International Centre for Genetic Engineering and Biotechnology

International Conference

Pandemic preparedness

Achievements, current challenges, and new frontiers

11-13 November 2024, Trieste
Savoia Excelsior Palace Hotel

ABSTRACT BOOK

With the patronage of

Sponsored By

Funded by the European Union NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

Summary

Keynote & Invited Speeches

Addressing the ongoing threat of emerging viral infections

Yoshihiro Kawaoka - University of Tokyo and National Center for Global Health and Medicine, Japan; University of Wisconsin-Madison, USA

yoshihiro.kawaoka@wisc.edu

Every year, influenza epidemics occur, causing increased morbidity and mortality, particularly in vulnerable populations, such as the very young and very old. In addition, pandemics, such as the 1918 pandemic, occasionally occur. Consequently, influenza has an enormous impact on the global economy. By contrast, Ebola virus has only been recognized since 1976, and, until recently, outbreaks of this virus had caused relatively few deaths because they occurred in rural, isolated areas. However, the 2014 outbreak in West Africa occurred over a large, densely populated urban area and changed our understanding of what constitutes an Ebola virus outbreak. In December 2019 in China, SARS-CoV-2 emerged and spread globally, causing the fifth pandemic since the 1918 pandemic. I will discuss our recent research on these viruses.

Preparing the world for climate-driven amplifications of infections

Rino Rappuoli - Biotecnopolo di Siena Foundation, Italy

rino.rappuoli@biotecnopolo.it

Climate change is a powerful amplifier of infectious diseases, and several tropical pathogens are already reaching the European continent. The increased risk of exposure to known pathogens and the possible emergence of unknown pathogens with pandemic potential, mandates science-driven investments in diagnostics, vaccines, antibodies, and therapeutics to mitigate the impact of the new diseases. At the Conference an overview will be discussed of how the world is preparing for this scenario.

Outbreak preparedness at the Institute of Microbiology and Immunology in Ljubljana

Tatjana Avšič-Županc - University of Ljubljana, Slovenia

tatjana.avsic@mf.uni-lj.si

The Institute of Microbiology and Immunology of the Faculty of Medicine of the University of Ljubljana (IMI MF UL) is the central and largest Slovenian institution for specialised microbiological and immunological medical research and teaching activities. The lecture will present the achievements of the Institute's laboratories over the past twenty years in preparedness and response to disease outbreaks.

Advancing European Research: The role of National Health Institutions in European Partnerships linked to One Health Anti-Microbial Resistance, Pandemic Preparedness and through the collaboration with ECRIN/ItaCRIN

Maria Josè Ruiz Alvarez - Research Coordination and Promotion Service (CORI) and Italian National Institute of Health (ISS)

mj.ruizalvarez-esterno@sanita.it

As a researcher from the Italian National Institute of Health, I am actively engaged in key European initiatives aimed at addressing some of the most pressing health challenges of our time, including antimicrobial resistance (AMR) with One Health vision, the pandemic preparedness, and briefly, the personalized medicine approach on infectious diseases.
This presentation will highlight the pivotal role of Italian research institutions in these European Partnerships, illustrating how collaborative research efforts are essential to achieving impactful health outcomes.
I will also discuss the importance of ECRIN (European Clinical Research Infrastructure Network) and its Italian node, ITACRIN, as vital infrastructures for enabling high-quality, multinational clinical research. By facilitating seamless cooperation among diverse European partners, ECRIN/ITACRIN provides the foundation for innovative, coordinated responses to public health threats, underscoring the strategic importance of robust, interconnected research infrastructures.
Through this talk, I aim to shed light on how these partnerships and infrastructures not only support evidence-based policy and promote research excellence, and drive advancements in public health care at both the national and European levels, but also strengthen Europe's capacity to respond to future pandemics/health crises.
By fostering collaborative research, supporting training activities and enabling rapid knowledge exchange, these initiatives drive critical advancements in healthcare, helping to build a resilient, innovative, and responsive healthcare ecosystem at both national and European levels.

Next generation sequencing approaches to enhance surveillance capacity for emerging viruses

Emma Thomson - MRC-University of Glasgow Centre for Virus Research and London School of Hygiene and Tropical Medicine, UK

emma.thomson@glasgow.ac.uk

This talk will focus on the utilization of NGS technologies to improve the detection and monitoring of emerging viral threats. It will explore the latest advances in NGS methodologies and their applications in genomics, diagnostics, and epidemiology. NGS can be used to identify new or emerging viruses, estimate growth rates of viruses (or viral variants) in a population, monitor seasonal trends (for example in wastewater), and for serology applications such as PhiP-Seq or MIPSA. The broader research background includes the growing need for rapid and accurate viral identification and characterization to prevent and control outbreaks.

Broader-acting antivirals for epidemic and pandemic preparedness

Johan Neyts - University of Leuven (KU Leuven), Belgium

johan.neyts@kuleuven.be

Broad(er)-acting antiviral drugs, active against entire genera or families of viruses, should be developed and stockpiled in epidemic/pandemic peacetime. They can be used to counter outbreaks as soon as the new virus has been identified and will also remain important pharmacological tools after the introduction of vaccines and monoclonal antibodies. For a review on the topic see *Jochmans, Laporte & Neyts (2023) Cell Host & Microbes*.

"Forecasting" protein evolution using data-driven sequence landscapes

Martin Weigt – Sorbonne Université, Paris, France

martin.weigt@gmail.com

In the course of evolution, proteins change their amino-acid sequences due to mutations, but natural selection keeps their three-dimensional structure and their biological functions remarkable conserved, even over long evolutionary times. I will show how to use families of homologous proteins, downloadable from sequence databases, to infer data-driven protein sequence landscapes, which in turn allow to predict mutational fitness effects. I will discuss an application to the receptor-binding domain of SARS-CoV-2: using pre-pandemic data of spike proteins from other coronaviruses, we are able to predict mutable sites, which turned out to be mutated in many of the variants emerging later during the pandemics, partially being responsible for SARS-CoV-2 immune escape. This information can be of interest when designing antibody therapies or vaccines targeting mutationally conserved epitopes.

The NMR contribution to Cellular Structural Biology: from protein structures and their interactions to functional processes

Lucia Banci - University of Florence and Instruct-ERIC, Italy

banci@cerm.unifi.it

I will present some applications of Structural Biology via NMR and in particular of in-cell NMR and their relevance for describing functional processes in the cell, for effective drug screening protocols, and for contributing to the design of effective, protein-based vaccines.

Coronavirus pandemic preparedness through the lens of a structural biologist

Daniel Hurdiss - Utrecht University, Netherlands

d.l.hurdiss@uu.nl

This talk will focus primarily on our studies of coronavirus spike proteins and the value of structural biology to provide fundamental insights into coronavirus cell entry mechanisms and immune evasion, identify vulnerable sites on coronavirus spike proteins and develop new and improved therapies for pandemic preparedness.

Pandemic preparedness with a focus on prevention

Marion Koopmans - Erasmus University Medical Center, Netherlands

m.koopmans@erasmusmc.nl

As the majority of emerging infectious diseases originate from animals, a next step in preparedness is to understand mechanisms of disease emergence. Fundamental insights in the disease ecology and factors that drive infection dynamics in ecosystems is needed to understand where and how emerging disease outbreaks occur, and thereby help target enhanced surveillance activities.

Investigating the origins and mammalian spillover potential of the ongoing H5N1 panzootic

Tom Peacock - The Pirbright Institute and Imperial College London, UK

tom.peacock@pirbright.ac.uk

My talk will go over recent data about how the ongoing H5N1 panzootic emerged in avian species and what the underlying genetic drivers of this were. It will also cover recent mammalian spillover events, looking for evidence of mammalian adaptation and evidence for mammal to mammal transmission. Finally, I will use this data, and data from human primary cell models, to assess the pandemic potential of these viruses.

The comprehensive response to autochthonous outbreaks of dengue virus: the approach of the National Institute for Infectious Diseases "L. Spallanzani" in Rome

Fabrizio Maggi - National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, and University of Insubria, Varese, Italy

fabrizio.maggi@inmi.it

Dengue virus (DENV) has been expanding its range to temperate areas that are not usually affected, where the spread of vectors has been facilitated by global trade and climate change. In Europe, there have been many cases of DENV imported from other regions in the past few years, leading to local outbreaks of DENV among people without travel history. Studying locally transmitted cases of DENV offers important information about transmission patterns, regional molecular changes, and the virus's adjustment to human hosts and its vectors. Grasping these elements enhances public health preparedness and helps devise plans to counteract the spread of DENV.

Fostering pandemic preparedness and response research: the case of European Research Infrastructure on Highly Pathogenic Agents (ERINHA)

Jonathan Ewbank - European Research Infrastructure on Highly Pathogenic Agents - ERINHA, Belgium

jonathan.ewbank@erinha.eu

ERINHA, a non-profit organisation, brings together European high and maximum containment laboratories, brokering access for research on high-consequence pathogens. It coordinates the cross-disciplinary programme ISIDORe that funds projects using the services of most of the life-sciences research infrastructures. It also coordinates or participates in large-scale research projects to develop medical countermeasures against deadly pathogens, including Nipah virus and Crimean-Congo haemorrhagic fever virus. With unique capacities and expertise, ERINHA occupies a central position in the European and global pandemic preparedness and response landscape as well as builds public trust, by promoting safe and secure research.

Oral presentations

Session 1 "*Emerging and re-emerging viruses with pandemic potential*"

Modulation of Zika Virus Replication in Human Monocytes and Monocyte-Derived Macrophages (MDM) Through Pro-Inflammatory (M1) Polarization

Isabel Pagani[1], Silvia Ghezzi[1], Giulia Aimola[2], Paola Podini[3], Guido Poli[2,4] and **Elisa Vicenzi**[1]

[1]Viral Pathogenesis and Biosafety Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

[2]Human Immuno-Virology (H.I.V.) Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

[3]Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

[4]Vita-Salute San Raffaele University, School of Medicine, Via Olgettina 58, 20132 Milan, Italy

vicenzi.elisa@hsr.it

Zika virus (ZIKV), an arbovirus mainly spread by mosquitoes and also transmissible through sexual contact, can lead to severe neurological conditions such as microcephaly in fetuses and Guillain-Barré Syndrome in adults. ZIKV effectively infects human monocytes, with an even higher efficiency in monocyte-derived macrophages (MDM). Building on our prior research on human immunodeficiency virus (HIV), we explored the effect of cytokine-induced polarization of human MDM into M1 (pro-inflammatory, induced by interferon-γ and tumor necrosis factor-α) or M2 (alternatively activated by interleukin-4) cells on ZIKV replication. Consistent with findings in HIV-1 infection, ZIKV replication was markedly reduced in M1-MDM, while M2 polarization did not impede the cells' capacity to produce the virus. Notably, M1 polarization of MDM resulted in the downregulation of MERTK (Mer Tyrosine Kinase), a potential receptor for ZIKV entry, and the upregulation of several interferon-stimulated genes (ISGs), indicating two potential mechanisms for the observed inhibition of virus replication. These results emphasize the protective role of M1 polarization in human macrophages against various viral infections, including HIV-1 and ZIKV, and suggest a possible therapeutic strategy to enhance the antiviral capabilities of the innate immune system.

Phenotypic evolution of SARS-CoV-2 spike throughout the COVID-19 pandemic

Wilhelm Furnon[1], Vanessa M Cowton[1], Giuditta De Lorenzo[1], Richard Orton[1], Vanessa Herder[1,2], Diego Cantoni[1], Georgios Ilia[1], Diogo Correa Mendonca[1,2], Karen Kerr[1,2], Jay Allan[1], Nicole Upfold[1], Gavin R Meehan[1,2], Siddharth Bakshi[1], Udeet Ranjan Das[1], Sergi Molina Arias[1,2], Marion McElwee[1,2], Sarah Little[1], Nicola Logan[1], Kirsty Kwok[1], Katherine Smollett[1], Brian Willett[1], Ana Da Silva Filipe[1], David L Robertson[1], Joe Grove[1], Arvind H Patel[1,2] and Massimo Palmarini[1,2]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

[2]CVR-CRUSH, MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Wilhelm.Furnon@glasgow.ac.uk

Variants of SARS-CoV-2, defined mainly by mutations in the spike glycoprotein, have emerged throughout the COVID-19 pandemic and will undoubtedly continue to emerge in the future. Spike strongly influences virus phenotype, and it is therefore critical to understand the evolution of this viral protein during distinct phases of the pandemic. Here, we generated a panel of recombinant viruses carrying spike from 27 of the most significant variants, circulating between 2020 and 2024, within an otherwise identical genomic backbone. We systematically assessed phenotypic traits of these viruses both in vitro, ex vivo and in vivo in hamsters. Overall,

we determined distinct phenotypic trajectories of spike among and between the variants circulating before ("pre-Omicron") and after ("post-Omicron") the emergence of Omicron. Spillover of SARS-CoV-2 in the human population was followed by a period of adaptation and fine-tuning to the host in the pre-Omicron variants. Omicron simultaneously "reset" several established phenotypes of previously circulating variants. Since then, spike of post-Omicron variants maintained its enhanced tropism for the upper respiratory epithelium but displayed over time several phenotypic traits typical of the pre-Omicron variants. These data suggest that it may be possible for spike with phenotypic features of both pre- and post-Omicron variants to emerge in the future.

SARS-CoV-2 damage to neuronal networks is mediated by the pro-inflammatory activation of the cGAS-STING pathway in the glia

Pamela Martinez-Orellana[1], Matteo Manzati[2], Diletta Pozzi[2], Yingying Xiao[1], Chiara Kalebić[1], Marika Mearelli[3], Alessio Di Clemente2, Valentina Perrera[2], Denise Ferrarini[2], Tea Carletti[1], Carmen Falcone[3], Michele Giugliano[2,4] and Alessandro Marcello[1]

[1]Laboratory of Molecular Virology, The International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano, 99, 34149 Trieste, Italy

[2]Neuronal Dynamics Laboratory, International School for Advances Studies (SISSA), Via Bonomea, 265, 34136 Trieste, Italy

[3]Laboratory of Evolutionary Neurobiology, International School for Advances Studies (SISSA), Via Bonomea, 265, 34136 Trieste, Italy

[4]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze Campus Scientifico, ed. Scienze Biomediche V. Campi 287, I-41125 – Modena, Italy

pamela.martinez@icgeb.org

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection has been associated to neurological symptoms characteristic of long-lasting post-acute coronavirus disease (COVID). However, the complex mechanisms involved in these clinical manifestations are still unclear. Activated glial cells are key players in response to central nervous system infection, yet are implicated in inflammation and neurodegeneration. To investigate which cell types are specifically associated in the activation of the immune response triggered by SARS-CoV-2, we infected cultures of induced pluripotent stem cells (iPSC) astrocytes and human microglial clone 3 (HMC3) cells. We found that the higher infection rate was observed in iPSC astrocytes, while HMC3 supported only marginal virus replication. A significant IFN-β response was induced in astrocytes, while both cell types showed some level of chemoattractant production. Interestingly, both glial cells showed signs of senescence and activation of the pro-inflammatory cGAS-STING pathway by endogenous DNA. Then, we investigated if glial cells activation could affect the function of neuronal networks. Primary rat cortical cultures seeded on microelectrode arrays (MEAs) were used to monitor the electrical activity after exposure to SARS-CoV-2. Effective SARS-CoV-2 infection of the glia led to a major loss of synaptic connections, an increase expression and production of pro-inflammatory cytokines and chemokines, and an increase of DNA damage foci. Intriguingly, the pro-inflammatory response was cGAS-STING dependent. Finally, we demonstrated that an antagonist of the cGAS-STING pathway was able to rescue the decrease in electrical activity in the first hours' post-infection. These data point to SARS-CoV-2 infection of the glia as a culprit for neurological complications during COVID.

Using genomics to understand climate amplified diseases and epidemics

Marta Giovanetti[1,2,3]

[1]Department of Sciences and Technologies for Sustainable Development and One Health, Università Campus Bio-Medico di Roma, Rome, Italy

[2]Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

[3]Climate amplified diseases and epidemics (CLIMADE) Americas, Brazil

giovanetti.marta@gmail.com

The increasing frequency and intensity of climate change-related events are reshaping the landscape of infectious diseases, particularly those transmitted by vectors such as mosquitoes. Genomic technologies have emerged as critical tools for understanding and responding to these climate-amplified diseases and epidemics. This work delves into the role of genomics in deciphering the complex interplay between climate change, vector ecology, and pathogen dynamics. By integrating genomic data with ecological and climatic models, researchers can gain insights into how environmental changes drive the evolution, transmission, and geographic expansion of pathogens. The discussion focuses on key examples such as dengue, Zika, and other arboviruses, demonstrating how genomic sequencing enhances our ability to monitor outbreaks in real-time, trace pathogen spread, and predict future epidemic hotspots. Furthermore, the integration of genomic surveillance with ecological and epidemiological data allows for a more comprehensive understanding of disease dynamics, which is crucial for developing targeted public health interventions and preventive strategies. This approach not only improves our preparedness for future outbreaks but also highlights the importance of a multidisciplinary response to emerging threats in the era of climate change. By leveraging genomics, public health systems can enhance their capacity to mitigate the impact of climate-sensitive diseases, ultimately contributing to global health security.

Expanding Access to Sustainable Diagnosis in Africa-EXPANDIA

Mariana Ulinici[1], Ursa Susnjar[1], Tea Carletti[1], Alessandro Marcello[1] and EXPANDIA Project Team

[1]Molecular Virology Lab, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

ulinici@icgeb.org

The COVID-19 pandemic, along with recurring outbreaks of Ebola, Zika, Dengue, and the ongoing Mpox emergency, underscores the critical need for robust surveillance systems and accurate diagnostic capabilities to prevent the spread of infectious diseases and enable timely interventions. In this global context, Africa's preparedness for pandemics remains a significant challenge, particularly in terms of diagnostic capacity. In resposnse to these challanges, we at ICGEB have taken proactive steps, beginning with comprehensive needs assessments across 9 African countries. Key issues indentified include inadequate supply chains that hinder access to essential reagents and equipment, a lack of local manufacturing capabilities for diagnostics, and a fragmented regulatory environment complicating the approval of laboratory diagnostic tests. Our strategy focuses on scouting and integrating high-quality, novel diagnostic tools into clinical trials, especially those developed by technology providers from regions facing similar infectious disease burdens, thereby broadening the range of diagnostics available at more accessible rates. EXPANDIA's efforts are focused on optimizing and field-testing RT-LAMP as a point-of-care diagnostic tool for arboviruses such as Zika and Chikungunya. Recognizing the importance of monitoring circulating viral strains to ensure the accuracy of diagnostic tools, EXPANDIA has also initiated the transfer Oxford Nanopore MinION whole genome sequencing technology to laboratories across Africa. Additionally, we are exploring local manufacturing of essential reagents, such as

enzymes with optimized cold-chain formulations, to better adapt technologies to local needs. We also provide support in navigating regulatory pathways to facilitate the rollout of validated assays in partner countries, ensuring that new diagnostic tools can be rapidly deployed where they are most needed. Through EXPANDIA, we aim to challenge the *status quo* in diagnostic access and quality, delivering top-tier diagnostic tools globally without compromising efficacy or accuracy.

