm isolates frequently differed in their Tn*1546* type harbouring *vanA*.[2] This study aims to investigate the genetic composition and insertion site of the *vanB* transposon in both CT71 and non-CT71 VREfm collected from six different German cities.

Methods: Long-read sequencing was performed for 16 CT71 and non-CT71 VREfm using the MinION platform (Oxford Nanopore Technologies) while short-read sequencing data were already available.[1] Hybrid assemblies were performed with Unicycler v.0.5 and the *vanB* cassette was annotated using the Bakta pipeline.[3] The *vanB* cassette location was determined by comparing the upstream and downstream regions of the *vanB* cassette between CT71 VREfm and vancomycin-susceptible *E. faecium* (VSEfm). SnapGene software (www.snapgene.com) was used for the alignments.

Results: By cgMLST (Ridom SeqSphere+) the CT71 VREfm differed by up to 13 alleles. All CT71-VREfm isolates harboured one copy of the *vanB* cassette in a *Tn1549-like* transposon that was integrated into the chromosome, between *sufB* (Fe-S cluster assembly protein) and a site-specific intergrase. Comparative analysis revealed that the Tn*1549*-like was inserted at the same position in all CT71 isolates and was highly similar (Fig. 1), query coverage >97% and identity >99%. An insertion of IS*Efa11* upstream of *vanY* was detected in a CT71/*vanB* isolate. SNPs were found throughout the *vanB* cassette, including mutations in the sensor kinase *vanS* or the regulator *vanR*, which could affect gene expression. Nonetheless, these SNPs did not establish a specific pattern for delineating closely related CT71 isolates from various German cities. The non-CT71 VREfm isolates had the same insertion site and also similar composition of the *vanB* cassette.

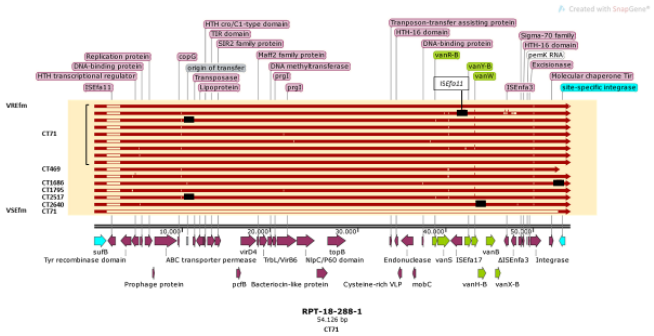
Conclusions: In conclusion, the insertion site of the *vanB* cassette was identical in the CT71, and its composition was highly similar, and therefore insufficient to delineate the closely related CT71 VREfm isolates circulating in Germany. Similar were the findings comparing the endemic CT71 clone to non-CT71 VREfm highlighting the importance of an in-depth investigation and comparison of closely related genotypes, such as CTs within the same ST.

References:

[1]Xanthopoulou et al. JAC 2020 1;75(10):2743-2751
[2]Brown et al. AAC 2001 45(4):1309-11
[3]Schwengers et al. Microb Genom. 2021 7(11):000685

Figure 1: Comparison of the insertion site and the composition of the *vanB* cassette of CT71 and non-CT71 VREfm. A CT71 VSEfm (short-read assembly) was also included to define the position of the *vanB* cassette; the genes flanking the region are coloured blue. The *van* genes are coloured green and black rectangles represent insertions.

Fig. 1



P-2-127

Compound V9 against Salmonella"s virulence - on the inhibition of T3SS-2 by the synthetic small molecule

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The enteric pathogen *Salmonella enterica* serovar Typhimurium utilizes a variety of effector proteins to invade and multiply within host epithelial cells, and can spread to reach other tissues, ultimately leading to a systemic infection. Specialized secretion systems facilitate the secretion of these effector proteins into host cells, playing a critical role in the bacterium's virulence. One of these systems is the Type III Secretion System 2 (T3SS-2), which is encoded on the *Salmonella* Pathogenicity Island 2 (SPI-2).

