

Abstractbook

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S-1-2

Development of inhibitors of *Pseudomonas aeruginosa* LasB for the treatment of acute lung and eye infections

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Question: *P. aeruginosa* is a major cause of acute and chronic infections, with rising antibiotic resistance limiting treatment options. As an alternative to bactericidal agents, antivirulence strategies aim to disarm the pathogen without exerting selective pressure for resistance. The zinc metalloprotease LasB is a key virulence factor that disrupts epithelial barriers and modulates host immune responses. Targeting LasB could mitigate tissue damage and enhance host defense, particularly in infections of the lungs, eyes and wounds.

Methods: LasB inhibitors were developed using structure-based design and synthesized via concise organic routes, enabling the generation of ~1k compounds. Inhibition was assessed using enzyme- and cell-based on- and off-target assays, and selected compounds underwent in vitro ADMET profiling. High-resolution co-crystal structures with LasB were obtained to guide optimization. Lead candidates were evaluated in pharmacokinetic studies in mice using various administration routes (IT, IP, IV, SC, inhalation), with lung exposure as a key parameter. Efficacy was demonstrated in murine infection models of the lung and eye.

Results: Among the synthesized candidates are ~570 inhibitors featuring a phosphonate moiety as the zinc-binding group, which we strongly favor due to its excellent biological performance. The compounds are small (<500 Da) and can be synthesized through straightforward and cost-effective routes. Our optimization strategy, based on iterative rounds of organic synthesis and biological evaluation, has been complemented by high-resolution co-crystal structures of LasB in complex with 70 of our inhibitors. For selected compounds, we observed no drug-drug interactions and no mutagenic potential, alongside a favorable safety profile with no red flags (including in vitro CEREP panel results and tolerability in mice). The compounds also exhibit excellent ADMET characteristics—such as lack of toxicity against human cell lines and strong metabolic stability—as well as potent activity against LasB derived from various clinical isolates. Promising in vivo PK profiles were observed in mice following different routes of administration, including excellent retention in plasma, lung tissue, ELF, and BALF, along with renal excretion. Importantly, in vivo efficacy was demonstrated in acute murine models of eye and lung infection, where co-administration with an antibiotic led to reduced bacterial burden, attenuated inflammation, improved clinical scores, and increased survival.

Conclusions: Our work provides novel pathoblockers targeting LasB with high potency, excellent safety and selectivity profiles, and demonstrated in vivo efficacy in acute lung and eye infection models in mice. Consequently, these inhibitors show strong translational potential. The next goal is to prepare a comprehensive preclinical data package.

S-1-3

Development of Corallopyronin A and Oxfendazole for the treatment of filarial infections

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Question: The goals of the WHO 2021-2030 Roadmap 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. Similarly, safe and effective drugs are required for the treatment of the filarial diseases loiasis and mansoniellosis as well as the intestinal helminth infection trichuriasis. Here, we present the development of Corallopyronin A (CorA), which kills the essential *Wolbachia* endosymbionts present in filariae, as well as the repurposed broad-spectrum veterinary anthelmintic oxfendazole (OXF).

Methods: Efficacy of CorA against filarial nematodes was tested in surrogate animal models and the PK/PD of CorA was assessed. Non-GLP 7-day toxicity studies in rats and dogs and GLP-toxicity studies in rats were performed. OXF is currently being tested in an adaptive phase 2 clinical basket trial in patients with onchocerciasis, loiasis, mansoniellosis and trichuriasis.

Results: In the *L. sigmodontis* gerbil model, CorA depleted >99% of *Wolbachia* and was macrofilaricidal with 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 CorA was confirmed in mice implanted with *Onchocerca ochengi*. Toxicity studies in rats and dogs demonstrated no prohibitive safety issues. PK/PD data modeling predicted a safety margin for the predicted human efficacious dose that supports a clinical trial. CorA production has been transferred to a GMP producer (Phyton, Hamburg).

The efficacy and safety of OXF is being assessed by the eWHORM consortium using a "basket trial" in onchocerciasis, loiasis, mansoniellosis and trichuriasis patients in four sub-Saharan African countries. The adaptive trial uses a harmonized master protocol and includes an interim analysis to drop non-efficacious treatment arms and allocate additional study participants to efficacious treatment arms or initiate a new treatment arm. This patient-centric approach allows the inclusion of co-infected patients and the detection of country-specific differences in efficacy. OXF is

currently administered using a 5-day regimen of 400mg or 800mg per day to patients with filariasis. Treatment of trichuriasis patients in Tanzania with a 1 or 3-day oxfendazole regimen has already been completed and data cleaning and analysis are ongoing.

Conclusions: CorA is a promising novel macrofilaricidal candidate in late preclinical/clinical development and the only 2nd generation anti-Wolbachial with a mode of action previously demonstrated to be safe and well tolerated. Oxfendazole is a pan-nematode macrofilaricidal candidate, which could also be used in combination with anti-Wolbachials, to improve drug efficacy and shorten treatment regimens.

S-1-4

Efficacy of Corallopyronin A against staphylococcal infections is based on a fC_{max}/MIC PK/PD driver

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Corallopyronin A (CorA), a natural product from myxobacteria currently under development against filariasis.¹ has beneficial *in vitro* ADME properties² and is active against *Staphylococcus aureus* with a favourable resistance profile.³ Previously, CorA was effective in the neutropenic thigh and lung infection models against methicillin-resistant *S. aureus* (MRSA). Moreover, we observed high concentrations of CorA in bone tissue.⁴ This opens the perspective for development of CorA in the indication of osteomyelitis. Given these properties and in light of the antimicrobial resistance crisis, underlined by the updated priority pathogens list of the WHO,^{5,6} we aim to qualify CorA as treatment option against staphylococcal diseases as a secondary indication. Several PK/PD indices have been established to understand which pharmacokinetic (PK) behaviour drives a pharmacodynamic (PD) effect.⁷ To inform the dosing regimen needed for maximal efficacy, we determined the PK/PD driver of CorA against MRSA in a neutropenic thigh infection model systematically by an in-depth dose fractionation study using the intravenous (IV) and the peroral (PO) routes. At the same time, we collected plasma samples at different time points from the infected animals to match them with our results from previous PK studies and to accurately calculate C_{max} , exposure and determine the %T above the *in vitro* determined minimal inhibitory concentration (MIC). Non-linear PK behaviour was observed after PO administration, necessitating employing different dosing regimens to achieve similar exposures. For the IV route, these effects were less pronounced. Next, we determined the bacterial load reduction and aligned it with the PK parameters for every dose group. Finally, we found that efficacy of CorA is mainly driven by its C_{max} levels. Thus, CorA achieving high C_{max} levels with a PK/PD driver for fC_{max}/MIC of around 0.5 already led to a 3- \log_{10} unit reduction in bacterial burden compared to the vehicle-treated group. That was in a similar range as observed for the positive control levofloxacin. In summary, this study informs that a C_{max} -based dosing regimen as the PK/PD driver of CorA. Thus, in future studies, we aim to achieve high levels in bone tissue to qualify CorA for the indication of staphylococcal osteomyelitis.

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References:

(1) Schiefer et al., 2020, PLoS Negl Trop Dis

(2) Ehrens et al., 2022, Front Trop Dis

(3) Balansky et al., 2022, Antibiotics

(4) Rox et al., 2023, Pharmaceuticals

(5) WHO, <https://www.who.int/publications/i/item/9789240093461>

(6) Bertagnolio et al., 2024, Lancet Microbe

(7) Anbrose et al., 2007, Clin Infect Dis

S-1-5

UNITE4TB – DECISION: A BTZ-043 phase IIB dose-ranging trial in combination with Bedaquiline and Delamanid

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Introduction: BTZ-043, a DprE1-inhibitor with proven bactericidal activity in phase IIA trials is one of six novel drug candidates currently under evaluation in phase II trials within UNITE4TB, a large European-led TB clinical trials network.

Methods: UNITE4TB-DECISION is a Phase IIB, Open-Label, Randomized Controlled dose-ranging trial to define the safety, tolerability, pharmacokinetics, and exposure-response relationship of three doses of BTZ-043 (T) in combination with Bedaquiline (B) and Delamanid (D). In Tanzania and South Africa, adults participant with newly diagnosed drug-sensitive pulmonary tuberculosis were randomized into one of four treatment arms: BTZ-043 500mg (BDT_{500mg}), 1000mg (BDT_{1000mg}), 1500mg (BDT_{1500mg}), or Moxifloxacin 400mg (BDM) each added to bedaquiline and delamanid for 16 weeks. Sputum samples were taken weekly to measure the change in bacterial load by time to positivity (TTP) using the mycobacterial growth indicator tube system (MGIT). Safety was assessed through weekly electrocardiography, safety blood tests, vision testing, and

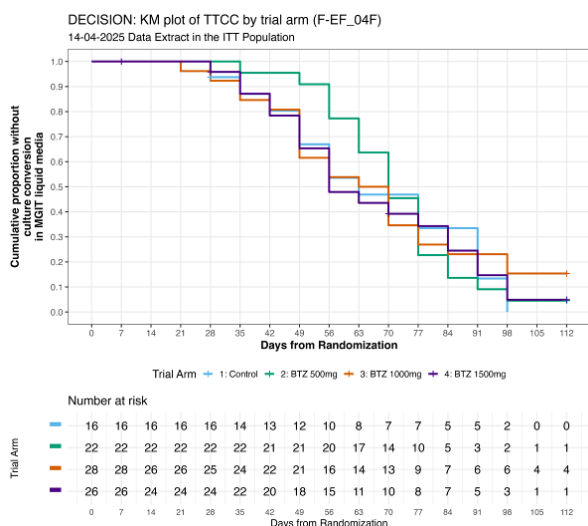
physical and neurological examinations. Pharmacokinetic measurements were done at week 4 and 8.

Findings: Between September 2023 and July 2024 183 individuals were screened for eligibility. 92 were enrolled and assigned to BDT_{500mg} (n=22), BDT_{1000mg} (n=28), BDT_{1500mg} (n=26) and BDM (n=16). 81.5% of participants were male, 90.2% were HIV negative. 64.1% had baseline grade 3+ smear results, and 77.2% had cavities. Three participants (one in each BTZ-043 arm) experienced serious adverse events: increased liver enzymes, hepatotoxicity and brief psychiatric disorder. Nine participants experienced grade 3 events. No grade 4 events occurred. The slope of increase of TTP over time was comparable between BDT_{1000mg}, BDT_{1500mg} and BDM;

the slope for BDT_{500mg} was lower than BDM, $p = 0.005$. Time to culture conversion showed consistent results (Figure).

Conclusion: Pending the final PK-PD model, we conclude that BTZ-043 1000mg is the appropriate dose based on tolerability, efficacy, and tablet burden for further clinical development in UNITE4TB.

Fig. 1



S-2-2

Evaluation of recurrent malaria after treatment with artemisinin-based combination therapies of uncomplicated or severe cases among children 10 years and younger in Ghana: A multicenter, randomized, open-label trial

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Background: Artemisinin-based combination therapies (ACTs) are the primary treatment for malaria. While efficacy

remains high in Africa, reports of artemisinin resistance in some African countries raise concerns, given the limited availability of alternative therapies. Continuous therapeutic efficacy monitoring is therefore critical. This study assessed the effectiveness of artemether-lumefantrine (AL), the most used ACT, artesunate-pyronaridine (AP), a recent addition to Ghana's treatment guidelines and parenteral artesunate followed by AL for the treatment of *Plasmodium falciparum* malaria.

Methods: A prospective interventional study was conducted between April 2024 and August 2025 at two sites in Ghana: Agogo (Ashanti Region) and Assin Foso (Central Region). Children aged ≤ 10 years with microscopy-confirmed uncomplicated or severe *P. falciparum* malaria were enrolled. Participants with uncomplicated malaria were randomized to receive AL or AP, while those with severe malaria were treated with parenteral artesunate followed by a full course of AL. All treatments were administered under direct observation, and participants were monitored for 42 days according to WHO therapeutic efficacy protocols. Adequate clinical and parasitological response (ACPR) was estimated using Kaplan-Meier analysis in R.

Results: In total, 450 children with uncomplicated malaria (AL: n=226; AP: n=224) and 113 with severe malaria were enrolled. No early treatment failures occurred in any treatment arm. For uncomplicated malaria, late treatment failures were more frequent with AL (n=56) than AP (n=28). Site-specific late failures were observed in 54/228 (23.7%) children at Agogo and 30/222 (13.5%) at Assin Foso. Uncorrected ACPR on day 28 was 85.8% (95% CI: 81.3-90.5) for AL and 97.3% (95% CI: 95.2-99.5) for AP, while day-42 ACPR was 74.9% (95% CI: 69.5-80.8) and 87.4% (83.1-91.8), respectively. Tolerability was comparable between the two groups. In severe malaria, uncorrected ACPR was 96.0% (95% CI: 92.3-99.9) at day 28 and 91% (95% CI: 85.8-96.8) at day 42. Results for PCR correction and detection of resistance markers will be ready by the end of the year.

Conclusions: The absence of early treatment failure suggests that artemisinin resistance is not yet established in Ghana. However, higher late treatment failure rates with AL indicate reduced post-treatment prophylaxis from lumefantrine. AP maintained high efficacy and may represent a suitable alternative ACT for national use. Parenteral artesunate followed by AL remains an effective regimen for severe malaria. The notable disparity in late treatment failure rates between the two study sites warrants further investigation to understand potential local epidemiological factors, such as transmission intensity, parasite genetics and patient characteristics.

S-2-3

An assessment of *Schistosoma haematobium* diagnostics comparing the performance of plasma qPCR, urine filtration microscopy and UCP-LF CAA using Bayesian Latent Class Analysis in a cohort of pregnant women from Lambaréné, Gabon

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Schistosomiasis, a neglected tropical disease caused by parasitic flatworms of the genus *Schistosoma*, is the second most impactful parasitic disease after malaria, affecting over 250 million people with significant morbidity and mortality. The WHO roadmap for NTDs by 2030 targets schistosomiasis elimination as a public health concern globally. Achieving this goal depends on developing novel, sensitive point-of-care (POC) diagnostics that improve disease mapping, treatment monitoring, and surveillance.

Current diagnostics are inadequate, especially for post-treatment surveillance and low-intensity infections. To address this gap, we compared different diagnostics using a clinical cohort of 379 pregnant women in the DFG HelmVit study in Lambaréné, Gabon. Each participant was tested using urine filtration microscopy, UCP-LF CAA, POC-CCA, and plasma qPCR on cell-free DNA. Our qPCR targets the Dral repeat (*S. haematobium*) or Sm1-7 (*S. mansoni*) and used a crude DNA extraction (Quantabio Extracta) requiring only 20µl input and minimal equipment. Though sensitivity was reduced, the qPCR still performed well as a predictor of infection, with potential for adaptation into a POC screening test. Importantly, the qPCR detected pre-patent infection, validated in *S. mansoni*-infected mice.

Given the lack of a gold standard, we applied Bayesian Latent Class Analysis (BLCA) to compare qPCR with other tests, estimate true prevalence, and assess test performance. This study underscores the need for more sensitive, specific, and scalable molecular diagnostics for schistosomiasis elimination. Further research should focus on POC molecular tools, including those for female genital schistosomiasis, praziquantel resistance detection, and xeno-surveillance.

S-2-4 Comprehensive Safety Evaluation of Takeda's Dengue Vaccine (TAK-003): Insights from Long-Term Clinical Trials and Postmarketing Surveillance

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The safety of TAK-003 was assessed in an updated integrated safety analysis (ISA) extending the previously published 36-month follow-up to 54 months post second dose. This analysis included healthy participants aged 4–60 years across five phase 2/3, double-blind, placebo-controlled trials, evaluated by baseline dengue serostatus and age. Here, 54 months of follow-up results are reported, including continued assessment of serious adverse events (SAEs), dengue-related hospitalizations, and outcomes in vulnerable subgroups (4–<6 and 50–60 years) for participants who received TAK-003 or placebo. In addition, a descriptive review of safety data up to 2 years post launch (February 18, 2025) captured in Takeda's Global Safety Database was conducted. ISA data were evaluated for 21,790 participants who received TAK-003 (seronegative, 4472; seropositive, 9808) or placebo (seronegative, 2063; seropositive, 4975). 54-month follow-up showed that SAE rates remained low and similar between TAK-003 (seropositive, 8.45%;

seronegative, 7.42%) and placebo (seropositive, 10.11%; seronegative, 9.02%). Dengue-related hospitalizations were consistently lower in TAK-003 (0.34%) than placebo (2.12%) recipients throughout the extended follow-up. There were no vaccine-related deaths, and no new safety signals were observed across age groups. Postmarketing data revealed 5480 case reports from 12.3 million doses distributed globally. The most frequently reported AEs were fever, headache, rash, myalgia, pain, vaccination site pain, arthralgia, pruritus, and fatigue. Most events were nonserious (91%). Newly identified safety signals resulting in label updates were anaphylactic reactions and transient events associated with vaccine viremia (eye pain, thrombocytopenia, petechiae).

In the 54-month ISA, TAK-003 was well tolerated, irrespective of baseline dengue serostatus and age, with no evidence of an increase in dengue disease severity. The TAK-003 postmarketing safety profile was largely consistent with clinical trial data. These combined data indicate that the benefit-risk profile of TAK-003 remains positive.

Funding: Takeda

S-2-5 Comparative immune profiling of T and B cells immune responses to Dengue, Zika and Chikungunya viruses: perspectives on climate-sensitive arboviral infections

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Question: Dengue, Zika and chikungunya viruses are infectious disease agents whose transmission vector, *Aedes* mosquito, thrives in warmer temperatures and has recently spread to previously cooler northern regions, including parts of Germany. They induce T cell effector response directed to viral envelope peptides and antibody response by B cells in people living in endemic areas. Moreover, prior exposure to dengue virus shapes cross-reactive T cell memory that amplifies responses against Zika virus while acute Zika infection modulates antigen-presenting cells and recalls cross-reactive memory B cells in flavivirus-experienced individuals. This dynamic interplay of cellular responses underpins the maintenance of immune memory crucial for protection. Hence, we aimed to investigate T and B cell responses to dengue, Zika, and chikungunya in populations affected by the northward spread of *Aedes* mosquitoes and identify immune differences that inform vaccine development amid climate-driven changes in arboviral diseases.

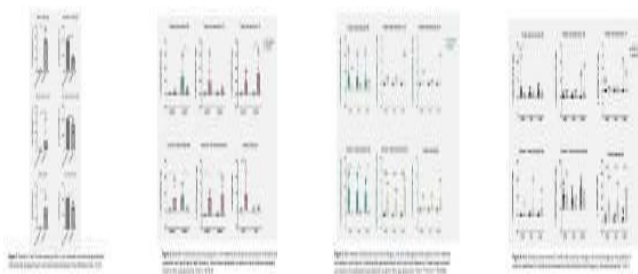
Methods: PBMCs from apparently healthy donors living in non-endemic (Tübingen) and endemic (Lambaréné) areas were isolated and cultured for 72 hours without stimulation, with α CD3/ α CD28 beads for T cell proliferation, CpG DNA+IL-15 for B cell proliferation, dengue, chikungunya and Zika peptides. Thereafter, using multicolor panel staining and flow cytometry, we characterize memory T cells using anti-CD3, anti-CD4, anti-CD8 and anti-CD45RO markers; intracellular cytokines and glycoprotein with anti-IFN- γ , anti-IL-17A, anti-IL-10 and anti-perforin. Moreover, we looked at memory B cells and their antibodies production using anti-CD19, anti-CD27 and anti-IgG markers.

Results: As expected, non-endemics had no T and B cell memory, but significant non-memory response compared to

endemics who showed 55% of memory CD4⁺ T cells, 10% of memory CD8⁺ T cells and 19% of memory CD19⁺ B cells (figure 1). Looking at the reactivity capacity, non-memory CD4⁺ and CD8⁺ T cells from non-endemics were prone to produce IFN- γ while endemics memory and non-memory CD4⁺ and CD8⁺ T cells were better producers of IL-17A and perforin respectively, while their memory B cells produced Ig (figure 2). Likewise, when stimulated with dengue, zika and chikungunya peptides, non-endemics PBMCs showed high frequencies of non-memory CD4⁺ and CD8⁺ T cells producing IFN- γ whereas high frequencies of CD8⁺ T cells producing perforin and B cells producing IgG were found with endemics (figure 3). In addition, endemics memory B cells were the most producers of IgG against viral peptides (figure 4).

Conclusions: This study highlights the polarization of T cells to produce IL-17A, perforin and memory B cells to produce IgG in dengue, zika and chikungunya infections in endemics versus non-endemics populations. This insight is pivotal for informing vaccine strategies targeting these emerging climate-sensitive diseases.

Fig. 1



S-3-2

Induced pluripotent stem cell (iPSC)-derived human retina organoids provide evidence for a role of Müller glia cells as niche for productive cytomegalovirus replication

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Human cytomegalovirus (HCMV) retinitis (HCMV-R) is a severe ophthalmic manifestation that can impair vision in immunocompromised individuals and congenitally infected infants. To study HCMV-R in a model recapitulating the *in vivo* complexity, we infect induced pluripotent stem-cell (iPSC)-derived human retina organoids containing various cell types such as photoreceptors, glia cells, as well as amacrine, bipolar, and horizontal cells after 180 days of differentiation. As expected, HCMV replicated in retinal pigment epithelial cells. Intriguingly, HCMV efficiently infected Müller glia cells in organoids and isolated 2D cultures. In addition to HCMV-TB40 and a clinical isolate derived from a HCMV-R patient, HCMV-AD169varS-based viruses were able to infect retina organoids, indicating that entry is not strictly dependent on the pentameric entry complex and the ULbⁿ region. Experiments using a panel of neutralizing antibodies targeting either the trimeric or

pentameric entry complexes indicated that HCMV enters Müller glia cells through both the trimer- and the pentamer-dependent route.

The treatment of HCMV-infected retina organoids and isolated Müller glia cells with a panel of antivirals including Maribavir, Letermovir, Ganciclovir, Cidofovir, and Foscarnet showed different levels of efficacy. We also assessed the antiviral activities of different interferons (IFNs) and inhibitors of Cullin RING ubiquitin ligases (CRLi). CRLi such as MLN4924 and TAS4464 as well as IFN β and IFN γ elicited potent antiviral activity against HCMV in retina organoids and Müller glia cells.

To optimize our model, organoids were populated with autologous mCherry-expressing microglia immune cells. We observed that microglial cells preferentially localize adjacent to HCMV-infected cells, suggesting that microglia cells recognize HCMV. Taken together, our retina organoid model enables studies regarding the role of different cell types including micro- and Müller glia cells during HCMV infections and the efficacy of approved and experimental antiviral drugs.

S-3-3

Clade-Specific Liver Tropism and Innate Immune Modulation by Mpox Virus

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Mpox virus (MPXV) is a zoonotic orthopoxvirus. Until 2022, it was largely confined to endemic regions in Central and West Africa. However, following the global outbreak in 2022 and a subsequent resurgence in the Democratic Republic of the Congo, MPXV has emerged as a significant global health concern. MPXV is divided into two major genetic clades: clade I and clade II. Further phylogenetic divergence within these clades has led to the identification of subclades: clades Ia and IIa, which comprise older African strains associated with sporadic outbreaks in endemic regions. Clades Ib and IIb include strains responsible for the 2022 and 2024 outbreaks. Clinically, MPXV infections present with a broad spectrum of symptoms, ranging from mild fever to severe systemic disease. While mpox is primarily recognized by its distinctive skin lesions, growing evidence suggests that the virus can disseminate to multiple organ systems. Recent studies have detected MPXV in liver tissue of patients with severe mpox, suggesting that hepatic involvement may play a role in disease severity. However, the mechanisms underlying MPXV tropism to these organs remain largely unknown. Here, we aim to investigate the determinants of MPXV liver tropism, examining differences in infection

dynamics among clades Ia, Ib, and IIb to gain deeper insights into viral pathogenesis and host interactions.

To assess hepatic infection dynamics, we infected liver cell lines Huh7 and HepG2 with different MPXV isolates from clade Ia, Ib, and IIb. Productive infection was established within 48 hours, with viral production comparable to VeroE6 cells. However, clade IIb exhibited inefficient infection in these cell lines, with infectious virus detected only in cell lysates. Similar to the tested cell lines, MPXV clades Ia and Ib replicate successfully within primary human hepatocytes (PHH), with time-dependent production of viral progeny. Successful infection of PHH was further confirmed by light microscopy showing cytopathic effect. Clade IIb shows delayed viral replication, with increasing titres observed at 72 hours post-infection.

Additionally, infection with MPXV interferes with the expression of IFN-stimulated genes and treatment with interferon-alpha 2 (IFN α 2) significantly reduced infectious viral loads for all isolates, indicating a role of innate immune responses in restricting MPXV replication. Transcriptomic analysis further revealed that PHH donors exhibit differential sensitivity to IFN α 2 treatment, with a high degree of variance in the effect of IFN α 2 on the percentage of mapped reads to MPXV.

In conclusion, *in vitro* studies using liver cell lines (Huh7 and HepG2) and PHH confirm susceptibility to infection with MPXV clades Ia and Ib, while clade IIb demonstrates inefficient infection, correlating with disease severity. Strain-specific differences in immune response activation and replication efficiency shed light in our understanding of MPXV liver involvement.

S-3-4

Optimizing WHO treatment decision algorithms for childhood tuberculosis: Analysing the added value of host transcriptomic and immunological markers

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Approximately 240,000 children <15 years die from tuberculosis (TB) every year, mostly because of missed diagnosis. In 2022, the World Health Organization (WHO) released interim recommendation for treatment decision algorithms (TDAs). A recent meta-analysis reported TDA had a sensitivity of 84.3% and specificity of 50.6%. We aimed to explore the optimization of TDA performance by including novel tests using previously collected data from the RaPaed-TB study.

RaPaed-TB was a prospective diagnostic accuracy study for paediatric TB conducted in South Africa, Mozambique, Tanzania, Malawi, and India. In this analysis, children aged 3 months -10 years were included and the diagnostic accuracy using the WHO-TDA was evaluated using a composite reference standard (CRS, confirmed + unconfirmed TB vs unlikely TB). Performance was reported as mean sensitivities and specificities estimated through bootstrapping. The added value of including two novel assays, a 3-gene transcriptomic assay using fingerstick blood, and a flow-cytometric T-cell activation marker (TAM-TB), based on whole blood, was explored. The 3-gene assay was evaluated at two positivity cutoffs (<1.5 and <1.9) for the TDA optimization. Novel assays were added at two diagnostic steps within the TDA ("mWRD" & "TB contact", Figure 1A,B) and the impact on diagnostic performance was assessed.

Of the 975 children enrolled, 732 were included in this analysis. Of which, 136/732 (18.6%) children were classified as confirmed TB, 227/732 (31.0%) as unconfirmed TB, and 369/732 (50.4%) as unlikely TB following NIH-consensus criteria. The baseline TDA sensitivity was 88.7% (95%CI 85.3%-91.8%) and specificity was 24.7% (20.5%-28.9%). The addition of the 3-gene signature assay or the TAM-TB assay alone did not significantly improve the WHO TDA performance at the mWRD step (3 gene assay in TDA had sensitivity of 88.7% and specificity of 23.1%; TAM-TB in TDA had sensitivity of 91.2% and specificity of 24.4%). When both assays were added, sensitivity increased to 91.5% (88.4%-94.2%) and specificity decreased to 21.7% (17.8%-25.9%). When adding assays to the TB contact step, sensitivities declined while specificities increased pronouncedly: the 3-gene assay had a sensitivity 67.9% (63.2%-72.7%) and specificity of 61.0% (56.1%-65.9%) at <1.5 cutoff, and a sensitivity of 68.6% (64.1%-73.5%) and specificity of 60.0% (54.9%-64.8%) at <1.9 cutoff. The addition of both novel assays had a sensitivity and specificity of 73.1% (68.6%-77.6%) and 56.7% (51.6%-61.7%), respectively (Table 1).

Optimization of WHO TDA by the addition of novel diagnostic assays suggests an improved specificity with minor compromise in sensitivity. The improved specificity of the optimized TDA may prevent overtreatment for a substantial number of children, thereby maximising efficient resource use in low resource settings. Further optimization of WHO TDA by the addition of other novel assays or using advanced computational methods are ongoing.

Fig. 1

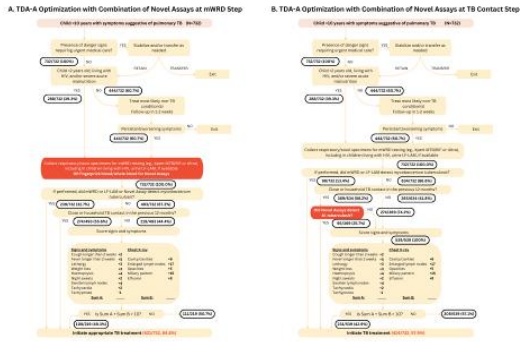


Fig. 2

	TP	FP	FN	TN	Sensitivity	Specificity	AUROC
WHO TDA	322	278	41	91	88.7% (85.3%–91.8%)	24.7% (20.5%–28.9%)	0.56 (0.53–0.56)
TDA mWRD Step							
3-gene signature (<1.5)	322	264	41	85	88.7% (85.3%–91.8%)	23.1% (19.1%–27.5%)	0.56 (0.53–0.56)
3-gene signature (<1.9)	323	288	40	81	89.9% (85.6%–92.1%)	21.9% (18.0%–26.2%)	0.55 (0.53–0.55)
T-cell activation marker TB	331	279	32	90	91.2% (88.1%–94.0%)	24.4% (20.2%–28.7%)	0.58 (0.55–0.58)
Combination of assays at TDA mWRD Step							
3-gene signature (<1.5) + T-cell activation marker	332	289	31	80	91.4% (88.4%–94.2%)	21.7% (17.8%–25.9%)	0.57 (0.54–0.57)
TDA TB Contact Step							
3-gene signature (<1.5)	246	143	117	226	67.9% (63.2%–72.7%)	61.0% (56.1%–65.9%)	0.65 (0.61–0.66)
3-gene signature (<1.9)	249	147	114	222	68.6% (64.1%–73.5%)	60.0% (54.9%–64.8%)	0.64 (0.61–0.64)
T-cell activation marker TB	259	148	104	221	71.4% (66.9%–75.9%)	59.7% (54.6%–64.6%)	0.65 (0.62–0.66)
Combination of assays at TDA TB Contact Step							
3-gene signature (<1.5) + T-cell activation marker	265	159	98	210	73.1% (68.6%–77.6%)	56.8% (51.6%–61.8%)	0.65 (0.62–0.65)

S-3-5

Impact of chronic infection with Wuchereria bancrofti on incidence and prevalence of viral infections in Southwest Tanzania, focusing on high-risk papilloma virus and HIV

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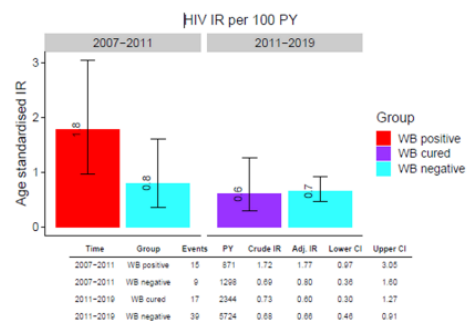
As part of a large-scale prospective cohort study conducted over a period of 12 years in southwestern Tanzania, we were able to recruit study participants prior to the introduction of anthelmintic treatment programs and continue to observe them during mass drug administration (MDA) campaigns. The prevalence of Wuchereria bancrofti (WB) in 2007 was 35.1% in individuals over the age of 14. During four annual follow-up examinations of > 2500 villagers between 2007 and 2011, we documented new HIV infections and found an

increased HIV incidence for the WB-infected subgroup aged 14 to 65 years. Overall, the HIV incidence was 1.91 cases per 100 person-years (PY) among villagers with WB and 0.80 cases/100 PY among those without filarial infection (age- and sex-adjusted incidence ratio (IRR) of 2.17 (95% CI 1.08–4.37, p = 0.030). After MDA for lymphatic filariasis (LF), we documented a decline in WB prevalence from 35.1% to 1.6%. In 1,299 former study participants who were revisited after successful MDA, we investigated whether a reduced WB prevalence could have an impact on HIV incidence. Of these, 74% had never been infected with WB, 24% had been infected previously but had since been cured, and 1.5% remained infected with WB.

Results: Among participants cured from WB, HIV incidence decreased significantly from 1.77 cases/100 PY in the period 2007–11 to 0.60 cases/100 PY in the period 2011–19, with an IRR of 0.41 (95% CI 0.2–0.8; p = 0.012). In contrast, the HIV incidence among individuals who tested consistently negative was 0.80 cases/100 PY in the period 2007–11 and 0.66 cases/100 PY in the period 2011–19 (IRR 1.07 (0.5–2.2); p=0.85). To investigate possible host factors that could explain the increased susceptibility to HIV in filarial-infected individuals, we compared the T-cell phenotypes of WB+ and WB- women in a subset of the above-mentioned cohort. Flow cytometry analysis of activation and differentiation markers on CD4 T cells and the HIV entry receptor CCR5 was performed on cervical and peripheral blood samples from 54 women without HIV (WLWoH), 28 of whom were infected with filaria. WB infection was associated with a significantly increased frequency of CD3+γδ2+ T cells in the cervical mucosa (median 4.0% vs. 1.4%, p = 0.012). Contrary to our expectations, we found a lower frequency of CCR5 on total, memory, and activated memory CD4 T cells in the WB+ group. However, the frequency differences decreased after adjusting for age in a multivariate analysis. In addition, HPV tests were performed on their samples and on 13 WLWH. WB and HIV infections were associated with a 3.8-fold and 3.2-fold increase in HR-HPV risk, respectively (WB status: p = 0.058; HIV status: p = 0.057).

Conclusions: Our results suggest immunological mechanisms by which WB increases HIV risk, albeit independently of the CCR5 receptor. With this work, we demonstrate the influence of WB on susceptibility to HIV and HPV, which can be reduced by eliminating the helminth.

Fig. 1



S-4-2

A single dose of a self-amplifying RNA vaccine protects mice against lethal CCHFV infection within 14 days

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Crimean-Congo hemorrhagic fever virus (CCHFV) can cause hemorrhagic fever in humans with a case-fatality rate of up to 30%. To date, there are no approved vaccines and CCHFV is listed by the WHO as a priority pathogen for research, with high potential to become a Public Health Emergency of International Concern (PHEIC). The development of effective vaccine candidates against CCHFV is therefore of utmost importance.

We developed and characterized self-amplifying RNAs (saRNAs) encoding the major viral glycoprotein (Gc) or the nucleoprotein (NP) of the CCHFV strain Afg09-2990. The saRNAs were co-formulated in lipid nanoparticles (saRNA Gc+NP) and induced humoral and cellular immune responses in mice. Subsequently, efficacy studies with saRNA Gc+NP-vaccinated interferon alpha/beta receptor knockout mice were performed. In contrast to vehicle-immunized control mice, all prime-boost (28-day interval) and prime-only immunized animals survived homologous CCHFV Afg09-2990 infection 56 days after prime vaccination. Due to the high genetic diversity of CCHFV lineages, we tested the saRNA Gc+NP vaccine against the phylogenetically distant CCHFV strain Kosovo Hoti using the same regimens as before. Prime only and prime-boost saRNA Gc+NP also provided cross-protection in the otherwise lethal mouse model. In both models tested, Gc+NP-immunized mice showed no clinical symptoms, apart from slight weight loss or transient ruffled fur in some animals. In a comprehensive post-mortem analysis, no CCHFV-specific RNA or infectious CCHFV were detected in the serum or organs of vaccinated animals.

To analyze the onset of protection induced by saRNA Gc+NP, a single dose of the vaccine candidate was administered either 14, 7 or 3 days before infection with CCHFV Afg09-2990. saRNA-Gc+NP provided 100% protection when administered 14 days prior to infection. Survival rates in mice that received saRNA Gc+NP 7 and 3 days prior to infection were 86% and 25%, respectively. Post-mortem analysis only detected CCHFV-specific RNA and infectious CCHFV in the serum and organs of mice vaccinated 3 days prior to challenge.

In summary, we demonstrated the efficacy of saRNA Gc+NP when administered as a prime-boost or prime-only regimen against homologous and heterologous CCHFV infection. The ability of a prime-only regimen of saRNA Gc+NP to confer protection against the homologous CCHFV strain Afg09-2990 within 14 days or less in individual animals further substantiates the potency and rapid onset of immunity of the saRNA Gc+NP. Overall, our data suggest further investigation of saRNA Gc+NP as a vaccine candidate against CCHFV is warranted.

S-4-3

Two-year persistence of MERS-CoV-specific antibody and T cell responses after MVA-MERS-S vaccination in humans

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MVA-MERS-S, a Modified Vaccinia virus Ankara (MVA) viral vector vaccine candidate against Middle East respiratory syndrome (MERS), was recently evaluated in a two-center, randomized, placebo-controlled phase 1b trial (NCT04119440) to assess its safety, immunogenicity, and optimal dosing in healthy adults¹. A two-dose regimen elicited robust spike-specific antibody responses, which were significantly enhanced by a late third dose. We extended this trial to assess the two-year durability of MERS-CoV-specific antibody and T cell responses in 48 study participants. Our findings show that antibody titers gradually wane after the third vaccination but remain detectable for at least 24 months at levels comparable to the peak response observed after the second vaccination. Two years after vaccination, 90% (36/40) of the participants remained S1 IgG seropositive, and 75% (30/40) and 50% (20/40) of the participants maintained detectable pseudovirus and live-virus neutralizing titers, respectively. Vaccine-induced antibodies cross-neutralized MERS-CoV spike mutants that emerged during the 2015 outbreak in humans. To assess whether vaccine-induced T cell responses persisted until two years after vaccination, we restimulated whole blood samples with an overlapping spike peptide pool and measured cytokine release using an automated ELISA. The median IFN- γ and IL-2 responses were significantly higher in MVA-MERS-S-vaccinated participants compared to the placebo group. Although the immune correlates of protection against MERS remain unknown, the observed durability of humoral and cellular immune responses supports the potential of MVA-MERS-S as a promising MERS vaccine candidate and highlights the importance of a booster dose in sustaining long-term immunity.

Raadsen, M. P. *et al.* Safety, immunogenicity, and optimal dosing of a modified vaccinia Ankara-based vaccine against MERS-CoV in healthy adults: a phase 1b, double-blind, randomised placebo-controlled clinical trial. *Lancet Infect. Dis.* **25**, 231–242 (2025).

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Braunschweig, Hannover, Germany), Verena Krähling (Institute of Virology, Philipps University Marburg, Marburg, Germany; German Centre for Infection Research, partner site Gießen-Marburg-Langen, Marburg, Germany) and Eric van Gorp (Department of Viroscience, Erasmus Medical Centre, Rotterdam, The Netherlands).

S-4-4 Identification of pan-Orthoebolavirus-reactive antibodies from rVSV-EBOV vaccinated individuals

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Orthoebolaviruses such as Ebola virus (EBOV), Sudan virus (SUDV) and Bundibugyo virus (BDBV) are highly contagious and can cause severe diseases with significant case fatality rates in humans. For EBOV disease few monoclonal antibodies (mAbs) or cocktails and three licensed vaccines are available. However, highly potent and cross-reactive neutralizing mAbs to all filoviruses are hardly described. To identify and develop pan-filovirus reactive antibodies, we aim to leverage the humoral immune response in rVSV-EBOV-vaccinated individuals. From these, we have already isolated highly potent EBOV-specific mAbs. We hypothesize that, despite the EBOV-specific immune response, the rVSV-EBOV vaccine may also induce broader neutralizing antibodies.

To gain insights into the development, duration and cross-reactivity of a filovirus-specific humoral immune response, we analyzed EBOV-specific mAbs induced by vaccination. Therefore, we collected samples of participants of a clinical phase I study vaccinated with the rVSV-EBOV vaccine in 2015, 24-/84-months post vaccination. Our preliminary analysis, suggests a long-lasting B cell response seven years after vaccination. To investigate cross-reactivity of the humoral immune response, we used a sorting strategy with BDBV-/SUDV-glycoprotein (GP) baits and performed single B cell sequencing, the *in vitro* production of corresponding antibodies and binding as well as neutralization studies with various filoviruses. To this end, a functional lentivirus-based pseudovirus neutralization assay was established to analyze neutralization activity of isolated mAbs. We were able to identify two pan-Orthoebolavirus-reactive mAbs neutralizing EBOV, BDBV and SUDV. Of note, they also neutralized authentic EBOV and SUDV, of which one shows higher potency against SUDV compared to cross-reactive mAbs such as REGN3479. Moreover, investigation of the pharmacokinetic profile showed good *in vivo* stability and preliminary epitope analysis suggests an epitope in the GP2 region of the EBOV-GP. In *in vivo* efficacy studies using

EBOV and SUDV mouse models, our lead candidate consistently delayed disease progression, mitigated disease severity, and resulted in substantial survival benefits.

These findings provide important insights into the development of a filovirus-specific immune response and the efficacy of the rVSV-EBOV vaccine, showing the development of filovirus cross-reactive antibodies induced by an EBOV-GP-specific vaccine. Additional investigation will include detailed structural analysis to further evaluate the lead candidate and explore its clinical potential.

S-4-5 Profiling a large HIV-1 elite neutralizer cohort reveals remarkable CD4bs bNAb for HIV-1 prevention and therapy

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Administration of HIV-1 neutralizing antibodies can suppress viremia and prevent infection *in vivo*. However, clinical use is challenged by envelope diversity and rapid viral escape. Here, we combined micro-scale antibody production with direct functional testing to perform detailed single-cell profiling of the largest cohort of top HIV-1 elite neutralizers studied to date (32 individuals) to identify broadly neutralizing antibodies (bNAbs) with highest antiviral activity. From 831 expressed monoclonal antibodies, we identified 04_A06, a VH1-2-encoded CD4 binding site bNAb with remarkable breadth and potency against multiclade pseudovirus panels (GeoMean IC50 = 0.059 µg/ml, breadth = 98.5%, 332 strains). Moreover, 04_A06 was not susceptible to classic CD4bs escape variants and maintained full viral suppression in HIV-1-infected humanized mice. Structural analyses revealed an unusually long 11-amino acid heavy chain insertion that facilitates inter-protomer contacts with highly conserved residues on the adjacent gp120 protomer. Finally, 04_A06 demonstrated high activity against contemporaneously circulating viruses from the Antibody Mediated Prevention (AMP) trials (GeoMean IC50 = 0.082 µg/ml, breadth = 98.4%, 191 virus strains) and *in silico* modeling for 04_A06LS predicted HIV-1 prevention efficacy of >93%. Thus, 04_A06 will provide unique opportunities for effective treatment and prevention of HIV-1 infection.

S-5-2 The hidden link between cefiderocol resistance and increased virulence in *Klebsiella pneumoniae*: Insights from a TraDIS-based investigation

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Background: The global spread of antimicrobial resistance (AMR) in Gram-negative bacteria, particularly *Klebsiella pneumoniae*, poses a major challenge for human health, severely limiting effective treatment options. New antimicrobials, such as the sideromycin cefiderocol (FDC), specifically approved for infections caused by extensively drug-resistant *K. pneumoniae*, offer new therapeutic avenues. However, resistance to FDC has emerged, with underlying mechanisms still largely unknown.

Methods: We used a saturated transposon-mutant library of a *K. pneumoniae* ST258 strain to identify FDC resistance genes through transposon-directed insertion-site sequencing (TraDIS). In duplicate, the library was exposed to 32 mg/L of FDC, and TraDIS was used to identify genes that become essential under FDC exposure. Following TraDIS analysis, we constructed gene deletion mutants to evaluate the contribution of each identified gene to FDC resistance and bacterial virulence using minimum inhibitory concentration (MIC) testing and a *Galleria mellonella* infection model, respectively.

Results: Our analysis identified 146 genes with increased transposon insertions following FDC exposure. Among these, previously reported FDC resistance genes were identified, including those involved in outer membrane channels and siderophore translocation, such as *ompK36* and *cirA*. In addition, TraDIS analysis revealed a strong association between FDC resistance and genes involved in capsule production. Deletion of the capsule degradation master regulator, namely *csrD*, confirmed this association, resulting in a 4-fold increase of FDC MIC and a 1.3-fold increase in mortality in the *in vivo* model.

Discussion: This study provides new knowledge on the genetic background of FDC resistance in *K. pneumoniae*, highlighting both known and previously unrecognized pathways, including genes involved in capsule synthesis. The observed association between FDC resistance and increased virulence underscores the potential risks that the development of FDC resistance may not only limit treatment efficacy but also increase the risk of severe infections. Ultimately, this highlights the importance of a multi-pronged approach that addresses not only resistance, but also the potential unintended consequences for bacterial pathogenicity associated with the development of AMR.

Question: Antimicrobial resistance (AMR) in *Enterobacter cloacae* complex (ECC) poses a significant threat to neonatal healthcare, particularly in resource-limited settings. ECC readily acquires ESBLs and carbapenemases and persists on hospital surfaces, yet its environmental reservoir and transmission pathways in African neonatal units remain under-characterized.

Methods: This study aimed at characterizing the molecular epidemiology, antimicrobial resistance, and transmission dynamics of ESBL-producing ECC isolates collected from neonates, mothers, healthcare workers, and hospital environments in two hospitals in Tanga, Tanzania.

Result: Between April 2022 and March 2023, a total of 132 ECC isolates were recovered, with environmental samples (65.90%, n/N = 87/132) yielding more isolates than human sources (34.10%, n/N = 45/132). ECC colonization was observed in 4.2% of neonates, of whom 62% acquired colonization during hospitalization, indicating nosocomial transmission. Multidrug resistance (MDR) was prevalent (73.4%), with high resistance rates to third-generation cephalosporins, and notable carbapenem resistance (11.3%). Genomic analysis identified a high diversity of beta-lactamase genes, dominated by *bla*CTX-M-15 (88%) and *bla*ACT genes (94%), alongside carbapenemase genes *bla*NDM-1 and *bla*NDM-5 (12.1%). Notably, colistin resistance gene *mcr-10.1* was exclusively associated with ST66 isolates, highlighting the emergence of locally significant ECC lineages with resistance against last-resort antibiotics like colistin not commonly used in Tanzania. ST346 was the most dominant sequence type (24.2%), circulating across human and environmental samples, demonstrating repeated transmission and persistent colonization in hospital wards. The co-occurrence of multidrug resistance and virulence factors in temporal clusters underscores the enhanced potential for invasive infections.

Conclusions: ECC circulation in these neonatal wards is strongly linked to environmental reservoirs and repeated human–environment transmission. Targeted environmental hygiene and reinforced infection prevention, coupled with sustained human–environment genomic surveillance and stewardship of third-generation cephalosporins/carbapenems, are immediate priorities. Focused monitoring of high-risk lineages (for example ST346 and ST66 carrying *mcr-10.1*) should guide interventions to curb spread and reduce neonatal risk.

S-5-3

Molecular epidemiology and antimicrobial resistance of extended-spectrum beta-lactamase-producing *Enterobacter cloacae* complex in neonatal units and hospital environments in Tanga, Tanzania

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Fig. 1



Fig. 2

S-5-5

Piloting Precision: Implementation of targeted next generation sequencing for antibiotic resistance testing into the diagnostic algorithm of Tuberculosis in Kyrgyzstan

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Targeted next-generation sequencing (tNGS) is an increasingly valuable tool in tuberculosis diagnostics, offering rapid, comprehensive detection of drug resistance mutations directly from clinical specimens or cultures. Its ability to guide individualized treatment and improve diagnostics makes it particularly beneficial in high-burden settings like Kyrgyzstan, where DR-TB poses major challenges to public health.

Here we report on the first successful implementation of tNGS at the Republican Tuberculosis Reference Laboratory. Using the WHO-endorsed Deeplex® Myc-TB assay, we conducted a pilot study on sputum samples collected from 2020 to 2022 from 281 people living with TB.

Overall, 266 samples were eligible for tNGS. 238 (89%) yielded full resistance profiles covering 15 drugs. 143/238 (60%) were rifampicin (RIF) resistant. 108 (76%) were MDR, 22 (15%) pre-XDR and 6 (4%) XDR. Bedaquiline resistance was detected in 11/143 (8%) samples which were recovered only from new cases. No resistance to linezolid was detected. Deeplex success rate correlated with smear positivity.

The concordance to phenotypic drug susceptibility testing was 90% for RIF. Noteworthy, 15/19 (79%) of the samples tested phenotypically susceptible towards RIF actually carried low level resistance mutations.

On average, the final tNGS results were available after 53h from the start of DNA extracts.

DR-TB remains a challenge in Kyrgyzstan, particularly due to high numbers of fluoroquinolone and bedaquiline resistance. Our findings support the integration of tNGS into the national diagnostic algorithm as a complementary test to the existing diagnostic methods to improve precise diagnosis of DR-TB.

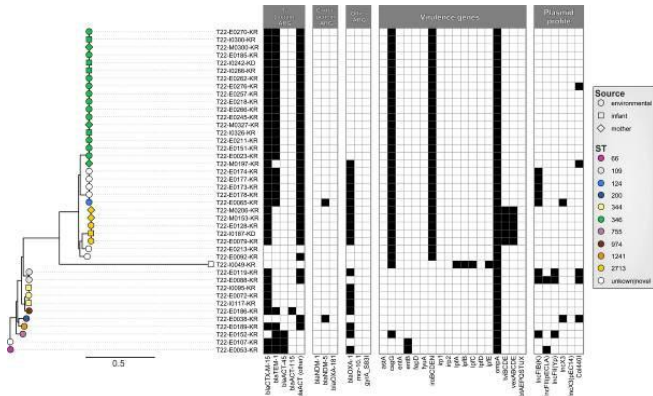
PEG-1-2

From First Contact to Challenge: Lung Immune Modulation by Single-Sex *Schistosoma mansoni* Infections

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The lung is a critical control point in the early phase of *Schistosoma* spp. infection, where migrating larvae



S-5-4

The evolution and global spread of drug-resistant *Mycobacterium tuberculosis*, as documented by over 200,000 isolate genome sequences

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Antibiotic-resistant *Mycobacterium tuberculosis* complex (MTBC) strains are WHO priority pathogens. They cause one of the deadliest infectious diseases in humans, i.e. tuberculosis, posing a significant threat to public health and placing a considerable strain on healthcare systems. Genomic surveillance is a powerful tool for monitoring the evolution and spread of drug-resistant *M. tuberculosis*, and provides essential data for global TB control.

Enterobase is a platform for genomic surveillance (<https://enterobase.dsmz.de/>) that integrates large-scale genome data available from the public domain by processing the data through standardized pipelines and providing advanced bioinformatics tools for subsequent data analysis. We have established a comprehensive *M. tuberculosis* database on the Enterobase platform that currently holds genome sequences and associated metadata from more than 200,000 MTBC isolates, with daily updates integrating newly published data (<https://enterobase.dsmz.de/>). All sequence data undergo automatic assembly, quality control and genotyping to facilitate large-scale comparative analyses. Recently, we have implemented an automated pipeline that systematically screens all available genome sequences for genetic variants known to be associated with drug resistance in *M. tuberculosis*, including single-nucleotide polymorphisms and small insertions or deletions. Our evaluation in a strain set with known resistance profiles indicated a high sensitivity and specificity for drug-resistance predictions.

As a result, this tool for the first time provides a global perspective on the evolution and spread of drug-resistant MTBC strains at such a large scale. We show that the genomic record has documented the repeated emergence of increasingly resistant MTBC strains, together with the timescales of their evolution and geographical spread, and the impact of human interventions, e.g. treatment regimens.

encounter robust inflammatory responses that may limit their further development. These processes are central to parasite defense by the host. We have clear evidence of the impact of unisexual infections - whether with male or female parasites - on local immunity, which is still poorly understood, as is their impact on subsequent infections.

Herein we characterized the pulmonary immune milieu in mice infected with male, female, or male+female (*bisexual*) cercariae at 4- and 16-days post-infection, analysing bronchoalveolar lavage and lung tissue by flow cytometry, qPCR, and multiplex analyses. Single-sex groups showed only minor differences compared with each other but displayed marked differences from bisexual infections, including distinct inflammatory signatures and shifts in immune cell composition.

We further assessed whether single-sex *Schistosoma mansoni* larvae can prime the immune system during lung migration and influence the outcome of a subsequent bisexual challenge infection. QPCR-based inflammatory profiling and detailed T-cell subset analysis by flow cytometry were used to characterize the immune milieu. First results revealed that prior single-sex infection was associated with differences in worm burden in the lung after challenge, suggesting functional consequences for host protection.

These findings underscore the importance of infection history and parasite sex in shaping pulmonary immunity and point toward new avenues for targeted intervention strategies against schistosomiasis.

PEG-2-2

E3 ubiquitin ligase LRSAM1 restricts intracellular *Staphylococcus aureus* survival

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Introduction and Question: Intracellular invasion and persistence of *Staphylococcus aureus* can result in chronic and relapsing infections enabling the pathogen to evade the host immune response and antibiotic treatment.¹ In addition, staphylococci are remarkably resilient, withstanding more than 100 daily applications of ethanol-based hand antiseptics.² Ubiquitination of bacterial surfaces by specific E3 ubiquitin ligases is a host cell strategy that targets intracellular bacteria for degradation via selective autophagy. This process requires a cascade of enzymatic reactions involving E1 activating enzymes, E2 conjugating enzymes and E3 ubiquitin ligases, with E3 ligases providing substrate specificity.³ However, the identity and function of E3 ligases involved in bacterial surface ubiquitination is very limited, especially for Gram-positive bacteria.

Methods: Lung epithelial cells represent the primary line of defence against infections of the respiratory tract. Therefore, we analysed A549 lung epithelial cells during *S. aureus* infection, with a particular focus on the role of the E3 ligase leucine rich repeat and sterile alpha motif containing 1 (LRSAM1). In LRSAM1-CRISPR-Cas9-deficient A549 cells we evaluated the host cell response to the infection compared to wild-type (non-targeted control, NTC) cells. We investigated the intracellular survival of *S. aureus*, activation

of host cell signalling pathways related to cytokine production as well as bacterial surface ubiquitination, induction of selective autophagy and host cell death.

Results: By analysing LRSAM1-deficient cells, a significant increase of intracellular bacteria and elevated secretion of the pro-inflammatory cytokine IL-6 was observed, which was accompanied by an enhanced host cell death mainly due to induction of apoptosis and necroptosis. In addition, LRSAM1-*knockout* led to a reduction in the ubiquitination of the bacterial surface, thereby preventing the elimination of intracellular *S. aureus* by selective autophagy, although the process of autophagy itself was strongly induced.

Conclusions: In conclusion, our results indicate a prominent role for LRSAM1 during *S. aureus* infection. Further experiments are needed to clarify the specific (bacterial) substrates of LRSAM1 and to elucidate the mechanism by which this E3 ligase affects intracellular bacteria recognition and how it directly or indirectly impacts intracellular bacteria survival and host cell death. Understanding the molecular mechanisms of LRSAM1 function will help to develop new therapeutic strategies against host cell persistence of e.g. *S. aureus*.

¹Kahl et al. Clin Microbiol Rev (2016) 29:401-27.

²Kramer et al. Am J Infect Control (2025) 53(4):426-33.

³Seifert et al. Cell (2010) 142(4):613-24.

PEG-3-2

Duration of protection from pneumonia after pneumococcal vaccination in haemodialysis patients – Results from the prospective observational DOPPIO stud

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Pneumonia is a leading cause of death in patients with end-stage chronic kidney disease receiving dialysis. Pneumococcal vaccination reduced pneumonia rates and mortality in this population. However, the clinical impact of titre decline observed in haemodialysis patients within 6 to 12 months after immunisation is uncertain. We thus compared vaccine efficacy and its immunological correlates in two strata of vaccinated patients: those recently vaccinated and those vaccinated more than two years ago.

We compared pneumonia incidence between those vaccinated within the previous two years and those vaccinated more than two years before enrolment. Secondary aims included characterising the kinetics of anti-pneumococcal antibody titres and opsonophagocytic activity over time, identifying factors influencing antibody decline, and determining the association between antibody levels and pneumonia incidence to extrapolate a potential protective threshold. Main inclusion criteria were haemodialysis and vaccination against pneumococcal infection in accordance with Robert Koch Institute (RKI) guidelines prior to enrolment. Data on baseline demographics, vaccination history, and underlying disease were assessed. Pneumococcal antibody titres were determined at baseline and every 3 months for 2 years. A total of 12 Clinical Trial

Units (CTUs) within the German Centre for Infection Research (DZIF) with 33 allocated dialysis practices participated in this study.

A total of 789 patients were enrolled within this prospective multi-centre study. Mean age was 66.5 years and most patients (63%) were male. Of 789 patients, only 128 had received vaccination more than 2 years before inclusion. Pneumonia rates and antibody titres are currently analysed and results will be available in Q3/2025.

This prospective study adds to the growing body of evidence that duration of vaccine protection is reduced in patients with end stage renal disease and suggests the need for clinical trials to elucidate optimal vaccination schedules.

PEG-4-2

Outcome of invasive *Candida* infections in Europe and the US: Results from an ongoing multinational study [2024-2026]

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Objectives: Invasive *Candida* infection cause significant morbidity and mortality, especially in immunocompromised patients. The FungiScope *Candida* Campaign 2024-2026 aims to analyze clinical data to better understand risk factors, treatments, and outcomes across different geographical regions.

Materials & Methods: Anonymized patient data were collected via an online questionnaire (www.clinicalsveys.net for Europe, Carelane for the USA), capturing demographics, clinical history, diagnostic findings, antifungal treatment, source control and outcome.

Results: By August 2025, 1141 patients with invasive *Candida* infection were included (median age 67 [18–102]; 62.2% male). Most cases were from Germany (597; 52%), followed by Spain (349; 30.6%), USA (84; 7.4%), Italy (80; 7%), UK (21; 1.8%) and Austria (11; 0.9%). The most frequent causative species were *Candida albicans* (44.6%), *Candida glabrata* (29.9%), and *Candida parapsilosis* (13.7%). Less common species included *Candida tropicalis* (7.3%), *Candida krusei* (2.9%), *Candida dubliniensis* (2.6%), and others (<2% for each).

Frequent risk factors included CVC use (57.6%), ICU treatment (38.3%), chronic cardiovascular disease (41.8%), hematological/oncological conditions (38.9%), and major surgery (35%). Chronic kidney diseases/acute kidney injury and uncontrolled diabetes were present in 26.6% and 13.5% of cases, respectively.

Most patients received antifungal therapy (92.1%; median 14 days [1–442]). First-line treatment most commonly included

casposfungin (46.7%), followed by anidulafungin (18.9%) and fluconazole (17.6%). Notably, 7.9% of patients did not receive antifungal therapy. Treatment distribution varied by country, with echinocandin use dominating in Germany, while fluconazole and micafungin were frequently used in Spain and the USA, respectively.

Survival was not significantly influenced by either the initial antifungal therapy ($p = 0.131$) or the *Candida* species (*albicans* vs. non-*albicans*; $p = 0.462$). The overall mortality rate was 42.3%.

Conclusion: In this cohort, echinocandins were predominantly used as first-line treatment and fluconazole for second-line. Neither initial antifungal choice nor *Candida* species significantly impacted survival.

PEG-5-2

Synergistic reduction of *Klebsiella quasipneumoniae* biofilm by phage vB_KpUKJ_2 and ceftazidime combination

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Biofilms formed by *Klebsiella pneumoniae* and related species exhibit high tolerance to conventional antibiotics, leading to persistent, difficult-to-treat infections. Bacteriophages represent a promising alternative, particularly in combination with antibiotics, where synergistic effects can enhance antibiofilm activity.

In this study, light sheet fluorescence microscopy (LSFM) was used for real-time, high-resolution imaging of *K. quasipneumoniae* biofilms treated with the lytic phage vB_KpUKJ_2¹, β -lactam ceftazidime, or their combination over 24 h. Biofilm viability was assessed via metabolic activity measurements², while automated volumetric analysis of LSFM datasets was performed using custom ImageJ macros. Confocal laser scanning microscopy (CLSM) was employed to evaluate alterations in the polysaccharide component of the extracellular polymeric substance (EPS) after phage exposure. Phage-resistant mutants were isolated and phenotypically characterized.

Quantitative LSFM analysis and viable cell counts showed that the phage–antibiotic combination rapidly and durably eradicated mature biofilms at sub-inhibitory minimum biofilm eradication concentration (MBEC) levels. Area-under-the-curve (AUC) analysis confirmed pronounced synergistic activity. Complementary CLSM imaging revealed swift and progressive degradation of EPS polysaccharides after phage exposure, consistent with depolymerase-mediated disruption of biofilm architecture. Notably, the combined regimen of phage and ceftazidime completely suppressed the emergence of detectable resistant mutants in both planktonic and biofilm states. In contrast, phage monotherapy yielded resistant isolates with fitness trade-offs, including reduced growth rate and impaired biofilm formation. Genome sequencing of these mutants is ongoing to identify genetic determinants of resistance.

These findings highlight the therapeutic potential of phage–antibiotic combinations for both eradication of *Klebsiella* biofilms and suppression of resistance, and establish LSFM as a powerful platform for quantitative, time-resolved evaluation of antimicrobial strategies.

References:

Mirza KA, Tchatchiashvili T, Marquet M, Nietzsche S, Pletz MW, Makarewicz O. Characterization and genome analysis of novel *Klebsiella pneumoniae* phage vbKpUKJ_2 isolated from hospital sewage water. *BMC Microbiol.* 2025 Feb 26;25(1):96.

Tchatchiashvili T, Jundzill M, Marquet M, Mirza KA, Pletz MW, Makarewicz O, et al. CAM/TMA-DPH as a promising alternative to SYTO9/PI for cell viability assessment in bacterial biofilms. *Front Cell Infect Microbiol.* 2025 Jan 21;14:1508016.

P-1-1 Redundant siRNA Pools Overcome Sequence Diversity in Coronaviruses

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RNA viruses pose a continuous threat to global health due to their rapid evolution, zoonotic potential and extensive sequence diversity. These features highlight the urgent need for antiviral strategies with cross-species activity that maintain efficacy across genetically diverse viral populations.

Short interfering RNAs (siRNAs) are potent inhibitors of viral replication, but their efficacy is often limited by viral sequence variability. To overcome this limitation, we introduce the concept of redundant siRNA pools, a novel design strategy in which multiple siRNA variants are combined to collectively target all known sequence variants of the same viral target site. Unlike conventional siRNA designs based on a single reference sequence, our approach explicitly incorporates viral diversity into the design to ensure sustained efficacy even across highly variable targets.

One representative group of RNA viruses with a high medical need and large diversity is the Orthocoronavirinae (CoV) subfamily, which comprises approximately 50 species including SARS-CoV, SARS-CoV-2 and MERS-CoV. A comprehensive conservation analysis of nearly 30,000 potential 19-mer siRNA target sites revealed extensive sequence variability across CoVs, with most target sites existing in 200–300 distinct variants. Even the most conserved target site, located in the RNA-dependent RNA polymerase gene, exhibited 37 naturally occurring variants, rendering conventional single-siRNA designs insufficient.

Two complementary strategies were employed to design redundant siRNA pools against this target site: (i) a screening-based approach, in which siRNAs were sequentially added based on empirical knockdown performance, and (ii) a computational optimization model based on integer linear programming (ILP) to identify the minimal set of siRNAs required to cover all 37 target site variants, while minimizing a position-specific weighted mismatch score.

The design strategies were validated using dual-luciferase reporter assays with a panel of 37 reporter constructs representing each target variant. Redundant siRNA pools achieved consistent and effective knockdown across all constructs. We are currently extending validation to a wide range of wild-type CoV infection models, including hCoV-OC43, hCoV-NL63, SARS-CoV, SARS-CoV-2, and MERS-CoV.

This work establishes a generalizable framework for designing siRNA pools resilient to viral sequence variability, enabling the development of RNAi antivirals with cross-species activity and supporting preparedness for future outbreaks.