Establishing robust and efficient system for surveillance of microorganisms in environmental waters

Nina Prezelj[1], Živa Lengar[1], Maja Ferle[1], Denis Kutnjak[1] and Ion Gutierrez-Aguirre[1]

[1]National institute of biology, Večna pot 121, SI-1000 Ljubljana

nina.prezelj@nib.si

Environmental water is an important source of information that can be used to identify the presence of pathogens, assess the risk of disease transmission and determine the presence and source of different kinds of microbial contamination in environment. However, to determine the presence of largely diluted viruses and other pathogens in water samples a concentration method is usually essential to increase the sensitivity of their detection. The objective of this study was to compare four different water concentration methods (monolith chromatography (CIM-QA, BIAseparations), combined use of Large volume concentration kit and Concentration pipette from Innovaprep, classic filtration through electropositive filters (EZ-Pak 0.45µm, Millipore) and passive sampling using torpedo passive samplers (Monash University Australia)) for their ability to concentrate viruses and bacteria.
Water samples (3-26 liters depending on the method) consisted of tap water spiked with inactivated avian influenza A virus and with wastewater effluent. Total nucleic acids were extracted from concentrated samples and the presence and relative amount of avian influenza A virus and different species of bacteria from *Bacteroides* genus were determined with quantitative PCR in each concentrated sample and in the loaded water sample to estimate the concentration factor and recovery.
Compared to the load sample, the relative amount of targets was higher in all concentrated samples. For bacteria from *Bacteroides* genus the best concentration factor was observed with Innovaprep Large volume concentration kit and Concentration pipet combination. Influenza A virus was concentrated most efficiently using classic filtration.
Innovaprep Large volume concentration method enables concentration of up to 100 liters of water and enables higher throughput compared to classic filtration. Further optimizations of the method are possible, to obtain even better concentration results also for viruses.

Near-Real-Time Detection and Identification of Airborne Viral Threats

Federica Armas[1], Mariagiulia Conte[1], Gabriella Schirinzi[1], Francesco Roncari[1], Ambra Maddalon[1], Cloè Desmet[1], Pascal Colpo[1], Andrea Barbaglia[1] and **Andrea Valsesia**[1]

[1]European Commission, Joint Research Centre, Technologies for Health Unit, Via Enrico Fermi 2749, 21027, Ispra, Varese, Italy, +39 0332786149

andrea.valsesia@ec.europa.eu

Airborne viruses pose a continuous threat to public health, with their potential for rapid spread and pandemic initiation. Traditional surveillance methods are resource-intensive and slow, necessitating innovative approaches for efficient monitoring. In this work, we present a cutting-edge technology that enables near real-time, autonomous detection and identification of viruses through an optical detection system. Using particle

scattering spectra with a machine-learning algorithm, the system identifies virus like particles and distinguishes them from common confounding airborne particulates.
The system consists of an aerosol sampler enabling the efficient collection of intact viral particles in a buffer. The sample is then transferred into a microfluidic device where the optical characterization of the collected particles is performed using bio-functionalized surfaces. The optical reading system measures and records the light-scattering spectra of the collected particles. The machine-learning algorithm then recognizes the spectra and the concentration of viral particles is then calculated. The machine-learning model, which incorporates a confusion matrix and a random forest algorithm, has been trained on a dataset of around 3000 spectra. The system has an automated mechanism for sample preservation, enabling the subsequent genetic material extraction and analysis as a final validation.
In conclusion, our optical detection method offers a transformative advancement in airborne virus surveillance. It enables surveillance in indoor settings like airports and hospitals and enhances public health preparedness by providing quick and accurate results with minimal human intervention. A near real-time and autonomous detection strengthens our ability to respond to emerging viral threats, underscoring its value in global health security.

Infrared spectroscopy - a stop shop technology for vector-borne disease surveillance?

Emmanuel Mwanga[1,2], Doreen Siria[1,2], Roger Sanou[3], Issa Mshani[1,2], Sophia Mwinyi[1,2], Bazoumana Sow[3], Ivan Casas Gomez-Uribarri[1], Klaas Wynne[1], Mauro Pazmiño Betancourth[1], Heather Ferguson[1,2], Abdoulaye Diabate[3], Mario Gonzalez-Jimenez[1], Simon Babayan[1], Fredros Okumu[1,2] and **Francesco Baldini**[1,2]

[1]University of Glasgow, UK

[2]Ifakara Health Institute, Tanzania

[3]Institut de Recherche en Sciences de la Sante, Burkina Faso

Francesco.Baldini@glasgow.ac.uk

Effective surveillance of vector-borne diseases, including dengue, Zika, chikungunya, is crucial to prevent outbreaks and epidemics. Monitoring vectors and their associated pathogens allows for timely interventions to mitigate transmission and protect vulnerable populations. However, measuring key demographic traits such as vector species composition, age structure, infection prevalence, blood meal preference and insecticide resistance status is often challenging, given the lack of accurate and scalable methods, resources and high costs. Our team is developing a technology to measure several vector key traits simultaneously, using a high-throughput, accurate and cost-effective technology based on infrared spectroscopy.
Using the malaria vector *Anopheles gambiae s.l.* as a study system, we have been measuring >50,000 mosquito spectra using an ATR-FTIR mid-infrared spectrometer (MIRS) from different laboratories and field sites in Africa. The biochemical information obtained with MIRS was then used to train machine learning (ML) algorithms to predict key traits in individual mosquitoes.
MIRS-ML was able to correctly classify *Anopheles gambiae s.l.* cryptic species, age classes, *Plasmodium* infection, insecticide resistance status and blood meal host species with high accuracy under laboratory conditions. Field validation using wild mosquito collections confirmed that the technology can be used under natural conditions, although with the need of local calibration. We are currently developing a generalisable, holistic approach to simultaneously predict all key traits from different ecological settings. Additionally, preliminary results from other taxa (Culicine mosquitoes, tsetse flies, blackflies) suggest that MIRS-ML can be applicable to different vectors and become a stop shop technology for vector-borne disease surveillance to rapid assess disease transmission risk and prevent outbreaks.

Enhancing Diagnostic Capacity for Viral Hemorrhagic Fevers in Guinea: Lessons Learned and Future Directions

Giuditta Annibaldis[1,2,3], Fara Raymond Koundouno[4], Youssouf Sidibe[5], Jacob Camara[6], Kékoura Ifono[4], Barré Soropogui[6], Julia Hinzmann[1,2,3], Hugo Soubrier[3,7], Mette Hinrichs[1,2,3], Sarah Ryter[1,2,3], Saa Lucien Millimono[4], Karifa Kourouma[4], Tamba Elie Millimouno[4], Fernand M'Bemba Tolno[4], Faya Moriba Kamano [4], Eugène Kolie[6], Moussa Condé[6], Nourdine Ibrahim[6], Bakary Sylla[6], Fanta Berete[6], Mamadou Alpha Baldé[6], Mamadou Dioulde Barry[5], Bely Sonomy[5], Soua Marie Koulemou[5], Mariame Traore[5], Kaba Keita[5], Emily Victoria Nelson[1,2,3], Beate Becker-Ziaja[1,2], Carolina van Gelder[1,2], Anke Thielebein[1,2], Lisa Oestereich[1,2], Meike Pahlmann[1,2], Philippe Lemey[8], Liana Eleni Kafetzopoulou[8], Stephan Günther [1,2,3], N'Faly Magassouba[6], Sanaba Boumbaly[6] and Sophie Duraffour[1,2,3]

[1]Bernhard Nocht Institute of Tropical Medicine, Department of Virology, Hamburg, Germany

[2]German Center for Infection Research, Hamburg, Germany

[3]The European Mobile Laboratory Consortium, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

[4]Laboratoire de diagnostic des fièvres hemorragiques et virales de Gueckedou, Gueckedou, Guinea

[5]Laboratoire de diagnostic des fièvres hemorragiques et virales de N'Zérékoré, Hôpital Régional de N'Zérékoré, N'Zérékoré, Guinea

[6]Centre de Recherche en Virologie - Laboratoire des Fièvres Hémorragiques Virales de Guinée, Conakry, Guinea

[7]Epicentre, Médecins Sans Frontières, Paris, France

[8]Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Leuven, Belgium

giuditta.annibaldis@bnitm.de

The ongoing challenges posed by viral hemorrhagic fevers, especially in resource-limited settings, underscore the critical need for robust laboratory preparedness and diagnostic capacity. Following the devastating Ebola virus outbreak in West Africa (2014-2016), we initiated a long-term laboratory capacity-building project with Guinea to support and strengthen national capacity for the detection and effective response to viral hemorrhagic fever outbreaks.

Targeted training programs, infrastructure improvements and continuous technical support have allowed for the successful establishment of a network of three laboratories in country, importantly in the remote area of Forest Guinea. Following establishment of rapid and accurate viral hemorrhagic fevers detection in each laboratory, surveillance was expanded to include community engagement and genomic surveillance techniques for rapid epidemiological tracking to understand disease dynamics.

Despite the challenges faced during implementation, the strengthened surveillance system has led to the identification of a resurgence of Ebola virus in 2021, with prompt containment measures implemented. Moreover, the detection of Guinea's first ever documented human case of Marburg virus the same year highlighted the system's effectiveness. Additionally, several cases of Lassa fever were reported and managed efficiently in the past 3 years, including a nosocomial outbreak in 2022, demonstrating the system's adaptability in responding to multiple viral hemorrhagic fevers.

The enhancement of viral hemorrhagic fevers surveillance in Guinea has proven effective in detecting and managing emerging viral threats post-Ebola. By sharing our experiences, we aim to contribute to the broader discourse on pandemic preparedness, highlighting the importance of building resilient health systems capable of addressing current and emerging infectious disease threats. Continued investment in these areas will be crucial for mitigating future epidemiological risks associated with viral hemorrhagic fevers in the region.

ACE2-modified graphene field-effect transistors, a new class of sensors for pandemic preparedness

Alessandro Silvestri[1], Maurizio Prato[2], Ivan Coluzza[3] and Alejandro Criado[4]

[1]Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy

[2]Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo de Miramon 194, 20014 Donostia-San Sebastián, Spain

[3]BCMaterials, Basque Center for Materials, Applications and Nanostructures, Bld. Martina Casiano, UPV/EHU Science Park, Barrio Sarriena s/n, 48940 Leioa, Spain

[4]Universidade da Coruña, CICA – Centro Interdisciplinar de Química e Bioloxía, Rúa as Carballeiras, 15071 A Coruña

Alessandro.silvestri@unive.it

COVID-19, caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), originated a global health crisis, causing over 2 million deaths and affecting human daily life all over the world. This pandemic emergency highlighted how the current diagnostic tests have important limitations. Therefore, it is urgent to develop faster, more precise, and sensitive sensors. Graphene field effect transistors (GFET) are analytical platforms that enclose several of these requirements. However, the design of a sensitive and robust GFET is challenging. In this work, we report a GFET-based analytical platform for the detection of SARS-CoV2 spike protein using as a receptor the human membrane protein involved in the virus internalization: Angiotensin Converting enzyme 2 (ACE2). By finely controlling the graphene functionalization, and tuning the Debye length, we have been able to detect the spike protein with a limit of detection of 2.94 aM. Such limit of detection is comparable with the ones obtained with GFET functionalized with selective antibodies. Furthermore, we deeply characterized the ACE2-spike protein interaction with several techniques such as AFM, PSR, QCM, and nanomechanical measurements. This work demonstrated that the human membrane proteins responsible for the internalization of viruses could be used as receptors for electrochemical analytical platforms, allowing the detection of a wide variety of pathogens, and being a powerful tool in the fight against future pandemics.

Oropouche virus: An emerging orthobunyavirus

Krista Gunter[1], Dorcus Omoga[1], James M Bowen[1] and **Natasha L. Tilston-Lunel**[1]

[1]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana, USA

ntilston@iu.edu

On February 2nd, 2024, the Pan American Health Organization (PAHO/WHO) issued an epidemiological alert on the rising Oropouche virus (OROV) infections in South America. By August 3rd, 2024, this alert level had escalated from medium to high. OROV, a public health concern in Central and South America since its emergence in Brazil in the 1960s, now presents a significant challenge with sustained transmission in non-endemic regions of Brazil, local transmission in Cuba, two fatalities, and several suspected cases of vertical transmission. As of July 2024, 8,078 OROV cases have been confirmed. The 2024 OROV outbreak underscores critical gaps in our understanding of OROV biology and our lack of high-throughput diagnostics, therapeutics, and vaccines.

Reporter viruses are indispensable tools for rapidly studying virus dynamics and developing neutralization and antiviral screening assays. However, OROV, a tri-segmented bunyavirus, poses challenges in generating such reporters due to the typical impact on viral fitness when foreign elements are introduced into the genome. We previously demonstrated that the non-structural gene NSm on OROV medium (M) segment is non-essential for replication *in vitro*. Leveraging this finding, we have now generated a recombinant OROV expressing the

fluorescent protein ZsGreen in place of NSm. This reporter OROV is both stable and pathogenic in IFNAR-/- mice, offering a powerful tool for OROV pathogenesis studies and assay development.

Host-directed therapy targeting proteostasis for Tick-borne Encephalitis Virus (TBEV) drug discovery

Federica Dattola[1], Rafaela Bonotto[2], Tea Carletti[1], Emanuele Orsini[1], Serena Maiocchi[3], Luca Braga[3] and Alessandro Marcello[1]

[1]International Center for Genetic Engineering and Biotechnology, Molecular Virology Laboratory, Località Padriciano 99, Trieste, Italy

[2]Virus Bank Platform, KU Leuven, Leuven, Belgium

[3]International Center for Genetic Engineering and Biotechnology, Functional Cell Biology Laborartory, Località Padriciano 99, Trieste, Italy

dattola@icgeb.org

Currently, there are no specific treatments for Flavivirus infection and very few vaccines are available for most of them. Host-directed Therapy (HDT) has been suggested as a new tool to identify antivirals, since it targets essential host cell factors, and it is indeed less prone to develop drug resistance phenotypes. We previously demonstrated that the Unfolded Protein Response (UPR) activation upon Tick-borne Encephalitis virus (TBEV) infection induces an early innate immune response leading to a strong cell-intrinsic antiviral defense. Such response is achieved by an increase of Interferon Regulatory Factor 3 (IRF3) nuclear translocation, activation of interferon-stimulated genes (ISG) followed by virus inhibition.
To take advantage of this pathway for HDT we designed a screen for drug discovery based on these findings. Lentiviral particles were used to create a stable cell line expressing a GFP-fluorescent form of IRF3 (U2OS eGFP-IRF3) for microscopy-based High-Content Screening (HCS). An antiviral assay based on a dual readout for both IRF3 nuclear translocation and TBEV inhibition was then developed for antiviral drug discovery.
We performed an HCS with FDA-approved drugs and a set of ER stress-related compounds. Results convergedon the identification of five drugs that showed the double phenotype, all of which belonged to a defined class of Integrated Stress Response inhibitors, highlighting eIF2α phosphorylation as a key player in the regulation of innate immunity upon TBEV infection. Dose-response experiments confirmed good anti-TBEV activity also at low nanomolar ranges without cytotoxicity. Moreover, kinetic studies revealed an early activation of the innate immune response and a potent antiviral activity against other flaviviruses. These findings clarify the intricate mechanism which links the cellular stress response to the innate immunity activation, suggesting a new druggable pathway that can be exploited for therapeutic purposes.

An orally efficacious coronavirus assembly inhibitor that targets the viral M protein

Manon Laporte[1], Dirk Jochmans[1], Dorothée Bardiot[2], Lowiese Desmarets[3], Oliver J. Debski-Antoniak[4], Giulia Mizzon[5,6], Rana Abdelnabi[1,7], Pieter Leyssen[1], Winston Chiu[1], Zhikuan Zhang[9], Norimichi Nomura[14], Sandro Bolland[2], Umeharu Ohto[9], Yannick Stahl[5], Jurgen Wuyts[2], Steven De Jonghe[1], Annelies Stevaert[1], Martijn van Hemert[10], Brenda W. Bontes[10], Patrick Wanningen[10], G. J. Mirjam Groenewold[10], Aneta Zegar[13], Katarzyna Owczarek[13], Sanjata Joshi[12], Mohamed Koukni[2], Philippe Arzel[2], Hugo Klaassen[2], Niels Cremers[1], Kim Donckers[1], Thibault Francken[1], Tina Van Buyten[1], Jasper Rymenants[1], Joost Schepers[1], Krzysztof Pyrc[13], Rolf Hilgenfeld[12], Jean Dubuisson[3], Berend-Jan Bosch[4], Frank Van Kuppeveld[4], Cecilia Eydoux[11], Etienne Decroly[11], Bruno Canard[11], Lieve Naesens[1], Eric Snijder[10], Sandrine Belouzard[3], Toshiyuki Shimizu[9], Ralf Bartenschlager[5,6,15], Daniel L. Hurdiss[4], Arnaud Marchand[2], Patrick Chaltin[2,8] and Johan Neyts[1]

[1]KU Leuven - Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium.

[2]CISTIM Leuven vzw, Leuven, Belgium

[3]Université de Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL- Center for Infection and Immunity of Lille, F-59000 Lille, France.

[4]University of Utrecht, Department Biomolecular Health Sciences, Utrecht, The Netherlands

[5]Heidelberg University, Medical Faculty Heidelberg, Department of Infectious Diseases, Molecular Virology, Heidelberg, Germany

[6]German Center for Infection Research (DZIF), Heidelberg partner site, Heidelberg, Germany

[7]KU Leuven - Department of Microbiology, Immunology and Transplantation, VirusBank Platform, Leuven, Belgium.

