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2ND ISIDORe USER CONFERENCE

Pandemic Preparedness & Response Through ISIDORe:

A Celebration of Users & Research Infrastructures

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ABSTRACT BOOK


ISIDORe

GLOSSARY OF KEY TERMS

Integrated Services for Infectious Disease Outbreak Research – ISIDORe

An EU-funded research infrastructure project providing fast, free-of-charge access to high-end services and resources to support research on epidemic-prone pathogens.

Research Infrastructure – RI

Facilities, resources, and services available for access to the scientific community to conduct high-level research. These are commonly referred to as *RI services*. ISIDORe integrated the services of 17 RIs to deliver a unified service offer in pandemic preparedness and response research.

Access Provider

A facility or institution that is part of a RI and delivers services and resources (e.g. laboratories, data, biobanking, etc.) to external researchers. In ISIDORe, the consortium gathered close to 150 access providers.

Catalogue of Services

The curated list of RI services made available to external users through ISIDORe.

User Project

A research project proposed by an external researcher (user) and selected for access to ISIDORe's RI services.

Transnational Access – TNA

A funding mechanism that enables external researchers (users) to access services offered by the project's access providers. In ISIDORe, TNA supported over 180 research projects across Europe and beyond.

Joint Research Activities – JRA

Internal collaborative efforts conducted by ISIDORe access providers to improve technologies, methodologies, and the readiness of the project's catalogue of RI services.

UNITED FOR PREPAREDNESS: THE ISIDORe EXPERIENCE

The ISIDORe (Integrated Services for Infectious Disease Outbreak Research) project comes to a close after three and a half years of unprecedented **collaboration, scientific progress, and service** to the global research community. **The 2nd ISIDORe User Conference marks the culmination of this collective effort** by more than 150 partners committed to strengthening pandemic preparedness and response across Europe and beyond.

With ISIDORe, Europe took a decisive step toward a **more connected, responsive, and purpose-driven research ecosystem** for tackling infectious disease threats. Operating amid not one, but two active health crises – COVID-19 & mpox – the project showed that fast, coordinated mobilisation of research infrastructures is not only possible – but essential – to meet the demands of high-impact outbreaks.

Over its duration, ISIDORe has supported over **180 user projects via Transnational Access (TNA)**, enabling researchers to access critical high-end services and resources free of charge. This conference highlights about a quarter of the supported TNA projects, yet already showcases the **remarkable scientific diversity, public health relevance, and disciplinary breadth** fostered through ISIDORe – a striking illustration of the scale and strategic value of its integrated approach.

Beyond supporting users, ISIDORe access providers conducted **Joint Research Activities (JRA)** to advance technologies, improve internal practices, and strengthen long-term infrastructure readiness. Together, TNA and JRA form the backbone of ISIDORe's **contribution to Europe's broader health preparedness agenda** and serve as a practical demonstration of what research infrastructures can deliver when mobilised with urgency, coordination and purpose.

This conference highlights the achievements of all these scientists – users and providers – who helped **advance our understanding of infectious diseases and develop better tools for their detection, treatment, and control**. It reflects the diversity and excellence of ISIDORe-supported research, firmly anchored in public health priorities and spanning multiple scientific disciplines and pathogen families.

As ISIDORe concludes, this abstract book serves not only as a scientific compendium but also as a **compelling case for embedding such integrated capabilities into Europe's long-term preparedness architecture**.

TNA SESSION 1

USER PROJECTS ON SURVEILLANCE, DIAGNOSTICS, VECTOR CONTROL & SOCIAL SCIENCES

Serological Evaluation of Crimean-Congo Haemorrhagic Fever in Humans with High-Risk Professional Exposure & in Residual Sera Collected in 2022-2023 Across Corsica (France)

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The Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne pathogen known for causing severe viral haemorrhagic fever.

We aimed to evaluate the potential CCHFV circulation in Corsica through a serosurvey among individuals consisting of anonymized residual sera (RS) and high-risk individuals exposed to animals and/or tick-bites due to occupational activities.

This cross-sectional study involved two groups: anonymized RS from medical biology laboratories, and sera from high-risk individuals (slaughterhouse workers, veterinary professionals, animal farmers, and rangers) collected across Corsica during 2022-2023. Antibodies targeting CCHFV viral nucleoprotein were detected using double-antigen ELISA. ELISA-positive samples underwent neutralizing antibody testing. Sociodemographic and epidemiological data were recorded using structured questionnaire in high-risk group.

Anti-CCHFV IgG seropositivity was of 0.08% (n=2) [95% Confidence Interval (CI): 0.06; 0.09] in RS and of 0.50% (n=1) [95% CI: [0.43; 0.56]) in high-risk groups (p=0.00). Lifetime tick bites was reported by 65.9% (n=118) of respondents, with higher rates among farmers (Odds Ratio (OR) = 3.4; 95% CI 1.4–8.5) and participants with >10 years of occupational exposure (OR = 3.8; 95% CI 1.7–8.5).

This study provides initial evidence of human exposure to CCHFV in Corsica, with rates consistent with those observed in other Western European regions. Our results indicate a risk of CCHF among the Corsican population, particularly among farmers and slaughterhouse workers. Continuous surveillance and public education are essential to mitigate this risk, especially among these targeted groups and healthcare professionals, ensuring prompt diagnosis and prevention of potential outbreaks.

Isolation of CCHFV in Spanish Ticks & Confirmation of Specific Antibodies in Animals

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Risk group 4 viral pathogens represent a public health problem. One of these pathogens is Crimean-Congo haemorrhagic fever virus (CCHFV), a tick-borne virus with limited therapeutic strategies and no vaccine. CCHFV is the only BSL-4 pathogen present in Europe, including Spain. Improving knowledge of Spanish CCHFV strains and classifying animal infections would help for a more accurate risk modelling and improve CCHF diagnosis.

We attempted CCHFV isolation from PCR-positive Spanish ticks (BSL-4) to characterize the complete genome of isolates. We started with multi-genotype RT-qPCRs and attempted CCHFV isolation by blind passages VeroE6 & SW13 cell lines. Cells were observed for cytopathic effects (CPE) and the supernatants were tested for viral genome presence by RTqPCR. We tested a selection of red deer sera (37 ELISA-pos and 18 ELISA-neg), serially collected from twenty 3-32- month-old animals in a CCHFV enzootic area of Spain, with two CCHFV isolates (IbAr 10200, G-III; Kosova Hoti, G-V) using virus-neutralization-tests (VNTs). We tried to adjust a human IgM ELISA against CCHFV to red deer. Neither CPE nor viral genome replication was detected. None of the 18 ELISA-neg sera showed neutralising activity for CCHFV G-III and G-V, while 21 of the 37 ELISA-pos sera (56.8%) showed ND50 values 8, mainly against G-III (ND50:8-42.5). Four out of nine 3-month-old ELISA-pos animals presented ND50 values 8, but all 9 were ELISA/VNTneg 4 months later, indicating a maternal origin of these antibodies and their neutralising activity.

We observed strong viral neutralising activity in three 7-month-old animals that was still evident 6 months later, indicating a probable infection at the autumn peak of Hyalomma lusitanicum. A fourth animal (ELISA/VNT-neg at 3 and 7 months of age) presented an increase in neutralising antibodies at its 13 months, after the spring peak of H. lusitanicum. Three of these 4 animals again presented neutralising activity at 19 months of age, suggesting re-infections during the autumnal peak of H. lusitanicum. In 2 of them, the neutralising antibodies persisted 6 months later. Four of the 20 animals (20%) remained ELISA-neg until 25-32 months of age.

The results of the VNTs suggest that neutralising antibodies may be good indicators of recent infections as initially hypothesized, supporting their usefulness to identify when and where CCHFV is more prone to be transmitted and prevent CCHF cases.

Development of Next Generation Respiratory Infection Diagnostics (NeResDia)

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The recent SARS-CoV-2 pandemic demonstrated the high demand for diagnostic tests to combat COVID-19, emphasizing the importance of pre-analytical factors like transportation duration and storage temperature for reliable results (Hardt et al., 2022, Hardt et al., 2024). Additionally, safe handling protocols, multiplex testing of respiratory viruses and adaptable diagnostic workflows for high throughput are crucial for addressing pandemics.

The project involves cell culture and spike in experiments under BSL-3 conditions in facilities experienced with ISO/CEN standards for pre-examination workflows. Based on the ERINHA project, we examined the effect of storage duration (up to 96 hours) and temperature (RT and 37°C) on the stability of respiratory viruses in a commercial swab system.

Tested viruses included SARS-CoV-2, RSV A&B and influenza A&B. Viral RNA was isolated from cell culture experiments to test viral inactivation and from stability studies to assess post-collection stability. Virus quantification was done by RT-qPCR after RNA extraction or directly using the QIAprep&amp system. We also studied how substances like nasal spray components could affect qPCR accuracy and interfere with results. Nucleic acids of all tested viruses spiked in UVT swab system medium remained stable for up to 96 hours at RT and 37°C. In contrast, TMA-D and TMA-P compounds from QIAGEN inactivated all tested viruses, though their concentrations were incompatible with the QIAprep&amp workflow. TMA-P, however stabilized viruses at RT and 37°C for up to 96 hours. Benzalkoniumchloride (BAC), a nasal spray component, inactivated viruses and is compatible with QIAprep&amp for high-throughput testing, but it did not stabilize viruses at 37°C. Dequaliniumchloride showed no viral inactivation potential. Additionally, nasal spray solutions interfered with qPCR outcomes using QIAprep&amp.

Previous studies showed the importance of pre-analytical factors. In the present project, we have developed two different workflows that can be adapted to various diagnostic needs especially during flu seasons and pandemics. BAC inactivates respiratory viruses and is compatible with QIAprep for high-throughput detection, while TMA-P inactivates viruses and stabilizes nucleic acids for up to 96 hours at 37°C. Furthermore, nasal sprays used prior sample collection can interfere with diagnostic tests, so patient information is required to avoid interfering substances and false qPCR results.

SARS-CoV-2 Variant Identification by RT-PCR vs. Whole Genome Sequencing: A Comparative Study in a Cohort of Spanish Healthcare Workers

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We aimed to compare two molecular techniques for determining SARS-CoV-2 lineages: whole genome sequencing (WGS) and PCR-based single nucleotide polymorphism (SNP) detection, within the framework of a prospective cohort of 428 Spanish healthcare workers enrolled in a multicentre study funded by the European Centre for Disease Prevention and Control (ECDC) and conducted from July 2021 to April 2024.

WGS, supported by Horizon Europe through the ISIDORe network and facilitated by EATRIS services, was performed using the Artic v5.3.2 protocol primers and an Illumina® MiSeq™ instrument. This was done on all nasopharyngeal samples (collected weekly) confirmed by PCR for SARS-CoV-2 (Ct < 28) according to routine hospital protocols. The pre-processed FASTQ files were analysed at the end of 2024 using HAVoC v1.0.0 software to identify variants. Alternatively, multiple RT-PCR mutation analysis (probe-based melting curve) for SARS-CoV-2 lineage identification was performed in the hospital microbiology laboratory on the same day as sample collection, using TIB MOLBIOL VirSNiP assays.

After removing duplicates (to ensure that only one sample per episode was analysed) and those failing to meet quality standards, 138 isolates with paired results were included in the analysis. Considering the maximum depth resolution of the VirSNiP kit, the overall agreement between the two techniques was 98.5%, with only two discrepancies attributed to the misassignment of BA.2-like lineages. However, when evaluated for accurate lineage assignment, the agreement dropped to 25.3%, with only 35 samples correctly categorised by PCR.

Notably, WGS proved more reliable for lineage assignment during periods when the PCR kit had not yet been adapted to the circulating strains. PCR-based SNP lineage assignment was particularly useful early in the pandemic for guiding treatment decisions, such as the administration of monoclonal antibodies for what the kits were tailored. However, its utility diminished due to increased viral diversity, evolving lineage definitions, continuous database updates, and delays in kit updates. Concurrently, WGS became faster and more accessible, offering superior accuracy and the potential for additional analyses. Further optimization of WGS will ensure its continued application in molecular epidemiology, particularly in the surveillance of emerging diseases and potential pandemics.

Information Practices & Needs of Individuals Living with Post-COVID-19 Condition

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Post-COVID-19 condition (Long COVID) is a major health issue affecting millions worldwide. Estimates suggest that 7–20% of individuals experience persistent or new symptoms beyond three months post-infection. With limited understanding of its causes, diagnostics, mechanisms, and treatments, many affected individuals seek information independently, often online. However, little is known about how they navigate available information, the barriers they face, and their specific needs. Given the uncertainties surrounding Long COVID and symptoms like fatigue and cognitive impairment that may hinder information processing, this study explores information-seeking behaviours and needs among affected individuals.

An online survey (preregistered at https://aspredicted.org/5DJ_L26) was conducted via Panelclix (September 3–17, 2024). Participants (N = 571; 60.6% female, mean age 45.5 years) met three criteria: being 16+, living in the Netherlands, and having post-COVID-19 for at least three months. Most (64.5%) had symptoms for over two years, with 83.1% reporting daily life limitations. Common symptoms included fatigue (53.1%), concentration issues (49.7%), and loss of physical condition (46.4%).

Nearly a third (29.6%) had searched for post-COVID-19 information in the previous two weeks. Key topics included symptoms, symptom management, daily life adjustments, and acceptance. The most-used sources were the internet, personal networks, healthcare professionals, and peers. Participants primarily sought information on treatments, symptoms, personal health (e.g., test results), and symptom management – favouring healthcare professionals as sources. Participants reported challenges in finding reliable information, citing a general lack of knowledge ($M = 5.10$, $SD = 1.70$) and difficulties accessing details on new treatments ($M = 4.38$, $SD = 1.89$) or relevant personal content ($M = 4.27$, $SD = 1.81$). Feeling well-informed was positively associated with mental well-being ($r = .11$, $p = .007$).