P-1-2 Identifying GPCR candidate targets by a novel RNAi approach in *Schistosoma mansoni*

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Schistosoma mansoni is a parasitic flatworm responsible for schistosomiasis, a neglected tropical disease and zoonosis, which affects >250 million people worldwide. Control of schistosomiasis is limited since a vaccine is not available, and Praziquantel is actually the only treatment option. This highlights the urgent need for alternative drugs. The reproductive biology of schistosomes is unique, as the female requires continuous pairing with a male to achieve sexual maturation. We investigated G protein-coupled receptors (GPCRs) as potential drug targets, given their crucial roles in biological processes and their druggability. However, despite extensive research on GPCRs in vertebrates, their roles in parasites like schistosomes remain poorly understood. We focused on the functional characterization of *S. mansoni* GPCRs differentially expressed in males and females, hypothesizing their involvement in male-female interactions and reproduction. To overcome low RNA interference (RNAi) efficiencies, we developed a novel RNAi approach, achieving knockdown (KD) efficiencies of 92–99% for candidate GPCRs. Physiological effects were monitored over 21 days *in vitro*, evaluating pairing stability, attachment capacity, stem-cell activity, and egg production. Following RNAi of candidate GPCRs we observed drastic phenotypes like body curling, tegumental damage, reduced motility, decreased stem-cell proliferation, and a decline in the number of mature oocytes. These results strongly suggest that the selected GPCRs play critical roles for vitality and reproduction of *S. mansoni*.

In conclusion, based on an optimized RNAi protocol, we identified GPCRs with essential functions for schistosome vitality and reproduction. Target-based inhibitor design against these GPCRs may open novel perspectives towards new drugs to combat schistosomiasis.

P-1-3 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, seven 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 aim to develop an inhaled drug consisting of our antiviral siRNA coupled to an integrin ligand. We coupled an $\alpha\beta6R$ -ligand to an anti-CoV siRNA via click chemistry. The $\alpha\beta6R$ is an epithelial-specific integrin, enabling specific uptake into lung epithelial cells where coronaviruses replicate.

Air-liquid interface (ALI) cultures mimic key aspects of airway epithelial development by promoting the differentiation of basal progenitor cells into specialized cell types, including ciliated, goblet, and club cells. This makes ALI models highly relevant for studying host-pathogen and host-drug interactions. To optimize and test our siRNA drug in a physiologically relevant airway epithelium, we established a coronavirus infection model of the human lung using primary human bronchial epithelial cells cultured in an air-liquid interface. Using a multifactorial design-of-experiment (DOE) approach in the software JMP18, we systematically evaluated the impact of seven variables on viral replication across intracellular, apical, and basal compartments. These variables included day of infection post-airlift, day of harvest, timing and frequency of mucus washing, infection route, donor passage number, and viral incubation time. The day of infection and harvest timing had the strongest influence on intracellular and apical viral loads, with peak copy numbers observed when infecting at day 0 (day of airlift) and harvesting after 3–4 days. Mucus removal shortly before infection increased apical viral copy numbers in harvests. At later stages of ALI culture, washing mucus up to three times and closer to infection significantly enhanced OC43 viral loads. Interestingly, an advantage of basal over apical infection was observed with increasing ALI differentiation. These data hint at the protective role of mucus, which is deposited on the apical side of differentiated, air-exposed lung cells, towards foreign particles including viruses and drugs. 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 research will enhance our understanding of siRNA delivery to the lung and has implications for pandemic preparedness.

P-1-4

An AI chest X-ray severity score is prognostic of time to culture conversion in a pulmonary TB clinical trial

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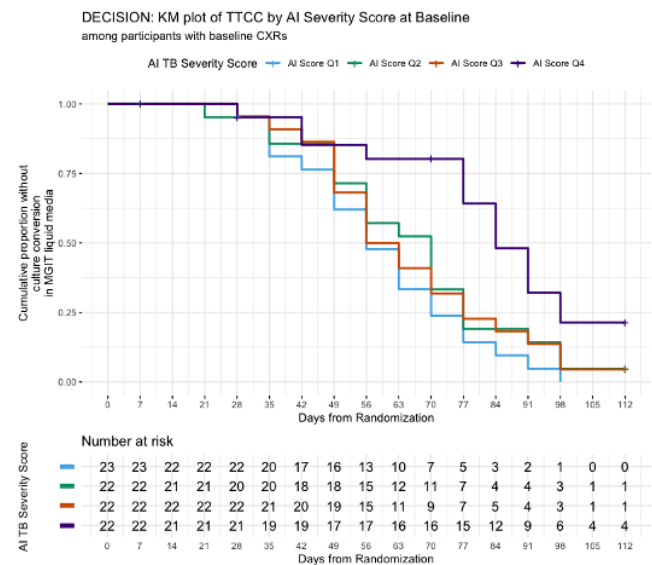
Background: Computer-aided detection (CAD) systems have demonstrated value in tuberculosis (TB) screening frameworks and have recently been endorsed by the WHO, but their value in describing disease severity and predicting treatment response remains unexplored. Our study evaluated the associations between a novel AI-generated chest X-ray (CXR) score, measured at baseline, and early microbiological response, measured as time to culture conversion (TTCC) in liquid media.

Methods: We performed a secondary analysis of CXRs from participants enrolled in DECISION, a 16-week phase 2 dose-finding clinical trial conducted by the UNITE4TB Consortium at four African trial sites. Of 92 enrolled participants, 89 had baseline CXRs available. Novel severity scores were generated by quantifying size and probability of active TB from heat maps generated by CAD4TB v7.0, an existing CAD screening tool. Cox proportional hazards models were used to assess the relationship between baseline AI severity score and TTCC, with both unadjusted and adjusted for baseline covariates: age, sex, HIV status, and BMI. Since trial results have not been publicly presented, all analyses aggregated data across trial arms.

Results: Baseline severity, quantified using an AI-derived score from CAD4TB-generated heatmaps, was a strong predictor of TTCC. Among the participants with available baseline CXRs, the median baseline severity score was 36.3 (range 7.1-66.1). In unadjusted analyses, the severity score was strongly associated with TTCC, with a hazard ratio of 0.77 per 10 unit increase in score (CI: 0.66-0.89, $p < 0.0001$); results were similar in the adjusted analysis (HR: 0.69 CI: 0.57-0.83, $p < 0.001$). When split into quartiles, participants with the highest 25% of scores converted more slowly than the least severe group, with a median difference in TTCC of 28 days (HR 0.32, CI: 0.16-0.62, $p < 0.0001$) (Figure 1).

Conclusion: An AI-generated CXR severity score at baseline offers a potentially powerful tool to identify patients at risk of delayed culture conversion. These findings, collected in a randomized control trial, highlight the tool's potential for early risk stratification in treatment of pulmonary TB, with the additional benefit of improving consistency in data collection within multi-site studies.

Fig.1



P-1-5

Risk identification and risk management in infectious tissue biobanking

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Background and Objectives: Quality controlled and assured human biosample handling is an essential

cornerstone of tissue biobanking. The DZIF Tissue Bank, part of the Translational Infrastructure for Bioresources, Biodata, and Digital Health (TI BBD), is one of the first biobanks accredited according to the biobanking standard DIN EN ISO 20387 and a unique tissue bank for infection research worldwide. The DZIF Tissue Bank thus plays a central role in quality assurance, risk assessment, and quality management consulting, and plays a key role in a qualified biobanking infrastructure at the DZIF. However, the process entails various risks that can compromise sample integrity, data accuracy, and compliance with regulatory requirements.

Methods: To identify and minimize the key risks associated with tissue storage in biobanks, including collection, storage, and processing conditions, as well as data security, we have implemented a systematic risk assessment framework as part of our quality management system. Potential vulnerabilities in all areas of the DZIF Tissue Bank (e.g., storage of biospecimens, IHC, IF, chemical staining, tissue microarray assembly, nucleotide extraction) were first identified and then assessed based on their probability of occurrence and extent of damage using evaluation and acceptance criteria.

Results: The identified and categorized risks were systematically categorized in a risk matrix according to the severity of potential damage and the need for immediate or optional action. For very high risks, risk mitigation measures, such as standardized operating procedures (SOPs), quality control measures, staff training, and robust data management systems, were implemented to ensure maximum quality, safety, and reproducibility.

Conclusion: Quality assurance and quality control in the context of tissue biobanking is a key part of the work of the DZIF Tissue Bank. Risk assessment of all services and performed procedures and biobanking process is one key factor of a sustainable and consistent high quality. The continuous review, revision and improvement of work processes contributes to the leading role and outstanding position in infectious disease research tissue biobanking of the DZIF Tissue Bank from which all DZIF scientist profit. This highlights the importance of proactive risk management to ensure the reliability of biobank resources, maintain donor trust, and support reproducible research results.

P-1-6

Selective Eradication of *Staphylococcus aureus* Using the Recombinant Lytic Agent HY-133: A Novel Strategy Against Antimicrobial Resistance

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Question: *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), remains a major threat in healthcare settings. Traditional decolonization approaches such as mupirocin are increasingly compromised by emerging resistance, disruption of the commensal microbiota, and demanding application protocols. These limitations highlight the urgent need for alternative strategies that are rapid, specific, and capable of minimizing resistance development.

Methods: HY-133 is a recombinant bacteriolytic agent developed to address this gap. It combines the CHAP domain from the endolysin of bacteriophage K, responsible for enzymatic cleavage of bacterial cell walls, and the cell wall-binding domain of the staphylolytic enzyme lysostaphin. This chimeric design enables highly specific and efficient targeting of *S. aureus*, while sparing coagulase-negative staphylococci and other commensals of the microbiome.

Results: In vitro, HY-133 demonstrated rapid and robust bactericidal activity against a broad collection of *S. aureus* clinical isolates including MRSA (n>1000), representing extensive genetic diversity across more than 100 *spa* types and phenotypic variants (1-4). Time-kill studies revealed a pronounced reduction in viable bacterial counts within two hours of exposure (5). These findings are complemented by in vivo studies in animal models, which confirmed both safety and efficacy of HY-133 following nasal application. HY-133 has been manufactured in GMP quality as a stable, application-ready nasal formulation. Its clinical development has advanced to a randomized, double-blind, placebo-controlled phase 1 trial. This first-in-human study evaluates single and multiple dose regimens, assessing safety, tolerability, local effects, and preliminary efficacy. An extended study phase is also investigating its impact on the nasal microbiome.

Conclusions: HY-133 represents a new class of antibacterial agents based on engineered bacteriophage-derived proteins. Its mechanism of action is independent of traditional antibiotic resistance mechanisms, including those conferring resistance to mupirocin or beta-lactam antibiotics. The agent's high specificity for *S. aureus*, rapid bactericidal effect, and low risk of resistance development make it a compelling candidate for targeted decolonization prior to hospital admission or surgical procedures. By preserving the resident microbiota, it may also reduce the likelihood of re-colonization and lower the risk of nosocomial infections. The ongoing clinical trial will provide essential data on the potential of HY-133 to transform *S. aureus* eradication practices, offering a novel, precision-based approach in infection prevention.

1. Schleimer et al. 2019. Int J Mol Sci 20:pii:E716.
2. Kaspar et al. 2018. Antimicrob Agents Chemother 62:pii:e00385-18.
3. Idelevich et al. 2016. Antimicrob Agents Chemother 60:2551-3.
4. Kaspar et al. 2022. Microorganisms 10:35208898
5. Knaack et al. 2019. Diagn Microbiol Infect Dis 93:362-368.

P-1-7

Addressing the challenges of developing reliable minimum inhibitory concentration testing for some drugs targeting the metabolic pathways of

Mycobacterium tuberculosis

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As the crisis of antibiotic resistance looms on the horizon, the scientific community is rallying for the next antibiotic renaissance. Developing new and repurposed drugs at the speed necessary will take major collaborative efforts from public and private sectors worldwide. *Mycobacterium tuberculosis* is a devastating disease killing over 1.8 million people in 2023 alone, and around 4% of new cases have some antibiotic resistance. Furthermore, efforts to control the spread of tuberculosis have been severely backtracked due to the Covid-19 pandemic. In this presentation I will share research performed within European Regimen Accelerator for Tuberculosis (era4tb.org), a consortium of more than 30 partners from publicly funded institutes, universities, non-profit organizations, and pharmaceutical companies, which has successfully brought new drugs into clinical trials at half the time and cost previously achieved.

Although there has been significant progress developing drugs with novel targets, some promising compounds have proven challenging to handle in the lab. This work shows the difficulties in establishing reliable minimum inhibitory testing for the novel compounds Q203 (telacebec) and TBD-11. These drugs target metabolic pathways – specifically, Q203 targets the cytochrome bcc-aa3 complex in the oxidative phosphorylation pathway; and TBD11 targets cholesterol metabolism by modulating the activity of a specific adenyl cyclase encoded by the Rv1625c gene, and increasing cAMP production. The European Committee on Antimicrobial Susceptibility Testing has developed a standardized phenotypic assay for setting epidemiological cut-off values for *M. tuberculosis*. However, this method has not worked well with these specific drugs. This work demonstrates the limitations of the "one size fits all testing" approach to antimicrobial testing for this complex bacterium and next-generation antibiotics.

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P-1-8

Preclinical evaluation of highly potent anti-PcrV monoclonal antibodies in a murine *Pseudomonas aeruginosa* bloodstream infection model

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Pseudomonas aeruginosa (PA) remains a major clinical challenge causing severe nosocomial infections including bloodstream infections and sepsis, which is further aggravated by the rising prevalence of drug-resistant strains. Among the various virulence factors contributing to the pathogenicity of PA, the type III secretion system (T3SS) plays a central role in acute infections. This syringe-like structure injects bacterial effector toxins directly into the cytosol of host cells, promoting cell death and tissue damage. Due to its strong association with increased morbidity and mortality in infected patients, T3SS has emerged as a promising target for antivirulence therapeutic strategies.

Recently, we developed highly neutralizing patient-derived antibodies targeting the PcrV protein, which is located at the extracellular tip of the needle complex and is crucial for its function. These mAbs showed potent T3SS-inhibitory activity across antibiotic-resistant strains and demonstrated superior efficacy compared to competitor antibodies developed from murine models. To identify a lead candidate for further clinical development, we conducted comprehensive efficacy studies of selected anti-PcrV mAbs in a murine bloodstream infection model. Several candidate mAbs displayed robust protective effects, showing a clear dose-response relationship with efficacy observed at remarkably low doses up to 0.1 mg/kg. Consistent with our previous observations, potency of patient-derived anti-PcrV mAbs exceeds that of competitor anti-PcrV antibodies, highlighting their translational potential for therapeutic application. Notably, treatment with the patient-derived mAbs was well tolerated with no detectable adverse effects or toxicities.

These results underscore the superior functional activity of patient-derived anti-PcrV mAbs and support their advancement as clinical candidates for therapeutic use. Further preclinical characterization will form the basis for the selection of a lead candidate and the initiation of Good Manufacturing Practice development toward the first patient-derived anti-PcrV mAb for the treatment of acute PA infections.

P-1-9

Efficacy and safety of pyronaridine/artesunate and pyronaridine/artesunate/praziquantel for treatment of uncomplicated *Schistosoma haematobium* infection in Gabonese adolescents and children: protocol of a single-centre, assessor-blinded, randomized, controlled trial (CORMA-BIL Study)

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Background: Urogenital schistosomiasis remains a major public health concern in endemic regions, particularly among children and adolescents, where malaria is frequently co-endemic. In 2023, according to the WHO, schistosomiasis was responsible for an estimated 253 million cases globally, with 53% affecting children and adolescents. Given the overlapping populations affected by these diseases, there is a potential for integrated treatment strategies using antimalarial drugs with anti-schistosomal activity. Pyronaridine/artesunate (PYR/ART), an antimalarial, has

demonstrated potential anti-schistosomal effects in preclinical studies.

Methods: As part of the CORMA-BIL project, two phase IIa randomized controlled trials were conducted in Lambaréné, Gabon, to assess the efficacy and safety of PYR/ART, alone or in combination with PZQ, for the treatment of *Schistosoma haematobium* infection in children and adolescents. A total of 125 participants (aged 5 to ≤ 18 years) with confirmed uncomplicated urogenital schistosomiasis were enrolled. Participants were randomized 1:1 within two trials: (I) PYR/ART vs placebo and (II) PYR/ART+PZQ vs PZQ alone. Follow-up was 6 weeks for trial I and 14 weeks for trial II. The primary outcome was the percentage of egg reduction, with urine egg counts between baseline and week 6 for trial I and time to egg re-appearance after initial egg clearance for trial II.

Results: In trial I, the per-protocol population included 56 participants, while trial II comprised of 48 participants. The median age was 8 years (IQR:7-11) in trial I and 9 years (IQR 7-11.5) in trial II. Males represented 75% of trial I and 53.6% in trial II. Median baseline egg counts were 80.4 (23.1-176) eggs/10ml in trial I, and 28.1 (11.3-122) in trial II.

PYR/ART significantly reduced egg burden at week 6 compared to placebo, with a median egg reduction rate of 74% (95%CI: -37 to 93%) versus -51% (-316 to 39%), respectively (p =0.0034). In trial II, the PYR/ART + PZQ showed a significantly longer median time to reinfection (69 days) compared to PZQ (54 days) (p=0.01) indicating more favorable protection than PZQ alone. No serious adverse events were reported.

Conclusions: The combination of PYR/ART and PZQ demonstrated favorable efficacy against *S. haematobium*. These findings suggest that the anti-malarial treatment of PYR/ART offers complementary activity against schistosomiasis, supporting its potential use in integrated treatment strategies in co-endemic settings to improve resource use and control efforts.

Table 1: Reduction in *Schistosoma haematobium* Egg Counts from Baseline to Week 6 between PYR/ART and Placebo Arm

Figure 1: Time to Reappearance of Eggs in PYR/ART/PZQ and PZQ Arms

Fig. 1

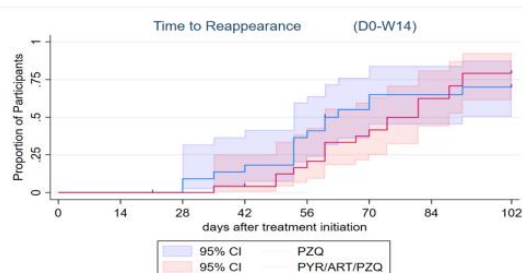
Population	N	Outcome	Placebo	PYR/ART	Wilcoxon ranksum test	
ITT	N=56*	% Egg reduction at W6 in reference to Baseline				
		Median [95% CI]**	-34% (-260% to 18%)	39% (-65% to 91%)	0.0495	
	Egg counts per 10 ml					
	N=56	Baseline	Median [IQR]	106 (46 to 189)	49 (11 to 161)	0.098
	N=56*	W4	Median [IQR]	126 (14 to 658)	45 (7 to 151)	0.06
N=56*	W6	Median [IQR]	126 (30 to 658)	21 (2 to 94)	0.002	
PP	N=47	% Egg reduction at W6 in reference to Baseline				
		Median [95% CI]**	-51% (-316% to 39%)	74% (-37% to 93%)	0.034	
	Egg counts per 10 ml					
	N=47	Baseline	Median [IQR]	106 (55 to 176)	71 (15 to 270)	0.32
	N=47	W4	Median [IQR]	136 (14 to 644)	48 (7 to 165)	0.12
N=47	W6	Median [IQR]	121 (26 to 683)	15 (1 to 114)	0.01	

ITT = intention-to-treat, PP = per-protocol

*Missing data was imputed based on the average egg counts of participants

**95% Confidence Intervals were obtained by non-parametric bootstrap (2000 resamples with replacement) and defined by the adjusted 2.5th and 97.5th percentiles of the bootstrap distribution

Fig. 2



P-1-10

Capture of viable bacteria from liquid samples to enhance sensitivity of classical microbiological methods

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Culturing of bacteria and fungi is a cost-efficient and well-established method for environmental monitoring, food & water safety testing, as well as clinical diagnostics. However, the methodology is limited by the sample volume that can be applied to the growth medium. This bottleneck is currently addressed by various concentration methods such as filtration and centrifugation protocols. In food testing it is circumvented by pre-culturing in liquid growth media. Each of these methods come with certain disadvantages.

The goal of this project is to develop a fast and user-friendly method to concentrate live bacteria from large volumes. Our aim is to capture bacteria with high cell release and survival rate without the use of filtration or centrifugation.

We implemented a workflow utilizing buffer-optimized magnetic separation. The performance of this workflow was tested using both Gram-positive and Gram-negative bacteria and capture efficiency was determined by comparing colony counts of plated bacterial populations before and after the capture process.

Preliminary results indicate a near-complete recovery of Gram-positive bacteria, showing strong potential for high-efficiency live bacterial capture. Ongoing work focuses on optimization of Gram-negative bacterial capture and maintaining viability across all bacterial types using common buffer conditions.

These findings demonstrate the feasibility of our approach to recover live bacteria from large-volume samples and even if present in very low concentration. With further optimization, this method holds significant potential for broad applications in rapid and accessible pathogen detection, microbiome studies, and various microbiological analyses where maintaining bacterial viability is essential and where classical microbiology's sensitivity is currently hampered by insufficient sensitivity.

P-1-11

Advancing Phage Therapy: Integrating Fitness Profiling of *Pseudomonas aeruginosa* under Phage-Pressure into Clinical Decision-Making

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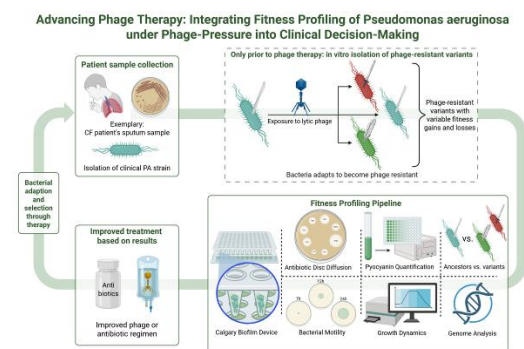
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Objectives: Multidrug resistant *Pseudomonas aeruginosa* (PA) poses a major clinical concern due to recalcitrant infections driven by its adaptability. While bacteriophages (phages) are a promising treatment option, phage resistance can develop and unpredictably alter bacterial fitness traits relevant to patient outcomes. This study aimed to establish and test a standardized pipeline to systematically assess such phage-driven changes, with the goal of improving phage therapy decision-making in clinical practice.

Methods: We developed a pipeline designed to be clinically applicable, enabling the evaluation of phenotypic changes in PA for risk assessment prior to treatment and real-time decision-making during phage therapy. To validate its use, clinical PA isolates were exposed to lytic phages, and resistant variants were selected. Subsequently, 16 pairs of phage-sensitive ancestor strains and their phage-resistant variants were compared across key traits: genome analysis, pyocyanin production, growth kinetics, motility, antibiotic susceptibility (disc diffusion), and biofilm formation (Calgary Biofilm Device with crystal violet staining and CFU quantification).

Results: The pipeline reliably identified diverse and clinically relevant fitness changes in phage-resistant PA variants, was well-suited for integration into routine phage therapy workflows, and required only standard lab equipment. These clinically relevant fitness changes differed markedly depending on the specific phage-bacteria combinations, affecting traits such as pyocyanin toxin production, which ranged from a 20.2-fold increase to a 56.3-fold reduction across different phage-driven variants of patient strains. Comparable diversity in phage-driven fitness costs and gains was observed for growth kinetics and motility. Antibiotic susceptibility profiles also shifted: some variants gained up to four new susceptibilities, while one acquired resistance to two additional antibiotics. Biofilm formation showed similar heterogeneity, being either unchanged or reduced in resistant variants.

Conclusion: The pipeline proved easy to handle, practical, and informative for evaluating clinically relevant phage-driven phenotypic changes. Given the strong variability between specific phage-bacteria combinations, systematic assessment using such a pipeline should become standard practice in phage therapy to optimize treatment and improve patient safety.



P-1-12

Repurposing Clomifene as a Small-Molecule Modulator boosting Antiviral Immune responses

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Background: CD8⁺ T cells are central effectors in immune defense against viral infections such as the lymphocytic choriomeningitis virus (LCMV). In chronic infections their activity is often impaired by exhaustion and immunosuppressive microenvironments. There is an unmet need for orally available, cost-effective agents that can enhance T cell responses through mechanisms complementary to existing therapies. Small-molecule modulators capable of directly activating CD8⁺ T cells represent a promising therapeutic approach.

Methods: A library of clinically approved compounds was screened using splenic T cells from LCMV-infected mice co-cultured with LCMV-gp33 antigen-expressing cells to assess T cell activity. Top candidates were further evaluated on isolated pan T cells stimulated with PMA/ionomycin to determine their direct effects on activation. Lead compounds were tested *in vivo* in chronic LCMV (clone 13) infection models. In addition, LCMV, VSV, and CMV *in vitro* infection assays were performed to distinguish whether compound activity was mediated through host cells or T cells.

Results: Clomifene, a selective estrogen receptor modulator was identified as a promising candidate that enhanced T cell activity against target cells. In the PMA/ionomycin activation system, clomifene directly increased T cell activation and dose-dependently upregulated TNF- α and IFN- γ production. *In vitro* virus plaque assays with LCMV (Armstrong), VSV, and CMV showed no direct antiviral effect on host cells, indicating that clomifene acts primarily on T cells. In LCMV-infected mice challenged with a high dose (5×10^6 PFU/mouse), clomifene treatment increased CD8⁺ T cell frequencies in blood and spleen and reduced expression of exhaustion markers TIM-3 and PD-1 on splenic CD8⁺ T cells. Under low-dose infection (1×10^6 PFU/mouse), clomifene increased effector CD8⁺ T cells, T follicular helper (Tfh) CD4⁺ T cells, and LCMV-specific CD8⁺ T cells (np396- and gp33-specific), without significant changes in LAG-3 or PD-1 expression.

Conclusion: Clomifene enhances antiviral immunity by promoting effector and antigen-specific CD8⁺ T cell responses and reducing T cell exhaustion in chronic viral infection models. These findings warrant further investigation into its mechanism of action and potential as a repurposed small-molecule immunomodulator for chronic viral infections.

P-1-13

Profound and immediate LMP1 effects on B-cell homeostasis are effectively downregulated by LMP1-TRAF2 inhibitors at the phosphoproteome level

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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 lymphoma (HL), and non-Hodgkin lymphomas (NHL). In 2020, EBV was responsible for at least 137,000 cancer deaths worldwide, highlighting the urgent medical need for new EBV-specific therapeutics. LMP1 is essential for the survival and proliferation of EBV-transformed B cells, thereby driving the development of most EBV-associated malignancies. By hijacking the cellular signaling network, it activates pathways that promote EBV-transformed cell survival. Phosphorylation is a key mechanism regulating signaling pathways. We therefore characterized the proteome-wide deregulation of host protein phosphorylation induced by LMP1 in lymphoblastoid cell lines (LCLs) to gain deeper insights into mechanisms of cell transformation. In addition, we analyzed the effects of SBL316, one of our in-house developed LMP1-TRAF2 small-molecule inhibitors which disrupts the interaction of TRAF2 with the LMP1 subdomain CTAR1, onto LMP1 induced phosphorylation.

Methods: LMP1 signaling was induced via crosslinking of an NGFR-LMP1 chimera protein in LCLs, with and without LMP1-TRAF2 inhibitor compound SBL316 treatment. SBL316 is part of a class of highly active, IP-protected LMP1-TRAF2 inhibitors that are currently in the lead optimization phase. A comprehensive proteome analysis of immediate phosphorylation events was performed. For validation, LMP1-induced phosphorylation of selected, high-interest targets, as well as their inhibition by the compound, was confirmed by immunoblotting. Phosphorylation events were then matched with databases of known phosphorylation sites in human signaling networks.

Results: Analysis of the immediate changes in the cellular phosphoproteome after one hour of stimulation already revealed broad effects of LMP1 onto signaling processes. These include phosphorylation of direct receptor-proximal signaling mediators involved in cellular signaling pathways such as NF- κ B and MAPK, as well as their downstream effector sites. Through the activation of these signaling pathways, LMP1 deregulates proteins and parts of cellular complexes that are necessary for cellular homeostasis such as components of the spliceosome, the SMN complex, and the HUSH complex. Even proteins that mediate cell-cell contacts are affected by LMP1. Treatment of the cells with SBL316 counteracted many LMP1-triggered phosphorylation

events, demonstrating that the inhibition of LMP1 signaling represents a valid therapeutic strategy.

Conclusions: Deregulation of the cellular signaling network by LMP1 can be monitored via phosphorylation changes in host signaling adaptor proteins. LMP1-TRAF2 interaction inhibitors effectively counteract LMP1 signaling and represent promising candidates for development into EBV-specific therapeutics.

P-1-14

Collating PK/PD data from multiple animal models for the pre-clinical development of Corallopyronin A

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Question: Corallopyronin A (CorA) is a novel antibiotic candidate with macrofilaricidal activity against filarial nematodes. Translating pre-clinical findings into human dose predictions requires integrating pharmacokinetic (PK) and pharmacodynamic (PD) data across diverse models that capture the complex host-parasite biology. Our aim was to compile PK/PD relationships for CorA from multiple animal systems to support the pre-clinical development of treatments for human filarial infections.

Methods: We conducted PK/PD studies in multiple models:

Litomosoides sigmodontis infections in BALB/c mice and gerbils allowing assessment against different developmental stages and PK parameters. *Dirofilaria immitis* in infected beagle dogs to evaluate PK/PD in a definitive host and investigate the macrofilaricidal activity of CorA. *Onchocerca ochengi* in gerbils, using (a) direct implantation of adult worms and (b) implantation of nodules containing *O. ochengi*, to compare drug distribution and efficacy in free versus encapsulated parasites.

In all models, plasma was sampled serially and analyzed via LC-MS/MS to determine PK parameters. PD endpoints included worm burden, *Wolbachia* load and changes in fertility.

Results: CorA exhibited measurable systemic exposure in all species, with notable interspecies variability. In *L. sigmodontis*-infected mice, CorA was effective against both developing and adult worms. Dose-fractioning experiments indicated a higher efficacy with multiple administrations per day in mice and gerbils. In addition, CorA was effective against adult worms in a mono- and co-

administrative therapy with benzimidazoles and long-term sterility of worms was confirmed in isolated worms up to 5 months after treatment in the gerbil model. In *D. immitis*-infected dogs, studies testing the efficacy of CorA against adult worms are ongoing. In *O. ochengi* models, directly implanted worms were highly susceptible to CorA. Collating PK/PD data revealed correlations between systemic and local exposure and macrofilaricidal effect with model-specific potency shifts.

Conclusion: Integrating PK/PD data from multiple animal models provides a translational framework for predicting effective CorA dosing in humans. Differences in drug distribution, parasite stage susceptibility and host physiology highlight the value of a multi-model approach for filarial drug development. These findings inform dose selection and trial design for advancing CorA into first-in-human studies targeting onchocerciasis and other filarial diseases.

P-1-15 Development of a repeated-batch process in shaking flasks (400 mL) for the corallopyronin A production

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Corallopyronin A, a polyketide antibiotic, originating from myxobacterium *Corallococcus coralloides*, is heterologously produced by *Myxococcus xanthus* DK 1622 [1]. It is being developed as an anti-*Wolbachia* drug to treat filarial infections as well as methicillin-resistant *Staphylococcus aureus* and fastidious intracellular bacteria, e.g., *Neisseria gonorrhoeae*. [2,3]

Currently, the production of corallopyronin A is a batchwise process, resulting in long processing times and high operational costs. To overcome these deficiencies, we developed a repeated-batch process. Process stability and reproducibility of the repeated-batch process were evaluated by characterizing product formation, substrate consumption, pH, cellular morphology, and behavior.

The most influential factors affecting product formation were identified as acetate concentration, cycle time, and inoculation volume. These factors were optimized using central composite face-centered design. Results indicate a global optimum at a cycle time of 100.8 h, an acetate concentration of 4.2 g/L, and an inoculum volume of 18.5%.

Compared to batch production, the repeated-batch process reduces overall process time by more than 40% and maintains ~ 83% of the product. The repeated-batch process increased productivity to 187% compared to four single-batch approaches, making it a powerful alternative for corallopyronin A production.

Long-term stability testing revealed the option to complete more than four cycles while maintaining product stability, which leads theoretically up to a 200% increase in productivity

[1] Pogorevc D, Panter F, Schillinger C, Jansen R, Wenzel SC, Müller R. (2019) Production optimization and biosynthesis revision of corallopyronin A, a potent anti-filarial antibiotic. *Metab Eng.* 09 55, pp. 201-211

[2] Krome AC, Becker T, Kehraus S, Schiefer A, Gütschow M, Chaverra-Muñoz L, Hüttel S, Jansen R, Stadler M, Pogorevc D, Müller R, Hübner MP, Hesterkamp T, Pfarr K, Hörauf A, Wagner KG, König GM. (2022) Corallopyronin A: Antimicrobial discovery to preclinical development. *Nat. Prod. Rep.* 09 01, 2022, pp. 1705-1720.

3] Edwards JL, Balthazar JT, Esposito DLA, Ayala JC, Schiefer A, Pfarr K, Hörauf A, Alt S, Hesterkamp T, Grosse M, Stadler M, Golparian D, Unemo M, Read TD, Shafer WM. 2022. Potent in vitro and ex vivo anti-gonococcal activity of the RpoB inhibitor corallopyronin A. *mSphere* 7:e0036222.

P-1-16 Serotonin agonist 5-Nonyloxytryptamine inhibits SARS-CoV-2 infection

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The COVID-19 pandemic has caused 7 million deaths globally (WHO, July 2025 <https://data.who.int/dashboards/covid19/deaths?n=o>) and continues to be a major cause of hospitalizations despite the presence of multiple vaccines. There is no clear seasonality in the number of SARS-CoV-2 infections, and new variants are constantly emerging necessitating the need for new therapies. 5-Nonyloxytryptamine (5-NL) is a serotonin receptor HTR1D agonist designed for the treatment of neurological disorders including depression and migraine. Using a co-culture screen of LCMV-primed splenic T cells and antigen presenting cells, we discovered novel immunomodulatory functions of 5-NL on antigen presenting cells in the context of the anti-tumoral setting (PMID: 37582744) and wondered whether the compound could additionally be repositioned as an anti-viral. Using in vitro based plaque assays, 5-NL did not suppress Lymphocytic Choriomeningitis Virus (LCMV) or Vesicular Stomatitis Virus (VSV, rhabdovirus) infection. This was corroborated in vivo where we showed that 5-NL did not improve the severity or survival of infected mice with LCMV (acute and chronic strains) or VSV.

Previously, we developed a deep learning-based platform for the in vitro classification of SARS-CoV-2 infection and drug toxicity, aimed at testing novel compounds against COVID-19 (PMID: 33918368). Specifically, using bright-field images of Vero cells infected with SARS-CoV-2, we developed a cytopathic effect (CPE) classification system. By excluding potential drug toxicity, we incorporated a toxic score (TOX) that detected cell death (CPETOXnet). When we tested the effect of 5-NL on SARS-CoV-2 infection using CPE, we found that a concentration of 2.5 µM reduced infection by half, and 5 µM almost completely blocked SARS-CoV-2 infection in Vero cells. This effect was confirmed using fluorescent microscopy with anti-SARS-CoV-2 specific antibodies. Mechanistically, 5-NL was able to decrease the

gene expression of TMPRSS2 in Vero cells, a cofactor used for SARS-CoV-2 cell entry. Furthermore, RNA sequencing in Vero cells revealed highly upregulated lipid synthesis pathways upon 5-NL treatment. Specifically, pathways related to sterol, cholesterol, and steroid biosynthesis were significantly upregulated, leading to increased lipid droplet formation. As increased cholesterol synthesis has been shown to interfere with the SARS-CoV-2 infection pathway (PMID: 33147445), and the accumulation of cholesterol within endosomes can reduce viral infection (PMID: 32975484), we suggest this to be a contributor of 5-NL's mode of anti-viral action.

Taken together, by implementing the CPE platform, we discovered that 5-NL decreases SARS-CoV-2 infection without causing collateral cytotoxicity. Mechanistically, 5-NL appears to increase lipid synthesis, potentially providing a novel mechanism for SARS-CoV-2 inhibition that warrants further exploration in additional strains and in vivo models.

P-1-17

Antimalarial Triple therapy with Atovaquone-Proguanil disrupts *Plasmodium falciparum* development in blood feeding infected mosquitoes

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Background: In addition to curative treatment against the pathogenic asexual stages of malaria parasites, targeting the transmissible sexual stages is essential to impede the spread of drug resistance. The effects of antimalarial drugs circulating in human blood on *Plasmodium falciparum* development in blood-feeding infected mosquitoes has been largely neglected. The present study relies on a phase III clinical trial testing the benefits of combining Atovaquone Proguanil (AP) with Artemether-lumefantrine (AL) in the

treatment of uncomplicated malaria. In this ancillary study, we blindly assessed the activity of plasma from African children treated with artemether-lumefantrine (AL) with or without atovaquone-proguanil (AP) in infected mosquitoes.

Methods: Eight time points (from Day 0 to Day 28) plasma from 17 patients treated with either AL+Placebo or AL+AP were collected for mosquito feeding. *Anopheles gambiae* laboratory-reared female mosquitoes were first exposed to infectious blood and four days later to a second blood meal reconstituted with the plasma from treated patients.

Results: A total of 7,961 mosquitoes were dissected, 4,187 at 7 days and 3,774 at 14 days post-infection (dpi). Plasma from AL+AP treated individuals significantly reduced oocyst infection prevalence and intensity up to 87% and 63% respectively. Similarly, for sporozoite infection prevalence and intensity, up to 80% and 89% were observed respectively. Extrinsic incubation period was also prolonged in the AL+AP treatment arm by producing smaller oocysts and reducing the prevalence and fraction of ruptured oocysts. Significant positive correlation was established between plasma atovaquone concentration and both transmission blocking activities ($\chi^2_1 = 33.45$, $p < 0.001$) and transmission reducing activity ($\chi^2_1 = 10.76$, $p = 0.001$). Similarly, reductions in oocyst size, prevalence and fraction of ruptured oocysts were positively associated with atovaquone concentrations. These inhibitory effects persisted up to 28 days post-treatment.

Conclusion: These results highlight a benefit of triple ACT containing atovaquone proguanil as when treated patients are bitten by infected mosquitoes, they may be partially "cured" of the infection and therefore reduce transmission.

Keywords: Transmission-blocking, *Plasmodium falciparum*, Triple ACT, Atovaquone

P-1-18

Target-based approaches to antischistosomal drug discovery

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Schistosomiasis is a neglected tropical disease caused by blood flukes (genus *Schistosoma*) and has significant medical and socio-economic impact in countries of the Global South. A vaccine is not available and praziquantel the only drug in use. This justifies the fear of emerging resistance and motivates the search for alternative treatments.

Using *in silico* approaches, worm *in vitro*-culture systems, animal models, and post-genomics tools, we explore novel strategies to identify antischistosomal molecules. In our target-based approach, genes known to be important for pathogen survival are targeted by different strategies. RNA interference (RNAi) is a highly specific approach for gene silencing and promises tremendous potential as novel therapeutic concepts for pathologic conditions. This includes infectious diseases, when genes of the pathogen are targeted. To explore siRNA therapy against *Schistosoma mansoni*, we prioritized genes known to be vital for the parasite. Efficacy of modified siRNAs with extended half-life was confirmed against worms *in vitro*. This way, we selected siRNAs against six target genes to be tested in a mouse

model. We achieved worm burden reduction of up to 60% using siRNAs targeting schistosomal ATPases. Future work will further optimize the protocol and test efficacy against juvenile parasites.

In another target-focused approach, we aimed to discovery natural products inhibiting the schistosomal thioredoxin glutathione reductase (SmTGR). Insects represent the most species-rich class of animals on earth with a wide spectrum of biologically active molecules, but have received only little attention as source for antiparasitic compounds. We designed a bioinformatics pipeline to create a virtual library of over 1000 insect molecules, which were subsequently used for a virtual screening against SmTGR. For one of the potential SmTGR inhibitors identified, buprestin H from jewel beetles, we confirmed activity against *S. mansoni* *in vitro*.

Our studies highlight the potential of siRNA as well as insect-derived molecules in antischistosomal drug discovery.

P-1-19
Meta-analysis of efficacy, effectiveness, and correlates of protection of vaccines across Africa, Europe, North America and India: an evidence-based approach to ascertain region-based differences of vaccine performance

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Background: The number of studies reporting differences in responses (immunogenicity & efficacy) between developed and developing countries is growing. The observed differences have been attributed to socioeconomic, epidemiologic, and genetic variations. Whether these factors, affect all vaccine platforms (Inactivated, Live attenuated, Toxoid vaccines, Subunit vaccines, DNA vaccines) and study design, will provide strong evidence of region-based differences in vaccine performance.

Methods: We designed a meta-analysis to compare the performance of vaccines across sub-Saharan Africa, Europe, North America (Canada/USA), and India. Co-primary endpoints were :1) efficacy, defined as randomized controlled phase 2/3 clinical trials assessing clinical disease, and/or infection ;2) effectiveness, defined as studies assessing vaccine responses against clinical disease, infection, infectiousness, severity of disease, or immunological markers; 3) correlates of protection, defined as phase 2/3 trials assessing validated thresholds of immune markers correlating to vaccine protection, Following PRISMA 2020 guidelines, four electronic databases were searched ,with exclusions for duplicates, irrelevance, or incomplete data.

Results: The search identified 11,365 records; 7236 duplicates and 2979 non-eligible records were excluded. Of 1,420 records reviewed, 34 studies met inclusion for quantitative synthesis so far in the Efficacy category comprising 14 live attenuated (mainly measles),5 subunit, 6 inactivated and 9 toxoids vaccines. Effectiveness included 57 studies, predominantly toxoid vaccines. Correlates included 103 studies, of which 35 were live attenuated, 35 subunit ,16 inactivated,17 toxoid vaccines. A total of 485 records remain for extraction after removing overlaps with efficacy and effectiveness records (Fig 1).

So far regional efficacy of live attenuated vaccines was higher in Europe (72.20%, CI 55.99-82.43), followed by India (60.74%, CI 51.44-68.27), North America (52.36%, CI 25.58-69.62), and Sub Saharan Africa (48.62%, CI 67.50-89.40). Effectiveness of toxoid vaccines showed a different pattern, North America leading (79.80% CI 67.50-89.40), followed by Europe (74.23% CI 61.23-90.30), India (57.20% CI 32.30-65.51), and Sub Saharan Africa (40.17% CI 21.63-54.80).

Across live attenuated vaccines, preliminary Seroprotection rate were higher in North America (91.1%, CI 85.0-95.0%), followed by India (90.5%, CI 82.0-95.0%), Europe (85.0%, CI 79.0-90.0%), and Sub-Saharan Africa (53.1%, CI 36.0-69.0%), corresponding to a relative risk of 0.58 (95% CI 0.46-0.73, I2 = 71%) North America versus Africa (Fig 2).

Conclusion: Preliminary results suggest lower post-vaccine performance in sub-Saharan Africa compared with Europe, North America, and India. These findings underscore the importance of investigating region-specific factors influencing vaccine performance and adapting immunization strategies accordingly.

Fig. 1

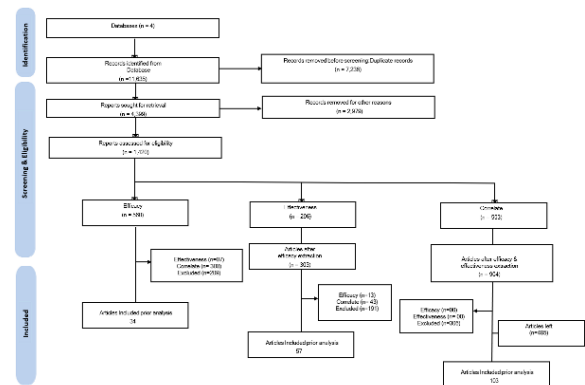
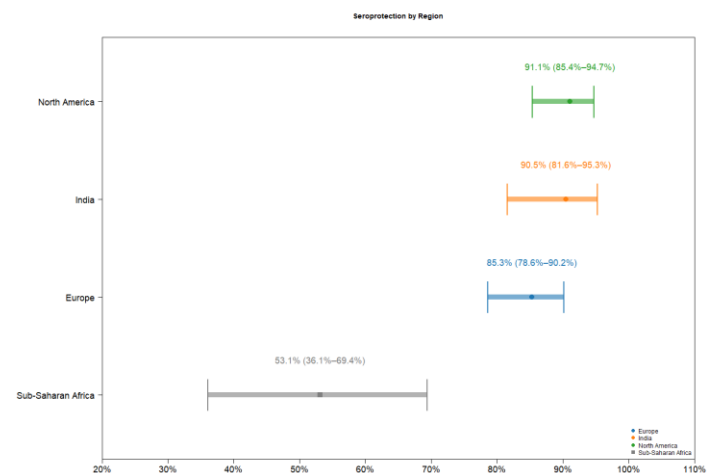


Fig. 2



P-1-20
Targeting Bacterial Biofilms with Coralopyronin A

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Biofilm-associated infections pose a persistent challenge due to their inherent resistance to conventional antibiotics. *S. aureus* biofilms are involved in osteomyelitis, skin and soft tissue infections, and implant-associated infections. Corallopyronin A (CorA), produced by soil myxobacteria, targets the DNA-dependent bacterial RNA polymerase by blocking the switch region [1]. It is active against MRSA and rifampicin-resistant *S. aureus* [2] as well as Gram-negative bacteria lacking efflux pumps and intracellular pathogens. It is in the transition from preclinical to clinical development for the treatment of filarial nematode infections [3].

To support its development for biofilm-associated infections, we assessed the efficacy of CorA against *S. aureus* biofilms, including MRSA and rifampicin-resistant *S. aureus*. We evaluated its ability to prevent biofilm formation and to eradicate pre-established *S. aureus* biofilms *in vitro*, using the MBEC system and confocal microscopy, complemented by an *in vivo* mouse model. With confocal microscopy, we screened a total of 43 staphylococcal strains (laboratory and clinical strains with different resistance profiles), of which 15 have been tested with the MBEC system.

We demonstrated that CorA inhibits biofilm formation and eradicates the preformed biofilm mostly in concentrations below or equal to the MIC, while the comparators dalbavancin and rifampicin require concentrations ≥ 4 -8-fold MIC. Viability test showed that CorA and rifampicin reduce biofilm biomass at concentrations lower than dalbavancin. In a murine foreign body infection model, CorA significantly reduced the bacterial load and edema caused by *S. aureus* infection.

These promising results support further evaluation of CorA in additional *in vivo* models, e.g., osteomyelitis and wound and soft-tissue infections, and in a hollow-fiber infection model to determine its PK/PD driver.

[1] Irschik H, Jansen R, Höfle G, *et al.* The corallopyronins, new inhibitors of bacterial RNA synthesis from Myxobacteria. *J. Antibiot.* 1985, 38, 145–152.

[2] Balansky J, Pfarr K, Szekat C, *et al.* The RNA Polymerase Inhibitor Corallopyronin A Has a Lower Frequency of Resistance Than Rifampicin in *Staphylococcus aureus*. *Antibiotics* 2022, 11, 920. <https://doi.org/10.3390/antibiotics11070920>

[3] Schiefer A, Hübner MP, Krome A, *et al.* Corallopyronin A for short-course antiwolbachial, macrofilaricidal treatment of filarial infections. *PLoS Negl Trop Dis.* 2020;14(12):e0008930. doi:10.1371/journal.pntd.0008930.

P-1-21

Challenges of dengue in haematological malignancy patients: Analysis from the DANGO registry during the 2023-2024 outbreak in Argentina

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Background: Dengue is a single-stranded RNA virus with four serotypes, transmitted primarily through the *Aedes aegypti* mosquito. Haematological malignancy (HM) patients are at heightened risk for severe dengue due to their immunocompromised state, yet the infection is often underdiagnosed in this group due to overlapping symptoms with their underlying condition or treatments.

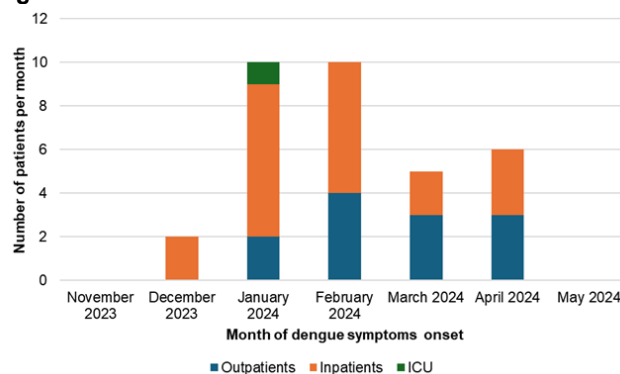
Methods: This retrospective multicentre study analysed HM patients diagnosed with dengue between November 2023 and May 2024. Using data from the DANGO registry, which documented cases from seven hospitals in Argentina during the dengue epidemic 2023-2024, the study included patients aged ≥ 16 years with confirmed dengue and existing HM, collecting data on clinical presentation, laboratory findings, and outcomes.

Results: The study included 33 HM patients diagnosed with dengue. The median age was 54 years. The most common comorbidities were hypertension (18%) and renal insufficiency (9%), and the most frequent HM was non-Hodgkin lymphoma (30%). Laboratory findings indicated severe/very severe thrombocytopenia (52%) and leukopenia (67%) in the majority of patients. The study found that 64% of patients required hospitalization, for a median duration of 7 days. The overall mortality rate was 6%, with all deaths associated with dengue complications.

Conclusion: Dengue in HM patients poses diagnostic challenges due to symptom overlap with other conditions. This study emphasizes the need to consider dengue in febrile illnesses during outbreaks in endemic areas, advocating for early diagnosis, adequate management, and possibly routine DENV testing in this population to reduce morbidity and mortality.

Figure 1. Patient distribution by symptom onset month

Fig. 1



P-1-22

Ceftazidime-avibactam in real-world clinical practice: results from a single center

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Background: During the last decade, the increase in multidrug resistance (MDR) of Gram-negative bacteria (GNB) has become an emergency worldwide and a challenge in clinical practice. This study evaluates clinical features, microbiological profiles, and outcomes, focusing on resistance patterns, antibiotic therapy, and mortality factors.

Materials and Methods: We conducted a retrospective, single center study to evaluate the clinical characteristics and outcomes of hospitalized patients treated with ceftazidime–avibactam (alone or in combination with aztreonam) for at least 72 hours. The study period covered from November 1, 2019, to September 30, 2024. Data collected included demographics, comorbidities, prior healthcare exposures (antibiotic use, hospitalizations, device utilization), clinical severity scores (PITT ≥ 4 , CCI ≥ 4 , INCREMENT ≥ 8), microbiological data on pathogens and resistance mechanisms, antibiotic therapies (empiric/targeted, duration, combinations), regimen adjustments (escalation/de-escalation) and mortality.

Results: A total of 109 patients were included (62% male, median age 49 years, range 16–88), with a median hospital stay of 31 days (range 4–248). Severe clinical scores were noted in 19% (PITT score ≥ 4), 32% (CCI ≥ 4), and 38% (INCREMENT score ≥ 8). CVCs were used in 75% of cases, prior antibiotic use was reported in 66%, prior hospitalization in 58%, and urinary catheter use in 49%. Hematologic malignancies were identified in 35%, with acute myeloid leukemia (17%) being the most common. *Klebsiella pneumoniae* was the leading pathogen (66%), predominantly isolated alone. Multidrug resistance was frequent, with MBL in 22% and MBL plus ESBL in 37%. All patients received empiric therapy (median duration 3 days), most commonly ceftazidime–avibactam with aztreonam (39%). Targeted therapy was administered to 77% (median duration 7 days), often with the ceftazidime–avibactam with aztreonam (48%). Antibiotic regimens were modified in 77% of cases, including escalation in 57% and de-escalation in 17%. Despite clinical cure was achieved in 74%, overall mortality reached 33%, with 16% attributed to infection. ICU admission ($p=0.034$, OR 4.1), INCREMENT score ≥ 8 ($p=0.035$, OR 3.5), and urinary catheter use ($p=0.025$, OR 4.6) were independently linked to higher mortality.

Conclusion: This study highlights the importance of early, targeted antibiotics and comprehensive management for multidrug-resistant (MDR) Gram-negative infections. Ceftazidime–avibactam, particularly with aztreonam, is effective against MDR pathogens, including MBL-resistant strains. ICU admission, high INCREMENT scores, and urinary catheter use are linked to higher mortality, stressing the need for vigilant risk assessment and tailored therapy. These findings offer real-world guidance for clinical decisions in this challenging setting.

P-1-23

Orthopoxvirus infection of the blood brain barrier – studies in a 3D spheroid model

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Orthopoxviruses (OPV) like monkeypox virus (MPOX), vaccinia virus (VACV) or variola virus (VARV), the trigger of smallpox, cause mainly dermal manifestation of varying severity. Neurological complications including altered mental status and seizures can be caused by OPV crossing the blood brain barrier (bbb), which subsequently leads to encephalitis and brain edema. In these cases viral DNA can be detected in cerebrospinal fluid. First studies performed in rodents lead to the suggestion that particularly MPXV can cross the blood brain barrier, which subsequently leads to neurological manifestation. For in vitro studies of OPV neurotropism and the efficacy of antiviral agents we have established a novel 3D cellular spheroid model of the blood brain barrier using primary human-derived pericytes and endothelial cells attached to an inner core of astrocytes. As shown by immunofluorescence staining of ZO-1, tight junctions between endothelial cells are formed. Particularly proteomics data will be analyzed for the further characterization of the heterocellular bbb spheroid regarding the establishment of bbb characteristics and cellular polarity in comparison to 2D cultivated cells. Cell type specific infection of orthopoxviruses and inhibition of the latter by the F13L phospholipase inhibitor tecovirimat (TPOXX) will be analyzed by flow cytometry and immunofluorescence staining. The 3D heterocellular bbb allows the investigation of virus trafficking in the brain and the inhibitory effects of antiviral compounds on that.

P-1-24

Identification and development of Sudan virus VP40 inhibitors with promising pan-filovirus activity using a crystallographic high-throughput screening

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The Sudan virus (SUDV) matrix protein VP40 plays essential roles in the viral replication cycle, mediating budding of virions and the regulation of viral genome replication and transcription.

Currently, no small molecule drugs are available for filoviruses, and existing vaccines and antibody therapies are limited to Zaire ebolavirus (EBOV), highlighting the urgent need for novel antiviral strategies.

Targeting of the essential VP40 dimers, which are precursors to higher oligomeric forms offers a promising therapeutic approach. In this study, we employed a high-throughput crystallographic screening in order to identify VP40-binding ligands. SUDV VP40 was expressed in *E. coli*, the fractions containing dimeric VP40 purified, and used for crystallization. Over 1,200 pre-formed protein crystals were soaked with more than 1,000 small molecules and analyzed via X-ray diffraction at Diamond Light Source, UK. We identified 50 binding molecules whose binding sites clustered on VP40's surface. These compounds were then tested for anti-filovirus activity under BSL-4 conditions, revealing that, at medium micromolar concentrations, four compounds effectively inhibited the replication of SUDV, as well as EBOV and Marburg virus, in a dose-dependent manner. Immunofluorescence analyses further showed that, upon treatment with the compounds, VP40 accumulated in NP-

induced inclusion bodies and was less present at the plasma membrane in SUDV-infected cells. This suggested that VP40 trafficking to the plasma membrane was impaired by the inhibitors.

In conclusion, our findings establish a strong foundation for the development of small-molecule inhibitors targeting VP40, paving the way for new therapeutic options to combat filovirus infections.

P-1-25

Novel Small Chemical Compounds Blocking Herpes Simplex Virus DNA Synthesis

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HSV-1 and HSV-2 cause disabling and life-threatening diseases of the oral and genital mucosa, skin, eye, and brain. Current antivirals in use or in clinical trials perturb viral DNA replication with amenamevir, pritelivir, ABI-1179, or ABI-5366 targeting the helicase-primase complexes, while acyclovir inhibits the polymerases. Despite their general safety and efficacy, there is an unmet clinical need for additional therapeutics, as toxicity and the rise of drug-resistant strains, particularly in immunocompromised patients, limit prolonged treatments.

Using a BAC-derived HSV1-GFP virus and phenotypic, microscopy-based screens, we identified six novel PANH compounds that reduced plaque formation of parental, acyclovir- and pritelivir-resistant HSV-1 and HSV-2 as well as VZV with IC₅₀ from 0.5 to 10 µM but not HCMV, KSHV, or adenovirus. As acyclovir and pritelivir, PANH_135, _173, and _174 inhibited DNA synthesis and reduced the formation of C-capsids. PANH_070, _128, and _184 had a minor effect on DNA synthesis and capsid formation, but impaired later steps of the infection. Further medicinal chemistry led to the identification of derivatives with IC₅₀ around 1 µM and CC₅₀ above 150 µM. All PANH compounds and these derivatives inhibited HSV-1 spread in a murine skin explant culture.

We isolated PANH-resistant HSV-1, sequenced their genomes, and reintroduced into the HSV1-GFP BAC single potential resistance-conferring mutations against different PANH compounds or known resistance-conferring mutations against acyclovir, pritelivir, or amenamevir. Testing these mutants indicated different modes of action for the PANH compounds than the other drugs. Moreover, we have synthesized clickable and photoactivatable derivatives that inhibit HSV-1 plaque formation at 100 to 300 nM in Vero cells and HaCaT keratinocytes. We will use these in biochemical assays to characterize the novel drug targets and potential host off-targets.

Our PANH-derived compounds might contribute to the development of novel therapeutic drugs for the combinatorial therapy of HSV-1, HSV-2, and VZV infections.

P-1-27

Targeting a cellular metabolic pathway with a broadly acting antiviral small molecule inhibitor

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Viral infections are still a huge burden in the immunocompromised host. Adenovirus (AdV), Kaposi-Sarcoma-Associated Herpesvirus (KSHV), and Epstein-Barr-Virus (EBV) infections can cause severe diseases especially in transplant patients and patients treated with immunomodulatory drugs while so far no antiviral treatment against these viruses is available yet. Host targeting antivirals (HTAs) typically target universal cellular functions, which are necessary for viral replication, often making them broadly acting inhibitors with low viral resistance. We identified a small molecule inhibitor (SMI) "1704", which is active against a broad range of DNA viruses, like EBV AdV and KSHV with an IC₅₀ in the high nanomolar range with almost no cell toxicity.

We could show that one of the mechanisms of action of 1704 is the inhibition of the Dihydroorotate dehydrogenase (DHODH) which is an essential enzyme in the *de novo* pyrimidine synthesis pathway. This pathway is important for the replication of different viruses, explaining the broad antiviral activity of 1704. Recent work published by others and our preliminary findings suggest that KSHV and AdV may reroute the pyrimidine synthesis and DNA-repair pathways to their own advantage and may therefore be particularly sensitive to its inhibition. Our findings also suggest a possible additional mode of action which involves targeting cellular DNA repair mechanisms as we could show that compound 1704 can interact with DNA-PK. This could contribute to the broad antiviral range and efficacy of our SMI.

Testing 1704 in *in vitro* and *in vivo* ADME studies we could show a promising metabolic drug profile and first *in vivo* experiments in an EBV/KSHV co-infected tumour mouse model show a reduction of EBV viral loads as well as limited growth of tumour cells in these mice. Mice treated with this SMI tolerated the treatment over a period of two weeks. Thus, our novel SMI 1704 could represent a promising candidate for the development of a novel broadly acting antiviral drug for the treatment of several DNA viruses of concern in immunocompromised patients. As a first step in this process we have synthesized about 50 derivatives of SMI 1704 and have obtained compounds with an approximately 10fold lower antiviral IC₅₀ concentration in the mid-nanomolar range.

P-1-28

ULBP2-expressing HCMV mutants modulate NKG2D expression and function of CD8+ T cells

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Solid organ transplant recipients with insufficient HCMV-specific memory T-cell responses are at high risk of viral reactivation and severe complications due to immunosuppression. This risk is especially pronounced in HCMV donor+/recipient- patients, where a protective HCMV vaccine, which is currently not available, would reduce the risk for HCMV reactivation and disease. Therefore, developing a live-attenuated HCMV vaccine that induces HCMV-specific protective T cells is crucial. We investigated HCMV mutants expressing the costimulatory NKG2D ligand ULBP2, in presence or absence of immune-evasins for HLA class I molecules (US2-6, US10, US11), to compare their ability to activate HCMV-specific CD8+ T cells. HCMV mutants were derived from the parental strains TB40 (Δ US2-US6) or TB40R (expressing US2-6), with ULBP2 inserted in the UL16 region under a weak or strong promoter. Fibroblasts (HFF) infected with these mutants were analyzed for ULBP2 and HLA class I expression. Co-culture experiments of virus-infected HFF and healthy human PBMCs served to assess CD8+ T-cell activation, NKG2D receptor modulation, and HCMV-specific responses using IFN- γ ELISpot assays. The presence of US11 resulted in downregulation of HLA class I molecules in all infected cells, with a stronger effect in TB40R-infected cells due to the expression of US2-6. After 24h of co-culture, CD8+ T cells were activated by HCMV mutants, as indicated by CD69 upregulation. Furthermore, ULBP2 expression induced significant NKG2D down-modulation on CD8+ T cells, correlating with the strength (weak, strong) of ULBP2 expression. In addition, HCMV-specific CD8+ T cells were found in response to our HCMV mutants, demonstrating the strong immunogenic potential of ULBP2-expressing mutants. Deleting HLA class I immune evasins while expressing the costimulatory ligand ULBP2 is a promising strategy for HCMV vaccine development. This approach effectively elicits robust T-cell responses, offering potential for high-risk transplant recipients.

P-1-29
Advances in the development of synthetic small molecules targeting the central regulator of *Salmonella* pathogenicity

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Salmonellosis is the second most reported foodborne gastrointestinal infection globally. *Salmonella enterica* represent a substantial health and economic burden in humans, livestock, and poultry. While most non-typhoidal *Salmonella* infections are self-limiting, invasive and antibiotic-resistant strains can lead to a systemic disease.

Here, we present advances in the anti-virulence strategy targeting *Salmonella* pathogenesis by inhibiting HilD, a transcriptional regulator essential for bacterial invasion and systemic dissemination in the host (1). We describe a series of synthetic small molecules inhibiting the transcriptional activity of HilD, subsequently blocking the expression of virulence gene clusters. A structural characterization of the drug-target complex unveiled the binding site and binding mode of the inhibitors. We then applied a structure-activity relationship analysis to reach activity at nM scale, and performed a pharmacological characterization of optimized

compounds for lead selection. Finally, we present advances towards an *in vivo* proof-of-efficacy.

Synthetic small molecules targeting HilD could be valuable options for the treatment of *S. enterica* gastrointestinal infections and the prevention of invasive *S. enterica* infections in humans and animals.

Reference (1) Boudrioua *et al.* Discovery of synthetic small molecules targeting the central regulator of *Salmonella* pathogenicity. *Sci.*

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P-1-30
Development of a robust and standardized progression cascade for compounds targeting host-pathogen interactions in tuberculosis within the ERA4TB consortium

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Over the past decade, our understanding of the host-pathogen interface in *Mycobacterium tuberculosis* (*Mtb*) infected cells and tissues has leapt forward dramatically. In addition, recent developments in phenotypic drug screening have allowed the identification of compounds that interfere with critical bacteria-derived virulence factors or function as immunomodulators of infected host cells. However, strategies for standardized characterization of these compounds and identification of their respective targets are lacking, which limits the rapid clinical development of hit or lead compounds derived from these drug discovery campaigns.

Here, we describe the development of a multi-assay progression cascade to investigate interventional aspects of host-pathogen interactions (HPI) targeting *Mtb* within the ERA4TB consortium.