SPI-2 is essential for intracellular survival, particularly within macrophages, as it enables *Salmonella* to evade the host immune response by manipulating vesicle trafficking and maintaining the integrity of the *Salmonella*-containing vacuoles (SCV), allowing the bacterium to persist within the host. Blocking the SPI-2-encoded T3SS-2 could prevent systemic infections. At the core of T3SS-2 is the major export apparatus protein SsaV, which plays a crucial role in the translocation of bacterial effector proteins into host cells. SsaV"s function is critical for *Salmonella*'s ability to establish infection and proliferate intracellularly.

We identified a novel small molecule SsaV inhibitor (V9), through virtual screening. We confirmed V9"s *in vitro* binding to SsaV using MST, followed by a functional characterization using a split-luciferase-based host cell invasion assay, where V9-treated *Salmonella* significantly reduced secretion of

effector proteins into HeLa cells. Western blotting further confirmed the impaired effector protein secretion in the presence of V9. We recently focus on identifying V9"s binding pocket using crosslinking experiments to covalently link the V9 to SsaV in combination with MS and molecular dynamics simulations.

Lastly, mRNA sequencing will help analyze changes in gene expression in both *Salmonella* and host cells following V9 treatment, providing valuable insights into how inhibition of SsaV affects virulence-associated genes and other key signaling pathways. This approach will also enable us to better understand the consequences of bacterial invasion on the host cell's defense mechanisms, revealing how the pathogen alters immune responses and intracellular survival strategies.

This inhibition mechanism offers a strong basis for developing anti-virulence therapies to treat *Salmonella* Typhimurium infections by specifically targeting the critical SPI-2/T3SS-2 pathway, without contributing to antibiotic resistance.

P-2-129

Immune phenotyping in very old or frail CLL patients treated with Acalabrutinib

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Common complications of chronic lymphocytic leukemia (CLL) are viral and opportunistic infections. Current treatment approaches however include treatment with targeted therapeutics, such as Bruton-Tyrosine kinase inhibitor (BTKi) and Venetoclax (a B-cell lymphoma [BCL-2] inhibitor).

Monotherapy with Ibrutinib has shown to positively impact particularly adaptive and also innate immune cell subpopulations. Immunological studies have shown a reversed immunosuppressive microenvironment in CLL under Ibrutinib treatment with activated T cells and decreased T-cell exhaustion markers. Inhibiting BTK impacts B-cell-, monocyte-, and macrophage-signaling. Regarding B cell immunity, it causes a reduction of B cells without completely eliminating CLL cells.

Acalabrutinib has a higher BTK selectivity than Ibrutinib. At the same time, it does not inhibit the epidermal growth factor receptor (EGFR), interleukin-2-inducible T-cell kinase (ITK) or TEC, resulting in fewer cardiac side effects. The CLL-FRAIL phase 2 study evaluates the efficacy and safety of acalabrutinib monotherapy in elderly patients (≥ 80 years and/or increased frailty).

We will perform immune surveillance to investigate the immune reconstitution with acalabrutinib monotherapy, to gain further insights into immune kinetics and their impact on infections in this subgroup of patients. To this end we will correlate B-/T-cell kinetics with clinical, laboratory and genetic risk factors. Samples were obtained before and during treatment at specific time points from 53 patients of the CLL-FRAIL study. We will perform flow cytometry analysis by using a comprehensive antibody panel including various markers of T cell exhaustion and activation. Analyses will be conducted in Q3/2024 and are currently prepared.

By further understanding of immune reconstitution, approaches can be tailored on an individual level in future and optimized infection prophylaxis can be adjusted upon the gained knowledge in future.

Short outlook and ideas for follow up studies: The here presented project is a pilot project. It aims to establish immune phenotyping for a structured accompanying program in the context of the CLL 18 study. Further CLL subpopulations will be investigated regarding their immune reconstitution, e.g., those receiving bispecific antibodies.