People with post-COVID-19 actively seek information but rely on the internet and social networks despite preferring healthcare professionals. The gap between available information and patient needs underscores the importance of improving healthcare communication. Additionally, the link between being well-informed and mental well-being highlights the need for accessible, accurate information to support affected individuals.

Testing Whether the Application of Sterile Males for Two Successive Activity Seasons Can Further Reduce the Risk of Transmission of Aedes Albopictus-Transmitted Diseases in Temperate Areas: Swiss Case

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The exotic invasive tiger mosquito, Aedes albopictus, appeared in Canton Ticino (southern Switzerland) in 2003. The spread of the mosquito has been surveyed constantly since then, and an integrated vector management, focused on the juvenile stages of the insect, has been implemented to control its numbers. Despite the system has proven its ability to contain tiger mosquitoes the densities in the territory of Ae. albopictus still present a risk factor for disease transmission. In addition, there is a growing practice by citizens to use automatic dissemination systems of products against mosquito adults thus running the risk of facilitating resistance processes.

The aim of this project was to test whether the application of the Sterile Insect Technique (SIT) can bring these densities below the risk threshold, reduce the nuisance at the climatic and urban conditions present in the region, while also evaluating if the technique is an effective and sustainable tool to integrate into the integrated control measures for the tiger mosquito already in place. The project analyses several data from two release seasons conducted between May and September in 2023 and 2024 in 45 ha. Preliminary results are promising, showing a reduction of Aedes albopictus adult female densities by approximately 64,9% in the release area during these years.

The project carried out by SUPSI is linked to the TDR Sterile Insect Technology Project in partnership with CDC, IAEA and NTD/WHO global project that is precisely about testing whether the application of SIT can effectively and sustainably reduce the incidence of diseases related to Ae. albopictus and Ae. aegypti, such as dengue, chikungunya and Zika. The field outcomes collected in the Canton Ticino setting will therefore made available to the wide vector control community to contribute to the overall evaluation of this technique when applied against invasive Aedes.

Mapping Malaria's Journey in the Mosquito: Quantifying Organelle Secretion & Parasite Dynamics During Plasmodium Falciparum Transmission Using Advanced 3D Electron Microscopy

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Plasmodium falciparum, the deadliest malaria parasite, undergoes key developmental transitions in the mosquito, including the gametocyte, ookinete, and sporozoite stages. Despite their importance to develop effective interventions against malaria, many aspects of these transmission stages remain poorly understood. We address two specific gaps: (1) the role of a protein, GEST, present in secretory organelles across transmission stages, and (2) the maturation and midgut traversal dynamics of ookinetes. To investigate these, we (1) examine GEST's role in secretory organelles by comparing wild-type (WT) and GEST-deficient (GEST-KO) parasites, and (2) map structural changes and cellular interactions of WT ookinetes during their maturation and midgut traversal.

Using Correlative Light-Electron Microscopy (CLEM) and SEM-Array at the ISIDORe-supported Prague ALM & EM Node, we acquired high-resolution datasets of gametocytes, ookinetes (from the blood bolus and midgut), and sporozoites. Immunolabeling enabled precise localization of parasites in low-density samples, facilitating targeted imaging and the generation of detailed 3D datasets. These datasets were subsequently analysed by the Finnish Advanced Microscopy Node, producing quantitative models across multiple instances of each studied stage.

Data show significant defects in organelle secretion in GEST-KO gametocytes and sporozoites despite normal organelle formation, highlighting GEST's role in secretory processes. Ongoing work focuses on quantitative comparisons across WT, GEST-KO, and GEST-KI ookinetes, and time-resolved imaging of WT ookinetes in both blood bolus and mosquito midgut.

We successfully applied advanced imaging techniques to quantitatively study the role of a key protein in P. falciparum transmission stages, uncovering its impact on organelle secretion across transmission stages. Simultaneously, we are building a detailed structural map of ookinete maturation and its interactions with mosquito midgut tissues across multiple time points. Beyond addressing specific research questions, this work is generating a high-resolution dataset that will serve as a valuable resource for the broader scientific community, advancing our collective understanding of malaria parasite biology.

TNA SESSION 2

USER PROJECTS ON NATURAL HISTORY, VACCINES & THERAPEUTICS

Intranasal Multiepitope Nanovaccine: A Next-Generation Immunotherapy for COVID-19

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Nanotechnology-based vaccines emerged as innovative solutions for COVID-19, with the potential for improved immune responses through mucosal immunity. Supported by ISIDORe services, our research aimed to evaluate a next-generation multiepitope nanovaccine in a challenge study using a SARS-CoV-2 infection model.

This intranasal nanovaccine platform, designed for flexible and rapid deployment, co-delivers immunogenic peptides alongside PD-L1 expression regulators and adjuvants to induce robust immune responses by enhancing dendritic cell (DC) maturation and antigen presentation. Through a comprehensive immunoinformatic workflow, SARS-CoV-2 peptides were selected as key antigens, enabling the platform to generate both systemic and local immune responses. The inclusion of PD-L1-siRNA led to a robust increase in T-cell proliferation, antibody production, and neutralizing activity against SARS-CoV-2 variants, including Omicron. In vivo, siPD-L1 NV-8 induced both strong systemic and mucosal immunity, evidenced by elevated IgA levels and T-cell responses.

Additionally, the intranasal (IN) administration of this nanovaccine triggered a significant Th1-shifted immune response, demonstrated by elevated IFN-γ and IL-1β cytokine levels. In the challenge study, our findings suggest that siPD-L1 NV-8 effectively induces protective immunity against SARS-CoV-2, showing promise for broad applicability in future vaccine strategies against respiratory infections. Moreover, the lyophilized form of the nanovaccine was shown to be stable at room temperature for extended periods, making it a viable option for widespread distribution, particularly in resource-limited settings. This study underscores the potential of our nanovaccine platform as a scalable, adaptable solution to combat SARS-CoV-2 and future viral threats.

Broadly Protective Mucosal Immunity Against SARS-CoV-2

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Although licensed COVID-19 vaccines provide excellent protection from severe COVID-19, hospitalization and death, protection from infection by emerging variants of concern, such as omicron, is substantially lower and waning.

The aim of this project is to decipher the best way to induce long-lasting mucosal immunity, which may provide protection against heterologous SARS-CoV-2 variants.

At the UK Erlangen, there are no facilities available allowing non-human primate studies. The number of institutes in Europe able to perform these BSL3 studies in NHP are limited and the BPRC is a very experienced partner with a long-standing expertise in viral respiratory tract infection models in NHPs.

Three groups of six macaques were immunized with the commercially available Wuhan strain mRNA vaccine Comirnaty, by intramuscular injection at a 10µg dose at weeks 0 and 4, as a priming to induce systemic immune responses. Subsequently, animals received a mucosal booster immunization using either an adeno serotype 5 (Ad5) vector encoding the S or N protein (in total 2x109 IU/animal) or a live attenuated vaccine SARS-CoV-2 DNsp16 (106 PFU), both based on the sequences of the original Wuhan strain, via an oropharyngeal spray application. As a comparison, one of the Comirnaty immunized groups was infected with SARS-CoV-2 Delta variant. Twenty-four weeks after the booster immunization animals were challenged with Omicron lineage EG.5.1.1 via combined intranasal and intratracheal inoculation.

Comirnaty induced strong systemic IFNg T cell responses as well as high titre IgG and modest IgA anti-Spike antibodies (Ab) in serum. Spike specific IgG, but not IgA Ab could also be detected in nasal fluid and bronchoalveolar lavage (BAL) after priming. Adenoviral vector boosting enhanced anti-Spike IgG levels in serum, nasal fluid and BAL. Furthermore, Spike specific IgA Ab were increased in serum and induced in nasal fluid as well as BAL fluid to comparable levels as achieved by infection with the SARS-CoV-2 Delta virus. In BAL, CD4 and CD8 Spike specific T cell responses were induced by the Comirnaty prime and enhanced by the adenoviral vector boost. After challenge with Omicron the Ad5 boosted animals and Delta virus infected animals had significantly reduced viral loads in nose, throat and BAL, reduced CD8 T cell activation and inflammatory cytokines in the blood. The live attenuated vaccine did not result in increased immune responses or protection against infection.

Adenoviral vector immunization against SARS-CoV-2, given as a spray on the tonsil, provides efficient boosting of systemic and mucosal Ab and local T cell responses in Comirnaty primed animals and protects against Omicron virus replication and cytokine storm.

Protection Against Lethal Crimean-Congo Haemorrhagic Fever Virus (CCHFV) Infection in Ifnar$^{-/-}$ Mice Induced by Different Prime/Boost Vaccination Regimens

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Crimean-Congo haemorrhagic fever virus (CCHFV) is an emerging zoonotic tick-borne pathogen widely distributed in Africa, Asia, Eastern and Southern Europe, and the Balkans. Increased geographical distribution of this virus is associated with the spread of the Hyalomma tick vectors, influenced by global warming, bird migration, international trade and human travels. The virus causes a severe disease in humans and numerous wild and domesticated animals act as reservoirs of the virus. Although livestock are asymptomatic, these are considered amplifying hosts for CCHFV. Unfortunately, there are not effective therapies or safe vaccines commercialized nowadays. As CCHF is a serious threat to public health, there is an urgent need to further investigate on the development of safe and effective vaccination strategies.

In this work, we will evaluate the immunogenicity and protective efficacy of recombinant plasmid DNA and modified vaccinia virus Ankara (MVA) vector vaccines encoding the polyprotein glycoprotein precursor (GPC) or the nucleoprotein (NP) against CCHFV following a heterologous prime boost in mice and a CCHFV challenge. In addition, in order to study whether the vaccines may protect against other genotypes, we will perform a challenge using a different strain. Preliminary results of mice immunized with these schedules have shown a strong and specific humoral and cellular responses against viral antigens. In addition, different epitopes against several viral proteins were found to elicit a T CD8+ specific cell-mediated immune response.

Monkeypox Virus Clade IIb Propagates in Human Placenta Explants

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Vertical transmission of monkeypox virus (MPXV), the causative agent of mpox disease, poses a significant but poorly understood risk to maternal and foetal health. Case series and reports suggest that MPXV infection during pregnancy may lead to adverse foetal outcomes, including miscarriage and intrauterine foetal death. Notably, placental pathological changes, such as vascular injury, have been documented, and MPXV DNA has been detected in foetal and placental tissues, suggesting the possibility of vertical transmission.

To address this knowledge gap, the PREGPOX project aims to investigate the life cycle and cellular tropism of MPXV using a preclinical model based on human placenta explants.

Our findings indicate that while cell-associated infectious titres increase exponentially until eight days post-infection, infectious virus release remains two to three orders of magnitude lower, suggesting a primary mode of cell-to-cell spread. Confocal microscopy of MPXV-infected explants reveals viral antigens concentrated mainly in the syncytiotrophoblast layer, with potential presence in cytotrophoblast cells, as confirmed by cytokeratin co-expression analysis. However, we did not observe evidence of infection in stromal or Hofbauer cells. To assess host responses to MPXV infection, we performed RNA sequencing on placenta explants from several donors, comparing mock-treated and MPXV Clade IIb-infected samples. Data analysis is currently ongoing, and the results will be ready for presentation at the ISIDORe User's Conference.

In conclusion, our data demonstrate the ability of MPXV to infect and propagate within the human placenta, providing a foundation for further investigations into MPXV biology at the maternal-foetal interface.

Determining the Monkeypox Virus Host Range

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Mpox (MPX) is a zoonotic disease caused by monkeypox virus (MPXV) and is currently the most prevalent orthopoxviral infection in humans after the eradication of smallpox and the cessation of universal smallpox vaccination. In endemic areas (African region), MPXV circulates among a number of mammals, and occasional spill-over events to humans frequently generated outbreaks, although sustained human-to-human transmission is rare, until recently. Since early May 2022, a multi-country outbreak of Mpox was reported, with cases identified all across the world. MPXV can infect a wide range of mammal species, but no known animal reservoir currently exists outside of Africa. A major concern with the current outbreak is that MPXV may enter a new host species and establish new animal reservoirs outside of Africa through spillback from infected humans to pets, pests or farm animals.

To determine whether an animal reservoir could be established in Europe, we inoculated rats, cats and calves with an isolate of the 2022/2023 Mpox outbreak. Animals were monitored for the development of clinical signs. Physical examinations included recording of temperature, blood draw, collection of skin, oral, rectal, and nasal swabs up to daily throughout the study.

Following exposure to a clinical isolate of Mpox from the 2022/2023 outbreak via intranasal inoculation and scarification, animals did not lose weight or show overt clinical signs. In rats and calves, skin lesions were observed at the site of inoculation. Viral genome was detectable in throat and nose swabs as well as skin swabs. Results in cats are pending.

In conclusion, these results show that rats and calves are susceptible to infection with MPXV in the absence of overt clinical signs. Virus was detected in both respiratory tissues and skin at the site of scarification, suggesting the potential for transmission to and from rats and cattle early after exposure.

However, based on the acute self-limiting infection, lack of viremia, and lack of prolonged shedding as shown in this study, there is a low risk for rats or cattle to establish as a reservoir for MPXV.

In addition, considering the ongoing multi-country outbreak of clade I MPXV, here we present a potential new infection and disease model in rats that recapitulates the disease seen in most human cases and can be used for preclinical evaluation of countermeasures against MPXV.

Extracellular Vesicles in Poxvirus Infection: A Revolutionary Solution for Disease Prevention & Treatment

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Extracellular vesicles (EVs) are crucial inter-cellular signalling organelles that contain diverse bioactive components, including proteins, lipids, and nucleic acids. EVs play a critical role in many homeostatic and pathophysiological processes, such as tissue development, immune modulation, and regulation of host-pathogen crosstalk. Poxviruses, a family of large double-stranded DNA viruses, including variola, mpox (MPXV), and vaccinia virus (VACV), have a strong evolutionary link with EVs. Cellular immune responses rely on active EV secretion to combat viral infections, while numerous viruses modify EV secretion pathways to promote productive infection. Our research aims to characterize poxvirus-induced EVs (PoxEVs) and reveal their role in poxvirus infection.