A streamlined flow of HPI assays was created to distinguish between compounds targeting host-directed effects or impairing bacterial virulence factors. Using a panel of HPI-targeting compounds with known mechanism of action, we were able to define the specificity and performance of the platform, which will help to better characterize compounds modulating HPI in future efforts of the consortium. This differentiation provides essential information needed to select downstream approaches aiming at target identification. After investigation of putative mechanisms of action, these approaches include determining the contribution of different HPI-targeting compounds to the antibacterial activity of classical anti-tubercular antibiotics in *ex vivo* combination assays. We are exploiting a comprehensive approach combining high-content based phenotypic screening of various drug combinations by automated confocal microscopy followed by classical colony forming unit (CFU) determination of infected human macrophages complemented by an high throughput human

neutrophil necrosis assay. Our comprehensive approach deciphers translational aspects of the *Mtb*-HPI interface, facilitating the rational progression of HPI hit and lead compounds. In the future, this platform will be used to improve host-directed therapies against tuberculosis.

P-1-31

The Cholesterol-Pneumolysin Interaction as a Therapeutic Avenue for Invasive Pneumococcal Diseases

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Background: Invasive pneumococcal diseases (IPD) caused by *Streptococcus (S.) pneumoniae* remain a leading cause of global infectious mortality, despite the availability of antibiotics. A key virulence factor in IPD is pneumolysin (*Ply*), a cholesterol-dependent cytolysin that disrupts host membranes, evades immune detection, and facilitates bacterial spread. Preclinical and clinical evidence suggests that host cholesterol dynamics influence disease severity, offering a potential therapeutic avenue through cholesterol modulation.

Hypothesis: We hypothesize that synthetic sterol derivatives can function as decoys for *Ply* to reduce cytolytic activity and invasiveness of *S. pneumoniae*. We further propose that structural optimization of these sterols can enhance both *Ply* scavenging and cellular protection, providing dual-mode therapeutic support in IPD.

Objective: To develop a library of structurally diverse, high-purity synthetic sterols. Characterize sterol-PLY interactions and their biological effects in vitro and ex vivo. Elucidate cholesterol's role in immune modulation and detoxification via nanoparticle delivery systems.

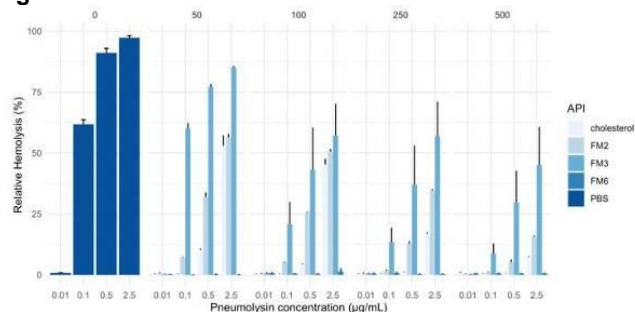
Methods: Cholesterol was chemically modified targeting its stability and *Ply* binding efficacy using Halogenation, generating a library of active pharmaceutical ingredients (API) candidates. HPLC-MS assessed the purity of the synthesized sterol derivatives. The API candidates were then formulated into liposomes using the ethanol injection method, employing dipalmitoylphosphatidylcholine (DPPC) as the lipid matrix. The resulting liposomal formulations were characterized for their physicochemical properties (dynamic light scattering, electron microscopy). To assess the biological activity of the API's were screened by a hemolysis assay and pneumolysin as CDC.

Results: Eight API candidates were successfully synthesized with varying, hydroxylation, fluorination and deuteration. Formulated liposomes with the candidates were 70 nm 140 nm range (at a constant API candidate concentration of 44.8 molar % and 30.9 wt/wt % had a polydispersity index (PDI) < 0.2, and zeta potential of -10 mV. Modifications (single fluorination and mesyl substitution at the terminal methyl, hydroxyl at the D-ring, deuteration) resulted in loss of activity and membrane integration of the

API candidate. Di-fluorinated and deuterated API's inactivated *Ply* inactivation in the 50 µg/mL liposome or more efficient as cholesterol.

Conclusions: These findings support the continued development of synthetic sterol-based formulations as a promising adjunctive strategy for mitigating the effects of IPD and to support the host defense.

Fig. 1



P-1-32

Unveiling the Function of LlpA in the Biosynthesis of Lysolipin I in *Streptomyces tendae* Tü 4042

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Polyketides represent a fascinating class of natural products, renowned for their structural complexity and diverse biological activities. Their biosynthesis typically involves modular polyketide synthase (PKS) assembly lines, embellished by a range of specialized tailoring enzymes. While ester and ketone moieties are common across polyketides, certain members of this family also feature amide bonds, which can significantly influence their chemical stability and bioactivity.

Lysolipin, a halogenated polycyclic xanthone produced by *Streptomyces tendae* Tü 4042, exemplifies such complexity, incorporating an amide linkage within its structure. It was previously proposed that this amide bond is introduced early in the biosynthetic pathway by a dedicated amidotransferase, which catalyzes the formation of a malonamide starter unit^[1] via the transfer of an amino group to malonyl-CoA, as found for oxytetracycline.

However, our recent findings challenge this assumption. Functional inactivation of the putative amidotransferase gene *llpA* in *S. tendae* generated a $\Delta llpA$ mutant that completely abolished production of Lysolipin I, while instead accumulating a biosynthetic intermediate lacking the characteristic amide bond. These results strongly suggest that the amide nitrogen in lysolipin I is incorporated at a later stage, following the formation of the polycyclic core.

This revised understanding of *llpA*'s function sheds new light on the enzymatic logic of lysolipin biosynthesis and points to an alternative role for *llpA* in the pathway. Elucidating these mechanisms not only enhances our knowledge of natural product biosynthesis but also paves the way for bioengineering efforts aimed at generating novel compounds with improved properties.

References:

[1] Lopez *et al.* Gene 2010, 5-14.
<https://doi.org/10.1016/j.gene.2010.03.016>

P-1-33

Estimating the Impact of Interethnic Differences in CYP2C9 Metabolism of Corallopyronin A using Physiologically Based Biopharmaceutics Modeling

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Corallopyronin A (CorA) depletes the essential *Wolbachia* endosymbionts of filarial nematodes and is a promising candidate for the treatment of lymphatic filariasis and onchocerciasis. Since these neglected tropical diseases predominantly affect patients of African descent, population specific metabolic differences must be considered during clinical development. Based on its chemical structure and properties, ADMET Predictor[®] (v10.3) predicted CorA metabolism to be mediated mainly by CYP2C9, an enzyme known to exhibit variable expression across populations.

In vivo efficacy was assessed in the *Litomosoides sigmodontis* mouse infection model by quantifying *Wolbachia* burden via qPCR. A physiologically based biopharmaceutics model was developed in GastroPlus[®] to simulate corresponding plasma concentrations after multiple-dose administration over a 14-day treatment period. A modeled effective dose of 16.5 mg/kg twice per day in mice was converted to an anticipated effective dose in humans of approximately 100 mg twice per day.

The hepatic clearance of CorA for a 70 kg human was estimated at 2.34 L/h using allometric scaling from preclinical species (mouse, rat and dog), assuming homozygosity for the CYP2C9*1 wild-type allele. A quantitative structure-activity relationship model predicted a 65 % reduction in CorA clearance for heterozygous mutations compared with wild type, based on literature data from 12 compounds. Extrapolation of the correlation between heterozygous and homozygous variants suggested an 86 % reduction in clearance for homozygous mutations.

The Hardy-Weinberg equation was applied to estimate allele and genotype frequencies in different populations, allowing integration of genetic diversity into the pharmacokinetic modeling. To account for the predicted clearances, the Michaelis-Menten constant of CYP2C9 was adjusted, and simulation of a single 100 mg oral dose resulted in different AUC_{0-48h} across populations: 35 µg*h/mL for wild type, 46 µg*h/mL for Caucasian, 38 µg*h/mL for African, and 36 µg*h/mL for Asian populations. These results indicate that when progressing from Phase I trials in Europe to later clinical phases in African endemic regions, approximately 20 % lower drug exposure may occur at the same dose.

These findings underscore the importance of considering CYP2C9 polymorphisms when interpreting Phase I results, translating them into later stages of clinical development, and assessing potential drug-drug interactions. Such an approach will help to optimize dosing strategies and ensure therapeutic efficacy across genetically diverse patient

populations, particularly in the context of neglected tropical diseases where clinical studies must reflect real world population genetics.

P-1-34

The influence of lysosomal ion channels on intramacrophage replication of high consequence bacterial pathogens

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Facultative intracellular bacteria whose lifecycle primarily occurs within macrophages, i.e. *Francisella tularensis*, *Yersinia pestis* and *Brucella spp.*, the highly pathogenic causative agents of tularemia, plague and brucellosis, respectively, are classified as potential bioterrorism agents due to their high misuse potential. Their low infectious dose and high lethality underscore the urgent need for research into effective prevention and treatment strategies. Since the replication inside macrophages is a critical phase in their pathogenesis, understanding this intracellular lifecycle is essential for unraveling mechanisms of intracellular replication and immune evasion, making them points of application for prophylactic or therapeutic intervention.

While the functional role of endosomal cation channels has been shown to counteract viral infections, their impact during the progression of bacterial intracellular infections remains poorly understood. Nevertheless, the interaction of bacteria with the endo-lysosomal pathway is a hallmark of intramacrophage replication of these bacteria. Here, intracellular replication as well as endosomal escape and intracytoplasmic replication strategies are employed by the bacteria. Our study aims to better understand the prerequisites for bacterial replication by analyzing the influence of ion channels on endosomal maturation and progression of bacterial infections at different stages.

To address these challenges, we have developed robust *in vitro* infection models for bacteria replicating in the intracellular environment of macrophages. Our models utilize *in vitro* differentiated macrophage-like cells, i.e. from THP-1 cells, to closely simulate the natural host environment. These systems provide a versatile platform for infection research and can be combined with down-stream analysis such as flow cytometry, fluorescence and electron microscopy, as well as transcriptomic analysis, e.g. by quantitative reverse transcription PCR or RNA-Seq.

Using these models, therapeutic interventions with antibiotics or agonists and antagonists of endosomal ion channels can be analyzed on the cellular level, and therefore, first *in vitro* efficacy data for new intervention strategies can be generated.

P-1-35

Targeting the master regulator of Salmonella pathogenicity: HiID

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Salmonella's virulence relies mainly on type III secretion system (T3SS), whose expression is centrally regulated by HilD, an AraC-like transcription factor that integrates environmental signals to activate pathogenicity islands SPI-1, SPI-2, and SPI-4. Deletion of HilD renders *Salmonella* avirulent in murine models, establishing this protein as a critical regulatory node and promising drug target. The global rise of multidrug-resistant bacteria demands alternative strategies to combat infection. Antivirulence approaches, which disarm pathogens without affecting their viability, represent a promising approach.

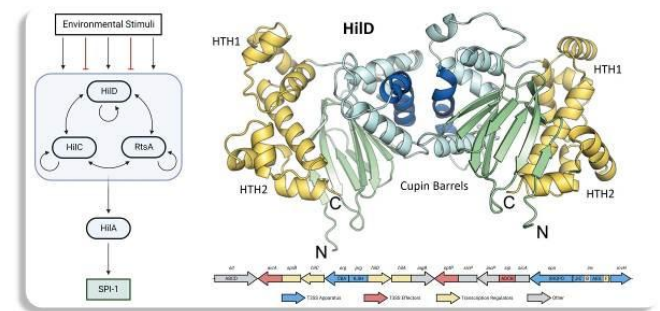
We applied complementary biochemical approaches such as EMSA, NanoDSF, and analytical SEC, to dissect HilD regulation by natural effectors and synthetic inhibitors. Long-chain fatty acids (LCFAs) directly bind HilD, preventing its dimerization and DNA binding, whereas HilE inhibits HilD through protein-protein interaction. These distinct mechanisms converge to tightly control HilD activity and thereby pathogenicity. During our studies, we identified the compound C26: a synthetic small molecule that occupies the conserved LCFA-binding pocket of HilD effectively inhibiting its activity as transcription regulator.

Our findings define the molecular mechanisms controlling HilD activity and demonstrate that this transcriptional regulator is druggable. This work illustrates how detailed mechanistic understanding of virulence regulation can be translated into tractable drug discovery strategies against high-priority pathogens.

Fig. 1



Fig. 2



P-1-36

The nucleoside analogue NITD008 in combination with Ribavirin strongly suppresses viral replication in immunodeficient humanized mice stably infected with the hepatitis E virus

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Background and Aims: 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 HEV, off-label treatment with ribavirin (RBV) is used in daily clinical practice, although it is effective in 80% of patients. Thus, there is an urgent need for effective antiviral drugs against HEV. The adenosine nucleoside analogue NITD008 was shown to be effective in preclinical studies against a broad range of RNA viruses. The aim of this study was to evaluate the efficacy of NITD008 alone or in combination with ribavirin in HEV-infected human liver chimeric mice.

Method: Immunodeficient human-liver chimeric mice were infected with HEV (GT-1) via the co-housing fecal-oral route. After achieving stable viremia (median 1.5x10⁷ IU/ml HEV RNA), mice received either 20mg/Kg/day of NITD008 alone by oral gavage for up to 2 weeks, or 5mg/Kg NITD008 in combination with RBV (50mg/Kg), while other mice received mock treatment or RBV monotherapy. Viral changes were determined by qPCR in faeces and blood. Intrahepatic analyses (immunohistochemistry, RNA-ISH and qPCR) were performed at sacrifice.

Results: NITD008 monotherapy effectively reduced fecal HEV loads ($\Delta\log$:-4log) already after 1 week of treatment. All mice had undetectable serum HEV titers (LloQ) after 7 days, while controls showed no decrease in fecal and serum titers. Intrahepatic analysis showed a $\Delta\log$:-2.5 reduction in HEV RNA levels in treated mice compared to controls after 14 days of treatment. RNA-ISH analysis confirmed the strong reduction of HEV-RNA-positive human hepatocytes in treated mice, whereas approximately 95% of human hepatocytes were positive in control animals. NITD008 administration did not induce ALT elevation. 11 days of combination therapy induced much stronger suppression of HEV RNA in faeces ($\Delta\log$:-5.02) than ribavirin monotherapy ($\Delta\log$:-1.08), despite using lower NITD008 amounts.

Conclusion: This study demonstrates the efficacy and non-toxicity of NITD008 towards human hepatocytes in HEV infected humanized mice. NITD008 displayed more potent antiviral activity compared to ribavirin and combination

regimens with ribavirin and lower NITD008 concentrations showed strong synergistic antiviral activity. The study indicates that HEV can potentially be cleared even in immunosuppressed individuals and highlights the importance of further developing this class of direct-acting antiviral agents.

P-1-37

Targeting Murein endopeptidases as novel targets to fight multidrug-resistant *Pseudomonas aeruginosa*

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Nosocomial infections with multidrug-resistant (MDR) Gram-negative bacteria are an increasing threat. Novel approaches to fight Gram-negative (GN) pathogens are needed but novel compounds addressing innovative targets remain scarce. GN bacteria have an outer membrane and an inner membrane, 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 chains are connected via peptide crosslinks. Assembly of novel glycan building blocks into the sacculus requires Murein endopeptidases (Mep) which cleave the crosslinks between the peptide side chains. We recently identified four Mep genes, encoding *mepM1*, *mepM2*, *mepM3*, and *mepH2*, as candidates contributing to β -lactam resistance in a MDR *Pa* bloodstream isolate [1]. The three putative MepM proteins identified in our screen, namely *PaMepM1*, *PaMepM2*, *PaMepM3*, are phylogenetically highly related to *EcMepM* and we could validate that *PaMepMs* contribute to β -lactam resistance. We have shown that MepM proteins are promising targets for novel drugs to re-sensitize MDR pathogens to β -lactam treatment, especially meropenem. The disruption of MepM proteins in MDR *P. aeruginosa* strains resulted in a decrease in the meropenem minimal inhibitory concentration in a range that would render treatment with meropenem a therapeutic option. Furthermore, we have collected evidence that MepM proteins have a significant influence on several biological processes associated with pathogenicity including, biofilm formation, cell motility, salt and bile resistance. As the active sites of *PaMepM1*, *PaMepM2* and to lesser extent *PaMepM3* are highly homologous, we performed a virtual screen to identify inhibitors targeting both *PaMepM1* and *PaMepM2*. For inhibitor testing, we developed an *in vitro* MepM activity assay. This assay uses a quenched fluorescence minimal endopeptidase substrate, resembling the muropeptide crosslinks. Using this assay, we could verify the activity of MepM inhibitors identified *in silico* and could show that it is indeed possible to simultaneously target both *PaMepM1* and *PaMepM2*. Furthermore, we already have tested a set of derivatives of our best performing compound to elucidate the

potential for compound optimization. The promising results open up a novel route to combat MDR *P. aeruginosa* by the inhibition of MepM proteins.

1. Sonnabend MS, Klein K, 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). doi: 10.1128/AAC.01771-19.

P-1-38

Designing metabolically enhanced next-generation probiotics to eradicate multi-drug resistant bacteria from the gut

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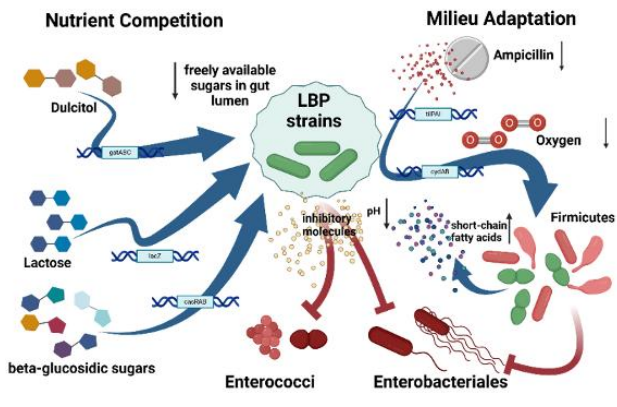
Healthcare-associated infections, especially those caused by multidrug-resistant (MDR) bacteria, are a leading cause of morbidity and mortality worldwide¹. Gut colonization with MDR bacteria often precedes infections and facilitates patient-to-patient transmission, necessitating costly isolation measures. While antibiotics effectively treat acute infections, their preventive use is limited due to detrimental impacts on the gut microbiota and promotion of further resistance in nosocomial pathogens. Consequently, innovative strategies to selectively eliminate MDR pathogens from the gut are urgently needed.

The human microbiome, a diverse ecosystem of microorganisms, offers a promising source for novel therapeutic agents. Microbiota-based interventions, such as fecal microbiota transplants (FMTs), have shown remarkable success in treating recurrent *Clostridioides difficile* infections (>90% cure rates)^{2–4}. However, FMTs are less effective against gram-negative MDR bacteria (GN-MDRs)⁵, likely due to the metabolic flexibility of these pathogens, which enables them to persist despite intervention.

Our project aims to address this limitation by developing live biotherapeutic products (LBPs)—defined microbial consortia designed to synergistically close the metabolic niches of MDR bacteria in the gut. LBPs improve upon traditional FMTs due to their controlled composition, reducing safety risks and simplifying production and regulatory approval.

We have identified specific strains within the Enterobacteriales family as critical components of these consortia 6-9. So far, Enterobacteria were largely excluded from probiotic strain mixtures due to safety concerns but we could prove that careful strain selection and genetic engineering shows that safe enterobacterial can be selected for use in humans. Our findings highlight their essential roles in suppressing GN-MDR pathogens. These strains perform key functions, including metabolizing carbon sources critical for pathogen growth, degrading antibiotic residues and oxygen to support the recovery of anaerobic bacteria, and contributing to down-regulation of intestinal inflammation⁶⁻⁹.

The efficacy of our LBP candidates has been demonstrated in multiple *in vivo* models, including immunocompromised mice, humanized microbiota models, and those consuming human-like diets. These results underscore the importance of interdependent interactions between Enterobacteria and strict anaerobes in restoring colonization resistance. This approach represents a significant advancement in microbiome therapeutics, enabling the development of precise bacterial cocktails to combat MDR pathogens effectively and sustainably.

Fig. 1**P-1-39****MALDI imaging of the anti-tuberculosis drug Q203 in mouse tissue***L. Grösche^{1,2}, C. Hölscher^{2,3}, K. Walter^{2,3}, A. Römpf^{1,2}¹University Bayreuth, Chair of Bioanalytical Sciences and Food Analysis, Bayreuth, Germany²German Center for Infection Research (DZIF), Brunswick, Germany³Research Center Borstel, Leibniz Lung Center, Division of Infection Immunology, Borstel, Germany

The potential cessation of USAID funding for **tuberculosis** (TB) control is projected to result in an increase in TB incidence rates. Modeling suggests that from 2025 to 2030, countries with a high dependency on this funding could experience a rise in TB cases ranging from 2.1 % to 36 %.(1) This highlights a critical need for international collaboration and investment in the development of novel anti-TB strategies and pharmacological agents. One promising drug candidate is **Q203**, which acts by inhibiting a crucial energy production pathway in *Mycobacterium tuberculosis*, thereby impeding bacterial growth.(2)

However, the formation of necrotic granulomas in the lung makes it difficult for antibiotics to penetrate.

To effectively analyze and visualize the spatial distribution and penetration of drugs within these granulomas, we applied **Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI)**. It is one of the modalities capable of providing detailed molecular maps of drug localization within tissue samples. Experimental parameters including matrix compounds and application have been systematically optimized. Several measures were taken to ensure the reliable and accurate detection of Q203. Prior to analysis, lung tissue sections were treated with γ -irradiation to inactivate the pathogen. A mimetic tissue model spiked with Q203 was used as model system to identify the limit of detection. Lung tissue of interleukin-13-overexpressing mice was then used to investigate the penetration of the drug into granuloma.

References:

(1) Mandal, S.; Nair, S.; Sahu, S.; Ditiu, L.; Pretorius, C. A Deadly Equation: The Global Toll of US TB Funding Cuts, 2025. DOI: 10.1101/2025.03.04.25323340.

(2) Jager, V. R. de; Dawson, R.; van Niekerk, C.; Hutchings, J.; Kim, J.; Vanker, N.; van der Merwe, L.; Choi, J.; Nam, K.; Diacon, A. H. Telacebec (Q203), a New Antituberculosis Agent. *The New England journal of medicine* 2020, 382 (13), 1280–1281. DOI: 10.1056/NEJMc1913327.

P-1-40**Virtual fragment docking to identify protein-protein interaction modulators against LASV infection***M. Pfeifer^{1,2,3}, A. Sweeney^{1,3}, B. Maertens^{1,2,3}, A. Rammelt^{1,3,4}, G. Diana^{1,3,4}, M. Rosenthal^{1,4,5}, M. Topf^{1,3}¹Centre for Structural Systems Biology (CSSB), Hamburg, Germany²Universitätsklinikum Hamburg-Eppendorf (UKE), Hamburg, Germany³Leibniz-Institute of Virology (LIV), Hamburg, Germany⁴Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany⁵Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP), Discovery Research ScreeningPort, Hamburg, Germany

Proteins are fundamental building blocks of life, and protein-protein interactions (PPIs) regulate essential processes in both normal physiology and disease. Modulating PPIs has therefore emerged as a key strategy in therapeutic development. However, targeting PPIs with small molecules remains challenging due to large, flat interface areas, high binding affinities of interaction partners, and the scarcity of successful reference compounds. Structure-based virtual screening offers a promising approach to these challenges, enabling the exploration of millions of compounds against models derived from structural data. PPIs play a particularly important role in viral infections. In Lassa virus (LASV), the matrix protein Z inhibits the viral L protein's polymerase activity, effectively blocking viral transcription and genome replication. This interaction is a compelling target for antiviral therapy. Structural studies suggest that conformational rearrangements underlie the inhibitory mechanism. Mimicking the L-Z interaction through orthosteric small molecule binding could provide a novel route to disrupt viral replication and to suppress infection. We developed a streamlined virtual screening workflow to discover small molecules targeting the L-Z interface. Using the cryo-EM structure (PDB: 7CKL) as a template, we first mapped surface pockets on the L protein, then docked a 735,000 fragment library into the L-Z site. To pinpoint critical interaction hotspots, we performed an *in silico* alanine scanning mutagenesis. Fragments engaging these key residues were retained, and the top 1000 candidates advanced to the next stage. Each was used as a substructure query against PubChem, and after comprehensive filtering, hits were clustered to yield a chemically diverse set of compounds for experimental testing. To test compound binding we developed a *bioluminescence resonance energy transfer* (BRET) assay able to measure L-Z interaction in cells. We applied the BRET assay to test competitive effects of 26 shortlisted compounds on L-Z interaction. Three out of 26 compounds showed at least a 25% reduction of nanoBRET ratios and presumably L-Z binding activity at screening concentration (10 μ M). These results highlight the feasibility of the discovery of molecules that bind to regulatory PPI interfaces by virtual screening. Next, compounds will be tested in *in vitro* polymerase activity assays and label-free kinetic assays to confirm their effect. Prospectively, lead compounds should be further developed and examined in a broader set of human pathogenic arenaviruses.

P-1-41**Biophysical characterization of bocaparvovirus gene therapy vectors***L. Ruck^{1,2,3}, Y. Sano^{1,2}, D. Grimm^{1,2,3,4}¹Heidelberg University, Medical Faculty, Department of Infectious Diseases/Virology, Section Viral Vector Technologies, Heidelberg, Germany²Heidelberg University, BioQuant, Heidelberg, Germany³German Center for Infection Research (DZIF), partner site Heidelberg, Heidelberg, Germany⁴German Center for Cardiovascular Research (DZHK), partner site Heidelberg, Heidelberg, Germany

Question: The approval of several AAV-based gene therapy products in the last two decades has consolidated AAV's status as one of the leading viral vector platforms. Concurrently, crucial drawbacks of AAV, namely, its high seroprevalence and limited packaging capacity, impede further advancements and confine the range of therapeutic applications. Bocaparvoviruses (BoV) have emerged as a potential alternative to AAV, mostly because BoV allow for packaging of larger transgenes such as CFTR, required for the treatment of cystic fibrosis. The creation of chimeric vectors via cross-packaging of an AAV genome into BoV capsids merges the well proven safety of AAV and the unique characteristics of bocaviral capsids of numerous serotypes with distinct tropisms. In prior studies from our lab, chimeric vectors based on capsids of HBoV1, HBoV4, or GBoV1 were shown to successfully transduce pHAe.[1] However, to be able to harness the full potential of BoV, it is imperative to study their biophysical characteristics and the ensuing implications for vector storage, dissemination and application.

Methods: To this end, we will employ a wide variety of methods comprising qPCR, luciferase assays after transduction of Huh7 cells, and silver staining. Concurrently, we will conduct genome ejection assays and differential scanning fluorimetry to determine the thermostability of selected BoV isolates.

Results: Our aim is to identify the optimal buffer formulation for BoV storage, measured by its potential to maintain vector stability and infectivity over longer periods of time, at different storage temperatures, and under freeze-and-thaw stress. For AAV vector products, the addition of cryoprotectants and surfactants was shown to reduce titer loss and enhance the therapeutic index by preventing denaturation, surface adsorption, and aggregation. Our preliminary data show that for BoV, too, long-term storage in PBS without any excipients results in vector loss and impairs transduction capability. Furthermore, the impact of purification via iodixanol density gradient on vector yield and purity will be assessed. First data indicate both higher vector yields and improved transduction efficiency when BoV is kept at higher concentrations of iodixanol, suggesting that iodixanol protects BoV against degradation. It will now be of interest to study whether this protective effect is unique to iodixanol or whether it can be mimicked by other excipients, too.

Conclusions: Taken together, the novel insights into the biophysical characteristics of BoV resulting from this study will provide a dual benefit, namely, a better understanding of its basic biology as well as an acceleration of the clinical translation of BoV vectors.

[1] Fakhiri J, Schneider MA, Puschhof J, et al. Novel Chimeric Gene Therapy Vectors Based on Adeno-Associated Virus and Four Different Mammalian Bocaviruses. *Mol Ther Methods Clin Dev*. 2019;12:202-222. Published 2019 Jan 18. doi:10.1016/j.omtm.2019.01.003

P-1-42

Tools for pandemic preparedness in Germany

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During the COVID-19 pandemic the *Center for Pandemic Vaccines and Therapeutics* (ZEPAI) was established to execute rapid vaccine distribution in crisis situation and to prepare in interpandemic times for future pandemics using vaccines and therapeutics. As the cause and time of a next

pandemic cannot be predicted it is of utmost importance to develop different scenarios for an optimal preparation. ZEPAI, mandated by the Federal Ministry of Health, implements concepts and measures to achieve this goal. Therefore, we have established, implemented and are accompanying several preparedness tools like (i) pandemic preparedness contracts, and different modes of procurement and stockpiling, (ii) horizon scanning of innovations and (iii) mapping of manufacturing sites to protect the German public in case of new health emergency.

(i) **The pandemic preparedness contracts** are signed with three companies in Germany covering two platform processes for mRNA- and vector-based vaccines and enabling to production of up to 80 Mio doses per year. These contracts comprise ready to use facilities for a pathogen agnostic approach. In the European context, ZEPAI supports national decision-makers on **procurement and reservation contracts** on specific products within the HERA framework for cross-border health threats. Particularly relevant is the Joint Procurement Agreement (JPA), which enables coordinated purchasing.

With the horizon scanning and technology scouting, as well as the mapping of production facilities ZEPAI started setting up a network of manufacturers and academia to implement innovative techniques in the field of vaccines and therapeutics.

(ii) With its **horizon scanning efforts**, ZEPAI focusses on new developments and innovations in the field of vaccines, therapeutics and delivery systems. Systematic technology scouting helps to identify critical gaps in preparedness landscape and to accelerate the timely integration of innovations within the pandemic.

(iii) **Mapping of manufacturing sites**, capabilities and if possible, capacities further strengthens preparedness. By linking research institutes and start-ups with manufacturers or Contract Development and Manufacturing Organizations (CDMOs), ZEPAI facilitates the advancement of prototype vaccines or monoclonal antibodies from preclinical testing to authorization.

Taken together, these tools provide a sound preparedness framework in which the different approaches reinforce each other. Horizon scanning can, for example, identify promising developers for preparedness contracts or inform procurement decisions, while manufacturing mapping supports technology transfer and capacity building. This strategy strengthens Germany's ability, within Europe, to respond rapidly and effectively to future pandemics.

P-1-43

SAM Synthetase Dysregulation as a Novel Antibacterial Strategy Against *Mycobacterium tuberculosis*

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The rise of multidrug-resistant *Mycobacterium tuberculosis* (*Mtb*) demands therapeutic strategies that exploit unrecognized metabolic vulnerabilities. Here, we describe **AvG64**, a non-cytotoxic, mycobacterial-selective small molecule that arrests *Mtb* growth by engaging the essential enzyme S-adenosylmethionine (SAM) synthetase, MetK. MetK catalyses the synthesis of SAM from methionine and ATP. Following resistant mutant screens and whole-genome sequencing, resistance mapped exclusively to *metK*, and biochemical assays confirmed direct target engagement. Unlike canonical enzyme inhibitors, **AvG64** does not block SAM synthesis but drives sustained SAM accumulation coupled to progressive ATP and methionine depletion. Metabolomics profiling further revealed extensive remodeling of one-carbon and sulfur metabolism, as methionine scarcity activated transsulfuration, leading to accumulation of cystathionine, cysteine, and γ -glutamylcysteine, alongside depletion of glycine and glutamate, consistent with a potential thiol-biosynthetic compensatory response. Growth rescue experiments revealed that exogenous methionine, but not SAM, restored growth, identifying methionine depletion as the immediate growth-limiting lesion. These findings uncover methionine/SAM homeostasis as a previously untapped metabolic vulnerability of *Mtb*, establish **AvG64** as a first-in-class MetK-directed chemotype, and highlight enzymatic dysregulation as a novel antibacterial strategy distinct from classical inhibition.

P-1-44

Targeting *Salmonella*'s virulence: Inhibition of the T3SS-2 major export apparatus SsaV by a synthetic small molecule

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The enteric pathogen *Salmonella enterica* serovar Typhimurium utilizes multiple effector proteins to invade and multiply within host epithelial cells. Bacteria can then spread to reach other tissues, ultimately leading to a systemic infection. Specialized secretion systems, such as the Type III Secretion System 2 (T3SS-2) encoded on the *Salmonella* Pathogenicity Island 2 (SPI-2), are essential for the translocation of these effector proteins into host cells. T3SS-2 is essential for intracellular survival, particularly within macrophages, enabling *Salmonella* to evade the host immune response by manipulating vesicle trafficking and maintaining the integrity of the *Salmonella*-containing vacuoles (SCVs), thereby allowing bacterial persistence within the host.

The major export apparatus protein SsaV, located at the core of T3SS-2, plays a crucial role in the translocation of bacterial effector proteins into host cells. A functional SsaV is essential for *Salmonella* to establish infection and proliferate intracellularly. Therefore, blocking T3SS-2 by targeting SsaV could prevent systemic infections.

Through virtual docking on SsaV, we report the identification of a synthetic small-molecule binder V9. We validated the *in vitro* binding of V9 to SsaV by purifying the nonameric protein to perform a Microscale Thermophoresis (MST). We then used a split-luciferase host cell invasion assay that showed significantly reduced effector protein injection in V9-treated *Salmonella*. Western blotting further confirmed impaired effector protein secretion upon V9 treatment. Additionally, fluorescence microscopy and bacterial counting assays in HeLa cells demonstrated a reduced intracellular replication of *Salmonella*.

To experimentally validate the predicted binding pocket of SsaV and improve the compounds efficacy, we performed an alanine scanning mutagenesis and identified key residues whose substitution significantly impaired the binding of the compound V9. Additionally, crosslinking experiments will be performed to covalently attach V9 to SsaV, further confirming the exact location of the binding site. Together, these complementary approaches will guide structure-activity relationship (SAR) studies and hit optimization.

Further we aim to analyze changes in gene expression in both *Salmonella* and host cells following V9 treatment using mRNA sequencing. Through this approach, we expect to uncover valuable insights into the mechanisms by which bacterial invasion alters host defense responses, including how the pathogen manipulates immune functions and intracellular survival strategies.

The inhibition mechanism of V9 offers a foundation for developing anti-virulence therapies targeting T3SS-2, with the potential to effectively treat *Salmonella* Typhimurium infections while minimizing the risk of promoting antibiotic resistance.

P-1-45

Phylogeny-aware, correlative metabologenomics for identifying links between natural products and biosynthetic gene clusters

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Tens of thousands of biosynthetic gene clusters (BGCs) have been identified in microbial genomes, but the vast majority of associated natural products (NPs) and their underlying biosyntheses remain unknown. Metabologenomics approaches integrate genomic and metabolomic datasets to statistically associate BGCs to their cognate NPs, yet often suggest many false links. Here, we show that incorporating information on the producer strains' phylogeny greatly improves accuracy.

We sequenced 72 *Sorangium* spp. genomes (myxobacteria), predicting 2,029 BGCs in 265 gene cluster families (GCFs). Mass spectrometry revealed 99 metabolite families (MFs) from the same strains. Using a phylogeny-aware statistical analysis, we identified 43 high-confidence associations between GCFs and MFs, correctly including 89% of previously characterized links and reducing spurious associations by 33-fold, compared to simple correlational analysis. Our approach identified previously unknown BGCs for rowithocin and an undescribed poly-glycosylated NP. It also identified a distinct BGC associated with the production of chlorotoniol C variants and refined the BGC for maracen.

This study demonstrates the effectiveness of phylogeny-aware metabologenomics as a scalable strategy for NP discovery and biosynthetic pathway elucidation.

P-1-46

Macromolecular prodrugs to treat intracellular persisting fungal pathogens

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Aspergillus fumigatus is the major causative of invasive aspergillosis in immunocompromised patients, a life-threatening disease with mortality rates up to 95%. Like many other pathogens, *A. fumigatus* has evolved mechanisms to avoid its elimination inside phagolysosomes (PLs) of alveolar macrophages. The treatment of intracellular persisting pathogens is challenging, because the utilized drugs have to cross two membranes, the cytoplasmic and the PL membrane. Drug delivery systems bear the potential to reach intracellular persisting microorganisms and, by applying macromolecular prodrugs (MPDs), can lead to high local concentrations of the drug.

The study was aiming to elucidate, whether nanoparticles (NPs) can target intracellular persistent pathogens and if a drug release specifically in these intracellular organelles is possible. Dye-labeled NPs with a size large enough for phagocytosis by macrophages were formulated and their internalization into RAW 264.7 macrophages was analyzed by imaging flow cytometry. Intracellular localization was confirmed by fluorescence microscopy and TEM. The MPDs were consisting of an antifungal drug, a peptide linker specifically cleaved by enzymes in PLs and a polymer. Drug release from MPDs was quantified by LC-MS analysis.

Macrophages internalized NPs efficiently and addition of NPs to prior infected macrophages confirmed co-localization of NPs and conidia in the same PL due to fusion of separate PLs. The number of phagolysosomes containing both conidia and PPs increased at elevated NP concentrations or after addition of the fusion enhancer Vacuolin-1. Dye-labeled MPD also showed co-localization with conidia in PLs and drug release was only occurring intracellularly.

In this study, the fusion of conidia- and NP-containing PLs was proposed as putative mechanism for NPs reaching intracellular conidia and fusion rate could be increased by certain methods. Furthermore, smart prodrugs were employed to release the drug only in the PL. These results represent the requisite for the development of advanced delivery systems reaching intracellular persistent pathogens.

P-1-47

ATHANA – a modular nanoparticle approach for every infection

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Infectious diseases caused by fungi represent a particular and often underestimated problem. Especially invasive fungal infections, affecting in particular immunocompromised patients, are associated with unacceptable high mortality

rates, up to 95%. Limited treatment options and an increasing number of resistant strains further highlight the importance of this topic.

The ATHANA Alliance aims to develop drug-loaded nanoparticles that can recognize pathogen-specific structures and attack pathogens in a targeted manner. Encapsulating the active pharmaceutical ingredient (API) within a 'nanocarrier' is a strategy employed to prevent premature degradation and facilitate controlled release at the targeted location. This approach should increase efficiency and reduce systemic side effects. The toolbox approach should facilitate the flexible, systematic and accelerated production of therapeutic nanoparticles with different functionalities for gradual expansion to accommodate new applications. Furthermore, the use of nanoparticles would open the application of pharmacologic problematic substances, as the pharmacological properties in these formulations are dominated by the carrier system and not by the API itself.

The primary objective of the ATHANA project is the development of nanoparticles with the capacity to target specifically fungal cells. These nanoparticles are to be loaded with a drug candidate that has the potential to combat invasive pulmonary aspergillosis. The focus is the development of an inhalable drug form that will reach patent maturity and meet all prerequisites for future preclinical and clinical studies.

P-1-48

Ion-dependent phage decolonization of vancomycin-resistant *Enterococcus faecium* (VRE) in an *in vitro* gut model

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Enterococcus (*E.*) *faecium* is an integral part of the human gut microbiome but simultaneously belongs to the infamous group of ESKAPE pathogens 5. This pathobiont exhibits a low level of virulence under eubiotic conditions and is therefore generally considered harmless to the host. However, if a disease alters the host's immune status or triggers dysbiosis, *E. faecium* can overgrow and cause life-threatening infections such as sepsis and endocarditis 5. In this context, vancomycin-resistant *Enterococcus* (VRE) strains, which the WHO considers as "high-priority" pathogens 6, are a major cause of nosocomial infections, especially in immunocompromised groups, such as oncology patients. These patients often show high levels of *E. faecium* colonization due to the vulnerable immune system and the application of chemotherapeutics, which can damage colonocytes, leading to dysbiosis and an impaired gut barrier 7. In general, VRE infections are often difficult to treat, and the main approved treatment option left is the use of linezolid, an expensive reserve antibiotic with serious side effects 8.

Therefore, alternative treatment options are urgently needed. As part of the DZIF Flagship project EVREA-phage (TTU08.926), we evaluated the activity of a specifically designated phage cocktail against clinically relevant VRE for preventive decolonization, consisting of four different bacteriophages. We used anoxic growth kinetic assays (lysis curves) and a bioreactor-based *in vitro* gut community model (ivGCM) to test the decolonization efficacy of the cocktail

under colon-like conditions, which are known to potentially alter phage activity against enteropathogens through metabolic shifts and phage receptor changes⁹. We could demonstrate that under these conditions, the phages strictly require divalent cations (Mg²⁺ and Ca²⁺) to sufficiently lyse VRE in single cultures and within a representative gut bacterial community. Overall, we were able to reduce VRE colonization inside the *iv*GCM by 2.5-log steps with a single dosage of the phage cocktail and the supplementation of divalent ions. The results deliver important preclinical data relevant for future formulations of a suitable phage product based on the selected bacteriophages. By demonstrating that the efficacy of the selected phages depends critically on the availability of divalent ions, we identified both a promising therapeutic candidate and an important ecological factor that influences the outcome of the treatment.

5 Miller, W. R. & Arias, C. A (2024) in: Nat Rev Microbiol 22, 598–616

6 Sati, H. et al. (2025) in: The Lancet Infectious Diseases 25, 1033–1043

7 Zhang, M. & Guo, H. (2024) in: Cell Host & Microbe 32, 1455–1457

8 Di Paolo, A. et al. (2010) in: Clin Pharmacokinet 49, 439–447

9 Hernández Villamizar, S. et al. (2023) in: Applied and environmental microbiology 89, e0149123

P-1-49

In vitro* effects of different antibiotic classes against *Wolbachia

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Anti-wolbachial therapy is a powerful strategy to treat the human infections lymphatic filariasis and onchocerciasis, neglected tropical diseases that are major drivers of morbidity and poverty. *Wolbachia*, obligate endobacteria of filarial worms, are essential for embryogenesis, larval development, and adult survival. Despite *Wolbachia*'s unique characteristics, such as a reduced genome and the absence of a canonical cell wall, key enzymes for lipid II synthesis are conserved. Fosfomycin, known for inhibiting the first dedicated cytoplasmic enzyme for lipid II synthesis (MurA), is active against *Wolbachia*. Notably, fosfomycin treatment induces the enlargement of *Wolbachia* cells from 0.76 µm (untreated) to 1.43 µm (512 µg/ml), emphasizing the

necessity of lipid II for division. To better understand the role of conserved cell wall enzymes and a putative cell wall, we studied the *in vitro* effects of several cell wall biosynthesis inhibitors (fosfomycin, d-cycloserine, teixobactin, muraymycins, beta-lactams) and the RNA polymerase inhibitor coralopyronin A (CorA), an antibiotic being developed to treat human filarial infections, against *Wolbachia* wAlbB in C6/36 insect cells.

We found that d-cycloserine, an inhibitor of the ligase (Ddl) that synthesizes the terminal dipeptide of the lipid II pentapeptide chain, induces the same phenotype as fosfomycin (512 µg/ml: 1.15 µm). Enlarged *Wolbachia* were also observed after treatment with teixobactin (51.2 µg/ml: 0.88 µm) and the muraymycins MRH-76 and MRH-92 (8 µg/ml: 1.54 µm and 1.28 µm, respectively, compared to untreated: 1.13 µm), while beta-lactams had no effect. Treating *Wolbachia* with CorA did not result in enlarged endobacteria, mirroring the results seen with rifampicin, another RNA polymerase inhibitor. Since anti-wolbachial *in vitro* and *in vivo* experiments have not indicated resistance to CorA to date, we studied the resistance development against CorA in *Wolbachia* for 245 days. No loss of sensitivity in *Wolbachia* towards CorA was observed and hence no resistance was selected. Our Omics analysis revealed that 163 genes and 32 proteins were significantly differentially expressed following fosfomycin treatment (102 genes and 30 proteins downregulated; 61 genes and 2 proteins upregulated; FDR <0.05, log₂ fold change >1), indicating disruption of multiple cellular pathways. These molecular changes correspond with the observed *Wolbachia* enlargement phenotype, further supporting the hypothesis that fosfomycin affects multiple pathways.

Further research is needed to understand *Wolbachia*'s cellular response to cell wall biosynthesis inhibitors and whether the enlarged phenotype represents a persistence-like state. This knowledge will enhance our grasp of cell morphogenesis and integrity in these endobacteria and open avenues for the discovery of novel anti-wolbachial targets that, like CorA, could be developed into new treatment options for filarial infections in humans.

P-1-51

Therapeutic Approaches Using Cyclophilin Inhibitors Against Filo- and Henipavirus Infections in Organotypic Tissue Cultures

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Cyclosporin A (CsA) is a well-characterized cyclophilin inhibitor (CI) with broad antiviral activity *in vitro* and *in vivo*. The underlying mechanisms are manifold and include modulation of cyclophilin-dependent pathways that are involved in the initiation of antiviral immune responses. We have previously demonstrated that CsA induces interferon (IFN) expression and subsequent activation of interferon-stimulated genes (ISGs), making it a promising candidate for treating infections where a dysregulated IFN response contributes to pathogenesis. As an approved immunosuppressant with decades of clinical use, CsA also holds potential for off-label application in antiviral therapy.

However, the antiviral effect of CIs against filoviruses (e.g., Sudan virus, SUDV) and henipaviruses (e.g., Nipah virus,

NiV)—both of which can cause severe, often fatal, multi-organ disease—has not been fully investigated. In initial experiments using human cell lines, we observed that both SUDV and NiV replication can be suppressed by CIs. To further assess the antiviral potential of CsA, we employed *ex vivo* organotypic cultures (OC) of murine origin, which allow infection studies in the context of tissue-specific cellular networks. This approach adheres to the 3R principle (Replacement, Reduction, Refinement), enabling meaningful analysis prior to animal experimentation.

We established OC from liver, brain, spleen, and kidney derived from individual wild-type (WT) and interferon α/β receptor knockout (IFNAR^{-/-}) mice. Cultures remained viable and metabolically active for up to 11 days. Upon NiV infection, all OCs supported robust viral replication. CsA treatment significantly reduced NiV replication in OC from WT mice but had no effect in IFNAR^{-/-} cultures, suggesting that CsA's antiviral effect is dependent on intact type I IFN signaling. Parallel analysis of IFN and ISG mRNA levels confirmed CsA-induced immunomodulatory activity. SUDV experiments are currently ongoing.

In summary, our findings highlight the utility of organotypic *ex vivo* cultures as a robust platform for preclinical antiviral research. They provide a valuable alternative to animal models, especially for investigating host-pathogen interactions and evaluating antiviral efficacy in tissue-specific contexts. This system facilitates the identification of promising therapeutic candidates such as CsA for combating infections with high-consequence pathogens.

P-1-52

Artificial Intelligence (AI) System for Automated Histological Evaluation of Onchocerca volvulus Nodules

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Question: Can an AI system automate the histological evaluation of *Onchocerca volvulus* nodules to address the critical bottleneck of limited expert readers and time-consuming manual analysis that delays drug development for onchocerciasis?

Methods: We developed a deep learning system using digitized histological slides as input to detect and classify individual worm sections and identify free microfilariae (Mf) in nodule tissue. Models were trained on data labeled by two independent expert readers. The system evaluates four key parameters: worm vitality (alive/dead), normal embryogenesis, *Wolbachia* (*Wb*) presence, and free Mf. Two aggregation methods were tested against data from original human assessments of clinical trials that the AI had not been trained on. A simple aggregation (≥ 1 positive worm section = positive nodule) was tested on a retrospective data set from the SCOTT trial (ISRCTN66649839). In a second approach, we used regularized logistic regression on engineered aggregation features (counts, thresholded ratios, statistical summaries, conditional threshold variants) derived

from section- and crop-level confidence scores. These regression models for nodule-level predictions were validated on DNDi-TYL-01 (NCT04913610), DNDi-EMO-04 (NCT05180461), and ASTAWOL (PACTR202009704006025). The benefits of this automated approach were assessed by simulating the AI end-to-end approach against histological analyses performed by two independent experts as part of the clinical trials.

Results: The simple aggregation method resulted in accuracies for the retrospective data set below 80% for all attributes (vitality 69.6%; normal embryogenesis 79.9%; *Wb* 62.8%; Mf 20.4%) with a high number of false positives. Changing to regression-based aggregation for the prospective validation achieved high accuracy across trials (Vitality 96.4-97.5%, normal embryogenesis 91.0-95.0%, Mf detection 77.7-89.2). For the *Wb* detection, the simple aggregation method already leads to 93.7% and 95.9% accuracy for ASTAWOL and DNDi-TYL-01 without needing a regression-based aggregation. In a nodule analysis simulation using 290 nodules from the DNDi-TYL-01 trial, implementing the AI reduced dual expert review requirements to 20% of the nodules, saving 45% of expert worktime. The user interface enabled rapid review of discrepant cases, reducing assessment time for e.g., 120 flagged MF detection samples from the ASTAWOL trial from ~20 to ~4 hours. Notably, 15/120 "false positives" were confirmed as true positives missed by human experts.

Conclusions: This validated AI system transforms onchocerciasis clinical trial evaluation, achieving >90% accuracy for most parameters while dramatically reducing analysis time from months to days. With 80% of nodules analyzable by AI plus single expert analysis, the system addresses the critical shortage of histopathology expertise. The tool is now ready for deployment in eight upcoming clinical trials, supporting faster drug development.

P-1-53

Testing the Reproducibility of RNA Sequencing in Mycobacterium tuberculosis: A Pilot Study on Isoniazid Treatment

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Standardization and reproducibility of RNA sequencing (RNAseq) data are critical to understanding gene expression in pathogens like *Mycobacterium tuberculosis* (Mtb), where replicate experiments are rarely performed due to high costs. Within the European Regimen Accelerator for Tuberculosis (ERA4TB) consortium, this study aimed to test the reproducibility of a standardized RNAseq workflow for transcriptomic profiling of Mtb challenged with isoniazid (INH). Identical protocols for bacterial culture and treatment were implemented across two independent laboratories, with minor deviations introduced in RNA extraction and library preparation.

In these experiments, the reference strain H37Rv was exposed to half the minimum inhibitory concentration (MIC) of INH, five-times the MIC of INH, or no INH (untreated control). Both laboratories showed consistent results, where exposure to half the MIC of INH did not elicit transcriptomic changes compared to the untreated control. Treatment with five-times the MIC of INH showed an overlap of 45 upregulated and 10 downregulated genes across both labs. Furthermore, both laboratories observed differential regulation of known INH-responsive genes involved in the fatty acid

synthase type II operon responsible for mycolic acid synthesis, such as *kasA*, *kasB*, *accD6* and *acpM*. Early results indicate consistency in the number of differentially expressed stress and metabolic associated genes across datasets, however 95 differentially expressed genes did not overlap between the two datasets.

Initial findings suggest encouraging cross-laboratory reproducibility, reinforcing RNAseq as a robust and reliable platform for detecting drug-induced transcriptional changes and potential resistance mechanisms during varied drug pressures. The presence of non-overlapping genes across datasets likely reflects expected biological variability, particularly among genes not strongly associated with INH stress. Overall, these results support the utility of RNAseq as a valuable tool for preclinical drug evaluation in Mtb research.

This work reflects only the author's views, and the JU is not responsible for any use that may be made of the information it contains.

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P-1-54

How are chikungunya outbreaks defined and reported? Challenges to public health management and prevention efforts

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Background: Chikungunya is a growing public health (PH) concern as its prevalence and geographic distribution increase. Detection and reporting of chikungunya outbreaks, however, are often inconsistent, complicating management and prevention efforts.

Objective(s): Describe inconsistencies in the criteria used by PH authorities to define and declare an outbreak and compare the number of outbreaks declared by PH authorities with those reported in the scientific literature.

Methods: Multiple sources of event-based and indicator-based surveillance were used to estimate total reported cases of chikungunya in 2019-2024 by country. The scientific literature reporting outbreaks from 2010-2024 was reviewed, as well as official outbreak declarations from PH authorities during this time and the criteria used to declare an outbreak against the backdrop of endemic disease.

Results: The reasons for defining an outbreak across PH organisations varied: increased case numbers, occurrence of a first case, documented local transmission and increased hospitalisations/morbidity. Often the reasons were not specified. Of the outbreaks reported in the literature since 2010, less than half were declared outbreaks by CDC, ECDC or WHO. Endemic countries like Brazil, reporting more than 1M cases over 5 years, reported rising case numbers year-on-year in the absence of official outbreak declarations.

Conclusions: Lack of standardised criteria for defining an outbreak, especially in endemic settings, and gaps in surveillance/reporting often result in delayed PH recommendations. The risk of exposure to chikungunya is therefore often hidden to travellers visiting endemic areas. Healthcare professionals should consider them for immunisation against chikungunya even when an outbreak is not formally declared.

P-1-55

Improving Tuberculosis Care for Migrant Children and Adolescents Implementing WHO Guidelines at the local level: The Pediatric Migrant and Public Health Center (PMPH) Munich

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In 2023, numbers of global asylum seekers reached record levels, with 38% aged less than 18 years. Europe experienced the largest rise in international migration in decades, while childhood tuberculosis (TB) remains a major public health challenge, particularly among migrant children from TB high-incidence countries. Following the COVID-19 pandemic, TB incidence has surged globally as TB programs suffered a significant setback. Although European countries have implemented screening programs, numerous barriers persist for migrant children and families to achieve adequate health care. These include limited health insurance coverage, language and cultural barriers, bureaucratic hurdles, and insufficient age-adapted TB care.

Pediatric TB management differs significantly from adult care and requires early diagnosis and – if necessary - prompt treatment initiation by means of child-adapted tools, and specialized pediatric expertise. In response to persistent gaps in care, the Pediatric Migrant and Public Health Center Munich (PMPH) was launched in March 2023. This model integrates the Munich Public Health Department for TB Control and the Infectious Diseases Unit at the Hauner Children's Hospital ensuring access to pediatric TB expertise and tailored support services.

A new pediatric bridging position was created linking both institutions and thus facilitating structured TB assessment, administration of chemoprevention, and access to translation services, social support, and mental healthcare. Key barriers identified included inadequate initiation of chemoprophylaxis in young children, limited access to pediatric radiology, and fragmented service pathways. Migrant-specific challenges such as high mobility and psychosocial stressors further complicated care. WHO recommendations on ending TB in children and improving migrant health in Europe served as the foundation for developing this novel integrated model of care.

Until now PMPH has markedly strengthened multisectoral collaboration, improved early access to preventive and diagnostic tools, and promoted family-centered, culturally competent care. It fully aligns with WHO's calls to scale up child-focused TB services and to create inclusive, responsive healthcare systems for migrant populations. Additionally, PMPH generates a framework for translational research in childhood tuberculosis in Germany, supporting the

development of evidence-based practices and informing national TB strategies.

PMPH represents an innovative, locally adapted, and scalable new model of care demonstrating how international guidelines can be operationalized locally to improve TB care for vulnerable pediatric populations in high-income countries. Ongoing data collection and stakeholder engagement will support continuous improvement and serve as a blueprint for other regional and national institutions.

P-1-56

Approach to merge individual RAA assays into a multiplex RAA design for simultaneous detection of multiple *Schistosoma* species

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Isothermal amplification methods such as LAMP and RPA/RAA are considered to be valuable tools for decentralized testing setups and field campaigns that require the use of simplified and rugged molecular diagnostic tools. The expiration of patent protection for these amplification and detection methods led to a further rise of interest in recent years. A major bottleneck of isothermal amplification methods, however, is their limited multiplexing capability. While countless single-plex isothermal assays for detection of pathogens are described in the literature, little is known how to improve the multiplexing capabilities of LAMP or RAA reagents.

Here we describe an approach to leverage the wealth of well-characterized assay designs described in the literature for a streamlined design process of duplex isothermal assays. For that, two well performing real-time RAA assays for detection of *Schistosoma mansoni* (Sman) and *Schistosoma haematobium* (Shae), respectively, were selected. After verifying the claimed performance for the individual assays, various parameters critical to assay performance were screened. Because we intend to apply the duplex Sman/Shae assay for environmental monitoring as well as clinical diagnostics, we prioritized assay sensitivity over other parameters such as assay speed.

This work proved that it is feasible to turn individually designed single-plex isothermal amplification assays into a duplex assay for simultaneous detection of more than one pathogen. Our approach allows to design a multiplex RAA assay with reduced capacities by building on the primer design work performed by other researchers, who have already screened primers and probes for optimal specificity and sensitivity.

P-1-57

Nucleic Acids on the go: Rapid, field-deployable nucleic acid extraction workflow from disease vectors

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The climate change crisis is expanding the landscape of disease vector populations causing disease outbreak. Adding to the concern, such drastic environmental changes are leading to rapid evolution in both vectors and pathogens against current control measures. This demands a need for xenomonitoring across different regions to constantly screen for rising vector populations or infectious microorganisms. Molecular techniques provide a more accurate estimation of

infectious vector populations and are effective in detecting low infection levels with high sensitivity. Such techniques can be applied in vector species identification, targeted screening for known pathogenic organisms, and to screen different vector habitats for pathogen prevalence. This is crucial to raise awareness of a possible epidemic and implement immediate transmission control measures. But, the available protocols for nucleic acid extraction from different vector tissues are time-consuming, unsustainable, and labor-intensive – making it difficult for field testing. Therefore, we aimed to develop a simplified, rapid protocol that can accommodate different vectors (ticks, mosquitoes, snails, etc.) and can be deployed at the point of testing. The working principle is a direct-lysis technique with a user-friendly homogenization method followed by a novel 'reverse-purification' technology that removes the complex impurities and pigments present in vector tissues. Our process co-extracts both vector and pathogenic nucleic acid content in under 30 minutes and the final lysate is compatible with various downstream analytical methods such as qRT-PCR (quantitative Reverse Transcription - Polymerase Chain Reaction), RAA (Recombinase-aided Amplification), LAMP (loop-mediated isothermal DNA Amplification), NGS (Next-Generation Sequencing) techniques, etc. which can provide real-time data, without the need for a laboratory setup.

P-1-58

Evaluating the diagnostic performance of UCP-LF-CAA for the detection of female genital schistosomiasis in comparison to colposcopy-based visual diagnosis: a cross-sectional study from rural Madagascar

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Female genital schistosomiasis (FGS) is a condition resulting from chronic infections with *Schistosoma haematobium*, affecting approximately 50 million women mainly in low- and middle-income countries. The standard screening for FGS recommended by the World Health Organization is colposcopy, visually detecting its typical signs. The laboratory methods conventionally used to detect schistosome infection have the potential to detect FGS but they have not been validated yet. Using these methods in highly endemic countries, such as Madagascar, might be advantageous to overcome limitations of colposcopy such as operator-dependency, invasiveness of the test and challenges in regard to its implementation at primary level of care, due to trained healthcare personnel and complex

equipment requirements. The objective of this study was to compare the diagnostic performance of colposcopy for FGS with the detection of the circulating anodic antigen (CAA), which is produced by the parasite and can be quantified using an immunochromatographic assay.

A cross-sectional study was conducted at three primary health care centres from March to August 2021 in the district of Marovoay in the Boeny region of Madagascar, endemic for *S. haematobium*. Socio-demographic information, clinical history, colposcopic images and urine samples were collected from women aged 18-49 years. Urine samples were subjected to upconverting reporter particle, lateral flow (UCP-LF)-CAA analysis at the *Centre d'Infectiologie Charles Mérieux* in Antananarivo. Clinical diagnosis of FGS was based on an agreement of two gynecologists on the presence of FGS-specific lesions on the colposcopic images during a blinded reconciliation process. The proportions of FGS-positive results for colposcopy and schistosome infection based on UCP-LF-CAA were estimated. To evaluate the performance of UCP-LF-CAA in comparison to the visual diagnosis of FGS, we computed measures of diagnostic agreement. Logistic regression will be used to calculate crude and adjusted odds ratios (OR) with 95% confidence intervals for the association of visual FGS diagnosis with the antigenic diagnosis of the parasite by UCP-LF-CAA.

From a convenience sample of 500 women, UCP-LF-CAA and colposcopy results were available for 413. From this, 17.4% (n = 72) were negative in both tests, 26.4% (n = 109) tested positive with UCP-LF-CAA, 20.1% (n = 83) were visually diagnosed with FGS and 36.1% (n = 149) were positive in both tests.

Preliminary results show a high prevalence of schistosome infection, which is in alignment with previous assessments of FGS in the area. This represents an ideal setting to further assess the diagnostic performance of antigen detection methods and thus opens perspectives for the improvement of FGS diagnosis in highly endemic contexts, such as Madagascar.

include PW in Mass Drug Administration (MDA) with praziquantel (PZQ) from the second trimester is being challenged by the reluctance due to a lack of safety data. In children under two years of age, a test-and-treat strategy is currently recommended as safety data are very limited.

Methods: We assessed the safety of PZQ among PW and 9-month-infants from a Cluster Randomized Clinical Trial which evaluated the potential impact of test-based schistosomiasis treatment with point-of-care Circulating Cathodic Antigen (POC-CCA) integrated into routine antenatal care. In the intervention arm (IA) all PW (second trimester) with a positive test were treated with PZQ 40 mg/kg. Testing was offered to their 9-month-infants and a fixed dosage of 300 mg crushed PZQ was administered in case of positivity. In the control arm (CA) no POC-CCA was offered to PW. If schistosomiasis was suspected, they were referred to the local health system. Safety in PW was assessed by measuring adverse perinatal and pregnancy outcomes. Perinatal outcomes were maternal death during pregnancy or within 42 days of pregnancy termination, and neonatal death within 28 days of birth. Pregnancy outcomes were preterm birth (born alive before 37 weeks' gestation) and foetal death occurring after 22 completed weeks' gestation. The occurrence of death within 30 days of receiving PZQ was the safety outcome chosen for infants. Outcomes were reported as a comparison of frequencies between IA and CA, using the Fisher exact test. Single outcomes were assessed by excluding participants with missing data.

Results: A total of 4613 PW were enrolled. Four pregnancy-related deaths (4/2081, 0.19% and 4/2392, 0.17%, p=0.9735) were reported in both IA and CA. Neonatal deaths were 14 in the IA (14/2094, 0.7%) and 25 in the CA (25/2406, 1%, p=0.1813). No differences were found for preterm births and foetal deaths between IA and CA (209/1354, 15.4% vs 375/1913, 19.6% p=0.0521 and 26/2094, 1.2% vs 38/2406, 1.6%, p=0.3399). Among 1242 infants in the IA one death was recorded versus 0 in the CA.

Conclusions: Our data show no differences in adverse perinatal or pregnancy outcomes after PZQ treatment in PW, supporting their inclusion in MDA. The good safety profile found for infants will help inform public health interventions in this vulnerable age group.

P-1-59

Safety and tolerability of praziquantel among pregnant women and infants: results from a cluster randomised clinical trial (freeBILy trial) in Madagascar

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Question: There is increasing evidence of morbidity and high prevalence of schistosomiasis in pregnant women (PW) and preschool aged children. The recommendation to

P-1-61

Acceptability of MDA with praziquantel among toddlers aged 9-24 months: results from a cross-sectional study from Madagascar

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Question: The World Health Organisation recommends mass drug administration (MDA) with praziquantel (PZQ) for children of pre-school age (PSAC), and a paediatric formulation is now licensed. Acceptance for PSAC can be influenced by a number of factors, so availability alone is not sufficient for successful uptake. This study aims to investigate the factors influencing the acceptability of PZQ for toddlers aged 9-24 months (mo) in the Boeny region, Madagascar.

Methods: A cross-sectional study was performed from February to December 2023. PZQ treatment was offered to the caregivers (CGs) in primary care facilities, hospitals and during community outreach. Data on socio-demographic characteristics, knowledge of schistosomiasis, awareness and previous experience in PZQ-MDA were collected. The prevalence of treatment acceptance and refusal was estimated. Factors associated with refusal were assessed, given the high prevalence of acceptance. To identify factors associated with treatment refusal, adjusted prevalence ratios (aPR) were estimated using Poisson regression.

Results: Of 5154 toddlers enrolled, 50.2 % (2586) were female, 33% (1703) were aged 9-12 mo, 30.9% (1592) aged 13-17 mo and 36.1% (1859) aged 18-24 mo. CGs were mostly mothers (90.7%, 4674); 1970 (38.2%) were farmers. Previous experience with PZQ-MDA was reported by 2108 (39.2%).

Treatment was accepted by 4446 CGs (86.3%) and refused by 708 (13.7%). Higher treatment refusal was associated with fear of adverse events (aPR 3.63, IC 2.95-4.46), lack of previous experience with PZQ-MDA (aPR 1.78, CI 1.47-2.15), and lack of knowledge about the risk of schistosomiasis in toddlers (aPR 1.5 CI 1.14-1.99). CGs other than the parents (1.70, CI 1.3-2.22) and CGs of younger toddlers (aPR 1.51, CI 1.29-1.77 for the age 9-12 mo and 1.26, CI 1.07-1.50 for the age 13-17 mo) refused treatment more frequently, while farmers had lower refusal (aPR 0.68, CI 0.57-0.80).