P-2-130

Sex-dependent variability of isoniazid and rifampicin serum levels in patients with tuberculosis

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Introduction: Drug-sensitive TB (DS-TB) is treated with isoniazid, rifampicin, ethambutol, and pyrazinamide. Factors like fast-metabolizing enzymes, malabsorption, and drug interactions can influence serum drug levels. Current TB treatment guidelines recommend weight-adapted dosing without considering sex differences. This study examines drug levels of isoniazid and rifampicin in TB patients treated between 2019-2023 at our center focusing on sex-specific aspects.

Methods: Patients diagnosed with TB and available serum levels of isoniazid or rifampicin between 2019-2023 were retrospectively identified. Serum levels were measured using liquid chromatography–mass spectrometry and high-performance liquid chromatography. Patients were stratified by sex and a linear regression mixed effect model was used to assess predictors for different serum levels.

Results: The study included 281 single therapeutic drug monitoring (TDM) measurements from 59 patients (28 women, 47.5%). Adverse effects were noted in 43.8% (42/96) of measurements in women and 29.5% (54/183) of measurements in men (p=0.03). For isoniazid, no sex-specific differences were identified. On the other hand, female sex was a significant predictor of higher rifampicin plasma levels (coefficient 4.16, 95% CI 0.74-7.59, p=0.009). Only 38.2% of rifampicin serum level measurements in male patients were within target range. Women displayed higher overall rifampicin serum levels than men (median 14.7 mg/ml vs. 7.1 mg/ml, p=0.04), although weight adjusted doses were not significantly different (median 10.0 mg/kg bodyweight vs. 9.8 mg/kg, p=0.56).

Discussion: Rifampicin levels were significantly lower in men compared to women, despite weight-adjusted dosing. Clinicians should consider TDM and potential sex differences when treating patients with TB.

Figure 1: Serum levels for isoniazid and rifampicin over time (A, D), stratified by gender (B, E) and weight adjusted dose stratified by gender (C, F). Dotted lines in figure panel A, B, D, and E represent target ranges of isoniazid and rifampicin

respectively. Repeat measurement from the same patients are connected by continuous lines in different colors (A, D). f = female; m = male.

Figure 2: Individual trajectories of drug levels in female and male patients (first column), their results (middle column) and consequence (right column) for isoniazid serum levels (A) and rifampicin levels (B). Colors indicate individual patients.

Fig. 1

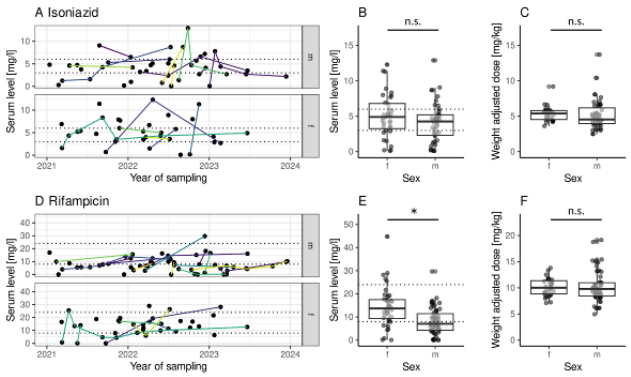
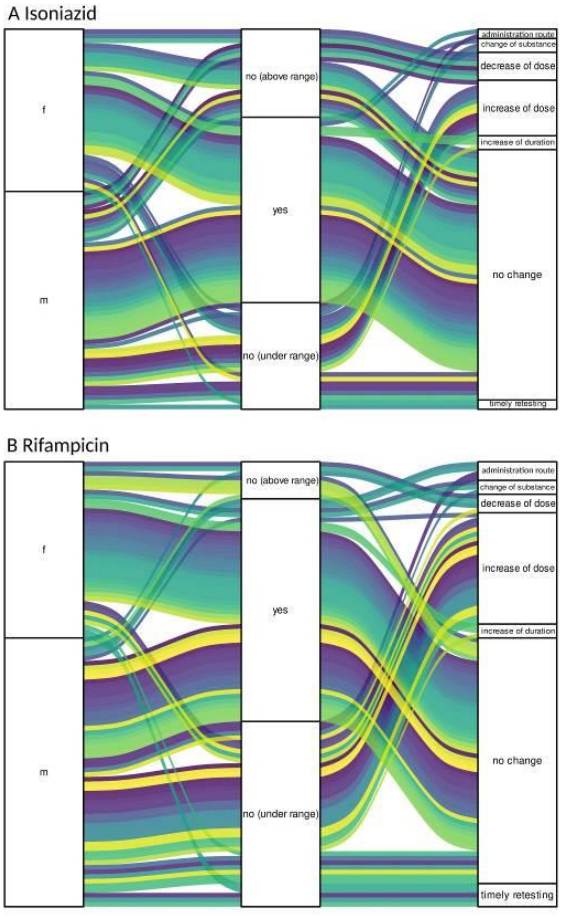