At this research stage, we have utilized state-of-the-art imaging techniques. Other methods, including biochemistry and modern precision analytics, will be employed later. Experiments were conducted using VACV as a reference virus, parallel to MPXV. Imaging techniques demonstrated that poxvirus infection initiates the production of PoxEVs, which are positive for poxvirus membrane proteins and the cellular membrane marker phosphatidylserine but devoid of the cellular EV marker, CD63. The Finnish Biomedical Imaging Node at Euro-Bioimaging, Turku, Finland, supported imaging and data analysis.

Our preliminary findings suggest that poxvirus infection increases EV production in host cells. PoxEVs contain viral and host cell proteins, lipids, and nucleic acids, which most probably regulate immune response formation and poxvirus infection efficiency. The imaging and data analysis services provided by the Finnish Biomedical Imaging Node greatly facilitated these discoveries.

The study will significantly enhance our understanding of MPXV-host cell interactions and the role of EVs in poxvirus infections. The findings will highlight the potential of PoxEVs in poxvirus disease prevention, treatment, and medical applications. The results will be particularly significant in the context of pandemic preparedness and response, as they provide new insights into the mechanisms of viral infections and potential therapeutic targets.

Comparative Analysis & ISG15´S Role in Clade II MPXV Infection

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The unexpected human-to-human transmissibility (HHT) of the 2022 outbreak of clade IIb MPXV has driven research into the host-virus interactions and viral form production that may underlie this phenomenon. Interferon-stimulated gene 15 (ISG15) encodes a ubiquitin-like protein with critical immune functions, including cytokine activity when secreted extracellularly. ISG15 binds covalently to de novo proteins through ISGylation, a reversible process. Previous studies suggest that ISG15 facilitates VACV spread, making its role in MPXV infection a topic of interest. Poxviruses produce two infectious forms: Mature Virus (MV), associated with localised infection and person-to-person spread, and Extracellular Enveloped Virus (EV), critical for systemic spread. ISG15 -/- cells showed reduced EV production during VACV infection, and changes in the MV/EV ratio may have contributed to the dynamics of the 2022 outbreak. To investigate this, we analysed three clade II MPXV strains - 2022 outbreak, 2003 USA outbreak and WRAIR 7-61 - in WT and ISG15 -/- mouse embryonic fibroblast (MEF) cells to assess strain-specific dynamics and the role of ISG15 in infection.

After infection, electron and confocal microscopy were used to quantify viral forms or actin tail formation. Proteomic and phosphoproteomic analyses provided further insight into host-virus interactions. With the support of the ISIDORe consortium, state-of-the-art facilities and expertise were used to achieve our goals.

The 2022 outbreak of MPXV showed a slower viral cycle, whereas the 2003 USA strain showed faster growth, increased extracellular viral titres and increased EV-related protein expression. ISG15 -/- MEFs showed higher viral titres, validating the electron microscopy and proteomics results. Interestingly, while most viral proteins were upregulated in ISG15 -/- MEFs, immune response inhibitory viral proteins were downregulated, suggesting a role of ISG15 in protein regulation. Additionally, actin tail formation was reduced, as was phosphorylation during MPXV infection.

These findings underscore the evolution of clade II MPXV and ISG15 potential role in the 2022 global outbreak. As rodents are MPXV reservoirs, understanding the virus's dynamics within these hosts is essential for preventing future outbreaks. A One Health approach is increasingly important as climate change and urbanisation drive zoonotic diseases such as the recent Congo clade Ib MPXV outbreak.

Spatial Analysis of Henipaviral Infections Across Scales of Biological Complexity & Optical Resolution

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Nipah virus (NiV) is a zoonotic pathogen characterized by systemic infections with a high mortality rate in humans and animals. NiV is a negative strand RNA virus able to interact with ephrin-B2 and -B3 present on the host cell surface. Elucidating the molecular mechanisms of virus-host interactions both at the plasma membrane and at different stages of the virus lifecycle in a relevant animal model is essential for informing the drug and vaccine development. We applied fluorescence imaging approach to reveal NiV infection and organization at different levels of biological complexity. Tissues from African green monkeys infected with NiV were used to visualize the viral phosphoprotein by large volume 3D Light sheet fluorescence microscopy (LSFM) and tissue optical clearing. 3D organization of NiV phosphoprotein and ephrin-B2 at the nanoscale level in host lung tissues was achieved with Stimulated emission depletion (STED) microscopy and tissue precision sectioning. To relate the virus tissue imaging observations with infections in cell culture, Vero E6 cells were infected with Cedar henipavirus (CedV). This fluorescence imaging approach allowed the analysis of viral protein organization and distribution in host cells at close to physiological environment. LSFM was applied to characterize NiV in lungs, spleen and kidneys. High- and super-resolution microscopy analysis showed NiV and CedV protein organization in different cell compartments inside tissues and cells, respectively. The spatial analysis contributes to better understanding of NiV interactions and organization in infected cells. Establishment of the novel virus imaging methodologies can open new avenues for studies of other zoonotic viruses.

STEP (Stop Tuberculosis Efflux Pumps): A Comprehensive Approach to Understand Antibiotic Efflux

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a major infectious disease, killing over 1 million people annually. Despite initial control through first-generation antibiotics, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has maintained an epidemic death toll. XDR strains heavily contribute to tuberculosis (TB) incidence and mortality and lead to disproportionate costs to diagnose, treat, and care for patients with drug-resistant TB. It is therefore imperative to shorten TB regimens to increase patient amenability and thus counteract the rapid spread of multidrug-resistant TB. As an obligatory pathogen, Mtb has evolved defence mechanisms to persist for lengthy periods within the host. One such mechanism is the upregulation of efflux pumps that extrude the administered antibiotics, enabling the transient drug tolerance necessary for mutations to accumulate, beneficial mutations to be selected, and highly resistant mutants to persist. While some efflux pump inhibitors have been tested in vitro, none are approved for TB treatment and, despite the potential contribution of drug efflux systems for tolerance mediation and the concomitant development of resistance in Mtb, mechanistic and functional information about efflux pumps is severely lacking and detailed characterization of relevant efflux pumps is vital to inform downstream drug development. Hence, the fight against drug resistance in TB demands the design of new pharmacological strategies that include inhibiting the efflux of anti-TB drugs.

Here, the high-throughput (HTP) platform for the production of membrane proteins at Diamond (Harwell, UK) was used to screen conditions for expression and purification of efflux pumps implicated in drug resistance. Five mycobacterial MFS efflux pumps were included in the HTP screening and subsequently, a detergent screen was performed. The conditions to express and purify four efflux pumps were obtained, though some fine-tuning will be required for large-scale production and characterization. Good amounts of monodisperse sample of one of the efflux pumps were obtained, and preliminary negative staining and cryogenic electron microscopy data look encouraging.

The biochemical and structural information resulting from this endeavour can be translated by academic and pharmaceutical drug discovery teams into novel efflux inhibitors that can be combined with and safeguard existing TB treatments.

Comparative Impact of Swine Influenza A Virus & Porcine Reproductive & Respiratory Virus Infections on Porcine Respiratory Lymph Nodes B Cells & Macrophages

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Several lines of evidence indicate that pigs serve as a source of new influenza virus variants by acting as mixing vessels for mammal and bird strains. Additionally, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) has a significant impact on the pig-rearing sector due to its persistence for months, largely attributed to delays in the development of neutralizing antibodies. PRRSV and swine influenza A virus (swIAV) are frequently co-associated in swine herds. By compromising the overall health and specific immune fitness of pigs, PRRSV is strongly suspected of facilitating influenza infections. A comparative analysis of the early immune responses to each virus could enhance our understanding of their specificities and pave the way for further research into the impact of PRRSV on influenza virus reassortment in pigs.

In 2021, we collaborated with CRESA (Barcelona) as part of the VetBioNet (MATB) initiative to compare immune responses at 8 days post-infection (DPI) with swIAV and PRRSV in the tracheobronchial draining lymph nodes. Complementary serum analyses were carried out through the ISIDORe project (CytokFluPRRSV). At 8 DPI, serum IL-5 levels were significantly higher in PRRSV-infected animals compared to those infected with swIAV. CXCL13 and BAFF levels were also elevated in PRRSV infections. In the LNs, swIAV-infected animals exhibited hypercellularity compared to the mock group. In contrast, PRRSV-infected animals showed a marked deficit in immune cell recruitment, which was associated with greater and prolonged viral shedding and the absence of neutralizing antibodies in the serum, unlike influenza infections. Beyond LN cellularity, all other LN immune parameters were comparable between mock and swIAV-infected animals at day 8 post-infection.

By comparing swIAV- and PRRSV-infected animals, PRRSV-specific mechanisms were identified. These included the expression of PD-L1 in efferent macrophages, the induction of extrafollicular plasmocytes, and the recruitment of inflammatory monocytes/macrophages. PRRSV's inhibitory mechanisms are comparable to those used by human immunodeficiency virus (HIV) and murine chronic lymphocytic choriomeningitis virus (LCMV) to evade the immune response.

It would be valuable to further investigate how the observed effects of PRRSV on innate and adaptive immune responses may influence the anti-influenza defences of pigs.

Unravelling the Adaptation of Avian Influenza to Wild Birds & Mammals

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The world is currently experiencing the most extensive and severe panzootic of H5N1, a highly pathogenic avian influenza (HPAI) strain from the clade 2.3.4.4b goose/Guangdong (Gs/GD) lineage. This outbreak has resulted in an unprecedented number of affected wild and domestic bird populations, high mortality rates, and increased susceptibility in mammalian species which had previously not been considered vulnerable. These developments pose a major threat to biodiversity, create economic challenges for the livestock industry, and raise significant public health concerns within the One Health framework. To address this critical issue, our ISIDORe project *WildFlu* investigates the increasing adaptation of HPAI viruses to wild birds and mammals.

As part of this study, we conducted experimental inoculations of yellow-legged gulls (Larus michahellis) and American minks (Neogale vison) with two different H5 HPAIV strains from the Gs/GD lineage: one carrying known genomic signatures of adaptation to mammals isolated from a mink, and another isolated from a gull without such markers. Our aim was to compare infectivity, transmission, and pathogenicity in both species by integrating data on viral shedding, environmental sampling, genomic adaptation using long-read nanopore sequencing data, and clinical outcomes.

Our results showed that in minks, only the mammal-adapted strain caused clinical signs and 100% mortality in inoculated animals, whereas the non-mammal-adapted strain led to the spontaneous occurrence of the PB2 T271A mutation in a subclinically infected mink—a mutation that had previously been associated with increased transmission and pathogenicity. In gulls, the mammal-adapted strain led to no infections, while the non-mammal-adapted strain resulted in 100% mortality in inoculated individuals and 75% mortality in direct contacts. These preliminary results suggest that once a strain is adapted to mammals, it might lose its efficiency of infecting wild birds, potentially reducing the subsequent risk of viral transmission via migratory flyways. We additionally showed that non-invasive environmental sampling of air and water can function as an early viral RNA detection tool.

In conclusion, our results highlight the host-specific adaptation of H5 HPAI viruses, emphasizing the importance of understanding infection dynamics in new hosts and exploring novel early detection methods. These insights are crucial for the control and prevention of future outbreaks.

Rational Design of SARS-CoV-2 Main Protease Inhibitors: From Molecular Models to In Vivo Evaluation

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused one of the greatest health crises of this generation and led to millions of deaths worldwide. Similar to common cold viruses, SARS-CoV-2 is expected to continue to circulate and pose a substantial threat to global health. Despite promising vaccination programs against COVID-19, antiviral drugs will be crucial to control future outbreaks of coronaviruses. At the onset of the pandemic, our research team at Uppsala University initiated a drug discovery project focused on identifying inhibitors of the SARS-CoV-2 main protease (Mpro). Inhibition of Mpro blocks the processing of polyproteins produced by translation of the viral RNA, which is an essential step in SARS-CoV-2 replication. The determination of high-resolution structures of Mpro enabled us to perform virtual screens for inhibitors. Molecular docking was used to screen a diverse library of 235 million molecules against the active site, and experimental evaluation of top-ranked compounds identified novel inhibitors. Hits from the screens were optimized for potency by utilizing structure-based design, leading to the discovery of inhibitors with nanomolar potencies.

An extensive medicinal chemistry program focused on a promising scaffold generated hundreds of inhibitors with high potency and improved in vitro and in vivo pharmacokinetic properties. The goal of the ISIDORe project was to identify a broad-spectrum antiviral that could be developed into a drug suitable for treatment of coronavirus infections. We have assessed the broad-spectrum antiviral activity (SARS-CoV-1, SARS-CoV-2, and MERS-CoV) of many our inhibitors and evaluated several promising compounds in human lung epithelial cells (SARS-CoV-2 variants). In the second phase of the ISIDORe project, the two most promising compounds were evaluated in vivo using a hamster model (SARS-CoV-1, SARS-CoV-2). We will present our approach to discovering the inhibitors and the results from cellular models and animal studies performed by ISIDORe.

LTX-109 as a COVID-19 Therapeutic & Prophylactic Drug

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LTX-109 is a small synthetic cationic peptidomimetic with a membrane-lysing mechanism effective against various bacteria. However, limited data exists on its efficacy against viruses. Through the Isidore project ISID 9661 (WP14), we tested LTX-109 against SARS-CoV-2 in vitro and in vivo, supported by the *Istituto Zooprofilattico Sperimentale delle Venezie* (IZSVE, Italy). The drug is produced as a nasal spray, with the ultimate goal of developing a broad-spectrum compound capable of treating respiratory viral infections.

Different concentrations of LTX-109 were incubated for 10 minutes with BQ.1.1 variant of SARS-CoV-2 virus and then all the samples were titrated onto a cellular substrate to evaluate the residual infectivity and calculate the inhibition curve.

LTX-109 efficacy was also tested in vivo. Golden Syrian hamsters were intranasally infected with the BA.5 variant of SARS-CoV-2 and treated following two different administration protocols: the drug was delivered intranasally at various dosages, using both therapeutic and post-exposure prophylactic approaches.