Conclusion: Overall acceptance of PZQ was high. The fear of adverse events, the lack of previous experience of PZQ-MDA and the lack of knowledge of the risk of infection in this age group represent barriers for the acceptance. Awareness campaigns on both PZQ-MDA and schistosomiasis among toddlers could overrun them.

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Background: The recent emergence and spread of *Plasmodium falciparum* partial artemisinin resistance in East Africa underscores the need for strategies to curb its spread further across the continent. Triple artemisinin-based combination therapies (TACTs) represent a promising approach. The ASAAP consortium is evaluating a novel TACT comprising artemether-lumefantrine (AL) and atovaquone-proguanil (AP), that has a multi-stage antimalarial activity, for treating uncomplicated malaria in African children.

Methods: This phase 3, multicentre, randomized, comparator-controlled, double-blind, non-inferiority trial was conducted in Mali, Ghana, Gabon, and Benin. Children aged 6 months to 10 years with uncomplicated *P. falciparum* malaria (1,000–200,000 parasites/ μ L) were randomized 1:1 to receive either AL+AP or AL. Treatment was administered twice daily for three days and adjusted according to weight. Participants were followed up for six weeks. The primary endpoint was day-28 PCR-corrected efficacy in the per protocol (PP) and intention-to-treat (ITT) populations.

Results: Between January 2022 and February 2024, 2,395 children were screened and 1,651 enrolled in mITT (AL+AP: 823; AL: 828). Fewer late parasitological failures (74 [8.99%, 95%CI: 7.13; 11.16] vs. 113 [13.65%, 95%CI: 11.38; 16.18]) and late clinical failures (13 [1.58%, 95%CI: 0.84; 2.69] vs. 36 [4.35%, 95%CI: 3.06; 5.97]) were observed by day 28 in the AL+AP group. Crude and PCR-corrected efficacy analysis are ongoing.

Vomiting within one hour of dosing occurred more often in the AL+AP arm (104 vs 51; $p < 0.001$), leading to 30 vs. 11 treatment discontinuation. Mali had the highest rate (15.1%), followed by Ghana (8.5%), Gabon (5.4%), and Benin (5.0%). Most other frequent adverse events related to the study drugs were rash (6 vs. 2), abdominal pain (4 vs. 3), anaemia (3 vs. 2), and decreased appetite (2 vs. 0). There were no cases of QTc prolongation or deaths.

Conclusion: The combination of AL+AP demonstrated an acceptable safety profile in the treatment of uncomplicated *P. falciparum* malaria in children. Preliminary efficacy results suggest that AL+AP may reduce the late treatment failure rate at day 28 compared to AL alone; however, there was an increased incidence of early vomiting warranting compliance to the treatment. These findings support the evaluation of using of AL+AP in areas of artemisinin resistance to delay or prevent the spread of antimalarial resistance in sub-Saharan Africa.

P-1-62

Efficacy and Safety of Artemether-Lumefantrine Plus Atovaquone-Proguanil for Uncomplicated Malaria in African Children: A Multicentre Phase 3 Trial

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P-1-63

Placental pathology in relation to other risk factors for low birth weight in a malaria-holoendemic area: results from the Malaria Birth Cohort

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Introduction: The perinatal period and the first 1,000 days of life are important for healthy development. Children born small-for-gestational age (SGA) are at risk of growth, psychomotor and intellectual impairments. Known risk factors for low birth weight are for example maternal factors, such as smoking, young age, nulliparity, and ethnicity. Furthermore, in endemic areas, malaria in pregnancy is a strong contributor for low birth weight.

We recruited 1,256 pregnant women for our Malaria Birth Cohort (MBC) from the Ashanti Region in Ghana, where *P. falciparum* malaria is holoendemic (NCT04050566). The aim of the MBC is to better understand antimalarial immunity development considering demographic, bio-medical, and socioeconomic factors of mother-child pairs. Here we report on the placental pathology results.

Methods: Mothers ≥ 18 years of age were recruited during pregnancy between April 2019 and April 2022 if they planned to deliver at our study site, the *Presbyterian Hospital Agogo* (PreHA). The gestational age (GA) of newborns was confirmed by ultrasound dating. During attended birth the placenta was weighed, and a sample for histopathological analysis was taken. Birth weight *P. falciparum* by PCR was performed using an assay targeting the high-copy var gene acidic terminal sequence (ATS) of *P. falciparum* using a HotStarTaq Mastermix (Qiagen) on a LightCycler 480II (Roche).

Risk factors for low placenta weight (≤ 330 g) were analysed using log-binomial regressions, while risk factors for lower birth weight were analysed using linear regression (both univariate and multiple).

Results: In mother-child dyads for whom all placental information was available (N = 485), the coverage of intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) was very high (481/485, 98%) with most participants (333/485, 70%) receiving all 3 SP courses. Still a high proportion of placental samples of asymptomatic mothers turned out to be positive for *P. falciparum* by PCR (166/485, 34%). Eleven percent (53/485) of placentas were of low weight (≤ 330 g) and a high proportion of babies were SGA (183/485, 26%). Presence of any placental pathology (low weight, fibrosis or infarction of $\geq 20\%$ or any inflammation) were very common (355/485, 72%). Placental malaria (defined as positivity by PCR) increased the risk for low placental weight but was not associated with low birth weight per se, while low placental weight, premature birth, female sex, and young mother's age (≥ 18 -<21 years) were associated with lower birth weight.

Conclusion: Despite full IPTp coverage, a high proportion of placenta samples were PCR positive for malaria, which slightly increased the risk of low placental weight. Other known risk factors for lower birth weight could be confirmed by our study.

P-1-65

Early Detection of SARS-CoV-2 Potential Variants of Concern through In Silico Genomic Surveillance with CoVerage

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The COVID-19 pandemic underscored the importance of timely detection of emerging viral variants, a task undertaken by global initiatives including WHO and national public health agencies. We present CoVerage (sarscoverage.org), an automated, real-time genomic surveillance platform that continuously analyzes over 16.5 million SARS-CoV-2 sequences from GISAID to identify and characterize potential Variants of Interest (pVOIs) before widespread transmission.

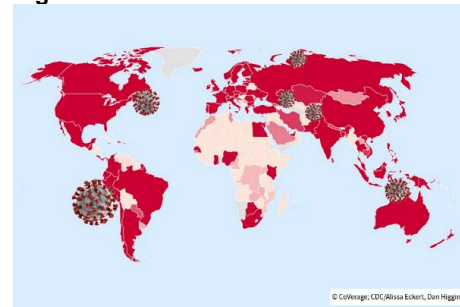
CoVerage integrates three analytical modules: (1) lineage frequency dynamics using statistical testing, (2) spike protein allele dynamics based on phylogenetic analysis, and (3) antigenic alteration scoring via a mutation-weighted matrix derived from influenza evolution. These methods enable early detection of variants with potential growth or immune escape advantages.

In retrospective validation, CoVerage predicted 89% of WHO-designated VOCs and VOIs with a mean lead time of 84 days and detected 73% of all designated lineages. It notably identified Omicron BA.1 shortly before its official designation and outperformed PyR0 in classifying variants of public health relevance (PRAUC 0.84 vs. 0.74), while also demonstrating high antigenic score correlation with neutralization and antigenic distance data.

CoVerage serves as a reproducible, web-based resource for global SARS-CoV-2 monitoring and is extendable to other rapidly evolving pathogens. It exemplifies how in silico analytics can complement epidemiological surveillance to support proactive pandemic response.

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Fig. 1



P-1-66

One year lung sequelae after SARS-CoV-2 infection: a prospective single-centre study of hospitalised patients in Johannesburg, South Africa

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Background: Evidence on post-COVID-19 burden in African regions is scarce. We examined one-year trends in pulmonary function, exercise capacity, and quality of life in a cohort of South African COVID-19 patients with high comorbidity burden and assessed risk factors for post-COVID-19 lung sequelae.

Methods: The prospective cohort study included adult hospitalised patients with confirmed SARS-CoV-2 infection, recruited at discharge between 20 March and 22 September

2021 at Chris Hani Baragwanath Academic Hospital in Johannesburg, South Africa. Participants were followed up within 40 days and at 3, 6, and 12 months post-discharge. Spirometry, St George's Respiratory Questionnaire (SGRQ) for quality-of-life measurement, and 6-minute walk test (6MWT) were performed in all participants (n=72). An in-depth analysed subset of participants (n=44) underwent additional plethysmography, diffusing capacity of the lung for carbon monoxide (DLCO) testing, and high-resolution chest CT.

Results: Pulmonary function improved during one year follow-up in most participants, but improvement was mainly limited to the first 6 months. Reduced FVC remained the predominant spirometry with 27.9% of participants below the lower limit of normal at Month 12. Median 6MWT distance was low (280.0m, IQR 220.0-340.0) and 52.8% had elevated SGRQ total scores after one year. The prevalence of inflammatory CT findings decreased during follow up from 79.6% to 25.0% whereas fibrotic-like CT findings slightly increased from 27.3% to 37.5%. DLCO was identified as most sensitive lung function outcome reflecting structural CT abnormalities and was lowest in participants with a mixed inflammatory-fibrotic CT result (mean DLCO: 68.6 %predicted, 95% CI: 62.2–75.0). A history of tuberculosis, HIV infection with CD4 <200 cells/mm³, COVID-19 severity, body mass index (BMI) <18.5 kg/m² and >25 kg/m² were associated with reduced lung function outcomes, with the effect of high BMI and COVID-19 severity diminishing over time.

Conclusion: Our study demonstrated structural, functional, and quality of life impairment one year post infection. The results emphasize the need for follow-up care to prevent fibrotic changes and long term impairment, especially in patients with severe COVID-19 disease, lung involvement after infection, and pre-existing comorbidities.

P-1-68

SARS-CoV-2 infection dynamics and disease impact in a prospective cohort study of sub-Saharan pulmonary tuberculosis patients

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Background: Longitudinal SARS-CoV-2 studies are rare in sub-Saharan countries with a high tuberculosis (TB) disease burden. The study aimed to better understand SARS-CoV-2 spread and its clinical presentation in (post-)TB patients.

Methods: The multi-centre TB Sequel study enrolled pulmonary TB patients from 2017 to 2019 at their time of diagnosis and followed up in regular time intervals for a minimum of 24 months. Blood samples collected within the study between September 2019 and November 2022 in South Africa, The Gambia and Mozambique were analysed for SARS-CoV-2 antibodies via Roche Elecsys® SARS-CoV-2 anti-Nucleocapsid (anti-N) and anti-Spike (anti-S) assays. Additional pre-pandemic samples from the cohort were analysed for specificity to account for potential cross-reactivities. Kaplan-Meier curves were generated to estimate infection dynamics. Cox proportional hazard regression was performed to identify risk factors for early infection-induced SARS-CoV-2 anti-N seropositivity.

Results: Analysis of 2732 samples from 887 participants indicated a strong increase in seropositivity for anti-N and anti-S from 2.9% (95% CI: 1.7–4.1) and 2.8% (95% CI: 1.6–3.9) at post-wave 1 (15 October 2020) to 90.8% (95% CI: 88.1–92.9) and 93.8% (95% CI: 91.5–95.5) at post-wave 5

(1 July 2022). Analysis of 359 pre-pandemic samples revealed a specificity of 99.7% (95% CI: 98.5 – 100.0) for both assays. Cox regression analysis including samples until post-wave 2 (15 April 2021) showed a higher hazard of early SARS-CoV-2 infection for participants from The Gambia (HR 2.12, 95% CI: 1.37–3.29) and South Africa (HR 1.22, 95% CI: 0.73–2.02) compared to those in Mozambique. TB treatment start prior 2019 (HR 1.48, 95% CI 1.07–2.04) and non-smoking (HR 2.63, 95% CI: 1.50–4.59) were associated with increased risk, whereas age, sex, educational level, marital status, and HIV status were not identified as risk factors for early infection. Currently, ongoing analyses investigate the effect of SARS-CoV-2 co-infection in (post-)TB disease course on various health-related outcomes.

Conclusion: The study showed a high SARS-CoV-2 antibody seroconversion during the pandemic course. This calls for a better understanding of the clinical relevance of SARS-CoV-2 infections in treatment and recovery of (post-)TB patients.

P-1-69

Genomic sequencing-based hybrid characterization of *Schistosoma* sp. worms from clinical cohorts- "GENOSCHIS"

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Introduction: In the backdrop of reemergence and spread of *Schistosoma* zoonotic hybrids in endemic areas; which disrupts existent diagnosis, treatment & control measures, there is an impetus for novel technologies to be able to detect and characterize hybrid infections (WHO, 2020). One way this can be achieved is by developing molecular tools to detect cell-free DNA (cf-DNA) from the parasite, circulating in the patient blood samples (Wichmann et al., 2009). And in case of hybrid infections, this can be done via sequencing and characterizing both nuclear and mt-DNA markers as shown by Cnops et al. 2021. For the characterization of *S. haematobium* hybrids, the targets *Dra1* (Hamburger et al., 2006) and 28S rDNA (Sondoval et al., 2006) were chosen as nuclear, and *cox1* as mt-DNA makers as well described genetic targets (Littlewood et al., 1997).

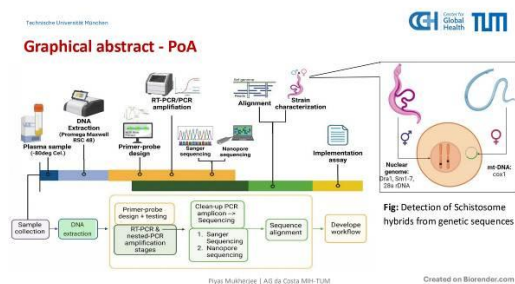
Methods: 174 peripheral blood (serum/plasma) samples were used in this project, a subset from the clinical study "HelmVit" (CERMEL, Gabon). We devised a workflow of qPCR/PCR assays for 3 chosen genes of interest. Of these, 28S *Isr* rRNA and *cox1* targets were used for amplicon sequencing by Sanger and Oxford Nanopore long-read sequencing, for detection and characterization of hybrid infection from samples in the cohort.

Results: 28S and *cox1* amplicons were successfully obtained from one *S. haematobium*-positive sample (helmv146). Sequencing (controls vs. helmv146) amplicons

with Oxford Nanopore was comparable to Sanger sequencing, in terms of median quality and length of passed reads as seen via sequence alignment. The 28S *Isr* rRNA (nuclear target) amplicon indicated clear alignment with *S. haematobium*. However, the result of *cox1* sequencing was inconclusive in determining the hybrid nature of our sample from the mt-DNA fragment, owing to below threshold quality of reads in species specific conserved and mismatch regions.

Outcome & Discussion: Limited success in sequencing *cox1* gene is likely due to constricted amount of mt-DNA fragments in cf-DNA. Exploring broader genomic sequencing of strains will help provide a novel overview of the F1 or later generation hybrid, and inform the development of novel diagnostics and therapeutics. Optimizing Oxford Nanopore sequencing for hybrid identification in field settings can fast-track research aims.

Fig. 1



P-1-70
kdr Mutations and Pyrethroid Resistance Undermine Control of Anopheles gambiae s.l. in Osun State, Nigeria

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Insecticide resistance in *Anopheles* mosquitoes poses a persistent challenge to malaria control programs. This study assessed the resistance status of *Anopheles gambiae* s.l. to pyrethroids (permethrin, deltamethrin, alpha-cypermethrin) and the organophosphate pirimiphos-methyl, alongside detection of the knockdown resistance (*kdr*) gene, across six locations in Osun State, Nigeria.

Larval collections were conducted weekly from January to December 2022, reared to adulthood under laboratory conditions, and identified morphologically. Standard WHO bioassays were performed with permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), and pirimiphos-methyl (0.25%). Molecular assays were conducted to detect the *kdr* mutation.

Results showed significantly higher knockdown and mortality with pirimiphos-methyl compared to pyrethroids ($p < 0.05$). No resistance to pirimiphos-methyl was observed across all study sites, with the highest susceptibility recorded in Ila (63%) and lowest in Inisa (40%). Conversely, resistance to pyrethroids was widespread, with mortality rates ranging from 90–97%. Confirmed resistance was observed in Ila (permethrin, 86% mortality), Ejigbo (alpha-cypermethrin, 60%), and Ejigbo (deltamethrin, 40%) ($p < 0.05$). The presence of *kdr* mutations further corroborated pyrethroid resistance across locations.

These findings highlight the continued efficacy of pirimiphos-methyl against *An. gambiae* s.l., contrasting with reduced effectiveness of pyrethroids. Strengthening insecticide resistance surveillance and incorporating pirimiphos-methyl into indoor residual spraying, alongside strategic rotation of pyrethroids in insecticide-treated nets, are essential to delay resistance development and sustain vector control interventions.

P-1-71
Characterization of Bibersteinia trehalosi in a rare zoonotic infection

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Like other *Pasteurellaceae*, *Bibersteinia trehalosi* is an important pathogen in ruminants such as sheep, goats, and cattle (1). Although *B. trehalosi* can asymptotically colonize the upper respiratory tract of domestic and wild animals (2, 3), it has also been described to cause pneumonia in sheep and severe systemic infections in lambs (2), thus *B. trehalosi* infections pose a significant risk to livestock and due to high mortality rates (4). Outbreaks are associated with relevant economic losses, considerably affecting regions relying on cattle and sheep breeding (5). While zoonotic infections of humans by *Pasteurella multocida* are common, *B. trehalosi* is not known as a zoonotic pathogen in humans yet. To the best of our knowledge, this is the first report of *B. trehalosi* being isolated from a clinical specimen. The wound swab originated from a 61-year-old male who cut his thumb while handling a knife preparing game meat. The isolate was grown on blood agar and identified by MALDI-TOF. Results were confirmed by nanopore-based third generation sequencing using the adaptive sampling approach. Antibiotic susceptibility testing revealed sensitivity for penicillin G, cefotaxime, meropenem, levofloxacin, doxycycline, and cotrimoxazole.

The relevance of *B. trehalosi* is mostly substantiated by research of infections in livestock but the findings in this patient reveal the potential as a zoonotic pathogen. As of today, little is known about the virulence factors and host-pathogen interactions of *B. trehalosi* so that data on its genome may provide valuable insights. Modern sequencing techniques may help to identify and improve our understanding of their epidemiological relevance in a One Health concept.

1. Smith GR. Isolation of Two Types of Pasteurella haemolytica from Sheep. Nature. 1959;183(4668):1132–3.
2. Blackall PJ, Bojesen AM, Christensen H, Bisgaard M. Reclassification of [Pasteurella] trehalosi as Bibersteinia trehalosi gen. nov., comb. nov. International Journal of Systematic and Evolutionary Microbiology. 2007;57(4):666–74.
3. Jaworski MD, Hunter DL, Ward ACS. Biovariants of Isolates of Pasteurella from Domestic and Wild Ruminants. J VET Diagn Invest. 1998;10(1):49–55.
4. Szeredi L, Rausch F, Szeleczyk Z, Jánosi S. High mortality caused by Bibersteinia trehalosi septicaemia in adult sheep – A case report. Acta Vet Hung. 2018;66(4).
5. Gizaw S, Desta H, Alemu B, Tegegne A, Wieland B. Importance of livestock diseases identified using participatory epidemiology in the highlands of Ethiopia. Trop Anim Health Prod. 2020;52(4):1745–57.

P-1-72

Detection of Schistosoma eggs from Potassium Hydroxide (KOH) macerated placental tissue at Zomba Central Hospital, Malawi

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Background: Schistosomiasis, also known as bilharzia, is a prevalent parasitic disease in Malawi caused by *Schistosoma haematobium* or *Schistosoma mansoni*. During pregnancy, it can affect the placenta and may contribute to poor maternal and neonatal outcomes. Microscopic detection in urine samples lacks sensitivity and requires experienced and well-trained personnel. We aimed to detect *Schistosoma* eggs from placental tissue at Zomba Central Hospital (ZCH), Malawi using a previously described potassium hydroxide (KOH) based maceration technique.

Methods: The placenta sample used was residual material taken from the bin of labor ward. It was sectioned into six circular pieces (about 5cm in diameter), five from the peripheral regions and one from the central region. The sections were put in individual containers filled with 0,9% saline and transported to the laboratory. Each section was further cut into 1 cm pieces and transferred into a 50 mL tube. Each tube was filled with 4% KOH to a final volume of 45 mL and incubated at 37°C for 24 hours while loosely capped. After incubation, samples were centrifuged at 2,500 rpm for 10 minutes at room temperature. The supernatant was discarded, and the remaining pellet was used to make a wet mount that was examined under microscope at 10x, 20x, and 40x objectives for the presence of *Schistosoma* eggs.

Results: Wet mount microscopic examination showed a typical background with blood cell debris. No *Schistosoma* eggs were observed in any of the slides.

Conclusion: Maceration of placental tissue offers a simple and affordable method for the detection of *Schistosoma* eggs. Even though no eggs were detected in this intent, ongoing examination may yield positive findings. This method can complement existing diagnostic strategies and may help improve detection of schistosomiasis during pregnancy.

P-1-73

Prevalence of Maternal Schistosomiasis and Associations with Adverse Birth Outcomes among pregnant women in Agogo, Ghana

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Background: Schistosomiasis during pregnancy is associated with increased risk of adverse birth outcomes (ABO) such as low birth weight (LBW) or small for gestational age (SGA) [1]. Possible underlying pathophysiological mechanisms include placental inflammation, which can be induced by placental schistosomiasis (PS) [2]. Reliable data on the prevalence of maternal schistosomiasis and its association with ABO are sparse. A recent meta-analysis from 2021 showed a prevalence of 20% [3], while observational studies showed contradictory evidence on the contribution on different endpoints of ABO [1].

Methods: The design of the initial project was a cross-sectional study carried out between 2000 and 2001 in Agogo (Ghana) with the purpose to analyze associations of maternal malaria with ABO. Detailed data on the cohort (n=839) and residual samples (maternal serum) are still available. Samples were tested for the presence of cell free circulating DNA using a PCR protocol targeting *Dra1* and *Sm1-7*. Prevalence of maternal schistosomiasis will be assessed and its impact on prematurity, SGA and LBW calculated using multivariate analysis. Ethical approval for the original study was approved by the University of Science and Technology Kumasi and for the current retrospective analysis by the Kwame Nkrumah University in Kumasi (Ghana).

Results: From 839 participants, residual samples of n=300 samples have been analyzed so far. Preliminary results show a prevalence of maternal schistosomiasis of 19% (57/300). Prevalence of low birth weight among the whole cohort was 16.2% (136/839). Maternal *Schistosoma* infection had the tendency to be more prevalent in women who delivered infants with LBW (56.9% vs. 44.7%), but final statistical analysis will be performed upon completion of qPCR analysis.

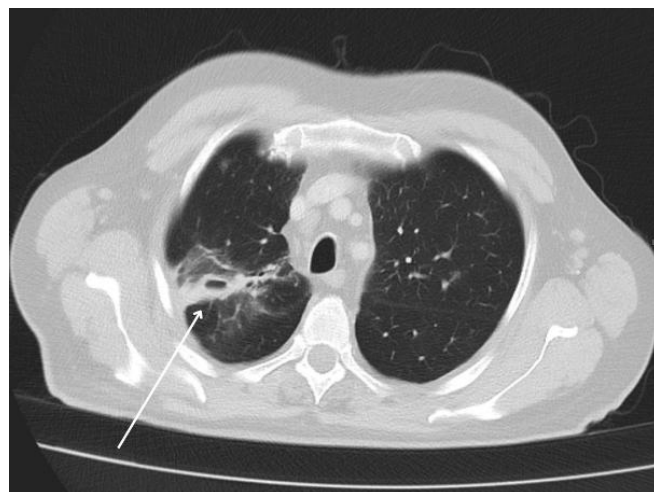
Discussion: Assessing the prevalence of maternal schistosomiasis in endemic regions is a challenge and epidemiological data remain scarce. Meanwhile, it poses a threat to maternal and newborn health. Better understanding of the relationship between maternal schistosomiasis and ABO would underline the importance of the urgently needed treatment of schistosomiasis in pregnant women and young women of reproductive age and provide further rationale for treatment recommendations.

References:

1. Freer JB, Bourke CD, Durhuus GH, Kjetland EF, Prendergast AJ. Schistosomiasis in the first 1000 days. *Lancet Infect Dis* **2018**; 18:e193–e203.

- Gerstenberg J, Mishra S, Holtfreter M, et al. Human Placental Schistosomiasis — A Systematic Review of the Literature. *Pathogens* **2024**; 13:1–8.
- Adam I, ALhabardi NA, Al-Wutayd O, Khamis AH. Prevalence of schistosomiasis and its association with anemia among pregnant women: a systematic review and meta-analysis. *Parasit Vectors*. 2021; 14.

Fig. 1



P-1-74

Melioidosis imported to Northern Germany: A Case Report Series

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Purpose: Melioidosis is a serious infection caused by *Burkholderia pseudomallei*, a bacterium found in soil, water, and plants in tropical and subtropical regions. The infection can present with a range of clinical symptoms and may be fatal without appropriate treatment.

Case presentation: We present four confirmed cases of melioidosis in patients with a history of travel to endemic areas. These cases illustrate the diverse clinical manifestations of the infection, ranging from respiratory tract involvement to multiple organ abscesses, as well as the therapeutic approaches employed.

Conclusion: Melioidosis is a severe infection with variable clinical presentations, including fever, night sweats, weight loss, dyspnea, and abscess formation at different sites. The broad spectrum of symptoms complicates diagnosis. Therefore, melioidosis should be considered in returning travellers from endemic regions presenting with compatible clinical features. Prolonged antibiotic treatment is essential for effective infection control. Importantly, recent evidence highlights that changing climate conditions and increased extreme weather events may further facilitate the geographical spread and importation risk of *B. pseudomallei*, underlining the importance of awareness regarding melioidosis.

P-1-75

Innovative malaria vaccination strategies using slow-growing genetically modified parasites

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Recently we investigated a novel malaria vaccination strategy using genetically attenuated, slow-growing *Plasmodium* parasites delivered as a single sporozoite injection (Sattler et al. 2024). By screening gene-deletion mutants of rodent (*P. berghei*) and human (*P. falciparum*) malaria parasites, we identified two rodent *P. berghei* mutants that retained mosquito transmissibility but showed significantly reduced virulence and blood-stage multiplication, resulting in only mild, self-limiting infections in mice. Mice immunized with these attenuated parasites demonstrated robust, long-lasting protection against challenge with wild-type sporozoites. Furthermore, the approach was extended to *P. falciparum*, where mutants with reduced blood-stage growth were generated, presenting promise for evaluating immune responses in humans.

Future experimental approaches will focus on generating and characterizing an expanded sets of single gene mutants in *P. berghei*, *P. falciparum* and *P. knowlesi*. Additionally, in *P. berghei*, promoter swap approaches and double gene deletions will be employed to further modulate parasite attenuation and test the effects on immunogenicity and protection. In *P. falciparum*, specific mutants will be created that lack the ability to adhere to the endothelium, which may improve safety and broaden vaccine applicability.

P-1-76

Lymphadenitis Colli in a Somalian Refugee: Overcoming Medical and Social Challenges in Infection Management

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Background: Lymphadenitis colli is common in pediatrics. In children with migration history, tuberculosis (TB) should be considered for early diagnosis and treatment. TB remains a global challenge, with 10.8 million new cases in 2023, 12% in

children. MDR-TB accounts for ~400,000 cases annually, posing treatment challenges. Historically, MDR-TB therapy required prolonged, toxic injectable regimens of 18–20 months, with poor adherence and adverse effects. Shorter, all-oral regimens, including 6-month BPaLM, improved outcomes but were initially unavailable for children due to pretomanid contraindications. Delamanid (Dlm) is now a safe pediatric alternative. In June 2024, WHO recommended a 6-month BDLLC regimen (Bdq, Dlm, linezolid, levofloxacin, clofazimine), proven safe in children by the BEAT-TB trial. Despite these advances, careful monitoring for neurological, gastrointestinal, musculoskeletal, ophthalmological, psychiatric, and cardiac toxicities is essential. Social vulnerabilities, such as unstable housing, limited healthcare access, and high costs, further complicate care, highlighting the need for holistic management.

Case: A 12-year-old Somali boy who was in Germany for one year presented with right cervical lymphadenitis and night sweats. Labs showed elevated CRP (3.1 mg/dL); serologies for Bartonella, CMV, EBV, and Toxoplasma were negative. Ultrasound revealed two abscessed lymph nodes; biopsy culture grew drug-sensitive *Mycobacterium tuberculosis*. Pulmonary TB was excluded. Standard therapy was started. By July 2024, after 3 months of treatment, worsening lymphadenitis prompted repeat biopsy, revealing dual infection with drug-sensitive and MDR-TB isolates. Insurance delays for Bdq and Dlm led to weight loss and persistent wound drainage. MDR-TB therapy per WHO BDLLC recommendations was then started. Follow-up included clinical, lab, neurological, ophthalmological, and psychiatric monitoring. Social factors, including trauma history, language barriers, unstable housing, and maternal illiteracy, required a multidisciplinary support network to maintain adherence.

Discussion: Deterioration despite initial drug-sensitive TB should prompt repeat testing for MDR co-infection. Implementation of WHO MDR-TB guidelines is challenging due to regulatory, financial, and logistic barriers. Successful management requires a patient-centered, multidisciplinary approach addressing medical and social determinants. Regular monitoring for adverse events ensures safety under short-course regimens. Holistic, multidisciplinary management is essential to overcome barriers and achieve favorable outcomes.

P-1-77

Severe Mediastinal Lymphadenopathy due to *Mycobacterium avium* in a 4-Year-Old Immunocompetent Child: Diagnostic and Therapeutic Challenges in Pediatric Non-Tuberculous Mycobacterial Disease

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Background: Non-tuberculous mycobacteria (NTM) are environmental organisms increasingly recognized as causes of pediatric disease. In adults, NTM infections have been increasingly reported worldwide over the past decades, for reasons that remain unclear. In otherwise healthy children, the most common presentation is cervicofacial lymphadenitis, whereas pulmonary disease is rare and usually occurs in the context of underlying conditions.

Case: We report a 4-year-old girl presenting with mediastinal lymphadenopathy, initially raising suspicion of lymphoma. The bronchoscopy revealed an endobronchial, exophytic lesion causing near-complete bronchial obstruction. We performed interventional recanalization and obtained biopsies from the lesion. Biopsy excluded malignancy but revealed *Mycobacterium avium* by PCR and culture. Due to marked bronchial compression and respiratory symptoms, treatment with rifampicin, azithromycin, and ethambutol was initiated. The patient showed early improvement in symptoms and radiological findings during the first six months. Based on scarce pediatric data, therapy was continued for a total of 12 months. Regular clinical and radiological follow-up was performed, alongside monitoring for potential toxicities, including liver function and vision. Over the course of therapy, the patient demonstrated sustained clinical improvement, resolution of cough, and radiological regression of lymphadenopathy. Immunological and genetic evaluation excluded underlying immune deficiency or genetic predisposition.

Discussion: This case highlights that severe NTM disease can occur in otherwise healthy children. It illustrates the challenges in pediatric NTM management. Prolonged therapy was justified by disease severity, airway compromise, and microbiological confirmation. Evidence gaps remain significant: most guidelines are based on expert consensus, derived from adult or cystic fibrosis literature as well as from children presenting with cervicofacial infection; optimal treatment duration and toxicity thresholds are not well defined, and species-specific guidance is limited. Clinicians must carefully balance the risks of long-term antibiotic therapy against watchful-waiting strategies in milder cases.

Conclusion: NTM infections should be considered in the differential diagnosis of mediastinal lymphadenopathy in children, even in immunocompetent individuals. This case underscores the need for further pediatric research to define evidence-based treatment strategies, optimal therapy duration, and predictors of clinical outcome.

P-1-78

Filling the Gaps in Surveillance Data: Detailed Demographic Insights into Paediatric Tuberculosis Cases in Munich, 2023–2025

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Background: In March 2025, the ECDC Paediatric Expert Group released a rapid communication analyzing surveillance data to explain the recent increase in notified paediatric tuberculosis (TB) cases in the European Union/European Economic Area. However, the report lacked detailed demographic and social determinant information—factors essential to understand underlying drivers, anticipate future trends, and guide public health policy. This study aims to address this gap by providing detailed demographic and clinical data on paediatric TB cases from a tertiary care center in Munich, Germany.

Methods: Since March 2023, paediatric TB care at the Hauner Children's Hospital, LMU Munich, has been

strengthened through a collaborative project with the Public Health Department of the City of Munich and the Pediatric Migrant and Public Health Center Munich. This initiative enabled systematic data collection and centralized TB care in the region. Clinical and sociodemographic data were prospectively collected for all children aged under 15 years presenting with confirmed, probable, or possible TB, as well as those with TB infection, between March 2023 and June 2025.

Results: During the study period, 18 children were diagnosed with TB disease. Of these, 10 (55%) were born in Germany, but none (0%) had parents of German origin. In addition, 93 children were diagnosed with TB infection; 31 (33%) of these were born in Germany, and only 3 (3%) had parents of German origin.

Conclusion: The majority of paediatric TB cases in Munich occurred among children with migrant backgrounds, even among those born in Germany. These findings underscore the critical role of social determinants in shaping TB epidemiology. Targeted approaches in paediatric TB care and prevention are needed, taking into account the specific vulnerabilities of migrant families. By providing detailed demographic information, this study adds important context to surveillance data and may support the refinement of public health responses to the recent rise in paediatric TB cases across Europe.

P-1-79

Development of field-applicable loop-mediated isothermal amplification (LAMP) assays for helminth infections

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Soil-transmitted helminths and filariae are among the most prevalent parasitic infections worldwide, collectively affecting over one billion people, predominantly in tropical and subtropical regions. These parasites contribute significantly to global morbidity, leading to blindness, dermatitis, anemia, malnutrition and reduced life expectancy. Despite their global impact, helminth infections often remain underdiagnosed due to limited access to reliable, sensitive and affordable diagnostic tools. This is especially the case in low-resource settings. Microscopy, the traditional gold standards for the diagnosis of helminths can have limited sensitivity for low-intensity infections and requires intensive training. Meanwhile, qPCR has a high sensitivity but requires sensitive and expensive machines. This highlights the need for field applicable, easy-to-use, sensitive and specific molecular diagnostics. This is in line with the current recommendation of the WHO for improved diagnostic tools to guide decisions when to halt elimination programs.

We developed several diagnostic assays based on loop-mediated isothermal amplification (LAMP), a rapid, sensitive, and field-applicable DNA amplification technique. Primer sets that target conserved regions, i.e., ribosomal genes, for several helminth species were designed. Genomic DNA was extracted from stool, skin biopsies, and blood clots from

infected individuals, arthropod vectors, as well as isolated parasites and was used for the validation of assays.

LAMP assays were developed for the human-pathogenic filariae *Loa loa*, *Onchocerca volvulus*, and *Mansonella perstans*—to detect parasites in both humans and vectors. Additional assays target soil-transmitted helminths: *Ascaris* spp., *Trichuris trichiura* and *T. incognita*, and hookworms (*Necator americanus*, *Ancylostoma duodenale* and *A. ceylanicum*). DNA from stool samples of 14 individuals were analysed for the congruence between eggs per gram, qPCR and LAMP results. For *Ascaris lumbricoides*, 6 out of 8 egg positive samples were detected as positive, while 1 egg negative sample was detected as positive. Overall 10 out of 14 samples were in line with the microscopy data, resulting in a congruence of 71.4%. Interestingly, the LAMP data agreed 100% with the qPCR data obtained from the same material. In line, results from microscopy and hookworm LAMP agreed in 11 out of 14 sample resulting in a congruence of 78.6%. That said, the LAMP results again overlapped 100% with the qPCR data. Moreover, this new LAMP assay can also be used to detect *Ancylostoma ceylanicum*, a potent zoonosis.

In summary, we demonstrate that LAMP is a highly sensitive and reliable molecular diagnostic tool, which facilitates diagnosis in resource-limited settings.

P-1-80

Clinical and immunological signatures of acute Lassa fever

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The Lassa virus (LASV), which belongs to the *Arenaviridae* family, is the causative agent of Lassa fever (LF) and is endemic to most West African countries. Although most cases are asymptomatic, approximately 900,000 infections occur every year, resulting in a case fatality rate of around 20% among hospitalized patients. The WHO therefore listed LF as a priority disease in their 2016 Research & Development Blueprint due to its epidemic potential and the lack of therapeutic treatment or vaccination options. It is known that the early activation of the adaptive immune response correlates with LF survival in non-human primates. However, the immune response in human LF patients has not yet been investigated in much detail, except for a few single cases.

In order to address this, our aim is to analyze immune cells such as antigen-presenting cells (APCs) and T cells, during LASV infection in human patients, in order to understand underlying immunological mechanisms that influence disease outcome. Additionally, we intend to correlate these findings with clinical parameters such as blood chemistry and enzyme levels that are affected by LASV infection.

Furthermore, we intend to investigate the organ manifestations and inflammatory markers of these patients.

In Nigeria, we have set up a glovebox-based immunology laboratory to process blood samples of LF patients together with our collaboration partner at the Irrua Specialist Teaching Hospital. Between 2022 and 2024, 502 participants were enrolled, with over 30 laboratory parameters and over 50 clinical parameters measured per time point.

Decreased survival rates and multiple organ dysfunctions in these patients were related to age, but not to sex. We observed variances in dendritic cell subtypes and altered frequencies of myeloid cells in the blood of LF patients. However, the analysis of APCs also showed no distinct sex-related differences between patients with moderate or severe disease. Comparing LF survivors and fatal cases revealed an increase in the expression of T cell exhaustion markers, accompanied by a reduction in activation markers. This might result in T cell dysfunction which could be a pivotal factor in severe disease progression.

Further analysis is ongoing to deepen our understanding of the immune response-driven pathology after LASV infection.

P-1-81

Expansion and diversification of West Nile virus circulation in Germany (2023/2024)

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The emergence of West Nile virus (WNV) around the world is closely connected to global warming trends. Higher temperatures shorten the extrinsic incubation period of this arbovirus in vector-competent mosquitoes. Furthermore, high rainfall creates water reservoirs and thus optimal breeding conditions for these vectors. The consequence are increased virus transmission rates to avian amplifying hosts but also to humans as dead-end hosts. Approximately 20% of human infections lead to West Nile fever, a mild febrile disease. In rare cases this zoonosis can progress to West Nile neuroinvasive disease with potentially lethal outcome. WNV lineage 2 (WNV-2) was initially detected in birds in north-eastern Germany in 2018 and developed a stable enzootic transmission cycle in the region afterwards.

The wild bird-associated zoonoses network (WBA-Zoo) was established in Germany more than a decade ago. It focuses on nationwide surveillance of WNV and the closely related Usutu virus (USUV) in birds. For the years 2023 and 2024, more than 2300 blood samples from live birds were collected countrywide by dedicated cooperation partners and analysed with WNV and USUV specific serological assays and reverse transcription quantitative polymerase chain reaction protocols. Additionally, whole genome sequences were generated from tissue material of 86 naturally WNV infected deceased birds using Oxford nanopore technology.

While the detection rates of neutralizing antibodies (nAb) against WNV in birds in known hotspots of circulation such as Berlin remained relatively stable (15-20%), silent circulation of the virus was once more indicated by presence of WNV nAb in resident wild birds further westward in 2023 as it had already been in preceding years. This was followed by the emergence of acute WNV infections in birds in almost

all previously unaffected German federal states in 2024. Phylogenetic and phylogeographic investigation of this dynamic situation revealed continued dominance (>70%) of an established WNV-2 variant (subcluster 2.5.3.4.3c) in well-known as well as emerging hotspots of virus circulation. Nonetheless, the local maintenance of a second variant (cluster 2.5.3.2) could also be proven, which had remained unclear during previous seasons due to a limited number of available sequences. On the whole, the geographic expansion of WNV circulation in Germany appeared to be driven by local transmission rather than new introductions for both aforementioned variants.

In conclusion, the WBA-Zoo is a well-established tool for WNV surveillance in Germany. The marked westward expansion of acute avian WNV infections in 2024 was accompanied by autochthonous human cases and had long been foreshadowed in the serological surveillance data of wild birds. This emphasizes the need for continued WNV monitoring, especially in a changing climate, and proves the suitability of an approach based on the avian population as an early-warning system in a One Health context.

P-1-82

From Case Reports to Comparative Genomics: Uncovering the Clinical Impact of *Wohlfahrtiimonas chitiniclastica*

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Wohlfahrtiimonas chitiniclastica, an emerging zoonotic pathogen originally associated with parasitic fly larvae, is increasingly reported in human infections, including wound colonization, bacteremia, and sepsis. Despite its growing clinical relevance, diagnostic awareness remains limited. In our study, we combined whole-genome and phenotypic characterization of 14 clinical isolates with comparative analysis of 26 publicly available genomes. Phenotypically, all isolates were susceptible to cephalosporins, levofloxacin, and trimethoprim/sulfamethoxazole, while showing intrinsic resistance to fosfomycin and aminoglycosides. Genomic screening revealed both conserved resistance determinants (e.g., macA/B) and mobile genetic elements (transposons, phages) potentially contributing to resistome expansion. Pan-genome analysis demonstrated high genetic plasticity, with 57% of genes being accessory, and indicated the presence of a distinct subspecies among the clinical isolates.

Drawing on insights from our recent systematic review, we highlight the diagnostic challenges and the clinical underrecognition of *W. chitiniclastica*. Its zoonotic origin and frequent association with neglected wounds underscore its relevance within a One Health framework. Together, our data support the need for improved diagnostic awareness, genomic surveillance, and evidence-based antimicrobial management of this emerging pathogen.

1. Kopf A, Bunk B, Riedel T, Schröttner P. The zoonotic pathogen *Wohlfahrtiimonas chitiniclastica* - current findings from a clinical and genomic perspective. BMC Microbiol. 2024;24(1):3.

2. Kopf A, Bunk B, Coldewey SM, Gunzer F, Riedel T, Schröttner P. Comparative Genomic Analysis of the Human Pathogen *Wohlfahrtiimonas Chitiniclastica* Provides Insight Into the Identification of Antimicrobial Resistance Genotypes

and Potential Virulence Traits. *Front Cell Infect Microbiol.* 2022;12:912427.

3. Kopf A, Bunk B, Coldewey SM, Gunzer F, Riedel T, Schröttner P. Identification and Antibiotic Profiling of *Wohlfahrtiimonas chitiniclastica*, an Underestimated Human Pathogen. *Front Microbiol.* 2021;12:712775.

4. Schröttner P, Rudolph WW, Damme U, Lotz C, Jacobs E, Gunzer F. *Wohlfahrtiimonas chitiniclastica*: current insights into an emerging human pathogen. *Epidemiol Infect.* 2017;145(7):1292-303.

P-1-83

MAMS_6 – a novel transcriptome-Informed Quantitative Real-Time PCR-Based Signature for Monitoring Tuberculosis Treatment Success

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Background: Timely diagnosis and effective treatment monitoring remain key challenges in tuberculosis (TB) care. There is an urgent need for reliable, sputum-independent biomarkers to support rapid diagnosis and assess treatment response.

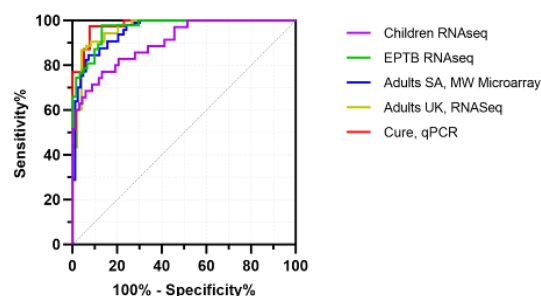
Methods: Through a multi-step process, we developed a parsimonious real-time quantitative PCR (RT-qPCR) signature for TB cure. Whole blood transcriptomes from participants in the Phase IIB PanACEA MAMS-TB-01 trial (n=21) were profiled using RNA sequencing at baseline and at weeks 2, 12, and 26 of treatment. We prioritised highly differentially expressed genes identified in this study and appearing in published signatures to develop an RT-qPCR-based assay. Gene expression was quantified using microfluidic RT-qPCR in a training (n=21) and validation cohort (n=39), followed by ROC curve analysis to identify genes discriminating between active and cured TB. The resulting six-gene signature, MAMS_6, was further validated using both in-house and public transcriptomic datasets.

Results: TB treatment induced rapid and dynamic transcriptomic shifts, particularly in genes related to leukocyte activation and inflammatory signalling. The MAMS_6 signature demonstrated 97% sensitivity and 82% specificity for predicting treatment completion. Its performance was consistent across clinical subgroups and correlated with stable culture conversion. Additionally, MAMS_6 showed diagnostic utility in external datasets, highlighting its broader relevance.

Conclusion: The MAMS_6 RT-qPCR signature holds promise as a dual-purpose tool for TB diagnosis and treatment monitoring. It may be particularly valuable for individuals unable to reliably produce sputum, supporting more inclusive and effective TB care.

Fig. 1

ROC curve: ROC of CompSIG>ROC_6_median_val+



P-1-84

Non-sputum biomarkers for on-treatment prediction of TB disease recurrence

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Introduction: TB disease recurrence represents a significant burden to persons on treatment and incurs long follow-up periods in clinical trials. On-treatment identification of individuals at greater risk of recurrence could guide alternative treatment and follow-up strategies. We performed a validation study of previously published treatment monitoring signatures in people with TB recurrence, to examine their treatment monitoring capabilities. We also examined the temporal differences in clinical covariates, to determine if any could represent easily accessible markers of treatment response.

Methods: From the PanACEA multi-arm, multi-stage trial, we identified 8 persons who experienced disease recurrence within 1 year of treatment initiation, and 8 controls with sustained cure, matched for biological sex, age, treatment arm, and baseline disease severity. We generated bulk RNA-sequencing from whole blood samples at weeks 0,1,2,4,9,14, and 26, and paired with clinical covariate data. We performed cross-sectional univariate analysis using t-tests and Kruskal-Wallis tests, and used linear mixed models and repeated measures correlation for longitudinal data, with Bonferroni correction. Model selection was performed with likelihood ratio tests (LRT). We validated the predictive performance of multiple transcriptomic signature scores using area under the receiver operating characteristic curve (AUROC) values.

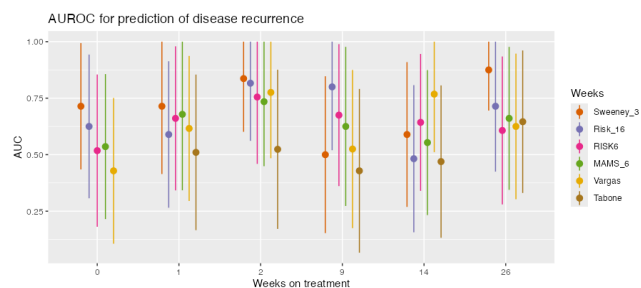
Results: Median time-to-negativity was significantly different between recurrence vs controls (adjusted $P=2.0 \times 10^{-2}$). Longitudinally, we also found significantly different temporal

changes in body mass index (BMI) (adjusted $P=3.82 \times 10^{-4}$) and heart rate (adjusted $P=6.71 \times 10^{-5}$) between outcome groups. Sweeney-3, Risk-6, MAMS6 and Tabone27 signatures were correlated with BMI over time, and MAMS6 and Risk-16 with neutrophil counts. At week 26, Sweeney-3 showed highest performance [AUROC = 0.87 (0.68-1 CI); Figure 1]. Interestingly, for 4 signatures, the highest AUC values were observed at Week 2 into treatment.

Conclusions: The Sweeney-3 signature shows promise for treatment outcome prediction at week 26. Temporal biomarkers may be more informative than cross-sectional observations for treatment monitoring, and HR and BMI could represent informative and easily measurable markers of treatment response. Ongoing work is focused on integrative modeling combining transcriptomic and clinical features to optimize early risk stratification.

Figure 1. Receiver operating characteristic curves of transcriptomic signature scores derived from original models throughout treatment. The scores were calculated from the gene expression counts in whole-blood samples from patients at weeks 0,1,2,9,14 and 26. The AUC value represents a performance metric for a binary classifier for treatment outcome label "recurrence" vs "sustained cure" using model scores. AUC, area under the curve; TPR, true positive rate; FPR, false positive rate; CI, confidence interval.

Fig. 1



P-1-85 The DZIF Transplant Cohort e.V.

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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 2.949 patients included in the data base and more than 270.635 biosamples aliquots collected, including PBMCs, RNA-stabilized blood, serum, urine, feces.

P-1-86

Intra-specific differences of *Schistosoma mansoni* influence immune response and organ pathology in a mouse model

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Although caused by the same species, infections with *Schistosoma* (*S.*) *mansoni* can lead to significantly different pathological outcomes. To investigate whether the origin of the parasite strain contributes to this variability, we analyzed three geographically distinct *S. mansoni* laboratory strains—Liberia, Belo Horizonte (Brazil), and Puerto Rico—in NMRI mice for parasite phenotype, immune responses, and organ pathology.

Our data show significant strain-specific differences in worm size, morphology, and egg production, as well as in liver and spleen pathology. The Puerto Rican strain exhibited a higher male-to-female worm ratio and induced particularly severe liver damage, characterized by increased organ-to-body weight ratios, higher collagen deposition, and a dominant Th2 cytokine profile. In contrast, the Liberian strain showed attenuated immune activation, smaller granulomas, and less organ pathology. Interestingly, granuloma size and collagen content did not consistently correlate across strains: while the Liberian strain induced the smallest granulomas, collagen levels were comparable to the Brazilian strain, highlighting the uncoupling of granuloma architecture and fibrogenesis.

Flow cytometry analyses revealed distinct strain-dependent shifts in immune cell populations in liver and spleen, including dendritic cells, eosinophils, and CD4⁺ T cell subsets (Tregs, Th17, Th1), further supporting differences in immune polarization. These systemic and tissue-level variations suggest that parasite strain geographic origin significantly shapes the host's inflammatory and fibrotic response.

We are currently extending the analysis to the intestinal region. Ongoing experiments aim to quantify intestinal tissue damage using blinded Histoscore and FITC dextran permeability assays. In addition, we are performing targeted qPCR analyses to investigate important pathological mechanisms, including type 2 immune polarization, inflammation and immune regulation, tight junction integrity, mucus production and myeloid cell migration.

We are using flow cytometry to investigate strain-specific differences in intestinal immune cell populations, including myeloid cells, ILCs and T cells. In parallel, 16S rRNA microbiome analyses are performed to characterize possible strain-specific shifts in the microbiome composition in the intestine.

Our results underscore that intraspecific variation of *S. mansoni* influences both the hepatic and intestinal immune response and pathology. These findings are crucial for the development of strain-specific and tissue-specific therapeutic and preventive strategies for schistosomiasis.

P-1-87

Evaluating novel sputum-independent diagnostic biomarker for assessing tuberculosis activity in children and adolescents

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Background: Tuberculosis (TB) in children is notoriously difficult to diagnose and stage. Especially in cases classified as TB infection (TBI), clinical and radiologic findings fail to reflect the true immunological activity of the disease. Yet TB is known to be a continuum of disease states differing individually, from exposure through incipient infection to active disease, rather than as a binary latent vs. active state. Differentiating children who are sub-clinically progressing to active TB from those who are stable is critical to improve individual treatment decisions and preventing missed opportunities for early intervention.

Objective: This study uses the TAM-TB assay, a flow cytometry-based blood test measuring activation markers on TB-specific CD4⁺ T-cells, to compare immunological disease activity with standard clinical classifications. We aim to demonstrate that children with similar clinical diagnoses, particularly those labeled as TBI, may differ substantially in their immune response profiles, indicating varying stages along the TB disease spectrum.

Methods: In this prospective cohort study, children and adolescents (<18 years) with suspected or confirmed TB or recent exposure were recruited at a tertiary pediatric hospital. Each participant underwent comprehensive clinical assessment including medical history, radiological imaging, IGRA testing, and where applicable, microbiological diagnostics. Simultaneously, the TAM-TB assay was performed on peripheral blood samples to measure IFN- γ , CD38, and CD27 expression on antigen-stimulated CD4⁺ T-

cells. Based on clinical criteria, participants were categorized as: no TB, TB exposure only, TBI, or active TB. We then analyzed how immunological profiles derived from the TAM-TB assay correspond to, or deviate from, these clinical classifications.

Preliminary Observations (Results expected in December/January): Early data analysis suggests that children with identical clinical diagnoses, particularly within the TBI group, may show markedly different TAM-TB profiles. This supports the notion that some children classified as TBI may, in fact, be in a preclinical or early active disease state and thus at higher risk for progression.

Conclusion: The TAM-TB assay revealed immunological differences not identified by conventional clinical and radiological evaluation. Our findings suggest that TB in children should be understood as a dynamic immunological process, and that children labelled as TBI may in fact represent a rather heterogenous group of various activity states of TB spectrum. Our findings are a call for more refined, biomarker-informed approaches to risk stratification, monitoring, and treatment decisions in pediatric TB care.

P-1-88

Diagnostic and predictive accuracy of a 3-gene *Mycobacterium tuberculosis* Host Response Cartridge in tuberculosis household contacts – updated results from the multi-centre prospective ERASE-TB study

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Question: WHO recommends tuberculosis (TB) screening for household contacts (HHCs). Non-sputum diagnostics based on mRNA expression show promise. We evaluated the diagnostic and predictive accuracy of the Cepheid Xpert *Mycobacterium tuberculosis* Host Response (MTB-HR) prototype for TB in the context of HHC screening.

Methods: 2,100 HHCs (≥ 10 years) from Mozambique, Tanzania, and Zimbabwe were screened for TB (clinical, radiological, microbiological) every 6 months over 24 months, including an MTB-HR test at every visit. The MTB-HR test gives a TB score based on the cycle threshold of three expressed genes, with a smaller score indicating TB. We analysed MTB-HR scores stratified by symptom and HIV status. We determined the diagnostic accuracy of MTB-HR (within 30 days of diagnosis) versus "confirmed" or "likely" TB diagnoses as ascertained by an expert endpoint review committee. We determined the predictive accuracy of incident TB diagnoses at 6 and 12 months. We calculated the area under the receiver operating curves (AUROC) with 95% confidence intervals. Optimal MTB-HR score thresholds were identified using the Youden method. Sensitivity, specificity, and positive and negative predictive values were calculated with 95% confidence intervals (95%CI) using the Wilson method.

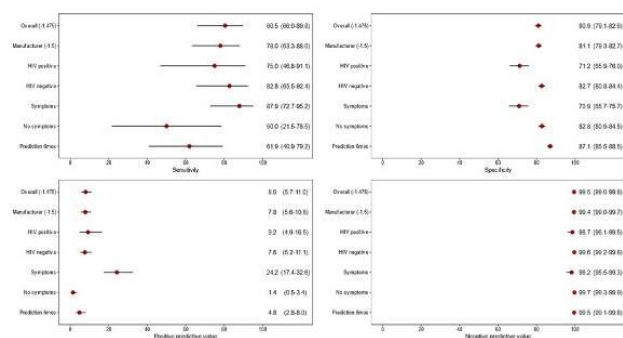
Results: We included 2,079 HHCs, median age was 27 years (IQR: 17–42), 1,294 (62%) were female. The median MTB-HR score was -0.9 (IQR -1.3–0.60) in HHCs without TB and -2.6 (IQR -3.1–1.7) in HHCs with TB. Symptomatic HHCs with TB had lower MTB-HR scores than asymptomatic HHCs: -2.6 (IQR -3.1–1.9) vs. -1.2 (IQR -1.7–0.84, $p=0.006$). Analysis of diagnostic accuracy included 41 HHCs with MTB-HR results within 30 days of TB diagnosis. AUROC was 0.86 (95%CI 0.79–0.92). At a cut-off of -1.475, sensitivity was 81% (95%CI 66–90) and specificity 81% (95%CI 79–83). Sensitivity was higher in HIV-negative and symptomatic HHCs, while specificity was higher in HIV-positive and asymptomatic HHCs. Negative predictive value exceeded 98% across subgroups; positive predictive value was low. Prediction analysis included 21 and 14 HHCs with incident TB at 6 and 12 months, respectively. AUROC was 0.77 (95%CI 0.65–0.89) at 6 months, and 0.56 (95%CI 0.41–0.71) at 12 months. At 6 months, a -1.675 cut-off yielded 62% sensitivity (95%CI 41–79) and 87% specificity (95%CI 86–89).

Conclusions: Our results indicate that the MTB-HR might be useful in identifying HHCs who need further testing or closer follow-up for the detection and management of TB. Further research integrating the MTB-HR into a screening algorithm for high-risk groups is needed.

Figure: Accuracy of the MTB-HR for TB diagnosis and prediction: sensitivity (a); specificity (b); positive (c) and negative predictive values (d).

Fig. 1

Figure: Accuracy of the MTB-HR assay for TB diagnosis and prediction: sensitivity (a); specificity (b); positive (c) and negative predictive values (d).



P-1-89 Serological Detection of circulating Vaccine-Derived Poliovirus Type 2: Recombinant Production of VP1 Antigens for Assay Development

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Recently, circulating vaccine-derived poliovirus type 2 (cVDPV2) was detected in wastewater samples from major European cities, including Munich, Barcelona, Warsaw, and London.[1] Despite the absence of clinical cases, this highlights the need for robust diagnostic tools and outbreak preparedness. To support surveillance and immunity monitoring, we are developing a serological assay targeting the immunodominant VP1 capsid protein of poliovirus.

Recombinant production of the VP1 antigen is currently being established in bacterial (*E. coli*), insect (HighFive), and human (HEK293) cell expression systems. These platforms are being evaluated for antigen yield, protein quality, and immunoreactivity.[2] Following antigen validation, an assay will be applied to the analysis of serum samples from a defined study cohort. The goal is to assess antibody prevalence against cVDPV2 and evaluate the feasibility of high-throughput screening for poliovirus vaccination and exposure to cVDPV2 on the Elecsys immunoassay platform (Roche Diagnostics), enabling automated serological testing.

This approach aims to facilitate early detection of immunity gaps and silent circulation. Here, we present current progress in antigen expression purification, and biophysical characterization, as well as preliminary assay design considerations, and the roadmap toward a sensitive and specific diagnostic tool for supporting poliovirus surveillance in Europe.

[1] Böttcher S, Kreibich J, Wilton T, Saliba V, Blomqvist S, Al-Hello H, Savolainen-Kopra C, Wieczorek M, Gad B, Krzysztozek A, Pintó RM, Cabrerizo M, Bosch A, Saxentoff E, Diedrich S, Martin J. Detection of circulating vaccine-derived poliovirus type 2 (cVDPV2) in wastewater samples: a wake-up call, Finland, Germany, Poland, Spain, the United Kingdom, 2024. *Euro Surveill*, **30**(3):2500037 (2025). doi: 10.2807/1560-7917.ES.2025.30.3.2500037.

[2] de Marco, A., Berrow, N., Lebendiker, M. *et al.* Quality control of protein reagents for the improvement of research data reproducibility. *Nat Commun* **12**, 2795 (2021). <https://doi.org/10.1038/s41467-021-23167-z>

P-1-90 Breaking the Bottleneck in Respiratory Pathogen Testing: Rapid, Robust DNA Extraction from Diverse Clinical Specimens

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Respiratory tract infections (RTIs) remain among the leading causes of morbidity and mortality worldwide. They account for 4 million deaths annually and impose substantial burdens on healthcare systems globally, but more significantly in low- and middle-income countries [1]. Timely and accurate pathogen detection is essential to monitor therapy, curb antimicrobial resistance, and improve patient outcomes. However, conventional nucleic acid extraction methods for upper respiratory tract specimens often require heavy instrumentation, complex manual steps, and lengthy turnaround times, limiting their impact in urgent diagnostic settings.

Within the framework of the HoliCare project, we developed and validated a rapid and broadly applicable DNA extraction protocol for Gram-positive and Gram-negative bacteria, fungi, and mycobacteria. The method was tested on sputum, bronchoalveolar lavage (BAL), and nasopharyngeal swabs containing reference strains, producing PCR- and LAMP-ready DNA in under one hour including liquefaction of sputum samples. The workflow demonstrated robust performance even with challenging matrices such as viscous sputum and low-biomass BAL samples. A further advantage from a field implementation perspective is the possibility to automate the sputum liquefaction and DNA extraction on open platforms for DNA/RNA extraction such as the SwiftXtractor.

This newly developed protocol manages to be rapid and easy to use without compromising on efficiency. Benchmarking against leading sample-to-result platforms from market competitors such as Cepheid's GeneXpert® System revealed equivalent analytical performance, while retaining flexibility for integration into diverse downstream workflows. This rapid, reliable, and versatile extraction method addresses key bottlenecks in respiratory pathogen diagnostics and aligns with HoliCare's mission to expand access to high-quality, affordable molecular testing [2].

References: [1] World Health Organization. Global Health Estimates 2023: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2022. WHO, Geneva. [2] HoliCare Project. Holistic & Integrated Care for Multidrug-Resistant Tuberculosis and Other Respiratory Infections.

P-1-91

Delayed Outbreak and Clinical Characteristics of *Mycoplasma pneumoniae* Infections in Children: A Retrospective Study from Tertiary Care Childrens Hospital (2024)

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Introduction: *Mycoplasma pneumoniae* (MPP) infections are known to occur in regular patterns every 1 to 3 years.[1] A new wave of infections was anticipated around late 2022 to early 2023.[2] However, data from the Dr. von Hauner Children's Hospital (DHCH) revealed a delayed onset, with a noticeable increase in cases emerging in 2024. This study characterizes affected patients in clinical presentation and treatment in 2024.

Methods: We performed a retrospective analysis of MPP the 78 cases at the DHCH in 2024, focusing on direct pathogen detection, patient characteristics, and clinical outcomes. Statistical analysis was conducted using SPSS. Associations between *Rhino-Enterovirus* (RV/EV) co-infection and symptoms were analyzed using Chi-square or Fisher's exact test, with odds ratios (OR) and 95% confidence intervals (CI).

Results: Case numbers remained stable in early 2024, followed by a sharp increase, peaking in November.

Hospitalization was required in 73.1% of patients, three needed intensive care. 57.7% exhibited pre-existing conditions, most commonly neurological, hematological, respiratory, and cardiac diseases.

Co-infections occurred in 37 of cases most commonly (RV/EV) (26; 70.3%). The RV/EV-positive status was associated with a slightly longer hospital stay (median: 6.5 vs. 5.5 d) and significantly less frequent fever (53.8% vs.

84.6%, $p < 0.003$; OR: 0.212; 95% CI: 0.072–0.623, $p < 0.05$).

Antibiotics were given in the majority of cases (93.7%) starting on aminopenicillins, switching to macrolides. Inhalation therapy was used in 55.3%, oxygen in 40.3% and non-invasive ventilation in three cases.

Conclusion: The 2024 MPP outbreak showed delayed onset, high hospitalization rates, and frequent co-infections, foremost RV/EV. Reduced fever in additionally RV/EV-positive patients may be a useful clinical marker. Further epidemiological monitoring is warranted.

[1] Beeton Michael L, Zhang Xu-Sheng, Uldum Søren A, Bébéar Cécile, Dumke Roger, Gullsby Karolina, Ieven Margareta, Loens Katherine, Nir-Paz Ran, Pereyre Sabine, Spiller O Brad, Chalker Victoria J, the ESCMID Study Group for Mycoplasma and Chlamydia Infections (ESGMAC) Mycoplasma pneumoniae subgroup. *Mycoplasma pneumoniae infections, 11 countries in Europe and Israel, 2011 to 2016*. Euro Surveill. 2020;25(2):pii=1900112. <https://doi.org/10.2807/1560-7917.ES.2020.25.2.1900112>

[2] Meyer Sauter PM, Beeton ML; European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Mycoplasma and Chlamydia Infections (ESGMAC), and the ESGMAC Mycoplasma pneumoniae Surveillance (MAPS) study group. *Mycoplasma pneumoniae: delayed re-emergence after COVID-19 pandemic restrictions*. Lancet Microbe. 2024 Feb;5(2):e100-e101. doi: 10.1016/S2666-5247(23)00344-0. Epub 2023 Nov 23. PMID: 38008103.