Fig. 2



P-2-131

Invasive fungal disease in chronic liver transplant failure – an underestimated burden

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Background & Objective: Invasive fungal infection (IFI) is a severe complication in organ transplant patients and a diagnostic challenge. To elucidate significance and occurence of IFI in chronic liver transplant failure, comprehensive histo- and molecularpathological analyses were performed in cooperation with the DZIF tissue bank at the Institute of Pathology of Heidelberg University Hospital.

Methods: FFPE tissue samples and pathological findings from all explanted liver transplants due to chronic transplant failure from the Heidelberg University Hospital (1991-2021, ≥90-day graft survival) were reexamined. Additional stainings with periodic acid-Schiff and Grocott methenamine silver were used in light-microscopic investigations to uncover occurrence, severity, and associated conditions of IFI. Molecular fungal species identification was performed chip-based by DNA hybridization.

Results: Light-microscopic examination revealed fungal infection in 41 (27.5%) of the 149 analyzed cases with 2/3 being newly specified. Female patients presented a slightly higher risk for IFI. Typically, the large bile ducts were affected, accompanied by acute inflammation with frequent abscess and concrement formation. In 35 cases, molecular identification of the fungal species was achieved. *Candida albicans* was the most common species (61%) with mixed infections in 14% of cases. The cohort included three autopsy cases from patients that died of septic multiorgan failure. For one case, a clear connection to the IFI causing species *Candida glabrata* could be demonstrated.

Conclusion: These data show the underestimated prevalence and high diagnostic and clinical relevance of IFI in chronic liver transplant failure. Adapted preparation protocols, molecular pathological analyses as well as medication guidelines are urgently needed to identify and prevent chronic transplant organ failure caused by IFIs. The contribution of structured registries and qualified biobanking is evident in such retrospective (but also prospective) studies and improves diagnostics and development of adequate therapeutic strategies. In future analyses, this approach will be transferred to other solid organ transplants like the kidney.

P-2-132

Milker's nodule after calf bite in a 23-year-old patient

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Question: Milker's nodule is an occupational viral skin disease caused by pseudocowpox virus, a virus of the genus parapoxvirus, within the poxvirus family. It is transmitted from infected cows to humans, usually through contact with the

animal's udder or nose [1]. We present the case of a 23-year-old female patient who developed a milker's nodule infection following a calf bite.

Methods: The patient presented to her GP with a hard nodule on her right index finger. She had been bitten by a calf at the same site 4 weeks (29 days) earlier. The wound had initially healed within a few days without any sequelae. The nodule was approximately 1 cm in size with a whitish border and central hemorrhagic erythema. She also had a swollen supraclavicular lymph node on the right side but no fever. The nodule puncture remained dry and a swab showed no bacterial growth. Empirical antibiotic treatment with amoxicillin/clavulanate was started. 6 days after starting antibiotics, she developed a macular pruritic rash starting on both distal extremities and spreading to the trunk. The antibiotic treatment was discontinued on the assumption that it was a drug related rash. A swab was sent to the Robert Koch Institute for analysis of poxviruses in view of the previous animal contact.

Results: The PCR for parapoxviruses showed a positive result. The sample was specified as pseudocowpox virus by sanger sequencing of the B2L gene. Parapox-specific IgM and IgG were detected by immunofluorescence assay in a serum sample taken 7 weeks after the bite. In context of the diagnosis of pseudocowpox, the rash is most likely to be classified as infection-induced erythema multiforme.