[8]KU Leuven, Centre for Drug Design and Discovery (CD3), Leuven, Belgium

[9]University of Tokyo, Graduate School of Pharmaceutical Sciences, Tokyo, Japan

[10]Molecular Virology Laboratory, Leiden University Center of Infectious Diseases, Leiden University Medical Center, The Netherlands

[11]Architecture et Fonctions des Macromolécules Biologiques, Centre National de la Recherche Scientifique and Aix-Marseille University, France

[12]Institute of Molecular Medicine and German Center for Infection Research (DZIF), University of Lübeck, Lübeck, Germany

[13]Virogenetics Laboratory of Virology, Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

[14]Kyoto University, Department of Cell Biology, Graduate School of Medicine, Kyoto, Japan

[15]German Cancer Research Center (DKFZ), Heidelberg, Germany

manon.laporte@kuleuven.be

We present the discovery of CIM-834, a potent small molecule SARS-CoV-2 inhibitor that targets the Membrane (M) protein. The coronavirus M protein is the key organizer of coronavirus assembly.
CIM-834 was obtained through high-throughput phenotypic antiviral screening followed by medicinal chemistry efforts and target elucidation. Initial µM active compounds were optimized towards compounds with single-digit nanomolar antiviral activity against SARS-CoV-2. The compound inhibits the replication of all SARS-CoV-2 variants of concern, including the current Omicron strains, and SARS-CoV. ADME properties of the compound series were optimized to improve metabolic stability, allowing to achieve good PK after oral administration. In SCID mice, intranasally infected with SARS-CoV-2, oral treatment with CIM-834 reduces lung viral titers to nearly undetectable levels, even when treatment is only started 2 days post-infection.
Serial passaging of SARS-CoV-2 in vitro in the presence of increasing concentrations of CIM-834 led to the selection of a P132S amino acid change in the viral M protein. Introduction of M:P132S into the wild-type virus confirmed the role of M:P132S in the phenotypic antiviral resistance. Further mechanistic assays showed that CIM-834 prevents oligomerization of the M protein and as a consequence the formation of infectious virus particles. Consistently, transmission electron microscopy studies demonstrate that the formation of replication organelles in the cytoplasm is unaffected by CIM-834, whereas virion assembly is completely absent. Single-particle cryo-electron microscopy finally revealed that CIM-834 binds and stabilizes the M protein in its short form thereby blocking its conformational switch to the long form which is required for successful particle assembly.
In conclusion, we discovered a novel druggable target in the replication cycle of coronaviruses and a potent small molecule inhibitor thereof which is being further developed towards a candidate drug.

Repurposing Heparin Derivatives: A Promising Strategy for Antiviral Treatment of Zika Virus Infect

Isabel Pagani[1], Paola Panina-Bordignon[2, 3], Gianvito Martino[2, 3], Sarah Taylor[4], Jeremy E Turnbull[4, 5], Edwin Yates[4, 5], Guido Poli[3, 6] and Elisa Vicenzi[1]

[1]Viral Pathogenesis and Biosafety Unit, Division of Immunology, Transplantation, and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

[2]Neuroimmunology Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

[3]Vita-Salute San Raffaele University, Via Olgettina 58, 20132 Milan, Italy

[4]Department of Biochemistry and Systems Biology, ISMIB, University of Liverpool, Liverpool L69 7ZB, UK

[5]Department of Life Sciences, Keele University, Keele, Staffordshire ST5 5BG, UK

[6]Human Immuno-Virology, H.I.V. Unit, Division of Immunology, Transplantation, and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

pagani.isabel@hsr.it

The severe impact of fetal Zika virus (ZIKV) infection highlights the urgent need for effective antiviral agents to treat infected pregnant women. Drug repurposing, a promising strategy for addressing emerging infectious diseases, has led to the investigation of heparin, an anticoagulant used during pregnancy to prevent thromboembolic complications, and its derivatives for their antiviral potential. Heparin has demonstrated activity against the attachment and entry of several enveloped viruses, including ZIKV. However, its direct use is limited by off-target anticoagulant effects. To address these challenges, we explored heparin derivatives (Der-Hs) with varying levels of anticoagulant activity and degrees of sulfation, as well as discarded fractions (F-Hs) generated during conventional pharmaceutical heparin production. Der-Hs and F-Hs were tested on neurospheres derived from human neural progenitor cells (NPC-NS) before ZIKV infection. Additionally, selected Der-Hs and F-Hs were combined with Sofosbuvir, an approved anti-HCV drug that has demonstrated both in vitro and in vivo efficacy against ZIKV. Pre-incubation of NPC-NS with Der-Hs and F-Hs one hour prior to ZIKV infection resulted in a potent inhibitory effect against ZIKV-induced cytopathic effects. Notably, only specific derivatives were able to inhibit ZIKV replication, irrespective of sulfation position or overall charge.

Picolinic acid a broad-spectrum inhibitor of enveloped virus entry

Rohan Narayan[1,2], Mansi Sharma[1,2], Rajesh Yadav[1,3], Abhijith Biji[1,2], Oyahida Khatun[1,2], Sumandeep Kaur[1,2], Aditi Kanojia[1,2], Christy M Joy[1,2], Raju Rajmani[4], Pallavi R Sharma[3], Sharumathi Jeyasankar[3], Priya Rani[2], Radha K. Shandil[6], Shridhar Narayanan[6], Chilakalapudi D. Rao[5], Vijaya Satchidanandam[2], Saumitra Das[2], Rachit Agarwal[3] and Shashank Tripathi[1,2]

[1]Emerging Viral Pathogens Laboratory, Infosys Wing, Centre for Infectious Disease Research, Indian Institute of Science; Bengaluru, 560012, India.

[2]Department of Microbiology and Cell Biology, Division of Biological Sciences, Indian Institute of Science; Bengaluru, 560012, India.

[3]Centre for BioSystems Science and Engineering, Indian Institute of Science; Bengaluru, 560012, India.

[4]Molecular Biophysics Unit, Indian Institute of Science; Bengaluru, 560012, India.

[5]Department of Biological Sciences, School of Engineering and Sciences, SRM University, Andhra Pradesh, 522240, India.

[6]Foundation for Neglected Disease Research, KIADB Industrial Area, Doddaballapur, Bengaluru - 561203

rohannarayan@iisc.ac.in

The COVID-19 pandemic highlights an urgent need for effective antivirals. Targeting host processes co-opted by viruses is an attractive antiviral strategy with a high resistance barrier. Picolinic acid (PA) is a Tryptophan metabolite endogenously produced in mammals. Here we report the broad-spectrum antiviral activity of PA against enveloped viruses, including Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), Influenza A virus (IAV), Flaviviruses, Herpes simplex virus, and Parainfluenza virus. Mechanistic studies reveal that PA inhibits enveloped virus entry by compromising viral membrane integrity, inhibiting virus-cellular membrane fusion, and interfering with cellular endocytosis. More importantly, in preclinical animal models, PA

exhibits promising antiviral efficacy against SARS-CoV-2 and IAV. Overall, our data establish PA as a broad-spectrum antiviral with promising preclinical efficacy against pandemic viruses SARS-CoV-2 and IAV.

Cross-neutralization of Morbillivirus by single-domain antibodies

Akanksha Shanbhag[1,2], Alexandre Lalande[3], Morgane Di Palma Subran[1], Marianne Wyss[1], Cyrille Mathieu[3] and Philippe Plattet[1]

[1]Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Bern, Switzerland

[2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

[3]Centre International de Recherche en Infectiologie (INSERM), Université Lyon, Lyon, France

akanksha.shanbhag@unibe.ch

Morbilliviruses include pathogens such as measles (MeV) and canine distemper virus (CDV), which are highly transmissible. They pose substantial health risks leading to respiratory infections and deadly encephalitis. Despite the availability of effective vaccines, MeV kills more than 140,000 people each year while CDV presents a serious risk of spillover to wildlife. This highlights gaps in vaccine coverage and the importance of novel alternative therapies such as post-exposure antivirals, potentially with broad-spectrum molecules. Here we report the discovery of cross-reactive single-domain antibodies (known as nanobody; Nb) against the viral fusion glycoprotein of CDV and MeV. The Nb library generated upon alpaca immunization with the recombinant F proteins was screened. Binders were primarily identified via phage display and further characterized by flow cytometry and ELISA. They were tested *in-vitro* for inhibition against multiple strains of MeV and CDV as well as *ex-vivo* in an organotypic brain cell culture model for MeV.
Two identified Nbs exhibited potent neutralizing ability (reaching IC50s of single-digit nanomolar range) towards multiple CDV and MeV strains, including hyperfusogenic MeV variants known to contribute to the induction of severe brain disorders. Remarkably, they also successfully inhibit cell-to-cell spread of MeV in the *ex-vivo* model. To improve inhibition and binding potency, both Nbs were fused to form tandem constructs via a flexible glycine-serine linker. The tandems showed an increase of about 10-100 times in viral neutralization assays (against both CDV and MeV strains), along with more potently inhibiting lateral spread of hyperfusogenic MeV variants in the *ex-vivo* model.
We identified and produced two Nbs that inhibit CDV and MeV. When combined in single molecules, they exhibited increased binding capacity and enhanced neutralization potency. Testing of these constructs against other morbilliviruses such as PPRV is currently underway.

The Democratization of Antiviral Drug Discovery for Pandemic Preparedness

M. Luis Rodriguez[1,2], Briana L. McGovern[1,2], Randy Diaz-Tapia[1,2], Jared Benjamin[1,2], Eric Marsan[3], Kunal Kumar[3], Robert J. DeVita[3], Adolfo García-Sastre[1,2] and **Kris M. White**[1,2]

[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Global Health Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3]Department of Pharmacological Sciences, Drug Discovery Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

kris.white@mssm.edu

The COVID-19 pandemic was likely the result of a zoonotic transmission event, and it has become clear that many viral families have animal reservoirs with pandemic potential. Antiviral therapeutics that target host proteins have the advantages of being refractory to resistance and having true broad-spectrum activity, both

excellent properties for pandemic preparedness. The translation elongation factor, eEF1A, has been identified as an important host factor in a diverse set of important human pathogens, including influenza viruses, coronaviruses, flaviviruses, paramyxoviruses, and retroviruses. We have identified plitidepsin, a clinically approved eEF1A inhibitor, as having picomolar antiviral activity and potent efficacy in mouse models of SARS-CoV-2 and influenza virus infections. This led to a successful phase I/II clinical trial for plitidepsin treatment of COVID-19 patients, establishing a clinical safety profile in this population. We believe the clinical safety data for hundreds of patients and broad-spectrum antiviral activity of plitidepsin validate eEF1A as a desirable host target for therapeutic development. Novel chemical matter targeting eEF1A will be critical to overcome the current liabilities of plitidepsin, a complex chemical structure and a lack of oral bioavailability required for an effective antiviral therapeutic. Here we performed a 1.2 billion compound *in silico* screen of the Enamine REAL library to identify novel chemotypes that could potentially interact the identified plitidepsin-binding pocket. The top 256 compounds from our preliminary *in silico* screen were ranked by binding energies (Ranging from -10.7 to -10.2 kcal/mol), Pan Assay INterference CompoundS (PAINS) were removed, and the docking poses visually inspected for artifacts. 195 compounds with unique scaffolds were selected for chemical synthesis followed by *in vitro* screening and characterization.

Enhancing predictions of protein stability changes induced by single mutations using MSA-based language models

Francesca Cuturello[1], Marco Celoria[1], Alessio Ansuini[1] and Alberto Cazzaniga[1]

[1]Area Science Park, Trieste, Italy

francesca.cuturello@areasciencepark.it

Protein language models offer a new perspective for addressing challenges in structural biology, while relying solely on sequence information. Recent studies have investigated their effectiveness in forecasting shifts in thermodynamic stability caused by single amino acid mutations, a task known for its complexity due to the sparse availability of data, constrained by experimental limitations. To tackle this problem, we introduce two key novelties: leveraging a protein language model that incorporates Multiple Sequence Alignments to capture evolutionary information, and using a recently released mega-scale dataset with rigorous data preprocessing to mitigate overfitting.

We ensure comprehensive comparisons by fine-tuning various pretrained models, taking advantage of analyses such as ablation studies and baselines evaluation. Our methodology introduces a stringent policy to reduce the widespread issue of data leakage, rigorously removing sequences from the training set when they exhibit significant similarity with the test set. The MSA Transformer emerges as the most accurate among the models under investigation, given its capability to leverage co-evolution signals encoded in aligned homologous sequences. Moreover, the optimized MSA Transformer outperforms existing methods and exhibits enhanced generalization power, leading to a notable improvement in predicting changes in protein stability resulting from point mutations.

Adapting broad protein language models to viruses with pandemic potential

Spyros Lytras[1], Adam Strange[1], Jumpei Ito[1] and Kei Sato[1]

[1]Division of Systems Virology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, 108-8639

spyros@ims.u-tokyo.ac.jp

The fields of Influenza A virus (IAV) and now SARS-CoV-2 have highlighted the unprecedented importance of genetic surveillance for pandemic preparedness. With the exponential increase in virus sequences it is imperative that we develop modern methods to interpret how these pathogens are evolving. The field of artificial intelligence has recently experienced a boom in popularity and commercial applications, particularly in the form of large language models (LLMs). LLMs are state-of-the-art algorithms trained on a large text corpus (set of words in the context of sentences) that aim to identify fundamental properties of grammar and meaning. Similarly, LLMs can be trained in any corpus of contextualised characters, such as amino acids in proteins; these are referred to as protein language models (pLM). We have "fine-tuned" an existing pLM, originally trained across all known proteins, to switch its focus on the influenza hemagglutinin (HA) protein using a comprehensive set of more than 10,000 unique IAV HA sequences. Given a new HA sequence, our model can infer the probability for how likely each amino acid is in each protein site. We summarise these as the entropy of probabilities per site and show that the model-inferred entropy correlates well with the per-site entropy of the HA sequence alignment. Unlike alignment-based entropy, however, this pLM method infers different per-site entropies for each unique protein sequence, corresponding to which sites are more mutable in that specific protein context. We map this novel metric on the phylogenies of four IAV serotypes with pandemic potential (H1, H3, H5 and H7) and show that it reveals unique trends in protein evolvability upon

major events, like host-switching. This approach can be implemented on any virus protein and paves the way for efficient, alignment-free forecasting of viral pathogen evolution.

Discovery and characterization of Novel Pan-Coronavirus Inhibitors Using Artificial Intelligence

Abdullah Algaissi[1,2], Aisha Ghazwani[3], Ahmed Shareef[3] and Anwar M Hashem[3,4]

[1]Department of Medical Laboratories Technology, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia

[2]Health Research Center, Jazan University, Jazan, Saudi Arabia

[3]Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21859, Saudi Arabia

[4]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah 21859, Saudi Arabia

aalgaissi@jazanu.edu.sa

The COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) led to devastating impact on global health and economy. SARS-CoV-2 belongs to the betacoronavirus (Beta-CoV) genus of the CoV family along with two other highly pathogenic viruses; SARS-CoV-1 and MERS-CoV, which highlights the importance of these viruses as a cause of pandemics. Yet, there is no approved effective antiviral drugs against these viruses. Therefore, there is an urgent need to control current CoVs as well as any potential future pandemics caused by such viruses. An antiviral drug with broad spectrum activity against multiple CoVs would be very valuable for future pandemic preparedness.

We previously have shown that the fusion step of the virus replication to be conserved among multiple Beta-CoVs. Based on the crystal structure of the heapted region 1 (HR1) within the S2 subunit of the viral spike protein, we identified critical residues for viral fusion. Then, we screened thousands of small molecule compounds using artificial intelligence (AI)-based design targeting the conserved regions of HR1 in the spike protein of all known human CoVs. The inhibitory effects of the lead compounds were tested using a pseudovirus- and live virus-based assays.

Using a well-developed drug screening assays, we found seven compounds that were able to block SARS-CoV-2, SARS-CoV-1 and MERS-CoV entry into target cells at very low micromolar concentrations. One of which, C45, showed high potency against SARS-CoV-2 (IC_{50}: 0.57 µm), SARS-CoV-1 (IC_{50}: 2.20 µm), and MERS-CoV (IC_{50}: 2.43 µm). The potent inhbitory effects and the safety profiles of the lead compunds were confirmed with mutiple additional assays.Thus, we present here promising novel pan-CoVs drugs that can inhibit not only current known CoVs but also future emerging CoVs that can cause pandemic.

New IR approaches to study sars-coV2 mPro conformations

Federica Piccirilli[1], Hendrik Vondracek[2], Lucia Silvestrini[3], Pietro Parisse[4], Francesco Spinozzi[3], Lisa Vaccari[2], Andrea Toma[5], Vincenzo Aglieri[5], Loredana Casalis[2], Antonio Palumbo Piccionello[6], Paolo Mariani[3], Giovanni Birarda[2] and Maria Grazia Ortore[3]

[1]AREA Science Park, Padriciano 99, Trieste, I- 34149, Italy

[2]Elettra Sincrotrone Trieste S.C.p.A., Strada Statale 14, Basovizza, Trieste, I-34149, Italy

[3]Department of Life and Environmental Sciences, Marche Polytechnic University, via brecce bianche, Ancona, I-60131, Italy

[4]CNR - Istituto Officina dei Materiali, s.s. 14 km 163.5 in Area Science Park, 34149 Basovizza, Trieste, Italy

[5]Istituto Italiano di Tecnologia, via Morego 30, Genova, I- 16163, Italy

[6]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, viale delle scienze, Palermo, I-90133, Italy

federica.piccirilli@areasciencepark.it

The emergence of SARS-CoV-2 has created an urgent public health crisis, with limited treatment options available due to the absence of targeted therapies. This study employs PIR-SEIRA (plasmonic internal reflection surface enhanced infrared absorption spectroscopy), a novel infrared technique using plasmonic antennas enhancing signals in mid infrared, to investigate the dimerization of the SARS-CoV-2 main protease (mPro) and its inhibition triggered by a specific ligand binding. Our spectral analysis was based on the observation of Amide I and Amide II bands (1710-1510 cm-1) of a mPro monolayer anchored to a IR antenna confining light to the monolayer large-scale and allowing for the study of the protein structure in liquid buffered environment. Being the monomeric forn of mPro more active in virus replication than dimeric, the focus was on the existing relationship between enzymatic and dimerization inhibition, at the basis of structure-function paradigm. We studied a selected inhibitor of the mPro enzymatic activity, the NV-1399, previously identified through in silico screening, that also demonstrated significant dimerization inhibition. Our approach enabled the detailed observation of structural changes at the secondary structure level in mPro upon NV-1399 binding and confirmed the ligand's preferential binding to mPro monomers over homodimers. Our findings suggest that combining IR spectroscopy with plasmonic technology provides a powerful tool to complement biological assays, to deepen the understanding of Mpro inhibition mechanisms and to aid in the development of future COVID-19 treatments.