P-1-92

New biomarker approaches for risk stratification of outcome development in patients with Primary Sclerosing Cholangitis (PSC) based on bacterial glycoalkal degradation

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Introduction and Aim: Primary Sclerosing Cholangitis (PSC) is a chronic progressive disease of the bile ducts, characterized by recurrent inflammation leading to fibrosis and bile duct strictures. The course of PSC is variable and unpredictable. Potential clinical endpoints include the development of cholangiocarcinoma (CCA), end-stage liver disease requiring liver transplantation (LTX) and death. At present, no predictive tools are available to identify high-risk individuals, limiting targeted surveillance and resulting in poorer prognosis due to delayed clinical detection and intervention.

The mechanisms driving PSC progression and outcome development remain unclear, but are thought to be multifactorial. One important contributor is the degradation of the cholangiocyte glycoalkal by biliary bacteria. Cholangiocytes are then exposed to toxic bile acids triggering apoptosis and inflammation.

In the present study, we aimed to quantify genes and mRNA of bacterial enzymes involved in the pathway of glycocalyx degradation as well as cytokines in bile as a new biomarker approach for risk stratification.

Methods: Bile samples were obtained from 106 PSC patients during routine endoscopic retrograde cholangiography (ERC). Cytokines were measured using the Bio-Plex Pro Cytokine and Chemokine Assay (Bio-Rad). In a subcohort, bacterial DNA and RNA were quantified using our newly developed in-house qPCR-assay. To assess transcriptional activity, an RNA/DNA-ratio was implemented. A ratio > 1.2 was considered high transcriptional activity; a ratio < 0.8 was considered low transcriptional activity.

Results: In the overall cohort, reduced levels of 10 cytokines in bile were associated with a significantly increased CCA incidence during the 5-year-follow-up. Among them, CTACK showed the best discriminatory power, with a CCA incidence of 15% in the low-level group and 0% in the high-level group ($\chi^2 = 9.2$; $p = 0.002$).

For the subcohort, Kaplan Meier analysis was performed: High transcriptional activity was associated with a shorter event free survival time compared to the low-transcriptional-activity group (mEFS: 1.5 years [95% CI: 1.1-1.9] vs. 7.6 years [95% CI: 3.9-11.3] post sampling; $p = 0.041$) with events defined as CCA, LTX and death.

Conclusions: Our analyses identified the transcriptional activity of bacterial enzymes involved in the degradation of the cholangiocyte glycocalyx as well as cytokines measured in bile as promising predictive tools for risk stratification in PSC patients. This is essential for targeted and effective surveillance and on-time intervention. Importantly, these findings also underscore the key role of the biliary microbiota in modifying PSC progression. However, further analyses and validation in independent cohorts is needed to validate these findings.

P-1-93

Multi-omics techniques decipher 3 distinct immunotypes of extrapulmonary tuberculosis and delivers a novel diagnostic gene expression signature

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Introduction: 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.

Objectives: While there is a high amount of research data on pulmonary TB (PTB), knowledge on EPTB remains scarce. Here, we aim to provide a deep and extensive immunological characterization of EPTB in relation to disease severity and clinical representation of EPTB.

Methods: Within the multicentric EX-TB cohort we used a broad range of state-of-the-art OMICS methods (bulk and single cell RNAseq, multicolor flow cytometry and cytokine profiling) to deeply characterize immunotypes of EPTB.

Results: Multi-OMICS profiling lead to the detection of three immunotypes (low, intermediate and high) of EPTB each characterized by a distinct immunological program. Within PBMCs, we identified monocytes as the major contributor to type I interferon signaling in mild EPTB, in addition to an expansion of pro-inflammatory monocyte states. NK cells showed the strongest immune effector activity in intermediate EPTB, which was interestingly diminished in severe EPTB. In contrast, T cells displayed a hyperactivated and cytotoxic phenotype within EPTB. Further, we were able to detect an activated CD4 T cell subset characterized by a unique transcriptomic profile distinct to EPTB in comparison to healthy controls and other infectious diseases. Further, intersecting genes constitutively upregulated in each EPTB immunotype, identified a core signature confidently dissecting EPTB patients from healthy controls and other diseases. To further dissect the different severity levels, we developed a rank-based gene prioritization algorithm using an iterative gene-additive model, revealing additional intermediate and severe EPTB-specific signatures.

Conclusion: EPTB presents with highly heterogeneous disease manifestations. Multi-OMICS revealed distinct immunotypes of EPTB disease and our data resulted in a novel gene signatures characterizing the underlying immunotypes. Future work will assess the treatment responses of the EPTB immunotypes and the application of the EPTB-core signature as a point-of-care triage test. Deep immune characterization will be in the future used to tailor and discover novel host-directed therapies, which are highly needed because of the emergence of multidrug-resistant strains.

P-1-94

Transcriptomic changes associated with lung function impairment after successful tuberculosis treatment point towards altered immune response and tissue remodelling

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Many tuberculosis (TB) survivors experience lung function impairment as part of post-TB lung disease (PTLD), which involves persistent respiratory issues after treatment. The pathological mechanisms behind this sustained impairment after successful TB treatment remain unclear.

The TB Sequel cohort, conducted in 4 African countries is one of the largest studies assessing PTLD. It investigates long-term lung function as one of its main objectives. As a sub study, RNA sequencing was performed on whole blood

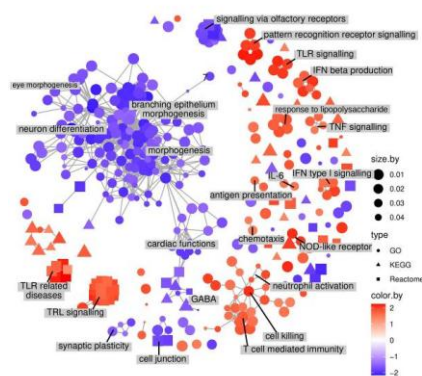
samples from 353 patients at three time points: treatment initiation, month 2 and month 6 (end of treatment, EOT). Differential gene expression using peripheral blood was performed, comparing the transcriptomic profiles of patients with most severe lung impairment vs. those with no impairment after treatment, categorised based on spirometry at EOT. Differentially expressed genes were aggregated using gene set enrichment analysis (GSEA) to understand the processes leading to different outcomes in TB survivors. Patients with moderate impairment were analyzed subsequently.

Our study revealed the highest number of differentially expressed genes at month 2 (DEGs, N = 1171). GSEA identified two clusters of biological processes in patients with severe lung impairment: tissue morphogenesis and differentiation, which were downregulated, and immune related processes, including neutrophil activation, T cell signalling, Toll-Like receptor, interferon, and TNF signalling, which were upregulated compared to those without impairment. At treatment initiation and EOT, the number of DEGs was considerably smaller (N_{INIT} = 96 and N_{EOT} = 92), the differential processes were primarily immune-related and upregulated in patients with lung impairment. The patients with moderate lung impairment showed intermediate activation levels, but high variance between the patients.

This study sheds light on processes leading to greater lung damage following TB in some individuals, highlighting both known immune pathways and previously unimplicated ones like olfactory receptor signaling (expressed by macrophages), cardiac and neurological processes. Understanding these mechanisms will open new routes for PTLD prevention and treatment.

Figure legend: Figure show networks of similar gene sets that are reported as significantly enriched among DEGs at month 2 between patients without and with severe lung function impairment; nodes represent gene sets (GS) from different sources, edges connect GSs that share > 50% of DEGs; NES – normalized enrichment score

Fig. 1



P-1-95
Comparative Study of Female Genital Schistosomiasis (FGS) and the Impact of Mass Drug Administration in Zanzibar and Madagascar: Unravelling the Disconnect Between Schistosomiasis and FGS Prevalence

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Background: Female genital schistosomiasis (FGS) is a chronic inflammatory disease caused by eggs of the parasite *Schistosoma haematobium* (*Sh*) in the female genital tract, affecting an estimated 40 to 56 million women worldwide. FGS causes gynaecological complications such as pain, abnormal bleeding, infertility, ectopic pregnancies, miscarriages, and increased susceptibility to HIV and HPV, the latter raising cervical cancer risk. These outcomes often lead to social stigmatization, discrimination, and mental health problems in women. The clinical inhomogeneity of FGS makes diagnosis difficult, especially in resource-limited areas. Regular mass drug administration (MDA) with praziquantel is the primary strategy for reducing the prevalence of schistosomiasis, and it is expected to reduce the severity and prevalence of FGS.

Question: This study examines the impact of targeted interventions on FGS in two endemic settings: Madagascar's Boeny region, with >50% schistosomiasis prevalence and irregular MDA mostly for school-aged children, and Pemba Island, Zanzibar, where sustained MDA campaigns reduced prevalence from >70% in the 1990s to <5%. It aims to assess MDA's effect on FGS, identify factors influencing its onset and progression, and map residual cases relative to schistosomiasis prevalence.

Methods: We recruited 634 female participants aged 18–49 years: 500 from three primary health centres in Boeny (March–August 2021) and 134 from one centre on Pemba (recruitment ongoing since April 2025). We collected and analysed colposcopy, clinical, demographic, and parasitological data. Colposcopy images were reviewed by expert-trained gynaecologists, and *Sh* infection was diagnosed using UCP-LF-CAA assays, with urine filtration microscopy additionally conducted in Zanzibar.

Results: So far, our preliminary results show no *Sh* infections detected on Pemba (n = 134, 0.0%, 95% CI 0.0–2.7), but a high prevalence in Boeny (n = 413, 62.4%, 95% CI 57.5–67.1). Nevertheless, the prevalence of FGS appears similar in both regions: 51.1% (95% CI 42.3–59.9) in Zanzibar and 56.1% (95% CI 51.0–61.1) in Madagascar. In Madagascar, 17.4% (95% CI 13.8–21.8) of participants were negative by both methods, 26.4% were positive only by UCP-LF-CAA, 20.1% only by colposcopy, and 36.1% by both.

Conclusion: The persistently high FGS prevalence in Zanzibar despite low *Sh* rates might indicate that FGS as a chronic condition, with pelvic mucosal changes taking years to resolve and explaining the discordance with *Sh* prevalence on Pemba. Ongoing analyses will assess whether MDA-driven reductions in schistosomiasis have improved FGS severity and progression and will examine the role of individual MDA history. These findings, in line with the conference theme on gender and precision medicine, highlight that infection-focused interventions, while reducing active infection, fail to address the persistent chronic sequelae of *Sh* in women.

P-1-96

HDV EVs facilitate infection and viral genome replication in vivo

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Background: Extracellular vesicles (EVs) have been shown to play a role in a myriad of hepatotropic viral infections, including Hepatitis D infections (Chu et al. 2024). In particular, Hepatitis D virus (HDV) EVs have been shown to contain HDV RNA (Cuhna et al. 2021) and can induce an immune response in non-permissive cells (Jung et al. 2020). However, the exact role of HDV EVs during the initial cell entry and infection progression remains unclear.

Methods: Human hepatocytic cell lines in cell culture were transfected with HDV plasmid and infected with EVs derived from HDV producing cell lines. Replication levels were measured via qPCR.

Results: Cells transfected with HDV plasmid showed an increase in HDV genomes 7 days post transfection. HDV EVs were also able to facilitate HDV genome entry into cells, and replication thereof.

Discussion: HDV EVs can infect human cells in vitro, and lead to viral replication in cell culture. They may play a role in the initial HDV entry and in the progression of the infection. The exact method of transmission remains to be investigated further.

P-1-97

Accelerated SARS-CoV-2 Clearance in Unvaccinated HLA-B*07:02+ Individuals Targeting a Highly Conserved Nucleocapsid CD8+ T Cell Epitope Shared Among Betacoronaviruses

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Viral clearance rates in acute SARS-CoV-2 infection vary widely among individuals, reflecting differences in both innate and adaptive immune responses. Human leukocyte antigen (HLA) genotype is a major determinant of epitope presentation to CD8+ T cells, yet its impact on viral kinetics in infection-naïve, unvaccinated individuals remains incompletely understood. HLA-B*07:02 presents a highly conserved SARS-CoV-2 epitope from the Nucleocapsid (NC) region, N₁₀₅₋₁₁₃ (SPRWYFYLY), which shares near-identical sequences with multiple human and zoonotic betacoronaviruses. This high conservation enables potential cross-reactivity of CD8+ T cell responses across divergent coronaviruses, raising the question of whether such responses contribute to more rapid viral control. The NC protein has been identified as a major target of early and persistent CD8+ T cell responses in COVID-19 (Eser et al., *Nature Communications*, 2023), making it a strong candidate for cross-reactive immunity.

We analysed previously infection-naïve, unvaccinated adults (n=25) with PCR-confirmed SARS-CoV-2 infection, recruited between May and December 2020, HLA-typed at class I loci. Eleven individuals were excluded due to absence of relevant HLA allotypes or insufficient PBMCs, leaving n=14 (n=7

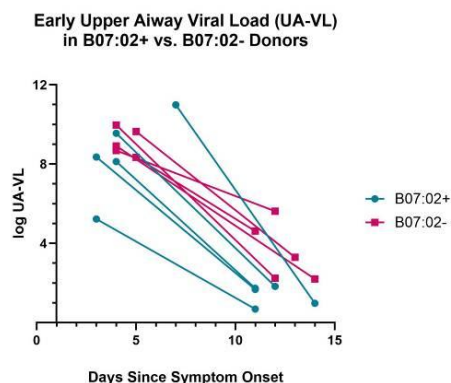
HLA-B*07:02+; n=7 HLA-B*07:02-). Upper airway viral loads (UA-VL) were quantified from nasopharyngeal swabs collected at multiple timepoints. SARS-CoV-2-specific CD8+ T cell responses were mapped by IFN- γ ELISPOT using 197 peptides spanning the proteome, including NC=14, Spike=34, Membrane=19, nsp3=49, RTC*=45, nsp13=15, and ORF3a=21. The 37 most immunogenic peptides were aligned in MEGA-X to identify homologues in Human Coronavirus (HCov) 229E, NL63, OC43 and HKU1, MERS-CoV, SARS-CoV-1, and Bat Hp-betacoronavirus Zhejiang2013 (Bat CoV Zh2013). Homologues corresponding to relevant HLA restrictions were tested in parallel with their SARS-CoV-2 counterparts.

HLA-B*07:02+ donors exhibited lower UA-VL when compared with HLA-B*07:02- donors as early as week 2 (W2) after symptom onset. The proportion of week 1 (W1) viral load remaining at W2 was compared between HLA-B07:02+ and HLA-B07:02- donors using the Mann-Whitney U test (p=0.0079) and further evaluated with ordinary least squares regression adjusting for baseline UA-VL (OLS β = -25.24, p=0.0298). After adjusting for baseline, HLA-B07:02+ donors had a markedly lower proportion of UA-VL remaining at W2 compared with HLA-B07:02- donors (partial Spearman ρ = -0.92, p=0.00016), consistent with faster viral clearance in the B07:02+ group.

HLA-B*07:02+ participants mounted robust CD8+ T cell responses to N₁₀₅₋₁₁₃. Individuals recognizing this epitope also exhibited high-frequency responses to homologous sequences from MERS-CoV, Bat CoV Zh2013, and HCoV-OC43, indicating the capacity for broad cross-reactivity across divergent betacoronaviruses.

*Replication-Transcription Complex=nsp7, nsp8, nsp12 (pooled)

Fig. 1



P-1-98

Targeting the pUL11 glycoprotein to restore T cell control of HCMV

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Human Cytomegalovirus (HCMV) can modulate the host's immune system in many complex ways. Infected cells are manipulated so that they avoid immune surveillance and, by less well studied mechanisms, uninfected immune cells are suppressed by the virus. The HCMV glycoprotein pUL11 is expressed on the surface of infected cells, where it suppresses both CD4 and CD8 T cell cytokine secretion and cytotoxic responses. pUL11 interacts with the tyrosine phosphatase CD45 and, notably, the presence of pUL11 in

HCMV infected cells correlates with decreased CD45 activity in both CD4 and CD8 T cells in co-culture experiments.

We have made use of a murine monoclonal antibody targeting pUL11 and shown that it significantly enhances T cell-mediated control of viral spread. By blocking the interaction between pUL11 and CD45, this antibody restores CD4 and CD8 T cell functionality, leading to improved inflammatory cytokine secretion and cytotoxicity against infected cells in our co-culture system. Using this antibody, we can also improve control of HCMV infection by T cell preparations used for adoptive therapy and also by T cells derived from HSCT patients within the first 100 days after transplantation.

To understand the mechanism by which pUL11 inhibits CD45, we have generated a model of the two proteins interacting. This will also inform our future plans to develop a humanised monoclonal antibody targeting pUL11.

While HCMV infections are generally asymptomatic in healthy individuals, they pose a serious risk to immunocompromised patients, such as hematopoietic stem cell transplant (HSCT) recipients, where functional T cells are critical for controlling viral spread. Our findings suggest

That disrupting the interaction between pUL11 and CD45 using a monoclonal antibody could provide a novel therapeutic strategy for immunosuppressed individuals with active HCMV infections.

P-1-99

Evaluating the Diagnostic Accuracy of the TAM-TB Assay for Paediatric Tuberculosis in five low-income and middle-income countries: a secondary analysis of the RaPaed-TB study

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Diagnosing paediatric tuberculosis (TB) remains challenging due to its paucibacillary nature and the limited sensitivity of sputum-based diagnostics. The T-cell activation marker (TAM)-TB assay, a sputum-independent blood-based test, detects *Mycobacterium tuberculosis* (Mtb)-specific T-cell activation via flow cytometry and has shown promise in TB diagnosis in children.

As part of the prospective, multi-country RaPaed-TB study conducted across five low- and middle-income countries, we evaluated a simplified TAM-TB assay based on CD38 expression on IFN γ ⁺CD4⁺ T-cells following ESAT6/CFP10 stimulation in children under 15 years of age examined for TB. Children were classified using NIH-adapted guidelines as confirmed TB, unconfirmed TB, or unlikely TB. Diagnostic accuracy was assessed using a strict reference standard (SRS: culture confirmed vs. unlikely TB) and a microbiological reference standard (MRS: Ultra or culture confirmed vs. unlikely TB).

TAM-TB was conducted on whole blood samples from the enrolled cohort (n=975) at baseline and longitudinally for up to six months post-treatment initiation. At baseline, 74.5% (726/975) TAM-TBs had available and valid results. Of those, 26.3% (191/726) had confirmed TB, 30.0% (218/726) had unconfirmed TB, 32.4% (235/726) were unlikely TB and 11.3% (82/726) were unclassifiable. Using SRS, the assay had a sensitivity of 63.7% (CI: 54.1–72.4) and a specificity of 86.8% (CI: 81.7–90.5). Sensitivity declined to 50.3% (CI: 41.2–55.9) when using MRS. Assay specificity was lower than anticipated, leading to further characterization of TAM-TB "false-positives". In the unlikely TB group (n=235), 30 children (12.8%) had positive TAM-TB results, potentially hinting towards concurrent Mtb infection. Most of these children had immunological and epidemiological evidence of Mtb exposure: 90.0% (27/30) had a positive tuberculin skin test (TST) and 93.3% (28/30) reported recent TB contact, primarily within the household. These children exhibited a significant reduction in CD38 expression (median 59.4% to 37.5%, p<0.002) by one month post-enrolment. This pattern mirrored the decline observed after 1 month of treatment in confirmed TB+ cases undergoing treatment, suggesting spontaneous immune-mediated self-control of active Mtb infection.

These findings support a high specificity of the TAM-TB assay and highlight its potential to detect microbiologically confirmed TB cases as well as concurrent Mtb infection. Further, it can reveal a subgroup of untreated children with signs of controlled active infection. However, the assay's technical complexity represented by the proportion of invalid results underscore the need for further refinement to enhance robustness and scalability for widespread use in paediatric TB diagnosis and monitoring.

P-1-100

Acute Hookworm Infection in naïve Europeans vs exposed Africans using a controlled human hookworm infection model

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Background: Hookworm are soil-transmitted helminths (STH) estimated to infect 230 million people worldwide, predominantly in the tropics. The highest prevalence of Hookworm is in sub-Saharan Africa, where *Na* is endemic; whilst it is relatively rare in the global north. The impact of previous exposure means that there are varying levels of exposure and immunity, which result in differing immunological observations in the global north vs the global south. Controlled Human Hookworm Infection (CHHI) allows for a detailed examination of differing responses to acute infection as determined by previous exposure to hookworm.

Methods: We enrolled 27 participants, 15 in Gabon and 12 in the Netherlands. Participants were challenged with 50 hookworm larvae. The Gabon group included participants in intervention and placebo groups, assigned in a 2:1 ratio. All participants in the Dutch group received the intervention. Participants were treated at week 16 of follow-up. Primary endpoints for our study analysis were (1) the occurrence of adverse events in the study groups, and (2) burden of infection in stool samples- median egg counts until 16 weeks post-challenge, and time to infection by Kato-Katz and PCR. Blood was collected for safety evaluations of blood count and differential, and additional peripheral blood mononuclear cells (PBMCs), serum, and plasma for immunological investigations.

Results: Participants in the Dutch cohort were younger than participants in the Gabonese cohort (28.5 [22.5-32.2] vs 34 [28.0-39.0] years; $p < 0.001$). BMI was similar between the groups (25.0 [21.8-32.0] vs 24.0 [22.0-26.0]). Rash and itching at inoculation site, and abdominal pain are significantly associated with acute hookworm infection. Asides from early time points at weeks 6 ($p < 0.01$) and 7 ($p < 0.05$), egg counts were similar between the cohorts, although egg counts were higher in the Dutch group. Egg detection by PCR were significantly different at weeks 6 ($p < 0.01$), 9 ($p < 0.05$), and 11 ($p < 0.05$). When comparing blood differential counts, the eosinophilic and leukocytic response to CHHI in the Dutch group was significantly different ($p < 0.01$) from the Gabon groups; from weeks 5-10 and weeks 4-14 after challenge, respectively. When comparing log difference in eosinophilia from baseline, there was no difference between the intervention groups, but significant difference with placebo. Immunologic response analyses are ongoing and will be presented.

Conclusion: Acute hookworm infection elicits differential responses in exposed vs non-exposed individuals that is observable both clinically and immunologically. These differing responses require further elucidation, as they might be consequential in the development of hookworm drugs and vaccines for use in hookworm endemic areas.

Fig. 1

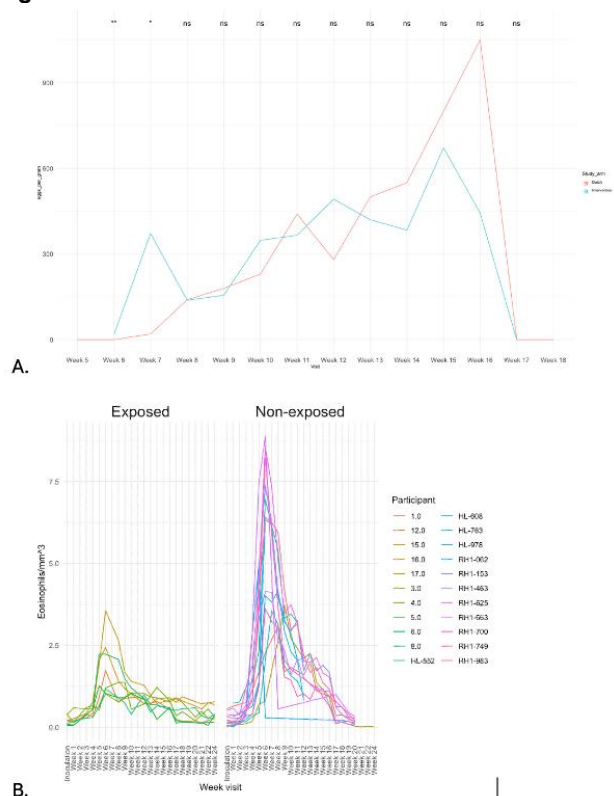
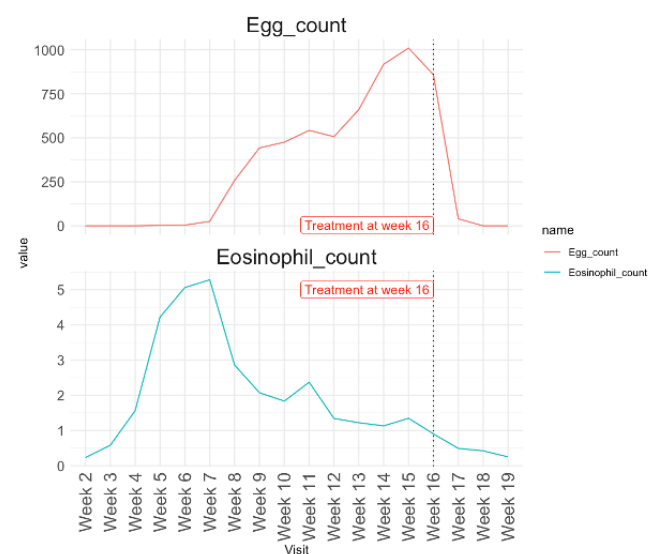


Fig. 2



P-1-101
Incidence and infection dynamics of the human papilloma virus in a 4-year cohort in rural Madagascar- A longitudinal study

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Question: Human papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide, though 90 percent of infections are self-cleared, high-risk (HR) carcinogenic types were responsible for 660,000 cases of cervical cancer (CC) in 2022. Vaccines preventing infections with HPV have been introduced since 2006, and have been shown to reduce CC incidence following high vaccination coverage. Madagascar is among the countries with the most delayed implementation of an HPV vaccination schedule. Further, there is scarce data on both the frequency of HPV infection and the dynamics of clearance and reinfection. This study aims to estimate the incidence and clearance of HPV to support and inform the implementation of vaccination programmes in the country. Additionally, this study will contribute to bring supporting information on the viral dynamics of HPV and the risk factors for persistence of high-risk infections.

Methods: This longitudinal study in the region of Boeny, Madagascar was implemented in three primary health care centers over four timepoints (TP) from March 2021 to March 2025. A Luminex bead-based PCR assay is performed on cervical-vaginal lavage specimens for HPV typing of 19 HR and 2 low risk subtypes. Statistical analysis in R will include descriptive statistics, measures of incidence and rate of clearance of infection. Regression analysis will be utilized to determine risk factors for persistence of HPV infection.

Results: Preliminary analysis of attendance showed 1906 person visits over all TPs, with 64 women attending all four TPs. First longitudinal results of TP1 and TP2 show that common HR- HPV types at TP1 (n= 481) were HPV45 (11% of women) and HPV52 (7.9%), while at TP2 (n=557), HPV52 (8.4%) and HPV68 (6.3%) were predominant. Among women joining both T1 and T2 (n=110), 12.7% cleared all existing infections, 17.3% acquired new infections and 31.8% of cases remained positive for any HPV subtype. The highest rates of HR-HPV one-year persistence were seen in HPV52 with 71.4% of infections persisting and HPV68 with 57.1% persisting.

Conclusion: Preliminary results show that different high-risk strains of HPV circulate in Madagascar with more new infections than clearances after one year, 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 programmes preventing infection with HPV as well as CC onset and progression in Madagascar.

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Community-based assessment of female genital schistosomiasis (FGS) in Gabon: prevalence, risk factors, and awareness

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Background: Female Genital Schistosomiasis (FGS), caused by *Schistosoma haematobium*, is a neglected tropical disease contributing to substantial reproductive morbidity, including infertility, cervical cancer, ectopic pregnancy, and increased HIV risk. Despite the high burden, FGS remains underdiagnosed and undertreated due to limited awareness, diagnostic tools, and resources. In Lambaréné, Gabon, urogenital schistosomiasis prevalence reaches 58%, with secondary infertility rates particularly high in rural areas where healthcare access is limited. However, no data exist on FGS prevalence, associated risk factors, or community awareness in this setting.

Methods: We are conducting an observational, prospective longitudinal study to assess the prevalence of FGS, its association with cervical dysplasia, and community knowledge, attitudes, and practices (KAP) regarding FGS. Sexually active women aged 15–50 years were recruited for *S. haematobium* using urine filtration. Among them, *S. haematobium*-positive women matched controls undergoes a gynaecological examination including colposcopy. Samples collection such as cervical biopsy, vaginal swab, and cervicovaginal lavage was done for FGS diagnostic. A KAP survey is administered to explore awareness of FGS symptoms, consequences, and prevention.

Results: The study will provide the first data on FGS prevalence and its relationship with cervical dysplasia in Gabon. Results of the KAP survey identifying knowledge gaps and misconceptions, and will be presented to highlight areas for targeted education and intervention.

Conclusions: Findings from this study will inform public health strategies to improve FGS diagnosis, management, and prevention. The integration of FGS screening into reproductive health services, along with enhanced community education, could reduce the burden of FGS and improve women's health outcomes in endemic areas.

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Use Case for Joining Routine Care and Study Data for Better Insights in Transplantation

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Integrating routine hospital data with research data holds significant potential to reduce study duration and costs while promoting secondary use of valuable study datasets. Routine hospital data reflect real-world patient care and can enrich study datasets with high-resolution, longitudinal information that is costly to capture in structured research documentation. Integrating both data sources enables cost reduction, helps to answer new clinical questions by data reuse, improve risk prediction, and support personalized treatment strategies.

Within this project we aim to demonstrate the feasibility and added value of combining routine hospital and research data by selecting a suitable study from the German Center for Infection Research (DZIF). The DZIF Transplant Cohort (TX-Cohort), a multicenter prospective cohort with nearly 3,000 transplant recipients across five German sites, was selected due to the available consent for secondary use of routine hospital data. In addition to setting an example of data integration, our aim is to evaluate whether patient-specific variables, such as demographics, transplantation details, longitudinal laboratory values, vital signs, and clinical procedures, can predict the risk of opportunistic infections (OIs) in the first 12 months post-transplantation.

Transplant recipients are particularly susceptible to OIs due to immunosuppressive therapy. Despite prophylactic interventions (e.g., valganciclovir for CMV), infection-related morbidity and mortality remain high. Prior research has indicated potential predictive value of biomarkers such as lymphocyte counts, yet large-scale, high-resolution, longitudinal analyses are scarce. Machine learning models have shown promise in infection prediction, particularly for sepsis and CMV infection, but are likely underutilized for broader OI risk in transplant patients.

For each TX-Cohort site, we completed the time-consuming process of presenting our study aim to site managers, ethics committees, and data integration centers. We received the ethics committee approvals. As soon as the approvals from the data integration centers are available, we will link routine hospital data locally in a privacy-preserving manner. The analysis will assess time-to-event and infection incidence by transplant type, as well as dynamic interactions between clinical markers and infection outcomes. Specific focus will be placed on routinely documented longitudinal parameters such as heart rate, temperature, blood pressure, laboratory markers, and administered medication.

This project will add evidence to the potential predictive value of diverse clinical markers for OI and serve as a first model showing the complexity of the data integration process in infectious disease research. It aims to improve clinical decision-making, support data reuse, and reduce patient burden in future studies, while contributing to the standardization and optimization of care processes in transplantation medicine.

P-1-104

On-site nanopore sequencing of microbial cell-free DNA for detection and monitoring of persistent pathogens in infective endocarditis

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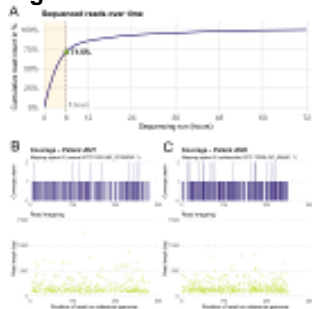
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Question: Infective endocarditis remains challenging to diagnose and monitor, particularly when blood cultures turn negative after antibiotic initiation. We evaluated whether on-site nanopore sequencing of microbial cell-free DNA (mcfDNA) can detect and monitor the disease-causing pathogen in this setting. **Methods:** An open-source multiplex Oxford Nanopore workflow for mcfDNA sequencing from plasma was first validated in spiking experiments using six bacterial species representing both Gram-positive and Gram-negative pathogens, achieving a detection limit of 0.03 ng/mL. In the second step, the protocol was tested in a clinical setting on a small cohort of patients with various infectious diseases to assess feasibility under real-world conditions, detecting, among others, *E. faecalis*, *S. pneumoniae*, *E. coli*, *K. oxytoca*, and *E. hormaechei*. Finally, the workflow was applied in a prospective feasibility study at Jena University Hospital in nine patients with infective endocarditis, one of whom also had concomitant spondylodiscitis. In these patients, plasma samples were collected alongside concurrent blood cultures, with additional longitudinal follow-up sampling during antibiotic therapy in some patients to monitor pathogen-specific mcfDNA dynamics. Quality control included a no-template control per run, a two-tube collection strategy to mitigate skin-flora contamination, and mapping-based validation to confirm even genome-wide coverage for candidate taxa.

Results: McfDNA was detected in all nine patients (8× *S. aureus*, 1× *S. epidermidis*), including two culture-negative cases (both *S. aureus*). Sequencing results matched conventional microbiology at genus level in all culture-positive cases. In selected patients, mcfDNA persisted for up to 16 days after targeted antibiotic therapy, declining to baseline only after surgical source control. Pathogens were reliably identified within ~6 hours of sequencing start. Uniform genome-wide coverage enabled confident species-level assignments. In the non-IE pilot cohort, mcfDNA correctly identified Gram-negative pathogens (e.g., *E. coli*, *K. oxytoca*, *E. hormaechei*) and Gram-positive species (*E. faecalis*, *S. pneumoniae*), demonstrating performance beyond staphylococci.

Conclusions: On-site mcfDNA sequencing delivers same-day (≤12 h), culture-independent identification and real-time monitoring of infective endocarditis, including culture-negative cases. Across nine patients it consistently detected *S. aureus* and *S. epidermidis*; in deep-seated infections, mcfDNA persisted under antibiotics and cleared only after source control, supporting its use as a practical liquid biopsy to guide antibiotic duration and timing of surgical intervention. A prospective validation study is planned to evaluate diagnostic accuracy and impact on clinical decision-making.

Fig. 1**P-1-105****Standard microbiological diagnostic results in bile reflect relevant structures of the biliary microbial community**

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Introduction and Aim: In patients suffering from PSC, the biliary microbial community is known to correlate with the course of disease. A predominance of streptococcus and staphylococcus spp. within the 16S profile (relative abundance > 33%) was associated with the need for liver transplantation, the development of a CCA and/or death. Communities with a predominating bacterial genus were termed Bilotype (BT). Staphylococcus BT and Streptococcus BT were associated to a poor prognosis, Enterobacteriaceae BT, Enterococcus BT and other BT to a good to intermediate prognosis. So far, standard microbiological cultivation method (SMC) has been shown to be insufficient in detecting main members of the biliary microbiota reliably. However, since SMC is available widely and cost efficient, we want to analyze, whether SMC is sufficient in order to draw conclusions on those relevant Bilotypes and to facilitate bedside diagnostics.

Methods: Clinical data and SMC results of bile samples of patients (patients n = 430, samples n = 732) with PSC, SSC, CCA, HCC and other malignant tumors, benign stenosis, gall stones and other diseases of the liver were analyzed retrospectively. Bile was collected between 2008 and 2015 via ERC and has been analyzed by NGS (n = 732) and by SMC (n = 351).

Results: In samples with Enterococcus BT, Enterococcus spp. are found in the SMC in 94% of cases. The samples with Streptococcus BT, Enterobacteriaceae BT and Other BT featured a lower correlation to its corresponding spp. in SMC (76%; 73%; 64%) with the lowest result being in the Staphylococcus BT (62%). Overall, the detection rate for the BT defining genera in SMC is low except for Enterococcus BT. In the next step, we assessed the potential of the SMC findings for Bilotype detection. BT detection rates based on SMC findings were very low. Samples containing Enterococcus spp. in SMC exhibited Enterococcus BT in 39% of cases. Samples in which SMC detected Enterobacteriaceae spp. showed an Enterobacteriaceae BT in 32% of cases, while Streptococcus spp. were associated with a Streptococcus BT in 31% of cases. The lowest

concordance was observed for Staphylococcus spp., with a Staphylococcus BT present in only 15% of cases. However, when focusing on BTs indicating a favorable prognosis, samples in which SMC found Enterococcus spp. and/or Enterobacteriaceae spp. were associated with such a BT (Enterococcus spp: 98%, Enterobacteriaceae spp: 90%).

Conclusion: Our results show a correlation between Enterococcus spp. and Enterobacteriaceae spp. in SMC results with BTs indicating a favorable prognosis. Thus, SMC results may serve as additional information for the estimation of disease progression in clinical settings. Still, we need further examination through prospective studies for validation.

P-1-106**Course and clinical outcomes of chronic hepatitis delta: A longitudinal analysis of 565 patients from the D-Solve and HDV-1000 consortia**

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Background and aim: Chronic hepatitis Delta (CHD) is considered the most severe chronic viral hepatitis. However, some studies challenged this accelerated course and the predictors of CHD progression are scarcely defined. This study aims to examine the clinical course and outcomes of CHD patients in a large real-life cohort.

Materials and Methods: CHD patients with ≥3 years follow-up from the multicenter retrospective D-SOLVE and HDV-1000 database (6 European centers) were enrolled. Longitudinal changes in biochemical and laboratory markers were analyzed. Time-to-event analysis was performed, and predictors of liver-related events (LREs) were assessed with univariable and multivariable Cox regression analysis.

Results: Among a total of 1,004 patients, 565 (56%) with ≥3 years follow-up were included. Patients had a mean (SD) age of 45 (12) years, 55% men, 60% of European origin. During a median (IQR) follow-up of 55 (46-62) months, 48 patients progressed to cirrhosis at 1-, 3- and 5-year cumulative incidence of 1.8%, 5.6% and 13.6%, respectively. De-novo liver-related events (LREs) occurred in 47 (9%) patients at 1-, 3-, and 5-year cumulative incidence of 0.8%, 2.4% and 10.8%, respectively. Cox regression analysis showed that anti-HCV+ (aHR=1.72, 1.22-5.88) and elevated GGT (aHR=2.77, 1.22-6.27) at baseline significantly

associated with cirrhosis onset, while older age (aHR=1.03, 1.00-1.07), elevated GGT (aHR=4.38, 1.81-10.57), detectable HDV RNA (aHR=10.32, 1.34-79.53) and cirrhosis diagnosis (aHR=2.23, 1.03-4.84) correlated with LREs. The risk for LREs increased from HDV RNA ≥ 1000 IU/mL, while HBsAg levels did not correlate with disease progression.

Conclusions: In a large real-life cohort of CHD patients, older age, GGT elevation, cirrhosis and detectable HDV RNA were the main determinants of liver-related outcomes, with worse prognosis noted from HDV RNA ≥ 1000 IU/mL.

P-1-107 Region-Specific Spatial Transcriptomics Reveal Distinct Immunological Functions in Human Tuberculosis Granulomas

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A detailed understanding of host-pathogen interactions is essential to develop innovative strategies against multi-drug-resistant tuberculosis (MDR-TB). To investigate pathogenic mechanisms at the site of infection, we assembled a cohort of partial lung resections from patients with drug-susceptible, MDR, and XDR tuberculosis. Lung tissue was stratified based on pathological patterns—such as unaffected areas, diffuse inflammation, cellular granulomas, and necrotic granulomas—and tissue microarrays were constructed to integrate multiple patient samples and lesion types within single paraffin blocks. Using multispectral imaging, we identified key immunological regions at the protein level, including multinucleated giant cell areas, T cell- and macrophage-rich zones, early and late-stage granulomas, and tertiary lymphoid structures. Spatial transcriptomics was then employed to obtain region-specific gene expression profiles, which were correlated with distinct immunological functions. For example, macrophage-rich regions displayed both type I and type II interferon responses, while giant cell areas showed gene signatures related to pH regulation and acidification. The outer rim of granulomas—enriched in T and B cells—were characterized by growth factor signaling and complement activation pathways. These findings reveal distinct molecular signatures within defined morphological niches that are associated with either protective or pathological outcomes. Our results shed light on the immunometabolic landscape of pulmonary TB lesions and may inform the development of targeted, host-directed therapies.

P-1-108 Organoids as experimental models for highly pathogenic viruses

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The emergence of novel highly pathogenic viruses (HIPAV) has a large impact on regional and global health and economics. In order to get more insight into infection dynamics and pathogenesis, suitable models are needed.

Usually, mouse models are commonly utilized to study such mechanisms however laboratory mice are resistant to infection with many HIPAV including Ebola virus (EBOV), Lassa virus (LASV) and Nipah virus (NiV). To fill this gap, we have established human brain and lung organoids to study HIPAV cell tropism, innate immune responses and persistence. Brain and lung organoids were generated from induced pluripotent stem cells (iPSCs) and cultured for 100 or 70 days, respectively. Organoids were characterized via immunofluorescence (brain and lung) and flow cytometry (lung) using organ-specific antibodies. Both organoids consist of a variety of organ-specific cell types for example neurons, microglia and astrocytes in brain organoids and basal, ciliated and goblet cells in lung organoids. We could recapitulate EBOV persistence in brain organoids for 120 days and explored the kinetics of NiV infection in human lung organoids.

Our results suggest that organoids may provide important insight into infection dynamics and persistence and may facilitate the identification of primary target cells and provide a platform for the evaluation of antiviral strategies.

P-1-109 Liver chimeric humanized mice with an enriched amount of HDV-mono-infected hepatocytes are more susceptible to therapeutic interference with pegIFN α and Bulevirtide

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Background and aim: HDV relies on concurrent HBV infection for its viral envelope, but HDV replication occurs independently of HBV. PegIFN α is often used to treat CHD patients, and studies in immunodeficient humanized mice have demonstrated the ability of pegIFN α to suppress patient-derived HDV strains (Giersch et al., JHEPRep 2023). Analysis of paired liver biopsies from three clinical trials investigating the efficacy of Bulevirtide (BLV) demonstrated a significant reduction of HDV-infected hepatocytes and innate immunity-related gene expression (ISGs) (Allweiss et al., JHEP 2024). Surprisingly, intrahepatic analysis revealed large populations of cells mutually exclusive for HBV and HDV infection in patients. Understanding of the dynamics of HBV/HDV interplay and antiviral effects of pegIFN α and BLV on the different cell populations in this diverse intrahepatic environment remains limited. Therefore, we aimed to establish a model with high ratios of HDV-mono-infected hepatocytes to investigate the impact of pegIFN α and BLV on HDV mono-infected versus HBV/HDV co-infected cells.

Methods: Immunodeficient humanized mice co-infected with HBV and HDV were treated with lamivudine (LAM) in the viral spreading phase to yield a large population of HDV mono-infected hepatocytes. Mice then received either (a) pegIFN α or (b) BLV for 8 weeks near the peak of HDV viremia. Serological and intrahepatic changes were evaluated by qPCR, ELISA, and immunofluorescence.

Results: LAM treatment initiated during the spreading phase of both viruses reduced HBV DNA in the serum ($\Delta 1.2 \log_{10}$ in 4w) and slowed down the intrahepatic spread of HBV but had no effect on the ramp-up phase of HDV viremia ($\Delta 2.9 \log_{10}$; median HDV RNA 9×10^6 copies/ml). IF imaging

showed strong HDV dissemination, resulting in large areas harboring mono-infected HDV+ cells. ALT levels transiently increased during HDV spread and strongly correlated with HDV RNA in serum ($r=0.8047$; $p<0.0001$). Adding pegIFN α reduced all HDV markers in serum and liver below the LLOQ by 8 weeks of treatment, while levels of intrahepatic HDV RNA did not decrease in animals not receiving pegIFN α . BLV-treatment of mice harboring a large population of HDV mono-infected hepatocytes (b) resulted in lower levels of intrahepatic HDV RNA ($-0.5\log_{10}$ compared to LAM and $-0.8\log_{10}$ vs. untreated, $p=0.0221$) at the end of treatment. BLV induced a more pronounced decrease in human ISGs compared to control mice receiving LAM (ISG15: $-1.2\log_{10}$, $p=0.0065$).

Conclusion: In mice with enriched numbers of HDV-mono-infected hepatocytes, few co-infected cells can sustain HBV-dependent spread of HDV. However, pegIFN α treatment profoundly impacted HDV, nearly clearing HDV infection. Bulevirtide-mediated blocking of new infections resulted in a more substantial decline of HDV intrahepatic RNA, compared to mice with mainly HBV/HDV co-infected cells (Volz et al., EASL 2023).

P-1-110

Exhaled Breath Sampling as a Patient-Friendly Alternative for Detecting *Pseudomonas aeruginosa* in Patients with Chronic Lung Diseases: A study protocol

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Background: Bacterial infections pose a significant burden for patients with chronic respiratory diseases. Early detection and targeted treatment are crucial for controlling infections and preventing further lung damage. However, many patients struggle to produce sputum for traditional culture, especially in the presence of thick mucus or unproductive cough. Exhaled breath aerosols (XBA) are considered a promising investigative material due to their simple, non-invasive sampling. While there is increasing evidence for the detection of respiratory infections using XBA, data on bacterial pathogens of the lower respiratory tract remain limited.

Pseudomonas aeruginosa is a common pathogen causing difficult-to-treat and recurrent infections in patients with chronic lung diseases such as cystic fibrosis and bronchiectasis. Within a clinical study we investigate whether the detection of *P. aeruginosa* in XBA represents a reliable and more patient-friendly alternative to conventional sputum sampling.

Objectives: To validate XBA sampling as a reliable diagnostic method by comparing *P. aeruginosa* detection via PCR in samples collected with two prototype devices, against standard sputum culture and eventually against Sputum PCR. Additionally, we will assess patient preferences and device usability.

Methods: A non-interventional, exploratory cross-sectional study is currently being conducted. The study will include approximately 30 adult patients with bronchiectasis and/or cystic fibrosis who provide sputum samples for diagnostic purposes during routine care at the Thorax Clinic of Heidelberg University Hospital. Each participant will provide one exhaled breath sample per prototype instrument. By the

end of the study, the collected XBA samples will be analyzed by PCR and will be compared to the results of sputum culture and, if available, an additional PCR analysis of the sputum will be conducted. Additionally, patients are asked to complete a short questionnaire on the usability of the prototype instruments. We will assess the accuracy of the prototypes via positive percent agreement and negative percent agreement between XBA samples collected with each instrument compared to sputum analysis. The results of the usability questionnaire will be reported in absolute numbers and percentages. Currently, 20 out of 30 participants have been recruited and the recruitment is expected to be finished by the end of the year.

Expected Outcomes: It is expected that exhaled breath aerosols will represent a non-invasive and reliable method for detecting *Pseudomonas aeruginosa* (Proof of Concept). In the long term, the insights gained could pave the way for more comprehensive studies to further validate XBA diagnostic methods. Additionally, valuable information on the usability of the two prototype devices could be obtained, supporting future product development.

Fig. 1



P-1-111

Targeting Intracellular Bacterial Reservoirs to Improve the Treatment of Urinary Tract Infections (UTIs)

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Background: Recurrent UTIs are driven by the ability of uropathogenic bacteria such as *Klebsiella pneumoniae* to invade bladder epithelial cells and persist within quiescent intracellular reservoirs (QIRs).¹ In this state, bacteria become tolerant to conventional antibiotics, promoting relapse after apparently successful treatment.² This challenge is aggravated by rising multidrug resistance, leaving few therapeutic options.³ While intracellular persistence is increasingly recognised as a key driver of chronic infections⁴, systematic approaches to identify compounds that eradicate these reservoirs are lacking. Targeting QIRs therefore represents a promising strategy to improve treatment, reduce recurrence, and mitigate resistance development.

Methods: We are establishing an automated high-throughput screening assay using a Hudson Robotics platform, which ensures high-throughput capacity, to identify compounds with intracellular activity against *K. pneumoniae* (RB120). Bladder epithelial cells (HTB-9) are infected with luminescent bacteria, followed by gentamicin to eliminate and suppress extracellular growth. Infected cells are then exposed to compound libraries, with an initial focus on the Enamine Diversity Set (~10,000 compounds, in collaboration

with Prof. Müller, HIPS Saarbrücken), with potential expansion to further synthetic and natural product libraries. Intracellular survival is quantified by luminescence after bacterial outgrowth, with hits defined as compounds achieving ≥ 1 log₁₀ reduction compared with controls. Cytotoxic compounds will be excluded early using CellTiter-Glo® viability assays. Confirmed hits will undergo CFU recovery, dose-response and time-kill studies, complemented by imaging, LAMP1 co-labelling, and bacterial stress reporters to characterise effects on pathogen-containing vacuoles and intracellular physiology.

Expected Results: We expect to identify compounds that directly kill intracellular bacteria or stimulate host defence pathways. Hits with selective, non-toxic activity will provide insight into persistence mechanisms and open new therapeutic avenues. Comparative evaluation with other intracellular pathogens may reveal broader applicability.

Conclusion: This project aims to establish a robust platform for systematic discovery of intracellularly active antimicrobials against *K. pneumoniae*. Beyond identifying candidates, the approach is designed to pioneer strategies that shorten therapy, reduce selective pressure, and slow resistance emergence. By addressing intracellular persistence as a critical but underexplored aspect of bacterial pathogenesis, the study aligns with DZIF priorities and contributes to sustainable therapies for recurrent UTIs.

References:

- 1 Mysorekar IU, Hultgren SJ. PNAS 2006. doi:10.1073/pnas.0602136103
- 2 Grant SS, Hung DT. Virulence 2013. doi:10.4161/viru.23987
- 3 WHO. GLASS Report. 2022
- 4 Ernst CM, et al. Nat Med 2020. doi:10.1038/s41591-020-0825-4

P-1-112

CD8⁺ T Cells in Urine are a Distinguishing Marker in Schistosomiasis

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Introduction: Schistosomiasis (Bilharzia) is a Neglected Tropical Disease caused by trematode parasites, with *Schistosoma haematobium* being a primary cause of urogenital Schistosomiasis in sub-Saharan Africa (SSA). After a prepatent period, paired adult worms produce eggs in the venous plexus of the bladder, which are shed in urine from infected humans. However, some eggs become trapped in host tissues, leading to granuloma formation and inflammation in urinary tract and renal damage.

Rationale: We aim to identify biomarkers in urine and blood which indicate disease progression as other easily assessable morbidity markers are not established.

Hypothesis/Methods: The underlying hypothesis is: Disease progression correlates with increased levels of immune cells, Tubular Epithelial Cells (TECs), and antibodies in urine or blood, which are expected to decline following effective treatment.

Here, we are going to investigate total counts of immune cells and TECs in urine via flow cytometry as well as hematological and biochemistry markers in blood samples in negative controls (n=20), recently infected returning travelers (RT) (n=22), and *S. haematobium*-infected (n= 9) and uninfected participants (n= 4) from an endemic region (EP).

Results: Our preliminary results revealed that CD8⁺ T cells in urine were exclusively detected in the EP group, especially in *S. haematobium*-infected EP (median: 96; IQR: 40-286.5), while they were nearly absent in negative controls (0.5; 0-2.8), recently infected RT (0; 0-2), and uninfected EP participants (3.5; 0-424).

Conclusion: This finding shows that CD8⁺ T cells in urine are a distinguishing biomarker between recently infected RT and EP participants.

P-1-113

Klebsiella pneumoniae brain abscess caused by evolution of heterovirulence in the urinary tract

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Persistent bacterial infections provide an opportunity for bacterial pathogens to evolve antibiotic resistance and virulence in patients with simple adaptive mutations. In the case of antibiotic resistance, adaptive evolution often leads to antibiotic resistant subpopulations that are maintained and difficult to detect in the clinic. While within-patient evolution of antibiotic resistance has been intensely studied, less is known about within-patient evolution of pathogenicity of bacterial pathogens, due to more complicated phenotypic readout, and the requirement of mechanistic investigations with *in vitro* and *in vivo* models of infection, and clinical associations, to establish an impact of adaptive mutations on clinically observed virulence.

However, we were recently able to identify globally occurring adaptive mutations in capsule biosynthesis genes that affected the virulence of carbapenem-resistant classical *K. pneumoniae* (cKp) (Ernst et al., Nat. Med. 2020). Gain of function mutations in the capsule biosynthesis gene *wzc* conferred a hypercapsule which promoted phagocytosis resistance and promoted dissemination in mice, which could also be seen in clinical associations with bloodstream infections in patients. We also detected subpopulations with hypercapsule mutations in 3 of 6 multidrug-resistant urine specimens.

Here we demonstrates a case of heterovirulence in a patient with a virulent clinical outcome which could be recapitulated with enhanced virulence in a mouse model of infection and a mechanistic investigation of adaptive mutations with isogenic mutants. We show how the stepwise acquisition of three non-synonymous mutations in capsule biosynthesis genes

led to the emergence of a highly virulent subpopulation in the bladder that disseminated to the brain of a patient. The study introduces the concept of heterovirulence, highlights the key role of *wzc* mutations in promoting hypercapsule-associated virulence and provides the missing link between the observation of our previously reported hypercapsule subpopulations in the bladder of patients and disseminated infection.

In analogy to heteroresistance, heterovirulence can be difficult to detect in diagnostics labs due to variable evolutionary adaptive trajectories, a potential fitness cost in the absence of selective immune pressures in the patient, and complicated phenotypic readout and interpretation due to a wide spectrum of potential virulence. Early detection of heterovirulence could lead to improved patient outcomes. For example, a more aggressive treatment of urinary tract infections or colonizations caused by heterovirulent *Klebsiella pneumoniae* populations with signature mutations in *wzc*, could prevent virulent trajectories and thus be effective against a wide spectrum of virulence enhancing mutations.

P-1-114

The hepatitis E virus ORF2 protein forms amyloid-like fibrils

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Hepatitis E virus (HEV) is the most common cause of viral hepatitis worldwide, affecting millions annually. Especially in immunocompromised patients, HEV infection can become chronic and the rate of extrahepatic manifestations - such as neurological disorders - increases. HEV genomic RNA has been detected in cerebrospinal fluid of chronic HEV patients and in brain tissues of HEV-infected animals.

We discovered that the ORF2 capsid protein contains an intrinsically disordered region (IDR) at its N-terminus using nuclear magnetic resonance (NMR), which is capable of inducing hexanediol-sensitive condensates by liquid-liquid phase separation (LLPS) in cells. We further confirmed droplet formation by recombinant IDR protein triggered by RNA using differential interference contrast microscopy, and this effect was correlated with increasing concentrations of both agents. By solid-state NMR we could further show that upon undergoing LLPS, the dense phase became more rigid upon aging. Using a novel full-length fluorescent HEV reporter, we performed correlative light-electron microscopy (CLEM) and electron tomography analysis of HEV-infected cells. These studies revealed that ORF2 protein matures into aggregates composed of orderly stacked tubular filaments with a periodicity of ~35 nm, reminiscent of amyloid fibrils. Ectopically expressed wild-type ORF2 formed the same structures, suggesting that it is the sole viral determinant responsible for these structures. We identified these filamentous structures in a range of HEV-infected cell types, including iPSC-derived hepatocytes and neurons. In HEV-infected neurons, we detected a significant reduction in number and length of dendrites. This phenotype was replicated by ectopic expression of wild-type ORF2, but not by an ORF2 mutant lacking the N-terminal IDR, which is essential for LLPS and fibril formation. Together, our findings uncover a novel structural and potentially pathogenic role for

the HEV capsid protein in neuronal cells, implicating it in HEV-associated neuropathogenesis and highlighting it as a promising target for therapeutic intervention.

P-1-116

Lymphogranuloma Venereum in a Patient on HIV Pre-Exposure Prophylaxis: Diagnostic Challenges

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Background: Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by invasive genotypes (L1–L3) of *Chlamydia trachomatis*. It is prevalent in several countries in Africa, Asia and South America and only occurs sporadically in Europe, mostly affecting men who have sex with men (MSM). LGV can present with a range of clinical manifestations, including inguinal lymphadenopathy, proctitis and genital ulcers.

Case Presentation: A 51-year-old man presented to the infectious disease outpatient clinic with a painful, swollen lymph node in the left inguinal region. He also reported pruritus of the penis without discharge approximately ten days before. He had been taking HIV pre-exposure prophylaxis (PrEP) with TDF/FTC 245/200mg for the past 16 months due to sexual risk exposure. He attends regular follow-up visits and was treated for rectal chlamydia and N. gonorrhoeae infection one year ago. Clinical examination showed a swollen lymph node of approximately 5 cm on the left side. Swabs were taken from urethra and rectum, and a multiplex PCR for sexually transmitted pathogens (STI panel) was performed. PCR analysis of the urethral swab detected *Chlamydia trachomatis* later identified as LGV by MOMP-genotyping, the rectal swab was positive for *Chlamydia trachomatis* genotype F, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. In addition, inguinal ultrasonography revealed a reactive lymph node with signs of abscess formation. According to the guidelines, the patient was treated with doxycycline 100 mg twice daily for 21 days. At a follow-up visit after 4 months, he was asymptomatic and the STI screening yielded negative results for *Chlamydia trachomatis*.

Discussion: This case highlights several clinically and diagnostically relevant aspects in the context of STI management in a patient on HIV pre-exposure prophylaxis (PrEP). Multiplex PCR for STIs can be a useful tool for detecting sexually transmitted infections; however, it may also lead to findings of uncertain clinical relevance, such as the detection of *Mycoplasma hominis* or *Ureaplasma urealyticum*, which are often found to be part of the commensal flora. In cases with suggestive clinical findings, such as inguinal lymphadenopathy in this patient, genotyping of *Chlamydia trachomatis* becomes particularly important, as detection of LGV strains guides treatment duration and approach.

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P-1-117

Establishment of a novel human liver chimeric mouse model to study HBV DNA integrations and HBV infection

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Background and aim: HBV integrates parts of its DNA into the host genome early upon infection. Although unnecessary for viral replication, integrations are found in all phases of acute and chronic hepatitis B. They contribute to hepatocellular carcinoma (HCC) development through multiple, not fully defined mechanisms, including viral protein expression, genomic instability and disruption of cancer-associated genes. We aimed to establish a mouse model to study the role of HBV DNA integrations in primary human hepatocytes (PHH).

Methods: The model uses human liver chimeric USG (uPA/SCID/IL2Rγ^{-/-}) mice, and introduces a prototypic HBV DNA integration via lentiviral gene transfer in PHH. This integration comprises HBs and HBx open reading frames under endogenous promoters and a downstream eGFP sequence. Integrations and integration-derived viral RNA and proteins were characterized by histology, qPCR and RNA or DNA in situ hybridization (ISH).

Results: PHH were transduced ex vivo (average efficiency 35%) and transplanted into naïve USG mice (n=10), leading to the repopulation of mouse livers with clusters of integration-carrying and integration-free PHH. The presence of integrations did not seem to hinder PHH proliferation, as the in vivo distribution of eGFP-positive cells resembled initial transduction rates. Intrahepatic viral RNAs and DNA were detected by qPCR. HBsAg was secreted into the mouse serum, but viral RNA or DNA was not. Integrated DNA and expressed RNAs were also visualized at the single-cell level by DNA- and RNA-ISH.

Conclusion: We created a unique model to study the role of integrations in HBV persistence and HCC development. It allows controlled introduction of specific integrations, with or without a concurrent cccDNA-driven HBV infection, enabling investigation of early carcinogenic events in primary cells in vivo. Additional applications include the evaluation of treatments targeting integrations or establishing HDV mono-infections.

P-1-118

Anti-hepatitis D virus (HDV) prevalences, virological and clinical features in the liver clinic of University Hospital Heidelberg (Germany)

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5% of the >250 million individuals chronically infected with hepatitis B virus (HBV) are estimated to be co-infected with hepatitis D virus (HDV). This prevalence is likely underestimated due to limited testing, including in high-income countries. Reasons include low awareness, restricted diagnostic availability and limited treatment options. HDV

infection causes the most severe viral hepatitis, which can lead to liver cirrhosis and hepatocellular carcinoma. Currently available treatment options are Pegylated interferon-alpha and the virus entry inhibitor Bulevirtide.

To facilitate broader access to HDV diagnostics, we have previously developed a lateral flow assay (LFA) capable of detecting anti-HDV antibodies in serum or plasma within 20 minutes. This prototype LFA was applied in parallel with the clinically approved Dia.Pro anti-HDV ELISA for screening. Anti-HBcAg-positive (indicating exposure to HBV) patients who presented in the liver clinic of university hospital Heidelberg for any reason were included. Patients testing positive for anti-HDV were assessed for HDV RNA, and further characterized regarding virological parameters, clinical features and courses of disease.

At the time of abstract submission, 310 anti-HBcAg-positive patients were included, of whom 153 (49.3%) were HBsAg-positive, indicating ongoing HBV infection. Among the 157 anti-HBcAg-positive but HBsAg-negative individuals, 17 (10.8%) were tested positive for anti-HDV, compared with 26 (17.0%) of HBsAg-positive patients. 14 of the 17 patients with evidence of past, but not ongoing, HBV and HDV co-infection had undergone liver transplantation. Of the HBsAg- and anti-HDV positive patients, 16 (61.5%) had detectable HDV-RNA. 5 patients who were tested positive for anti-HDV in the ELISA were negative in the LFA. All of those patients were HDV RNA negative, and, based on clinical features, had undergone HDV exposure or infection several years ago. Assuming 100% accuracy of the ELISA, this would result in 88.4% sensitivity of the LFA, "missing" only patients who were HDV RNA negative and likely had very low anti-HDV levels.

Wider implementation of anti-HDV LFA testing, particularly in settings with limited diagnostic access, may be essential to increase testing for a better epidemiological understanding of HDV hotspots, and to identify infected patients; to ultimately reduce the disease burden of chronic hepatitis D.

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HDV infection and infection inhibition by HBV subviral particles using patient samples

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Background and aims: Chronic hepatitis D develops after co- or superinfection of hepatitis B virus (HBV) with hepatitis D virus (HDV). HDV recruits HBV envelope proteins consisting of hepatitis B surface antigen (HBsAg) for viral entry and egress from the host cell. Entry is mediated by attachment of viral particles to heparan sulfate proteoglycans (HSPGs) on the cell surface, and binding to the NTCP transporter on hepatocytes via the preS1 domain of the large HBsAg (L-HBsAg). HBV subviral particles (SVPs) - formed as spheres or filaments with different amounts of L-, medium (M-) and small (S-) HBsAg - are produced in excess over HBV infectious virions. While non-infectious, SVPs might contribute to exhaustion of T cells and prevent antibodies from binding to virions; and HBsAg is a target for novel therapeutic approaches. This study aims to characterize the

effect of HBV SVPs on HDV infectivity, using infected patient sera.

Methods: HBV- or HBV/HDV-infected patient sera were collected in the liver outpatient clinic of Heidelberg University Hospital. SVPs were produced via transfection of Huh7-NTCP cells with the pT7HB2.7 plasmid, which encodes all forms of HBsAg, and subsequently purified by heparin affinity chromatography; or were isolated from patient sera via affinity chromatography followed by isopycnic gradient centrifugation. Separation into spherical and filamentous SVPs was performed via size exclusion chromatography. Infection experiments were conducted using T1 (Taylor strain) HDV in Huh7-NTCP cells.

Results: HDV infectivity of patient sera positively correlated with HDV RNA and HBsAg levels. Additionally, a strong correlation between HDV RNA and HBsAg was observed. Infectivity of both laboratory-derived virus and HDV-positive patient sera was reduced significantly when co-incubated with SVPs. When separated, SVPs enriched either for filaments or for spheres, and unseparated SVPs, inhibited HDV infection. Laboratory-derived unseparated SVPs and patient-derived SVPs showed similar inhibitory potential. SVPs deficient in L-HBsAg were only slightly less effective in inhibiting HDV infection. Furthermore, SVPs not binding to heparin during purification did not inhibit HDV infection. Analysis of the HBsAg composition in the used HDV, SVPs, and patient sera, as well as analysis of the distribution of filaments and spheres in the SVPs, is ongoing.