In vitro, LTX-109 at 1% reduced SARS-CoV-2 infectivity by more than 3 log10 TCID50/ml, with the full maximal inhibitory concentration (IC99.9) calculated at 0.3%.

In vivo, molecular analyses of oropharyngeal swabs and nasal turbinates collected during the study revealed a significant viral load reduction in samples from animals treated with different administration protocols (prophylactic & therapeutic at various drug concentrations), compared to controls. Therapeutic treatment at different doses demonstrated a dose-dependent efficacy of LTX-109, with the compound effectively inactivation of viruses at the inoculation site at the highest doses. None of the animals treated with 3% LTX-109 tested positive in oral swabs, and only a few viral genomic copies were detected in nasal samples in the 1% group.

LTX-109, in its 3% formulation, effectively treated SARS-CoV-2 infection using both therapeutic and postexposure prophylaxis protocols. The therapeutic treatment protocol also demonstrated a dose-dependent effect on inactivation of viruses at the inoculation site (with lower concentrations of LTX-109 still reducing viral loads by more than 99%). These findings support moving LTX-109 into clinical trials to evaluate the use of LTX-109 as a virucidal against respiratory viral infections.

Heliscreen – Screening Efforts to Delve into the Features & Therapeutic Potentials of SARS-CoV-2 Helicase (NSP-13)

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Among SARS-CoV-2 non-structural proteins (NSPs), NSP13 helicase is a promising and still scantly studied target since it is involved in essential steps of viral replication as a part of the replication-transcription complex. In addition, this superfamily 1B helicase is highly conserved among different coronaviruses and therefore it could be targeted to develop broad-spectrum antiviral drugs.

Notwithstanding the above, few inhibitors are hitherto reported, and this hampers a precise evaluation of both the mechanistic details of the enzyme and the therapeutic efficacy of its inhibition. Hence, large screening campaigns as provided by ISIDORe services are required to expand the portfolio of NSP13 inhibitors and to better characterize the druggable binding site(s) of the enzyme.

In this work a FRET assay proposed by Adedeji and reported by Zeng and Corona was used to detect NSP13 helicase activity. When the helicase is active, it opens the two strands in the 5'-3' direction and the leader strand with the fluorophore is separated. The Cy3 fluorophore emits photons, and a fluorescence signal can be detected at the proper wavelength. The substrate is the annealed DNA substrate.

When the helicase is inactive (e.g., when inhibited by an active compound), the two strands remain annealed, and the photons emitted by the leader strand are absorbed by the BHQ-2 of the other strand. As a result, a reduced fluorescence signal is measured. The assay is also performed on a positive and a negative control. In this study, suramin was used as the positive control and DMSO 100% as the negative control. Also, structure-based computational approaches (including virtual screening campaigns) were performed to rationalize the obtained results and to develop AI-based predictive models to support the identification of new inhibitors.

To find new inhibitors for SARS-CoV-2 nsp13, we have screened the European Chemical Biology Library (ECBL) of about 100k molecules, including 2464 bioactive compounds. The screen showed a hit rate (> 50 % inhibition) of 0.31%. 271 compounds were re-ordered from EU-OPENSCREEN and confirmed under screening conditions in triplicates. To remove false positives confirmed hits were evaluated in counter assays for interference with the fluorescence readout and for intercalation in dsDNA (dsRNA). Compounds that showed activity in counter assays were removed from the final hit list. In total 15 compounds were selected for further

Targeting RNA of SARS-CoV-2 – From RNA Structure to Viral Replication Inhibitors

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Respiratory tract RNA viruses are recognized as the most dangerous viruses worldwide. SARS-CoV-2, which is causing the COVID-19 pandemic, belongs to that family. This virus is responsible for 777 million illnesses and more than 7 million deaths (WHO, January 2025), and these numbers are growing. SARS-CoV-2 possesses a single-stranded RNA genome with positive polarity, and its replication cycle depends solely on RNA.

Viral RNA of SARS-CoV-2 has become a promising target for various types of inhibitors since the relationship between RNA structure and function has been identified. We aim to understand better the role of RNA structure in the replication process of SARS-CoV-2 and to use this knowledge to design potential inhibitory tools. Based on experimental investigations of SARS-CoV-2 RNA secondary structure, we selected conserved motifs as a target for small molecules (SMs).

Under the ISIDORe grant using high throughput screening (HTS), we selected around one hundred compounds from the European Chemical Biology Library (ECBL, EU-OPENSCREEN) (consisting of about 100,000 compounds) that bind tightly to two motifs of viral RNA in a dose-dependent manner. The chosen method was a fluorescent indicator displacement assay

(FID). HTS was also conducted on Enamine and LOPAC libraries, ending with a selection of 13 SMs considered to test in cells.

Selected small molecules were evaluated against a non-infectious SARS-CoV-2 replicon created in our laboratory. The SARS-CoV-2 replicon is only depleted of E and S while containing green fluorescent protein (eGFP), which allowed us to monitor the antiviral effect of validated compounds in the HEK293T cell line. Flow cytometry, Western Blot Assay, and RTqPCR were used.

Results showed inhibition of reporter expression for many of the tested small molecules, showing their potential as antiviral agents. High-throughput cell culture tests against SARS-CoV-2 under ISIDORe funds are underway to show small molecule inhibitors of SARS-CoV-2 replications in native virus assay.

In conclusion, carefully choosing RNA targets followed by high throughput screening of small molecular library such as ECBL, allows the selection of small molecules with high potency to become future drugs.

Application of AlphaScreen for High-Throughput Screening of Inhibitors Targeting the PALS1/SARS-CoV-2 E Interaction

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The COVID-19 pandemic has highlighted global vulnerabilities in public health and economic stability, emphasizing the urgent need for new treatments against SARS-CoV-2 and the development of fast and robust screening tools for emerging zoonotic threats.

Many viruses use the same cellular pathways for replication, making host protein targeting a promising strategy for developing broad-spectrum antivirals with low resistance potential. In coronaviruses, the Envelope (E) proteins interact with the PDZ domain of human PALS1, a key protein in maintaining epithelial polarity, leading to tight junction disruption—a crucial factor in COVID-19 pathogenesis. Inhibiting PALS1 / E-protein interaction could serve as a promising therapeutic strategy to prevent tight junction disruption and mitigate COVID-19 pathogenesis.

Thanks to the support of ISIDORe (Integrated Services for Infectious Disease Outbreak Research), we were able to screen the European Chemical Biology Library (ECBL), which contains more than 100,000 diverse compounds. For this, we proposed screening the library against PALS1-PDZ using a well-established and robust miniaturized AlphaScreen assay to identify small-molecule inhibitors of this interaction. AlphaScreen offers high sensitivity and a wide dynamic range for detecting protein-protein interactions, even in complex biological samples, without requiring protein fusion. Additionally, it provides lower background noise, making it particularly well-suited for high-throughput screening. The full ECBL library was screened in batches consisting of 21–24 plates, with an average intra-plate quality control parameter of Z’ = 0.84 and a CV of 4.59. A compound was selected for validation if the corresponding percent inhibition value (at 10 µM) was equal to or greater than 30% in the primary screening. A total of 344 inhibitor compounds were selected for the validation experiment. These compounds were tested at eight concentrations (50 µM, 10 µM, 3 µM, 1 µM, 300 nM, 100 nM, 10 nM, and 1 nM) in three biological replicates. Out of these hits, 185 compounds were chosen for follow-up experiments.

This screening will identify lead compounds for the development of novel antivirals that are effective against current and future coronavirus strains while minimizing the risk of resistance.

A Polarized Airways Epithelial Cells Culture Model to Assess the Role of Vitamin D in the Early Host Response to SARS-CoV-2 Infection

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Epidemiological studies and biological plausibility suggest that a low vitamin D (vitD) status enhances COVID-19 risk and severity. Yet, laboratory data showing vitD effects on the host cell response to SARS-CoV-2 are lacking. Our goal was to set up an in vitro model to define whether and how cell response to SARS-CoV-2 is conditioned by long-term exposure to physiological doses of 25-hydroxyvitamin D (25OH), the blood metabolite defining the vitD status.

The support of ISIDORe allowed to complement the user expertise on vitD with that of the access provider on epithelial cells differentiated at the air-liquid interface (ALI) and SARS-CoV-2 infections. We performed three sets of experiments with primary human nasal epithelial cells (hNEC) obtained from three different donors: (i) kinetics of 25OHD treatments, up to four weeks, in the absence of virus challenge; (ii) challenge with live attenuated SARS-CoV-2_OTS-206; (iii) challenge with wt-SARS-CoV-2 (Wuhan prototype strain). For all sets of experiments, hNEC exposed or not to 25OH were characterized for morphology, epithelial permeability, mRNA expression of vitD effector and target genes, antimicrobial peptides and inflammatory cytokines/chemokines. For the infection experiments, infectious virus shedding and viral RNA loads were also assessed.

Long term 25OH treatment did not affect hNEC morphology and barrier function nor significantly alter basal expression of inflammatory cytokines/chemokines. The cells did respond to 25OH, as shown by robust induction of the VDR target genes CYP24A1 and CAMP/LL-37. The best induction was observed in the first two weeks of treatment, and we chose this time window for subsequent infection experiments. Replication of live attenuated SARS-CoV-2_OTS-206 was not affected by 25OH treatment, despite a strong mRNA induction of the antimicrobial peptide CAMP/LL-37. Remarkably, infection with wt-SARS-CoV-2 showed a trend toward reduced replication following 25OH treatment.

We successfully established an ALI culture model with long-term exposure to physiological doses of 25OH, and we showed that 25OH did not affect replication nor cellular response to live attenuated SARS-CoV-2, while it reduced wt-SARS-CoV-2 replication, suggesting potential antiviral activity against pathogenic strains of the virus. The new experimental model is extensible to other emerging or re-emerging infections.

Safe & Effective Aerosol Delivery of Aloxistatin in Mice Infected with Nebulized SARS-CoV-2 Variants & a Mouse-Adapted Strain

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In a subset of individuals, SARS-CoV-2 infection induces severe disease, characterized by extensive inflammation and excessive wound healing, leading to lung fibrosis. The secretion of pro-inflammatory cytokines and pro-fibrotic mediators involves cysteine cathepsins. In vitro studies with Aloxistatin (Aloxi), a broad cathepsin inhibitor, revealed anti-inflammatory and anti-fibrotic potential and inhaled Aloxi was proven to be safe in clinical phase I studies. Thus, we aimed to evaluate the anti-inflammatory and anti-fibrotic effects of inhaled Aloxi in mice during acute infection and in a SARS-CoV-2-induced fibrosis model. Intranasal infection of aged Balb/c mice with the mouse adapted SARS-CoV-2 strain (MA10) is the only animal model leading to fibrotic lung lesions 4 weeks post-infection (p.i.). However, it's associated with high mortality in the acute phase of infection. To improve animal welfare, we established a virus nebulization protocol that leads to high virus replication and inflammation in the lungs without early-phase mortality. We used a nose-only exposure system to deliver virus and Aloxi treatment. Virus titres, qPCR, cytokines and pro-fibrotic markers were assessed at various timepoints.

Furthermore, computer tomography (CT) scans of fixed lungs and histopathology will help to evaluate fibrosis and inflammation of the lung parenchyma. Metabolomic profiles of lung homogenates will be assessed by mass spectrometry. Virus nebulization at 105 PFU/mouse led to high replication in the upper respiratory tract and the lungs, similar to 5x103 PFU/mouse intranasally (IN) pipetted virus. In contrast to IN inoculated, aerosolized virus led to survival of 100% of aged Balb/c mice. Notably, the Aloxi metabolite E64c was detected in mouse lungs 40 min and in plasma up to 4 h after aerosol delivery of Aloxi. At 4 days p.i., Aloxi had no effect on viral replication and cytokine levels compared to the control groups using three different virus variants (Wildtype, Delta, Omicron-BA5) in hACE2-expressing transgenic mice. While not significant, cytokine release tended to be lower in Aloxi-treated MA10-infected Balb/c mice. The analysis of the effects of Aloxi on lung fibrosis is ongoing.

Aerosol delivery of virus to lungs improves animal welfare and the reliability of infection models, that can be applied for any other respiratory virus with pandemic potential. Further, Aloxi is safe in infected mice with no detrimental effect on infection and virus titres.

Antibody-Based & CAR-T Cell-Based Therapeutic Approaches Against SARS-CoV-2 Infection

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Monoclonal antibodies (mAbs) present a valuable therapeutic opportunity for treating viral infections, including SARS-CoV-2. However, SARS-CoV-2's ability to mutate has resulted in variants of concern (VOC) that evade many existing mAbs, underscoring the need for new mAbs capable of targeting emerging VOCs. In this study, mAbs against the SARS-CoV-2 Spike protein were generated using phage display technology. Their affinity and binding properties were characterized through ELISA and SPR, with initial experiments conducted using Wuhan and Omicron strains. Two promising mAbs demonstrated efficient recognition of infected cells expressing Spike protein. Further characterization of these mAbs focused on epitope mapping to evaluate their capacity to target conserved regions, which is crucial for broad reactivity and high therapeutic potential.

To advance mAb characterization, ISIDORe services were utilized for access to high-capacity screening platforms integrated into EU-OPENSCREEN. These platforms offered expertise in mAb characterization, computational simulations, and tools for assessing mAb interactions with diverse VOCs.

Epitope mapping was performed through loss-of-binding assays with SARS-CoV-2 mutants and SPR cross competition studies. Computational modelling guided by experimental data was used to construct structural models of mAb-antigen complexes, providing mechanistic insights.

ISIDORe services led to the identification of the C10 mAb, with broad reactivity against all tested VOCs including Omicron variants. Its epitope appears to reside in a highly conserved RBD region, making it an ideal therapeutic candidate for current and future VOCs. This pan-SARS-CoV-2 mAb efficiently binds infected lung epithelial cells and induces their lysis via NK cell-mediated antibody-dependent cellular cytotoxicity. Additionally, we engineered C10-based, Chimeric Antigen Receptor (CAR)-T cells which demonstrated efficient killing of infected lung epithelial cells and significant viral titre reduction.