Label-free biochemical characterization and antiviral susceptibility of enveloped mammalian RNA virus in physiological-like conditions. A Road to Fast Response in Pandemic Preparedness and Countermeasure program

Denis Rajnovic[1,2,3], Fatima Matroodi[1], Lamyaa Almehmadi[4], Igor Lednev[4], Claudio Masciovecchio[1], Alessandro Marcello[2] and Barbara Rossi[1]

[1]Elettra - Sincrotrone Trieste S.C.p.A. S.S. 14 km 163,5 in AREA Science Park IT-34149 Basovizza, Trieste, Italy

[2]International Centre for Genetic Engineering and Biotechnology (ICGEB) Padriciano, 99 34149 Trieste, Italy

[3]Area Science Park - Padriciano, 99 34149 Trieste – Italy

[4]Department of Chemistry, University at Albany, SUNY, 1400 Washington Avenue, Albany, New York 12222, United States

denis.rajnovic@areasciencepark.it

Since 2019 SARS-CoV-2 pandemic there is a compulsory need in innovations that can in rapid time elucidate virus biochemical architecture and test for antiviral susceptibility. Here, we have designed a virus research workflow based on DUVRR spectroscopy which shows how to perform bottom-up and top-down virus biochemical characterization assignments and test the antiviral susceptibility in remarkably short time, in a label-free and non-destructive approach, and in physiological like conditions. Our study reports the spectra position and degree of contribution of key biochemical components (lipids, amino acids and nucleotides), how to use database sequences to design Virus spectrum, determines biochemical composition in Virus spectrum by using Multilinear Regression (MLR) analysis, and study the effect of antiviral and its mechanism of inactivation, with molecular sensitivity. These findings may facilitate the progress in overall pandemic preparedness and countermeasure program in which this workflow can be transferred to other types of enveloped and non-enveloped viruses, to provide a fast response upon emergence of deadly pathogens.

Real-time surveillance and pandemic prediction using GPS-nanobiosensor technologies in an interdisciplinary framework - Prediction2Response

Annelin Seppola[1], Michael Patten[2], Daniel A. Heller[3], Martin Steinert[4], Christian Sonne[5], Ma Nyuk Ling[6], Su Shiung Lam[7], Duong Vesna[8], Malen Chan[8] and **Courtney Waugh**[9]

[1]Faculty of Social Sciences, Nord University, Universitetsalléen 11, 8026 Bodø, Norway

[2]Faculty of Biosciences and Aquaculture, Nord University, Kongens Gate 42, 7713 Steinkjer, Norway

[3]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA; Department of Pharmacology, Weill Cornell Graduate School of Medical Sciences, New York, NY, USA

[4]Department of Mechanical and Industrial Engineering, Norwegian University of Science and Technology, Høgskoleringen 1, 7034 Trondheim, Norway

[5]Department of Ecoscience, Aarhus University, Arctic Research Centre; Frederiksborgvej 399, DK-4000 Roskilde, Denmark

[6]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[7]Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[8]Institute Pasteur du Cambodge, No. 5 Monivong Boulevard P.O Box. 983, Phnom Penh, Cambodia

[9]Faculty of Biosciences and Aquaculture, Nord University, Kongens Gate 42, 7713 Steinkjer, Norway

Courtney.waugh@nord.no

We propose an interdisciplinary approach that advances beyond current methods towards better predicting virus spill-over events from wildlife and life-stock that could lead to pandemics, while generating a timely and effective response to prevent or contain new outbreaks. Given the current lack of real-time access to viral RNA, DNA or miRNA data, we need to go beyond the state of the art to develop new methods and technologies. Here we present how intellectual exchange between biologging and biomedical sensor fields can address this challenge. Single-walled carbon nanotube-based implantable sensors have recently demonstrated their ability to detect viral RNA, proteins, and miRNAs in real time, in biofluids and in vivo. For application in animals, these sensors would need to be custom-designed to integrate with GPS tracking sensors, enabling the transmission of real-time data to laboratories for analysis. Further, for Prediction2Response, we need to elicit an adequate response from policy makers and the public to prevent and/or adequately respond to pandemics. It is clear from the current state-of-the–art and the lack of prevention and inadequate response to COVID-9, that an interdisciplinary approach is needed to facilitate communication from the Natural Sciences to the Social Scientists in a way that can inform and cause action.

Revision of a repurposing screen led to a new invalidation pipeline and identified a true novel inhibitor against papain-like protease from SARS-CoV-2

Stefano Morasso[1], Maria Kuzikov[2], Jeanette Reinshagen[2], Markus Wolf[2], Vittoria Monaco[3,4], Flora Cozzolino[3,4], Simona Golič Grdadolnik[5], Primož Šket[6], Janez Plavec[6], Daniela Iaconis[7], Vincenzo Summa[8], Francesca Esposito[9], Enzo Tramontano[9], Maria Monti[3,4], Andrea R. Beccari[7], Björn Windshügel[2,10], Philip Gribbon[2], Paola Storici[1] and Andrea Zaliani[2]

[1]Protein Targets for Drug Discovery Lab, Elettra-Sincrotrone Trieste S.C.p.A., SS 14 - km 163,5 in AREA Science Park 34149 Basovizza, Trieste, Italy

[2]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Discovery Research ScreeningPort, Schnackenburgallee 114, 22525 Hamburg, Germany

[3]Department of Chemical Sciences, University of Naples "Federico II" Comunale Cinthia, 26, 80126 Naples, Italy

[4]CEINGE Advanced-Biotechnologies "Franco Salvatore", Via Gaetano Salvatore, 486, 80145 Naples, Italy

[5]Laboratory for Molecular Structural Dynamics, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

[6]Slovenian NMR Center, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

[7]EXSCALATE - Dompé Farmaceutici SpA, via Tommaso De Amicis 95, 80131, Napoli, Italy

[8]Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, 80131 Naples, Italy

[9]Dipartimento di Scienze della vita e dell'ambiente, Cittadella Universitaria di Monserrato, SS-554, Monserrato, 09042 Cagliari, Italy

[10]Constructor University, School of Science, Campus Ring 1, 28759 Bremen, Germany

stefano.morasso@elettra.eu

The SARS-CoV-2 papain-like protease (PLpro) is a crucial target for antiviral drug development due to its role in viral replication and immune evasion. Despite extensive research, the development of PLpro-specific inhibitors has been hindered by the redox-sensitivity of the enzyme and the unspecific nature of previously reported compounds. Indeed, GRL-0617 is currently the only proven chemical framework for developing powerful inhibitors.

This study aimed to identify novel PLpro inhibitors through a rigorous screening and validation process. We employed a comprehensive approach, including enzymatic assays, direct-binding studies, and cellular assays, to ensure the specificity and efficacy of identified compounds. We emphasize the critical importance of rigorous validation in primary screening, as we identified redox-sensitive compounds that proved to be false positives. Our findings revealed a novel non-covalent inhibitor, CPI-169, that demonstrated micromolar range inhibition of PLpro's proteolytic activity. Ligand-observed NMR experiments confirmed CPI-169's competitive binding with GRL-0617, suggesting a similar binding site. CPI-169 effectively inhibited viral replication in Vero-E6 cells, highlighting its potential as a promising scaffold, offering a new avenue for the development of PLpro-targeting antiviral drugs.

Engineering, structure, and immunogenicity of a Crimean–Congo hemorrhagic fever virus pre-fusion heterotrimeric glycoprotein complex

Elizabeth McFadden[1], Stephanie R. Monticelli[2,3], Albert Wang[4], Ajit R. Ramamohan[1], Thomas G. Batchelor[2,5], Ana I. Kuehne[2], Russell R. Bakken[2], Alexandra L. Tse[4], Kartik Chandran[4], Andrew S. Herbert[2] and Jason S. McLellan[1]

[1] Department of Molecular Biosciences, The University of Texas at Austin, Austin, Texas, USA

[2] United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA

[3] The Geneva Foundation, Tacoma, WA, USA

[4] Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA

[5] Oak Ridge Institute of Science Education, Oak Ridge, TN, USA

elizabethmcfadden@utexas.edu

Crimean–Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that can cause severe disease in humans with case fatality rates of 10–40%. The virus is primarily spread by ticks of the *Hyalomma* genus, which are distributed widely throughout parts of Europe, Africa, and Asia. CCHFV is a World Health Organization priority pathogen, but no vaccine or therapeutic has yet been approved for use. The glycoproteins are major targets for vaccine and antibody therapeutic development, yet little is known about their structural organization and function in the viral life cycle. Although structures of CCHFV glycoproteins GP38 and Gc have provided insights into viral entry and defined epitopes of neutralizing and protective antibodies, the structure of glycoprotein Gn and its interactions with GP38 and Gc have remained elusive. Here, we used structure-guided protein engineering to produce a stabilized GP38-Gn-Gc heterotrimeric glycoprotein complex (GP38-GnH-DS-Gc). A cryo-EM structure of this complex provides the molecular basis for GP38's association on the viral surface, reveals the structure of Gn, and demonstrates that GP38-Gn restrains the Gc fusion loops in the prefusion conformation, facilitated by an N-linked glycan attached to Gn. Immunization with GP38-GnH-DS-Gc conferred 40% protection against lethal IbAr10200 challenge in mice. These data define the architecture of a GP38-Gn-Gc protomer and provide a template for structure-guided vaccine antigen development.

Preclinical efficacy of an engineered VLP-based vaccine candidate against Zika virus

Giuditta De Lorenzo[1,2], Rapeepat Tandavanitj[1,3], Maria Vittoria Mancini[1,4], Lorena Preciado-Llanes[5], Ricardo Sanchez-Velazquez[1], Thomas H. Ant[1], Shivan M. Murdochy[1], Daniel D. Gingell[1,4], Chayanee Setthapramote[1,6], Piyatida Natsrita[1,7], Raissa Prado Rocha[5], Young Chan Kim[5,8], Alain Kohl[1,9], Steven P. Sinkins[1], Arturo Reyes-Sandoval[5,10] and Arvind H. Patel[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, Scotland, UK

[2]Istituto di Ricerca e Innovazione Tecnologica, Area Science Park, Trieste, Italy

[3]Biologicals research Group, Research and Development Institute, Government Pharmaceutical Organization, Bangkok, Thailand

[4]Dipartimento di Biologia e Biotecnologia, Università di Pavia, Pavia, Italy

[5]The Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK

[6]Department of Clinical Pathology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand

[7]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[8]Division of Structural Biology, Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK

[9]Departments of Tropical Disease Biology and Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

[10]Instituto Politécnico Nacional, IPN. Av. Luis Enrique Erro s/n. Unidad Adolfo López Mateos Mexico City, Mexico

Giuditta.DeLorenzo@areasciencepark.it

Zika virus (ZIKV) remained poorly studied until an outbreak of infections in 2015 established its association with severe neurological disorders and congenital malformations. Currently, there are no antiviral drugs or vaccines available. The envelope (E) protein is the major target of neutralizing antibodies in infected hosts and thus represents a candidate of interest for vaccine design. However, it is also the target of many cross-reactive poorly neutralizing antibodies that can cause antibody-dependent enhancement (ADE) of infection of close pathogens, such as dengue virus, thus requiring an effort in immunogen engineering.
In this work we developed a virus-like particle (VLP)-based vaccines displaying E locked in a covalently linked dimeric (cvD) conformation to enhance the exposure of E dimers to the immune system and elicit virus-specific strongly neutralizing antibodies upon vaccination.
Since the primary route of Zika virus (ZIKV) transmission is through the bite of an infected Aedes mosquito, we performed a preclinical validation of the vaccine candidate in a mosquito-mouse-mosquito transmission model using both Asian and African ZIKV lineages showing that the vaccine protected the animals from developing the disease, strongly reduced viremia and was able to inhibit virus transmission from the host to the vector.
Finally, when combined with a simian adenovirus vector vaccine (ChAdOx1 prMEΔTM) in a heterologous prime and boost vaccination strategy. The combined vaccine technologies elicited a strong cellular response and high levels of neutralising antibodies, attributed to ChAdOx1 prMEΔTM and VLP-cvD respectively, providing useful insights with important implications for the development of effective vaccination strategies against ZIKV and other emerging viruses.

Comparison of the humoral immune responses induced by vaccination or natural infection by SARS-CoV-2

Marne Azarias Da Silva[1], Anne Bull-Maurer[2], Pierre Nioche[1], Calaiselvy Soudaramourty[1], Sonia André[1], Ricardo Silvestre[3], Pierre Corbeau[4], Jerome Estaquier[1] and **Fabrizio Mammano**[2]

[1]Inserm UMR-1224, Université Paris-Cité, Paris, France

[2]Inserm UMR-1259, Université de Tours, Tours, France

[3]CNRS UMR9002, Université de Montpellier, Montpellier, France

fabrizio.mammano@inserm.fr

The continuous emergence of SARS-CoV-2 viral variants represents a major challenge for the control of the epidemic, in particular in the context of vaccination. Antibodies play a central role in the containment of the infection. In addition to their neutralizing capacity, antibodies also drive cell-mediated immune responses that participate in virus clearance.
In this study we compared the humoral responses of patients who were either vaccinated (2 and 3 doses of mRNA vaccine) or naturally infected (convalescent), or infected and vaccinated (hybrid immunity). We measured the quantity and persistence of virus specific IgA and IgG, their avidity for the S and N antigens, as well as their capacity to bind the viral proteins expressed on the cell surface and to neutralize the infection by different viral strains.
Pre-infected vaccinated individuals displayed the strongest and most durable humoral responses, coupled with high avidity antibodies production. Three doses of mRNA vaccine were necessary to match the quantity and avidity of antibody production, but the durability of the response remained lower. Given the importance of antibody-dependent cell-mediated immunity against viral infections, we next measured the capacity of IgG to recognize spike variants expressed on the cell surface. We found that cross-reactivity was also strongly

improved by repeated vaccination, without however reaching the breadth conferred by natural infection. Finally, we found low levels of CXCL13, a surrogate marker of germinal center activation and formation, in vaccinees both after two and three doses as compared to infected individuals, providing a potential explanation for the short duration and low avidity of the immunoglobulins induced by vaccination.
Altogether, our results demonstrated that repetitive vaccinations are required for maintaining high levels of good quality immunoglobulins.

A plug-and-play method to produce pseudotyped Bunyaviruses and their application in neutralisation assay

Federica Marchesin[1,2], Emma M Bentley[2], Francis Mutuku[3], Victor Jeza[3], Bryson Ndenga[4], Sarah Kempster[1], Stuart D Dowall[5], Neil Almond[1], Edward Wright[6], Ashley C. Banyard[7], Nicola J Rose[1], Yasuhiro Takeuchi[1,2] and Giada Mattiuzzo[1]

[1]Medicines and Healthcare products Regulatory Agency, South Mimms, UK

[2]University College London, London, UK

[3]Technical University of Mombasa, Mombasa, Kenya

[4]Kenya Medical Research Institute, Nairobi, Kenya

[5]UK Health Security Agency (UKHSA), Porton Down, UK

[6]School of Life Sciences, University of Sussex, UK

[7]Animal and Plant Health Agency, Addlestone, UK

federica.marchesin@mhra.gov.uk

Accelerated development of tools to assess immune responses to vaccines and therapeutics against emerging pathogens is required to support the 100 Days Mission and pandemic preparedness. Pseudotyped viruses have shown to be a suitable alternative to authentic virus in serological assays. They alleviate the need to acquire and amplify viral isolates and do not require high containment facilities. Pseudotyping of viruses in some families of the class *Bunyaviricetes* is challenged by lack of co-localization of viral glycoproteins and vector budding site.
A pseudotyping system based on a non-replicative, recombinant vesicular stomatitis virus vector, was used. The same conditions were optimised for the production of pseudotyped virus for two prototype viruses: Rift Valley fever virus (RVFV, *Phenuiviridae*) and Crimean-Congo haemorrhagic fever virus (CCHFV, *Nairoviridae*). The use of RVFV and CCHFV pseudotyped viruses in neutralisation assays was evaluated against monoclonal antibodies and plasma/serum from convalescent individuals.
The optimised pseudotyping protocol succesfully produced RVFV and CCHFV pseudotypes with an average titre of 4.06×10^4 and 2.42×10^4 TCID50/mL respectively, and was also tested for the production of Oropouche virus (*Peribunyaviridae*) and Dabie bandavirus (*Phenuiviridae*) pseudotypes with average titres of 5.08×10^4 and 3.85×10^4 TCID50/mL. The pseudotypes were neutralised in a dose-response manner using specific monoclonal antibodies. The use of the RVFV pseudotyped virus in a neutralisation assay generated results with a statistically significant correlation with those from a microneutralisation method using authentic virus (Spearman's $\rho = 0.65$, 95% C.I. 0.51-0.76; $p < 0.0001$).
The optimised 'plug-and-play' pseudotyping system for internally budding bunyaviruses offers an example of an adaptable approach which enables rapid response to emerging viral threats, contributing to global pandemic readiness.

How to ensure rapid supply of safe and efficacious vaccines in pandemic scenarios? A company perspective

Margherita Berni[1], Giovanna Campanella[1], Carine De Kesel[2], Sandrine Dessoy[2], Jean- Francois Dierick[2], Hervé Gressard[2], Carine Letellier[2], Nathalie Lenz[2], Xiangming Li[3], Claudia Magagnoli[1], Ivan Pisoni[1], Daniela Stranges[1], Laurent Strodiot[2], Monica Tello Soto[2], Magdalena Zwierzyna[1] and **Cristiana Campa[1]**

[1]GSK, Siena (Italy)

[2]Rixensart (Belgium)

[3]Rockville (US)

cristiana.x.campa@gsk.com

This presentation will provide an overview of key enablers for rapid supply of vaccines, to support clinical development in pandemic scenarios, covering innovative strategies and technologies in the context of chemistry, manufacturing, and controls (CMC). Concrete examples will be shared, grounded on GSK's experience with multiple vaccine platforms and integrating digital transformation. Implementation of such acceleration opportunities is considered beneficial not only for preparedness for the next pandemic but also to efficiently address unmet medical needs today. These reflections will evidence the importance of

- A holistic, cross-disciplinary framework with clear expectations and actions for each relevant area, from (academic) discovery/ target antigen identification to fast registration and launch.
- The establishment of vaccine platforms, for DS (drug substance), DP (drug product), and Analytical testing, ready for immediate use in both development and GMP (Good Manufacturing Practice) areas.
- The implementation of innovation and digital tools for fast development and innovative control strategies. Such opportunities include, for instance, product design, stability predictions, automation, and process digital twins.