Conclusions: HDV infectivity of patient samples was variable, but correlated well with HDV RNA levels within individual patients. Our results hint at HDV infection inhibition by HBV SVPs, at least in part, depending on the attachment to HSPGs on the cell surface, as heparin-binding, but not heparin-non-binding SVPs inhibited the infection.

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Genomic epidemiology of clinical *Enterobacter*: salmochelin marks a clinically successful lineage

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Enterobacter cloacae complex (ECC), a WHO-priority ESKAPE pathogen, is a major cause of hospital infections. Treatment is hindered by broad antibiotic resistance and unclear species classification, resulting in poorly defined epidemiology and species distribution. To gain clarity on the diversity and resistance of ECC, we analysed a historical (n = 202) and contemporary (n = 87) set of clinical *Enterobacter* isolates using long-read sequencing. We identified eight clinically relevant species, with *E. hormaechei* (also referred to as *E. xiangfangensis*) being the most common (68.5%), mainly represented by the subspecies *steigerwaltii* (34.95%) and *hoffmanii* (22.15%). Resistance genes for phenicolones, quinolones, and β -lactams were widespread, while fosfomycin and colistin-resistance genes (*arnBCDATE*) were mainly found in *E. hormaechei/xiangfangensis* subspecies *steigerwaltii*. All isolates carried virulence genes for capsule, enterobactin, flagella, and a Type-6 secretion system, defining a core virulence gene set of ECC. Additionally, *E. hormaechei/xiangfangensis* subspecies *steigerwaltii* uniquely encoded salmochelin, a siderophore necessary for blood infections. We also extended our observations to a global ECC genomes dataset (n = 15,081), which confirmed the predominance (65.6%) of *E. hormaechei/xiangfangensis*, particularly the subspecies *steigerwaltii* (27.1%) lineage carrying *arnBCDATE* and salmochelin, underscoring its clinical relevance. The exclusive presence in the clinically relevant and predominant isolates suggests that salmochelin may mark a clinically successful lineage.

P-1-121

Diagnostic Performance of Published Tuberculosis Transcriptomic Signatures in Children – a secondary analysis of the RaPaed-TB Study

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Tuberculosis (TB) diagnosis in children is challenging due to paucibacillary disease, unspecific clinical presentation and difficulty to obtain sputum samples. Microbiological confirmation remains the diagnostic reference standard but often performs sub optimally in the paediatric population. Transcriptomic host-response signatures have shown promising diagnostic potential in adults but their performance in children is underreported. To address this, we evaluated the diagnostic performance of published transcriptomic signatures in samples of one of the largest paediatric cohorts: RaPaed-TB, a prospective multi-country diagnostic accuracy study across five low- and middle-income countries.

We here evaluated the diagnostic performance of published transcriptomic signatures in children with presumptive TB. RNA for whole transcriptome profiling by RNA sequencing was extracted from whole blood collected in PAXgene RNA tubes. Enrichment scores were calculated using the TBSignatureProfiler R package for published TB diagnostic signatures. Children were classified as confirmed TB and unlikely TB using NIH-adapted guidelines. Diagnostic accuracy was assessed against the microbiological reference standard (Xpert Ultra or culture confirmed vs. unlikely TB).

We selected children with close-to-certain diagnostic classification at baseline (n=158). Of these children, 40.5% (64/158) were classified as confirmed TB and 59.5% (94/158) as unlikely TB. 54.4% (86/158) were female, 6.3% (10/158) were living with HIV and 68.9% (109/158) had a recent TB contact. The median age across all categories was 3.2 years old (CI: 1.5-7.2). From the TB signatures assessed, the highest performing were the Roe_3-gene signature (Roe_3) with an AUC of 0.71 (95% CI: 0.62-0.79), Maertzdorf_15-gene signature (Maertzdorf_15) with an AUC of 0.69 (95% CI: 0.6-0.8) and Sweeney_3-gene signature (Sweeney_3) with an AUC of 0.69 (95% CI: 0.6-0.8). Sensitivities were 50.0% (95% CI: 37.2-62.8) for Roe_3, 59.4% (95% CI: 46.4-71.5) for Maertzdorf_15, and 54.7% (95% CI: 41.7-67.2) for Sweeney_3, with corresponding specificities of 89.4% (95% CI: 81.3-94.8), 81.9% (95% CI: 72.6-89.1), and 84.0% (95% CI: 75.0-90.8). Notably, across sex and age groups, Roe_3 had a higher performance in children ≥ 2 years (AUC 0.75 vs. 0.64 in < 2 years) and in females compared to males (AUC 0.73 vs 0.69). Similar trends were observed in the other signatures.

Our results demonstrate a low or moderate performance of published TB diagnostic signatures in children and highlight the need for paediatric-specific signatures that account for age-related immunological differences, variations in gene expression and disease presentation.

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The Association Between Hepatitis E Virus Seroprevalence and Neurodegenerative Disorders in the Rhein-Neckar Area

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Background/Aim: Dementia, including Alzheimer's disease (AD) is a clinical syndrome most commonly resulting from neurodegeneration and currently affects over 55 million people worldwide. In addition to genetic and vascular risk factors, infectious agents have been proposed as potential contributors. Notably, a case-control study in Spain found a higher hepatitis E virus (HEV) seroprevalence in dementia patients compared to controls. HEV is a zoonotically transmitted RNA virus that can cause both acute and, in some cases, chronic infections. Although the liver is considered the primary site of viral replication, HEV infections have been increasingly associated with neurological complications. While definitive evidence of HEV RNA in the human brain is still lacking, studies in animal models have demonstrated that HEV can cross the blood-brain barrier, and genome compartmentalization of HEV within the human central nervous system has been reported. Given the frequent association of HEV with neurological symptoms and its possible neurotropic behavior, this study aims to investigate whether HEV exposure or active infection is associated with AD or non-AD dementias in the Rhein-Neckar area.

Methods: Serum was collected from patients with AD and non-AD dementia. The AD cohort (n=170) was defined by having an amyloid- β (A β) 42/40 ratio < 0.5 . The non-AD cohort (n=170) included patients with other types of dementia. Controls (n=340) were collected from age- and sex-matched individuals. HEV prevalence was assessed using the Wantai HEV IgG ELISA and HEV viremia by RT-qPCR using specific primers.

Results: We found that 59 of 124 (47.58%) AD patients and 54 of 124 (43.55%) age- and sex-matched controls were positive for anti-HEV IgG. The odds ratio for HEV IgG positivity in the AD patients vs. controls was 1.177 (95% CI: 0.723-1.924, p=0.52).

Conclusion & Outlook: Our preliminary results suggest that the prevalence of HEV is higher in both AD patients and controls than has been reported for these age groups in previous studies (~20-25%). Next, we will test the non-AD cohort and complement our analysis by assessing HEV viremia in our cohorts.

P-1-123

Wildling Mice as a Next-Generation Model for Immunity and Infection

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Wildling mice, generated by transferring embryos of inbred strains into wild-caught surrogate mothers, are emerging as a powerful model system in infection biology. They combine defined genetics of classical inbred laboratory strains with a naturally complex microbial exposure. In contrast to widely used specific pathogen-free (SPF) mice, which carry a simplified and highly standardized microbiota, Wildlings harbor diverse bacterial, viral, fungal, and helminth communities that drive immune development under conditions much more similar to the natural environment. SPF mice often display immature immune states and exaggerated pathogen susceptibility, and their limited microbiota varies substantially between institutions, reducing reproducibility and external validity of experimental findings.

In contrast, Wildlings maintain a diverse and resilient microbial ecosystem that closely resembles the microbiota of wild mice. Their gut microbiota reliably out-competes SPF-type communities and remains stable even under environmental perturbations such as antibiotic treatment or high-fat diet. This microbial stability reduces inter-facility variation, increases data reproducibility, and provides a more reliable platform for studying immune responses and host-microbe interactions. Consequently, observations made in Wildlings are more likely to reflect biological processes and may improve translation of preclinical results.

Because Wildlings harbor natural microbiota and endemic pathogens, they require specialized housing and strict separation from SPF colonies to avoid cross-contamination. A new dedicated Wildling facility at the LIMES Institute in Bonn will become operational in 2026, offering researchers controlled access to Wildling colonies and enable systematic comparative studies with SPF mice, including infection models. Standardized availability of the model, combined with harmonized experimental conditions, will establish an important platform for assessing how natural microbial exposure shapes immunity and host-pathogen dynamics.

By bridging the gap between SPF conditions and ecologically realistic microbial ecosystems, Wildlings provide a model with superior external validity for studying fundamental principles of immunity and infection, ultimately improving the interpretability and translational potential of experimental research.

P-2-1

Adaptive mutations in RSV fusion protein following monoclonal antibody exposure *in vitro*: Implications for antibody-based interventions

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Introduction: Respiratory Syncytial Virus (RSV) is a widespread respiratory pathogen, causing illnesses ranging from mild common cold-like symptoms to severe respiratory diseases. While no specific antiviral treatments for RSV exist, recent advancements include licensed vaccines for pregnant women and individuals over 60. Although, monoclonal antibodies (mAbs) that target the viral fusion protein such as Nirsevimab and Palivizumab are available to prevent severe disease in infants and young children, RSV continues to pose a significant health risk. Like other RNA viruses, RSV can rapidly adapt to immune pressure or antiviral therapies, allowing it to develop resistance. The recent STIKO recommendation to administer monoclonal antibodies (mAbs) to all newborns during their first RSV season increases this risk. While this policy is an important measure for protecting infants, it could also exert increased selective pressure on the virus, potentially accelerating the emergence of treatment-resistant variants.

Objectives and Results: To address this concern, our project aims to anticipate and characterise mAb-associated RSV variants. Thus, we passaged an RSV-A isolate in the presence of Nirsevimab or Palivizumab in cell culture leading

to a significant increase in the mAb concentrations the virus can tolerate while maintaining productive infection. Transcriptomic analysis revealed the emergence of resistance-associated mutations in the σ binding region of Nirsevimab (L204I and K209E) and the mutation N262Y, already described *in vivo* associated with palivizumab breakthrough infection. The adapted viruses present reduced susceptibility to the respective mAbs, but do not show enhanced replication fitness.

Ongoing work includes characterising the adapted viruses and determining the persistence of the observed mutations. Additionally, we are leveraging a recently established tonsil-derived immune cell organoid system. Using the approved vaccines, we aim to stimulate RSV-specific plasmablast formation to investigate i) whether currently licensed vaccines can induce antibodies capable of neutralizing variants that escape Nirsevimab or Palivizumab neutralisation, and ii) whether the resulting B-cell clones can be utilized to develop mAbs that, in combination with the existing immunoprophylaxis, provide a more robust defence against escape variants.

Conclusion: Here, we have established a system that provides an end-to-end framework for studying RSV resistance. Identifying potential resistance mutations before they become widespread offers an opportunity to design targeted interventions. A synergistic approach combining multiple antibodies may reduce the emergence of escape variants and improve the overall efficacy of RSV prevention strategies.

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P-2-2

Structural characterization of antibodies targeting novel gH/gL epitopes for synergistic HCMV neutralization

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The HCMV poses a significant health challenge 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 understanding of HCMV infection and neutralization impedes antibody application for HCMV prevention and therapy.

To address this knowledge gap, in our previous study (*Immunity* 2023), we isolated 136 unique monoclonal antibodies (mAbs) targeting the gH/gL-surface complexes of HCMV, including highly neutralizing mAbs. Thereby, comprehensive epitope mapping identified distinct clusters of antibodies binding to eight novel sites on gH/gL.

In the current study, we aimed to further characterize these novel epitopes and their associated antibodies. To this end, we selected representative antibodies from each identified epitope group and performed high-resolution cryo-EM analysis on 17 antibody-gH/gL complexes. Remarkably, cryo-EM resolved the structure of eight non-overlapping antibodies binding simultaneously to the same protein complex. This analysis provided detailed insights into binding modes, spatial orientations, and potential neutralization mechanisms for each novel antibody and epitope tested.

Importantly, by combining non-competing antibodies, we identified synergistically acting cocktails significantly enhancing neutralization compared to individual mAbs. Interestingly, the synergistic effects follow epitope-specific patterns, suggesting functional cooperativity depending on spatial arrangement of the targeted sites. Moreover, these effects were consistently observed across various HCMV-susceptible host cells and proved effective against multiple HCMV strains. Furthermore, synergistic cocktails strongly inhibited rapid emergence of HCMV antibody escape observed with different mAbs in *in vitro* settings. This highlights the potential of combined antibody approaches to enhance antiviral efficacy and limit viral escape.

In summary, our study advances the understanding of HCMV neutralization by characterizing novel gH/gL epitopes and demonstrating a previously undescribed across-epitope synergistic effect of specific antibody combinations. These findings provide valuable insights for the rational design of next-generation HCMV-vaccines and antibody-based therapies.

P-2-3

Sequential serum galactomannan as outcome marker for invasive aspergillosis – an exploratory study from the FungiScope® registry

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Question: Galactomannan index (GMI) is established as a diagnostic tool for invasive aspergillosis (IA), while its utility in monitoring antifungal treatment (AFT) response and prognostic value remains unclear. This study evaluated the validity of GM as a biomarker for AFT monitoring and its prognostic significance by correlating GMI with clinical response and survival of IA.

Methods: Patients with IA and at least two sequential serum GMI measurements were identified from the FungiScope® registry. Joint models of event-time and longitudinal outcome were used for imputing missing GMI to account for the relationship between GMI and time to death, as well as GMI and time to drop-out for the survival analysis and AFT response analysis, respectively. Cox proportional hazards models and logistic regression models assessed survival on GMI changes at day 7 as a primary predictor, with baseline log GMI as an adjustment covariate.

Results: Among 66 patients with IA, correlation between day 7 GMI predictions and observed values was 92% and 88% in the survival and AFT analysis, respectively. GMI decreased in both patients who died within 42 days and those who survived but maintained higher in patients who died. Patients who died within 42 days showed an increase or less decline[OC1] in GMI compared to the continuous decline observed in survivors. Patients with baseline GMI <1 were more likely to survive until day 42 (17/21, 81.0%) compared to those with GMI ≥1 (31/45, 68.9%). For those, risk of death was about twice as high as with GMI <1 (HR=2.107, p=0.19).

Conclusions: Serum GMI has potential as a non-invasive, predictive tool for estimating survival probability at onset of IA. Early GMI changes correlate with survival and could prompt timely AFT adjustment, potentially improving clinical outcomes. Additionally, GMI could serve as surrogate endpoint in clinical trials, facilitating development of new antifungal strategies.

P-2-4

The connection of the DZIF Tissue Bank in regional, national and international networks – an opportunity to profit from expertise and infrastructures beyond the DZIF

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The DZIF Tissue Bank (TB), as part of the **German Center for Infection Research (DZIF) Translational Infrastructure Bioresources, Biodata and Digital Health (TI BBD)**, supports multiple infectious diseases research projects and various studies with biosamples, biodata and latest technologies. To accommodate these tasks the DZIF TB is locally, nationally and internationally connected to and active in multiple networks.

Locally, the DZIF TB is affiliated with the **Heidelberg University Hospital** and the **Institute of Pathology (IPH)** which provides access to the pathological archive with >1.8 million cases and >5 million FFPE samples, many of them in the context of infectious diseases, and therefore the possibility to support DZIF Researchers in multiple research questions with tissue biosamples. With its affiliation to the IPH the DZIF TB is also connected to other platforms at the Heidelberg site such as computational pathology, diagnostic trail center and comparative pathology (animal model pathology). In addition, the DZIF TB is part of the **BioMaterialBank Heidelberg (BMBH)** and has access to all infrastructural measures such as quality management, IT solutions like the biobanking laboratory information system STARLIMS, as well as joint educational training and outreach activities. On national level, the TB is - besides the TI BBD - also embedded within several key research networks and infrastructures, such as the interactive biobanking platform of the German Centers for Health Research (Deutsche Zentren der Gesundheitsforschung, **DZG**), the German Biobanking Network (**GBN**) with active participation in the GBN Working Group "Tissue", the Netzwerk Universitätsmedizin (**NUM**), and the Technology and Methods Platform for Networked Medical Research (**TMF**) Working Group "Biobanking", fostering standardization, cooperation, and innovation in biobanking practices. Through this extensive networking activities, the DZIF TB is well connected and profits from multiple expertise, assistance, and innovation. Internationally, the DZIF TB is involved in the activities of the Biobanking and Biomolecular Resources Research Infrastructure - European Research Infrastructure Consortium (**BBMRI-ERIC**) and recently taking actively part in establishing tissue-based research infrastructures as a new platform and working group seated in the **European Society of Pathology**. This will consequently expand its potential access to expertise in multiple fields and enabling cross-border cooperation that facilitate large-scale biomedical studies.

This multi-level networking framework and the described interactions and involvement in multiple networks ensures that the DZIF TB remains a cornerstone in translational infectious disease research, driving advancements in personalized medicine and improving patient outcomes and enhances the efficiency and outreach of biobanking for infectious diseases research and thereby the possibilities of supporting DZIF scientist and their research projects.

P-2-5

Rapid and specific detection of pathogenic bacteria using recombinant receptor binding proteins of bacteriophages

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For infections caused by highly pathogenic bacteria, such as *Bacillus anthracis* or *Yersinia pestis*, timely antibiotic therapy for infected patients is paramount. To ensure the correct treatment regimen, rapid and unambiguous pathogen detection is essential. While PCR is the gold standard for diagnostics of most infectious diseases, antibody-based assays that detect specific antigens of the pathogen are commonly used for rapid POC testing or as confirmatory methods in diagnostic laboratories. Nevertheless, 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 derived from various phages targeting *Bacillus anthracis* and *Yersinia pestis*. These RBPs were engineered into bio-probes and recombinantly expressed, incorporating fluorescent proteins or enzymes to enable specific detection of the target pathogens. Additionally, the RBPs were coupled to magnetic beads to serve as highly specific capture molecules for the enrichment and isolation of bacterial pathogens from different matrices.

We are currently expanding our library of RBP bio-probes to specifically target other pathogens of interest, including *Burkholderia spp.*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis*.

P-2-6

Antibody Alternatives from Bacterial Viruses: Phage Receptor Binding Proteins as Novel Diagnostic Tools for Bacterial Infections

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Phage receptor binding proteins (RBPs) are emerging as an innovative alternative to antibodies for the detection of pathogenic bacteria. While antibody-based assays are widely used for identifying specific bacterial antigens, they often suffer from limitations in specificity, particularly due to the close genetic relationship of target pathogens to their non-pathogenic relatives. RBPs, on the other hand, have evolved over millennia to bind to specific bacterial surface receptors, providing a more precise and, *a priori*, real-life-tested approach for pathogen detection. We focus on the identification and engineering of RBPs that may serve as highly specific detection tools in clinical and biodefense-related settings for a wide range of pathogenic bacteria including notorious biothreat agents. To achieve this objective, we employ *in silico* RBP prediction tools and structural analyses to uncover diverse RBPs with most likely optimal binding capabilities. For initial testing of new RBPs, their respective genes are funneled as synthetic open reading frames into our standardized heterologous protein production pipeline and coupled with reporter moieties, either fluorophores or chromogenic enzymes. If enrichment of target bacteria from clinical or environmental matrices is required, RBPs can be easily coupled to magnetic beads to enhance the capture and isolation of target bacteria from such complex samples. From there, options arise to improve sensitivities in diagnostic tests or to obtain pure live cultures for further study. In summary, by leveraging the unique

properties of RBPs, we seek to advance the field of pathogen detection and to overcome key limitations associated with traditional (antibody-based) diagnostic methods.

P-2-7

Chronic infection with *Pseudomonas aeruginosa* induces a broadly neutralizing antibody response to elastase B

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Chronic pulmonary infection with *Pseudomonas aeruginosa* (PA) drives persistent inflammation and contributes to progressive lung damage in individuals with respiratory diseases such as cystic fibrosis (CF). Among its virulence factors, the secreted zinc metalloprotease elastase B (LasB) plays a central role in pathogenesis of lung infections by promoting tissue damage, immune evasion, and sustained inflammation. Although the immunogenicity of LasB in humans has long been recognized, a comprehensive understanding of the humoral immune response against this enzyme has remained limited.

In this study, we investigated the humoral immune response to LasB in a cohort of chronically infected individuals with CF. Elevated titers of anti-LasB antibodies were associated with improved lung function, suggesting a protective role of the humoral response against this virulence factor. To further explore this observation, we performed single B cell analyses from six individuals with high anti-LasB antibody levels. These analyses revealed a diverse B cell receptor repertoire targeting LasB and enabled the generation of monoclonal antibodies (mAbs) that potently neutralize its proteolytic activity.

Functional characterization identified broadly reactive mAbs that retained efficacy against naturally occurring LasB

variants, including those from globally prevalent PA strains. Structural studies using cryo-electron microscopy elucidated distinct epitope-specific mechanisms of neutralization. *In vivo* evaluation of selected anti-LasB mAbs in murine PA infection models demonstrated reduced lung inflammation and enhanced bacterial clearance when used in combination with antibiotics.

Together, these findings demonstrate that chronic PA infection can induce a protective, broadly neutralizing antibody response against LasB. Human B cell-derived anti-LasB mAbs represent a promising antivirulence therapeutic approach and a potential adjunct to conventional antibiotic treatment in PA lung infections.

P-2-8

Vaccine adjuvants can boost oxfendazole efficacy in the *Litomosoides sigmodontis* filarial rodent model

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Introduction: Infections with filarial nematodes such as *Onchocerca volvulus*, *Wuchereria bancrofti* or *Brugia* spp. can cause debilitating neglected tropical diseases, affecting millions of people worldwide. Drugs currently used for mass drug administration lack a macrofilaricidal (i.e., adult worm killing) effect and the only macrofilaricidal treatment that is currently available, doxycycline, requires a 4-6 week daily treatment, restricting it for individual therapy. Oxfendazole, a promising clinical candidate, has demonstrated macrofilaricidal efficacy in pre-clinical models and is currently being tested in phase II clinical trials against onchocerciasis, loiasis, mansonellosis and trichuriasis. Previous research has shown oxfendazole efficacy to be dependent on the immune system, and stimulation with IL-5 during oxfendazole treatment to improve the treatment outcome, allowing shorter treatments.

Aim: We investigated the potential of different adjuvants and modified antigens to boost the efficacy of oxfendazole against *Litomosoides sigmodontis*, a rodent filarial nematode, and determined whether combination therapy allows for a shorter treatment regimen.

Methods: 6-8 week old female BALB/c wild type mice were naturally infected via mite bite with *L. sigmodontis*. Mice were treated either 1 day (against L3 stages) or 35 days post infection (against adult worms) and sacrificed 35 to 63 days post infection to determine the effect of the combination treatment. Additionally, 6-11 week old female Mongolian gerbils were naturally infected and treated after onset of microfilaraemia. Following regular blood-draws gerbils were sacrificed 20 weeks after treatment to determine the long term effects of the combination treatment.

Results: While three day oxfendazole monotherapy was insufficient for a reduction of adult worms (24%), combination therapy of three day oxfendazole with either MF59/LsAg (62%) or CpG-ODN1826/LsAg (55%) led to a reduction of total adult worm burden similar to a full five day oxfendazole monotherapy (66%). Additionally, the combination treatment led to a near complete clearance of microfilariae from the peripheral blood and a significant reduction of all embryonal stages, while the three day oxfendazole monotherapy was insufficient. Furthermore, changes in adult worm length and morphology could be observed. The combination treatment could also boost the efficacy of oxfendazole when treating

against the infective L3 larvae in mice and increase the initial microfilariae reduction in the gerbil model.

Conclusions: The results of our study indicate that combining oxfendazole with adjuvants and modified antigens can boost the treatment efficacy, which allows shorter treatment regimens that may further support the success of elimination programs. Additional experiments are ongoing, focusing on testing different adjuvant/antigen formulations and concentrations in combination with oxfendazole to further improve the treatment efficacy.

P-2-9

CXCR5⁺ Anti-Env CAR T Cells Targeting HIV-Infected Cells in Peripheral Blood and Secondary Lymphoid Tissue

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Question: Despite the success of antiretroviral therapy (ART), HIV remains a global health challenge due to the need for lifelong treatment, associated side effects, and persistent societal stigma. Viral rebound following ART interruption is driven by latent HIV reservoirs, particularly in long-lived memory CD4⁺ T cells within secondary lymphoid tissues. Our research addresses the question of how to target these viral reservoirs to achieve a functional cure following ART cessation. To this end, we aim to perform a proof-of-concept study generating anti-Env-specific chimeric antigen receptor (CAR) T cells co-expressing the lymphoid homing receptor CXCR5, and evaluating their antiviral activity against HIV-1 in-vitro.

Methods: Expression of hCXCR5, an anti-Env CAR, or both was achieved via lentiviral vector transduction of primary CD8⁺ T cells. Plasmids for anti-Env CARs incorporating the antigen-binding domain of different broadly neutralizing antibodies (bnAbs) were kindly provided by Henning Gruell. CXCR5 functionality was assessed using transwell migration assays to evaluate chemotaxis toward CXCL13. Antiviral activity will be evaluated in cytotoxicity and viral inhibition assays. For this purpose, HIV-infected primary CD4⁺ T cells or tonsil-derived human lymphoid aggregate cultures (HLACs)—which mimic secondary lymphoid tissue—will be infected with GFP⁺ or luciferase-expressing HIV-1 strains. Co-cultures of HIV-1-infected target cells with CXCR5⁺ or CXCR5⁻ anti-env CAR T cells will be analyzed for reduction of infected cells at various effector-to-target ratios and time points.

Results: Lentiviral transduction resulted in stable expression of either CXCR5 or a 3BNC117 (bnAb)-based CAR in approximately 10–15% of primary CD8⁺ T cells up to 12–14 days post-transduction. CXCR5-transduced cells exhibited significantly enhanced migration toward CXCL13, the cognate ligand of CXCR5, compared to mock-transduced controls. However, sequential transduction with two distinct vectors resulted in co-expression of CXCR5 and the CAR in fewer than 1% of CD8⁺ T cells, highlighting the need for further optimization of this approach. To model secondary lymphoid tissue, HLACs were successfully established from human tonsillar tissue and remained viable in culture for 5–7 days. Infection with an X4-tropic, GFP-expressing HIV-1 strain yielded stable infection rates of 5–10%.

Conclusions: Collectively, these results establish a robust experimental system to assess the cytotoxic potential of CXCR5⁺ versus CXCR5⁻ CD8⁺ T cells against HIV-infected targets from peripheral blood and secondary lymphoid tissues. This approach may contribute to the development of immunotherapeutic strategies toward a functional cure for HIV.

P-2-10

Generation and Characterization of Human Anti-YFV Monoclonal Antibodies from Booster Vaccinated Individuals

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The Yellow Fever Virus (YFV) remains a persistent health threat, particularly to populations in Africa and South America, although the YF17D vaccine is one of the most effective and safest live-attenuated vaccines. One reason is that its distribution and administration to vulnerable unvaccinated groups remains challenging in remote areas and resource-limited settings. This highlights the need for alternative therapeutic strategies for YFV-infected individuals in these regions. Monoclonal antibodies (mAbs) derived from human memory B cells have emerged as a promising approach for targeted antiviral therapy, further supported by advanced single-cell technologies in recent years. Therefore, we aimed in this study to establish a robust pipeline for isolating YFV-specific mAbs from the B cells of YF17D booster-vaccinated individuals and their functional characterization. By utilizing the in-house produced YFV soluble envelope (sE) protein as the target antigen, we successfully identified memory B cells producing specific antibodies by single-cell sorting. We examined and evaluated all obtained mAb sequences of which first candidates were produced and quantified validating our high-throughput system. Further we are currently characterizing their affinity and neutralization capacity to evaluate their therapeutic potential. The obtained and characterized mAbs could pave the way for the development of antibody-based therapeutics, providing a potential solution for individuals in regions where vaccine availability remains challenging.

P-2-11

Implementing mRNA technology at DZIF: Evaluation of mRNA-based vaccine candidates against Middle East respiratory syndrome coronavirus in mice

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The emergence of mRNA technology has marked a significant turning point in vaccine development. This is particularly evident in the pivotal role mRNA technology played during the COVID-19 pandemic and in the subsequent marketing authorization of the first mRNA-based anti-COVID-19 vaccine. The applicability of mRNA

technology should be systematically evaluated for its potential to enhance vaccine research activities of the German Center for Infection Research (DZIF), including preclinical, translational, and clinical applications.

A pilot project uniting three different DZIF partner sites was initiated to compare mRNA-based vaccine candidates against bacterial and viral pathogens of public health importance: *Helicobacter pylori*, hepatitis C virus and Middle East respiratory syndrome coronavirus (MERS), with well-established comparator vaccines. To integrate mRNA technology within the DZIF framework, lipid nanoparticle (LNP)-formulated mRNAs targeting specific pathogens were obtained from a contract research organization (CRO) with GMP manufacturing capabilities.

At Marburg University, the immunogenicity of a codon-optimized mRNA encoding the MERS spike (S) protein formulated with three different LNP formulations (LNP1, LNP3, LNP4) was compared to that of the well-established vector-based vaccine Modified Vaccinia virus Ankara (MVA)-MERS-S. Balb/C mice were immunized using a prime-boost schedule with an interval of 28 days. Serum samples were collected every 14 days until day 56, when spleen cells and final serum samples were obtained to evaluate humoral and cellular immune responses.

All three LNP formulations induced anti-MERS-S binding antibodies after just one vaccine dose, comparable to or exceeding the levels elicited by the MVA-MERS-S vaccine. Crucially, two doses also resulted in the generation of neutralizing antibodies. ELISpot assays confirmed the presence of T cells reactive to the MERS-S protein in vaccinated mice. Of the tested LNP-formulations, LNP3 induced significantly higher humoral and comparable cellular immune responses to MVA-MERS-S and was therefore selected for subsequent studies.

Additionally, a heterologous prime-boost vaccination regimen combining mRNA-MERS-S and MVA-MERS-S was tested. Regardless of the order of vaccine administration, humoral and cellular immune responses were comparable and in a similar range to the homologous mRNA-MERS-S vaccination regimen.

Finally, mice vaccinated with homologous or heterologous vaccination regimens were challenged with authentic MERS-CoV. Preliminary results show a significant reduction in viral load in vaccinated mice, while histological studies on lung damage are still pending.

In conclusion, our study successfully demonstrated the immunogenicity of the tested mRNA-MERS-S vaccine candidates. Moreover, by procuring these candidates via a CRO, DZIF has laid the foundation for incorporating mRNA vaccine technology, thereby enhancing its preparedness for future emerging pathogens.

P-2-12 Genome-wide Association Study in the Transplant Cohort of the German Center for Infection Research (DZIF Tx-Cohort) identifies novel candidate genes associated with primary graft dysfunction

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Since its establishment in 2014 within the framework of the Transplant Cohort of the German Center of Infection Research (DZIF Tx-cohort) up to date (May 2025) clinical data and biosamples have been collected from 2,900 patients who received a donor organ. Those serve for research on interrelations of medication and infectious events with transplantation outcome and therefore improved options for prevention and treatment within the collective.

In July 2021 (state of recruitment: n=1,866 patients) a genotyping study has been initiated to investigate the genetic impact on the immune system and transplantation outcome in the patients.

Therefore, around 1,700 buffy coat samples were requested from the 5 study sites Hannover, Heidelberg, Munich (KUM, MRI) and Tuebingen. DNA was extracted and a final set of 1,612 DNA samples was genotyped using the InfiniumTM Global Screening Array v 3.0 from Illumina.

After quality control, remaining samples (n=1,514, 94%) were subjected to a genome wide association study (GWAS). First results indicate an association (p-value <1.00e-05) of almost 30 single nucleotide variations (SNVs) with primary graft dysfunction considering a minor allele frequency (MAF) of > 5%. Those are scattered over the whole genome. Several SNVs are located in regions coding for long, partially intergenic, non-coding RNAs (lncRNAs) with potential or already known regulatory function in gene expression as e.g. rs4376937 (chr11:97432286-97432486, p-value 2.76e-07, novel transcript), rs35277443 (chr20:52616361-52616561, p-value 8.00e-07, novel transcript) or rs115263661 (chr3:179098612-179098812, p-value 7.68e-06, *PIK3CA-DT*). Further SNVs are associated with protein coding genes with so far mostly indirect, but potentially important implications in organ transplantation due to their roles in immune modulation (*BARHL2*, *ENPEP*, *MEF2C*, *IGSF22*), inflammation and oxidative stress response (*AKR1B15*, *RAB11FIP4*, *SLC48A1*, *SLC1A5*), organ reperfusion and vascular integration (*RAPGEF3*, *SLC14A2*, *KCNE1*), tissue regeneration and wound healing (*WFDC1*) as well as post-transplant malignancies (*GLANT13*, *PIK3CA*, *TTC28*), the most significant ones being rs11652450 (chr17:31445065-31445265, p-value 7.09e-07, *RAB11FIP4*), rs700585 (chr5:88856200-88856400, p-value 7.32e-06, *MEF2C*) and rs4817656 (chr21:34468788-34468988, p-value 2.17e-06, *KCNE1*).

In summary the present study identified several novel candidate genes that might play a role in primary graft dysfunction. Future studies have to further elucidate the interrelations between those genetic variants and allograft function, rejection and survival after organ transplantation and potentially develop diagnostic or therapeutic applications. In this context also lncRNAs are increasingly recognized as key regulators of immune responses, including allograft rejection and tolerance and could serve

as non-invasive biomarkers for predicting or diagnosing graft rejection, potentially replacing or supplementing tissue biopsies.

P-2-13

StuVac: A cross-sectional study on gender differences in COVID-19 vaccine side effects

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Research question: Do gender-specific differences exist in the incidence of adverse drug reactions (ADRs) following COVID-19 vaccinations depending on the vaccine dose administered? This study expands on previous gender-related research by focusing on a young adult cohort. The relationship between female and male gender and specific ADRs was analysed, taking into account dose adjustment for booster vaccinations.

Methodology: To address the research question, we conducted a cross-sectional study using a web-based survey among a cohort of medical and psychology students. Inclusion criteria were age ≥ 18 years and a history of at least one documented COVID-19 vaccination. The recruitment period was 12 weeks. Of 854 initiated surveys, 527 were completed in full (completion rate: 61.71%). Anonymized data were extracted in RStudio and analyzed through separate logistic regression models to examine the research question.

Results: The sample comprised 77.3% women and 22.7% men; 75.1% were aged 18–24 years. The median number of COVID-19 vaccinations received was 3 (IQR=1), corresponding to a range of 2 to 3 vaccinations among the middle 50% of participants. Overall, specific ADR analysis revealed significant gender differences for muscle pain after the second dose (OR=0.49, $p=0.006$), with a higher prevalence in women. After the fourth vaccine dose, men experienced significantly fewer ADRs (OR=2.10; $p=0.048$) (Fig. 1). Both significant results were confirmed in a combined model (Fig. 2). Vaccine-specific analyses showed that after the first dose of the Vaxzevria vaccine (OR=4.53; 95% CI:[0.83; 2.21]; $p<0.001$) and the Janssen COVID-19 vaccine after the first/second dose (OR=3.97; 95% CI: [0.37; 2.39]; $p=0.007$ and OR=4.66; 95% CI: [0.27; 2.81]; $p=0.016$), respectively), women reported higher severity of their ADRs. No statistically significant correlation was found between gender and COVID-19 infection after vaccination ($p>0.05$).

Conclusion: The results of this study identified gender-specific differences in the incidence and severity of ADRs following COVID-19 vaccinations. Men consistently showed lower ADR rates, particularly with higher vaccine doses and vector-based vaccines. These findings support the relevance of gender-specific research and pharmacovigilance strategies and could be further investigated in future studies with larger sample sizes.

Attachments:

Figure 1: Odds Ratios (dots) with p-values for doses 1–4. Reference group: female gender (OR = 1, dotted line). OR > 1 = higher probability of no side effects in men.

Figure 2: OR with 95% confidence intervals for the absence of side effects after the 4th dose and the occurrence of muscle pain after the 2nd dose. (OR=1, dotted line). OR>1 = more frequent occurrence in men, OR<1 = more frequent occurrence in women

Fig. 1

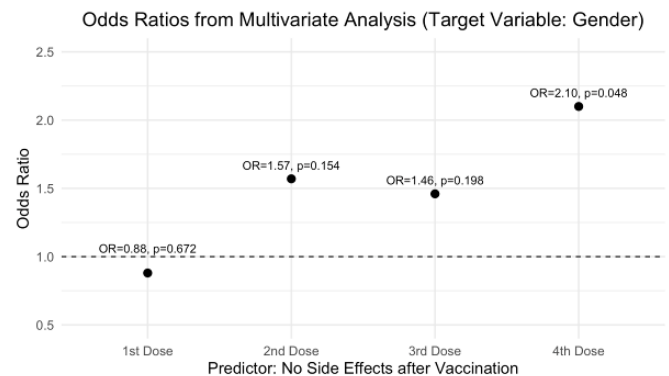
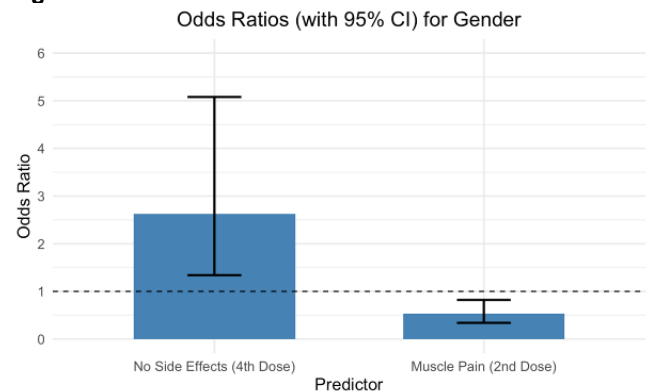


Fig. 2



P-2-14

Improved Immunogenicity and Protective Capacity of Single Dose Vaccination with an MVA-based vaccine expressing a Stabilized Recombinant MERS-CoV Spike Antigen

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The Middle East respiratory syndrome coronavirus (MERS-CoV) causes a severe respiratory disease in humans and still poses a significant threat to human health. The accessibility of a safe and rapidly protective vaccine will contribute to the effective prevention of MERS-CoV infections.

The favored antigen of MERS-CoV is the spike (S) glycoprotein. Here, the effect of a candidate vaccine delivering a prefusion-stabilized version of the MERS-CoV-S protein with an inactivated S1/2 cleavage site should be evaluated for immunogenicity and efficacy.

We used the Modified Vaccinia virus Ankara (MVA) to generate vaccine candidates against MERS-CoV. For this, we generated different MVA vaccines expressing the native, full-length S protein (MVA-MERS-S) or the prefusion-stabilized MERS-CoV-S protein (MVA-MERS-ST). We tested their immunogenicity and protective capacity in a lethal challenge mouse model (hDPP4-tg mice).

When tested in a single vaccination strategy, MVA-MERS-ST induced improved immunogenicity and efficacy, by robustly protecting mice against a lethal MERS-CoV challenge. In contrast, MVA-MERS-S failed to induce protection after single immunization. No differences between MVA-MERS-S and MVA-MERS-ST vaccination were determined using a prime-boost vaccination scheme. Importantly, protective efficacy, as seen by the lack of infectious MERS-CoV in lungs and brains, could be correlated to neutralizing antibodies (nAB). For MVA-MERS-ST, significant titers of nAB were induced already after single vaccination, while titers following single MVA-MERS-S vaccination were significantly lower. However, prime-boost vaccination with MVA-MERS-S and MVA-MERS-ST resulted in similar titers of nAB.

Our results suggest that MVA-MERS-ST represents an improved clinical candidate vaccine and that the prefusion-stabilized S antigen is highly beneficial for induced protective antibody levels.

P-2-15

Vaccination intentions, readiness, and preferred attributes of a future Lassa fever vaccine among staff at a tertiary hospital in Edo State, Nigeria: a cross-sectional survey

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Background: Lassa fever (LF), a viral hemorrhagic disease endemic to West Africa, presents a major public health challenge in Nigeria with a case fatality rate of around 18% in the 2025 outbreak. Healthcare workers face elevated infection risk and are therefore a priority group for the Lassa fever vaccine currently in development. However, vaccine hesitancy may impede uptake. This study assessed vaccination readiness, intentions and product preferences for a prospective LF vaccine among staff at Irrua Specialist Teaching Hospital.

Methods: A cross-sectional survey was conducted between February and May 2025 among clinical and non-clinical staff. Participants were selected using multistage sampling with proportional representation from all hospital departments. Ethical approval was obtained from the relevant institutional review board, and all study procedures adhered to established ethical standards. Data was collected through interviewer-administered questionnaires capturing sociodemographics, risk perception, vaccination intention, preferred vaccine attributes, and the 7C vaccination readiness scale. Primary outcomes were LF vaccination readiness and vaccination intention.

Results: A total of 485 participated (median age 41; IQR 35–48), with 52.0% female. The overall readiness to receive a LF vaccine was moderately high, with a mean 7C score of 3.41 (SD 0.44) out of 5. 70.1% expressed the intention to receive a future LF vaccine, 20.6% were uncertain, and 9.3% were unwilling. Vaccination intention was highest among nurses (81.9%) and declined slightly with age. Higher vaccination readiness was positively associated with vaccination intention. More than half were willing to accept a vaccine with at least 80% effectiveness. Vaccination intention decreased given certain scenarios: if multiple doses were required (62.7%), if no incentives were provided (61.0%), if the vaccine was not free of cost (48.0%), if mild side effects were expected (43.7%), or if it does not provide immunity for life (43.5%). Participants showed the highest trust in the LF vaccine if recommended by a doctor (70.3%), followed by the WHO (67.6%) and the Nigeria Center for Disease Control (63.5%).

Conclusion: Effective LF vaccine roll-out among hospital staff will require targeted strategies to ensure free and equitable access and endorsement by trusted sources, and tailor vaccine product characteristics and communication strategies to the preferences and concerns of specific staff groups.

P-2-16

Strong and early monkeypox virus-specific immunity associated with mild disease after intradermal clade-IIb-infection in CAST/EiJ-mice

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Monkeypox virus (MPXV) is a zoonotic poxvirus long endemic in West and Central Africa. Outbreaks, first the global spread of clade II outside Africa in 2022, and since 2023 the accelerating spread of clade I in central Africa, point to MPXV adaptations that pose the risk of it becoming more transmissible in humans. Animal models mimicking the clinical disease outcome in humans are important to better understand pathogenesis, host tropism, and the contribution of genetic mutations. Here, we demonstrate that MPXV infection via tail scarification in CAST/EiJ mice is an appropriate animal model to mimic human mpox. In our study, disease outcome is milder in clade IIb than clade IIa-infected mice, which is associated with enhanced immunogenicity early during infection. This suggests that clade IIb more efficiently activates host immune responses, highlighting how this animal model could facilitate studying new MPXV variants to help develop efficient antivirals and preventive measures.

P-2-17

MHV-68 Infection in ITK-deficient Mice Reveals a Lung-Dominant Immunopathology Resembling Features of Human ITK Deficiency

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Background: Patients with mutations in *inducible T cell kinase* (ITK) often present with Epstein-Barr virus (EBV)-driven lymphoproliferative disease, hemophagocytic lymphohistiocytosis (HLH), and immunodeficiency. To model this immunodeficient condition and investigate the underlying mechanisms driving immunopathology as well as therapeutic interventions to alleviate it, we used the murine analog of EBV, the murine γ -herpesvirus 68 (MHV-68), in ITK deficient (*Itk*^{-/-}) mice.

Methods: *Itk*^{-/-} and WT (C57BL/6J) mice were intranasally infected with 5X10⁴ PFU MHV-68, respectively. Lymphoid organs and bronchoalveolar lavage fluid (BAL) were analyzed at multiple time points (1–24 weeks post-infection) via flow cytometry, ELISA and histopathological analyses. Qupath software was used to quantify lung immunopathology. To alleviate the observed lung pathology, cohorts received anti-IFN- γ (monoclonal antibody that specifically binds to IFN- γ) and Ruxolitinib (JAK1/JAK2 inhibitor) therapy.

Results: Infected *Itk*^{-/-} mice displayed reduced CD8⁺ effector T cell in the BAL at 1 week post-infection, and decreased MHV-68 specific splenic CD8⁺ cytotoxic T lymphocytes at 2 weeks post infection. At later time points (4–24 weeks post-infection), the spleens of *Itk*^{-/-} mice were characterized by significant splenomegaly and increased frequencies of splenic effector T cells. Lung histology revealed massive infiltration and fibrosis in *Itk*^{-/-} mice compared to WT controls. Peak IFN- γ and CXCL9 in the BAL were observed 7 days post-infection. In the serum, the peak was reached 14 days post-infection in WT mice, whereas *Itk*^{-/-} mice had decreased IFN- γ and CXCL9 levels at these time points. Treatment with Ruxolitinib (40 mg/kg, twice a day, orally), but not anti-IFN- γ , elevated the levels of IFN- γ and CXCL9 in serum and BAL and significantly reduced pulmonary immune cell infiltration in *Itk*^{-/-} mice.

Conclusions: Itk deficiency impairs early antiviral CD8⁺ T cell responses and reduces IFN- γ and CXCL9 production, contributing to worsened lung pathology after MHV-68 infection. Ruxolitinib treatment partially restored cytokine levels and improved lung immune regulation. Further studies will explore MHV-68 re-activation at later time-points and explore the potential of immune training vaccine to ameliorate the observed pathology.

P-2-18

Mapping Point-of-Care HIV Viral Load Tests: A Scoping Review

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Background: In 2023, an estimated 1.3 million new HIV infections were reported worldwide, with the WHO African Region accounting for half of all cases. Early diagnosis and prompt initiation of antiretroviral therapy (ART) are critical to halting the progression to AIDS and curbing transmission. To achieve this, accurate, rapid, and affordable viral load (VL) testing is essential for monitoring treatment success and detecting treatment failure. However, conventional laboratory-based VL tests remain out of reach for many people living in low-resource settings, where access to centralized labs is limited. In these contexts, point-of-care (POC) tests offer a vital alternative, bringing life-saving diagnostics closer to patients. This scoping review aims to map the current landscape of available POC HIV VL tests and provide a comprehensive overview of their performance characteristics, operational feasibility, and practical usability in resource-limited settings.

Methods: We systematically searched MEDLINE (via PubMed), EMBASE (via Elsevier), the Cochrane Library (via Wiley), and Web of Science (via Clarivate) for POC assays that provide quantitative or semi-quantitative HIV-1 or HIV-2 viral load (VL) results using automated systems requiring minimal laboratory infrastructure. Laboratory-based tests, non-molecular tests detecting viral enzymes (e.g., reverse transcriptase) or proteins (e.g., p24 antigen), and methods for monitoring antiretroviral therapy (ART) response such as CD4 testing were excluded. Two reviewers screened titles/abstracts and the eligible full texts, resolving discrepancies by consensus or with a third reviewer. One reviewer extracted data, which a second reviewer is currently validating. Missing data will be sought from grey literature sources.

Results: Our search strategy identified 1,327 titles for screening, of which 78 publications were included for data extraction. This process yielded seven tests meeting the inclusion criteria: six already commercialized and one in development. Five tests require plasma—necessitating phlebotomy and centrifugation—while two can use whole blood. Five tests are quantitative and two are semi-quantitative. All required systems can be operated by minimally trained personnel; however, some require stable electricity or refrigeration and are costly, limiting their use in resource-limited settings.

Conclusions: Preliminary results indicate that the number of point-of-care (POC) tests available for measuring HIV viral load remains limited. To ensure equitable access to reliable HIV care, increased efforts are necessary to develop and implement viral load POC tests that are genuinely practical and feasible for use in resource-limited settings.

P-2-19

Estimating Averted Influenza Hospitalizations among Older Adults in European: A Simulation-Based Analysis of Vaccination Scenarios

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Introduction Seasonal influenza disproportionately affects older adults and is highly sensitive to climate conditions. Vaccination is a key defense against severe outcomes, yet its effectiveness at the population level hinges on both coverage and vaccine performance. This study aims to estimate how many hospitalizations could be prevented among individuals aged 65 and over across varying

vaccination coverage scenarios, to help guide public health strategies.

Methods We used simulation-generated weekly ILI+ incidence data from multiple European nations, analyzing six distinct scenarios. Our primary comparison was between Scenario A (typical disease burden with 15% higher vaccination coverage) and Scenario E (no vaccine, typical burden). For each country, we calculated mean ILI+ incidence for the 65+ demographic based on sample draws and converted these to hospitalization figures using baseline age-specific rates and conditional vaccine effectiveness against severe outcomes. Averted hospitalizations were determined by subtracting Scenario A outcomes from those in Scenario E. We assessed the relationship between vaccine coverage and hospitalizations averted using linear regression, comparing models with and without intercepts.

Results Across ten EU countries, higher vaccination coverage consistently correlated with reductions in influenza-related hospitalizations among older adults. The zero-intercept regression model suggested that each 1% increase in vaccine coverage led to an average 2.2% reduction in hospitalizations (95% CI: 0–4.5%). Though the free-intercept model showed wider uncertainty, it mirrored the overall trend. Despite differences across countries, the relationship remained roughly linear.

Conclusions Our findings underscore the significant potential of expanded influenza vaccination coverage in older adults. Simulation-based modeling reveals a robust and nearly linear relationship between increased coverage and fewer hospitalizations, reinforcing the importance of proactive seasonal vaccination strategies within the broader context of climate-sensitive infectious diseases.

P-2-20

Anti-tumor efficacy characterization of a multi-epitope therapeutic HPV16 vaccine in an HPV16-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 anogenital and oropharyngeal cancers in women and men, which account for approximately 5% of cancer cases worldwide. Many therapeutic vaccines targeting HPV16-associated malignancies have shown to be highly effective in preclinical studies, often tested against subcutaneous tumors in C57BL/6 mice. Evaluation in human patients, however, failed to reproduce these results. To overcome this discrepancy, our group focuses on the use of MHC-humanized mice, allowing assessment of vaccine platforms containing clinically relevant HLA-A2-restricted epitopes. Furthermore, we developed HPV-oncogene-dependent orthotopic tumor models, namely in the female genital tract and at the base of the tongue.

Here, we present a vaccine platform based on silica nanoparticles (SiNPs), which has already shown promising results as single-CD8⁺ T cell epitope vaccine in a

subcutaneous tumor model in MHC-humanized mice. We further optimized this platform by adding the CD4⁺ T cell epitope PADRE (pan-DR epitope) in the SiNP vaccine formulation, resulting in a significant enhancement of the induced immune response. Incorporation of further epitopes binding to different HLA class I supertypes created a multi-epitope vaccine applicable to the broad population irrespective of their HLA repertoire.

As HPV-associated malignancies occur exclusively in mucosal tissues, we determined several prime-pull approaches by inducing local inflammation or using a topical immune modulator, to guide the systemically induced immune response towards the mucosa of the female genital tract. Finally, the multi-epitope vaccine was applied with the best prime-pull strategy to assess anti-tumor efficacy in the genital tract tumor model.

Taken together, the orthotopic tumor models represent a platform for more meaningful preclinical assessment of new therapeutic HPV vaccine formulations and application routes. The obtained results of the multi-epitope SiNP-based vaccine validate this formulation for further development towards clinical translation.

P-2-21

Systematic collection, assessment and reporting of Rheumatic syndromes in vaccine trials: insights from rVSV-ΔG-ZEBOV-GP Ebola vaccine trials

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Background: There has been a concern associated with some vaccines, specifically for live attenuated vaccines, to induce rheumatic arthritis. However, rheumatic syndromes are rarely systematically investigated during clinical trials of vaccines. Instead, targeted signs are only monitored when a safety signal has already been reported. As part of a phase 2 trial to evaluate the safety and immunogenicity of the rVSV-ΔG-ZEBOV-GP Ebola vaccine compared to the shingles vaccine, we developed a comprehensive approach to systematically collect, evaluate and report rheumatic syndromes, which can be generalized.

Methods: We collected arthritic signs and symptoms in children who received rVSV-ΔG-ZEBOV-GP Ebola vaccine or Varilrix. Volunteers underwent a medical history review, symptom assessment, and physical examination during pre-selection/enrolment, on days 1, 3, 7, 14, 30, 60, 90, 180, 365, and during any outpatient visits/hospitalizations. We then generated an algorithm with the data collected to track septic arthritis, toxic synovitis, reactive arthritis, acute rheumatic fever, systemic juvenile idiopathic arthritis (SJA), oligoarthritis, polyarthritis (rheumatoid factor negative), polyarthritis (rheumatoid factor positive), psoriatic arthritis, and enthesitis-related arthritis at baseline and post vaccine. An incidence matrix incorporating the specific characteristics of each syndrome has been established

Results: Among the 120 children enrolled in clinical trials 97.1% completed D1 visits, medical histories, and physical examinations. The remaining participants completed the following visits: 94.2% D2/3, 93.3% D7, 90% D14, 91.7% D30, 91.7% D60, 87.5% D90, 85% D180, and 77.5% D365.

For cases suspicious of septic arthritis, juvenile arthritis, and reactive arthritis, the most common symptoms were joint swelling and fever in both vaccinees groups.

Conclusion: Following our investigations, none of the rheumatic syndromes occurred during the trial conduct consisting of one-year follow-up. Our findings support a systematic collection, assessment and reporting of rheumatic disorders during any vaccine trial.

P-2-23

Clinical and Paraclinical Correlates of Severe Lassa Fever: First Results of the Irrua Lassa Fever SEPSIS Study

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Background: Lassa fever (LF) is a severe zoonotic disease caused by the Lassa virus. LF is endemic to West Africa, with Nigeria experiencing annual outbreaks. Clinical presentation ranges from asymptomatic to severe illness, with case fatality rates exceeding 20% in hospitalized patients. Actual hemorrhage is rare, and the actual clinical pathophysiology is poorly understood. The SEPSIS study investigates whether LF triggers a hyperinflammatory response, either directly or via secondary bacterial infections, to identify potential therapeutic strategies.

Methods: Adult RT-PCR confirmed LF cases are recruited at the Irrua Specialist Teaching Hospital (ISTH), Nigeria, since January 2024. Study visits conducted bidaily include clinical assessment and laboratory investigations including inflammation (CRP, IL6 etc.) and bleeding (thrombocytes, INR, aPTT, etc.) markers. In addition, blood cultures are collected and, if positive, subjected to species identification diagnostics. Here, we present a preliminary analysis of the clinical and paraclinical outcomes of SEPSIS study patients with severe Lassa fever admitted to the intensive care unit at ISTH.

Results: As of 23 July 2025, 46 (24.5%) of enrolled patients were admitted to the ICU and thus were included in the preliminary analysis. The mean age was 43 (\pm 17) years, 65% were male. Patients had symptoms for 11.5 (\pm 5.3) days before admission. The case fatality rate in this population was 47.8% (n=22 deaths). Ribavirin was administered to 45 patients; 41 also received dexamethasone. Supportive care included blood transfusions (n=25), hemodialysis (n=27), and oxygen therapy (n=33). AKI was observed in 85%, bleeding, neurological symptoms, and severe anemia were also frequent. Inflammatory markers were markedly elevated in fatal vs non-fatal cases (mean CRP: 117 vs 73 mg/L; mean WBC: 25 vs 11 103/mm³). Sepsis biomarkers (IL-6, PCT) also showed a pronounced elevation correlating with severity. Blood cultures were positive in 10 of 28 ICU

patients; *Staphylococcus spp.*, *E. coli*, *Acinetobacter baumannii* and other pathogens were detected.

Conclusion: Severe cases of LF treated at the ICU at ISTH suffered from hyperinflammation, organ dysfunction, and frequent secondary bacterial infections, sometimes with (multi-)drug resistant pathogens. We recommend increased efforts for the development of host-directed, anti-inflammatory therapy for Lassa fever, in addition to evaluation and treatment of secondary infections.

P-2-24

Identification of T & B cell antigens for use in a multi-epitope subunit vaccine against *Rickettsia typhi* infection

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Rickettsioses are neglected febrile, potentially fatal infectious diseases that are emerging worldwide and represent a serious global health threat. Causative agents are small, obligate intracellular bacteria of the genus rickettsia. Treatment options are limited as the bacteria only respond to very few antibiotics. The drug of choice is still doxycycline. Delayed treatment or treatment with the wrong antibiotic usually leads to more severe or even fatal courses of the disease. In addition, the development of antibiotic resistance is a major concern due to the lack of alternative treatment options. A preventive vaccine does not exist and is urgently needed. In previous work using *Rickettsia (R.) typhi* as a model organism, we found that protection from disease and long-term control of persisting bacteria in infected mice depends on T cells. Therefore, T cells are the primary targets for a potential vaccine against *R. typhi*. In recent years, multi-epitope subunit vaccines consisting of multiple antigen sources have become increasingly popular as a novel approach to vaccine design. Employing *in vitro* and *in silico* techniques, we identified a series of potential T & B cell antigens and their epitopes in *R. typhi* infection. These epitopes were selected for *in vitro* restimulation of T cells from *R. typhi*-infected mice to elucidate their immunogenic potential. Subsequently, multi-epitope subunit vaccines will be constructed and used for the immunization of resistant wild type mice. Adoptive transfer of antigen-specific T cells into *R. typhi*-susceptible immunodeficient mice will further demonstrate their protective potential.

P-2-25

Outer membrane vesicle 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-2-26

Balanced co-stimulation and co-inhibition via immune checkpoint modulation enhances the efficacy of therapeutic hepatitis B vaccine in high-titer HBV carrier mice

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Although effective prophylactic vaccines are available, nearly 4% of the world population remains chronically infected with hepatitis B virus (HBV). Chronic HBV infection is characterized by the absence of neutralizing antibodies and scarce, dysfunctional HBV-specific T cells. Therapeutic vaccination is therefore considered a promising approach to

restore antiviral immunity. Our clinical candidate protein-prime/MVA-boost therapeutic vaccine, *TherVacB*, showed strong efficacy in preclinical mouse models with moderate HBV levels but failed to eliminate infection in high-titer HBV carriers. This study aimed to investigate the factors determining T-cell function by comparing *TherVacB*-induced CD8 T cells in low- vs high-titer HBV carrier mice, and to identify potential targets to overcome T-cell dysfunction in high-titer infection.

Low- and high-level persistent HBV infection was established in C57BL/6J mice using AAV-HBV. *TherVacB* was then administered, and liver-associated vaccine-induced HBV-specific CD8 T cells were isolated at multiple time points. Comparative phenotypic and transcriptomic analyses were performed to characterize immune responses in low- and high-titer HBV carrier mice.

Transcriptome and phenotypic analyses showed that *TherVacB* induced comparable numbers of effector CD8 T cells in both low- and high-titer mice; however, their functionality and long-term survival were determined by HBV antigen levels in the liver. In low-titer mice, resolving the persistent infection was followed by the establishment of robust effector and tissue-resident memory CD8 T-cell subsets. In contrast, in high-titer mice, vaccine-induced CD8 T cells expressed high levels of co-inhibitory molecules associated with T-cell exhaustion, such as PD1 and LAG3. Interestingly, we also found that the co-stimulatory molecule 4-1BB was the most strongly expressed in high-titer mice. Therefore, we assessed whether combining *TherVacB* with monoclonal antibodies (mAbs) targeting 4-1BB and PD-1 could improve antiviral efficacy under high HBV antigen load. We immunized high-titer mice with *TherVacB* and treated them *in vivo* with mAbs targeting 4-1BB, PD1, or their combination, and compared HBV-specific immune responses and infection parameters. *In vivo* treatment of high-titer mice with 4-1BB mAbs and *TherVacB* enhanced cytokine production in CD8 T cells, while only PD1 blockade had no additional benefit. A combination of PD1 blockade and 4-1BB stimulation further improved viral control. Treatment with 4-1BB alone or in combination with PD1 significantly reduced serum HBeAg and HBsAg levels, accompanied by transient ALT flares and efficient elimination of HBV-infected hepatocytes.

Combining *TherVacB* with 4-1BB stimulation, with or without PD-1 blockade, improved HBV-specific CD8 T-cell functionality and enhanced the antiviral efficacy of vaccination. Thus, targeting 4-1BB represents a promising therapeutic approach to overcome T-cell dysfunction in chronic hepatitis B.

P-2-27

Prostaglandin E2-modulation of TNFRSF9⁺Treg cell development governs symptom outcome during brain-inflammatory human helminth infection

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Background: The development and specialization of regulatory T cells (Tregs) are intricately shaped by disease context and tissue-derived mediators, a complexity that holds particular significance for diagnostic and therapeutic strategies in chronic inflammation. In neurocysticercosis (NCC), an inflammatory parasitic brain disease, we previously identified (viable)parasite-derived glutamate dehydrogenase (TsGDH)-induced prostaglandin E2 (PGE2) as a central driver of Treg development, essential for immune tolerance in asymptomatic, non-epileptic patients. Here, we characterize a unique PGE2-dependent induction of TNFRSF9⁺ Tregs as a key signature of NCC clinical outcome.

Materials and Methods: A cohort of healthy controls, asymptomatic NCC patients, and symptomatic patients with epilepsy and neurological disease was recruited and stratified by CT-imaging of parasite stage. Targeted lipidomics, GDH activity assays, and LC/MS/MS profiling assessed arachidonic acid metabolism. Monocytes/macrophages were pulsed with recombinantly expressed TsGDH to assess PGE2 induction and Treg differentiation. Transcriptional profiling (RNA-seq) and chromatin accessibility mapping (ATAC-seq) of *in vitro*-induced and patient-derived Tregs defined epigenetic and transcriptional determinants. Functional assays assessed the impact of TNFRSF9 modulation on brain microglial inflammation.

Results: Elevated TsGDH-driven PGE2 production in asymptomatic patients correlated with enhanced Treg development. Accordingly, TsGDH-pulsed monocytes transcriptionally upregulated PTGES2, driving PGE2 synthesis, and EP2/EP4 signaling, to convert naïve CD4⁺ T cells into Tregs uniquely expressing TNFRSF9, in contrast to symptomatics. Integrative ATAC-RNA-seq analyses revealed distinct CNS migratory and adhesion profiles (e.g. CEACAM1, RGS1, ITGA4) of asymptomatic CD69^{hi}CCR7⁺TNFRSF9⁺ Tregs, and JAK-STAT signaling pathway as an important regulator controlling the GDH-PGE2-driven Treg differentiation, absent in symptomatic patients. Importantly, the loss of TNFRSF9 expression or blockade of its ligand (TNFRSF9-L) on microglia limited Treg-mediated suppression of microglial inflammation.

Conclusions: This study highlights important insights into lipid mediators as novel regulators of the development of Treg-expressing TNFRSF9 with distinct features to maintain immune tolerance in NCC with relevance for other inflammatory brain disorders.

P-2-28

Video Frame Reconstruction to Detect the Malfunctioned Regions in Tissue Organoids

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Tissue organoids are three-dimensional microtissues derived either from stem cells or tissue-specific progenitor cells. They represent a simple version of complex human or animal organs such as the brain, stomach and intestine, which provides a more physiologically relevant system to study the process of cellular evolution, disease spread to other organs and evaluating the drug response in a controlled laboratory environment. And three-dimensional imaging is required to capture sequentially dynamic processes to record the responses to stimuli. Quick and reliable analysis of organoid images helps in precise modelling of disease phenotype and better overview of the organ development.

Recently, we developed a method which processes a video to extract the frames at user-specified time points while highlighting the posture of the object of interest by distinguishing it from the surrounding environment. Whereas this method helps to detect the object in the frame, it also provides a system to assemble the frames in a simplified system to introduce the cutting-edge frames that play a significant role in the process of organ development or disease progression. Based on our method, the crucial player frames can be easier captured for the quick and accurate incident recognition of the disease or evolution.

P-2-29

Sequential priming immunization with recombinant HBsAg efficiently improves antibody responses upon DNA prime – MVA boost therapeutic hepatitis B vaccination

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Chronic hepatitis B virus (HBV) infection affects 250 million people and causes more than 1 million deaths per year due to HBV-associated liver cirrhosis and hepatocellular carcinoma. Current treatment of chronic hepatitis B relies on nucleos(t)ide analogs, which suppress, but do not eliminate the virus, and only slightly reduce the risk of liver cancer. Thus, novel treatment approaches are urgently needed.