Conclusions: Although milker's nodules are a self-limiting condition, it is important to make the correct diagnosis as misdiagnosis can lead to invasive diagnostic procedures and overtreatment.

References:

[1] Poudel GP, Agrawal S, Dhakal S: Milker's nodule: An under-reported and under-diagnosed occupational infection. Clin Case Rep. 2020 Apr 14;8(7):1162-1165. DOI: 10.1002/ccr3.2850.

P-2-133

Somatic deficiency of human CBL in leukocytes impairs B cell but not T cell development and function and predisposes to severe infections

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The casitas B lineage lymphoma proto-oncogene (CBL) promotes positive selection and antigen responses in murine T lymphocytes by ubiquitinating ZAP70. However, murine CBL and CBLB are redundant for ubiquitination of SYK and regulation of B cell receptor signaling in B cells. We studied lymphocyte development, maturation and function in patients with somatic homozygosity for *CBL* loss-of-function variants in leucocytes. Surprisingly, human CBL is largely redundant for the development and function of human T cells. Conversely, CBL is critical for B cell development and function. Patients with somatic, hematopoietic CBL deficiency have a 10-fold increase in transitional B cells during childhood and are susceptible to bacterial infections. CBL deficiency impairs B cell maturation in a cell-intrinsic manner through reduced apoptosis and dysregulated B cell receptor signaling. These defects enhance survival and differentiation of autoreactive B cell clones, impair B cell tolerance, and underlies the production of autoantibodies. Our study surprisingly reveals the critical role of human CBL in B cells and its redundancy in T cells.

P-2-134

Assessment of protracted courses of EBV-associated infectious mononucleosis – First data from the IMMUC cohort study

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Background: Epstein-Barr virus (EBV) is a ubiquitous human gamma-herpesvirus that infects over 90% of the global population. Primary infection in children, adolescents and young adults may cause infectious mononucleosis (IM). Although typically self-limiting within one month, IM can result in post-viral syndromes, including severe forms of myalgic encephalomyelitis/chronic fatigue-syndrome (ME/CFS). The IMMUC Cohort Study aims to identify biomarkers and risk factors for post-viral syndromes in young people with EBV-associated IM (EBV-IM). This subproject describes the proportion of participants experiencing protracted courses following EBV-IM using a symptom diary.

Methods: A total of 150 participants aged 0-24 years are to be recruited in the IMMUC Cohort Study. During the current study period, 25 participants have been enrolled (Table 1: Age and sex distribution), the recruitment is still ongoing. One of 25 participants (4%) dropped out after the first visit.

The inclusion criteria required the presence of at least two of five typical IM symptoms: fever, fatigue, tonsillopharyngitis, lymphadenopathy, or splenomegaly lasting for ≤ 1 month at time of recruitment, along with serological evidence of a recent EBV infection. Complex clinical (questionnaires, olfactory test, hand grip strength measurement, passive 10-minute standing test) and laboratory (blood, saliva) data were collected at three consecutive visits (TR = time of recruitment, T3 = 3 months after IM onset, T6 = 6 months after IM onset) within 6 months after IM onset (T0). Furthermore, patients were provided with a diary to track data on bedridden status, housebound status, participation in kindergarten/school/work and ability to engage in sports weekly from T0 to T3, and monthly from T3 to T6. Additionally, at TR, patients were asked to retrospectively report on these variables for the period T-3 (last three months before EBV-IM onset) once, which corresponded to their baseline condition.

Results: To quantify disease protraction, the time taken for participants to return to their baseline condition was calculated based on the symptom diary (Fig. 1: Disease Protraction). At T3, 3 out of 14 females (21.4%, mean age 18.0 ± 2.65 years) and 4 out of 10 males (40%, mean age 15.25 ± 4.50 years) had not returned to their baseline condition, resulting in a total of 7 out of 24 participants (29.2%, mean age 16.43 ± 3.82 years). At T6, 2 out of 14 females (14.3%, mean age 17.5 ± 3.54 years) and 1 out of 10 males (10%, aged 15 years) had not returned to their baseline condition, totaling 3 out of 24 participants (12.5%, mean age 16.67 ± 2.89 years).