Besides the obvious operational benefit in accelerated context, building and use of prior knowledge is also critical to mitigate risks associated to (clinical) manufacturing and product quality, facilitating decisional process in emergency situations. Extent of platform knowledge re-use and of digitalization opportunities may depend on the nature of the vaccine type (e.g., mRNA, sub-unit, inactivated virus, etc.), and some considerations will be shared on pros' and cons' associated to the selection of different vaccine technologies.

CMC Platform Best Practices for Comparability Assessments and Manufacturing Process Validation - Simultaneous submission to multiple Health Authorities using Accumulus Synergy platform

Hassan Askary[1], Olga Rovira[1], Murtuza Ali[1] and Antonio Guzzi[1]

[1]CEPI, London, UK

hassan.askary@cepi.net

The "CMC Platform Best Practices" project is a collaborative effort from Vaccine Industry CMC and regulatory experts across the globe (DCVMN; IFPMA; Vaccines Europe/EFPIA) and Academia under CEPI's leadership. They have jointly developed a set of documents collectively referred to as "CMC Platform Best Practices" that could be applied to expedite execution of comparability studies and manufacturing process validation. The "CMC Platform Best Practices" provide a strategy-oriented CMC framework with approaches for newly developed vaccines pertaining to a vaccine platform technology and intended to assist in responding to public

health emergencies (PHE). The same principles could also be applied for situations requiring expedited development, e.g. when addressing an unmet medical need by leveraging prior knowledge utilizing platform technologies. This strategy can significantly reduce delays and risks associated with accelerated development. Generally speaking, best practices are optimally grounded if/when they are shared and aligned across the globe, not only between vaccine developers but also amongst regulatory bodies, ideally well ahead of PHEs. The pilot described in this presentation is one of the first activities comprised in CEPI's Regulatory Preparedness Framework, which aims at fostering further regulatory harmonization, collaboration and coordination to maximize preparedness for future PHEs.
CEPI intends to conduct a pilot and seek regulatory input simultaneously from multiple Health Agencies using "CMC Platform Best Practices" documents. A cloud-based technology platform developed by Accumulus Synergy will be used to test the simultaneous submission process. This product- and developer-agnostic project will demonstrate the value of transparent, parallel regulatory reviews in improving collaboration versus traditional scientific advice models.
CMC Platform Best Practices documents will be publicly available after regulatory consultation.

How to accelerate the supply of vaccines to all populations worldwide? Part III: Reflections after the Pandemic

Mic McGoldrick[1], Tran Ba Quang Truong[2], Cristiana Campa[3], Murtuza Ali[4], Antonio Guzzi[4], Mark Van Ooij[5], Cecile Feuillet[2], Jennifer Eck[6] and Nathalie Dubois[7]

[1] Merck & Co., Inc., Kenilworth, NJ, USA

[2] Sanofi Pasteur

[3] GSK

[4] CEPI

[5] Johnson and Johnson Innovative Medicines

[6] AstraZeneca

[7] Pfizer

mic_mcgoldrick@merck.com

When Covid began, a team of vaccine manufacturers worked with the COVAX Manufacturing SWAT to propose regulatory agilities for accelerating access to vaccines. The cooperation and success demonstrated during the pandemic proved the ability to simplify requirements and the benefits of reliance. This resulted in the creation of two papers which outlined some of the hurdles and solutions to accelerate development of vaccines, and ultimately access.
Since those papers were published, vaccine developers, manufacturers, the Coalition for Epidemic Preparedness Innovations (CEPI), along with other stakeholders have engaged in discussions to highlight the lessons learned and find solutions to address those challenges. This presentation covers the efforts of CEPI in initiatives that were either put in place or are ongoing to accelerate pandemic preparedness. It also will compare the regulatory and developmental experiences from manufacturers early in the pandemic compared to the experience manufacturers (both established and new) encountered at the end of the pandemic.
Some stumbling blocks identified were post-approval burden and additional stability studies early on and setting specifications and conducting comparability studies with limited clinical experience and commercially manufactured lots later.
Overall, we concluded that while the initial work and acceptance of risk-based approaches were valuable in accelerating vaccine development, there is a need to normalize these accelerated pathways to ensure timely access to vaccines in the future. Flexibility and reliance testing are essential to maintain product availability

and address emerging variants. As well as continued efforts towards knowledge management, collaboration, and digitalization to ensure regulatory efficiency and effectiveness in future health emergencies. The cooperation and success demonstrated during the pandemic should continue to simplify requirements, promote reliance and assure lessons learned are not forgotten.

Developing a manufacturing and supply chain strategy to support the 100 Days Mission

June Kim[1], Dimki Patel[1], Ramin Sabet-Azad[1], Syed Hassan Askary[1] and **Anna Särnefält**[1]

[1]CEPI (Coalition for Epidemic Preparedness Innovations), Post box 1030 Hoff, 0218 Oslo, NORWAY

anna.sarnefalt@cepi.net

Preparedness is essential to achieving the 100 Days Mission. This is underscored by the rapid response framework developed by CEPI, which outlines timely activities related to chemistry, manufacturing, and controls, including supply chain and facilities. By structuring activities to be done in preparation during interpandemic periods this framework enables assessment of platform readiness, identification of gaps, and development of mitigation strategies for rapid response in the event of an outbreak, where robust manufacturing and release of vaccine candidates are key elements.

Considering regulatory guidelines, CEPI has defined preparatory activities and deliverables for rapid response and further structured manufacturing/related efforts to be undertaken from outbreak recognition to release of the first clinical trial material, initial emergency use licensure, and anticipated marketing approval requirements.

The framework presents three scenarios, depending on previous vaccine development and familiarity of the outbreak pathogen, though all assuming that the concept of platform technology and prior knowledge is applicable. The activities have been categorized into five groups: Drug Substance/Drug Product process, Formulation and stability, Analytical, Materials and Supply Chain, and Facilities and Manufacturing. All these components contribute to development of a manufacturing and supply chain strategy for a potential vaccine candidate to be used in a possible pandemic situation.

The faster an effective vaccine is developed and deployed, the quicker an incipient pandemic can be contained and controlled. CEPI has developed a tool that outlines peacetime preparatory and outbreak response manufacturing-related activities in support of the 100 Days Mission, which aims to ensure rapid availability of vaccines in an outbreak or health threat.

Insight into the scope of alphacoronavirus receptor usage

Giulia Gallo[1], Aghnianditya Kresno Dewantari[1,2], Antonello Di Nardo[1], Stephen C Graham[3] and Dalan Bailey[1]

[1]The Pirbright Institute, Guildford, Surrey, GU24 0NF, UK

[2]Department of Infectious Disease, Imperial College London, W2 1NY, UK

[3]Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

giulia.gallo@pirbright.ac.uk

Alphacoronaviruses (alphaCoVs) are emerging viruses with zoonotic potential. While the majority of alphaCoVs have been found associated to bats, they can also infect other mammals, such as humans, cats, dogs, and pigs. In these hosts, viruses can cause different degrees of pathology, occasionally resulting in lethal outcomes. It has been showed that some alphaCoVs infect their hosts through binding of their Spike attachment protein to the receptor aminopeptidase N (APN). However, the broad zoonotic potential of these viruses has not been exhaustively evaluated. In our study, we selected Spike representatives of the alphaCoV genus and used pseudotyping to study their tropism for APN of different mammalian species. Additionally, we also investigated their usage of a library of ACE2. We found that most of our S could pseudotype, but only few of them would interact with our bank of APNs and ACE2s. Interestingly, we reported for the first time that two bat alphaCoV use APN to enter cells, broadening our understanding of S/APN interaction within the viral genus. We also observed host range differences among human, canine and bat alphaCoVs. Our data shed light on receptor usage among alphaCoVs and identified viruses with broad receptor usage ability – generalists – versus viruses restricted to a small number of mammalian species – specialists. From a One-health perspective, our results support the importance of studying zoonotic potential at a broad scale, both in terms of viral sequences and receptors' origin, providing information about likelihood of spill-over and potential intermediate reservoirs.

Assessing Stakeholder Collaboration within Highly Pathogenic Avian Influenza Risk Governance Strategies in the UK and USA

Kimberly Lyons[1], Yasmin Abdalla[2], Samantha Lycett[3], Paul Digard[4] and Lisa Boden[5]

[1]Global Academy of Agriculture and Food Systems, University of Edinburgh

[2]Global Academy of Agriculture and Food Systems, University of Edinburgh

[3]Roslin Institute, University of Edinburgh

[4]Roslin Institute, University of Edinburgh

[5]Royal (Dick) School of Veterinary Studies, University of Edinburgh

klyons@ed.ac.uk

Outbreaks of highly pathogenic avian influenza (HPAI) have led to a global rise in deaths of wild birds and poultry, as well as an increase in HPAI detected in mammals. With the recent outbreaks of HPAI in dairy cattle and the virus's pandemic potential, it is important to understand what processes are in place to mitigate the risks of HPAI. Risk governance frameworks for infectious diseases encourage policies to be grounded in different stakeholder perspectives and transparent communication. However, outbreaks such as that of Covid-19, exemplify that this collaboration is not always put into practice, leading to response processes that do not function as they should. There is no current research assessing the stakeholder landscape and risk governance processes in the United Kingdom (UK) and United States of America (USA) in response to the recent HPAI outbreak.

Key stakeholders in the UK and USA involved in HPAI decision-making and response were asked to provide insight into the stakeholder landscape, communication pathways, and challenges experienced during HPAI outbreaks. Hour-long semi-structured interviews were conducted with 12 participants from the UK and 8 from the USA. Participants included policy advisors, veterinarians, researchers, and industry representatives. Participants were initially recruited by reaching out to known contacts, and then using a snowball approach. A thematic content analysis was used to code and analyse interview data in the software NVivo by Lumivero.
Participant interviews identified the overall need to improve the mobilisation of resources, such as the inclusion of varied stakeholder perspectives, knowledge and expertise, data and information, and human and economic resources, across the science-policy-industry interface within the UK and USA to ensure efficient HPAI outbreak response processes.

Investigating the replication of BANAL-236 and related viruses in bat cells

Ségolène Gracias[1], Léa Vendramini[1], Flora Donati[1], Sandie Munier[1], Javier Juste[2], Carles Martinez-Romero[3], Arturo Marin[3], Lisa Miorin[3], Magdalena Rutkowska[3], Anastasija Cupic[3], Adolfo Garcia-Sastre[3], Jyoti Batra[4], Nevan J Krogan[4], Olivier Schwartz[1], Vincent Caval[1] and Nolwenn Jouvenet[1]

[1]Institut Pasteur, Paris, France

[2]Estación biológica de doñana, Seville, Spain

[3]Icahn School of Medicine at Mount Sinai Hospital, New York, USA

[4]J. David Gladstone Institutes, San Francisco, CA, USA

segolene.gracias@pasteur.fr

Bats serve as natural reservoirs for many emerging zoonotic viruses, the mechanisms underlying bats' ability to control viral replication are not well understood.
We aimed to study the replication of bat betacoronaviruses in bat cells, with a focus on viral entry mechanisms and innate immune response, using SARS-CoV-2 and BANAL-236, which was isolated from Rhinolophus bats in Southeast Asia. This study also includes sequences from BANAL-52, another virus discovered in Asian Rhinolophus bats, for which we do not have an isolate.
SARS-CoV-2 and BANAL-236 did not replicate in wild-type Rhinolophus cell lines. To determine whether this restriction could be overcome by expressing known human viral entry factors, we generated Rhinolophus cell lines stably expressing hACE2 and hTMPRSS2. Entry assays using pseudoviruses expressing the spike proteins (S) of SARS-CoV-2, BANAL-236, and BANAL-52 showed that entry of the pseudoviruses into Rhinolophus cells required the expression of both factors. Conversely, hACE2 expression alone permitted pseudovirus entry into human cells, likely due to endogenous TMPRSS2 expression. Rhinolophus cells expressed low levels of endogenous rACE2 and rTMPRSS2, potentially explaining BANAL-236 replication restriction.
While viral RNA and proteins were produced in Rhinolophus cells expressing hACE2 and hTMPRSS2 infected with BANAL-236 or SARS-CoV-2, no infectious virions were released. This suggests that Rhinolophus cells lack cellular factors necessary for viral infectivity or express antiviral factors blocking virion production. Despite efficient BANAL-236 replication, no induction of interferon-stimulated genes was detected in Rhinolophus cells. Since these cells are immunocompetent, this suggests that the viruses have evolved potent mechanism(s) to evade the interferon response in these bat cells.
This study provides valuable insights into bat betacoronavirus replication in their reservoir species and may contribute to our understanding of zoonotic virus transmission.

Attitudes and Practices of Humans to Flying Foxes and Viral Threats in Battambang Province, Cambodia: a Pilot Study

Malen Chan[1], Veasna Doung[1], Sowath Ly[1], Michael Allen Patten[2], Courtney Alice Waugh[2] and **Annelin Seppola**[2]

[1]Institute Pasteur du Cambodge, No. 5 Monivong Boulevard P.O Box. 983, Phnom Penh, Cambodia

[2]Nord University, Postboks 1490, 8049 Bodø, Norway

annelin.seppola@nord.no

Pandemics almost always begin with a spill-over event; the transmission of an animal microbe to a human, which in turn spreads from human to human. Combining the knowledge on the sources of zoonotic pandemics and how societies respond to and prepare for them is crucial to develop awareness and defence against pandemics. The Covid-19 pandemic emphasizes the need for innovative approaches thorough interdisciplinary research. We hypothesise that by integrating the natural and social sciences and improving communication and understanding within the public, this can inform local and global responses on how to go from predicting pandemics to providing adequate responses. Battambang province in Cambodia provides a model location for a pilot study due to the overlapping habitation of humans and flying foxes, as the attitudes and practices toward bats are largely unknown and unquantified. Zoonotic viruses have been previously detected in these (and other) bat roosts in Cambodia (and worldwide), and provide potential sources of pathogen spill-over (see Doung et al. 2020).

This pilot study provides opportunities to gather preliminary data on attitudes and behaviours toward flying foxes among people living in proximity to roosts. Combining these data with data on pathogens, we aim to build a test model which predicts the likelihood of spill-over of pathogens through Bayesian modelling. This template model will be tested and retested with the aim of being able to apply the model to further case studies. The data will be collected during August 2024.

At the time this abstract was submitted, the results are not yet ready. Data will be analysed during September-October 2024.

Conclusions will be drawn after data analysis and collection.

Impact of routine prophylaxis with monoclonal antibodies and maternal immunisation on respiratory syncytial virus burden in the Lombardy region, Italy

Francesco Menegale[1], Luigi Vezzosi[2], Marcello Tirani[2], Simona Scarioni[2], Stefano Odelli[2], Federica Morani[2], Catia Borriello[2], Elena Pariani[3], Ilaria Dorigatti[4], Danilo Cereda[2], Stefano Merler[5] and Piero Poletti[5]

[1]Fondazione Bruno Kessler, Trento, Italy; University of Trento, Trento, Italy

[2]Lombardy Region Welfare General Directorate, Milano, Italy

[3]University of Milan, Milan, Italy

[4]Imperial College London, London, United Kingdom

[5]Fondazione Bruno Kessler, Trento, Italy

fmenegale@fbk.eu

Respiratory syncytial virus (RSV) stands as a leading cause of acute lower respiratory tract infections (LRTI) and hospitalisations in infants worldwide. Recent approvals by regulatory agencies for using monoclonal antibody (mAb) nirsevimab (Beyfortus, Sanofi and Astrazeneca) and maternal immunisation with RSVpreF vaccine (Abrysvo, Pfizer) offer promising approaches to mitigate RSV- associated morbidity.
We applied a catalytic model to evaluate the effects of routine prophylactic campaigns based on mAbs or maternal vaccination on RSV hospitalisations in Lombardy, Italy. The model was informed by data on RSV-attributable cases and patients in the past, while accounting for changes in susceptibility caused by the lower RSV circulation experienced during the COVID-19 pandemic. Alternative scenarios were explored, considering the uptake levels observed in infancy and among pregnant women. The efficacy of nirsevimab and the RSVpreF vaccine in preventing the RSV infection was assumed between 62%-85% and 30%-75%, respectively.
Yearly administration of mAbs to 80% of infants, corresponding to uptake levels recently observed in Spain, was estimated to avert on average 41.5-56.6% hospitalisations in the overall population per year. Coverage levels close to those observed for childhood vaccines (95%) could result in an additional 18% reduction of hospitalisations. RSVpreF vaccine administered to 65% of pregnant women, resembling the Diphtheria, Pertussis, and Tetanus coverage for this target in the region, was estimated to avert on average 16.4-40.8% of hospitalisations. Considering a flu-like coverage (12%), less than 6% of hospitalisations could be averted by maternal immunisation.
Routine administration of nirsevimab in infants demonstrates significant potential to reduce the hospital burden associated with RSV. Maternal immunisation can play a complementary role in achieving high levels of protection in at-risk populations.