Induction of hepatitis HBV-specific immunity by therapeutic vaccination holds the promise to cure chronic HBV infection. We developed a clinical candidate therapeutic HBV vaccine, TherVacB, which employs priming with adjuvanted recombinant proteins and a boost with pan-genotypic Modified Vaccinia Ankara vector expressing five HBV antigens (MVA-HBVac). Protein vaccines require complex purification, which becomes costly when several antigens of interest are needed, and require Th1-based adjuvant to elicit potent effector CD8⁺ T cell responses in therapeutic use. These limitations can be overcome by DNA vaccines, leading to the production of the antigens directly *in vivo*. Here, we aimed to investigate the potential of DNA vaccination as an alternative to protein priming to broaden and improve HBV-specific immunity.

We generated DNA-HBVac, a plasmid DNA vaccine containing the same polycistronic expression cassette as

MVA-HBVac, optimized to induce a broad response by covering the immunodominant epitopes of the five most prevalent HBV genotypes, A-E, covering 95% of global circulating isolates. Immunogenicity and antiviral efficacy of the DNA-HBVac prime – MVA-HBVac boost regimen were compared to a classical TherVacB regimen with protein priming in HBV-naïve and HBV-carrier mice.

Immunization of HBV-naïve and HBV-carrier mice employing a DNA-prime and MVA-boost elicited robust HBV-specific CD4 and CD8 T-cell responses but poor anti-HBs titers. Thus, the vaccination effectively reduced serum HBeAg in several HBV-carrier mice but had only a minor effect on serum HBsAg levels. The addition of adjuvanted HBsAg into the priming regimen significantly improved anti-HBs response and the reduction of serum HBsAg. However, simultaneous immunization with DNA-HBVac and HBsAg completely abrogated plasmid-mediated immunity. By contrast, sequential immunization with DNA-HBVac and recombinant HBsAg followed by MVA-HBVac induced excellent multi-specific HBV-specific CD8 T-cell response and simultaneously high anti-HBs titers, thereby resulting in a reduction in circulating HBsAg and HBeAg levels. Interestingly, the order of the priming immunizations with DNA and HBsAg significantly influenced the immunogenicity of the vaccination.

Our results demonstrate that DNA-HBVac can successfully prime vigorous and broad HBV-specific T-cell responses, but it requires additional immunization with recombinant HBsAg to effectively reduce circulating HBsAg.

P-2-30 L-arginine metabolism is crucial for the outcome of intestinal *Salmonella* Typhimurium infection

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Arginase 1 (Arg1) and inducible nitric oxide synthase (NOS2) compete for L-arginine as common substrate and exhibit various, sometimes opposing effects on immune responses, tissue regeneration and microbial survival¹. While the role of NOS2 in *Salmonella* Typhimurium (*S. Tm*) infection is well-characterized, the function of Arg1 is less understood.

Since *S. Tm* infection disrupts amino acid metabolism² and intestinal inflammation interferes with L-arginine metabolism³, we investigated the effects of Arg1 on colonization resistance in the well-established streptomycin *S. Tm* infection model. We assessed the composition of intestinal microbiota by 16S rRNA and shotgun sequencing, the metabolome by HPLC and the immune response by scRNAseq and flow cytometry.

Following oral *S. Tm* infection, predominantly myeloid cells expressed Arg1. Unexpectedly, Arg1 driven L-arginine depletion promoted intestinal *S. Tm* infection and colitis (Fig. 1A-C). A delayed recovery of intestinal microbiota, an altered intraluminal metabolome and an expansion of myeloid cells with enhanced inflammatory signatures accompanied this unexpected phenotype. Dietary L-arginine supplementation restored L-arginine levels, diversity and richness of intestinal microbiota, and thus, restrained *S. Tm* replication and colitis (Fig. 1D-F). Similarly, fecal microbiota transplants (FMTs) from donor mice with a deletion of Arg1 in myeloid cells into wild-type recipients restrained *S. Tm* infection, while FMTs from wild-type littermates into Arg1-deficient mice prevented an advanced recovery from colitis and infection.

In summary, the Arg1-mediated regulation of L-arginine availability and the subsequent consequences on microbiota composition determine the outcome of *S. Tm* infection and infection-driven colitis (Fig. 2). The identification of specific, L-arginine dependent microbiota and/or metabolites opens new avenues for therapeutic intervention against infectious pathogens.

Figure legends:

Fig. 1: **L-arginine ameliorates *S. Tm* infection.** Arg1-expressing Cx3Cr1 Cre^{-/-} x Arg1fl/fl and Cx3Cr1 Cre^{+/-} x Arg1fl/fl mice which are deficient for Arg1 in myeloid cells (A-C) or 129SV mice supplemented with a 0,3%, 1% and 3% L-arginine (D-F) were pretreated with streptomycin and infected 6 hours later with 1x10⁶ CFUs of DaroA *S. Tm* (A-C) or strain SL1344 (D-F) per oral gavage. (A+D) The concentration of L-arginine was determined by high-performance liquid chromatography (HPLC) in the feces at day 20 after infection. Bacterial replication in the feces (B+E) and the severity of colitis (C+F) were monitored by CFU plating assays and histopathological analyses of H&E-stained tissue sections at day 20.

Fig. 2: Arg1-expressing myeloid cells cause dysbiosis due to L-arg depletion, subsequently aggravating *S. Tm* infection.

Literatur:

- Nüse et al. (2023). Gut Microbes. PMID: 37358082
- Radlinski et al. (2024). PNAS 121: PMID: 39546570
- Baier et al. (2020). J. Clin Invest. PMID: 32721946

Fig. 1

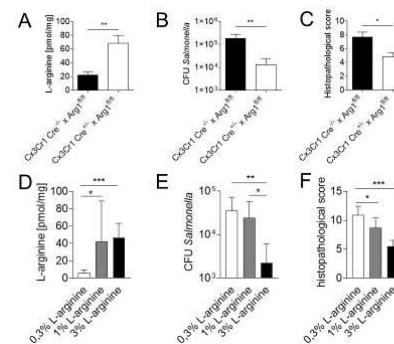
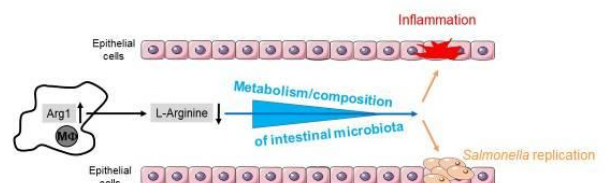


Fig. 2



P-2-31

HCMV vaccine candidate identification for solid organ transplant recipients: ULBP2-expressing HCMV mutants affect NK cell function and activation

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Purpose: HCMV infection elicits protective T and NK cell responses. Patients with inherited or acquired T/NK cell impairments, are at high risk of HCMV infection. A protective HCMV vaccine would not only greatly reduce the risk for HCMV reactivation and disease in those patients, but also lower the incidence of graft rejection. We hypothesize that a live-attenuated vaccine will elicit a broader and more efficacious immune response. In this work, we aim to achieve immune cell activation as well as viral attenuation by generating HCMV strains expressing the NKG2D ligand ULBP2.

Methods: ULBP2-expressing HCMV mutants were constructed using either the TB40 (Δ US2-US6) or the TB40R containing the immune-evasins US2-US6 and replace the UL16 region by ULBP2 which is expressed under a weak or strong promoter. Fibroblasts were infected with the respective virus mutants and analyzed by flow cytometry regarding ULBP2 expression. Cocultures of infected HFF with NK cells revealed immune cell activation and viral dissemination. Moreover, cytotoxic function of NK cells was analyzed by degranulation assays via CD107a expression.

Results: Fine-tuned, increased surface expression of ULBP2 was observed for the weak- and strong ULBP2-expressing mutants, but not in parental strains due to UL16-mediated retention. In viral dissemination assays, trends became observable that NK cells exhibit improved control regarding virus transmission upon contact to ULBP2-expressing infected cells. Furthermore, ULBP2 expression in HCMV-infected HFF resulted in a fine-tuned NKG2D downregulation depending on ULBP2 cell surface expression levels, confirming graded interaction between NKG2D and ULBP2. Moreover, NK cells get activated by the ULBP2-expressing mutants as evidenced by CD69 upregulation. Besides, the expression of ULBP2 significantly enhanced NK cell cytotoxicity indicating a beneficial effect of ULBP2 expression regarding NK cell function.

Conclusion: Overall, expression of NKG2D ligands like ULBP2 to achieve NK cell attenuation represents a promising approach for a live-attenuated HCMV vaccine development.

P-2-32

Association between *Staphylococcus aureus* carriage and individuals' neutrophil and monocyte responses to Panton-Valentine leukocidin (PVL)

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Background: Panton-Valentine leukocidin (PVL)-positive *S. aureus* are frequently associated with severe skin and soft tissue infection, particularly in sub-Saharan Africa. Nasal carriers of *S. aureus* have an increased risk of infection. However, it is unknown whether *S. aureus* carriers are particularly more susceptible to PVL than non-carriers. The aim was to compare the susceptibility of polymorphonuclear (PMN) leukocytes to PVL and inflammasome activation between *S. aureus* carriers and non-carriers.

Method: This cross-sectional study was conducted in Lambaréné, Gabon from November 2021 to July 2022. Two hundred healthy volunteers (≥ 18 years) were included and screened for nasal and pharyngeal *S. aureus* colonization. PMNs were isolated from all participants to measure their susceptibility to recombinant PVL using propidium iodide (PI) and monocytes were isolated to measure IL-1 β secretion after exposure to sub-lethal concentrations of PVL (inflammasome activation).

Results: 33 *aureus* colonization was present in 67 of 200 participants (33.5%). The proportion of PI-positive PMNs increased with increasing PVL concentration (0-5 nM). The proportion of dead cells was significantly higher in PMNs from non-carriers than in PMNs from carriers particularly at PVL concentration of 0.5 and 1 nM (Fig. 1). Similarly, inflammasome activation was stronger in non-carriers vs. carriers.

Conclusion : *S. aureus* colonization is associated with a reduced susceptibility of PMN to PVL and impaired inflammasome activation in monocytes, suggesting a certain degree of adaptation to PVL during colonization.

Figure 1: Susceptibility of neutrophils from *S. aureus* carriers and non-carriers to recombinant PVL. Symbols and error bars represent mean and standard error

Figure 2: Association between *Staphylococcus aureus* colonization and inflammasome activation in response to PVL (IL-1 β production). Red bars represent the mean. "0.02" is the p. value; Student's t-test

Fig. 1

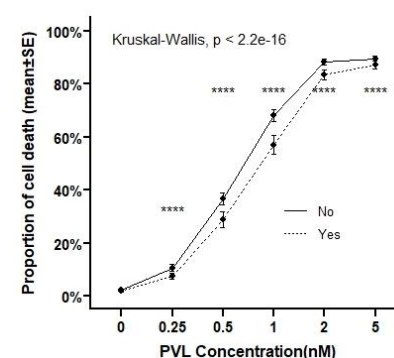
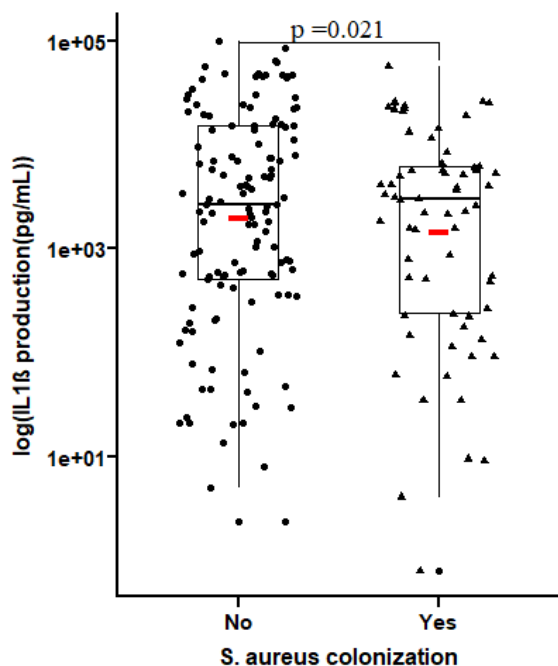


Fig. 2



P-2-33

From Gamete fusion to intervention: evaluating the transmission blocking vaccine target Hap2 in Plasmodium

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Transmission-blocking vaccines against malaria aim to prevent the parasite from infecting the Anopheles mosquito, thereby interrupting transmission. Hap2 is a fusogen expressed on male gametes and is essential for the fertilization of female gametes. Because of this central role, Hap2 is considered a promising candidate for transmission-blocking vaccines. Despite its importance, many molecular details of its function remain poorly understood. The goal of this project is to investigate the mechanism of gamete fusion and the role of Hap2 in sex-specific gamete fusion in *Plasmodium berghei*, a model organism for malaria research. The focus is on the sex-specific expression of Hap2 and the mechanisms that prevent double fertilization of female gametes. The knowledge gained is expected to provide new approaches for targeted blockade of primary fertilization. A genetically modified parasite line that produces only female gametocytes and in which Hap2 is artificially expressed will be used. This will test whether Hap2 on a female gamete is sufficient to trigger fusion with another female gamete. In addition, it will be examined whether simultaneous expression of Hap2 on both female gametes leads to inhibition of fusion. Furthermore, karyogamy—the fusion of nuclei after plasmogamy—will be analyzed. It is unclear whether this process is regulated in a sex-specific manner. Observations suggest that development up to the oocyst stage is possible with only half of the male genome, but sporozoites are not formed. This indicates that completion of the life cycle might also be possible through the fusion of two haploid nuclei.

P-2-34

Investigating the impact of the vaccination interval on antibody functionality using systems serology

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In the past, the analysis of vaccine-induced immune responses has mostly focused on binding and neutralizing antibodies. However, non-neutralizing antibody functions have also been suggested to contribute to protection against pathogens. Systems serology refers to a comprehensive analysis of the biophysical features of antibodies as well as neutralizing Fab and non-neutralizing Fc functionality. It combines high-throughput methods with computational analyses to gain a more profound understanding of vaccine-induced immune mechanisms and the identification of correlates of protection. At the University Medical Center Hamburg-Eppendorf, the viral vector vaccine candidate MVA-MERS-S against the Middle East respiratory syndrome coronavirus is currently being evaluated in a phase 1b clinical trial. As part of this trial, blood samples are collected from study participants longitudinally post vaccination, providing the opportunity to obtain information about the temporal course of the vaccine-induced immune response.

In a preceding first-in-human MVA-MERS-S phase 1a study, it was shown that a booster vaccination one year after the first vaccination series led to the enhancement of not only neutralizing but also Fc-mediated antibody functions compared to the primary vaccination series. Building on this finding, the impact of different time intervals between the first and second vaccination is now being investigated in the present phase 1b study. First analyses revealed that a longer vaccination interval induced higher binding and neutralizing antibodies, as well as higher antibody-dependent neutrophil phagocytosis (ADNP) capacity.

Additional non-neutralizing antibody functions, such as antibody-dependent cellular cytotoxicity (ADCC), cellular phagocytosis (ADCP), and complement deposition (ADCD), are currently being analysed using a systems serology approach. Comprehensive characterization of the humoral immune response following MVA-MERS-S vaccination will contribute to a better understanding of vaccine-induced immune mechanisms, which may inform future vaccination strategies.

P-2-36

Development and characterization of a novel T-cell-targeting hepatitis C virus vaccination strategy

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Question: After both cured and spontaneously resolved hepatitis C virus (HCV) infections, reinfection is possible and thus, the need for a prophylactic HCV vaccine is evident. We are developing a novel prime-boost vaccination strategy aimed at T-cell activation, with the priming step utilizing standard mRNA constructs encoding consensus sequences of non-structural proteins that entail immunodominant T-cell epitopes. In the boost step, a subgenomic replicon (SGR) of HCV is used as a non-infectious, but replication-sufficient vaccine candidate.

Methods: For efficacy evaluation, we established a simplified system where Huh7-Lunet cells with ectopic HLA-A2 expression are transfected with *in vitro* transcribed vaccine candidate RNA and subsequently cocultured with HCV-specific CD8⁺ T-cells. T-cell activation is assessed via cytokine staining and for SGR candidates, antiviral effects are evaluated through quantification of luciferase expression, a reporter protein in the SGR.

Results: We compared two vaccine candidates for the priming step – one encoding NS3-NS4A-NS5B resulting in membrane-bound proteins and one encoding a polyprotein comprising NS3 and NS5B lacking the anchoring domain, making the polyprotein soluble and potentially inducing stronger T-cell activation. In the coculture system, we achieved stronger T-cell activation with the membrane-bound candidate. For the boost step, we evaluated SGRs with low and high replication fitness, and demonstrated that SGRs with high replication fitness induce a more pronounced T-cell response and antiviral effect, indicating a stronger adaptive immune response.

Conclusion: This project explores a novel HCV vaccination approach with the goal of eliciting a robust CD8⁺ T-cell response. In the next steps, we are conducting *in vivo* experiments to explore the immunogenicity of our mRNA vaccine candidates in immunocompetent mice. Replication competence and delivery of our SGR candidates will be studied in human liver chimeric mice and to further establish the replicon vaccination candidate, we will use the rodent hepatitis virus (RHV) as a model system.

P-2-37

Class switch toward IgG2 and IgG4 is more pronounced in BNT162b2 compared to mRNA-1273 COVID-19 vaccinees

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Objectives: Vaccination against SARS-CoV-2 induces antibodies that reduce the risk of severe disease. Because IgG subclasses differ in their ability to activate complement, to bind Fc receptors and neutralize viruses, it is crucial to understand how IgG subclass responses differ between vaccine platforms.

Design: IgG1, IgG2, IgG3, and IgG4 binding antibodies against SARS-CoV-2 trimeric spike protein, receptor-binding domain, and S1/S2 subunits responses were quantified using a multiplex immunoassay, after a booster dose of either BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) in a healthy cohort (n = 165) who had received two previous vaccine doses.

Results: Boosting increased all subclass IgG levels, except for S1-specific IgG1 and S2-specific IgG2. However, IgG2 and IgG4 levels were significantly higher in BNT162b2 than in mRNA-1273 vaccinees ($P = 0.0313$ [IgG2 S] and $P = 0.0106$ [IgG4 RBD], $P = 0.0070$ [IgG4 S1]). Individuals who had previously received a non-mRNA vaccination showed no significant increase in IgG2 ($P = 0.4909$ [S]) and IgG4 ($P = 0.0607$ [S]) post-boost.

Conclusions: Vaccine-specific differences post-booster vaccination were identified and may drive the class switch between IgG2 and IgG4 responses. Given their different roles, these subtle differences may ultimately also affect long-term immunity and protection.

P-2-38

Pneumococcal serotype distribution among people living with HIV (PLWH) in the German CAPNETZ-Cohort

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Objectives: Community-acquired pneumonia (CAP) is a major cause of hospitalization among people living with HIV (PLWH) [1,2], with *Streptococcus pneumoniae* identified as the leading pathogen [3]. Identifying pneumococcal serotypes is critical for optimizing vaccination strategies, as different vaccines target distinct serotypes. While pneumococcal conjugate vaccines (PCVs) and polysaccharide vaccines (PPSVs) have been widely used, their serotype coverage has not been investigated within the subpopulation of PLWH. This study investigated the distribution of pneumococcal serotypes among PLWH in the German CAPNETZ cohort and compared it with an age- and sex-matched HIV-negative control group.

Methods: A total of 73 HIV-positive participants with CAP were matched in a 1:3 ratio with 218 HIV-negative controls. Pneumococcal detection was conducted using microbiological tests, including blood cultures, sputum analysis, nasopharyngeal swabs, conventional pneumococcal urine antigen tests (PUAT), with additional serotype-specific urine antigen detection (SSUAD) assays carried out at Pfizer's vaccine research center (NY).

Results: Of the 291 participants, 55 were positive for *Streptococcus pneumoniae*, mostly identified by nasal swab PCR (n=40) followed by SSUAD (n=19). From these, the most identified serotype was serotype 3, followed by serotype 4. No significant differences in serotype distribution were observed between the case and control group. All serotypes detected in the case group were covered by PPSV23, but not by PCV13 or PCV20, although it was only one case outside this coverage.

Conclusion: Distribution of pneumococcal serotypes among PLWH with CAP in the CAPNETZ cohort was similar to that of HIV-negative individuals. The study findings confirm that the vast majority of detected serotypes could be covered by both PPSV23 as well as PCV 20. More studies with larger sample size are needed, to confirm these preliminary data.

P-2-39

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) Influenza

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Infections by respiratory viruses, such as influenza viruses remain significant international public health concerns despite the availability of vaccines and anti-viral agents. While patients with hematological and oncological diseases remain amongst the most vulnerable groups of patients with regard to morbidity and mortality, they show poor vaccine response concurrently. Immunologic data in this population are limited and mainly focus on serologic parameters. However, cellular responses including, most notably, T cell responses often seem to be induced more reliably in those patients than humoral response.

To gain further insights into vaccine-induced immune responses and thereby improve protection from influenza infection in hematological and oncological patients, we are currently establishing a structured prospective research program (RESPONSE). Cooperation partners include the Institute of Virology (Prof. F. Klein, Cologne, Germany), the

Institute for *Infection Research and Vaccine Development* (IIRVD; Prof. M. Addo, Hamburg, Germany), and the *Systems Immunology Lab Tübingen* (Prof. F. Wimmer, Tübingen, Germany). 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 February 2025. The established cohort comprises patients with further hematological malignancies and follow-up up to 3 years.

By this research program we aim to improve vaccination schedules for our patients and hence promote prevention of vaccine-preventable infections by respiratory viruses, especially influenza viruses.

P-2-40

Targeting *Enterococcus faecalis* virulence with patient-derived monoclonal antibodies against EbpA pilus protein

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Background: The Gram-positive nosocomial pathogen *Enterococcus faecalis* (EF) accounts for 10–20% of infective endocarditis (IE) cases and represents the second leading cause of nosocomial urinary tract infections. Its pathogenicity is mediated by different virulence factors, including the pilus protein EbpA, which facilitates adhesion to fibrinogen and mediates biofilm formation. Importantly, EbpA has been shown to play a role in the establishment of endocarditis and catheter-associated urinary tract infections in animal models.

Methods: We characterized the human antibody response to EbpA across different cohorts, encompassing patients with enterococcal bacteremia (including cases of IE) as well as patients with cystic fibrosis (CF), a population prone to polymicrobial infections and recurrent antibiotic exposure. We isolated EbpA-specific B cells from individuals with high antibody titers and performed single-cell sequencing of immunoglobulin variable regions. This enabled the generation of patient-derived monoclonal antibodies (mAbs) with confirmed specificity to EbpA by enzyme-linked immunosorbent assay and Western blot.

Results: IE patients demonstrated elevated titers against EbpA compared to CF patients, healthy controls, and

immunocompromised patients following EF infections. Antibody repertoire profiling revealed a broad B cell receptor diversity with distinct clonal clusters. Patient-derived mAbs exhibited high affinity to EbpA.

Conclusion: Patient-derived mAbs targeting the EF pilus EbpA may be used to inhibit enterococcal virulence upon further characterization of their ability to inhibit biofilm formation and adhesion to fibrinogen. Potential clinical applications include therapeutic intervention in acute EF infections as well as the implementation of passive immunization strategies for high-risk individuals susceptible to invasive enterococcal disease.

P-2-41

Durable HIV-1 suppression by AAVMYO-expressed potent broadly neutralizing antibody

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Broadly neutralizing antibodies (bNAbs) represent a promising approach for HIV-1 treatment and prevention. While bNAbs demonstrate antiviral activity and favorable pharmacokinetics in humans, their use can be limited by the need for repeated administrations. Recombinant adeno-associated viruses (rAAVs) provide a potential solution by enabling sustained protein expression following a single gene transfer. However, early attempts using AAV2- or AAV8-based vectors for HIV-1 bNAbs yielded low neutralization titers. To advance the concept of AAV-mediated bNAb expression, we developed rAAV vectors using the myotropic AAVMYO capsid that encode for the highly potent CD4 binding site antibody 04_A06.

In NRG mice, a single i.v. injection of 10^{12} rAAVMYO-04_A06 genomes based on an optimized bicistronic AAV plasmid achieved high A06 serum levels (geometric mean of 862 µg/ml) that were maintained for >9 months. To assess the antiviral activity of rAAVMYO-bNAb therapy, we treated HIV-1_{YU2}-infected humanized mice with constructs encoding for A06, the less potent bNAb VRC01, or the SARS-CoV-2-specific antibody DZIF-10c. In virologically suppressed mice pre-treated with antiretroviral therapy (ART), viral rebound was observed in all rAAVMYO-DZIF-10c-treated mice within 3 weeks of ART interruption. In contrast, in the rAAVMYO-VRC01- and rAAVMYO-04_A06-treated groups only 2/7 and 0/8 mice rebounded within 8 weeks after stopping ART, respectively. In addition, we investigated rAAVMYO therapy in the more challenging setting of active viral replication at baseline. In viremic mice, rAAVMYO-VRC01 failed to control viremia, and single-genome sequencing of plasma viruses revealed the emergence of HIV-1 envelope amino acid substitutions associated with resistance to VRC01. In contrast, a single injection of rAAVMYO-04_A06 resulted in viral suppression in all treated mice within one week of administration that was maintained for >230 days without viral rebound.

Our findings highlight the potential for combining myotropic AAVs with highly potent HIV-1 neutralizing antibodies to achieve long-term viral suppression after a single-injection.

P-2-42

Immunogenicity of repeated COVID-19 vaccination in immunocompromised patients (Auto-COVID-VACC): Preliminary results from a multicentre, prospective, non-interventional study

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Background: Immunocompromised patients are at an ongoing high risk for severe COVID-19. Vaccination has been proven to be an effective measure in preventing severe SARS-CoV-2 infections, while data on B-cell and T-cell response are lacking in this population.

Methods: This multicentre, prospective, non-interventional study aimed to determine the immunogenicity and reactogenicity of a COVID-19 vaccination strategy in immunocompromised patients. Patients received up to eight COVID-19 vaccinations in four-week intervals depending on their individual anti-SARS-CoV-2-spike-IgG level as standard of care (SOC). Blood samples were drawn immediately before each vaccination and during a 6-months follow-up.

At each study visit, humoral and cellular immunity was analysed. Humoral immunity was investigated by measuring anti-SARS-CoV-2-spike-IgG levels, anti-SARS-CoV-2-nucleocapsid antibodies and Omicron-BA.1-specific neutralising antibodies. Cellular immunity was measured by T-cell-specific IFN- γ and IL-2 secretion upon SARS-CoV-2-spike-protein stimulation using interferon-gamma release assay, Fluorospot- and FACS analysis. All invasive procedures were performed as SOC.

Results: The study has enrolled 49 patients. Antibody responses were measured in all patients after each visit. Anti-spike-IgG levels show a steady incline in most patients following repeated vaccination and remained stable during follow-up. (**Figure 1**). Patients required a mean of 3.5 vaccine doses to reach an adequate immune response, which was defined as an anti-SARS-CoV-2-spike-IgG level

≥847 BAU/ml. Two female patients (31 and 55 years old) with an aggressive B-cell lymphoma, who received CAR-T cell therapy, failed to reach an anti-spike-IgG level ≥847 BAU/ml after 8 vaccine doses. With regard to cellular response, preliminary results also show an increase in T-cell immune response in most patients following vaccination. We here show results of one patient with a decline in antibody levels despite repeated vaccination, while T-cell response increases (**Figure 2**; patient 01-007).

Conclusions: Our preliminary findings show a boosted B-cell response following repeated vaccination in most patients. However, even with minimal antibody response, T-cell response may still offer protection. 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 leading to low immune responses in immunocompromised patients vaccinated against COVID-19, as well as the impact of repeated vaccination on B-cell and T-cell responses.

Fig. 1

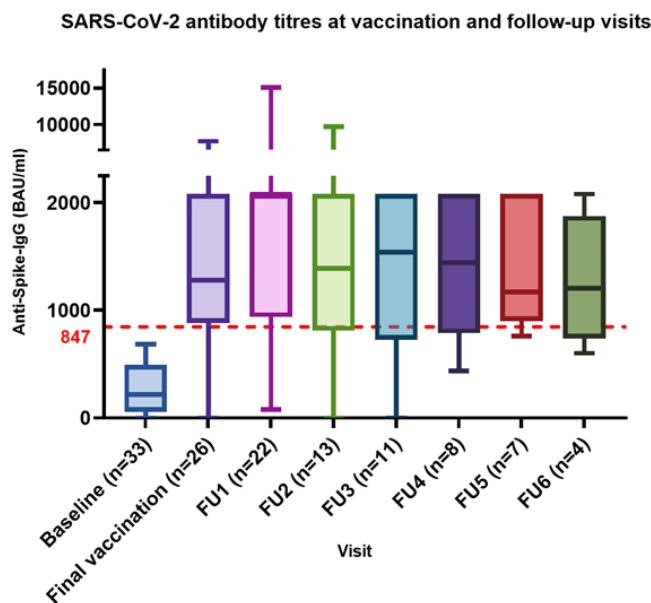
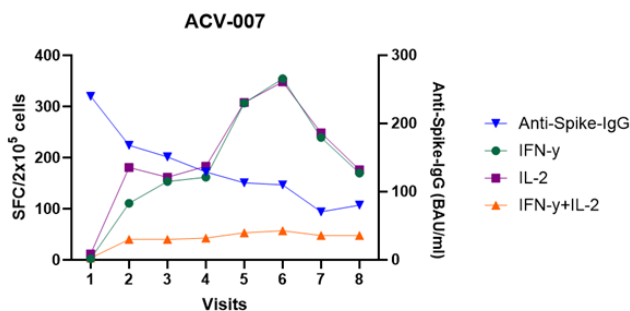


Fig. 2



P-2-43

Addressing a novel immune escape of Hepatitis B virus, reducing T cell killing of infected hepatocytes by multiomics analysis and target molecule screening
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Introduction: Chronic hepatitis B virus (HBV) infection currently affects around 296 million people worldwide, and is caused by a failure of virus-specific CD8 T cells to eliminate HBV-infected hepatocytes. The reason for the failure of anti-viral CD8 T cell immunity is so far ill-defined. We have previously identified that adenoviral or lymphocytic choriomeningitis virus infection shifts the balance from survival to apoptosis in virus-infected hepatocytes upon TNF- or Fas-ligand stimulation. This allows for more efficient elimination of virus-infected hepatocytes by CD8 T cells using TNF or Fas-ligand as effector molecules. However, HBV-infected hepatocytes escape viral sensitization and consequently cell death induction. This might explain the delayed clearance of HBV from the liver or even the development of chronic Hepatitis B.

Methods: To unravel the mechanisms underlying viral sensitization and escape of HBV, we performed systematic proteomic and transcriptomic analysis of virus-infected livers across five conditions: Ad-HBV, Ad-CMV-GL, Ad-CMV-GL/HBV, Ad-Empty, and uninfected controls. To study the biological variances in all omic layers, we employed Multi-Omics Factor Analysis (MOFA) and combined it with protein-protein interaction network pathway enrichment analysis and predicted therapeutic target. We employed a transformer language model, a generative AI method, to predict novel small molecules and dock them with the target.

Results: Multi-Omics Factor Analysis (MOFA) identified HBV-specific factors linked to mitochondrial regulation. Comparative pathway enrichment revealed that oxidative phosphorylation (OXPHOS) and the electron transport chain (ETC) were strongly upregulated in HBV-infected hepatocytes, whereas adenoviral infection drove their downregulation. Network integration with the MitoCarta dataset highlighted ETC-calcium signaling cross-talk as central to HBV-mediated protection. Atp5f1d, an ETC subunit, emerged as an HBV-associated hub gene strongly connected to calcium signaling. Critically, Atp5f1d interacted with Vdac1, a mitochondrial channel regulating calcium flux and apoptosis. Literature supports that Vdac1 upregulation enhances mitochondrial stability, placing the Atp5f1d-Vdac1 axis at the core of HBV-induced apoptotic escape. To translate these findings towards therapy, we commissioned a generative artificial intelligence (AI) drug discovery pipeline trained on 2 million chemical compounds and predicted 221 compounds.

Conclusion: HBV preserves mitochondrial resilience by reinforcing ETC and calcium signaling networks, thereby preventing TNF- and Fas-ligand-mediated apoptosis of infected hepatocytes. By combining multi-omics integration, network analysis, and AI-based drug discovery, this study provides mechanistic insights into HBV's immune evasion and establishes a framework for therapeutic strategies aimed at restoring apoptotic sensitivity, thereby supporting immune-mediated viral clearance.

P-2-44

Broadly reactive S2-antibodies in individuals vaccinated against MERS-CoV and SARS-CoV-2 infection

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Since the COVID-19 pandemic, there has been a decline in MERS-cases with 682 reports 2017-2020 vs. 44 reports 2021-2024; moreover, in recent outbreaks in 2025, 9 of 11 infections were non-fatal, among them asymptomatic cases. Understanding how prior immunization may shape coronavirus-specific immunity is essential for designing pan-coronavirus vaccines and therapeutics.

evaluated spike S2-specific B cell- and antibody responses in distinct cohorts of individuals i.) vaccinated with MVA-MERS-S, ii.) immunized against SARS-CoV-2, iii.) hybrid MERS-CoV/SARS-CoV-2-immunized, or iv.) naive for MERS-CoV and SARS-2-CoV-2 (prepandemic controls). We evaluated plasma antibody reactivity by spike ELISAs and neutralization assays. Moreover, we isolated and sequenced 768 single B cells with cross-reactivity to MERS-CoV and SARS-CoV-2 from 10 SARS-CoV-2-immunized donors and two individuals after sequential MVA-MERS-S and COVID-19 vaccination.

We found low but distinct serum antibody binding to MERS-CoV spike protein in 57 of 61 post-SARS-CoV-2-pandemic plasma samples. This cross-reactive plasma antibody response was predominantly focused on the S2-subunit. Follow-up of two MVA-MERS-S-vaccinated subjects over 4 years revealed declined RBD/S1 antibody titers, with a MERS-CoV-S2-antibody boost after SARS-CoV-2 immunization. Notably, we detected MERS-CoV/SARS-CoV-2-S-protein cross-reactive B cells in both the COVID-19-vaccinated as well as MVA-MERS-S and COVID-19-vaccinated individuals (0.018%±0.017 and 0.013%±0.004 of IgG+ B cells). To decipher these findings on a molecular level, single cell sorting and B cell-receptor sequencing were performed, revealing a polyclonal response biased towards the usage of κ light chains (93.86%).

Characterization of 126 monoclonal antibodies derived from memory B cells revealed the presence of cross-neutralizing antibodies in SARS-CoV2 single-immunized as well as cross-immunized individuals. The most broad and potent belonged to a public clonotype facilitating gene-segment IGHV1-46 and a 9-residue CDRH3.

Our preliminary results reveal the induction of MERS-CoV-S2 cross-reactive antibodies and memory B cells after SARS-CoV-2 immunization and may inform future vaccination strategies and the design of a pan-coronavirus therapy.

P-2-45 **mRNA-encoded broadly neutralizing antibodies for HIV-1 immunotherapy**

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Despite effective antiretroviral therapy, HIV remains a major public health burden with 1.3 million new infections and 630,000 deaths globally in 2024. Broadly neutralizing antibodies (bNAbs), such as the potent CD4 binding site-targeting antibody 1-18, present a promising approach for treatment and prevention. However, bNAbs utilization and accessibility remain challenging due to viral diversity and escape, long manufacturing processes, and high costs. mRNA-based delivery of bNAbs is a potential solution to overcome some of these hurdles. To investigate the concept of mRNA-encoded bNAbs for HIV-1 therapy, we determined pharmacokinetic parameters, antiviral activity, and viral escape pathways of an mRNA construct encoding for the half-life-extended 1-18-LS variant *in vivo*.

To confirm systemic and functional expression of mRNA-encoded bNAbs, we performed serum ELISAs and neutralization assays after intravenous injection of mRNA lipid nanoparticles in humanized mice. Following the administration of LNP-formulated mRNA encoding for 1-18-LS, serum antibody levels reached >200 µg/mL on day 1 and mean levels of 63 µg/mL on day 7 post injection. Antibody levels determined by ELISA strongly correlated with high serum neutralization against the HIV-1 YU2 and X2278 strains (r=0.92 and r=0.83, respectively). Notably, 1-18-LS-mRNA injection resulted in higher mean antibody levels on day 7 than intravenous administration of 0.5 mg recombinant 1-18-LS protein.

To determine the antiviral activity of mRNA-encoded 1-18-LS *in vivo*, we longitudinally analyzed plasma viral loads in HIV-1_{YU2}-infected humanized mice. While untreated mice maintained high levels of viremia, weekly injections of formulated 1-18-LS-mRNA resulted in a mean reduction of viral loads by 2 log₁₀. Importantly, all mice remained suppressed during the treatment period without viral rebound. Single-genome sequencing analysis of viruses emerging after the end of treatment did not indicate selection of escape mutations, highlighting that application of mRNA-encoded 1-18-LS can effectively prevent HIV-1_{YU2} resistance.

Our results provide evidence for the potential of mRNA-encoded HIV-1 bNAbs therapy. To further validate our findings, the efficacy of LNP-formulated 1-18-LS-mRNA will be determined in humanized mice infected with patient-derived primary HIV-1 isolates.

P-2-46 **Isolation and characterization of comprehensive antibody candidates targeting Human Cytomegalovirus glycoprotein B**

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HCMV is a highly prevalent dsDNA virus that can pose a great risk to patients with immune-related suppression events, e.g., after SOT, and unborn children by congenital infection. However, therapeutic options are limited and despite repeated efforts, no vaccine candidates have been approved yet.

In recent years, the B-cell response has gained importance as a future-oriented approach for both the treatment and prevention of infectious diseases. Given the complexity of the HCMV infection machinery and the virus' high cell tropism, comprehensive analyses of the humoral response are crucial to understand the key characteristics of protective monoclonal antibodies (mAbs).

One important antibody target site is the membrane fusion protein gB, which is indispensable for all infection pathways. It mediates membrane fusion during HCMV infection and transitions irreversibly from the reactive pre-fusion to a stable post-fusion conformation. Thus, it represents one of the most important surface structures for infection and hence, an interesting target for new therapeutic approaches.

We aim to close gaps in our understanding of gB-directed immunity by establishing a large panel of gB-targeting mAbs and identifying broadly neutralizing candidates. To this end, we applied a new pre-fusion-stabilized gB protein to isolate pre-fusion specific B cells for the first time and therefore enabled the novel analysis of a prominent HCMV target site.

To do so, we screened 100 buffy coats for HCMV serum reactivity and included six individuals that were pre-selected from a group of 9,000 HCMV+ donors due to their outstanding neutralization capacity. To guarantee a successful sort, we set up a novel gating strategy and labelled both pre- and post-fusion gB with two fluorophores each, which allowed us to sort double positive cells. In total, we isolated 2,979 pre- and post-fusion specific as well as cross-reactive IgG+ memory B cells using FACS at single cell level. After isolation, we decoded the DNA sequences and analyzed them by defined criteria such as clonal relationships and mutation rates, including overlapping clones between different donors, to choose 185 promising candidates for *in vitro* production. We verified binding via Elisa and an FC-Receptor assay and began assessing the neutralization ability, already having identified some prospective neutralizers.

Next, we aim to uncover novel epitopes by epitope mapping and structural analyses of the strongest candidates. Additionally, we work on establishing a fusion assay, that serves us to analyze the influence of the isolated gB antibodies on the membrane fusion of HCMV and the host cell.

The data obtained from this project will be a further advance in the understanding of HCMV biology and the resulting immune response and will provide the basis for further research projects. Paired with the identification of novel promising candidates, it will offer the foundation for new preventive and therapeutic strategies.

P-2-47

Immunogenicity of MVA-based vaccines targeting Nipah virus fusion and matrix proteins

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Introduction: Nipah virus (NiV) is zoonotic virus that is a member of the *Paramyxoviridae* family and causes a rare but severe illness in humans and pigs. The reservoir species are fruit bats of the *Pteropus* genus, which are found in Asia, Africa and Australasia. Outbreaks have occurred in Malaysia, Bangladesh and India, with the most recent one taking place in Kerala in July 2025. Human infections are characterised by severe respiratory and neurological symptoms and

encephalitis (case fatality = 40-75%). To date no vaccines and therapeutics have been approved, which highlights the need to develop vaccines against NiV. Previous studies indicated that strong T cell responses correlate with better disease outcomes.

Objective: We previously developed two candidate vaccines targeting the glycoprotein (G) of NiV using our Modified Vaccinia virus Ankara (MVA) viral vector platform. In this study, we developed two additional candidate vaccines that target the fusion protein (F) and the matrix protein (M) of NiV, which we tested for the activation of NiV-specific T cell responses in mice.

Materials & Methods: We generated recombinant MVA viruses expressing NiV-F and NiV-M by cloning the modified target gene into a MVA vector plasmid and introduced it into the MVA genome by homologous recombination, followed by serial plaque passaging. Recombinant viruses were then characterised by standard techniques to confirm protein expression, genomic stability and genomic integrity. To test immunogenicity, IFNAR-/- mice were immunised once or twice (28-day interval) with 10⁸ PFU of MVA-NiV-F or MVA-NiV-M. Mice were sacrificed at specific times after immunisation, and splenocytes were isolated and restimulated with NiV-F and NiV-M-specific peptides. T cell immunity was measured by IFN- γ ELISPOT assay and intracellular cytokine staining plus FACS analysis.

Results: T cell immunity was observed after a single and two immunisations with MVA-NiV-F and MVA-NiV-M. At 8 days post-prime MVA-NiV-F stimulated IFN- γ -producing CD8 T cells, while MVA-NiV-M stimulated IFN- γ producing CD4 T cells. At 14 days post-boost we observed NiV-F-specific IFN- γ producing CD4 and CD8 T cells after MVA-NiV-F vaccination, and NiV-M-specific IFN- γ producing CD4 T cells after MVA-NiV-M immunisation. Importantly, after both vaccination protocols, NiV-specific T cells mostly produced both IFN- γ and TNF- α (mean > 60% for CD4 T cells and mean > 70% for CD8 T cells) after both immunisation protocols, indicating polyfunctionality.

Conclusion: In conclusion, our MVA-NiV-F and MVA-NiV-M candidate vaccines induce robust, polyfunctional T cell responses, even after a single immunisation which provides a promising approach to develop improved vaccination strategies against Nipah virus. In the next step, their efficacy will be analysed in a mouse challenge model.

P-2-48

Adaptive immune responses following RSV infection and immunization in human immune organoids derived from children and adults

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Introduction: Respiratory syncytial virus (RSV) causes a significant burden of acute respiratory illness, particularly for older adults and infants. During the last three years, three vaccines have been developed and approved for adults: the two subunit vaccines RSVpreF (Abrysvo) and RSVpreF3 OA (Arexvy) and the mRNA vaccine mRNA-1395 (mResvia).

Currently, there are no approved vaccines for infants. Vaccination during pregnancy as well as monoclonal antibodies are mediating passive protection. However, breakthrough infections can occur. RSV vaccination of children may offer a more durable protection.

Our understanding of the human vaccine induced immunity, relies on animal models and analysis of peripheral blood mononuclear cells (PBMCs) of vaccinated individuals. However, analysis of peripheral blood cells cannot paint a full picture of immune priming and the interaction of immune cells in lymphatic tissue, which is responsible for the development of a long-lasting protective immunity. This gap can be bridged by using human immune organoids, such as tonsil organoids, as an in vitro model for adaptive immune responses to infections and vaccine candidates.

Methods: Tonsil specimens of infants and adults were collected from routine procedures. Immune organoids were co-cultured with the human lung cell line A549, enabling us to investigate immune responses towards both, virus infection and vaccination.

Results: RSV infection resulted in an increase in plasmablast counts in immune organoids derived from tissue specimens of both - adults and infants. Following immunization with the different RSV vaccines, differences in immune cell populations and activation were observed. Furthermore, an increase in RSV-specific B cells was observed following infection and vaccination.

Conclusion: RSV infection and immunization with different RSV vaccines induces distinct immune signatures in immune organoids derived from adults and infants.

P-2-49

Detection of pre-existing humoral immunity against influenza virus H5N1 clade 2.3.4.4b in unexposed individuals

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The spill-over of Influenza A virus H5N1 clade 2.3.4.4b from cattle to humans highlights the risk of a human H5N1 pandemic. Given the impact of pre-existing immunity on the course and severity of viral infections, we comprehensively assessed the humoral immunity against the H5N1 A/Texas/37/2024 isolate in H5N1-naïve individuals. To this end, we performed complementary binding and neutralization assays on 66 subjects and ranked activities among a panel of 76 influenza A virus isolates. We detected low but distinct cross-neutralizing titers against A/Texas/37/2024 with a 3.9 to 15.6-fold reduction compared

to selected H1N1 or H3N2 strains. By cloning and evaluating 136 monoclonal antibodies from memory B cells, we identified potent A/Texas/37/2024-neutralizing monoclonal antibodies in five out of six investigated individuals. These antibodies cross-neutralize H1, compete with antibodies targeting the HA stem, and protect mice from lethal H5N1 challenge. Our findings demonstrate partial pre-existing humoral immunity to A/Texas/37/2024 in H5N1-naïve individuals.

P-2-50

Screening Pipeline for Predicting bNAbs Sensitivity in People living with HIV Reveals Divergent Profiles of Elite vs. Non-Neutralizers and Extensive Inpatient Variation

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Background: Broadly neutralizing antibodies (bNAbs) that target the HIV-1 envelope glycoprotein (HIV-1 env) represent a promising strategy for both treatment and prevention of HIV-1 infection. However, the high genetic variability of HIV-1 env poses a major obstacle to the clinical application of bNAbs. To overcome this, pre-screening of people living with HIV (PLWH) for bNAbs sensitivity is essential. Currently, rapid and scalable assays for such pre-screening are lacking. To address this gap, we developed a pipeline for the rapid amplification and characterization of HIV-1 env sequences from the proviral reservoir.

Methods and Results: We established a well-matched cohort of elite neutralizers and non-neutralizers, characterized by significant differences in mean neutralizing activity (EN: 75.1%; NN: 15.0%, $p < 0.0001$). The cohorts were comparable in gender distribution and time since HIV infection, but differed slightly in CD4 count and geographic origin. Using PBMCs, we performed single-genome amplification of HIV-1 env sequences and applied PhytClust, a phylogenetic clustering algorithm, to identify representative sequences from each individual. These were subsequently cloned into envelope expression vectors to generate pseudoviruses carrying patient-derived envelopes.

To date, we have isolated 630 HIV-1 env sequences (EN: 326; NN: 304), with a median of 9 sequences per patient. Of these, 86% ($n=540$) have been successfully sequenced, and 67% ($n=360$) were fully functional and free from ambiguities in next-generation sequencing, confirming their origin from single proviruses. The next phase will involve testing these sequences for sensitivity to a panel of bNAbs.

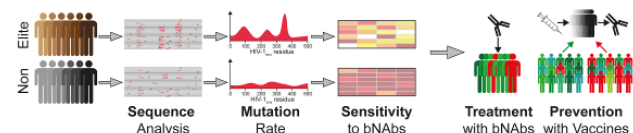
Conclusion and Outlook: We have established a high-throughput, sequence-based assay pipeline to study HIV-1 env diversity and bNAbs sensitivity in PLWH. This approach enables detailed comparisons of proviral landscapes between neutralizing and non-neutralizing individuals. In the future, we aim to integrate these datasets with computational tools to predict individual bNAbs susceptibility directly from env sequence data, supporting personalized HIV cure strategies.

Fig. 1

Aims



Aim 1: This study aims to develop a HIV_{env} screening tool using SGA and bioinformatics-based neutr. assays for characterization



Aim 2: This study investigates how elite and non-elite neutralizers impact the viral reservoir and viral fitness in HIV persistence and evolution.

P-2-51

Human microRNome and transcriptome profiling identifies a dose-dependent signature induced by the Ebola vaccine rVSV-EBOV

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Introduction: The recombinant vesicular stomatitis viral vector vaccine expressing the EBOV, (Ebola virus, species *Orthoebolavirus zairensis*, formerly Zaire Ebola virus) glycoprotein (rVSV-EBOV, represents a major advance in vaccine development and prevention of Ebola virus disease. The replicating viral vector vaccine demonstrated a high level of protection during ring vaccination in recent Ebola virus epidemics. Despite its proven efficacy, the molecular mechanisms underlying its protective function remain incompletely understood. MicroRNAs (miRNAs), which regulate gene expression by targeting messenger RNAs (mRNAs), have been implicated in modulating immune signaling pathways and may play a role in vaccine-induced responses.

Methods: A Phase I clinical trial was conducted in thirty healthy adult volunteers who received one of three vaccine doses (3×10^5 , 3×10^6 , or 2×10^7 plaque-forming units), assessing safety and immunogenicity.

In this study, we evaluated expression profiles of intracellular miRNAs at four different time points (day (d) d0, d1, d3, d14) following rVSV-EBOV immunization. For this approach, we isolated RNA from PBMCs and evaluated expression level via qPCR and NGS. To further investigate expression levels of miRNAs we performed Small RNA Seq. Next, identified microRNAs were correlated with neutralizing antibody responses.

Results: Distinct dose-dependent expression patterns of intracellular miRNAs and mRNAs were observed, particularly at day 1 post-vaccination. Fourteen miRNAs were differentially expressed, most of which were downregulated, while approximately 680 differentially expressed genes (DEGs) were identified in the high-dose group. Ingenuity Pathway Analysis suggested that roughly 160 DEGs could

be linked to the differentially expressed miRNAs, indicating potential regulatory interactions between these molecules.

Conclusion: This study provides new insights into the immunological networks and regulation of gene expression following rVSV-EBOV vaccination. The identification of distinct miRNA and mRNA signatures following immunization highlights potential molecular mechanisms that contribute to vaccine-induced protection. These findings may support the identification of predictive immune markers and inform future vaccine strategies.

P-2-52

Engineering of myotropic AAV vectors for high in vivo expression of broadly neutralizing anti-HIV-1 antibodies

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Broadly neutralizing antibodies (bNAbs) have the potential to reshape HIV-1 management across treatment-, prevention- and cure-focused research. A major prerequisite is to maintain sufficient bNAb levels in the blood, which is currently realized by repeated administration and which is associated with numerous drawbacks and challenges. Recombinant adeno-associated virus (rAAV)-mediated gene transfer offers an alternative delivery mechanism, which can provide long-term bNAb expression. However, intramuscular injections of vectors based on wild-type AAV1 or AAV8 resulted in only low bNAb titers in humans. This illustrates that improved vectors based on optimized viral capsids and bNAb expression cassettes will be critical to further advance this concept.

Here, we describe the development of novel rAAV vectors, based on the myotropic AAVMYO capsid protein, for the continuous 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 (WPRES) coupled to a SV40 polyadenylation signal. Transcription levels were initially assessed by RT-qPCR in transfected HEK293T cells, resulting in a ~2-fold higher expression in comparison to our "in house" primal construct, which was used in previous proof of concept studies (data not published). In transfected HEK293T and Huh7 cells, functional bNAb expression was confirmed by HIV-1BG505 ELISA, showing an up to ~8-fold increase of bNAb expression in the cell supernatants. Under the control of different promoters, a single intravenous (i.v.) injection of 1012 rAAV genomes led to a high (geometric mean of ~900 µg/ml) and durable bNAb expression in NOD-Rag1null IL2rgnull (NRG) mice that was maintained for >9 months. To add up to these promising results and to even further levy

the safety profile of our vectors, a subsequent CRISPR/Cas9 based inactivation of administered AAVs is currently investigated.

HIV-1YU2-infected mice were successfully treated with a myotropic rAAV, encoding for the latest anti-HIV-1 bNAb isolate A06 (Abstract: Chiara Hornung – Cologne). Our findings highlight the great potential of myotropic AAV-mediated gene transfer in general and in particular towards the aim to achieve long-term immunological control of infectious diseases (e.g. HIV-1) after a single intravenous administration.

P-2-53
Developing a Prototype Tool for Harmonized Data Discovery in DZIF Studies: The CoreDataSet Explorer

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Question: How can we improve the visibility and accessibility of collected data across DZIF studies to enable better data harmonization and facilitate cross-study research? Heterogeneous data collection practices across different studies currently limit the ability to compare, integrate, and reuse valuable research data. Researchers need an efficient way to discover which studies contain relevant data before accessing detailed databases.

Methods: We developed a prototype web-based platform, the DZIF CoreDataSet (CDS) Explorer, using data from the TIARA study as a proof-of-concept. The Explorer was designed to systematically present and explore the core data elements defined in the DZIF CDS. The platform allows researchers to discover which basic data elements are collected across studies, visualize data structures, and understand standardized data collection parameters. We focused on implementing an intuitive interface that showcases how standardized core elements can be consistently captured across different research contexts, serving as an entry point for data discovery.

Results: The TIARA study prototype successfully demonstrates the feasibility of systematically exploring DZIF CDS elements through an interactive platform. Notably, even though TIARA was initiated before the CDS was established, we successfully mapped most elements from the existing study data, demonstrating backward compatibility. The Explorer provides clear visibility into which basic data elements are available and displays stratified participant data. Researchers can quickly identify studies containing specific data elements matching their research criteria. Early feedback indicates strong interest in this standardized discovery approach. The prototype serves as a first contact point, enabling researchers to identify relevant studies before being directed to appropriate specialized tools or databases for detailed data access. This demonstrates how implementing mandatory basic data elements in all future DZIF studies could significantly enhance data FAIRness (Findable, Accessible, Interoperable, Reusable).

Conclusions: The DZIF CDS Explorer represents a crucial step toward improving data discovery, visibility, and harmonization across DZIF studies. By establishing and visualizing core data elements, researchers can efficiently identify studies collecting data based on their specific search criteria before proceeding to detailed data repositories. This discovery-first approach streamlines the research process and prevents unnecessary database queries. Implementing the CDS as a standard requirement for all new DZIF studies will facilitate cross-study analyses, improve research

efficiency, and maximize the value of collected data. Future development will expand the Explorer to encompass additional DZIF studies, enhance search functionality, and strengthen integration pathways to downstream databases and analytical tools.

P-2-54
Deciphering the antibody response against Candida virulence factors

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Introduction: *Candida* spp. possess various secreted or cell surface-associated antigens that contribute to commensalism and pathogenicity. This includes adhesion factors, factors conferring host cell damage, nutrient acquisition and immune evasion. Deciphering the humoral response during invasive infection may serve as a basis for diagnostic and therapeutic strategies.

Methods and Materials: To profile the immunoglobulin G host response to commensal colonization and invasive candidiasis, we screened sera of health volunteers (n=27) and patients with invasive *Candida* infections (n=53) for antibodies against recombinantly purified secreted aspartyl proteinases (Sap) 1, 2, 6 and 9, agglutinin-like sequence 3 (Als3) and hyphally regulated cell wall protein 1 (Hyr1) by serum ELISA.

Results: Both cohorts exhibit detectable antibody responses against all proteins tested. In median, antibody titres were statistically significantly higher in patients after invasive infections compared with healthy volunteers for Sap 1, 2, 4 and 6 and Als3. The difference was most pronounced for Als3 (p<0.001). In Hyr1, titers did not differ significantly between the groups. The median half-maximal effective dose (ED50) for Als3-reactive antibodies was 1772.7 in the infected cohort and 134.8 in the volunteer cohort, reflecting a 13.2-fold titer increase after infection.

Conclusions: All individuals displayed detectable levels of antibodies against *Candida* antigens, indicating a permanent host-pathogen interaction with commensal *Candida* spp. Invasive infection and subsequent convalescence were associated with a marked boost in antibody responses, potentially reflecting a protective immune response.

Outlook: For other infectious diseases, fully human mAbs have been isolated from individuals after infection or vaccination to develop new therapeutic strategies. Based on the result of our serum screening, we aim to isolate antigen-specific B cells from selected donors with strong humoral response. We will subsequently perform B cell receptor repertoire analysis and recombinant production of mAbs. Recombinant mAbs will undergo functional assays to evaluate their mechanisms and therapeutic potential in vitro.

P-2-55
Nontyphoidal *Salmonella* transmission reservoirs in sub-Saharan Africa: a genomic assessment from a One Health Perspective

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Background: Nontyphoidal *Salmonella* causes more than 1.2 million annual deaths worldwide, the majority in resource-limited countries such as sub-Saharan Africa. Nontyphoidal *Salmonella* have also become increasingly resistant to antibiotics and are the most frequent cause of bacteraemia in sub-Saharan Africa. Recent data suggests that this typically livestock-associated pathogen has genetically developed and adapted to different hosts and environments, proposing anthroponotic transmission.

Methods: Within this study, we collected *Salmonella* from humans (stool and blood), animals and the environment (dust and soil), in Tanzania and in Ghana. Strains were identified by biochemical methods and confirmed using the VITEK 2 System. Serotyping and antibiotic susceptibility testing was performed. Further, isolates were subjected to sequencing using a NextSeq 500 Illumina sequencer.

Results: 9,099 samples were collected. From these, 222 Nontyphoidal *Salmonella* were identified comprising 58 serovars. The highest level of resistance was in humans with fluoroquinolone resistance on the increase and multidrug resistance highest in isolates from blood cultures (24%, n/N=11/46). Of the invasive strains, MLST analysis confirmed the serovars and sequence types *S. Typhimurium* (ST313/ST19) being most common followed by *S. Enteritidis* (ST11/ST1479) and *S. Dublin* (ST10). A sequence type overlap amongst humans and livestock or environmental strains was detected for ST19.

Conclusions: Our study demonstrates a broad serovar distribution of *Salmonella* from livestock and the environment not typically associated with human infections. The substantially high level of multidrug resistance and emerging fluoroquinolone resistance seen in the invasive nontyphoidal *Salmonella* poses a challenge to current treatment strategies. Interestingly, we found ST19 more common in invasive human disease but also prevalent in samples from livestock compared to ST313, only seen in human samples. These findings strongly support the hypothesis of anthroponotic transmission of ST313 but not of ST19 in sub-Saharan Africa.

P-2-56

Investigation of antimicrobial susceptibility in *K. gyiorum*

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Introduction: *gyiorum* is a rarely encountered bacterium in routine diagnostics, yet it has the potential to cause human infections. As a result, data on this organism, particularly regarding its antimicrobial susceptibility, remain limited from a clinical microbiology perspective. This study presents phenotypic antimicrobial susceptibility data in conjunction with *in silico* findings for this species.

Methods: A total of 14 isolates previously identified as *K. gyiorum* during routine diagnostics were included. Whole

genome sequencing of all strains was performed using the PacBio platform. Species confirmation was carried out via digital DNA-DNA hybridization (dDDH), which reliably assigned all isolates to *K. gyiorum*.

Antimicrobial susceptibility was assessed using gradient diffusion testing for: amoxicillin/clavulanic acid, ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, azithromycin, erythromycin, clarithromycin, cefepime, ceftazidime, cefuroxime, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, doripenem, ertapenem, imipenem, meropenem, aztreonam, colistin, doxycycline, fosfomycin, nitrofurantoin, rifampicin, tigecycline, and trimethoprim/sulfamethoxazole.

To enable comparison to phenotypic results, *in silico* analysis was conducted using the Comprehensive Antibiotic Resistance Database (CARD). Phenotypic resistance profiles were then compared with corresponding genomic data to identify potential resistance genes.

Results: Antimicrobial susceptibility testing results are presented as minimum inhibitory concentration (MIC) values. Low MIC values were observed for amoxicillin/clavulanic acid (0.125-0.25 µg/ml), ampicillin (0.125-0.5 µg/ml), piperacillin/tazobactam (0.25-0.5 µg/ml), ertapenem (0.004-0.016 µg/ml), imipenem (0.25-1 µg/ml), and meropenem (0.032-0.125 µg/ml). In contrast, elevated MIC values were recorded for fosfomycin and nitrofurazone (64–256 µg/ml), azithromycin (1-8 µg/ml), erythromycin (4-8 µg/ml), clarithromycin (4-16 µg/ml), ciprofloxacin (1-2 µg/ml), levofloxacin (0.25-8 µg/ml), and moxifloxacin (0.125-8 µg/ml).

The analysis of resistance genes using the Comprehensive Antibiotic Resistance Database (CARD) revealed exclusively 'strict hits', with no 'perfect hits' detected. This suggests the presence of gene variants related to known resistance determinants. Among these, a variant of the "*catB3*" gene was identified, which is associated with resistance to phenicol antibiotics. In addition, a variant of the "*adeF*" gene, encoding a multidrug efflux pump, was detected (contributes to resistance against fluoroquinolones and macrolides).

Conclusion: The increased MIC values for fluoroquinolones and macrolides may be attributed to the presence of the "*adeF*" gene variant. High MIC values were observed for fosfomycin and nitrofurazone, resistance to these agents is likely. However, the CARD analysis did not identify any known resistance gene that could account for this observation.

P-2-57

From Virus to Vaccine: Fast, Non-Toxic Pathogen Inactivation with LEEI

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The continuous and safe inactivation of pathogens remains a key challenge in biopharmaceutical production, particularly for vaccines.

This presentation introduces low-energy electron irradiation (LEEI), an innovative, chemical-free technology for the rapid and effective inactivation of pathogens. The approach reliably destroys genetic material in liquid solutions. At the same time, the gentle irradiation process largely preserves surface proteins, a key factor in generating effective immune

responses. This opens new possibilities for vaccine development. Its ability to rapidly (≤ 1 second) generate safe, immunogenic pathogen preparations makes it a valuable tool in infection research, particularly in emerging infectious diseases and pandemic preparedness.

Developed over more than a decade of research, numerous studies and publications have demonstrated that LEEI can serve as a universal inactivation platform to treat bacteria, eukaryotes (parasites), and enveloped and non-enveloped viruses successfully. The inactivated pathogens proved highly effective as potential vaccines in animal models, inducing strong cellular and humoral immune responses in mice — for example, against Respiratory Syncytial Virus (RSV) and Tick-borne Encephalitis Virus (TBEV).

The LEEI technology is now commercially available and has been implemented in the electron Fast Inactivation Technology (eFIT) system, which is already used at European research institutions. The platform is suitable for exploring and optimizing LEEI-based inactivation across different applications, ranging from vaccine antigen preparation to viral clearance in biological therapeutics and blood-derived components.

The presentation will conclude with an outlook on ongoing developments, including the design of a high-throughput system for industrial-scale applications. Ongoing studies using *Escherichia coli* as a model organism aim to optimize process parameters, with future research planned for many more microbial organisms in partner laboratories.

P-2-58

Antimicrobial Resistance of *Salmonella typhi* and paratyphi among European and North American travellers: A Systematic Literature Review

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Background: *Salmonella typhi* and paratyphi infections are common among travellers returning from many low- and middle-income countries. Multi-drug resistance (MDR) for these infections is increasing and of concern due to their complex management and poorer clinical outcomes.

Objective(s): Our objective was to characterise reported *Salmonella typhi* and paratyphi infections among travellers by antimicrobial resistance pattern.

Methods: Following PRISMA guidelines, we conducted a systematic literature review (SLR) on MedLine and Embase in the period 2014-2024, selecting studies focused on European and North American travellers. We extracted and analysed data on their personal characteristics and demographics, travel destination and antibiotic resistance patterns.

Results: Of 301 studies screened, we included 39 in the SLR. No obvious underlying personal characteristic emerged as risk factor for MDR infections, although visiting friends and relatives was a frequently reported reason of travel; South and Southeast Asia were commonly reported destinations. Aside from ubiquitously high proportions of resistance to ampicillin, chloramphenicol and cotrimoxazole, we report varying degrees of resistance to second-line antimicrobial agents including cephalosporins, macrolides and carbapenems.

Conclusions: This SLR underscores the risk posed by MDR *Salmonella typhi* and paratyphi infections to travellers and the urgent need for holistic antimicrobial stewardship strategies. Immunisation of eligible travellers against *Salmonella typhi* infection should be considered in order to reduce use of antimicrobial agents and to help preventing the burden of MDR infections.

P-2-59

Dynamic transcriptional responses to bedaquiline in *Rv0678* mutants implicate sigma factors and metabolic rewiring in drug resistant *Mycobacterium tuberculosis*

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Drug-resistant and multidrug-resistant tuberculosis (MDR-TB) remain a major global health challenge, driven by limited treatment access, diagnostic delays, and therapeutic failures. While the introduction of a shorter, all-oral bedaquiline (BDQ)-based regimens represent a critical advancement, emerging BDQ resistance threatens its long-term effectiveness.

Understanding the molecular mechanisms of resistance is essential for improving diagnostics, therapies, and novel drug development. In *Mycobacterium tuberculosis* (*M. tuberculosis*) strains, BDQ resistance often arises from mutations in the *Rv0678* gene, which typically confer moderate resistance. To investigate their functional impact, we performed transcriptomic analyses of three laboratory-derived BDQ-resistant clones, each harbouring distinct mutations (A99V, inversion, or 274insA) in *Rv0678*, with and without BDQ exposure.

Following 0.5 h of BDQ exposure, 297 differentially expressed genes (DEGs) were common to both mutants and the wild-type strains, rising to 842 DEGs by 4 h. Among the *Rv0678* mutants, 308 DEGs were shared at 0.5 h, while 8 were unique, including the transcriptional regulator *oxiR* (*Rv0067c*) and the sigma factor-regulated genes *dipZ* (*Rv2874*, regulated by *sigK*) and *vapC45* (*Rv3180c*, regulated by *sigG*). At 4 h, 858 DEGs were shared across mutants, with 16 unique to them, notably *prpC* (*Rv1131*; a key methyl citrate cycle entry gene), *gyrA* (*Rv0006*; linked to fluoroquinolone resistance), and *sigK*-regulated genes (*dipZ* (*Rv2874*), *Rv0448c*).

These findings demonstrate that *Rv0678* mutations drive temporally distinct transcriptional responses, featuring early and late sigma factor-mediated adaptations (*sigK/sigG*) followed by metabolic remodelling. Together, our results reveal the dynamic and multifaceted adaptation strategies of *M. tuberculosis* under drug pressure and highlight the value of time-resolved transcriptomics in dissecting resistance mechanisms. This work expands the molecular understanding of BDQ resistance and identifies potential targets, including sigma factor networks and metabolic pathways, for future adjunctive treatments.

P-2-60

Ultrasensitive Pathogen DNA extraction from whole blood

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Sepsis remains a leading cause of mortality worldwide. Sepsis is a life-threatening systemic response to infection requiring prompt and accurate pathogen identification.

Conventional diagnostic workflows are often time-intensive (typically ≥ 24 hours), prompting the urgent need for molecular alternatives that are both rapid and sensitive.

This project aimed to develop and validate a rapid, efficient DNA extraction protocol tailored for bloodstream infections, enabling same-shift molecular diagnostic turnaround. Our vision is to replace traditional blood cultures by enabling direct DNA extraction from large volumes of blood, capable of detecting trace amounts of bacterial and fungal DNA.

A novel extraction workflow was developed, combining high-efficiency pathogen capture, heat driven lysis, and magnetic bead-based purification. The protocol was optimized for large-volume (10 mL) whole blood samples, minimizing processing time while maximizing sensitivity. Performance was assessed using spiked blood with low concentrations of clinically relevant pathogens, and downstream detection was validated via qPCR and isothermal methods.

The optimized protocol achieved high-quality DNA extraction in under 45 minutes, cutting time to result by several hours in comparison to conventional methods. DNA yield and purity were comparable to leading commercial kits. Extracted DNA demonstrated robust amplification in qPCR and was compatible with isothermal amplification methods. Sensitivity was evaluated using spiked blood samples with clinically relevant pathogens (e.g., *E. coli*, *S. aureus*, *Candida albicans*, *K. pneumoniae*, *S. pneumoniae* and *P. aeruginosa*). The protocol consistently recovered pathogen DNA from blood samples containing $< 10^2$ FU/mL, matching or exceeding the analytical sensitivity of blood culture

We present a breakthrough in sepsis diagnostics: a scalable, rapid DNA extraction method designed to process 10 mL of blood with sensitivity on par with blood culture. This method represents a major step toward real-time detection of bloodstream infections, enabling earlier intervention and improved patient survival.

P-2-61

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: Bacteriophages (phages) are viruses that infect and lyse bacteria with high specificity. They are increasingly explored as a targeted therapeutic alternative to antibiotics (phage therapy) in the fight against recalcitrant bacterial infections.

Problem Statement: Despite their therapeutic potential, clinical deployment of phage medicinal products (PTMPs) faces challenges, notably phage adsorption to manufacturing and delivery device surfaces. This surface adherence leads to substantial phage loss, impairing both production yield and clinical efficacy.

Objective: PhageSurf aims to investigate phage adsorption (PA) in medically relevant devices including infusion systems and urinary catheters targeting *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We assess phage adsorption during simulated therapeutic administration to optimize PTMP handling.

Methods: We used simulated infusion (IV) and urinary catheter models with three clinically relevant stained and unstained phages ($\phi 1-3$) to quantify post-administration phage concentrations. For unstained phages, adherence was determined by passing phages through the tubing systems, collecting two effluents, first with just the phages and second after rinsing the tubing system with sodium chloride, and comparing them to the initial phage concentrations.

Results: In IV systems with unstained phages, only 1–3% ($\phi 1$) and 1% ($\phi 2$) of the input phage concentration reached the end of the tubing ("patient"), suggesting 97–99% was retained due to phage adsorption. Fluorescent tracing confirmed these high losses. In urinary catheter models, overall, less phage loss was detected. In detail, $\phi 3$ showed minimal retention (87–92% delivered) in urinary catheter models, while $\phi 1$ and $\phi 2$ showed substantial retention (up to 77% and 60%, respectively).

Conclusion: Our findings confirm significant phage loss due to PA, especially in IV systems. These insights will guide improved PTMP [AYC1] administration protocols, enhancing future phage therapy implementation in Germany.

P-2-62

FormPhage – Phage Therapy in Frankfurt in Stable Pharmaceutical Formulations

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Background: Bacteriophages (phages) are viruses that infect and lyse bacteria with high specificity. They are being increasingly explored as a targeted therapeutic alternative to antibiotics (phage therapy) in the fight against hard-to-treat bacterial infections. As phage therapy moves toward clinical implementation, including personalized treatments where patients may self-administer phages at home, pharmaceutically acceptable formulations become essential. Despite increased interest, formulation stability data remains limited.

Problem Statement: Instability of phage concentrations in suboptimal formulations can compromise their therapeutic effectiveness and shelf-life, especially under pharmaceutical manufacturing constraints.

Objective: To evaluate pharmaceutically viable phage formulations for intravenous, inhalative, and topical (including nasal spray and hydrogel-based) applications, using two GMP (Good Manufacturing Practice)-produced phages that are intended for therapeutic administration to patients and to assess their stability over a four-week storage period under refrigerated conditions.

Methods: Two well-characterized lytic phages (ΦA and ΦB) were formulated individually for IV and inhalative use and incorporated into hydrogels and nasal spray bases. Phage concentrations were measured at day 0, 21, and 28 via double-layer plaque assays. Optimization experiments evaluated the effect of adding 8% human albumin (HA) as a potential stabilizer at different time incubations (15 min and 24 h) with subsequent testing over 28 days. Both in-house (phage lab) and pharmacy-stored stock solutions were included in the tests.

Results: Over the 4-week testing period, pharmaceutical IV formulations of ΦA and ΦB remained at high titers suitable

for therapeutic applications, with ΦA maintaining 1×10^8 PFU/mL and ΦB at 1×10^9 PFU/mL. Addition of 8% Human Albumin did not enhance IV formulation stability. Hydrogel formulations of ΦA and ΦB also preserved phage activity throughout the 4-week testing period with concentrations remaining at 3×10^8 PFU/mL and 3×10^9 PFU/mL, respectively, supporting their suitability for topical application. The spray mechanism of the nose spray formulations did not reduce phage concentration relative to the original reference solution it was made from. Due to formulations, phage concentrations had an expected potency from 3×10^9 to 1×10^8 PFU/mL for ΦA and from 3×10^{10} to 1×10^9 PFU/mL for ΦB for IV formulations. Similarly, the step of hydrogel formulations led to an expected potency of 3×10^9 to 3×10^8 PFU/mL for ΦA and from 3×10^{10} to 5×10^9 PFU/mL for ΦB .

Conclusion: We can use our GMP-phages for personalized human therapies, and we can even use them over a period of four weeks, facilitating outpatient management and patient self-administration.

P-2-63 Evaluation of Two IVD/CE-Approved Microdilution Assays for Cefiderocol Susceptibility Testing in Gram-Negative Bacteria: A Comparative Study Using Clinical Isolates and Whole Genome Sequencing

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The increasing trend toward the spread of multidrug-resistant bacteria poses a significant challenge for clinical therapy. Cefiderocol, a novel cephalosporin siderophore, offers a promising therapeutic option for the treatment of infections with multidrug-resistant Gram-negative pathogens due to its unique mechanism of action. The aim of this study was to compare two IVD/CE-approved microdilution tests for determining the minimum inhibitory concentration (MIC) of cefiderocol: UMIC (Bruker) and comASP (Liofilchem). The test procedures were compared with the results of the NARA reference laboratory and with established methods such as the E-test and disk diffusion using a total of 60 clinical isolates. In addition to methodological agreement with the reference laboratory, practical aspects such as handling, reproducibility, and the influence of iron concentrations were also taken into account. In addition, an analysis of the genotype-phenotype correlation of resistance-associated genes was performed. Compared to the comASP test, the UMIC test showed higher agreement with the reference laboratory (EA: 85%, CA: 91.7%, VME: 5%, ME: 1.7%, mE: 3.3% compared to EA: 68.3%, CA: 82.3%, VME: 11.7%, ME: 0%, mE: 1.7%) and met the acceptance criteria better overall, but not completely. Even slight variations in iron concentration led to deviations in the MIC values, which can be interpreted as a possible main factor for discrepancies with the reference laboratory. Resistance-associated genes such as *tonB*, *porin* genes, *PBP-3*, and various *beta*-lactamase genes (including *NDM*, *KPC*, *OXA*) were detected by whole-genome sequencing. However, a clear genotype-phenotype correlation was not found in all cases. Reproducibility analyses confirmed the stability of the measurement results over a longer period of time. In addition, the test results were consistently readable across different evaluators and species. The present results support the introduction of the UMIC test into routine diagnostic laboratory operations, particularly due to its higher accuracy, stability, and user-friendliness

Legends:

Figure 1: Workflow (left) and comparison of UMIC versus comASP (right)

Figure 2: Determination of the effect of iron(III) concentration (top) and measurement of iron content in the used broth (bottom).

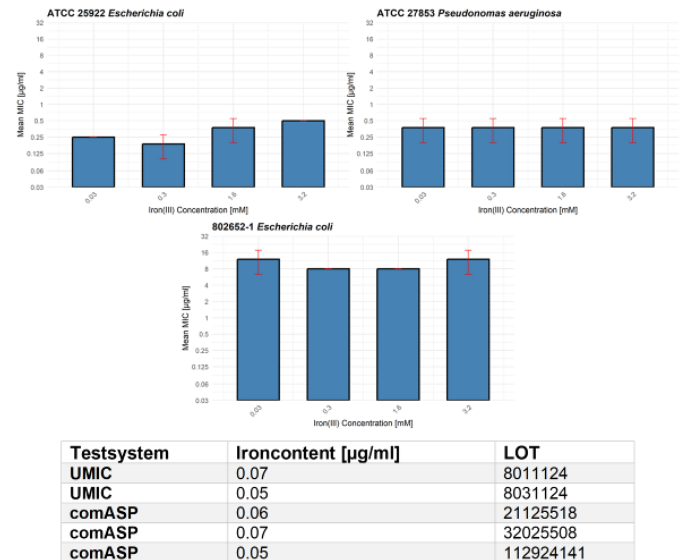
References:

https://drive.google.com/file/d/1VydoxO_9rToglickfahKFCMq3LYDJ9gS/view?usp=sharing

Fig. 1

Comparison with NARA reference results					
Test	EA	CA	VME	ME	mE
UMIC	85%	91.7%	5%	1.7%	3.3%
comASP	68.3%	82.3%	11.7%	0%	1.7%
Disk	-	65%	7.5%	7.5%	2.5%
E-Test	45%	75%	20%	0%	-

Fig. 2



P-2-64

Optimised screening and consecutive treatment control of urinary tract infections by flow cytometry

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Background: Urinary tract infection (UTI) is a major cause of disease in hospitals. However, low sensitivity and low specificity of initial dipstick (DS) analysis leads to underdiagnosis of UTI on the one hand and overtreatment on the other hand. Rapid quantitative examination with flow cytometry (FC) may improve diagnostic screening. Moreover, direct detection of bacteria could open the possibility of monitoring of treatment response.

Methods: Regular diagnostic urine samples of a major regional hospital (Germany) were screened in parallel by DS and FC. Semi-quantitative culture was performed if bacteria count (BC) was $\geq 100/\mu\text{l}$. For a limited number of patients with

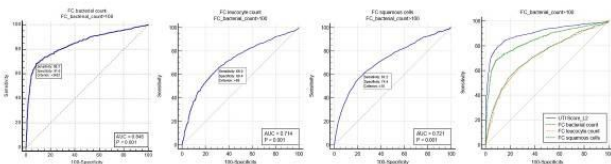
suspected UTI, the early treatment response (<24 hours) was tested in a second sample.

Results: Of 5,124 urines, 2,281 had increased BC with culture based follow up. 1,368 cases with UTI were confirmed by culture. Initial screening by FC (cutoff BC $\geq 100/\mu\text{l}$) was superior to DS (combination leukocytes/nitrite) which was reflected by higher sensitivity (100.0% vs. 90.3%) and specificity (75.7% vs. 58.2%). Diagnostic sensitivity of FC was primarily related to quantitative BC; however, leukocyte (inflammation) and squamous cell count (contamination) may further discriminate for relevant samples. Discrimination between infection vs. contamination is of particular importance for women. Early treatment response in <24 hours (selected patients only) was reflected by >10-fold decrease of BC. Inadequate decrease of BC was associated with persistent growth in culture due to resistance profiles (e.g. ESBL) or difficult-to-treat uropathogens (e.g. *P. aeruginosa*).

Conclusions: Optimised UTI screening requires quantitative detection of BC (FC) which is the most important diagnostic parameter. Indirect measurement (leucocytes, nitrite) by DS is insufficient for appropriate diagnostics. Early treatment monitoring (paired samples) could be established as promising marker to predict treatment response in <24 hours, which is 1-2 days before culture. The concept is still experimental and requires confirmations in larger prospective studies.

Figure 1: Sensitivity and specificity for different screening parameters in FC to predict UTI. Quantitative bacterial count (AUC 0.846) (A) was the best parameter to detect UTI in suspected samples (2281 samples with BC $\geq 100/\mu\text{l}$). Leucocyte count (AUC 0.714) (B) and inverse squamous cell count (AUC 0.721) (C) were also appropriate. However, an UTI-Score combining the different parameters of FC (AUC 0.901) (D) shows best performance.

Fig. 1



P-2-65

Nasal application of *Staphylococcus lugdunensis* to reduce nasal *Staphylococcus aureus* colonization – a first-in-human proof-of-concept microbiome intervention study

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Background: *Staphylococcus aureus* frequently colonizes the human nasal cavity as a commensal. Under certain conditions, such as immunosuppression, advanced age, or hospitalization with surgical interventions, it can cause

severe infections. Treatment is further complicated by the emergence of antibiotic resistance. *Staphylococcus lugdunensis*, another commensal, colonizes approximately 60% of the human population on the skin but only about 9% in the nasal cavity. Notably, individuals nasally colonized with *S. lugdunensis* have been shown to be at a lower risk to carry *S. aureus* in the nose.

Objective: We will conduct a phase 1 clinical trial in which a GMP-produced *S. lugdunensis* (IVK28 strain) will be administered intranasally to assess the safety and tolerability of escalating doses. Furthermore, the ability of *S. lugdunensis* to colonize the nasal cavity, and to investigate its potential to decolonize *S. aureus* will be investigated.

This study aims to provide critical insights into the use of *S. lugdunensis* as a novel approach with a live biotherapeutic product (LBP) against nasal *S. aureus* colonization, potentially opening new opportunities for infection prevention.

We report on the study design, the challenges of developing and testing a novel LBP, and—if available—the first results of the planned trial.

P-2-66

Cysteine-stabilized $\alpha\beta$ -defensins as promising antimicrobial agents against drug-resistant bacteria

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Antimicrobial resistance (AMR) has emerged as one of the biggest global health challenges of our time. The lack of newly approved antibiotics, combined with the rapid rise of resistant pathogens, has created an urgent need for new antimicrobial strategies. Antimicrobial peptides (AMPs), produced by both prokaryotic and eukaryotic organisms, have gained attention as promising candidates for the development of new antibiotics. Among these, cysteine-stabilized $\alpha\beta$ -defensins (CS $\alpha\beta$ -defensins) form a structurally conserved class of AMPs, defined by a characteristic α -helix and β -sheet motif, with activity against a broad range of bacteria, including AMR strains. Here we focus on two major subfamilies of CS $\alpha\beta$ -defensins: the GXGCP and XTCD subgroups. Plectasin and actifensin are members of the GXGCP subgroup, both binding the cell wall precursor lipid II and showing high structural similarities, although differ in mechanistic aspects (Sugrue et al., 2025). Within the XTCD subgroup, lucifensin—also interacts with lipid II, while sapecin has shown affinity for cardiolipin. This highlights a gap in understanding of mechanism-linked structural differences between the two groups of peptides. Here we elucidate the structural and mechanistic aspects and the potential role of CS $\alpha\beta$ -defensins in the fight against antimicrobial resistance.

Reference:

Sugrue, I., Ade, C., et al. (2025). Trans-kingdom conservation of mechanism between bacterial actifensin and eukaryotic defensins. *NPJ Antimicrob Resist*, 3(1), 66. doi:10.1038/s44259-025-00135-x

P-2-67

Molecular characterization of hypervirulent *Klebsiella pneumoniae* among hypermucoviscous strains

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Since the first reports of hypervirulent *Klebsiella pneumoniae* (hvKp) in Asia, hvKp has been recognized as an emerging pathogen in Europe as well. Infections are mostly found in young immunocompetent individuals and are often invasive, affecting liver, lungs, eyes, central nervous system, and the urogenital tract. (1, 2). The genetic determinants correlating with hypervirulence can be used as biomarkers (3) and phenotypic properties to identify hvKp have been proposed (4). Although research is increasingly gaining insights into the epidemiology of hvKp in Europe (5), data is still limited, impeding strategies for diagnostics, treatment and prevention strategies.

We phenotypically screened clinical *K. pneumoniae* isolates from invasive infections by string test to select them for molecular detection of virulence genes. $n = 2$ isolates originated from liver abscess samples, $n = 4$ from positive blood culture samples ($n = 1$ additional patient with liver abscess, $n = 2$ patients with urogenital foci, $n = 1$ patient with a pulmonary focus) and $n = 1$ isolate from a throat swab screening for multidrug-resistant bacteria of an asymptomatic patient. To investigate multi-locus sequence types (ST), capsule locus (KL), further virulence associated genes and resistance genes, the isolates were investigated by whole genome sequencing. Out of $n = 7$ analyzed isolates, the most common ST23 and genetic relation between the isolates was not detected. Isolates could either be assigned to other hypervirulent strains or did not harbor hypervirulence-associated genetic determinants despite a hypermucoviscous phenotype. The analyzed clinical isolates showed a heterogeneous genetic profile, suggesting that multiple different ST of hvKp circulate in the population. Since the string test is only of limited use to decide on whether further molecular biological work-up is justified, we emphasize that clinical precaution, severity of disease, and epidemiological as well as individual risk factors be considered.

1. Choby JE, Howard-Anderson J, Weiss DS. Hypervirulent *Klebsiella pneumoniae* – clinical and molecular perspectives. *Journal of Internal Medicine*. 2020;287(3):283–300.
2. Marr CM, Russo TA. Hypervirulent *Klebsiella pneumoniae*: a new public health threat. *Expert Rev Anti Infect Ther*. 2019;17(2):71–3.
3. Brisse S, Fevre C, Passet V, Issenhuth-Jeanjean S, Tournebise R, Diancourt L, et al. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS One*. 2009;4(3):e4982.
4. Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT. A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. *J Exp Med*. 2004;199(5):697–705.
5. Wahl A, Fischer MA, Klaper K, Muller A, Borgmann S, Friesen J, et al. Presence of hypervirulence-associated determinants in *Klebsiella pneumoniae* from hospitalised patients in Germany. *Int J Med Microbiol*. 2024;314:151601.

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-R₆C 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 ¹²⁵I 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 hepatocytes), 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-R₆C. 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.

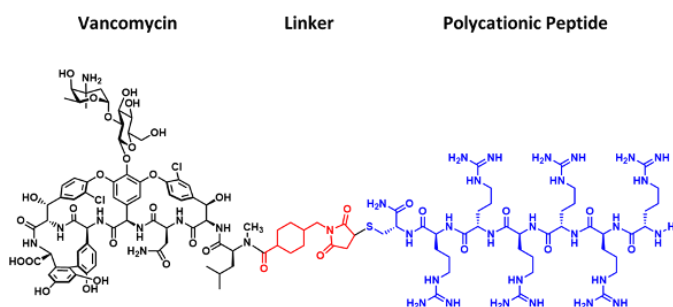
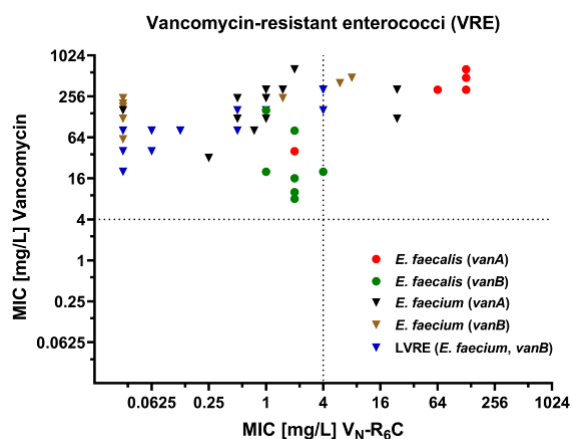
Figure 1: Structure of V_N-R₆C. First, the vancomycin-linker precursor is synthesized using the hetero-bifunctional linker SMCC. Second, the polycationic hexa-arginine is coupled to the maleimide via a thioether bond.

Figure 2: Minimal inhibitory concentration (MIC) of vancomycin and the polycationic-peptide conjugate V_N-R₆C 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.

P-2-68

Preclinical development of vancomycin polycationic peptide conjugate (V_N-R₆C) with high antimicrobial activity *in vitro* and *in vivo*

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Fig. 1**Fig. 2****P-2-69****The DZIF Pathogen Repository – A Resource for Medical Research and Antimicrobial Development***B. Abt¹, Y. Mast¹, S. Gronow¹¹Leibniz-Institut - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Brunswick, Germany

Medical research and antimicrobial development depend on access to diverse microbial strains for different applications, ranging from standardized testing to specialized experimental designs. Standard test strains are essential for routine procedures and quality control, while specialized strain panels play a crucial role in addressing specific questions in medical research.

Beyond the well-established type strains maintained at the DSMZ, the associated DZIF Pathogen Repository – as a part of the DZIF infrastructure "Bioresources, Biodata and Digital Health" (TI BBD) - hosts several microbiota collections, including mouse, human, pig, and chicken microbiomes, as well as specialized strain panels for antimicrobial testing and compound screening. In line with the *WHO Priority Pathogen List*, which highlights a catalogue of 15 bacterial families that pose the greatest threat to human health and is intended to guide and promote research and development (R&D) of new antibiotics, we have significantly expanded our collection of multi-resistant reference strains. Currently, the collection is being extended to include fungal pathogens, in alignment with the recently published *WHO Fungal Priority Pathogens List*.

Researchers are encouraged to explore the DSMZ strain catalogue and the BacDive database to identify appropriate strains for their experimental needs, including strains for antimicrobial testing, compound screening, or resistance profiling. Additionally, we ask the scientific community to contribute to the expansion of the collection by depositing

rare strains and strains with clinically relevant resistance profiles identified in their research.

Furthermore, we invite you to discover the whole range of services offered by the TI BBD infrastructure, including improved access to pathogens and clinical biosamples, as well as to databases, analysis tools, and apps facilitating translational infection research (<https://www.dzif.de/en/infrastructure/bioresources-biodata-and-digital-health>).

P-2-70**Carbapenem on the rise in Germany - Data from the National Reference Centre for Multidrug-resistant Gram-negative Bacteria***N. Pfennigwerth¹, S. Möller¹, L. M. Höfken¹, S. Gatermann¹¹Ruhr-Universität Bochum, Abteilung für Medizinische Mikrobiologie, Bochum, Germany

Question: Multidrug-resistance in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug against gram-negative bacteria will be introduced within the next years. Among all resistance mechanisms the spread of carbapenemases is the most worrisome. However, the correct identification of carbapenemases is still challenging and molecular epidemiology of carbapenemases is required.

Methods: The German National Reference Centre for Multidrug-Resistant Gram-negative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs according to the NAK criteria. Since March 2025, a positive result in an unspecific carbapenemase detection assay is also mandatory. Isolates with a positive result in a specific test (e.g. PCR, immunoassay) do not need a positive phenotypic test. At the NRC, all isolates are tested by a wide array of phenotypic and molecular methods, including synergy assays with boronic acid, EDTA, clavulanic acid and PCR analysis. A bioassay based on cell-free extracts and WGS methods allow the detection of still unknown β -lactamases.

Results: In 2025, 6,267 isolates were investigated for carbapenemase production until September 29th. One or multiple carbapenemases were found in 3,964 strains of *Enterobacterales*, 427 of *Pseudomonas aeruginosa*, 302 of *Acinetobacter baumannii* and 158 of other species. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 633), OXA-244 (n = 576), NDM-5 (n = 455), KPC-2 (n = 437), NDM-1 (n = 413) and VIM-1 (n = 386) which were also found in various combinations, e.g. NDM-1/OXA-48 (n = 160). In total, 89 different carbapenemases or combinations of carbapenemases were found in *Enterobacterales*. In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 227), followed by NDM-1 (n = 77), IMP-1 (n = 24) and GIM-1 (n = 22). OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 159), followed by OXA-72 (n = 88).

Conclusions: Compared to 2024, the number of isolates analyzed in the NRC decreased due to the significant change in the inclusion criteria. As the capacities of the NRC are more and more limited, it was necessary to reduce the number of carbapenem-resistant, but not carbapenemase-producing isolates analyzed. Nonetheless, a large variety of different carbapenemases was again detected, especially in *Enterobacterales*. Regarding the percentage of carbapenemase producers among the analyzed isolates, this

number increased to 77.4 % (status 2025/09/29), compared to 57.3 % in 2024, reflecting the new inclusion criteria.

P-2-71

Overcoming Resistance via Reactivation – Mode of Action of Vancomycin conjugates

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Glycopeptide antibiotics (GPAs) are drugs of last resort to treat infections caused by (multi-) resistant Gram-positive pathogens. They inhibit peptidoglycan biosynthesis mainly by binding to the D-alanyl-D-alanine terminus of lipid II peptide stem, thereby blocking transglycosylation and transpeptidation reactions in a sterical manner. Vancomycin (VAN) and teicoplanin (TEIC) represent the prototype GPAs in clinical use today. However, widespread GPA resistance has become a serious threat to global health. Resistance resulting from alteration of the PGN precursor, e. g. by exchange of the terminal D-alanine to D-lactate or D-serine, is conferred by the acquisition of a van-resistance operon. Enterococci, which harbour a vanA or vanB cluster, are most significant in the clinical setting. Research is focused on structural modification of glycopeptide antibiotics to overcome resistance. Lead candidate FU002, a vancomycin-hexa-arginine derivative, with high potency against vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) was generated by conjugation of polycationic peptides to VAN. Cell-based and *in vitro* assays are used to elucidate the precise mechanism of action to build a rational basis for targeted structure optimization.

P-2-72

Overcoming resistance – Targeting enterococci with β -lactam conjugates

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Due to the rising prevalence of antimicrobial resistance and the expected number of up to 40 million deaths within the next 25 years, there is an increasing demand for highly active antimicrobial therapies [1]. One of the high priority pathogens listed by the WHO is vancomycin-resistant *Enterococcus faecium* (VRE) that shows an intrinsic resistance against β -lactam antibiotics [2]. A promising way to overcome this burden is to sensitize these strains to modified approved antibiotics [3]. Therefore, we synthesized peptides and lipopeptides and coupled them to selected β -lactam antibiotics in a simple two-step synthesis using a hetero-bifunctional linker moiety. Afterwards the new conjugates were screened for *in vitro* efficacy using the antimicrobial susceptibility assay (MIC), selecting two promising and highly active candidates. The general procedure is depicted in figure 1. Ceftazidime-R6 and ceftazidime-R6-C12 showed a broadened spectrum and up to 1000-fold higher efficacy including VRE without increasing cytotoxicity [4]. These findings might represent the potential as potential platform technology to broaden the efficacy spectrum of cell-wall addressing β -lactam antibiotics, particularly against enterococci.

Figure 1 Synthesis and selection of lead compounds. The graphic depicts the general procedure. Susceptible strains are shown as rod-shaped bacterial cells in light green indicating no resistance gene against β -lactam antibiotics, enterococci are shown as diplococci with vancomycin resistant enterococci depicted in red.

References:

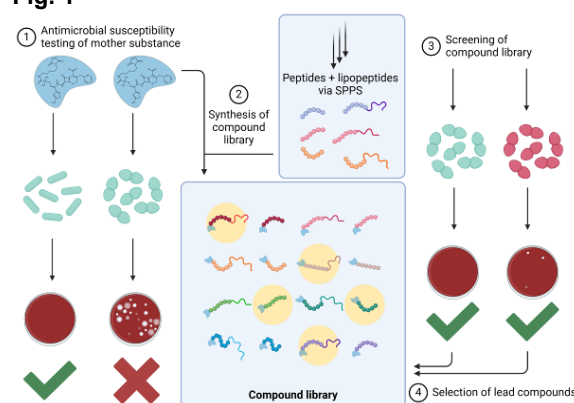
[1] Naddaf M, Nature 2024, 633, 747-748.

[2] WHO: WHO publishes list of bacteria for which new antibiotics are urgently needed

[3] Narendrakumar L *et al.*, Front. Microbiol. 2023, 13, 1092556.

[4] Werner J *et al.*, Adv. Sci. (Weinh) 2024,11(48):e2411406.

Fig. 1



P-2-73

Comparison of *Escherichia coli* isolates with resistance to third generation cephalosporins from hospitalised patients and outpatients in Germany, 2016-2022

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Introduction: *Escherichia coli* is the leading cause of community acquired urinary tract infections and multidrug-resistant strains pose a threat to hospitalised patients. Production of extended-spectrum beta-lactamases (ESBL) and carbapenemases is the main cause of these resistances that emerged in different clonal lineages worldwide. This study aimed to analyse the nationwide phylogeny of *E. coli* with resistance to third generation cephalosporins from hospitalised patients and outpatients.

Methods: *E. coli* isolates were collected prospectively in the scope of the resistance surveillance study performed by the Paul Ehrlich Society for Infection Therapy at 45 diagnostic laboratories, each in three time periods (October - March in 2016-17, in 2019-20 and in 2022-23). For whole genome sequencing (Illumina) all isolates with third generation cephalosporin resistance (study period 2016/17, n=160; study period 2019/20, n=145; study period 2022/23, n=121) were included. These isolates were from hospitalised patients (2016/17, n=123; 2019/20, n=96; 2022/23, n=84) and outpatients (2016/17, n=37; 2019/20, n=49; 2022/23, n=37). Genome sequences were assembled using unicycler and further analysed using core genome multilocus sequence typing (cgMLST). Resistance genes were identified using Kleborate.

Results: A total of 1038, 1031 and 1005 *E. coli* isolates were collected in 2016/17, 2019/20 and 2022/23, respectively. Resistance to third generation cephalosporins decreased from 2016/17 - 2022/23 and main cause of resistance were ESBL of the CTX-M family (Table 1). ESBL gene *bla*_{CTX-M-15} was stably present in 50-52% of all isolates from hospitalised patients and 40-62% of all isolates from outpatients. The proportion of *bla*_{CTX-M-27} increased in general, but the proportion of *bla*_{CTX-M-14} and *bla*_{CTX-M-1} decreased in hospitalised patients. Further causes of third generation cephalosporin resistance than CTX-M production, e.g. CMY, DHA and SHV-1 beta-lactamase overproduction, were found in only a few isolates.

MLST enabled assignment of various sequence types (STs) and a close genetic relationship was detected only for a few isolates (cgMLST-based). The most frequent ST was ST131 with a higher proportion in the study period 2016/17 (47%-56%) compared to 2022/23 (24-30%). Further, worldwide spread of *E. coli* "high risk clones" like ST10, ST38, ST69, ST73 and ST1193 were present in more than one isolate with slightly increasing numbers in the last study period among both hospitalised patients and outpatients.

Conclusions: *E. coli* isolates with resistance to third generation cephalosporins belong to various clonal lineages, with a clear dominance of ST131 in both hospitalised patients and outpatients. Apart from ST131, several STs that are known as "high risk clones" were detected in this study. The increased occurrence of ESBL gene *bla*_{CTX-M-27} in the last two study periods is mainly attributed to a shift of *bla*_{CTX-M-15} to *bla*_{CTX-M-27} in ST131 isolates.

Fig. 1

Table 1: CTX-M-type ESBL and sequence type ST131 in *E. coli* isolates from patients in hospitals and clinical practices in community medicine (ESBL, third generation cephalosporin resistance (resistance to ceftazidime and/or ceftazidime))

Study period	2016/17 community	2019/20 community	2022/23 community	2016/17 hospital	2019/20 hospital	2022/23 hospital	
3GCR Isolate number	37/460 (8.0%)	49/460 (10.7%)	37/450 (8.2%)	123/118 (105.9%)	96/118 (81.3%)	114/118 (96.6%)	
CTX-M-type ESBL	CTX-M-15 n=23	CTX-M-15 n=20	CTX-M-15 n=18	CTM-15 n=9	CTM-15 n=10	CTM-15 n=11	
	CTX-M-27 n=6	CTX-M-27 n=14	CTX-M-27 n=6	CTM-27 n=1	CTM-27 n=1	CTM-27 n=1	
	CTX-M-1 n=3	CTX-M-1 n=6	CTX-M-1 n=5	CTM-1 n=0	CTM-1 n=0	CTM-1 n=0	
	CTX-M-14 n=2	CTX-M-14 n=1	CTX-M-14 n=2	CTM-14 n=0	CTM-14 n=0	CTM-14 n=0	
	CTX-M-3 n=0	CTX-M-3 n=2	CTX-M-3 n=0	CTM-3 n=0	CTM-3 n=0	CTM-3 n=0	
	CTX-M-8 n=0	CTX-M-8 n=1	CTX-M-8 n=0	CTM-8 n=0	CTM-8 n=0	CTM-8 n=0	
	CTX-M-55 n=0	CTX-M-55 n=1	CTX-M-55 n=1	CTM-55 n=1	CTM-55 n=0	CTM-55 n=1	
	CTX-M-65 n=0	CTX-M-65 n=0	CTX-M-65 n=0	CTM-65 n=0	CTM-65 n=2	CTM-65 n=1	
	Sequence type	ST131 n=21	ST131 n=24	ST131 n=9	ST131 n=58	ST131 n=33	ST131 n=25

P-2-74

From mechanism to clinic: Iron deprivation and bacterial metabolite reactivation underpin nitroxoline's efficacy and self-limiting resistance

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The global rise of antimicrobial resistance (AMR) requires renewed attention to established but underused antibiotics such as nitroxoline (NTX). We combined proteomics, isothermal titration calorimetry and size-exclusion ICP-MS in *Escherichia coli* to show that NTX induces cellular iron deprivation, with upregulation of iron import and Fe-S cluster assembly proteins and marked loss of protein-bound iron, resulting in impaired respiration and metabolism. The primary urinary phase-II metabolites, NTX-sulfate and NTX-glucuronide, were biologically inactive and lacked metal-chelating capacity; ex vivo incubation in human urine revealed efficient pathogen-mediated reconversion to active

NTX, notably by *E. coli* and *Klebsiella pneumoniae*. Importantly, NTX-resistant mutants exhibit pronounced fitness loss and reduced virulence in a zebrafish infection model. Together, mechanistic evidence for iron-deprivation, pathogen-dependent metabolite activation, and self-limiting resistance behaviour provide a molecular rationale for reinforced therapeutic use of NTX, also beyond its use as drug against uncomplicated urinary tract infections (UTI).

P-2-75

Deciphering the modes of action of novel antibiotics targeting the bacterial cell envelope

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The increasing prevalence of infections caused by multidrug-resistant bacterial pathogens calls for novel antibiotics with innovative mechanisms of action. To identify, characterize, and prioritize promising antibacterial candidates for further development, detailed knowledge of their modes of action is essential.

Using our integrated mode-of-action platform, we combine *in vitro* studies with advanced whole-cell screening approaches to investigate antibacterial activity at multiple levels. A central focus is the bacterial cell envelope as a critical and underexplored target structure. Phase-contrast and fluorescence microscopy are employed to monitor how candidate compounds affect cellular morphology, membrane integrity, and protein localization. Complementary bioreporter strains help to link antibacterial effects to specific cellular pathways, including peptidoglycan, protein or nucleic acid biosynthesis.

Recent work has focused on natural product antibiotics, such as cyclic lipopeptides, as well as (semi-)synthetic derivatives of established compounds, like glycopeptides, which interfere with cell wall biosynthesis by targeting essential lipid intermediates. By elucidating antibacterial target structures and resistance mechanisms, our approach provides a foundation for rational antibiotic design and the development of next-generation agents to combat multidrug-resistant infections.

P-2-76

Antimicrobial Resistance Surveillance in Rural Burkina Faso: Insights from Nouna District Hospital

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Background: Antimicrobial resistance (AMR) poses a growing threat to global health, especially in low- and middle-income countries. LMICs at the same time remain underrepresented in AMR surveillance efforts, particularly in rural areas. This study presents six years (January 2019 to December 2024[SB1]) of laboratory-based AMR data from Nouna District Hospital, Burkina Faso offering critical insights

into resistance patterns outside urban centres to guide antibiotic use.

Methods: A hospital-based AMR surveillance system was established at Nouna Health District Hospital. From January 2019 to December 2024, clinical samples including urine, stool, blood, vaginal swabs, and pus were processed using standard microbiological techniques. Bacterial identification and susceptibility testing followed EUCAST guidelines were done using the Kirby-Bauer method. Data were analysed via WHONET software. Extended-Spectrum β -lactamase (ESBL) production was detected phenotypically.

Results: Out of 1,379 clinical samples analysed, urine (55.6%) and stool (34.4%) were the most frequent specimen types. *Escherichia coli* was the predominant pathogen isolated (12.2%), followed by *Klebsiella pneumoniae* (2.9%), *Staphylococcus aureus* (2.5%), *Acinetobacter baumannii* (2.2%), *Pseudomonas aeruginosa* (0.7%), *Salmonella* spp. (1.5%) and other bacteria (1.8%). For ESBL producers, *Escherichia coli* (41.6%) and *Klebsiella pneumoniae* (27.5%) were the most prevalent ESBL producers. Alarming high resistance rates were observed for commonly used antibiotics: Trimethoprim-sulfamethoxazole (SXT): 77.5% (*Escherichia coli*), 75.6% (*Klebsiella pneumoniae*); Amoxicillin-clavulanic acid (AMC): 73.3% (*Escherichia coli*), 72.5% (*Klebsiella pneumoniae*); Ciprofloxacin (CIP): 66.7% (*Escherichia coli*), 63.8% (*Klebsiella pneumoniae*). Imipenem retained high efficacy but remains largely inaccessible in rural settings. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 20.5% of *Staphylococcus aureus* isolates.

Conclusion: This study highlights an urgent need to revise empirical treatment protocols in Burkina Faso's rural health facilities. The high prevalence of ESBL and MRSA strains underscores the importance of strengthening AMR surveillance, investing in diagnostic capacity, and implementing antimicrobial stewardship programs. These findings support national efforts to align with WHO's GLASS initiative and inform targeted interventions in underserved regions.

P-2-77

The DZIF Natural Compound, Extract, and Fraction Libraries

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For years, the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) and the Helmholtz Centre for Infection Research (HZI), in close collaboration with the German Centre for Infection Research (DZIF), have been driving the discovery and systematic evaluation of natural products to find novel anti-infective compounds. Backed by the DZIF projects TTU 09.721 (Fermentation, Down-Stream Processing, Compound Production, Storage and Distribution) and TTU 09.826 (PAACT – Precision Access to Antibiotic Compounds and Targets), we have built libraries of extracts and natural products exhibiting antibacterial, antifungal, antiviral, or antiparasitic activity.

Building on this foundation, our new extended fractions library initiative will generate 20,000 samples from 1,000 mycobacterial strains in our unique strain collection by mid-2027. To support this effort, we have optimized cultivation

and extract processing, increased material availability for screening, enhanced the sample complexity, and improved the prediction of bioactive compounds from primary datasets. These developments are complemented by novel automation and high-throughput technologies, including LC-MS, fractionation, liquid handling, bioactivity assays, sample storage, and integrated data management.

P-2-78

Strengthening Antimicrobial Resistance Surveillance in Ghana: Laboratory-Based Evidence from Two Referral Hospitals

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Antimicrobial resistance (AMR) is a major global public health threat, causing 1.27 million deaths annually and further contributing to nearly 5 million worldwide. Projections estimate up to 10 million deaths per year by 2050 if AMR goes unchecked, with the heaviest burden in low- and middle-income countries. In Ghana, as in much of sub-Saharan Africa, limited diagnostic capacity and frequent reliance on empirical treatment contribute to poor outcomes, prolonged hospital stays, and excess mortality. Surveillance systems are scarce, particularly in rural hospitals, leaving critical gaps in resistance data and hindering evidence-based treatment decisions.

To address this, we established a prospective AMR surveillance system. The network comprises microbiology laboratories embedded in referral hospitals in Ghana, with capacity building through staff training in diagnostics and antimicrobial stewardship. Blood samples were processed using the BACTEC automated culture system, urine specimens on selective media, and isolates identified by biochemical methods. Antimicrobial susceptibility testing followed EUCAST guidelines, with results reported to clinicians within 24–48 hours to support evidence-based treatment. Only resistance rates for pathogen-antimicrobial combinations with at least 15 tested isolates were calculated.

Between April 2019 and December 2024, 9,477 clinical samples were processed from two hospitals in Ghana, Assin Foso and Agogo, including 6,020 blood and 3,457 urine cultures. Of these, 477 blood (7.9%) and 1,029 urine (29.8%) cultures were positive. Pathogen distribution showed site-specific differences. In Agogo blood cultures, *Staphylococcus aureus* (24.4%), *Klebsiella* spp. (19%), and *Escherichia coli* (15.5%) predominated, while in Assin Foso, *Streptococcus* spp. (21.3%), *S. aureus* (19%), and *Acinetobacter* spp. (13.1%) were most frequent. Urine cultures in both hospitals were dominated by *E. coli* (58.8% in Agogo, 53.7% in Assin Foso), followed by *Klebsiella* spp. (18.6% and 20.8%). Resistance analysis showed that extended-spectrum β -lactamase (ESBL) production in *E. coli* and *Klebsiella* spp. ranged from 70–80%, carbapenem resistance from 0–9%, and methicillin resistance *S. aureus* (MRSA) prevalence from 45–56% across both hospitals and specimen types.

This surveillance program demonstrates the feasibility of sustainable, laboratory-based AMR monitoring in Ghanaian referral hospitals. Findings highlight high rates of ESBL producers and MRSA, as well as the presence of carbapenem resistance. Locally generated antibiograms

provide essential evidence to guide treatment, hospital guidelines, and stewardship interventions. At the policy level, the data support the national AMR action plan and contribute to global monitoring through WHO GLASS. Strengthening microbiology capacity in referral hospitals shows that high-quality resistance data can be produced, offering a scalable model for regional surveillance.

P-2-79

Bacterial cell wall biosynthesis as target for novel antibiotic compounds

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Antimicrobial resistance represents a major global health threat as the treatment options for bacterial infections caused by (multi-) drug-resistant pathogens are hardly limited. The majority of antibiotics in clinical use today are derivatives of molecules discovered in the early to mid-20th century and novel antibiotics with new modes of action or targets are urgently needed (1). The cell wall biosynthesis pathway was the first one to be utilized for antibiotic intervention and is still the most important target despite the development of resistance (2).

Resistance to daptomycin, a cyclic lipopeptide frequently used for the treatment of complicated bacteremia, is a prime example of this alarming situation. As the restricted number of antibacterial drug targets limits *de novo* development, chemical modification of existing compounds represents an alternative development option for future antimicrobials. This approach involves altering compounds to target bacteria through multiple mechanisms and/or to reinforce them against resistant strains. Herein, the conjugation of polycationic peptides to daptomycin enhanced its effectiveness against a highly daptomycin-resistant laboratory strain of *Staphylococcus aureus* and clinical isolates of *Enterococcus faecium* with reduced daptomycin sensitivity.

References:

(1)Lewis, Kim. The Science of Antibiotic Discovery. In: Cell 181 (1), S. 29–45. 2020. DOI: 10.1016/j.cell.2020.02.056.

(2)Schneider T, Sahl H-G. An oldie but a goodie - cell wall biosynthesis as antibiotic target pathway. Int J Med Microbiology 2010; Vol. 300 (2-3): S. 161–169.

P-2-80

Development of a rapid test for the detection of antibiotic resistant *Neisseria gonorrhoeae*

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The WHO estimates that *Neisseria gonorrhoeae* (NG) is the third most common sexually transmitted infection in the world, with approximately 106 million new infections annually. Detection of NG is performed by time-consuming and expensive real-time-PCR-analysis, requiring specialised equipment and skilled personnel. Hence, there is an unmet medical need for the development of a rapid and easy-to-use antibody-based Lateral Flow Assay (LFA), performed directly by the attending physician. Tests of this type already exist for NG, but they usually have low specificity or sensitivity, cannot detect antibiotic resistance, and are not approved for routine diagnostics.

To identify better target antigens for NG detection by LFA, whole-genome-sequences of 70 NG strains were analyzed and screened for conserved genes. Out of 1567 alleles, 88 ORFs with 100% identity were determined. Of those, the most highly expressed ORFs were identified by qRT-PCR against a collection of unrelated NG strains with distant relationships (based on core-genome MLST analysis). The genes were ranked and five were selected, cloned and expressed in *E. coli* pET29b-expression system. Mice were immunized with these recombinant antigens and monoclonal antibodies (moabs) against each of these target proteins were generated by hybridoma technology. These moabs will be screened for binding to the endogenous NG protein and ultimately one antibody pair has to be selected to bind to one NG antigen in the NG-LFA.

Antibiotic resistance in NG against penicillin, quinolones, cephalosporins, tetracyclines, and aminoglycosides is based on single amino acid mutations in the *gyrA*, *penA*, *mtr*, *norM*, or *penB* genes. Based on Wang et al. (2020), a novel LFA format will be developed to detect these mutations in the corresponding mRNAs by using single-stranded DNA sequences immobilized in a line on the LFA strip. The mRNAs of the lysed bacterial isolate to be tested form a heteroduplex strand with the complementary DNA, which is then detected by a gold-conjugated monoclonal antibody (mAb S9.6, Bou-Nader et al. 2022) and thus displays a visible line when the target mRNA is present. The DNA-RNA hybridization conditions must be combined with the antibody-based detection of the selected NG antigen so that only the mRNAs of mutated target genes bind, thus indicating "resistance." Due to the simple and rapid adaptation of the DNA sequence to be immobilized, new mutations or variants can be quickly included in the heteroduplex LFA test, thus offering a high degree of flexibility.

Our approach will allow a fast and specific detection of NG combined with its antibiotic resistance determinants by using an affordable LFA. The implementation of antimicrobial resistance (AMR) in the NG-LFA will ensure appropriate treatment of patients with effective antibiotics and prevent AMR development against last-resort antibiotics.

P-2-81

Uncommon genomic rearrangements drive vancomycin resistance in *vanB*-positive *Enterococcus faecium*

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Introduction: The *vanB* cassette is composed of the *vanY*, *vanW*, *vanH*, *vanB* and *vanX* resistance genes and regulated by the two-component regulatory system *vanR* and *vanS*. As

part of routine diagnostic and surveillance studies, we have sequenced over 3,000 vancomycin-resistant *Enterococcus faecium* (VREfm) isolates on the MiSeq platform and noticed that some isolates lacked regulatory genes. We aimed to investigate the *vanB* expression of these isolates.

Material and Methods: Raw-reads were assembled using Velvet and the *vanB* cassette was detected using ARMFinderPlus (Ridom SeqSphere+). SPAdes and SKESA assemblies were used for confirmation. Vancomycin-resistance was confirmed by disc diffusion and broth microdilution. Van-gene presence was tested by PCR. Long-read sequencing was performed on MinION followed by Flye or Unicycler assembly. Endogenous protein expression of VanB was detected with the aid of monoclonal antibody generated by hybridoma technology and used in dot blot ELISA.

Results: We investigated the *vanB* cassette of >1700 isolates collected between 2013-2023. 12 isolates were missing regulatory genes or the ligase; 11 lacked *vanR* and *vanS*, while one isolate lacked *vanB*, confirmed also by different assembly algorithms. Susceptibility testing revealed that 10 of the 12 isolates were vancomycin susceptible, linked with the absence of the regulatory genes or the ligase. However, two ST117/CT71 isolates were vancomycin-resistant, despite lacking *vanR* or *vanS*. Expression of the *vanB* cassette was confirmed by dot blot ELISA in the absence and presence of vancomycin. Long-read sequencing identified in one isolate up to 3 copies of the *vanB* gene cluster (*vanY*, *vanW*, *vanB* and *vanX*) bracketed by *ISEnfa3*. In the second isolate, phage genes were detected upstream of the *vanB* gene cluster (*vanY*, *vanW*, *vanH*, *vanB* and *vanX*). In both cases, alternative promoters could regulate the expression of the VanB cluster leading to the observed resistant phenotype.

Conclusion: Retrospective analysis identified genotypically susceptible isolates lacking the response-regulator and sensor-kinase, but were phenotypically vancomycin-resistant, mediated through alternative mechanisms of gene expression due to uncommon genomic rearrangements. Our findings highlight the diversity of vancomycin resistance mechanisms in *E. faecium* and underscore the need for further investigation.

P-2-82

Prevalence of Repeat Positivity following Diagnosis with Chlamydia trachomatis, Neisseria gonorrhoeae, or Mycoplasma genitalium: A Systematic Review

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Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and Mycoplasma genitalium (MG) are common sexually transmitted bacterial pathogens. Infections may contribute to various morbidities of the reproductive tract in men and women and continue to be a major public health concern, as antibiotic resistance, coinfections and thus treatment failure are frequently observed. The aim of the present systematic review is to evaluate the prevalence of repeat positivity among people with one of these infections (CT, NG, MG) following a previous diagnosis through, for example, recurrent or persistent infection.

The review was registered on PROSPERO (CRD42024567220) and follows the PRISMA guidelines.

Multiple databases (EMBASE and MEDLINE via OVID, Scopus, The Cochrane Library) and registers (ClinicalTrials.gov, WHO International Clinical Trials Registry platform) were systematically searched using key terms related to repeat positivity and the pathogens of interest. In total, 17,063 references published between April 01, 2008 and June 30, 2025 were collected and de-duplicated using the reference manager Endnote. The starting date was selected to capture studies conducted after the search period of two systematic reviews on reinfection rates among men (1) and women (2) published in 2007 and 2009, respectively. Assisted with the software Rayyan, 9,007 titles and abstracts will now be screened for relevance followed by full text screening according to predefined eligible criteria. Observational and intervention studies on populations of any age with self-reported or lab-confirmed repeat positive test result for CT, NG, or MG and known prior infection with at least one of the pathogens will be included. Screening of and data extraction from relevant studies will be performed independently and in a blinded manner by two reviewers. Publications identified in the citations of included studies will further be screened for eligibility. If available evidence allows, we will stratify our prevalence estimates by relevant subgroups that are (hypothesized to be) associated with risk of infection and/or re-infection with the sexually transmitted infections of focus, i.e.: age, gender, populations of special interest (i.e. women in antenatal care, men who have sex with men), sexual behavior risk factors (i.e. HIV status, partner management use), asymptomatic repeat infection, presence of an antimicrobial resistant CT, NG or MG pathogen, presence of coinfection at index diagnosis or anatomical site of infection.

(1) Fung, M. et al (2007). Chlamydial and gonococcal reinfection among men: a systematic review of data to evaluate the need for retesting. Sexually transmitted infections, 83(4), 304–309.

(2) Hosenfeld, C. B. et al (2009). Repeat infection with Chlamydia and gonorrhoea among females: a systematic review of the literature. Sexually transmitted diseases, 36(8), 478–489.

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P-2-83

Challenges in the implementation of complex health interventions in clinical research: experiences from MAP-FGS in Madagascar

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Female genital schistosomiasis (FGS) is a gynaecological condition caused by chronic infection with *Schistosoma haematobium* (Sh), affecting approximately 56 million women and girls across sub-Saharan Africa (SSA) and causes serious complications such as infertility. In endemic regions, mass drug administration (MDA) with praziquantel (PZQ) is the primary strategy to control and eliminate schistosomiasis. Although PZQ clears adult worms, its impact on the prevention or resolution of FGS remains uncertain. Moreover, as MDA mainly target school-aged children, the long-term impact on adolescent girls and adult women is still poorly understood. Thus, the main aim of this study (MAP-FGS) was to assess the burden of FGS in women and girls between 10 and 60 years in SSA through a multi-country approach. In Madagascar, a cross-sectional study was conducted in two regions with different Sh transmission settings: Boeny and Diana, with high (>50%) and low Sh prevalence (0–10%), respectively. A target of 1200 women (18–60) and 276 girls (10–17) were to be recruited to capture exposure before and after the roll-out of the national MDA program. For diagnosis, FGS or probable FGS cases were confirmed by the identification of parasite eggs combined with colposcopy examination in women, and based on clinical symptoms in girls. Between April and July 2025, participants from 15 fokontany in each region were recruited, surrounding the three study sites at primary healthcare centres for each region. Overall, we visited 4407 households. From those, 1368 women and 293 girls took up the proposed service and visited the study sites. However, several challenges were encountered. Despite the high participation rate, the main challenge was reaching and recruiting participants far from healthcare centres. Furthermore, procurement and logistics of study materials proved difficult in these settings. In addition, permanent electricity supply was not always provided, posing a significant challenge for the study team performing colposcopy and microscopy, as well as maintaining the appropriate local storage conditions for collected samples. Working hours have also been adjusted to accommodate the participant's free time, as most work in rice fields during the day, especially the age group 18–25. Despite significant challenges encountered during the implementation of MAP-FGS in Madagascar, the Recruitment was successfully completed in 15 weeks. By documenting the burden and characteristics of FGS in this context, this study will ultimately inform national and regional schistosomiasis control strategies including women and girls who are not reached by school-based MDA programmes.

P-2-84

Effects of Sub-MIC Antibiotic Exposure on Staphylococcal Biofilm Formation

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Question: Biofilm-forming and multidrug-resistant bacteria pose a major challenge, particularly in implant-associated infections. In addition to developing new antibiotics, optimizing the use of existing agents is increasingly relevant. Dalbavancin, a lipoglycopeptide with strong activity against gram-positive bacteria, including MRSA, and a long half-life, is promising for both systemic and local use [1]. We have previously shown that local release of dalbavancin reduces inflammation and bacterial spread after biomaterial implantation in vivo [2]. Here, we mimicked declining antibiotic levels during local release and investigated the effect of subinhibitory concentrations on bacterial adherence and biofilm formation, compared to rifampicin and minocycline.

Methods: *Staphylococcus aureus* ATCC 35556 was grown in antibiotic concentrations ranging from 2x MIC to 1/32x MIC in 96-well polystyrene plates using broth microdilution. In accordance with EUCAST recommendations, susceptibility testing for dalbavancin was performed using final concentrations of 0.002% Tween 80. After 24 h of incubation in tryptic soy broth, wells were washed three times with phosphate-buffered saline. Biofilm biomass was then quantified using 0.06% crystal violet staining, referenced to an untreated control, or serial dilutions were plated to determine colony-forming units (CFU/ml).

Results: While bacterial cell counts remained stable across all tested antibiotic concentrations, noticeable differences in biofilm formation were observed. Rifampicin slightly increased relative biofilm mass at all tested concentrations. Minocycline reduced adherent biofilm mass at lower sub-MIC concentrations (1/8x and 1/4x MIC), while higher selective pressure (1/2x MIC) resulted in biofilm mass comparable to the untreated control. Although the combination of rifampicin and minocycline increased bacterial susceptibility, no additional inhibition of biofilm formation was observed compared to minocycline exposure alone. Finally, dalbavancin demonstrated a strong, concentration-dependent reduction in biofilm mass, particularly at 1/4x and 1/2x MIC.

Conclusions: Our data underscore the importance of determining both CFU/ml and biofilm mass to gain a comprehensive understanding of antibiotic-induced changes in biofilm formation. The results suggest a specific anti-biofilm effect for dalbavancin at subinhibitory concentrations. While rifampicin is often used in combination with minocycline to reduce the development of resistance, our data indicate that its presence may attenuate anti-biofilm effects. Ongoing qPCR and SEM analyses will help elucidate the mechanistic and structural basis for these observations and guide the rational design of future anti-infective, drug-eluting biomaterials.

References:

[1] J. R. Smith et al., *Infect. Dis. Ther.* **2015**, 4, 245–258. DOI: 10.1007/s40121-015-0077-7

[2] M. Kloss et al., *Front Bioeng Biotechnol.* **2022**, 10, 1021827 DOI: 10.3389/fbioe.2022.1021827

P-2-85

Ureaplasma parvum selects for a higher volatility of lactobacillus species which is directly connected to vaginal dysbiosis

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Introduction: The vaginal microbiome displays the first line of defence against sexually transmitted pathogens and plays a key role in human reproduction. One of the most common pathogens colonizing the vagina is *Ureaplasma parvum*, whose role in vaginal health and the development of reproductive tract diseases is not yet fully understood. We wanted to delineate what significance the presence or

acquisition of *U. parvum* has for the composition and stability of the vaginal microbiome.

Methods: We, therefore, longitudinally sampled cervical swabs from women aged 18- 22 years of age as part of our ESTIMATE study, recorded data on demographics, sexual behaviour and previous sexually transmitted infections (STI), and performed molecular testing of STI twice a year. The vaginal microbiota was analysed using 16S rRNA gene-based analysis in conjunction with predictive modelling of metabolic pathway abundances within the microbiome. A logistic regression model was built on the basis of microbial volatility to enable prediction of *U. parvum* acquisition.

Results: We detected *U. parvum* in 81 of 240 women (33.75%) at baseline visit. Women that tested positive for *U. parvum* show significantly increased numbers of sexual partners and duration of sexual activity compared to women without *U. parvum* colonisation. *U. parvum* presence is recorded in all CST apart from CST II. However, CST IV-B shows significantly enhanced prevalence of *U. parvum* (75 %) compared to all other CST (up to 36%). *U. parvum* positivity is accompanied by significantly higher α -diversity and the presence of specific bacterial taxa (mainly *Gardnerella* spp. and *Atopobium* spp.). As a functional consequence the microbiome of *U. parvum*-positive women displays an increased presence of the bifido shunt than *U. parvum*-negative women, a mechanism that is centrally involved in acetate production. Our data show, that in women with increased number of sexual contacts and longer duration of sexual activity an increase of volatility of the two most abundant vaginal *Lactobacillus* species predicts the risk of *U. parvum* acquisition.

Conclusions: The presence of *U. parvum* is associated to functional changes within the microbiome transferred majorly through *Gardnerella* spp. and *Atopobium* spp. These taxa presumably transmit an unbalanced acetate vs. lactate availability in the vagina in otherwise asymptomatic and healthy women. Importantly, we centrally highlight the role of volatility of most prevalent vaginal microbes as a predictor to acquisition of sexually transmitted microbes in asymptomatic women of our healthy cohort. Our data opens a new chapter in trying to dissect the role of microbiome function and instability with a specific focus needed to be set on sexually transmitted pathogens such as *C. trachomatis* or *N. gonorrhoea*.

P-2-86

Mode of action analysis of a vancomycin-teixobactin conjugate that restores activity against VRE

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The emergence of vancomycin-resistant enterococci (VRE) poses a major clinical challenge, compromising one of the last-line glycopeptide therapies. Here, we report the characterization of a novel vancomycin-teixobactin conjugate that exhibits markedly enhanced antibacterial potency against Gram-positive pathogens, including both vancomycin-susceptible and clinical VRE isolates. While neither vancomycin nor the inactive cyclic teixobactin fragment alone shows activity against VRE, the Cbp-lys10-teixo7-11-vanco conjugate restores potent antibacterial efficacy. Compared to natural, unmodified vancomycin, the hybrid displays up to 10-fold greater activity against *vanA*-type and 40-fold against *vanB*-type VRE. Mechanistic studies revealed that the hybrid retains high-affinity binding to peptidoglycan precursors such as Lipid I and Lipid II, thereby blocking their utilization in cell-wall assembly. Under

vancomycin-induced conditions, VRE cells accumulated the cell-wall precursor UDP-MurNAc-pentapeptide, demonstrating efficient inhibition of cell wall biosynthesis in cells synthesizing the modified Lipid II d-Lac precursor. These findings highlight the potential of rationally redesigned glycopeptides to circumvent established resistance mechanisms and restore the therapeutic utility of vancomycin-like antibiotics.

P-2-87

Metagenomic analysis of gut microbiome composition dynamics on strain level in humans and implications for future antimicrobial therapies

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The human gut microbiome confers colonization resistance against potentially pathogenic bacteria, including those capable of expressing or transmitting antibiotic resistance mechanisms such as extended-spectrum beta-lactamases (ESBLs). Antibiotic-resistant bacterial pathogens (ARBPs) can cause endogenous infections that require antibiotic treatment. Antibiotic-driven selection pressure promotes the spread of resistances creating a vicious cycle. To interrupt this cycle, novel microbiome-based strategies are needed to enhance resilience against colonization by and spread of ARBPs, including strain displacement strategies in which ARBPs are eliminated by better-adapted, non-resistant commensal strains.

To date, little is known about strain-level dynamics of microbiome composition, including the displacement of individual species. Elucidating these processes requires deep metagenomic sequencing of the gut microbiome.

To investigate this, we leveraged a Swiss study cohort in which stool samples from 38 healthy travelers returning from Southeast Asia were collected before travel and for up to one year thereafter. Samples were analyzed using metagenomic shotgun sequencing (MSGs; 50-100 Gb/sample) to resolve microbiome composition across taxonomic levels up to strain level and to investigate potential displacement dynamics of gut microbiome phylotypes.

In a first step focusing on *Escherichia coli* (*E. coli*), MSGS data were compared with whole-genome sequencing (WGS) data from isolated ESBL-positive and ESBL-negative *E. coli* strains generated by the laboratories in Zürich. To assess feasibility, stool samples from two participants with high or low ESBL-*E. coli* CFU counts after travel and over the period of one year were sequenced and analyzed.

Metagenomic sequencing at 50 and 100 Gb yielded sufficient numbers of reads assignable to *E. coli*, with read counts closely correlating with CFU measurements. Bioinformatic analyses enabled reconstruction of *E. coli* metagenome-assembled genomes (MAGs), which were identical to the WGS of corresponding *E. coli* sequence types (ST). However, only a subset of *E. coli* STs detected by culture and WGS could be reconstructed from MSGS data.

Together, MSGS combined with MAG reconstruction provided insights into *E. coli* displacement dynamics in the

gut microbiome and established a ground truth for extending this approach to other species across different phyla.