Conclusion: Future analyses of the IMMUC Cohort data will determine whether the measured laboratory parameters, collected questionnaires, and conducted functional tests provide indications of risk factors or serve as predictive

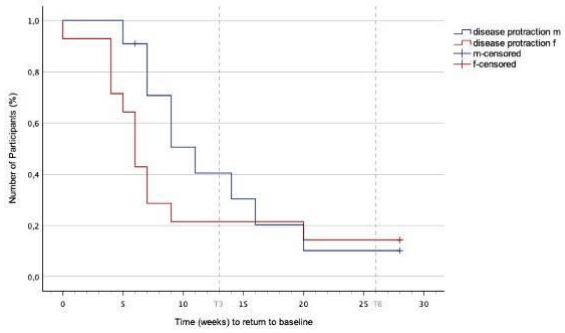
biomarkers for protracted courses up to post-viral EBV syndromes.

Fig. 1

Age groups	Male (n/total %)	Female (n/total %)	Total (n/total %)
Children 0-11 years	2/8%	2/8%	4/16%
Adolescents 12-17 years	5/20%	7/28%	12/48%
Adults 18-24 years	4/16%	5/20%	9/36%
Total	11/44%	14/56%	25/100%

Table 1: Age and sex distribution

Fig. 2



P-2-135

Patients with cardiovascular infections report a low HRQL one year after diagnosis – preliminary data of the prospective DERIVE cohort

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Question: Even though the assessment and influence on treatment options of patients health related quality of life (HRQL) has become increasingly important in the medical field in recent years, data on HRQL of patients with life threatening bacterial infections are rare. For cardiovascular infections, a few studies have recognized a reduced HRQL after cardiac surgery for endocarditis as well as a high number of patients suffering of post-traumatic stress disorder after surgery (Verhagen et al. 2009, Nayak et al. 2011, Saha et al. 2022). However, larger cohort studies on patients managed conservatively as well as surgically are lacking.

Methods: For this study data of 712 patients included in a German multicenter cohort study on cardiovascular infections (DERIVE study) between 11/2019 and 09/2023 have been analyzed. In this prospective cohort study patients with endocarditis, left-ventricular-assist-device (LVAD) infections and vascular prothesis infections are observed over a 12 month period, with follow up telephone calls 3 and 12 months after diagnosis. Besides baseline, clinical and microbiological data, data on the HRQL after 365 days post diagnosis is collected. HRQL is assessed with the EQ-5D-5L questionnaire and statistics were performed using IBM SPSS.

Results: Of the 712 patients included in the study, 307 (256 patients with infectious endocarditis, 27 patients with vascular prothesis infections, 24 patients with LVAD infections and) had reported their HRQL in the day 365 follow up. Median age was the highest for patients with endocarditis (67 years), while patients with LVAD infections had the highest median Charlson Comorbidity Index (4). One year after the diagnosis, around 15% of endocarditis patients reported to have moderate to severe problems in caring for themselves and over 30% in fulfilling daily activities. Moreover, 15% were suffering from moderate to severe anxiety. Similar results could be observed for patients with vascular prothesis infections, however, they reported a high impairment in their mobility as well. On the subjectively rated visual analog scale of the EQ-5D-5L (EQ VAS: values 0 – 100) endocarditis patients reported their HRQL in median around 69, while patients with vascular prothesis infections reported a median value of 52.

Conclusions: Patients with cardiovascular infections suffer to a great extend from a reduced HRQL one year after diagnosis. Compared to a general German population with similar age (Grochtdreis et al. 2019, Marten et al. 2021) patients with cardiovascular infections primarily report a low HRQL in the dimensions of self care, usual activities and anxiety and as well as on the EQ VAS. Further analyses on factors correlating with a decreased HRQL as well as on the development of the HRQL of these patients over the disease course are ongoing.

P-2-136

Carbapenem resistant Melioidosis in a traveller returning from Thailand

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Background: Melioidosis is a common cause of pneumonia and septicaemia in South-East-Asia and Australia, but is rarely diagnosed in travelers returning from endemic areas. While *Burkholderia pseudomallei*, the gram-negative bacteria causing melioidosis, is naturally resistant to a range of antibiotics, carbapenems are the gold-standard for severe infections. Ceftazidime is used for non-critically-ill patients. Carbapenem resistances have only rarely been described.

Case: A 57-year-old female with no known pre-existing condition presented to our outpatient clinic with fever up to 39,7 °C, headache and dyspnea. One week prior to consultation she had returned from a seven-week travel to Thailand and Cambodia, where she spent majority of her time in Bangkok, Phuket, Koh Samui and Siem Reap. Laboratory results revealed CRP-elevation of 218 mg/L, white blood count of 11.0 ´ 109/L and an increased blood sedimentation rate of 81 mm. A chest X-ray showed a consolidation in the left upper lung. An empirically initiated treatment with amoxicillin and clavulanic acid for suspected community acquired pneumonia did not lead to improvement of the patient"s clinical condition. The radiographic finding in the chest X-ray presented as a solid consolidation with a caverna in a consecutive CT-scan. The patient was admitted to our clinic and antibiotic therapy was escalated to meropenem. Notably, fever, dyspnea and headache continuously worsened and CRP increased under meropenem treatment. In a bronchoalveolar lavage *Burkholderia pseudomallei* was identified. Due to unresponsiveness to meropenem antibiotic treatment was changed to high-dose Ceftazidime. Subsequently, the

patient"s clinical condition rapidly improved and laboratory parameters normalized. A control CT-scan showed regression of the cavernous consolidation in the left upper lung.

Discussion: Carbapenems are the treatment of choice for severe cases of melioidosis, in particular in cases of disseminated melioidosis and of neurologic involvement.

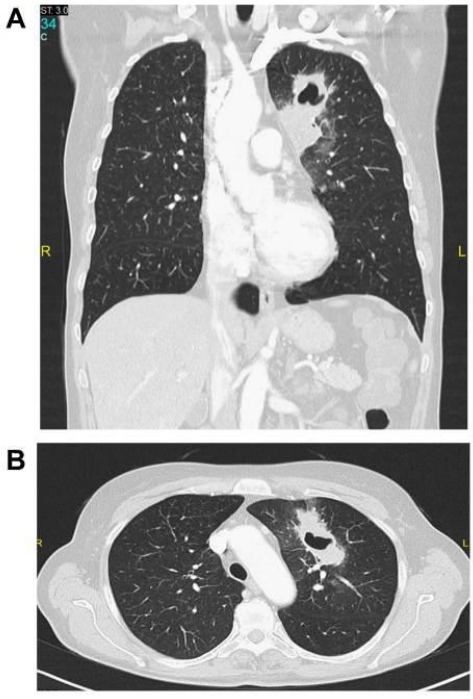
Natural resistances of *Burkholderia pseudomallei* towards carbapenems have not been reported previously, but carbapenem resistances occasionally evolves during treatment. Since our patient did not have any history of previous carbapenem treatment and did not respond to meropenem since the beginning of therapy, this case represents a rare case of natural carbapenem resistance.

Previous studies showed superiority of carbapenems compared to ceftazidime in treatment of severe melioidosis. Interestingly, this is the first reported case in which a ceftazidime treatment regimen was superior to carbapenems.

Carbapenem resistance represents a public-health threat, particularly for infection with *Burkholderia pseudomallei*, which is naturally resistant to most other antibiotics.

This case emphasizes that in carbapenem resistant melioidosis, susceptibility to ceftazidime should be tested and a treatment regimen with high-dose ceftazidime can be considered.

Fig. 1



P-2-137

Systematic screening reveals high prevalences of viral hepatitis B, C, D, and HIV antibodies in a medical practice specialized in addiction medicine in Germany

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Background: Intravenous drug users have high prevalences of infectious diseases, with frequent co-infections being human immunodeficiency virus (HIV) and viral hepatitis. Especially testing for hepatitis D virus (HDV) infection – which occurs as a co- or superinfection with HBV – has been insufficient, and awareness is still lacking. Of the ~254 million people worldwide infected with hepatitis B virus (HBV), about 5% are estimated to also be affected by HDV. In Germany, the described prevalence of anti-HDV in HBsAg-positive patients ranges from 0 to 7.4%. While there are two major cohort studies on HDV prevalences in HIV-positive individuals, only few studies focus on people who inject drugs. To more efficiently target populations of high risk for these infections, more data on prevalences in different settings is needed.

Methods: This study was conducted in a practice specialized in addiction medicine, which focuses on opioid-addicted patients. We screened patients for antibodies against HBV, hepatitis C virus (HCV) and HIV and conducted follow-up diagnostics and treatment according to current guidelines. All anti-HBcAg-positive patients (current or previous HBV infection) were also tested for anti-HDAg, using ELISA (DiaSorin) and in parallel a point-of-care test based on a lateral flow assay that we have recently developed. When patients were anti-HDAg-positive, we tested for HDV RNA to distinguish between ongoing and past infection.

Results: At the time of abstract submission (October 2024), 376 patients were included, of which 272 were enrolled in the opioid substitution program. 4 patients (1.1%) were HBsAg-positive (ongoing HBV infection) and 110 patients (29.3%) were anti-HBcAg positive with seroconversion to anti-HBsAg (past HBV infection). Among patients with prior HBV exposure, 16 (14.0%) had anti-HDV antibodies, and 1 (0.9%) had active HDV infection. Of the patients with anti-HDV antibodies, 4 (25.0%) were co-infected with HIV. 243 of all patients (64,6%) were positive for anti-HCV, of which 62 (25.5%) had detectable viral loads. 45 of all patients (12.0%) had anti-HIV antibodies, of which 4 patients (8.9%) had detectable HIV RNA. More detailed analysis including clinical courses and co-morbidities is ongoing.

Conclusion: We found high prevalences of antibodies against all tested viruses. The particularly high anti-HCV prevalence was in line with previous studies. While data is scarce regarding anti-HDV in Germany, in this setting we found a distinctly higher prevalence than previously described. Especially anti-HIV antibodies coincided frequently with anti-HDV antibodies in patients. These findings highlight the need for more frequent and systematic screening for HBV, HCV, HDV and HIV, especially in settings with high-risk patient groups, and especially if risk factors persist. Moreover, consistent screening at the first patient contact and prompt HBV vaccination (which also confers immunity against HDV) may be critical.

P-2-138

Analysis of HEV infection rates in immunocompetent MPGN patients compared to controls

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Background: Hepatitis B and C, are well-known causes of membranoproliferative glomerulonephritis (MPGN). Recently, hepatitis E virus (HEV) infections in immunosuppressed patients have been linked to the development of MPGN. This study aims, to investigate a possible association of HEV infections and MPGN in immunocompetent individuals.

Methods: A retrospective cohort of 73 MPGN patients was tested for anti-HEV IgG and IgM. A cohort of 1000 random blood donors and a cohort of 73 age- and sex-matched-pair blood donors served as control groups.

Results: In the MPGN cohort, 21 patients (29%) tested anti-HEV IgG positive (1.4%), while in the random blood donor cohort, only 17% of patients (n=166) tested IgG positive ($p<0.01$) and 25% of patients in the matched-pair cohort of 73 blood donors were positive for anti-HEV IgG ($p=0.71$). MPGN patients were significantly older and more often males compared to the unmatched blood donor cohort. In the MPGN cohort, anti-HEV IgG positivity was found in 36% of males (17/47) but only 15% (4/26) of females ($p=0.05$). Anti-HEV IgG positive MPGN patients were older when compared to anti-HEV IgG negative MPGN patients, albeit the difference did not reach statistical significance (median 63 years vs. 53 years, $p=0.06$).

Conclusion: Although anti-HEV IgG positivity is more common in patients with MPGN compared to healthy blood donors, this difference does not hold up when a cohort of blood donors of similar age and gender is studied. Thus, previous HEV infections are not a relevant trigger for the development of MPGN in immunocompetent patients.