Diagnostic contribution of a medicine laboratory in the recent pandemics

Giuseppe Castaldo[1], **Alessandro Pezzella**[1] and Alberto Aloisio[1]

[1]University of Naples Federico II, Carso Umberto I 80135 Naples

alessandro.pezzella@unina.it

The recent COVID-19 pandemic has committed the health systems of all countries in an unimaginable effort to contain and combat the disease. At the same time, the research structures have put all their efforts into the development of new approaches, to facilitate the diagnosis of the disease, evaluate its prognosis and the development of an effective immune response also towards the vaccine and to monitor its complications. Many of these results may also have an impact in the event of other epidemic/pandemic events. The Laboratory

Medicine research area of the Federico II University of Naples in collaboration with CEINGE-Advanced Biotechnologies (Naples), has conducted research on the following aspects:
a) Receptor mechanisms that regulate the entry of the virus into cells, distribution of receptors in different tissues and in subjects of different age groups.
b) Genes predisposing to COVID infection and related to its severity.
c) Methodologies for the detection of the virus and for the study of its variants; study of the microbiome related to infection.
d) Study of the flow cytometric profiles associated with COVID in the different pandemic waves.
e) Study of diagnostic biomarkers with particular attention to biomarkers of inflammation and endothelial damage.
f) Potential therapeutic approaches.
g) Study of predisposing factors and diagnostic biomarkers for pediatric COVID complications (pediatric systemic multiinflammatory syndrome).
h) Development of platforms for the preparation of vaccines.
i) Innovative methods for testing cellular immunity to vaccines and validation in both healthy and fragile subjects.

The challenges of pandemic preparedness and biosecurity – act locally to protect globally

Alessandro Marcello[1]

[1] Laboratory of Molecular Virology, The International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano, 99, 34149 Trieste, Italy

marcello@icgeb.org

In our interconnected world infectious diseases find the perfect conditions to spread quickly across countries and continents. A novel pathogen may emerge as a zoonotic disease anywhere without warning. In addition, deliberate or accidental release of pathogens is an increased risk that deserves attention. In this scenario, our global capacity to detect and respond to an emerging infection remains highly heterogeneous, with large areas unsupervised due to a combination of lack of resources and low perception of bio-threats. At the forefront of pandemic preparedness is the capacity to detect a (re)emerging pathogen as close as possible to the site of initial spread. This means enabling capillary distribution of robust and cost-effective diagnostics and surveillance tools at the periphery to reduce the turnaround time of results. In the context of global health, we are working in Africa within the EXPANDIA framework to deliver molecular assays for the detection of viral infections of global importance, while within BIO-GUARD we focus on the creation of a network of multidisciplinary bio-thereat units equipped to assess, detect and coordinate a response to a wide range of bio-risks. Only by reinforcing our capacity to identify an emerging risk we will be able to contain the spread of the disease.

Centralised Diagnostic and Surveillance Laboratory Capacity for Public Health Emergency Preparedness and Response - A Case Study Analysis of the National Pandemic Center at Karolinska Institutet, Sweden

Salome Steinke[1,2], Dorina Ujvari[1], Fredrik Boulund[1,3], Helena Nordenstedt[2,4] and Jessica J Alm[1,2]

[1]National Pandemic Center, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solnavägen 9, Biomedicum, SE-17165 Stockholm, Sweden

[2]Centre for Health Crises, Karolinska Institutet, Tomtebodavägen 18A, SE-171 77 Stockholm, Sweden

[3]Centre for Translational Microbiome Research, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solnavägen 9, Biomedicum, SE-17165 Stockholm, Sweden

[4]Department of Global Public Health, Karolinska Institutet, Tomtebodavägen 18A, SE-171 77 Stockholm, Sweden

salome.steinke@stud.ki.se

The COVID-19 pandemic underscored the importance of effective laboratory capacity management for public health emergency preparedness and response. In Sweden, the National Pandemic Center at Karolinska Institutet was established to meet surging demands for diagnostics and surveillance which had surpassed the standing capacity of decentralized laboratories. The impact of such centralisation on effective crisis management remains unclear. This study elucidates the role of centralised diagnostic and surveillance laboratory capacity for public health emergency preparedness and response by analysing Sweden's COVID-19 response and the contribution of the National Pandemic Center.
Nine semi-structured interviews were conducted with medical laboratory professionals to explore the perceived need for centralised laboratory capacity for Sweden's public health emergency preparedness and response. Reflexive thematic analysis was employed to investigate the experiences of these professionals from various

regional and state actors during the COVID-19 pandemic, with a special focus on the National Pandemic Center.
This study emphasises the value of complementing decentralised routine healthcare diagnostics and sequencing with centralised, high-capacity facilities for large-scale population testing and surveillance during health emergencies. High-throughput centralised facilities, like the National Pandemic Center were considered critical for Sweden's future emergency preparedness and response. Additionally, the importance of centralised governance, leadership, and interoperable national systems for effective laboratory surge capacity management was highlighted.
This study represents an important evaluation of laboratory capacity management for public health emergencies in Sweden. By highlighting the benefits of an integrated centralised and decentralised approach, it informs policy reformation for effective crisis management in Sweden and comparable settings worldwide. Key areas for further research are highlighted to strengthen capacity building for public health emergencies.

The Role of Reference Materials for Outbreak Preparedness

Emma M Bentley[1], Yann Le Duff[1], Nassim Alami-Rahmouni[1], Mark Hassall[1], Adrian Jenkins[1], Sarah Kempster[1], Edward Mee[1], Jane Mitchell[1], Emma N Summersgill[1], Nicola J Rose[1] and **Giada Mattiuzzo**

[1]Medicines and Healthcare products Regulatory Agency, Blanche Lane, South Mimms, United Kingdom

Giada.mattiuzzo@mhra.gov.uk

Public health emergencies of international concern have highlighted the lack of prophylactic treatments and effective diagnostics for emerging viruses. Reference reagents support assay development through the calibration and harmonisation of data and ensure robust evaluation of vaccines/treatments. World Health Organization (WHO) International Standards (IS) are the highest order calibrant and undergo extensive evaluation during multi-laboratory collaborative studies. Collecting and handling material for emerging pathogens is challenging and consequently, a WHO IS cannot be established rapidly at the beginning of an outbreak, we therefore sought to establish alternative strategies.
Chimeric lentiviral particles containing the RNA of a priority pathogen, such as Ebola virus, Lassa virus or SARS-CoV-2 have been generated to provide reference materials for molecular diagnostics. For serological methods, characterised plasma/sera from convalescent patients has been prepared as interim reference materials for SARS-CoV-2 and Mpox.
The performance of the chimeric lentiviral particles, as reference materials and ability to reduce inter-laboratory variation, has been proven in International collaborative studies for Lassa virus and SARS-CoV-2. They can be rapidly produced once a viral sequence is published. For antibody standards, the COVID-19 pandemic showed the value of an interim reagent strategy, such as 20/130 which was made available early in the pandemic to support serological assay development and later back calibrated to the WHO IS.
We have developed strategies which can be applied for the rapid preparation of reference materials to support the 100 Days Mission in response to future outbreaks, including Disease X. Additionally, several WHO IS are now available, or in development, for priority pathogens.

Suit-case mobile laboratory for international response to outbreak and epidemics

Mikaty Guillain[1], Balière Charlotte[1], Brisebare Angela[1], Cappuccio Lucie[1], Caro Valérie[1], Fichenick Frédéric[1], Garcia Laura[1], Grassin Quentin[1], Hoinard Damien[1], Hourdel Véronique[1], Kwasiborski Aurelia[1], Le Flêche Anne[1], Lomprez Fabienne[1], Mathéus Séverine[1], Siby-Diakite Dieyenaba[1], Vanhomwegen Jessica[1], Vincent Rémi[1], Dacheux Laurent[1] and Manuguerra Jean-Claude[1]

[1]Institut Pasteur, Université Paris Cité, Environment and Infectious Risks expertise research unit (ERI); Laboratory for Urgent Response to Biological Threats (CIBU), Paris, France

guillain.mikaty@pasteur.fr

During outbreaks, rapidity of response and identification of the threats are key in the management and containment of the epidemics. Emerging diseases often appear in countries with limited resources within isolated locations, distant from laboratories capable to identify the pathogens or diagnose the patients. For teams dedicated to survey and respond to outbreaks, it is essential to develop an efficient and amenable capacity to project themselves directly in the field with adapted diagnostic capabilities.
The Ebola outbreak in West Africa was an opportunity to demonstrate the efficacy of mobile laboratories in the field. Since its creation in 2002, the Laboratory for Urgent Response to Biological Threats (CIBU), at Institut Pasteur in Paris, has developed a comprehensive strategy to survey, investigate and tackle biological outbreaks using a mobile laboratory. This strategy is based on a modulable suitcase-based mobile laboratory, easily transportable worldwide, and adaptable to a large spectrum of biological threats and situations.
The CIBU developed a comprehensive and modulable Type II mobile laboratory (WHO's Rapid Response Mobile Laboratory classification). This laboratory is based on small transportable equipment, point-of-care and multiplex approaches, and cutting-edge tools, such as metagenomic. The laboratory is adaptable to most situations with multiple modules that can be shipped separately: point-of-care tools; molecular diagnostic; serological diagnostics; or NGS sequencing. The laboratory is equipped with a state-of-the-art Glovebox under depression and allows to handle any type of biological sample from BSL2 to BSL4 pathogens. The laboratory aims to be self-sustainable and autonomous with field furniture, electric generators and communication tools.
The CIBU Mobile Laboratory proved to be deployable and efficient in the field through past experiences and multiple field exercises abroad over the past decade. The team is ready to face the next outbreak.

Establishing a Biorisk Management Committee in an International Aid Agency

Miranda Smith[1]

[1]US Agency for International Development, 1300 Pennsylvania Ave NW, Washington, DC 20004 USA

mirsmith@usaid.gov

The COVID-19 pandemic underscored the critical importance of robust biorisk management, particularly within organizations that operate on a global scale. International aid agencies often fund programs that directly engage with high-risk biological materials in diverse and resource-limited settings. Despite this, many lack a centralized framework to ensure consistent adherence to both domestic and international biosafety and biosecurity standards. This abstract discusses the development of a Biorisk Management Cooperation Committee within an international aid provider agency. The Committee's objectives include the alignment of federally-funded international projects with domestic biorisk management standards and the enhancement of global biosafety practices.
A multi-phase approach was employed in the establishment of the Committee. Phase one involved a comprehensive review of existing biosafety and biosecurity policies within the agency. Phase two engaged key stakeholders across various departments to identify gaps and areas of risk in current practices. The final phase focused on the development of a cooperative framework, ensuring compliance with domestic guidelines while adapting to the specific needs of international projects.
The establishment of the Biorisk Management Cooperation Committee has resulted in the creation of standardized procedures for biorisk assessment and mitigation across all agency-funded projects. The Committee also facilitated the integration of international biosafety standards into domestic protocols, ensuring that all projects, regardless of location, meet a consistent standard of safety and security.

The creation of the Biorisk Management Cooperation Committee marks a significant step forward in the agency's commitment to global health security. By ensuring that federally-funded international work adheres to both domestic and international standards, the Committee enhances the agency's ability to mitigate biorisks in a coordinated and effective manner. This model could serve as a template for similar organizations aiming to strengthen their pandemic preparedness frameworks.

Planetary Health at Risk: Emerging Threats and Future Preparedness

Felix Moronta-Barrios[1] and Wendy Craig[1]

[1]Regulatory Science Group, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

moronta@icgeb.org

Strategic foresight is essential in pandemic preparedness, enabling the anticipation of future risks and the development of proactive measures to address them. The 21st century has seen significant progress in addressing global health threats, but new challenges continue to emerge, necessitating forward-looking strategies. This presentation will share insights from our work with the UN Environment Programme (UNEP) – International Science Council (ISC) Foresight Expert Panel, highlighting a comprehensive review of critical issues expected to impact planetary health by 2050. Key concerns include the escalating threat of antimicrobial resistance (AMR), the rise of emerging zoonotic diseases, the increased risk of biological warfare, and the potential health impacts of thawing permafrost.

AMR is a major challenge, with drug-resistant pathogens potentially rendering current treatments ineffective, posing a significant threat to global health. Concurrently, emerging zoonotic diseases, facilitated by environmental changes and increased human-animal interactions, present substantial pandemic risks. Advances in biotechnology and synthetic biology, while promising, also raise concerns about the misuse of these technologies in biological warfare. Additionally, the thawing of permafrost, potentially releasing ancient pathogens, further complicates the global health landscape.

This presentation will emphasise the interconnectedness of these issues and their potential to converge into complex, multifaceted threats. By reviewing current knowledge and identifying knowledge gaps, we will discuss strategic approaches to enhance global preparedness. Emphasis will be placed on interdisciplinary cooperation, robust surveillance systems, innovative research, and international policy frameworks. By incorporating strategic foresight, stakeholders can identify vulnerabilities, enhance resilience, and implement effective countermeasures before crises occur, ultimately aiming to mitigate future pandemic risks and safeguard planetary health.

Poster presentations

1. Nipah Virus Immunogen Design

Nasim Abdi[1]

[1]University of Texas at Austin, Austin, USA

nabdi2000@utexas.edu

Nipah virus (NiV) is a pathogenic Henipavirus of the Paramyxoviridae family that can cause infections, with case fatality rates of up to 75% in humans. NiV has two envelope glycoproteins that facilitate infection: the receptor-binding protein (RBP), involved in attachment, and the fusion protein (F), responsible for membrane fusion. Due to its high mortality rate and lack of approved treatments or vaccines, the World Health Organization (WHO) and the Coalition of Epidemic Preparedness Innovation (CEPI) have designated NiV as a high-priority pathogen with pandemic potential.

NiV's envelope glycoproteins are important targets for vaccine design, with both F and the RBP shown to elicit neutralizing antibodies that inhibit NiV infection. During F-mediated membrane fusion, NiV F undergoes a substantial conformational change from the metastable prefusion F (preF) to highly stable postfusion F (postF) conformation. The preF conformation has been shown to elicit higher titers of neutralizing antibodies than its postF counterpart in vaccine studies. Using structure-based rational design and computationally driven vaccine design, we have engineered and characterized over 254 single amino acid substitutions designed to stabilize the preF conformation in both native and tethered-ectodomain formats. Our results identify numerous substitutions that boost expression of F and increase the proportion of preF to postF relative to WT, as indicated by binding to preF-specific antibodies. A total of 95 combinatorial variants of the best individual substitutions have been designed to produce optimized preF variants and are currently under evaluation. We plan to iterate on designs with structural validation of exemplar variants and elucidate mechanistic insights of prefusion stabilization by key substitutions. Evaluation in preclinical models will assess the immunogenicity and protection potential of top variants. This work will advance our knowledge of stabilizing substitutions for Henipavirus F proteins and provide critical insights to inform structure-based vaccine development efforts.

2. Performance evaluation of dipstick-based wastewater surveillance of viruses through multi-operator Gage R&R study

Shruti Ahuja[1], Avani Kulkarni[2], Kiran Kondabagil[3] and Siddharth Tallur[2]

[1]Centre for Research in Nanotechnology & Science (CRNTS), IIT Bombay, Mumbai 400076, India

[2]Department of Electrical Engineering, IIT Bombay, Mumbai 400076, India

[3]Department of Biosciences and Bioengineering, IIT Bombay, Mumbai 400076, India

204366005@iitb.ac.in

The COVID-19 pandemic accelerated the development of low-cost assays for automated wastewater surveillance, essential for early warning of frequent disease outbreaks and future pandemics. Microbial concentration remains a major challenge in wastewater monitoring due to sample variability and low pathogen loads. For large scale testing in densely populated low- or middle-income countries (LMICs) such assays need to be low-cost, rapid with high recovery efficiency. To address this constraint, we have devised an easy and rapid dipstick method for RNA isolation suitable for sub-millilitre sample volumes alleviating the complexities of existing RNA isolation methods. The dipstick method involves RNA capture by immersing the dipstick in the sample followed by a washing step to eliminate cell debris and impurities and finally eluting the RNA in mastermix by rigorous bending and compression. Our key contributions are:

1. We have validated the dipstick method for successfully concentrating and isolating nucleic acids from different microbes such as SARS-CoV-2, PMMoV, and Phi6 from a wide range of wastewater samples with minimal sample pre-processing, with recovery efficiency at par with commercially available kits.
2. We have studied variability of the dipstick method by performing a multi-operator Gage repeatability and reproducibility (Gage R&R) study for wastewater samples collected from a sewage pumping station at IIT Bombay. This study demonstrated that the dipstick method has Gage R&R <30%, and could detect variations in PMMoV load associated with changes in population density due to summer break.
3. We further simplified the dipstick procedure by automating dipstick preparation and assay operation steps that contributed to variability. Our analysis revealed that the simplified method does not adversely impact Gage R&R, with variability arising mainly due to sample variation, confirming overall acceptability.

3. Utility of the Taqman Array card for detection of etiologies of Acute febrile illness in patients suspected to have Viral Hemorrhagic Fever Infections

Gloria Grace Akurut[1], Luke Nyakarahuka and Stephen Balinandi

[1]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

akurutgloria@gmail.com

Attributing a causative agent to acute febrile illnesses remains challenging yet important towards clinical care and public health response. We used a Taqman Array card (AFI-TAC) diagnostic assay for multi-pathogen detection on samples testing PCR-negative for Viral Hemorrhagic Fever.
A cross-sectional retrospective study design was employed, 182 viral hemorrhagic fever (VHF)-negative samples archived at Uganda Virus Research Institute (UVRI)-VHF Laboratory collected from August 2018 to March 2019 during routine surveillance were included. The inclusion criteria was defined as patients with a body temperature of ≥37.5 ºC, were bleeding and any other febrile symptom. Samples were tested on AFI-TAC. VHF positive samples were used for assay verification.
Among the 182 samples tested, the distribution was as follows: 152 (83.52%) from Uganda, 22 (12.09%) from the Democratic Republic of Congo, 7 (3.85%) from South Sudan, and 1 (0.55%) from Kenya. The median age of the patients was 27 years, with the majority being adults (79.7%). Seven pathogen targets were detected: Plasmodium spp. were identified in 49 samples (26.92%), with 69.4% (34/49) of these being Plasmodium falciparum. Other pathogens detected included Yellow fever virus (2 cases, 1.10%), non-typhus Salmonella (3 cases, 1.65%), Salmonella typhi (2 cases, 1.10%), Leptospira spp. (1 case, 0.54%), Streptococcus pneumoniae (1 case, 0.55%), and Rickettsia spp. (1 case, 0.55%). All previously positive samples (CCHF virus = 5, RVF virus = 1) tested positive on the AFI-TAC.
The AFI-TAC is a practical tool for multi-pathogen detection during surveillance or outbreaks of AFIs with high specificity and capacity for timely differential diagnosis.