ISIDORe services facilitated the discovery of a pan-SARS-CoV-2 mAb capable of targeting cells infected by diverse VOCs. The therapeutic potential of C10 could be harnessed in its full-length format or through C10-based CART cells. These findings pave the way for innovative antibody- and cell-based therapies, offering expanded treatment options for high-risk populations and severe COVID-19.

Inhibitors of Viral Late Domain/ESCRT Interactions as Novel Broad-Spectrum Antivirals

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Molecules blocking the interactions between viral proteins containing P(T/S)AP, LYP(x)nL, or PPxY viral Late domains and ESCRT proteins TSG101, ALIX, and NEDD4 have been shown to efficiently inhibit egress of multiple enveloped viruses, showing promise as novel broad-spectrum antivirals. Screening of drug repurposing and small compound libraries have allowed us to identify a set of inhibitors of these protein-protein interactions able to block the interactions between the full length viral and human proteins in human cells and to inhibit budding of Ebola and Marburg virus in Virus-Like Particle assays.

Within the ISIDORe project (ISID_2304) we aim to 1) assess the spectrum of antiviral activity against several group 4 pathogens (Ebola, Marburg, Nipah and Lassa virus) of the identified inhibitors 2) investigate their impact on cellular functions through mass spectrometry-based proteomic studies, and 3) evaluate synergistic effects associated to the simultaneous inhibition of different ESCRT targets for broad-spectrum antiviral activity.

The antiviral activity of the most promising compounds was evaluated against risk group 4 pathogens under BSL-4 conditions. Before each inhibitor treatment, cell viability assays, the optimal inoculation dose and incubation time for each virus were determined. For antiviral activity assays, different detection methods were used, such as Tissue Culture Infectious Dose and RT-qPCR. Some samples generated from these assays were exported for proteomic analysis outside the BSL-4 facility.

We have confirmed inhibitory activity against Ebola, Marburg and Lassa virus at micromolar range for some inhibitors. In particular, UEV-10 (Tsg101), ALIXEN-6 (Alix) & WWTM-1 (Nedd4), each targeting one key regulator of the ESCRT pathway, showed great impact on virus titres confirming their antiviral activity and potential use as broad-spectrum antiviral. This finding sets the basis for testing synergistic effects of selected compounds on viral infections which are under way. Also, first proteomic studies have been successfully performed for the most promising compound (UEV10) to evaluate the impact on the cellular and viral proteome upon treatment.

In conclusion, ISIDORe's services have enabled us to validate the broad-spectrum antiviral activity of several compounds, highlighting that targeting ESCRT protein-virus interactions is a promising strategy for combating emerging highly pathogenic and potentially pandemic pathogens.

JRA SESSION

NEW CAPABILITIES DEVELOPED BY ISIDORe'S ACCESS PROVIDERS FOR PANDEMIC PREPAREDNESS RESEARCH

VIRAL – Viral Infectiology Research with Advanced Laboratory Models

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An important requirement to better understand the pathogenesis of infectious diseases and to effectively test novel interventions, is the establishment of representative model systems. Traditionally, in vitro model systems in virology used transformed or cancerous cell lines, which have drifted extensively from their natural in vivo counterparts. This may cause for example cell culture adaptive mutations in the viral genome or induce different host responses to the infection. Animal models on the other hand are in general labour intensive, expensive and may have ethical issues. Primary cell models offer an attractive alternative to cell lines and animal models. Over the past years, human stem cell-derived organoids have shown also to bridge the gap between cell lines and in vivo models. A big advantage of 3D organoid technology is that cocultures with all kinds of relevant cell types are possible. Although organoid studies can greatly advance our mechanistic understanding of virus infections, further improvements are needed to cover applications for different animal species and a more diverse range of cell types. The aim of the VIRAL (Viral Infectiology Research with Advanced Laboratory models) JRA was to bring together different expert groups that develop novel ex vivo models (which includes the use of ex vivo tissues and organoids) to study viral infections to fill gaps in the currently offered ERINHA services. An overview of the activities and outcomes of the project will be provided.

RES4PRIOPATH – An Integrated Antiviral Discovery Platform of Broad-Spectrum Small Molecule Inhibitors of Viral Targets from Priority Preparedness Pathogens

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We present here a novel fluorescence-based HTS workflow that enables the rapid identification of Broad-Spectrum Antivirals targeting Viral Proteases and Polymerases. Recent outbreaks highlighted the global consequences of the scarcity of marketed antivirals. Broad-spectrum antivirals (BSA) are the way forward to responding early to variants of concern without specific treatment. BSAs can block viral proteins targets like proteases and polymerases, which have high fitness barrier to mutation, are conserved across viral genera, but show differential specificities to human orthologous enzymes. Current antiviral research in proteases and polymerases present important pitfalls, including limited throughput/ resolution at discovering new inhibitors, which modest outcomes were also justified by the limited structural diversity of the synthetic small-molecule libraries explored so far. To fill these gaps, the ISIDORE-JRA Res4PrioPath project (https://isidore-project.eu/about-us/) has enabled the assembly of a quick-response High Throughput Screening (HTS) platform based on fluorescent enzymatic assays that can quickly converge within weeks towards, structurally novel, low micromolar, synthetic inhibitors of flaviviruses / coronaviruses / picobirnaviruses / alphaviruses / ebolaviruses targets, demonstrating the universality of the quick-response methodologies developed; and confirming the readiness of this new service to respond to the necessities of real-life users bringing new targets, libraries and advanced antiviral candidates.

OptiGuideMalVac – Dissecting Protective Malaria Immune Responses: Optimizing & Expanding Guidance of Preclinical Malaria Vaccine Evaluation

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Malaria liver stage vaccine development is difficult to steer as the basis of vaccine-induced protective immunity remains obscure. Liver immunity is key in malaria, but an understanding of the underlying immune mechanisms is lacking. Knowledge of the mechanisms of vaccine-induced protective immunity is difficult to obtain in humans but would be crucial for guiding preclinical human malaria liver stage vaccine development. The establishment of signatures of protective liver immunity (model improvement) would make preclinical models more attractive to prospective users and broaden its application offering: 1) the provision of a detailed understanding of protective immune responses to malaria vaccine candidates tested in NHP models; 2) a better steering of malaria vaccine development through the early selection of vaccine candidates eliciting protective liver signatures in NHP models.

Immunity to liver stage malaria was studied in the P. cynomolgi-rhesus macaque model in which partial protection post-vaccination with P. cynomolgi sporozoites was established at BPRC (Pasini et al. NPJ Vaccines, 2022). In a holistic approach we used a combination of single cell RNA sequencing for TCR repertoire profiling and clonal expansion detection, untargeted NMR-based metabolomics and FACS analysis to identify protective liver and blood signatures to malaria.

Interesting differential signatures were detected in plasma of protected and non-protected animals involved in the vaccine experiments using NMR-based metabolomics and cytokine multiplexing technology when treatment and outcome were used as a read-out. Furthermore, an attempt was made to define differentially expressed infection-specific makers between protected and non-protected individuals using FACS analysis after PBMC (both circulating and tissue-resident) restimulation with malaria parasites. The RNA-sequencing of liver PBMCs and NMCs was performed to detect differential signatures and correlated them to treatment group and vaccination outcome (protected/non-protected).

This holistic approach allowed us to gain insights on the difference between markers of protection and of infection (vaccination with live parasites); what emerges is an overall complex immunological picture. The data on the induction of protective immune signatures obtained will be used to guide malaria liver stage vaccine development in the NHP models from the BPRC.

STICK-EM – Situated Tickborne Encephalitis Preparedness & Prevention: Education Measures for Europe

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The continued expansion of tick-borne encephalitis (TBE) in Europe, driven by climate change, changes in wildlife populations, increased outdoor activities and land-use change including urban greening, has resulted in small scale outbreak events. As these factors continue to evolve, together with rising vaccine fatigue, human TBE cases are predicted to increase. Up to 30% of cases can suffer severe neurological disease, long-term sequelae, or death. To date, however, very little social sciences research has been published on TBE, and thus there is little insight into how human interactions with ticks and forested environments may be facilitating or preventing TBE outbreaks, nor about the possibilities for prevention of them.

The project therefore evaluated comparatively the experiences of human-tick-environment relations in urban green spaces in Austria, France, and the Netherlands. We brought together anthropological approaches involving semi-structured interviews, participant-observation, and online social media analyses of public discourses about TBE, together with entomological assessments on the densities of ticks and tick-borne pathogens in our three sites. Entomological analyses found highly varied tick densities and tick-borne pathogen infection rates across sites and identified specific urban “hot spots” with high tick densities that require additional investigation. Across all sites, experts expressed substantial concern about the expansion of TBE incidence. Although knowledge and vaccination coverage for TBE was relatively high in Vienna, we found that the social emergence of TBE (that is, how knowledge about this pathogen is emerging among affected publics: what they know or don’t know and the foundations that affected publics use to produce that knowledge) is relatively inchoate in France and the Netherlands.

These findings have important implications for prevention and health education approaches should be undertaken within the three countries, as well as within Europe as a whole. There is a need to balance educating lay publics on the risks of TBE with the health benefits of using nature in urban spaces.

VECTORED – Vector-Borne Challenge Models

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A key gap in knowledge for the development of interventions against arbovirus infection is whether or not vaccines and/or antivirals protect against infection by vector bite – the natural route of arbovirus infection. There is accumulating data suggesting that virus challenge via a vector bite may differ from needle inoculation and requires higher vaccine efficacy because of immune-modulating substances and pro-viral factors in vector saliva. However, solid in vivo evidence for this phenomenon is currently lacking, because these experiments are technically and logistically highly challenging. We propose to develop vector-borne challenge models for a range of vectors (mosquitoes, and ticks) and different emerging medically important arboviruses on the list of ISIDORe priority preparedness pathogens (West Nile virus (WNV), and tickborne encephalitis virus (TBEV)).

In this JRA we performed vector competence studies for specific arboviruses and established optimal vector feeding conditions on laboratory mice at 2 different institutes. Next, we compared the viral replication kinetics and disease progression following vector-borne transmission challenge with that of the Gold standard needle inoculation in mice. Finally, we evaluated a micro-injection robot for automated injection of vectors.

The protocols and challenge models developed in this project will be important for preclinical evaluation of intervention studies. In addition, the models can be used to study the role of the vector in arboviral infection. Finally, by establishing these models for a range of vectors, this will also be a platform for preparing for arbovirus X.

WNED-X – Surveillance & Early Detection of West Nile Virus

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West Nile (WN) is the most widespread and deadliest mosquito-borne disease in Europe. The causative agent, West Nile virus (WNV), is transmitted by mosquitoes and maintained in the environment through an enzootic cycle involving mosquitoes and birds.

There are no vaccines or antivirals to prevent or treat WN in humans. Seasonal epidemics are increasing in size and frequency in Europe. Identifying areas where WNV circulates before human cases appear is crucial for informing authorities, the public, and pest control operators, contributing to disease risk estimation and epidemic prevention.

Screening field-collected mosquitoes reveals WNV two to five weeks before the first human cases, making early detection pivotal for preparedness and mitigation measures.

We present here WNED-X, a joint research activity funded under the ISIDORe project (Horizon Europe project funded by the European Commission, Grant Agreement 101046133), which gathers partners from five EU countries (Spain, Serbia, Belgium, France and Switzerland).

The project aims to create a transnational and operational framework combining field expertise, genomics, and modelling for WNV surveillance in strategic European locations.

The consortium has developed and validated a new methodology for early WNV detection, based on the assumptions that: a) WNV vertical transmission in mosquitoes contributes to virus circulation and amplification during spring/summer; b) The abundance of sampled mosquitoes affects the sensitivity of WNV detection in mosquito saliva.

The outcomes of the project are:
- A new TNA item is to be added to the ISIDORe catalogue
- Protocols, field and laboratory training for early WNV detection (larvae, adults, or saliva).
- A WNED-X External Quality Assessment for ring testing.
- Duplex Lyoph P&P(+ArRNA) for WNV, Usutu and “X” virus detection and discrimination.
- Circulating WNV isolates available through the EVAg catalogue.
- Modelling training course.

The strategy, objectives and preliminary results are presented here.

PATH2XNAT – Integrated Analysis of Digital Pathology & Spatial Transcriptomics Data for Research on the Pathogenesis of Infectious Diseases

Stefan KLEIN
Erasmus Medical Center, the Netherlands

PATH2XNAT will develop a data management and analysis platform that aims to accelerate investigations into the pathogenesis of infectious diseases, by leveraging existing data management (XNAT) and bioinformatics (Galaxy) tools for the analysis of Digital Pathology (DP) and Spatial Transcriptomics (ST) data. This ISIDORe Joint Research Action merges the capabilities of 3 European research infrastructures: Euro-BioImaging, ELIXIR, and EATRIS.

(M1) Data Generation: Histopathology sections of SARS-CoV2 infected lung tissue were scanned to obtain DP images. ST data was generated from serial sections using the Xenium ST platform.

(M2) Data management: DP images were converted into DICOM format and stored in XNAT; ST data were stored in Galaxy.

(M3) Workflows: We developed (i) a Galaxy tool to transfer DICOM data from and to XNAT; (ii) Galaxy notebook to classify DP images for SARS-CoV2 severity, and (iii) Galaxy notebook to support ST analysis. All workflows run in a secure Galaxy environment, which is part of the European Open Science Cloud (EOSC).

(M4) Pilot use case: 10 SARS-CoV2 infected lung tissue samples were used to demonstrate the utility of PATH2XNAT.

We have implemented an end-to-end workflow for DP and ST data generation (M1), data management (M2) and data analysis (M3) which is easily adaptable to any solid tissue and pathogen. Moreover, we have developed an optimized Xenium ST gene panel for investigating SARS-CoV2 molecular perturbations in lung tissues, in processes such as complement activation, in key cell types like myeloid cells and platelets during COVID-19 infection, aiming to understand their implication in acute disease and potential role in the development of Long COVID.

PATH2XNAT offers Galaxy services for the analysis of SARS-CoV-2 data, including DP image management (XNAT), deep learning prediction on DP images, and ST analysis in lung tissue. The PATH2XNAT platform is deployable as a scalable EOSC service when required for future pandemics. We are currently demonstrating the efficacy of PATH2XNAT through a study on SARS-CoV-2 pathogenesis, generating the largest spatial high-plex molecular atlas of COVID-19 infection to date, focusing on the interplay between inflammation and tissue damage during acute infection and its link to Long COVID symptoms. By identifying key biomarkers, we aim to improve treatments for acute infection and minimize post-acute sequelae.

CENTAUR – Genomic Taxonomy of Bacterial Pathogens for Outbreak Response

Sylvain BRISSE
Institut Pasteur, France

Surveillance, epidemiological tracking and outbreak investigation of microbial pathogens was revolutionized by the power of genomics, allowing the identification of chains of transmission or of new variants with altered virulence or transmissibility. In the CENTAUR project, we aim at addressing analytical challenges in the use of bacterial genomics, by automatizing the development of genotyping methods, tools and databases.

Gene-by-gene approaches, such as whole- or core-genome multilocus sequence typing (wg/cgMLST), leverage genomic information while building on MLST, the standard for microbial typing in most bacterial species. Still, developing new, high-quality genotyping schemes (i.e., dedicated wg/cgMLST methods) for emerging bacterial pathogens, and integrating their usage in nomenclature platforms, remains a challenging barrier. Within CENTAUR, the CRBIP team at Institut Pasteur is developing a novel hierarchical method that will facilitate the creation and adoption of genotyping schemes, with increased resolution (with the trade-off of decreasing applicability breadth) from species groups to emerging strains causing outbreaks.

Using the key priority bacterial pathogen Klebsiella pneumoniae (a prominent multidrug resistant hospital pathogen), we have developed a process of multiple cgMLST schemes creation. The process relies on recurrent core genome definitions, which can be applied in cascade to genotype subsets of the entire diversity of a species. We have used this novel process to develop schemes for genotyping of Klebsiella pneumoniae species complex, then for K. pneumoniae sensu stricto, then for particular sublineages of this species. The schemes are then incorporated into the strain taxonomy platform BIGSdb-Pasteur (https://bigsdb.pasteur.fr/) and will be benchmarked for outbreak investigations and strain nomenclature purposes.

This work will enable to rapidly genotype with maximal resolution power, emerging bacterial pathogens, and to quickly define for emerging outbreak strains, standardized and accessible nomenclatures that will facilitate communication among stakeholders involved in outbreak response and epidemiological surveillance.

POSTER SESSION

TNA & JRA PROJECTS

POSTER 01 – Metagenomic Insights into Viral Diversity in Mosquitoes in Serbia

Ana VASIC
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Mosquitoes are key vectors for zoonotic viruses, posing significant risks to both animal and human health. Our study aimed to investigate the viral diversity in mosquito populations, focusing on three species: Culex pipiens, Anopheles albopictus, and A. maculipennis. The objective was to detect viruses with potential zoonotic implications, enhancing surveillance efforts for pandemic preparedness and response.

Fifty pools of mosquitoes were used for nucleic acid (RNA and DNA) extraction. With ISIDORe's support, the pooled mosquito samples were prepared for viral metagenomic sequencing (amplification of both DNA and RNA viruses, Illumina's library preparation and sequencing). The bioinformatics analysis consisted of the following: trimming adapters with bcl2fastq and further quality filtering using Trimmomatic; Viral reads were extracted through alignment to mosquito reference genomes (for A. albopictus and C. pipiens) and Kraken2 annotation for A. maculipennis; Reads were aligned with BWA-MEM, and unmapped viral reads were extracted with Samtools; De novo assembly was performed with metaSPAdes, taxonomic classification with Kraken2 for reads and Kaiju for contigs; Taxonomic visualization was generated with kaiju2krona, and metagenome annotation was conducted with Prokka.

The sequencing generated a total of 634,879,492 raw reads, with an average of 12,697,590 reads per sample. After the removal of host sequences, between 0.42% and 54% of the reads remained unmapped, of which 0.001% to 66% were classified as viral reads. The de novo metagenomic assembly yielded an average of 18,186 contigs per sample, with approximately 1.44% classified as viral contigs.

The most abundant viral taxa identified across the samples were Riboviria, Monodnaviria, Duplodnaviria, and Varidnaviria. Several samples contained unclassified RNA viruses, including Culex negev-like virus 1 and Anopheline-associated C virus. A notable percentage of contigs in a few samples were classified as Naldaviricetes, while certain samples showed no presence of RNA viruses. Additionally, Alphamesonivirus, associated with respiratory infections in horses, and Meridalike virus (a rhabdovirus first detected in Mexico) were identified in some samples.

This study highlights the critical role of metagenomic surveillance in understanding viral diversity in mosquito populations. Results underscore the importance of monitoring to identify emerging zoonotic threats, thereby enhancing pandemic preparedness.

POSTER 02 – The MPOX Diagnostic in a Rapid & Point-of-Care Format: Development of an Isothermal DNA Amplification Assay

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Coris BioConcept, Belgium

The Mpox virus, currently of international concern, is active in Africa since many decades. The 2023 outbreak on this continent, mediated by the new Ib variant and targeting mainly children, highlights once more the need of diagnostic tests able to detect the virus in human samples to limit human-to-human transmission and to help the management of infected persons more rapidly. In this context, Coris BioConcept, with the *Istituto Nazionale Malattie Infettive Lazzaro Spallanzani* (INMI) as a service provider, is developing a point-of-care (POC) loop-mediated isothermal amplification (LAMP) molecular assay for rapid detection of viral DNA in patient samples (Project LAMPOX ISID_9992).

The development of this LAMP assay involves the selection of specific primers (West African and Congo Basin clades), the optimization of the reaction mix, the design of a drying method to stabilize the reaction mix in PCR microtubes, and the set-up of a rapid and easy preanalytical treatment of the sample.

During these development steps, regular verifications involving viral DNA and clinical samples are needed. Therefore, Coris BioConcept called on ISIDORe project support to obtain the necessary DNA material and access to a laboratory that could validate the developed prototypes on clinical samples.

During the LAMPOX project, INMI provided Mpox viral DNA and inactivated virus cultures for the early stages of development of the assay and the assessment of the analytical performances. Afterwards, the clinical performances (sensitivity and specificity) were verified at INMI with clinical samples (positive and negative).

The limit of detection of the LAMPOX assay has been determined at 150 copies of viral DNA/reaction. The LAMPOX assay was shown to have high sensitivity: 100% of Mpox positive samples (skin lesion and throat swabs) with a qPCR Cts below 32.6 (INMI homemade qPCR assay) were detected as positive for a time-to-result of less than 20 minutes. Concerning the specificity, none of the 112 Mpox negative samples (HPV and HIV positives anal swabs and oral rinses) were detected.

The LAMPOX assay's high sensitivity and specificity combined with its ease of use, rapid time-to-results, compatibility with fluorometric portable isothermal devices and the availability of a quick and simple preanalytical DNA extraction method (in its final stages of development) will bring this assay even closer to its future use as a POC test.

POSTER 03 – Ultrastructural Characterization of a Novel Type of Plasmodium Oocyst Division

Nathan RIBOT
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Plasmodium oocysts grow extracellularly at the abluminal side of the mosquito midgut. After successive rounds of DNA and organelle replication followed by a final budding step, one oocyst can generate thousands of sporozoites, the malaria parasite stage responsible for infecting the mosquito salivary glands and the liver of the vertebrate host.

Using dynamic and in vivo imaging on P. berghei – a rodent malaria model – we have reported phenomenon with characteristics of a novel type of plasmodial asexual division. This division involves the budding of a satellite, leading to the production of a multinucleated daughter cell. Budding is found in 0 to 20% of the oocyst population in fixed midguts, occurs mostly in the early days of infection, and its percentage inversely correlates with the oocyst density. However, the spatial organization of this phenomenon remains poorly understood and requires further characterization.

To investigate this phenomenon at the ultrastructural level, we utilized 3D volume electron microscopy (EM) with support from ISIDORe/Euro-BioImaging. Given the rarity of oocyst budding events, we refined a protocol combining fluorescent microscopy, X-ray imaging, and serial block-face scanning electron microscopy to identify and localize budding oocysts within the mosquito midgut, enabling their imaging at an ultrastructural resolution.

Using this approach, we successfully imaged budding oocysts and observed some connected by filaments composed of capsule and membrane. These findings suggest that dividing oocysts may remain transiently connected by these filaments. The 3D reconstruction of oocysts will provide deeper insights into the mechanisms underlying oocyst budding and division, advancing our understanding of oocyst biology.

POSTER 04 – Evaluation of the Contribution of the 271A, 590S & 591R Mutations in the PB2 Polymerase to the Replication & Transmission of Influenza A H1N1 in a Ferret Model

Romain VOLMER
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Mutations in the polymerase genes play a major role in avian influenza virus adaptation to mammalian hosts. The H1N1 virus that emerged in 2009 causing a pandemic contains an avian-origin PB2 protein with 271A, 590S and 591R which are considered as the main mammalian adaptation mutations in this PB2. Their individual contribution to mammalian adaptation has been investigated in various cell culture and animal models, but how the combination of these three amino acids modulate replication and transmission in vivo has never been investigated. With ISIDORe's support, we designed an experiment in the ferret model to characterize the replication and transmission of a wild-type pandemic H1N1 virus, A/Netherlands/602/2009 (H1N1), and a reverse-genetics engineered loss-of-function mutant carrying the typical avian amino acids 271T, 590G and 591Q (NL09-PB2av). Although the characterization of these experiments is in progress, preliminary results indicate that the wild-type NL09-PB2hu did not present any detectable selective advantage over the NL09-PB2av virus in ferrets, raising interesting questions, currently under further investigation, about the evolutionary process leading to the emergence of pandemic influenza viruses.

POSTER 05 – Characterisation of Acute Respiratory Distress Syndrome in Nipah Virus-Infected Primates

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Nipah virus (NiV) is a highly lethal zoonotic paramyxovirus that can be transmitted from person to person through the respiratory tract. NiV causes encephalitis and severe respiratory disease, and there are currently no approved therapeutics for this emerging infection. Several viral strains have been implicated in human outbreaks over the past two decades, one of which is more encephalitic (Malaysian strain) and some of which are responsible for human-to-human transmission and cause mixed encephalitic and respiratory disease (Bangladeshi and Indian strains). In recent years, our teams have been able to perform several in vivo therapeutic protocols using the Nipah Bangladesh strain (NiV-B) in the African Green Monkey (AGM) model. We now have a collection of samples that allow in-depth analysis of the pathological mechanisms. As the animals succumb to acute respiratory distress, we are focusing our study on lung invasion and lesion description to understand the mechanistic underlying lung damage. We aim to better characterise the pathology, in particular by assessing the activation status and damage of lung infected cells using histological imaging and transcriptomics. We expect that this series of experiments will provide a better description of NiV-B pathology in the lung and thus help clinicians to target their supportive treatments.

POSTER 06 – Characterization of Cell Type-Dependent Virus Replication, Assembly & Egress Strategies of Lassa Virus by Cryo-Electron Tomography

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Lassa fever is a life-threatening systemic viral illness endemic to West Africa, caused by Lassa virus (LASV), a member of the *Arenaviridae* family. Due to the lack of available vaccines or effective therapeutic treatments and its severe to often fatal outcome, LASV is classified as a Biosafety Level 4 (BSL-4) pathogen, posing significant challenges for studying its infection cycle and disease progression.

As part of the ISIDORe program, we conducted cryo-electron tomography (cryo-ET) studies on human type II pulmonary epithelial cells (A549 cells) to investigate the life cycle of the virus and its interactions with host cells at molecular resolution. Cryo-ET is the method of choice for obtaining high-resolution structural insights into native cellular environment, enabling the study of viral infection in unperturbed cells. In addition, we aimed to characterize the differences between LASV and its BSL-2 surrogate, ML29, to understand how their interactions with host cells differ and what distinguishes these two strains in terms of cellular entry, replication, and assembly.

For this purpose, A549 cells were cultured on EM grids before infection. Following vitrification, the samples were screened using cryo-light microscopy at the EMBL Bioimaging facility, and suitable grids were selected for cryo-focused ion beam scanning electron microscopy (cryo-FIB-SEM) to prepare thin lamellae. These lamellae were then transferred to a cryotransmission electron microscope (cryo-TEM) for tomographic data acquisition.

Through the ISIDORe program, we established a versatile cryoET pipeline that enables imaging of both BSL-2and BSL-4 samples. This pipeline allows us to work with unmodified virus-infected cells under BSL-2 conditions, as well as to conduct experiments with fixed cells from the BSL-4 laboratory, enabling comprehensive analysis of viral infection processes across different biosafety levels. Over multiple cryoET sessions, we captured tomograms from LASV- and ML29-infected cells to identify potential differences in viral release mechanisms and uncover structural distinctions between the human pathogenic LASV and its vaccine candidate, ML29. These insights will be crucial for advancing our understanding of the LASV life cycle and may inform future antiviral and vaccine development strategies.

POSTER 07 – Production & Immunogenicity of a Virus-Like Particle Vaccine Candidate Against Nipah Virus

Alexander JERMAN
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To bolster global pandemic preparedness, a diverse range of robust vaccine platforms is crucial. Nipah virus is a high-risk pathogen with pandemic potential. The scalability and manufacturing transferability is crucial to allow future stockpiling near areas with increased risk of deadly spillover events.

We developed enveloped Nipah virus-like particle (NiVLP) and nanoSFERIC™ recombinant nanoparticle vaccine platforms, which are produced in insect cells to ensure dependable production scale-up for preclinical and clinical trials.

The multi-valent NiVLP vaccine candidate is based on pathogen structural proteins: fusion (F), glycoprotein (G), and membrane (M). nanoSFERIC™ is a self-assembling nanoparticle vaccine design with planar NiV Gecd antigen presentation (nSf-NiVac). In this study, we tested the immunogenicity of NiV vaccine candidates with or without squalene oil-in-water adjuvant in a hamster model. We compared the prime-and-pull immunisation between the two vaccine platforms. We first immunized the animals intramuscularly and the boosting was performed with intranasal application. The immunogenicity was evaluated by anti-NiV IgG ELISA and neutralization potential was determined in an in vitro neutralization assay.

The results from prime-and-pull study revealed the superiority of the nanoSFERIC™ nanoparticle platform. In contrast to the immune response after NiVLP, the adjuvanted nSf-NiVac elicited further increase in the systemic antibody response, which peaked at 28 days post-prime (dpp). The humoral response for both adjuvanted and non-adjuvanted nSf-NiVac candidate maintained boosted levels by 50 dpp. However, the neutralization tests were suboptimal, thus highlighting the necessity for further optimization of the NiV antigen design, potentially in mammalian-based production systems.

Our nanoparticle and NiVLP vaccine candidates demonstrated a rapid antibody response, which is critical for the swift mediation of protection in the event of an outbreak. The true correlates of protection against Nipah virus remain to be elucidated, but a neutralizing antibody response could be pivotal for the control of disease in a naïve host. This evaluation is still in progress. Compared to NiVLP, the nanoSFERIC™-based nSf-NiVac exhibits a superior immunogenicity. This is a safe and cost-effective vaccine platform that is ideally suited for global deployment in light of the increasing threat posed by emerging Nipah virus outbreaks.

POSTER 08 – Application of Sequencing Techniques to Characterize a DNA Vaccine to Prevent Klebsiella Pneumoniae Infections

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Klebsiella pneumoniae is among the most worrisome antibiotic-resistant bacteria identified by the WHO, causing infections in the healthcare and community setting. Over the past five years, we have been working in the pre-clinical development of KlebsiGene, a prophylactic DNA vaccine, as a novel strategy to prevent infections caused by K. pneumoniae. The main aim of this study is to genetically characterize KlebsiGene vaccine prototypes, and the immune response elicited to facilitate future regulatory assessment and market entrance.

Eight vaccine prototypes encoding different proteins related to K. pneumoniae virulence were studied. Their immunogenicity and efficacy of protection against K. pneumoniae sepsis was tested in vivo. A prior service provision (ISID_ec15) evidenced regulatory points related to efficacy, safety, and quality applicable to KlebsiGene during proof-of-concept phase to address. Thus, access to ISIDORe EATRIS Immunomonitoring services was needed to accomplish two tasks.

First, plasmid chromosomal integration was studied by sequencing of total DNA from HEK 293 cells transfected with the eight vaccine candidates at 48h and 72h (Illumina NovaSeq S4 NS4-300 run). Second, an analysis to decipher changes in gene expression in these cells due to vaccine expression was done. RNA was extracted at 48h post-transfection and an Illumina Stranded Total RNA library followed by NovaSeq S1 NS1-200 run was performed. Analysis of differentially expressed genes compared to non-transfected cells was carried out.

Significant differences in antibody titres elicited and percentage of mice survival were observed among vaccine prototypes. No integration of the full plasmid nor the antigenic sequence was detected by PCR with vaccine-specific primers. Sequencing data would allow to decipher if partial integration occurred and differences among constructs exist. Differences in the transcription of the antigens by HEK293 cells were observed. By the end of the project, we expect to decipher the transcriptomic changes produced by the vaccine platform, and those that are antigen specific.

We benefit from the ISIDORe EATRIS services to complete critical development milestones related to regulatory of the technology, positioning KlebsiGene closer to clinical phase trials and market entrance. In addition, given the flexibility of the technology in development, this can set the basis for similar products addressing other applications.

POSTER 09 – Interaction of the Hypericin Loaded Metal-Organic Framework with Spike Protein & ACE2 Cell Receptors

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Metal-organic frameworks (MOFs) have been developed to be loaded with hypericin, a potential antiviral and photoactive molecule. The fluorescence of MOF enables the application of these structures in bioimaging, and the porous material is able to bind a large number of hypericin molecules. Using confocal fluorescence microscopy and time-resolved fluorescence microscopy, the interactions of hypericin with angiotensin-converting enzyme 2 receptor (ACE2r)-positive cells (labelled with EGFP) were investigated. Hypericin can be transported to cells expressing ACE2r. This cellular model mimics the conditions under which SARS-CoV-2 can enter the cells. The same method was used to detect changes in the expression of ACE2r after incubation of these cells with SARS-CoV-2 RBD-SD1 protein. We found that hypericin can easily interact with the SARS-CoV-2 RBD-SD1 protein and can be transported into the cell with the protein. In addition, we observed that the SARS-CoV-2 RBD-SD1 protein and hypericin silenced ACE2r to some extent in the cells. The impact of these effects may be related to autophagy of the infected cells and strongly influence tissue regeneration.

POSTER 10 – High-Throughput Screening of a Diverse Library of Academic Research Substances for SARS-CoV-2 Antiviral Properties

Savumiga SHANMUGAMANI
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The ongoing emergence of SARS-CoV-2 variants underscores the need for novel antiviral therapeutics. While vaccines mitigate disease severity, effective small-molecule inhibitors remain crucial, particularly for high-risk individuals and in the face of potential resistance. Identifying antivirals with distinct mechanisms of action can expand treatment options and strengthen pandemic preparedness.

The Molecule Archive of the Compound Platform at the Karlsruhe Institute of Technology serves as a repository for academic chemical substances, aiming to promote sustainability and transparency of research in chemistry. Thanks to ISIDORe Transnational Access we screened the entire collection of 11,580 compounds for antiviral activity against SARSCoV-2. Hits were defined as compounds inhibiting viral cytopathic effects by >60%, yielding a hit rate of 0.5% (55 compounds). Two promising drug-like classes — chiral bisindolylpiperidines (7 actives) and benzo-1,2,3-triazines (9 actives) — were selected for further evaluation.

Subsequent dose-response studies determined antiviral potency and cytotoxicity. A chiral nitrile-substituted bisindolylpiperidine exhibited an IC50 of 3.5 µM with no detectable cytotoxicity (CC50 > 100 µM), suggesting a favourable selectivity index (>28). The activity was enantiospecific, indicating a selective mechanism of action. Similarly, a 4-phenylsubstituted benzo-1,2,3-triazine displayed an IC50 of 1 µM and a CC50 of 19 µM (SI = 19). Structure-activity relationship studies identified key modifications influencing activity and toxicity.

Testing in A549 lung epithelial cells, a more physiologically relevant model, showed a loss of activity for bisindolylpiperidines and a tenfold reduction in potency for benzo-1,2,3-triazines. Mechanistic studies confirmed that benzo-1,2,3-triazines inhibit the endosomal viral entry pathway, while bisindolylpiperidines likely target an intracellular factor specific to VeroE6 cells.

POSTER 11 – Discovery of Novel Antiviral Compounds Inhibiting Coronavirus Infections

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COVID-19 is caused by the coronavirus SARS-CoV-2, which infects lung cells expressing angiotensin-converting enzyme 2 (ACE2) as its receptor. The virus binds via the spike (S) protein to defined residues of ACE2 without interfering with its peptidase activity. Since the interaction between the viral S protein and ACE2 is essential for infection, inhibiting it by small molecules is a valid antiviral strategy. We have developed and already successfully employed a high-throughput screen (HTS) for the discovery of compounds blocking the binding of the receptor binding domain (RBD) of the SARS-CoV-2 S protein to ACE2, which is novel since it is based on the enzymatic activity of ACE2. This method was used to screen an EU-OPENSCREEN library of 95.000 substances at Palacký University Olomouc, Institute of Molecular and Translational Medicine (IMTM), Czech Republic. Several hit compounds were detected and tested in follow-up experiments. One compound could be confirmed with a low µM IC50 for inhibiting RBD binding to ACE2 and also blocked the infection in a pseudovirus assay in cell culture. Further development by medicinal chemistry is now essential to allow preclinical and clinical trials eventually leading to a compound which can be used as prevention for infections caused by coronaviruses.

POSTER 12 – Progressing Toward Characterization of SARS-CoV-2 PLpro Hits-To-Lead Compounds

Paola STORICI
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New antiviral agents are critical for addressing coronavirus infections, particularly due to the emergence of resistant mutants. Among the pharmacological targets for SARS-CoV-2, the cysteine proteases, especially the main protease (Mpro) and the papain-like protease (PLpro), are of significant interest. PLpro is highly conserved across coronaviruses and functions both as a viral protease and as a modulator of host immune responses, making it an attractive target for broad-spectrum antiviral development.

Despite extensive drug screening efforts, only a few compounds, including the repurposed GRL-0617, have been validated as PLpro inhibitors. While recent studies have demonstrated the efficacy of targeting this protease in mouse models, progress has primarily focused on GRL-0617 derivatives, highlighting the need for novel inhibitor scaffolds to expand therapeutic options against PLpro.

Our research, initiated under the EU-funded EXSCALATE4CoV project (www.exscalate4cov.eu) and currently supported by the AVITHRAPID project (www.avithrapid.eu), aims to develop hit-to-lead compounds. We identified promising candidates through two screening campaigns: one involving 9,000 repurposed inhibitors and another in collaboration with the European Lead Factory (ELF) using a proprietary library of 550k molecules. Among these candidates, we identified a class of potent but poorly soluble compounds that require further investigation.

With a 6-months support from ISIDORe, we could collaborate with Ana Martinez's medicinal chemistry lab at CSIC to synthesize these compounds in both base and salted forms. The improved solubility of the salified compounds will facilitate detailed biochemical, biophysical, and structural characterization. Preliminary bioassays' results confirm their in vitro potency, and we are now conducting a complete characterization which includes extensive screenings to obtain valuable co-crystals.

Given the limited chemical diversity among existing PLpro inhibitors derived from the GRL-0617 scaffold and the challenges in elucidating structural details of new molecules within the PLpro binding sites, we are optimistic that our ongoing research will enhance understanding of inhibition mechanisms, ultimately contributing to the development of pan-antivirals against coronaviruses.

POSTER 13 – Targeting Senescent Cells to Treat Post-COVID-19 Syndrome

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A significant percentage of SARS-CoV-2 survivors experience persistent sequelae months after infection, known as post-COVID-19 syndrome (Long COVID). There is growing concern that national health systems may eventually be overwhelmed by the chronic diseases caused by this syndrome. Consequently, it is crucial to continue investigating the long-term effects of SARS-CoV-2 infection.

Recent research has shown that senescent cells play a central role in the short-term progression of COVID-19 in mice (DOI: 10.1111/acel.13771). Our hypothesis is that, over time, SARS-CoV-2-infected mice not treated with senolytics will develop severe, chronic lung and brain damage, which closely mimics the symptoms of post-COVID-19 syndrome observed in humans. We believe that senescent cells may also play a central role in triggering the long-term symptoms associated with Long COVID.

In this project, we propose an experimental design using aged animals susceptible to SARS-CoV-2 infection through the expression of the hACE2 receptor. We aim to selectively eliminate senescent cells as a treatment for the long-term sequelae of SARS-CoV-2. The study assesses clinical scores, viral load, respiratory function, and survival in mice over long-term monitoring. Additionally, serum samples were collected for cytokine/chemokine analysis using Luminex technology. Immunohistochemistry (IHC) assays on lung tissue will examine: (i) senescence markers (p21CIP1, p16Ink4a, p19ARF), (ii) relevant proteins involved in COVID-19 development, and (iii) fibrosis detection using Masson's trichrome staining. Senescence levels in each tissue will be quantified using SA-β-Gal assays.

These analyses will provide a clearer understanding of the inflammatory response to SARS-CoV-2 and the potential efficacy of senolytic therapy. While behavioural and cognitive assessments were initially proposed to investigate cognitive deterioration associated with post-COVID-19 syndrome, they could not be performed in the BSL3 facility. However, this behavioural platform will be available soon at the CIPHE BSL3 core facility.

This study underscores the need to refine viral dosing strategies, particularly with respect to animal age and sex, to develop more effective treatments for Long COVID. Unfortunately, due to financial constraints, we were unable to perform the comprehensive analysis needed to fully explore these variables. Increased funding would allow for a more thorough investigation, which could advance our understanding of the complex interactions at play and ultimately lead to more targeted senolytic therapies for Long COVID.

POSTER 14 – Activation of Mycobacterium Tuberculosis ClpP1P2 Protease & its ATPase Complexes

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Supramolecular assemblies are integral to cellular biochemical processes, relying on their dynamic nature to fulfil essential functions. The protease ClpP1P2, paired with ATPase partners ClpC1 or ClpX, is vital for the survival of

Mycobacterium tuberculosis (Mtb). While the ClpP1P2 complex requires activation by specific N-blocked dipeptides (e.g., Z-Leu-Leu) to exhibit proteolytic activity in vitro, the mechanism of in vivo activation remains unclear. In this study, we identified novel activators that enabled the structural determination of the ClpC1P2P1 complex, providing insights into its assembly. These findings propose a new model of Clp system activation in vivo, offering promising avenues for therapeutic targeting in tuberculosis treatment.

POSTER 15 – High-Throughput Screening of the European Chemical Biology Library to Identify Compounds Targeting Mycobacterium Tuberculosis Caseinolytic Protease

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Tuberculosis (TB) remains a major health concern, particularly for Africa. While treatment is available, the rapid emergence of drug resistance has escalated the need to identify new potent anti-TB drugs with novel mechanisms of action. In addition, the current treatment of TB is lengthy requiring 6 months, with a four-drug regimen, for drug-susceptible Mycobacterium tuberculosis (Mtb) to avoid disease relapse. In the case of multi-drug resistant (MDR) TB, treatment is extended up to 2 years with a concoction of up to ten antibiotics.

The Mtb caseinolytic protease (Clp) is an attractive drug target due to essential role for the growth and viability of Mtb. The Clp machinery is composed of two components, the proteolytic core (ClpP) and the regulatory ATPases (ClpC/ClpX), which operate to unfold and degrade damaged or misfolded proteins. Dysregulation of the Clp complex, either genetically or by small molecule binders, is detrimental to Mtb. The identification of new small molecule binders for this target is a potentially alternative effective strategy to identify new anti-TB agents without pre-existing clinical drug resistance. One analytical strategy for identifying binders of the Clp complex is investigate the effect of these compounds on the catalytic capacity of the full ClpC1P1P2 complex. For this purpose, the fluorescein isothiocyanate-labelled casein assay was proposed as the most biologically relevant assay able to identify inhibitors/activators binding to any of the Clp components. Using ISIDORe's services, we have expressed and purified ClpP1, ClpP2 and ClpC1. All expression protocols were optimised for large scale protein production at the service sites, IGBMC and Robotein. The Clp proteins were expressed and purified separately using a combination of ion exchange and size exclusion chromatography. Proposed assay has been adapted for high-throughput screening format by IBCH-PAS (a part of Polish consortium POL-OPENSCREEN and screening partner site of EU-OPENSCREEN). This assay is now being used to screen the European Chemical Biology Library (ECBL) to identify novel hits that target the Clp machinery. Following validation and mode of inhibition studies, prioritized hits will undergo medicinal chemistry optimisation, serving as new chemical starting points for H3D's TB Drug Discovery portfolio, which aims to identify pre-clinical drug candidates for treating MDR-TB with the potential to contribute to combination therapy for treatment-shortening.

POSTER 16 – A Phase III Trial of Two Adjunctive Drugs, Metformin & N-Acetyl Cysteine, to Promote Lung Function Recovery & Prevent Post-Tb Lung Disease in Patients with Rifampin-Susceptible Tuberculosis (Tb-Metnac)

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A Phase IIB randomized trial (https://doi.org/10.1093/cid/ciab964) was conducted to assess the impact of adding metformin to the standard anti-tuberculosis treatment regimen for adults with pulmonary tuberculosis. Although the trial found that metformin did not expedite the conversion of sputum culture, it did result in significantly reduced lung inflammation, as evidenced by faster radiographic clearance and decreased inflammatory markers.

The study sought ISIDORe EATRIS resources to enhance research and access external expertise. The ISIDORe advisory committee advised developing a Target Product Profile (TPP) for Metformin and N-acetylcysteine (NAC), crucial for strategizing development, designing a Phase III trial, and facilitating dialogue with regulatory authorities and experts. The expert committee suggested employing a double-blind, placebo-controlled trial design with the primary efficacy focus on mitigating lung function impairment. This is measured by the 1-second forced expiratory volume (FEV1) using spirometry. The FEV1% is evaluated at the 18-month mark after successful treatment, adjusted for baseline covariates such as baseline FEV1, in a superiority comparison.

The guidance provided by the ISIDORe advising committee was instrumental in establishing a global trial involving Metformin and NAC. By clearly defining the desired characteristics of the target product, the development strategy for both metformin and NAC could be specifically tailored to address the needs of treating Post TB Lung Disease (PTLD). This led to successful collaborations with countries such as Brazil, China, and Indonesia, significantly expanding the trial's global reach. Notably, the trial protocol received approval from the Government of India's Scientific Board and obtained ethical clearance, marking significant milestones within a relatively short timeframe.

Leveraging the expert advice from the ISIDORe advising committee facilitated the effective design of a global trial evaluating Metformin and NAC's efficacy in treating PTLD. This approach is expected to significantly contribute to advancing the development of new treatments for PTLD, improving patient outcomes globally. The strategic guidance illuminates a promising path towards innovative therapeutic solutions for PTLD, highlighting the potential impact of well-coordinated, expert-driven clinical research on addressing global health challenges effectively.

POSTER 17 – Targeting the Dengue Virus 5'-Untranslated Region Stem Loop A by Fragment-Based Drug Design

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Targeting RNA structures with small molecules is an emerging approach in drug discovery to combat infectious diseases. Several viral infections lack efficient treatment, especially neglected tropical diseases caused by flaviviruses. One example is Dengue Virus (DENV), the most prevalent mosquito-borne pathogen worldwide. Its RNA genome comprises highly conserved structural elements with suitable druggability for small molecules (Song et al. 2019). We address the three-way junction motif of 70-nucleotide stem-loop A (SLA) at the 5'-untranslated region. SLA functions as a promotor for viral replication by recognizing the non-structural protein NS5 (Lee et al. 2021), is present among flaviviruses including DENV, Zika (ZIKV), West Nile (WNV) and Yellow Fever virus (YFV) and sensitive to mutations. This makes SLA a promising target for anti-flaviviral agents. We aim for the identification of small molecules that bind SLA to disable the crucial interaction between RNA and protein and thereby stop viral replication.

Supported by ISIDORe, a nuclear magnetic resonance (NMR) fragment screening was conducted by the Schwalbe laboratory, Frankfurt University (Sreeramulu et al. 2021) to identify hits as potential starting points for ligand development. A library of 607 fragments was screened against DENV1 SLA resulting in 12 hits that were evaluated by chemical shift perturbations, waterLOGSY and T2-relaxation. These hits were confirmed via microscale thermophoresis (MST) by screening against DENV SLA and a truncated 40-nt RNA construct containing only the putatively druggable three-way junction (Sun et al. 2022). Three hits were confirmed as binders on both constructs with affinity in the high micromolar range which is a valid starting point for further optimization. A second fragment screening via surface plasmon resonance spectroscopy (SPR) is currently finalized by the Brenk laboratory, University of Bergen using the EUopenscreen library with 1056 fragments (Jalencas et al. 2024) to find further fragment hits.

The structure-activity-relationships (SAR) of the fragment ligands are currently elucidated by chemical derivatization to improve on-target affinity, selectivity and physicochemical properties. Supported by structural biology and computational studies, fragment growing, merging and linking will be applied to design optimized RNA binders for subsequent antiviral testing. This is the first reported approach to target DENV RNA elements with small molecules.

POSTER 18 – Synthesis of Anti-Inflammatory Drugs' Chalcone Derivatives & a Study of Their Conformational Properties Through a Combination of Nuclear Magnetic Resonance Spectroscopy & Molecular Modelling

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In this study, two chalcone analogues were synthesized through in silico and experimental methods, and their potential to inhibit the lipoxygenase enzyme, which plays a role in the inflammation pathway, was assessed. Specifically, this study is a continuation of previous research in which chalcone derivatives were synthesized and characterized.

In the current work, we present the re-synthesis of two chalcones, with a focus on their docking studies, NMR analysis, and dynamic simulations. The structure of each chalcone was elucidated through a combination of Nuclear Magnetic Resonance (NMR) and Density Functional Theory (DFT). The substituent effect on the absorption spectrum of the two chalcone derivatives was studied. Results: A "LOX–chalcone" complex, predicted by docking studies, was further examined using molecular dynamics (MD) simulations to evaluate the stability of the complex. After fully characterizing the "LOX–chalcone" complexes in silico, the atomic details of each chalcone's interaction with LOX-1 and 5- LOX were revealed through Saturation Transfer Difference (STD) NMR (Nuclear Magnetic Resonance) using ISIDORe services. Finally, their selectivity profile was investigated against human 15-LOX-1 and general lipoxidase activity.

This study, with ISIDORe's vital contributions, underscores the promise of chalcones as therapeutic candidates for inflammatory diseases. The findings are particularly significant for pandemic preparedness, as targeting inflammation pathways can play a crucial role in managing severe inflammatory responses associated with infections.

POSTER 19 – FLIP_COVID (JRA) – Engineering & Validation of Protease-Sensor Cell Lines for Coronaviruses Mpro & PLpro

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SARS-CoV-2 pandemic revealed once more the *Coronaviridae* as a putative source for emerging viral pathogens. It also showed the need for sensitive, fast-adaptable and low-biosafety level antiviral assays for fast detection of virus replication and development of antiviral therapies. In the FLIP_Virus JRA, Fraunhofer ITMP and KU Leuven develop a cell-based coronavirus protease sensor assay to be used by the scientific community for protease activity. The assay can be used to measure transduction and antiviral activity for coronavirus isolates. The assay uses FLIP-GFP protease substrate proteins that include the highly conserved PLpro or Mpro proteases cleavage sides. The proteolytic cleavage restores the fluorescence of the GFP. The amount of conversion can be detected and quantified by plate reader or fluorescence microscopy and correlates with viral load in the cell and protease activity, making it suitable for virus replication and protease inhibition studies. We will report on validation of the model at ITMP with transduction of SARS-CoV-2 PLpro and Mpro and inhibition of the proteases using reported inhibitors, such as GRL-0617 and nirmatrelvir (PF-07321332), as well as on usability of the cellular model for application in antiviral assays at KU Leuven.

POSTER 20 – HenipaVal (JRA) – Validation of Molecular & In Vitro-Based Diagnostic Methods for Nipah & Hendra Virus Detection

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Nipah virus (NiV) and Hendra virus (HeV) are negative single strand RNA viruses within the genus henipavirus in the *Paramyxoviridae* family. Both viruses have the potential to cause lethal infections in humans and animals. Fruit bats have been discovered as the reservoir host. In 1998/99, NiV infections were first diagnosed in farmed pigs in Malaysia, and almost 300 farm and slaughterhouse workers developed severe respiratory and nervous symptoms, with a fatality rate of 36%. Since then, outbreaks of human NiV infections have been reported almost yearly in India and Bangladesh, caused by transmission from fruit bats directly to humans via contaminated date palm sap or fruits. HeV was first detected 1994 in Australia, causing severe respiratory and central nervous symptoms in horses, followed by > 80 outbreaks in horses, and seven human cases with four fatalities. As henipaviruses are on the WHO and CEPI R&D priority list, comparing methods can help improve the diagnostic quality.

In this JRA, we are comparing molecular and serological diagnostic assays for the detection of henipavirus infections. In particular, we are assessing the diagnostic sensitivity of the applied PCR methods by including defined copy controls. Moreover, we are comparing the sensitivity and specificity of the serological assays that are being used by both partners. Likewise, we are exchanging cell lines that are being used in the Serum Neutralization Test (SNT). The performance will be compared using samples from animal challenge studies performed at both partner institutes.

First of all, diagnostic protocols used in both partner laboratories are being exchanged, showing some relevant differences. Our next step is to identify the impact of individual steps within the protocols on the outcome of the analysis. This step is still ongoing. To get a better understanding of the diagnostic routines in each laboratory, exchange visits will take place before the Annual ISIDORe meeting.

By the end of this project, we will have a thorough understanding of the sensitivity and specificity of the applied molecular and serological assays used in both partner laboratories, as an initial step towards harmonization of diagnostic methods.

POSTER 21 – DEMAR (JRA) – Development of a Cell-Based Assay for Detection of DeMARylation Activity of Viral Macrodomains & Antiviral Drug Discovery

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Targeting viral macrodomains (MDs) is a promising strategy for antiviral drug development. These MDs possess hydrolase activity that removes mono-ADP-ribosylation (MAR) modifications from proteins, thereby interfering with host defence mechanisms and disrupting interferon (IFN) signalling. Our project focuses on the development of a cell-based assay to study the deMARylation activity of viral MDs and to evaluate the efficacy of MD inhibitors using an established deMARylation detection system based on the PARP10 catalytic domain and the MAR-binding domains of PARP14.

We have successfully generated A549 ACE2+TMPRSS2 cell lines that express the PARP10 probe protein either constitutively or by doxycycline (Dox) induction. A robust protocol for cell lysis under mild non-denaturing conditions has been established, which is essential for maintaining the protein-protein interactions necessary for accurate capture and labelling of the PARP10 probe and detection of its MARylation status.

In addition, the PARP10 tool protein was heterogeneously expressed in E. coli and subsequently MARylated in vitro to facilitate control experiments. This allowed us to demonstrate an appropriate assay window between non- and fully MARylated tool protein, providing a reliable benchmark for our ongoing studies.

Current experimental efforts are focused on subjecting the A549 cells to various stimuli, including infection with SARS-CoV-2, to investigate the global changes in MARylation and to understand the extent to which these changes involve the ARTD10 tool protein. These experiments are critical to validate the functionality of our assay and its potential applications in drug discovery.

The successful establishment of this assay will provide a valuable tool for users of the ISIDORe platform, accelerating drug target validation and the development of innovative antiviral therapies. By improving our understanding of the interplay between viral macrodomains and the host immune response, we aim to identify new antiviral compounds that can effectively combat viral infections and thus contribute to pandemic preparedness.

Keywords: Viral macrodomains, antiviral drugs, mono-ADP-ribosylation, PARP10, deMARylation, drug discovery.

TOGETHER, WE DELIVERED

The 2nd ISIDORe User Conference marks the close of a unique scientific and collaborative journey that mobilised more than 150 partners over three and a half years. It offers a compelling and representative glimpse into the diversity, scale, and impact of what was achieved together – from accelerating research on priority pathogens to reinforcing Europe's long-term capacity to respond to infectious disease threats.

This event is not just a celebration of science. It is a reminder of what the research infrastructure community can achieve when mobilised with shared purpose, as well as the collective effort, expertise, and dedication that made it all possible.

We warmly thank:

- The **Research Infrastructures' staff and Access Providers** who delivered high-quality services across Europe and beyond,
- The **scientists** who proposed and carried out projects tackling key infectious disease challenges, supported by ISIDORe's Access Providers,
- The **reviewers** who evaluated proposals with rigour and fairness,
- The members of ISIDORe's **Strategic, Scientific, Ethics, and Security Advisory Boards**, whose guidance helped shape the project's direction and integrity,
- **The Research Executive Agency and the European Commission** for their trust and support in enabling this unprecedented research infrastructure mobilisation.

ISIDORe may be ending, but its legacy lives on – in new knowledge, new collaborations, and a strengthened community ready to meet future challenges together.

With appreciation,
ERINHA, Coordinator of ISIDORe

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