4. Epidemiology of Toxoplasma gondii Infection in Animals of the Arabian Peninsula: A Systematic Review and Meta-Analysis

Ebtisam Al-Mslemani[1], Asmaa Abdelgadier[2], Nada Assaad[2] and Zaynab Elhussein[2]

[1]Epidemiology and Infectious Diseases, Animal Resources, Ministry of Municipality, Doha, Qatar

[2]Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

e_almuslmani@yahoo.com

Toxoplasma gondii (T. gondii) is a zoonotic parasite that can be transmitted from animals to humans, with felids acting as its definitive host. Thus, understanding the epidemiology of this parasite in animal populations is vital to controlling its transmission to humans as well as to other animal groups.
This systematic review and meta-analysis aims to summarise and analyse reports of T. gondii infection in animal species residing in the Arabian Peninsula.
It was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), with relevant studies being retrieved from MEDLINE/PubMed, Scopus, Cochrane Library, Google Scholar and ScienceDirect. All articles published in Arabic or English languages between January 2000 and December 2020 were screened for eligibility. Random effects model was used to calculate the pooled prevalence of T. gondii infection in different animal populations which were found to harbour this infection. The critical appraisal tool for prevalence studies designed by the Joanna Briggs Institute (JBI) was used to assess the risk of bias in all included studies.
A total of 15 studies were retrieved, reporting prevalence estimates from 4 countries in this region and in 13 animal species. Quantitative meta-analysis estimated a pooled prevalence of 43% in felids [95% confidence interval (CI) = 23-64%, I2 index = 100%], 48% in sheep (95% CI = 27-70%, I2 = 99%) and 21% in camels (95% CI = 7-35%, I2 = 99%). Evidence of possible publication bias was found in both felids and sheep.
This meta-analysis estimates a high prevalence of T. gondii infection in animal species which are of high economic and cultural importance to countries of this region. Hence, these findings provide valuable insight to public health authorities as well as economic and animal resources advisors in countries of the Arabian Peninsula.

5. Isolation and characterization of patient-derived monoclonal antibodies that neutralize SARS-CoV-2 variants

Mathilde Couteaudier[1], Astrid Musnier[3], Marianne Maquart[1], Roxane Lemoine[2], Anne Bull[1], Yannick Corde[3], Saheli Mitra[3], Christophe Dumet[3], Abelraouf Keskes[3], Adrien Verdier[3], Zakaria Omahdi[3], Mélanie Cortes[3], Anne Poupon[3], Vincent Puard[3], Denys Brand[1], Hervé Watier[2], Thomas Bourquard[3] and Fabrizio Mammano[1]

[1]INSERM U1259 – MAVIVHe - Morphogenesis and antigenicity of HIV, hepatitis and emerging viruses – Université de Tours et CHRU de Tours, Tours, France

[2]PST-ASB, Université de Tours et CHRU de Tours, Tours, France

[3]MAbSilico SAS, Tours, France

mathilde.couteaudier@univ-tours.fr

The COVID-19 pandemic has highlighted that it was possible to select, develop and produce vaccines against SARS-CoV-2 in less than one year. But vaccination is less effective for immunocompromised patients. The use of neutralizing monoclonal antibodies directed against the S protein of the SARS-CoV-2 virus emerged as an effective complementary approach. The continuous emergence of viral variants, however, challenged their efficacy. The objectives of our study were to identify patient-derived broadly neutralizing antibodies, and to quickly adapt the antibody selection process to target the variants as they emerge.
We combined of high-throughput isolation of paired VH and VL sequences (clonotypes) from patients' circulating B cells captured by the trimeric spike protein from the strains Wuhan or BA-5, with artificial intelligence (AI)-based selection. This unique computational approach allows to evaluate the binding of the clonotypes to chose epitopes, here the epitopes at the interface with ACE2. We thus isolated four cross-binding and cross-neutralizing antibodies against several strains of SARS-CoV-2, including the Omicron variants BA.1, BA.2, BA.5, BQ.1.1. The IC50 values ranged from 30 to 200 ng/ml for Wuhan, 100 to 200 ng/ml for BA.1 and BA.2, and more than 5 µg/ml for BA.5 and BQ1.1. Antibodies selected using the BA.5 spike protein trimer displayed improved neutralization capacity against Omicron variants.

Overall, we showed that the adaptation of the antibody selection procedure to target Omicron variants allowed the rapid isolation of broad-spectrum neutralizing antibodies. This approach can improve the development of treatment strategies to protect patients who cannot benefit from vaccine immunization.

6. Susceptibility of brown rats (*Rattus norvegicus*) to mpox virus infection

Lucy Flett[1], Stephen Findlay-Wilson[1], Linda Easterbrook[1], Emma Kennedy[1], Francisco J. Salguero[1], Susan Fotheringham[1] and **Stuart Dowall**[1]

[1]UK Health Security Agency (UKHSA), Porton Down, Salisbury, Wiltshire, SP4 0JG. UK

stuart.dowall@ukhsa.gov.uk

Mpox (formerly known as monkeypox virus) is the zoonotic pathogen of mpox disease (MPXD) in humans. Its increasing emergence outside of its endemic area has heightened the importance to investigate the virus' prevalence and maintenance in sylvatic reservoirs.
During the global outbreak which began in 2022, different clinical manifestation were observed, including lesions around groin and anal regions. As such, the risk of mpox virus entering the sewerage system is increased. The common brown rat (*Rattus norvegicus*) can inhabit almost anywhere in the UK, posing a threat to zoonotic transmission to humans. This study investigated the susceptibility of brown rats to mpox virus infection with a clade IIb mpox strain via two challenge routes: intranasal and intradermal. Intranasal was chosen to represent infection through exposure of contaminated waste and intradermal to assess if infection could be passed through animal behaviour, such as biting.
All mpox-challenged animals were asymptomatic, although ELISA assays confirmed subclinical infection in both challenge groups. RT-PCR detected mpox DNA in the lung tissue and throat swabs within the intranasal inoculated group in addition to viable virus observed from the intranasal throat swabs. In contrast, no virus was detected in either tissues or swabs in the intradermal inoculated group or control group.
In conclusion, brown rats could act as a reservoir to transmission of MPXV to humans, as they have been demonstrated to maintain viable virus in the absence of clinical signs.

7. X-Ray Irradiation of Human Serum Samples for Downstream Serological Assays

Pedro Ferreira[1], Sahil Dugg[1], Scott Jones[1], Elle Campbell[1], Ashley Otter[1] and Roger Hewson[1]

[1]UK Health Security Agency, Porton Down, Salisbury SP4 0JG, UK

pedros.ferreira@ukhsa.gov.uk

The changing environment and modern human behaviour, such as global travel and urbanisation, has contributed to the increase in observed pandemics of high consequence infectious diseases (HCIDs). This has created pressure on the limited global high-containment facilities, making research and development capabilities challenging. Traditional inactivation methods of heat and chemical treatment perturb structural epitopes. Whilst gamma irradiation maintains epitopes, it is accompanied by security and safety concerns. X-ray inactivation provides similar structural benefits to gamma irradiation, without the same drawbacks linked to the use of radioactive materials. Successful viral inactivation, coupled with maintenance of immune epitopes, would allow immunological profiling of HCIDs at lower containment laboratories, progressing research and contributing to rapid start-up of pandemic diagnostic platforms. This study aims to use X-ray technology to irradiate ambient and frozen human serum samples spiked with Vaccinia and Mpox antibodies to evaluate the downstream serological detection of inactivated clinical samples.

Samples were initially irradiated at ambient using the MultiRad225 with methodology developed at UKHSA, and then analysed via Luminex and indirect ELISA techniques for antibody-binding activity.
Irradiated samples exhibited a measurable reduction in detection by ELISA and Luminex assays compared to non-irradiated controls. The addition of a radioprotectant had no effect in restoring the detection of irradiated antibodies, apart from enhancing detection in a small subset of antibodies.
These results led us to believe the production of free radicals, a consequence of ionising irradiation on water, could have impacted the antibody binding activity. As such, the irradiation processes were repeated at frozen temperatures and subsequently exhibited no loss in detectability when compared to controls.
X-ray-based inactivation methods are promising as a tool to safely handle high-containment clinical samples at lower levels of containment.

8. Sialic acids are cellular attachment factors for EV-D111

Typhaine Filhol[1,2], Alice Mac Kain[1], Marie-Line Joffret[1,2], Nolwenn Jouvenet[1], Vincent Caval[1] and Maël Bessaud[1,2]

[1]Virus Sensing and Signaling Unit, Institut Pasteur, 25-28 rue du Dr Roux, 75015 PARIS, France

[2]World Health Organization Collaboration Centers (WHO-CC) – Epidemiology & macro-evolution of polioviruses and non-polio enteroviruses, Institut Pasteur, 25-28 rue du Dr Roux, 75015 PARIS, France

typhaine.filhol@pasteur.fr

Enteroviruses (EVs) belong to the Picornaviridae family and have been studied since the 1950s. However, the vast majority of studies focused on just a few of the hundreds of EVs infecting humans. EV-Ds, despite infecting humans, are among the poorly studied EVs. Of the five known serotypes, EV-D68 is a respiratory virus, EV-D70 has an ocular tropism and EV-D94, EV-D111 and EV-D120 seem to be enteric viruses. Despite this diversity of tropism, EV-D68, EV-D70 and EV-D94 likely use sialic acids (Sia) as cellular attachment factors.
To deepen our knowledge of the biology of the EV-D species, we focused on EV-D111, a virus that has been isolated from humans and NHPs, suggesting a zoonotic origin.
We investigated the role of Sia in EV-D111 infection using sialidase treatments and loss-of-function experiments in human RD cells. More specifically, we knocked out the gene coding for CMAS, an enzyme essential for Sia metabolism using CRISPR/Cas9 approaches. Viruses with Sia-dependent or Sia-independent entry were used as controls. Assessing viral RNA yield by RT-qPCR analyses and infectious viral particle production by titration assays showed that the absence of Sia at the cell surface significantly slowed down EV-D111 infection kinetics without abolishing it. This suggests that Sia act as an attachment factor, but not as a receptor allowing cellular entry of viral particles. While Sia are not used by most EVs infecting humans, their use seems to be the norm in EV-Ds. This could therefore be an ancestral trait.
As a preference for Sia α2,3 or α2,6 could influence the virus' tissue tropism, we aimed to identify more precisely the isoform used by EV-D111.
Our work provides a better understanding of the biology of EV-D111, which is essential to determine its tropism and its potential to emerge in humans.

9. Experimental characterization of saliva aerosols containing SARS-CoV-2 in an indoor chamber

Jaeyoun Jang[1] and Artur Summerfield[1]

[1]Institute of virology and immunology, Sensemattstrasse 293, 3147 Mittelhäusern

jaeyoun.jang@ivi.admin.ch

The COVID-19 pandemic has brought high demand for a better understanding of the role of viral aerosols in the spread of diseases in indoor environments and the protection against them. The current research on the characteristics of SARS-CoV-2 aerosols using an aerosol chamber in controlled laboratory conditions still lack detailed elaboration. This study aims to characterize saliva aerosols containing SARS-CoV-2 to mimic respiratory aerosol particles using a novel measurement system in a containment facility. The system employs a 1 m^3 stainless steel chamber, an aerosol generator (TOPAS), an optical particle counter (FLUKE 985), and a six-stage Anderson cascade impactor (TECORA). A stock solution of virus diluted with artificial saliva was atomized into the chamber using the nebulizer, and aerosol number concentrations and size distributions were monitored with the particle counter in real time. Viral aerosols were collected by the impactor for 35 minutes using a pump at 28.3 L/min. It was found that saliva aerosols containing SARS-CoV-2 are in the size range of 0.5-2 µm with an average aerosol mass concentration between 154-175 µg/m^3. With the current chamber set up, the half-life of the aerosols was 200 minutes, and 5% of them can remain suspended in the chamber for 10 hours. SARS-CoV-2 concentrations recovered from samples collected by the impactor were 44±4.4 RNA copies/µg. This study supports airborne transmission of SARS-CoV-2 via saliva particles less than 2 µm, which remains airborne for sufficient time to transmit the virus. Moreover, the introduced measurement system facilitates future experimental design for a variety of bioaerosol research and aerosol reduction measures such as air ventilation and purification systems.

10. Role of Tick-borne encephalitis virus non-structural proteins upon infection

Chiara Kalebić[1], Alves Laís Nascimento[1], Yvette Kazungu[1], Federica Dattola[1], Tea Carletti[1] and Alessandro Marcello[1]

[1]Laboratory of Molecular Virology, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

kalebic@icgeb.org

Tick-borne encephalitis virus (TBEV) is an enveloped single-stranded positive RNA member of the *Orthoflavivirus* genus endemic in Central Europe. Like most arthropod-borne viruses with animal reservoirs, its spread depends on climatic and societal changes thus colonizing new areas including Norther Italy. While a vaccine is available, no antivirals have been approved for treatment of infected patients. TBEV is neurotropic with risk of meningitis and meningoencephalitis. TBEV encodes three structural and seven non-structural (NS) proteins. The role of several NS proteins has not been elucidated so far. Understating their role upon infection may lead to a better understanding of the disease and exploited as drug targets.
NS2B, NS4B and NS5 proteins have different roles in modulating the cellular response to infection. NS4B and NS5 have roles in inhibiting stress granules (SGs) formation. SGs are cytoplasmic protein-RNA aggregates formed upon cellular stress to stall translation and allow the cell to restore a homeostasis. The mechanism involves downmodulation of the PKR kinase to provide a conductive intracellular environment for virusreplication. On the other hand, NS2B was able to induce antiviral interferon-stimulated genes in a IRE1α-independent manner, which differs from other NS proteins. The mechanism involves modulation of IRF3 signalling. The tradeoff between virus replication and antiviral signaling is at the core of viral pathogenesis and a potential target for therapy.

11. Development of a Target Amplicon Sequencing -based test for the simultaneous diagnosis and surveillance of viruses associated with neurological disorders

Mohd Jaseem Khan[1], Camila Romano[2] and Ester Sabino[1]

[1]Faculty of Medicine/Institute of Tropical Medicine, University of Sao Paulo, Sao Paulo, Brazil

[2]Department of virology/Institute of Tropical Medicine, University of Sao Paulo, Sao Paulo, Brazil

mjkhan10@usp.br

Since almost 70% of viral encephalitis cases are left without an etiologic agent identified. Arboviruses, Herpesviruses, and Enterovirus present a serious challenge to public health and one of the most important viral agents nearly worldwide. Due to current political, social, and economical situations, a high migratory movement could fuel local outbreaks. In this study, we developed a assay for the diagnosis of viruses from human CSF or other body fluid samples.
The objectives of the study were to design primer panels, validate with laboratory reference viruses and test human clinical samples.
A total of 468 sequences corresponding to different strains or isolates of each viral species were searched from GenBank. A highly conserved region of the targeted gene individually was retrieved, and each target gene was manually checked and artificial sequences along with duplicates sequence were removed for a final list. Multiple sequence alignment was performed for the one-by-one viral gene of each species by using multiple alignment software programs SeaView v4.7.
A total of 24 primers (12 pairs) were selected and synthesized by Thermofisher Scientific. The primers were validated with reference viruses, all were detected specific in a pool of primers as well in a pool of viral genome (Pool-1, DENV-1 DENV-4 and CHIKV, Pool-2, HSV-1, HSV-2, VZV, EBV, CMV and HHV-6, and Pool-3, mix RNA/DNA of arboviruses and Herpes virus). We also tested some human clinical positive and negative clinical samples.
In summary, we developed a highly specific and sensitive sequencing- based test that could be used for the detection and surveillance of viruses associated with encephalitis/meningitis.

12. Genomic Surveillance of Dengue Virus (Serotype 3) in the Afar Region, Ethiopia

Feleke Mekonnen[1], Bilal A. Khan[2,3], Endalkachew Nibret[1], Abaineh Munshea[1], Daniel Tsega[5], Demeke Endalamaw[6], Senait Tadesse[1], Gizachew Yismaw[6], Damtie Lankir[6], Jemal Ali[7], Mariana Ulinici[2,4], **Emanuele Orsini**[2], Ursa Susnjar[2], Tea Carletti[2], Danilo Licastro[11], Molalegne Bitew[8], Marta Giovanetti[9,10] and Alessandro Marcello[2,11]

[1]Bahir Dar University, Bahir Dar, Ethiopia

[2]International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

[3]Dow University of Health Sciences, Karachi, Pakistan

[4]Nicolae Testemitanu Uniresity of Medicine and Pharmacy, Republic of Moldova

[5]Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia

[6]Amhara Public Health Institute (APHI), Bahir Dar, Ethiopia

[7]J.Ali (Afar Public Health and Research Institute)

[8]Bio and Emerging Technology Institute (BETiN), Addis Ababa, Ethiopia

[9]Department of Sciences and Technologies for Sustainable Development and One Health, Universita Campus Bio-Medico di Roma, Italy

[10]Oswaldo Cruz Institute, Rio de Janeiro, Brazil

[11]AREA Science Park, Trieste, Italy

orsini@icgeb.org

Ethiopia has been experiencing annual dengue outbreaks since the first cases were reported in 2013. In 2019 an outbreak occurred in the Afar Region in Noth-Eastern Ethiopia. Dengue serotypes 1 and 2 (DENV-1 and DENV-2) have been reported in the country, while dengue virus serotype 3 (DENV-3) was detected only recently in the Afar Region. Despite the increasing number of cases, much is still unknown about the genomic diversity and evolution of DENV lineages currently circulating in the country. To address this gap, a cross-sectional study was conducted from June to October 2023 on 384 acute febrile patients attending three healthcare facilities in the Afar Region.
This study underscores the dynamic and complex nature of DENV-3 transmission within Ethiopia. The introduction of at least three independent DENV-3 lineages into the Afar region, as indicated by genomic evidence, highlights a significant epidemiological pattern. These introductions have connections with distinct global regions including Asia and Europe, particularly Italy, suggesting international travel and movement as potential vectors for disease spread. Temporal and phylogenetic analyses reveal that while some of the identified strains suggest recent introductions, others indicate more established, ongoing local transmission within the country. These findings are critical for the Ethiopian public health response, emphasizing the need for robust surveillance systems that incorporate genomic epidemiology to track virus evolution and spread effectively. Additionally, this study produced near-complete genomes from the Afar region for the first time using Oxford Nanopore sequencing technology. This approach could be implemented in Ethiopia and other African countries to enhance genomic surveillance and management of DENV transmission in the region.

13. Tecovirimat for Severe Mpox: Assessing Its Clinical Effectiveness

Pierluigi Francesco Salvo[1], Domenico Benvenuto[1], Simona Di Giambenedetto[1,2] and Carlo Torti[1,2]

[1]Dipartimento di Sicurezza e Bioetica, Sezione Malattie Infettive, Università Cattolica del Sacro Cuore, Rome, Italy

[1]Dipartimento di Scienze Mediche e Chirurgiche, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Pierluigi.salvo01@icatt.it

Mpox has recently caused a significant outbreak in non-endemic regions. Although mpox is considered less severe than smallpox, various complications have been documented, and more severe cases may require specific antiviral treatment. Tecovirimat, an FDA-approved antiviral for smallpox, showed promise against mpox in animal models, but human data are still limited. We present two cases of severe mpox treated with tecovirimat, focusing on the challenges and outcomes of its use in individuals with severe skin involvement.
We collected clinical and demographic data from two unvaccinated patients diagnosed with mpox. Both patients provided informed consent.
The first patient, a 55-year-old man living with HIV on antiretroviral therapy and with optimal viroimmunological control, presented with a widespread necrotizing penile lesion, complicated with a bacterial superinfection. Despite initiating tecovirimat (600 mg twice daily), monkeypox virus DNA remained detectable for 35 days, though clinical improvement was documented. The second patient, a 31-year-old man with no comorbidities, showed an extensive nasal lesion. After starting tecovirimat within 48 hours of diagnosis, he experienced significant clinical improvement within 12 days, with viral clearance reached by day 15. In both cases, the medication was well tolerated without adverse effects.
These cases highlight both the potential benefits and limitations of tecovirimat in treating severe mpox. In the first case, tecovirimat was associated with clinical improvement but did not expedite viral clearance, indicating that it may not always lead to rapid virological resolution. The second patient responded more favorably, with clinical and virological improvement within two weeks. The findings suggest a need for further research to define tecovirimat's role in managing severe mpox, therefore we feel that the drug should be more accessible for prescription, at least in selected patients.

14. Developing RT-LAMP for broad-spectrum detection of Zika virus

Ursa Šušnjar[1], Alessandro Marcello[1] and EXPANDIA Project Team

[1]Molecular Virology Lab, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

ursa.susnjar@icgeb.org

The recent pandemic underscored the critical need for timely and reliable local virus identification to effectively manage disease spread on a global scale. Most testing worldwide was conducted in central reference labs using qPCR-based assays. While these molecular tools are powerful, they cannot be scaled to meet the demands of widespread testing, particularly in low-resource settings where access is limited by factors such as geography, cost, reagent shortages, supply chain disruptions, availability of laboratory equipment, and lack of trained personnel. Some of the limitations of gold-standard qPCR can be addressed by alternative diagnostic techniques.

RT-LAMP, an isothermal amplification-based molecular tool with a colorimetric readout, emerges as a promising candidate due to its sensitivity, robustness in detecting viruses in crude samples, ease of use, short turnaround time, and lack of need for specialized equipment.

Leveraging on SARS-CoV-2, we developed a colorimetric RT-LAMP assay using a 2X colorimetric master mix from New England Biolabs for broad-spectrum detection of Zika virus (ZIKV). Phylogenetic analysis identified several phylogenetically distant ZIKV strains from Africa, leading us to design multiple primer versions to ensure high primer homology with a wide range of ZIKV genomes. The final primer mix included 40 primers, allowing detection of ZIKV genotypes belonging to both, the African and Asian lineage. Primers against 18S rRNA were used as a reaction control. We assessed analytical sensitivity and specificity using serial dilutions of ZIKV RNA extracted from spiked human sera. The assay showed no cross-reactivity with other flaviviruses, and no nonspecific amplification was observed after 35 minutes of incubation.

RT-LAMP demonstrated good performance compared to the gold-standard RT-qPCR. Importantly, the colorimetric assay was developed in response to requests from African partners and holds the potential for adoption by community labs in Sub-Saharan Africa.

15. Inhibition of NSB-NS3 Proteases from all the four Serotypes of Dengue Virus

Raheem Ullah[1]

[1]Elettra Sincrotrone Trieste, Trieste, Italy

raheem.biotech@gmail.com

Dengue virus infections causing millions of deaths worldwide, is a major threat to the public health in tropical and subtropical regions. Despite of the growing threat, there is still no approved antiviral therapy or effective vaccine available for the prevention of these infections. As the available treatment is only supportive, so there is an urgent need to develop such therapeutic molecules which can cure epidemic diseases like dengue. Dengue virus NS2B-NS3 protease is considered as a key target to control dengue replication and hence play critical role in the development of antiviral drugs. The objective of the current study was to find specific antiviral molecule which can inhibit infections caused by all four serotypes of dengue, which is itself a challenging task. To accomplish this objective, plant extracts were evaluated to check their inhibitory activity against dengue protease using Fluorescence Resonance Energy Transfer (FRET) assay. The results showed that the three tannins named ellagic acid, punicalin and punicalagin identified from *Punica granatum*, were the potent inhibitors of NS2B-NS3 proteases from dengue serotypes 1-4. The highest inhibition was exhibited by punicalagin with an IC_{50} of 0.91 ± 0.1, 0.75 ± 0.05, 0.42 ± 0.03, 1.8 ± 0.16 µM against DENV1, DENV2, DENV3, DENV4 proteases respectively, followed by punicalin. Further, docking studies suggested a binding

mode of these molecules at the active site of the enzyme through hydrogen bonding and hydrophobic interactions. From our FRET screening assays and molecular docking studies, we can anticipate that these identified molecules can act as effective inhibitors of dengue NS2B-NS3 protease and can help to develop future anti dengue cost-effective drugs.

16. Biochemical characterization of zonulin inhibitor AT1001 derivatives as potential anti SARS-CoV-2 drugs

Vincenzo Vestuto[1], Simone Di Micco[2], Rahila Rahimova[3], Marina Sala[1], Maria Carmina Scala[1], Giovanni Vivenzio[1], Simona Musella[1], Graciela Andrei[4], Kim Remans[5], Lea Mammri[3], Robert Snoeck[4], Giuseppe Bifulco[1], Francesca Di Matteo[1], Pietro Campiglia[1], José A Marquez[6] and Alessio Fasano[7]

[1]Dipartimento di Farmacia, Università Degli Studi di Salerno, Via Giovanni Paolo II 132, 84084, Fisciano, Salerno, Italy

[2]European Biomedical Research Institute of Salerno (EBRIS), Via Salvatore de Renzi 50, 84125, Salerno, Italy

[3]European Molecular Biology Laboratory, EMBL, 71 Avenue des Martyrs, CS 90181, Grenoble Cedex 9, 38042, France

[4]Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, KU Leuven, 3000, Leuven, Belgium

[5]European Molecular Biology Laboratory, EMBL, Meyerhofstraße 1, 69117, Heidelberg, Germany

[6]European Molecular Biology Laboratory, EMBL, 71 Avenue des Martyrs, CS 90181, Grenoble Cedex 9, 38042, France; ALPX S.A.S. 71, Avenue des Martyrs, France

[7]Mucosal Immunology and Biology Research Center, Massachusetts General Hospital-Harvard Medical School, Boston, MA, 02114, USA

vvestuto@unisa.it

Anti SARS-CoV-2 vaccines are considerably addressing the global pandemic, nevertheless, the development of new therapeutics remains critical in controlling the viral spreading. The current most promising strategies involve the use of monoclonal antibodies, although small molecules remain the most investigated option, considering the cost and complications deriving from monoclonal antibodies administration. Here, we describe the development and biochemical characterization of new tripeptide derivatives of AT1001 active against SARS-CoV-2 M^{pro}. Molecular docking studies drove the design and synthesis of a small series of compounds that were then filtered by FRET enzymatic assays, leading to the identification of compound 4 as the most active one. X-ray crystallography studies demonstrated that compound 4 interaction with M^{pro} involved the formation of a covalent bond. *In vitro* antiviral analysis showed that 4 exhibited an improved activity against SARS-CoV-2 M^{pro} compared to the lead compound AT1001. In addition, its efficacy was evaluated in Vero cells, using different viral variants (Wuhan, UK, South African variants). Although compound 4 showed an inhibitory activity against Wuhan and UK variants, it was unresponsive against the South African one; so, additional structural modifications led to compound 58, that showed a significant antiviral activity against all SARS-CoV-2 variants and a valuable safety and a good pharmacokinetic profile after *in vivo* administration. The encouraging results obtained led us to consider compound 58 a starting point for the development of new series of compounds as adjuvant drugs for the treatment of SARS-COV-2 infections.

17. A Humanized-Mouse Model for Assessing Pathogenicity of Emerging Filoviruses

Molly A. Vickers[1], Beatriz Escudero-Pérez[1,2], B.S. Bodmer[3], Monika Rottstegge[1], Estefanía Rodríguez, T. Hoenen[3] and César Muñoz-Fontela[1,2]

[1]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany

[2]German Center for Infection Research (DZIF), Partner Site Hamburg-Borstel-Lübeck-Riems

[3]Institute for Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany

molly.vickers@bnitm.de

Emerging filoviruses, in particular members of the *Orthoebolavirus* and *Marburgvirus* genera, pose a significant zoonotic threat with epidemic potential. The six known species within the *Orthoebolavirus* genus exhibit stark variations in disease severity in humans, from the highly pathogenic Ebola virus to Reston virus, a seemingly nonpathogenic species. The reasons behind these differences in human pathogenicity remain unclear. Understanding the mechanisms behind these variations is crucial for developing vaccines, therapeutics, and assessing the risks of newly discovered filoviruses. A critical need in this research is a suitable model to assess these parameters.
Traditional animal models present limitations: mice and hamsters are not susceptible to filovirus disease, while guinea pigs do not recapitulate febrile illness with wild-type virus. Non-human primates are the current gold standard; but alongside ethical constraints, present with complications in species-specific comparisons, as Reston virus, despite being nonpathogenic in humans, may be lethal in this model. To circumvent these issues, our lab has developed a humanized-mouse model based on HLA-A2 transgenic, NOD-scid-IL2γ receptor knockout mice reconstituted with human hematopoietic stem cells (huNSG-A2).
We have demonstrated that huNSG-A2 mice accurately replicate the ebolavirus-specific case-fatality rates observed in humans, with severity correlating with viremia and a dysregulated, hyperinflammatory response. We also show that, based on their pathogenicity in huNSG-A2 mice, emerging filoviruses such as Bombali virus are likely non-pathogenic for humans. Specifically, huNSG-A2 mice infected with Bombali virus showed fatality rates of 20%, identical to that observed with Reston virus, while Ebola virus was uniformly lethal.
Ultimately, this model provides a valuable tool to add to the pile of pandemic preparedness resources to rapidly assess the potential pathogenicity of newly emergent filoviruses in humans.

18. AVITHRAPID – A European Consortium for the Development of Novel Broad-Spectrum Antivirals

Björn Windshügel[1], Giovanni Chillemi[2], Francesca Incardona[3], Paola Storici[4], Vincenzo Summa[5], Claudio Zamperini[6], Emanuele Nicastri[7], Maurizio Zazzi[8], Miguel Castanho[9], Enzo Tramontano[10], Fabrizio Mammano[11], Aigars Jirgensons[12], Francesca Ceccherini-Silberstein[13], Jan Martinovic[14], Carmine Talarico[15], Mickaël Riou[16], Sonja Bernhard[17] and Silvano Coletti[18]

[1]Fraunhofer ITMP, Schnackenburgallee 114, 22525 Hamburg, Germany

[2]University of Tuscia, Via S. Camillo de Lellis s.n.c., 01100 Viterbo, Italy

[3]EuResist Network GEIE, Via Guido Guinizelli 98/100, 00152 Rome, Italy

[4]Elettra Sincrotrone Trieste S.C.p.A., SS 14 - km 163.5 in AREA Science Park, 34149 Basovizza, Trieste, Italy

[5]Universita Degli Studi Di Napoli Federico II, Via Domenico Montesano 49, 40, 80131 Naples, Italy

[6]First Health Pharmaceutivals B.V., Science Park 406, 1098 XH Amsterdam, Netherlands

[7]National Institute for Infectious Diseases, Via Portuense, 292, 00149 Rome, Italy

[8]Universita Degli Studi Di Siena, Viale Bracci 16, 53100 Siena, Italy

[9]Instituto De Medicina Molecular Joao Lobo Antunes, Avenida Professor Egas Moniz, 1649-028 Lisbon, Portugal

[10]Universita Degli Studi Di Cagliari, Cittadella di Monserrato, SS554, 09042 Cagliari, Italy

[11]Université de Tours, 10 Boulevard Tonnellé, 37032 Tour, France

[12]Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006 Riga, Latvia

[13]Universita Degli Studi Di Roma Tor Vergata, Via Montpellier 1, 00133 Rome, Italy

[14]VSB – Technical University of Ostrava, 17 Listopadu 2172/15, 708 00 Ostrava Poruba, Czechia

[15]Dompé farmaceutici S.p.A., Via Tommaso de Amicis 95, 80131 Naples, Italy

[16]Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, Centre Val de Loire, 37380 Nouzilly, France

[17]Swiss Tropical and Public Health Institute, Kreuzstraße 2, 4123 Allschwil, Switzerland

[18]Chelonia SA, Piazzetta San Carlo 2, 6901 Lugano, Switzerland

bjoern.windshuegel@itmp.fraunhofer.de

The consortium "Antiviral Therapeutics for Rapid Response Against Pandemic Infectious Diseases" (AVITHRAPID). Funded by the European Union and the Swiss State Secretariat for Education, Research and Innovation (SERI), aims to support the development of novel broad-spectrum antiviral compounds by progression of a broad set of pre-existing bioactive small molecules along the drug discovery value chain. AVITHRAPID combines the expertise of 18 partners from 8 countries for the development of several pre-clinical candidates using medicinal chemistry, molecular modeling, biochemical and cell-based assays, X-ray crystallography, in vitro and in vivo PK as well as different animal disease models. Moreover, the consortium aims to identify and validate further viral targets and thereby contribute to the search for novel antiviral targets. The AVITHRAPID activities will allow to establish am early-stage drug discovery pipeline that can be used to rapidly identify and develop novel antiviral compounds against emerging diseases. The talk will provide an overview of the project, the workflow as well as the main objectives and tasks.

19. CIDR: Centre for Infectious Disease Reagents – Advancing Epidemic and Pandemic Preparedness through an Expanded Collection of Research Reagents for Emerging Viruses

Iliana Georgana[1], Rob Cunliffe[1], Nassim Alami-Rahmouni[1], Diane Cook[1], Giada Mattiuzzo[1], Philip J. Hogarth[1] and Yann Le Duff[1]

[1]Vaccines Division (R&D), Medicines and Healthcare products Regulatory Agency (MHRA), South Mimms, United Kingdom

Iliana.Georgana@mhra.gov.uk

The availability of specialised reagents is critical for epidemic and pandemic preparedness, facilitating rapid development of diagnostics, therapeutics and vaccines. The Centre for Infectious Disease Reagents (CIDR), based at the MHRA, was established to address this global need. Expanding on the framework of the Centre for AIDS Reagents (CFAR), CIDR now focuses on a broader range of emerging viruses. The scope of CIDR is guided by priority pathogens' lists from CEPI, WHO, and the UK Vaccine Network, which currently encompasses viruses such as Lassa, Nipah, Marburg, and Ebola.

CIDR is expanding its reagent portfolio through constant engagement with the scientific community to anticipate needs. CIDR is actively producing and commissioning new reagents and encouraging scientists to deposit cutting-edge materials, which are then validated and characterised. These reagents are distributed globally, ensuring researchers have timely and affordable access to essential tools, with a prioritisation to low- and middle-income countries.

Our repository has provided globally over 8,500 vials of reagents in the last decade, including more than 2,700 for SARS-CoV-2 since 2022. These reagents, including virus isolates, antibodies, cell lines, recombinant proteins, and clinical samples, have assisted projects important for advancements in viral research, therapeutic

development, and diagnostic innovation as shown during the COVID-19 pandemic. Currently, CIDR is focusing on producing new pseudotyped viruses and sourcing mAbs for Marburg and Sudan viruses.
CIDR is an indispensable resource, that contributes to the 100 Days Mission by accelerating research on emerging viral diseases and enhancing preparedness for epidemics and pandemics. We invite researchers to utilise and contribute to CIDR (cfar@nibsc.org), facilitating its expansion and driving scientific innovation. Our goal is to continue developing CIDR and supporting research that addresses the world's most pressing pathogen threats, while remaining responsive to changes in pathogen prioritisation.

20. Rational design of novel peptidomimetics against influenza a virus: biological and computational studies

Maria Carmina Scala[1], Mariangela Agamennone[2], Magda Marchetti[3], Fabiana Superti[3], Pietro Campiglia[1], and Marina Sala[1]

[1]Department of Pharmacy, University of Salerno, Fisciano, SA, Italy

[2]Department of Pharmacy, University of Chieti "G. d'Annunzio", Chieti, Italy

[3]Department of Technology and Health, National Institute of Health, Rome, Italy

mscala@unisa.it

The effective treatment of Influenza virus infection is still an unmet goal. Even though some antivirals are available, the alarming increase in virus strains resistant to them highlights the need to find new antiviral compounds.1 An ideal target for new anti-influenza therapy should be a viral component, whose function is essential for virus infection. In this contest, the influenza A virus hemagglutinin (HA) represents a very promising target.
Previously, we identified two tetrapeptides, SKHS (1) and SLDC (2), derived from bovine lactoferrin (bLf) C-lobe fragment 418-429, which were able to bind HA and inhibit cell infection in a concentration range of picomolar.2
Considering the above highlighted, the aim of this study was to synthesize a new library of peptidomimetics active towards influenza virus.3 In order to test their ability to bind HA, we carried out a preliminary screening by biophysical assays such as surface plasmon resonance (SPR) and the orthogonal immobilization-free microscale thermophoresis (MST) assays. Biological and computational studies on most interesting compounds were carried out.
All applied methods agreed upon the identification of a N-methyl peptide, S(N-Me)LDC, able to bind hemagglutinin with high affinity and inhibit influenza virus hemagglutination and cell infection at picomolar concentration.4 This small sequence, with high and broad-spectrum activity, represented a valuable starting point for the design of new peptidomimetics. This work opens the way to new perspectives for the development of new anti-influenza drugs.

With the patronage of

Sponsored By

Funded by the European Union NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA