

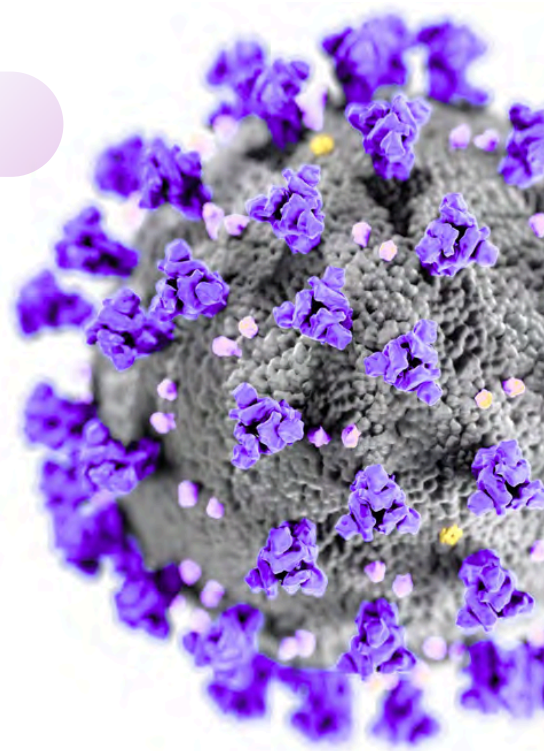
Karolinska Institutet
Campus Flemingsberg
Stockholm

VIROLOGY NEXT 2026

1st -2nd October

Venue:

Jan-Åke Gustafsson
Auditorium,
Neo Building



FLEMINGSBERG
SCIENCE



Karolinska
Institutet

Region
Stockholm



Directions to NEO (Blickagången 16, 141 52 Huddinge)

- From T-centralen, take the commuter train (pendeltåg), either **line 40 towards Tumba** or **line 41 towards Södertälje**
- Take the train to Flemingsberg station and take the exit towards **Utgång Sjukhus/Högskola Rättcentrum**
- Take the escalators up and continue straight across the courtyard towards the pedestrian bridge
- Continue over the bridge and in about 200m, the entrance to NEO can be found towards the right-hand side

Route from Flemingsberg station to NEO



Map: <https://share.google/Vpj9OemUHAGtuVGK2>

Entrance to NEO



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Keynote Speakers



Adolfo García-Sastre, ISMMS



Maria Van Kerkhove, WHO

Invited Speakers



**Stefan Pöhlmann
(DE)**



**Debby van Riel
(NE)**



**Nerea Irigoyen
(UK)**



**Susana Guerra
(ES)**



**Isabelle Imbert
(FRA)**



**Leo Hanke
(SWE)**



**Jayanta Bhattacharya
(IND)**



**Magnus Evander
(SWE)**



**Jonas Klingström
(SWE)**



**Elena Martinelli
(USA)**



**Clive M. Gray
(ZA)**



**Asier Sáez-Cirión
(FRA)**

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Supporting Sponsors

Virology Next 2026 gratefully acknowledges the support of Pixelgen Technologies, Metabolon, and Thermo Fisher Scientific. We thank them for their valuable contributions to the success of the meeting.



Virology Next 2026 Conference

Scientific Programme

1st and 2nd October 2026

Campus Flemingsberg, Jan-Åke Gustafsson Auditorium, Neo building

Day 1

TIME	SESSION / SPEAKER	TOPIC
08:30–09:00	REGISTRATION	
09:00–09:15	Opening Remarks Soham Gupta & Iva Filipovic	
SESSION 1	VIRUS ENTRY AND TROPISM	
09:15–10:00	KEYNOTE Adolfo García-Sastre <i>Icahn School of Medicine</i>	New determinants of host tropism of SARS-CoV-2 and influenza viruses
10:00–10:30 Coffee break		
10:30–11:00	INVITED TALK Stefan Pöhlmann <i>German Primate Center</i>	Targeting Sec61 and Furin for Antiviral Therapy
11:00–11:30	INVITED TALK Debby van Riel <i>Erasmus MC</i>	Neuropathogenesis of emerging viruses: influenza A viruses and SARS-CoV-2
11:30–11:45	SELECTED TALK Meaghan Flagg <i>Umeå University</i>	Induction of Strong Innate Immune Response in Human Alveolar Epithelium is Correlated with Pathogenicity of Avian Influenza Viruses.
11:45–12:00	SELECTED TALK Akhee Sabiha Jahan <i>Statens Serum institut</i>	DNA Vaccination with Inferred Ancestral Antigens: A New Strategy Against Evolving Swine Influenza Viruses
12:00–13:00 Lunch		
SESSION 2	HOST-VIRUS INTERACTION	
13:00–13:30	INVITED TALK Nerea Irigoyen <i>Cambridge University</i>	What can ribosome profiling teach us about Zika virus?
13:30–14:00	INVITED TALK Susana Guerra <i>Universidad Autónoma de Madrid</i>	ISG15-mediated antiviral restriction of emerging MPXV strains
14:00–14:15	SELECTED TALK Ana Cindrić <i>Genos d.o.o.</i>	Immunoglobulin G glycosylation as a modulator of antiviral immunity: from acute respiratory virus infection to maternal and infant RSV immunisation.
14:15–14:30	SELECTED TALK Mariano A Molina <i>Karolinska Institutet</i>	Multimics profiling reveals distinct cervicovaginal states across high-risk HPV infections.
14:30–15:00 Coffee break		

TIME	SESSION / SPEAKER	TOPIC
SESSION 3 · ANTIVIRALS, ANTIBODIES AND VACCINES		
15:00–15:30	INVITED TALK Isabelle Imbert <i>Aix Marseille Université</i>	Deciphering molecular replication in RNA viruses: paving the way for new antiviral strategies
15:30–16:00	INVITED TALK Leo Hanke <i>Karolinska Institutet</i>	Structural basis of henipavirus fusion neutralization
16:00–16:30	INVITED TALK Jayanta Bhattacharya <i>THSTI</i>	Monoclonal antibodies as rapid medical countermeasures for priority pathogens
16:30–16:45	SELECTED TALK Lara Sweetapple <i>Karolinska Institutet</i>	A Pre-GMP mRNA–LNP Platform to Accelerate Vaccine Development in the Nordics.
16:45–17:00	SELECTED TALK Britta Wahren <i>Karolinska Institutet</i>	Therapeutic vaccine regimens result in reduction of viral reservoir in pediatric HIV
17:00–20:00 Poster session + mingle + dinner		

Day 2

09:00 – 09:45 · INAUGURATION OF CSIB

09:00 - 09:03	Matti Sällberg <i>Campus Vice President KI South, Karolinska Institutet</i>	
09:03 - 09:13	Ujjwal Neogi, Iva Filipovic <i>Director/Co-director, Center for Systems Infection Biology, Karolinska Institutet</i>	
09:13 - 09:16	Maria Van Kerkhove <i>Director, Department of Epidemic and Pandemic Management, World Health Organization</i>	
09:16 - 09:19	Sara Heelge Vikmång <i>Kommunstyrelsens ordförande, Huddinge Kommun</i>	
09:19 - 09:21	Jonas Fuxe <i>Head of Department, Department of Laboratory Medicine, Karolinska Institutet</i>	
09:21 - 09:24	Christian Giske <i>Head of AMR and Healthcare-Associated Infection, European Centre for Disease Prevention and Control</i>	
09:24 - 09:27	Annika Karlsson <i>Division Head, Division of Clinical Microbiology, Labmed, Karolinska Institutet</i>	
09:27 - 09:30	Natasha Bank <i>Cluster Manager, Flemingsberg Science</i>	
TIME	SESSION / SPEAKER	TOPIC
09:45 – 10:30	KEYNOTE Maria Van Kerkhove <i>WHO</i>	Global pandemic preparedness
10:30–11:00 Toast, mingle + photo		

SESSION 4 · ZOO NOTIC AND EMERGING VIRUS PREPAREDNESS		
11:00–11:30	INVITED TALK Magnus Evander <i>Umeå University</i>	Rift Valley Fever at the Human-Animal-Environment Interface
11:30–12:00	INVITED TALK Jonas Klingström <i>Linköping University</i>	Hantaviruses on the move - preparedness and possible novel treatments
12:00–12:15	SELECTED TALK Peter Sjö <i>Drugs for Neglected Diseases initiative (DNDi)</i>	Identification of Host-Directed Therapies (HDTs) to prevent dengue disease escalation.
12:15–12:30	SELECTED TALK Léna Vandenabeele <i>Karolinska Institutet</i>	Comparative analysis of hantavirus infection in human and natural host cells.
12:30–13:30 Lunch		
SESSION 5 · MUCOSAL AND CELLULAR ANTIVIRAL IMMUNITY		
13:30 – 14:00	INVITED TALK Elena Martinelli <i>Northwestern University</i>	Host programs shaping viral persistence and antiviral immunity in HIV/SIV: from IFN to TGF-β
14:00–14:30	INVITED TALK Clive M. Gray <i>Stellenbosch University</i>	The Immune Landscape of the Placenta at the Maternal: Fetal Interface: The Case of Maternal HIV and CMV Co-Infections
14:30–15:00	INVITED TALK Asier Sáez-Cirión <i>Institut Pasteur</i>	Resilient CD8 T cells: An alternative differentiation pathway associated with optimal response against persistent viruses
15:00–15:15	CLOSING REMARKS	



Oral Presentations

Induction of strong innate immune response in human alveolar epithelium is correlated with pathogenicity of avian influenza viruses

Meaghan Flagg^{1,*}, Brandi Williamson¹, Johan Ortiz-Morales¹, Tessa Lutterman¹, Bridget Brackney¹, Christopher Winski¹, Emmie de Wit¹

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*Present address: Department of Clinical Microbiology, The Laboratory for Molecular Medicine Sweden (MIMS), Umeå University, Umeå, Sweden

Background: Human infections with avian influenza A viruses (AIVs) historically resulted in severe disease marked by respiratory distress, cytokine storm, and mortality in >50% of cases. Currently, clade 2.3.4.4b H5N1 AIVs are widely distributed in North America, and 70 human cases have been reported in the U.S. since 2024. In contrast to previous outbreaks, human infections with clade 2.3.4.4b H5N1 AIVs have largely resulted in mild disease. However, several severe cases requiring hospitalization have been reported. Notably, severe cases were frequently associated with infection by genotype D1.1 H5N1 viruses, as opposed to the co-circulating B3.13 genotype. The host and viral factors that contribute to differences in pathogenicity between AIVs are not understood.

Methods: We utilized human lung organoids and air-liquid interface cultures to compare virus replication and host responses to AIVs in primary human type 1 and type 2 alveolar epithelial cells. We compared two contemporary clade 2.3.4.4b genotype B3.13 viruses (A/Texas/37/2024, A/bovine/Ohio/B24OSU-342/2024), two clade 2.3.4.4b genotype D1.1 viruses (A/Wyoming/01/2025, A/Nevada/10/2025), and two historical AIVs (A/Vietnam/1203/2004 H5N1, A/Anhui/1/2013 H7N9).

Results: In type 2 alveolar epithelial cells, D1.1 genotype viruses and historical AIVs replicated to similar titers, while B3.13 genotype viruses were slightly attenuated. Distinct patterns of virus replication were observed in type 1 alveolar cells, where all clade 2.3.4.4b replicated to higher titers compared to historical AIVs. Activation of innate immune responses, indicated by up-regulation of interferon-stimulated genes, differed between viruses. Isolates obtained from severe or fatal cases (A/Wyoming/01/2025, A/Vietnam/1203/2004, A/Anhui/1/2013) induced significantly stronger innate immune responses compared to viruses isolated from mild cases, even when virus replication was very similar.

Conclusions: These data suggest that induction of strong innate immune responses in human alveolar epithelium may contribute to pathogenicity of AIVs and highlight the potential applications of human organoid systems for risk assessment of emerging viruses.

DNA vaccination with inferred ancestral antigens: a new strategy against evolving swine influenza viruses

Akhee Sabiha Jahan¹, Klara Marie Anker¹, Charlotte Kristensen², Bjørg Skovmand Helstad², Charlotte Torp², Ria Lassaunière¹, Anders Fomsgaard¹, Ramona Trebbien¹, Richard Webby³, Lars Erik Larsen², Charlotta Polacek¹

¹*Department of Virus and Microbiological Special Diagnostics, Statens Serum Institut, Copenhagen, Denmark*

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³*Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA*

Background: Swine influenza A viruses (swIAVs) continuously evolve through mutation and reassortment, generating antigenically diverse viruses with zoonotic potential. Vaccines capable of providing broad protection against evolving swIAVs could therefore improve disease control in pigs while reducing the risk of zoonotic transmission. We evaluated a DNA vaccine based on inferred ancestral hemagglutinin (HA) and neuraminidase (NA) sequences as a strategy to broaden protection against swIAV.

Methods: Piglets were immunized twice by needle-free delivery with DNA constructs encoding inferred ancestral HA and NA (HA/NAanc) or H1N1pdm09 HA and NA (HA/NApdm09). Control groups received DNA encoding internal IAV proteins, an unrelated glycoprotein, or PBS. Animals were subsequently challenged with a heterologous zoonotic Danish swine-origin H1N1 virus. Humoral and cellular immune responses were assessed by ELISA, ELISpot, virus neutralization, and ELLA. Viral loads in nasal swabs and lung tissue were quantified by qRT-PCR and plaque assay, and lung inflammatory responses were assessed by gene-expression analysis.

Results: Both HA/NA vaccines induced cross-reactive humoral and T-cell responses. HA/NAanc generated recall and neutralizing antibody responses comparable to HA/NApdm09. Following heterologous challenge, HA/NAanc improved viral clearance from the lower lungs and shortened the duration of nasal viral shedding compared with PBS controls. Lung gene-expression analysis additionally indicated reduced airway inflammation in HA/NAanc-vaccinated pigs.

Conclusions: Vaccination with inferred ancestral HA and NA induced cross-reactive humoral and cellular immunity and improved control of heterologous swIAV infection. These findings support ancestral-sequence-based DNA vaccination as a promising strategy for developing broader vaccines against evolving swIAVs.

Immunoglobulin G glycosylation as a modulator of antiviral immunity: from acute respiratory virus infection to maternal and infant RSV immunization

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¹Genos Glycoscience Research Laboratory, Zagreb, Croatia

²Queen Mary University of London, London, United Kingdom

³Faculty of Pharmacy, University of Zagreb, Zagreb, Croatia

Background: Immunoglobulin G (IgG) is a central effector of antiviral immunity, and its activity is significantly influenced by the conserved N-glycan at Asn297 of the Fc region, which modulates FcγR and complement binding and tunes the immune response. Variation in galactosylation, sialylation, bisecting N-acetylglucosamine (GlcNAc) and core fucosylation shifts antibodies along the pro- to anti-inflammatory axis, making the IgG glycome an informative readout of antiviral immune function.

Methods: Total IgG N-glycomes were profiled by released-glycan UHPLC analysis across independent and longitudinal cohorts spanning mild to fatal COVID-19, seasonal influenza and healthy controls. Derived IgG glycosylation traits were compared and related to clinical course and outcome.

Results: In COVID-19, severity was most consistently distinguished by reduced bisecting GlcNAc; longitudinal analysis across four cohorts showed that severe disease associates with decreased galactosylation, sialylation and bisecting GlcNAc and increased fucosylation of total IgG, a signature of reduced immunosuppressive capacity that scaled with severity. These changes were functionally relevant, as IgG glycovariants from poor-prognosis patients elicited stronger natural killer cell activation, and traits measured at diagnosis carried prognostic value. Comparing infections, galactosylation remained stable in influenza patients and COVID-19 survivors but fell in fatal COVID-19, while influenza showed time-dependent shifts in sialylation, fucosylation and bisecting GlcNAc; the survivor and influenza profiles may reflect an adequate response to enveloped viruses, whereas fatal COVID-19 marks its deviation.

Conclusions: These findings highlight the IgG glycome as a functional marker distinguishing protective from pathological antibody responses across respiratory viruses, with the strongest pro-inflammatory shifts associating with fatal disease. Whether immunisation reshapes the same effector-relevant glycan features is a question we are currently answering through the longitudinal profiling of maternal and infant antibodies within the Horizon PIPELINE RSV immunisation platform, which also holds implications for early-life antiviral protection and pandemic preparedness.

Multiomics profiling reveals distinct cervicovaginal states across high-risk HPV infections

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²*Department of Cellular Therapy and Allogeneic Stem Cell Transplantation (CAST), Karolinska University Hospital, Huddinge, Sweden*

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⁵*Center for Molecular and Biomolecular Informatics, Radboud University Medical Center, Nijmegen, 6525 GA, The Netherlands*

Background: High-risk human papillomavirus (hrHPV) infections show substantial heterogeneity in persistence and oncogenic potential. While viral genotype is an important determinant of risk, the contribution of the local cervicovaginal microenvironment remains incompletely understood. We investigated how hrHPV infection is associated with coordinated changes in the cervicovaginal microbiome and host transcriptome.

Methods: We performed integrated microbiome and host transcriptomic analyses in hrHPV-positive women to characterize relationships between bacterial communities, host gene expression, and viral features using circular probe-based RNA sequencing (ciRNAseq). Multiomics associations were assessed across 300 women, while HPV genotype-risk analyses were performed in a subset of 97 women.

Results: Distinct cervicovaginal microbial profiles were associated with different hrHPV genotype-risk groups, with specific bacterial species enriched in lower- or higher-risk infections. Host transcriptional profiles also varied across the cervicovaginal microenvironment, revealing differences in pathways linked to immune regulation, epithelial responses, and cellular stress. Integration of both data layers identified coordinated relationships between individual bacterial species and host transcriptional programs. Several of these microbial and host features were also associated with clinically higher-risk HPV states, suggesting that viral genotype, local microbial composition, and host response are biologically interconnected.

Conclusions: hrHPV infections are associated with distinct cervicovaginal microbial and host transcriptional states. These findings support a model in which viral genotype interacts with the local microenvironment to shape HPV infection biology and potentially influence subsequent disease risk. Multiomics profiling may therefore provide new insight into the mechanisms underlying heterogeneity in hrHPV infection outcomes.

A pre-GMP mRNA–LNP platform to accelerate vaccine development in the Nordics

Lara Sweetapple^{1,2}, Miina Ojansivu^{1,2}, Jingy Yan¹, Christian An Binh Nordentoft¹, Gustaf Ahlén^{1,2}, Matti Sällberg^{1,2}

¹*Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden*

²*Karolinska ATMP Center, Stockholm, Sweden*

mRNA–lipid nanoparticle (LNP) technology offers a powerful and adaptable approach to vaccine development, enabling rapid design, scalable production, and potent immune responses. However, limited regional infrastructure for mRNA–LNP manufacturing and analytics remains a barrier to translating promising vaccine candidates towards clinical development.

Here, we present the development of a Swedish Pre-GMP mRNA–LNP platform designed to bridge early-stage vaccine research and future GMP manufacturing. The platform integrates mRNA synthesis by in vitro transcription and HPLC purification with reproducible microfluidic LNP formulation, alongside analytical workflows assessing mRNA integrity, identity and modifications, as well as LNP size, encapsulation efficiency and stability.

The platform has demonstrated robust and reproducible production of mRNA–LNPs with high encapsulation efficiency and narrow particle-size distributions, as well as strong mRNA expression and immunological responses. Following successful preclinical evaluation of a luciferase reporter mRNA–LNP, the platform was applied to the development of a Crimean-Congo Hemorrhagic Fever (CCHF) mRNA–LNP vaccine. The candidate recently demonstrated strong neutralising activity against CCHF in a pseudovirus assay and conferred protection against lethal Hoti and IbAr CCHFV challenge in mice. The platform is now supporting the development of additional vaccines against infectious viral diseases, including hepatitis, dengue, Ebola and hantavirus.

This Pre-GMP platform establishes an integrated foundation for mRNA–LNP vaccine development in Sweden and the wider Nordic region. Building on the current capabilities, the platform is being advanced towards full GMP manufacturing and analytical capabilities in 2027, with the aim of accelerating vaccine candidates from discovery through preclinical development and ultimately into clinical translation.

Therapeutic vaccine regimens result in reduction of viral reservoir in pediatric HIV

Britta Wahren¹, Paolo Palma²

¹Karolinska Institutet, Stockholm, SE with the HIVIS-DNA study group

²Bambino Gesù Children Hospital IRCSS and University of Rome Tor Vergata, Rome, IT, with the HVRRICANE study group

Background: HIV is the most deleterious chronic disease, mortal if not continuously treated. Over 100.000 prenatally HIV-infected children are born each year, and over 2.4 million children and young adults are living with HIV in 2026 (UNAIDS, WHO). Children born by HIV-infected mothers mount weak, if any, immune responses to their virus, generating a condition similar to immunological tolerance. The genetic variability of HIV-1 is extreme, and the defective immune system cannot cope with the constant stream of new and variable viruses.

Methods: To address the genetic heterogeneity, we developed a multigene, multi-subtype vaccine HIVIS-DNA containing sequences from subtypes A, B and C.

Results: This vaccine, followed by pox-virus-based MVA-CMDR induced broad natural killer, cellular and humoral responses in therapeutic and prophylactic clinical studies in adults. During therapy interruption the virus relapsed, but the strains were still susceptible to antiretroviral drugs. A first childhood study in 5-9-year-old perinatally HIV infected children evaluating solely HIVIS-DNA as an immunotherapeutic strategy elicited augmented HIV-specific T- and B-cells. In a second study in older children (age range 9-16y), everybody responded with new HIV-specific cellular and humoral immunity. The addition of MVA-CMDR resulted in additional T-cells and augmented antigen-specific antibodies. The HIVIS-DNA together with the MVA-CMDR vaccination and an adjuvant consisting of a TLR agonist, generated HIV-specific CD8 T-cell immunity that correlated with significant reduction in HIV intact proviruses exclusively in the adjuvanted arm. The inducible viral reservoir was lower compared to those of children exclusively on antiretroviral treatment.

Conclusions: These results lend to hope, that sequential immunization strategies with varied genetic vaccine compositions including adjuvants, will sustain and improve innate and adaptive immune responses to complement and improve long-term antiretroviral therapies.

Identification of host-directed therapies (HDTs) to prevent dengue disease escalation

Peter Sjö¹

¹*Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland*

An estimated 400 million people are infected by dengue virus yearly, with almost half of the world's population living in areas at risk of dengue. During dengue epidemics, management of severe cases rapidly overwhelms fragile healthcare systems in disease-endemic countries. Despite the staggering numbers and impact on society, efficient and affordable dengue antivirals as well as treatments preventing disease escalation are still missing.¹

Most people infected by dengue virus develop mild symptoms, but some develop a severe and sometimes life-threatening form of the disease. Severe dengue is associated with plasma leakage, bleeding, and organ dysfunction. Today, no treatment exists to prevent or reverse progression to severe disease. Current management is limited to symptomatic support, including IV fluids, with round-the-clock monitoring for patients showing warning signs. Vaccines and vector control reduce transmission but face immunological limitations and do not treat established infection. Despite the scale of dengue, the therapeutic development pipeline remains severely underpopulated: few antivirals have progressed from preclinical into clinical studies and, apart from the SII dengue mAb none has moved to Phase III clinical studies. The Dengue Alliance aims to identify host-directed therapies (HDTs) to prevent disease escalation caused by dysregulation of the host immune response during early-stage dengue virus infection. Ultimately the HDT therapy will be combined with a dengue antiviral to increase the treatment window for incoming patients with symptoms.

In this study, we have used a combination of Machine Learning and Artificial Intelligence-based approaches, together with in vitro and in vivo validation studies to identify host-directed therapies (HDTs) with the potential to reduce the risk of disease escalation in patients with dengue infection, by maintaining vascular integrity.

Comparative analysis of hantavirus infection in human and natural host cells

Léna Vandenabeele¹, Benjamin Nilsson-Payant¹

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Hantaviruses are zoonotic, negative-sense, segmented RNA viruses. They are transmitted by rodents, shrews and other small mammals to humans, where they can cause either hantavirus cardiopulmonary syndrome (HCPS) or haemorrhagic fever with renal syndrome (HFRS). It is not yet known why hantaviruses cause disease in humans but are asymptomatic in their respective natural hosts.

To elucidate the mechanisms behind this, we are studying hantavirus infections *in vitro* by using relevant human and natural host species cell models and their respective hantavirus species, including Puumala virus (PUUV), Tula virus (TULV) and Seoul virus (SEOV). We compare the antiviral and general transcriptional host response to hantavirus infection by RT-qPCR and bulk RNA-seq to explore differences between host species that could explain differences in viral pathogenesis. In addition, we investigate the effects of host adaptation and host species context on the viral replication landscape and its consequences for innate immune induction. This project improves our understanding of the molecular mechanisms underlying virus-host interactions and hantavirus diseases and could shed new light on why hantaviruses cause disease in humans but not in their natural host species.



Poster Presentations

Poster 1

Dual-track antiviral discovery for mpox preparedness using a high-throughput phenotypic screening platform

Bert Vanmechelen¹, Rana Abdelnabi¹, Thibault Francken², Erik Nijs¹, Jana Meerkens¹, Leni Van Eyck¹, Stijn Verschoren¹, Bertrand Van Schoubroeck¹, Winston Chiu², Joost Schepers², Viktor Lemmens², Pieter Leyssen², Nelleke Cloet³, Wim Smets³, Hugo Klaassen³, Laurens Liesenborghs^{2,4}, Patrick Chaltin³, Johan Neyts² and Koert Stittelaar¹

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⁴*Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.*

Monkeypox virus (MPXV) is an orthopoxvirus closely related to variola virus (smallpox). Although MPXV infection (mpox) is usually a self-limiting disease, severe illness and death can occur in immunocompromised individuals. Recent outbreaks of MPXV involved sustained human-to-human spread across multiple continents, leading WHO to declare it a Public Health Emergency of International Concern in July 2022 and August 2024. Despite available vaccines and some active antivirals like tecovirimat, therapeutic options are scarce and resistance remains a concern. Here, we report the development and application of a large-scale phenotypic assay to identify MPXV inhibitors using a cytopathic effect (CPE)-based readout in human foreskin fibroblasts expressing a nuclear mRFP670 reporter. Infection with MPXV induced ~80% CPE within five days with a robust $Z' > 0.7$. In the frame of pandemic preparedness, this novel assay was used to screen two compound libraries: the KU Leuven Repurposing library (KURE), containing ~7,000 approved or clinical stage small molecules, and a large, chemically diverse library of ~350,000 molecules. This latter screen identified ~700 preliminary hits, currently undergoing confirmation and subsequent prioritization ranking to identify promising novel chemical scaffolds for future therapeutic development. In addition, hits from the repurposing screen, primarily classified as nucleoside analogues, anti-infectives (e.g. tecovirimat) or kinase inhibitors, are being evaluated for their potential use in combination therapies. Leveraging the advanced clinical development of these molecules supports a fast-track approach to improve preparedness for future outbreaks, complementing the longer-term development of next-generation antivirals against MPXV and related orthopoxvirus threats.

Poster 2

Advancements in high-throughput plasma proteomics using Olink® Reveal

Raza Ahmed¹, Ryan Lamers¹, Paula Perin¹, Agnese Bolognesi-Winfield¹, Kerhan Woo¹

¹Olink Proteomics (part of Thermo Fisher Scientific), Waltham, MA, USA

Background: Large-scale cohorts like the UK Biobank are advancing precision medicine by integrating proteomics with other omics data. Scalable plasma proteomics enables minimally invasive, systemic profiling across populations, however, broad coverage—especially of immune-related proteins—remains limited by cost and complex workflows. Olink's Proximity Extension Assay (PEA™) addresses these challenges with high sensitivity, specificity, and scalability. To expand access, Olink developed the Olink® Reveal panel: an easy to use, 1,034-protein assay including 537 immune targets. Informed by data from ~54,000 UK Biobank participants, the panel features over 850 disease-associated proteins, enabling deep biological and genetic insights.

Methods: Olink® Reveal uses a novel lyophilized multiplex assay format that leverages the affinity-based PEA™ technology, combined with next generation sequencing (NGS) readout from 4 µL of sample. The streamlined workflow delivers data in as little as 1.5 days with only 2 hours of hands-on time, using standard lab equipment and NGS infrastructure.

Assay performance and reproducibility of the platform was evaluated including a pilot study of EDTA plasma samples across four independent test sites. Detectability metrics and correlation analyses were performed to assess assay robustness.

Results: Assay performance and detectability exceeded 86% for most proteins, with inter- and intra- assay variabilities (CVs) of <10%. Pearson correlation coefficients of all data generated between the four independent test sites ranged from R=0.96 to 0.98; thus, demonstrating robust assay performance.

Conclusions: Olink® Reveal represents a breakthrough in accessible, large scale discovery proteomics. Its simplified workflow and broad protein coverage lower barriers to entry, enabling integration of proteomics into population-scale studies. With deep representation of inflammation-related pathways, researchers are empowered to uncover novel disease mechanisms and biomarkers with unprecedented ease.

Poster 3

Deep immune profiling delineates lymphoid and myeloid cellular population remodeling during long-term suppressive antiretroviral therapy

Panagiotis Andriopoulos¹, Anoop T Ambikan¹, Iva Filipovic¹, Magda Lourda¹, Ujjwal Neogi¹

¹Center for Systems Infection Biology, Department of Laboratory Medicine, Karolinska Institutet, Sweden

Background: Despite effective antiretroviral therapy (ART), people with HIV may continue to experience persistent immune activation and incomplete restoration of immune function. The relationship between alterations in circulating myeloid cells and broader lymphocyte remodeling remains insufficiently understood. Comprehensive profiling of circulating immune populations may provide further insight into the immunological state associated with treated HIV.

Objectives: To characterize persistent innate and adaptive immune alterations in people with HIV receiving long-term suppressive ART.

Methods: High-dimensional spectral flow cytometry was used to profile circulating myeloid and lymphoid populations in people with HIV receiving suppressive ART for a prolonged duration and in HIV-seronegative healthy controls. Multiple immune lineages and differentiation, activation and functional phenotypes were evaluated using conventional gating and multidimensional analytical approaches. Publicly available transcriptomic datasets were used to provide complementary biological context.

Results: Long-term treated HIV infection was associated with coordinated changes across multiple immune compartments. Altered cellular composition or phenotypic and functional states were observed within circulating myeloid populations, together with population-specific remodeling of T cells, B cells, and NK cells. Analysis of independent transcriptomic datasets provided complementary biological context and helped relate the flow-cytometry observations to broader pathways associated with immune regulation and cellular function. Of note, the myeloid cell populations also showed altered metabolic state identified by the genome scale metabolic models. These findings indicate that viral suppression does not fully normalize the peripheral immune landscape.

Conclusions: Our study identifies persistent multicompartiment immune alterations in people with HIV receiving long-term suppressive ART and provides biological insight into residual inflammation and immune dysfunction despite effective viral suppression.

Chikungunya virus induces a cell-autonomous, rheumatoid arthritis-like transcriptional programme in primary human skeletal muscle cells

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Background: Chikungunya virus (CHIKV) has resurged globally, with over 1.6 million suspected cases reported in 2024-2025. Myalgia and myositis are cardinal features, and murine studies restricting CHIKV replication in skeletal muscle show that muscle infection is required for acute disease, whereas recent single-cell and spatial transcriptomic work implicates joint-associated macrophages, rather than muscle, in chronic persistence. How human muscle cells themselves respond remains poorly defined.

Methods: Primary human skeletal muscle cells (HSkMC) were infected with CHIKV ECSA strain MY/08/4567 at MOI 1 and harvested in biological triplicate at 0, 6, 12- and 24-hours post-infection (hpi). Twelve poly(A)-selected bulk RNA sequencing libraries (RIN > 7) were sequenced on an Illumina platform, 150-bp paired-end. Differential expression used a two-fold threshold at $q < 0.05$, with Gene Ontology and KEGG enrichment, hierarchical clustering, and qPCR validation.

Results: HSkMC supported robust replication, peaking at 10^8 PFU/mL. Reprogramming was steeply time-dependent: 638, 4,777 and 10,132 genes were differentially expressed at 6, 12 and 24 hpi respectively (5,676 up and 4,456 down at 24 hpi), with 417 genes altered at every timepoint. Early responses were dominated by cytokine-cytokine receptor interaction, TNF, NOD-like receptor and NF- κ B signalling; late responses shifted towards mitophagy and cellular senescence, consistent with muscle atrophy in chronic alphavirus models. Thirty rheumatoid arthritis pathway genes, including IL6, TNF, MMP1, IL1A, CCL3 and CCL20, were induced alongside CXCL10, CXCL11 and CCL5. qPCR confirmed approximately 20-fold IL-6 induction.

Conclusions: Without any immune cells present, CHIKV-infected human muscle cells autonomously generate the chemokine and arthritis-associated signature usually attributed to infiltrating leukocytes, positioning muscle as an early amplifier rather than a passive target. This is the most comprehensive muscle-intrinsic human CHIKV transcriptome reported to date and nominates IL-6, senescence and mitophagy as host-directed targets.

Characterizing the structural and functional aspects of the henipavirus glycan shield

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Introduction: Enveloped viruses hijack the glycosylation machinery of the host cells during secretion, decorating themselves with N-linked and O-linked glycans that support viral entry, propagation, and immune evasion. The EU SHIELD consortium aims to identify new strategies to disrupt viral entry and glycan shielding to prepare for a future pandemic. By combining glycoanalysis with structure-guided CRISPR/Cas9-based glycoengineering, we here aimed to evaluate the functional importance of key glycans to guide vaccine design for threats such as henipaviruses, for which there are currently no available vaccines.

Methods: We employed a comprehensive glycomic and glycoproteomic approach to investigate glycan structures and their relative distribution at glycosylation sites. Total released N- and O-glycan profiles were determined on recombinant purified fusion (F) and attachment (G) proteins from Hendra and Nipah viruses, using a sensitive mass spectrometry-based glycomics protocol. Subsequently, we evaluated site-specific heterogeneity using two different approaches. First, we harnessed the differential potential of Endoglycosidase H and PNGase F in a sequential enzymatic approach to classify each N-glycosite as complex or hybrid/high-mannose. Second, to further elucidate the microheterogeneity at individual N-glycosites we performed intact glycopeptide analysis. A similar strategy was used for intact O-glycopeptide analysis, with a reduction of analyte complexity by removing N-glycans and sialic acid by PNGase F and neuraminidase, respectively.

Having identified glycan identities and location, we next dissected their functional roles. To this end, we used glycoengineered cell lines with defined differences in one or more elaboration steps of glycan synthesis, for example, MGAT1 KO, which abrogates the formation of any complex glycans, or ST6GAL1+2 KO, which abrogates the addition of 2,6 sialylation. The functional contribution of the glycans to fusion was evaluated by a transient transfection of F and G proteins in the glycoengineered cell lines and analyzing syncytium formation upon engagement of endogenous entry receptors.

Preliminary Results: We report more than 200 different N-glycan and 14 O-glycan structures decorating viral proteins. We furthermore map the location of the N- and O-glycans and show the abundances of complex and non-complex N-glycans in F and G proteins from Hendra and Nipah virus. Moreover, intact N-glycopeptide analysis demonstrated that some N-glycan sites have more heterogeneous glycan profiles than others, which may influence epitope accessibility and potential functional implications. Finally, fusion assays in glycoengineered cell lines demonstrate the importance of multiple glycan classes, highlighting their potential relevance for viral protein or cellular entry interactions, which will be explored further.

Japanese encephalitis virus exploits a mitochondrial glutamine–GOT2 axis to sustain replication

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Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in Asia, yet the host pathways supporting its replication in the central nervous system remain incompletely understood, and no specific antiviral therapies are available. Here, we integrated time-resolved transcriptomic and proteomic profiling using directionality-penalized multi-omics analysis to map host responses to JEV infection in mouse neuroblastoma (Neuro-2a) cells and the brains of infected mice. JEV induced a coordinated, temporally structured response characterized by progressive upregulation of interferon-stimulated antiviral proteins alongside extensive reprogramming of central carbon and energy metabolism, including the tricarboxylic acid cycle, glycolysis, oxidative phosphorylation and glutaminolysis. Temporal clustering of the brain proteome associated oxidative phosphorylation remodelling with increased reliance on glutamine metabolism. Pharmacological inhibition of glutaminolysis with 6-diazo-5-oxo-L-norleucine (DON) suppressed viral RNA, whereas inhibition of glycolysis or modulation of AKT signalling had no comparable effect. Moreover, silencing the mitochondrial transaminase GOT2 reduced viral RNA, protein abundance and infectious-virus production in human SH-SY5Y cells. Together, these findings establish the glutamine–GOT2 axis as a metabolic dependency of JEV replication and nominate it as a candidate host-directed therapeutic target for JEV and potentially other neurotropic flaviviruses.

Serological investigation of West Nile virus in dogs

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West Nile virus (WNV), of the genus *Flavivirus*, is a zoonotic mosquito-borne virus with birds as a reservoir. WNV has been detected on most continents. It can cause meningoencephalitis in humans and horses and also affects other animals as cat, dog, sheep and llama. Even though most species appear to be an epidemiological dead end, the detection of the virus in so many different hosts is concerning, especially when some of these animals are in such close proximity to humans and other species.

Furthermore, climate change is increasing the emergence and spread of infectious diseases, highlighting the need for effective One Health surveillance. In this context, dogs represent promising sentinel animals for West Nile virus infection.

In this context, we investigated the prevalence of WNV in the domestic dog population. A total of 552 serum samples collected in the Apulia region of southern Italy between 2023 and 2025 were screened using a qualitative ELISA. All the positive and borderline samples, together with a subset of negative samples, were further analysed by live-virus microneutralization (MN) assay using the WNV Lineage 1 Italy/2009 strain.

Of 552 samples tested, 36 were positive by ELISA and 3 were doubtful. All samples that tested negative by ELISA were also negative by MV assay. Of the 3 borderline cases, 2 tested positive in the MN assay. Of the 36 positive cases, 31 also tested positive in the MN assay. This brings us to a prevalence of 5,98% in our population, with highly variable ID50 values.

In conclusion, these findings suggest the circulation of WNV in the dog population of the Apulia region. The use of dogs as sentinel animals, together with validated serological tools, could represent a valuable component of surveillance programs within a OneHealth framework.

Modeling alphavirus neuropathogenesis in region-specific human brain organoids

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Introduction: Chikungunya virus (CHIKV), a re-emerging alphavirus transmitted via mosquito vectors, causes severe joint pain, fever, and arthritis in humans. In a subset of infected individuals, CHIKV also reaches the brain and can lead to neurological symptoms. However, tractable and physiologically relevant human models for CHIKV neurotropism remain underdeveloped, limiting mechanistic insight into CHIKV replication in the CNS and hampering drug development. Therefore, we established region-specific human brain organoids as a novel model system for alphavirus neuropathogenesis and antiviral drug testing.

Results: First, we infected cortical organoids with wild-type SFV – a related alphavirus that is extremely neurotropic in mice – and found that low levels of infectious virus were only recovered at high MOI. In contrast, infection of cerebellar organoids led to higher levels of infectious virus release. Using immunofluorescence, we confirmed that SFV replication was restricted to the periphery of cortical organoids but widespread throughout cerebellar organoids. Inclusion of a viral mutant that is mildly impaired in cell culture, unexpectedly led to higher level of virus replication in both organoid types.

Similarly, when cortical organoids were infected with CHIKV, infection was rapidly controlled, whereas mature cerebellar organoids supported efficient replication, suggesting that brain region influences alphavirus replication. Finally, we used an antiviral compound that was effective in cell culture, and showed it had profound antiviral effect in brain organoids. These results highlight the potential of this model system as an animal-free platform for evaluating antiviral drugs.

Conclusion: In summary, human brain organoids are a tractable and disease-relevant model to investigate alphavirus neuropathogenesis. Region-specific differences in SFV and CHIKV replication highlighting potential determinants of alphavirus replication, while viral mutants revealed distinct replication phenotypes and thus facilitate mechanistic insight. Finally, we used brain organoids to evaluate antiviral efficacy, establishing an animal-free platform for mechanistic studies and preclinical evaluation of novel antiviral strategies.

Species-specific expression of ACE2 in multiciliated airway cells determines SARS-CoV-2 susceptibility

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Background and objectives: SARS-CoV-2 is a zoonotic virus that infects a diverse array of animal species, and this host range is usually determined by binding of the viral Spike protein to the angiotensin converting enzyme 2 (ACE2) receptor. However, some species such as pigs are remarkably resistant to SARS-CoV-2 infection, despite the ability of Spike to bind porcine ACE2 and the ability of the virus to replicate in porcine cell lines, suggesting that additional barriers limit cross species transmission. Therefore, we aimed to investigate the resistance of pigs to SARS-CoV-2 infection.

Methods: We investigated the resistance of pigs to SARS-CoV-2 infection using cell lines and well-differentiated porcine and human airway organoid-derived epithelial cultures. We used MERS-CoV as control with validated tropism for pigs. We performed immunohistochemistry and immunofluorescent staining on airway cultures and tissues to assess the expression of coronavirus receptors in the airway cells of both species.

Results: In contrast to porcine cell lines and human airway cultures, porcine airway cultures were resistant to SARS-CoV-2 infection. Whereas human airway cultures and tissues expressed ACE2 in multiciliated cells at the base of the motile cilia, porcine airway cultures and tissues expressed ACE2 in the cytoplasm of multiciliated airway cells. Ectopic expression of human ACE2 led to the apical localization in porcine ciliated cells and conferred permissibility to SARS-CoV-2 infection. In contrast, DPP4 expression was efficiently expressed on the surface of both porcine and human airway cells, correlating with the tropism of MERS-CoV in these cells of both species.

Conclusions: These data indicate that the lack of susceptibility of pigs to SARS-CoV-2 is related to the expression patterns of the ACE2 receptor, and suggest that pigs are no viable reservoir host for ACE2-utilizing coronaviruses but are for DPP4-utilizing coronaviruses. Overall, our studies indicate that receptor localization must be considered carefully for evaluating species susceptibility and risk posed by zoonotic coronaviruses.

Multicellular human intestinal mucosa modelling reveals stromal and immune determinants of host–virus interactions

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Background: The gastrointestinal mucosa is a major interface between the host and the external environment and an important site of viral exposure. However, conventional intestinal *in vitro* models largely comprise epithelial cells and therefore fail to capture stromal and immune contributions that can critically shape viral infection. Improved human models are therefore needed to better study host–virus interactions and support research on emerging enteric viruses and pandemic preparedness. Here we developed a multicellular human intestinal mucosa model to study host–virus interactions in a relevant micro physiological modular system.

Methods: Adult- and foetal-derived intestinal epithelial cells were co-cultured with intestinal fibroblasts and monocyte-derived dendritic cells or macrophages on standard cell-culture inserts. Barrier, stromal, and immune functions were characterised by immunostaining, transepithelial electrical resistance, molecular analyses, flow cytometry, ELISA, and single-cell RNA sequencing. Cytomegalovirus (CMV) and enterovirus A71 (EV-A71), CMV and EV-A71 were used as proof-of-concept viral infections, alongside pro-inflammatory stimulation.

Results: Direct epithelial–fibroblast contact promoted organised extracellular matrix deposition, epithelial differentiation, and prolonged barrier stability, while modulating cytokine signalling and dendritic-cell phenotype. Viral infection studies revealed distinct contributions of the stromal and immune compartments. CMV replication occurred only in cultures containing intestinal fibroblasts, identifying the stromal compartment as a permissive target. Following apical EV-A71 inoculation, cultures containing dendritic cells showed significantly increased viral RNA and infectious virus production compared with epithelial monocultures, accompanied by increased metabolic activity without loss of cell viability. Dendritic cells remained highly viable and responded to inflammatory stimulation by upregulating CD80, CD83, CD86, and CD108, reducing CD103, and increasing IL-12p40 secretion, demonstrating preserved immune responsiveness within the model. Macrophages also retained polarisation capacity.

Conclusions: Incorporating epithelial, stromal, and immune compartments substantially alters intestinal viral infection dynamics and reveals host-cell contributions that are not captured by epithelial-only systems. This modular human model provides a platform for mechanistic studies of viral pathogenesis, host responses, and emerging enteric viruses, while also showing potential for pandemic-preparedness and preclinical evaluation of countermeasures.

AI- and physics-guided affinity maturation of an H5N1-neutralizing antibody with experimental validation

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Background: Rapid optimization of antiviral antibodies could strengthen pandemic preparedness, yet the combinatorial scale of sequence space limits experimental affinity maturation. Machine learning can generate and rank large libraries, while atomistic simulations provide mechanistically grounded refinement at greater computational cost. We therefore sought to integrate AI, physics-based simulation, and experimental testing in a focused workflow for improving an antibody against a high-consequence viral target.

Methods: We developed a hybrid framework for affinity maturation of H5.3, a human monoclonal antibody targeting hemagglutinin (HA) from the A/Vietnam/1194/2004 H5N1 influenza strain. Starting from the wild-type H5.3–HA complex, variants were generated by controlled, CDR-restricted inverse folding. A sequence-based affinity predictor trained on large-scale antibody–antigen data was fine-tuned using physics-derived mutation labels. Candidates were prioritized using ensemble uncertainty estimation and structural confidence assessment, then evaluated by molecular dynamics simulations and relative binding free-energy calculations.

Results: The resulting AI funnel explored more than 500,000 generated variants and prioritized 500 candidates for subsequent physics-based evaluation. Molecular simulations and free-energy calculations further refined this population, yielding a selected set of promising variants that were advanced to production. Relative binding free-energy ($\Delta\Delta G$) calculations predicted 20–30-fold improvements in binding affinity over wild-type H5.3. Separately, early experimental validation identified variants with up to a tenfold improvement in functional potency, measured as a lower IC₅₀.

Conclusions: This workflow combines scalable AI exploration, uncertainty-aware prioritization, atomistic refinement, and experimental testing to navigate vast antibody sequence space efficiently. The results provide a proof of concept for concentrating experimental resources on variants with measurable functional gains. Such integrated workflows could accelerate optimization or adaptation of existing antibodies against emerging viral threats and thereby contribute to pandemic preparedness.

Beyond surveillance: human macrophage responses as a tool for functional risk assessment of emerging influenza viruses

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Influenza A viruses continue to emerge from animal reservoirs and challenge preparedness efforts. Current risk assessment strategies rely largely on genetic and epidemiological data, providing limited information on how emerging viruses interact with the human immune system. Macrophages are among the first immune cells encountered during infection and play a key role in antiviral and inflammatory responses. Functional characterization of virus-macrophage interactions may therefore complement surveillance efforts and improve our understanding of the consequences of human infection.

In this study, human monocyte-derived macrophages from six healthy donors were used to compare the replication dynamics and immunomodulatory properties of influenza A viruses representing different pathogenic and ecological backgrounds. Viral replication was quantified by qRT-PCR and infectivity assays, while flow cytometry was used to determine infection rates and characterize changes in immune markers. Integrating virological and immunological readouts revealed distinct response profiles associated with different influenza viruses.

These findings highlight the value of human macrophage models as a complementary approach for functional risk assessment while providing insights into how emerging influenza viruses replicate, modulate innate immunity and contribute to disease development. Together, this work contributes to a broader One Health framework for the evaluation of emerging influenza threats.

From screening to lead discovery: type 2-pyridone derivatives target human parainfluenza viruses

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Background: Human parainfluenza viruses (PIV1-4) are major respiratory pathogens causing significant morbidity and mortality, particularly in children, older adults, and immunocompromised individuals. Despite their global burden, no approved antiviral therapies are available. This study evaluated a library of type 2-pyridone derivatives as potential antivirals against PIV, leveraging the versatility of the 2-pyridone motif as a privileged scaffold in drug discovery.

Methods: 158 type 2-pyridone compounds were screened in LLC-MK2 cells infected with recombinant, green fluorescent protein-expressing PIV3 (PIV3-GFP). Antiviral activity on infection was quantified using fluorescence-based imaging. Active compounds were further evaluated in dose-response assays to determine half-maximal inhibitory concentrations (IC₅₀). Cytotoxicity was assessed using the CellTiter-Glo® luminescent cell viability assay.

Results: Several compounds showed anti-PIV3 activity. SN121 demonstrated potent antiviral activity, reducing infection to 11.4% and 4.2% at 10 and 50 µM, respectively, and exhibited an IC₅₀ of 0.80 µM in dose-response analyses, exceeding the potency of remdesivir (IC₅₀ = 3.03 µM). Additional active compounds included GB24 (IC₅₀ = 3.79 µM), SN405 (IC₅₀ = 5.16 µM), SN450 (IC₅₀ = 5.28 µM), JeG821 (IC₅₀ = 5.39 µM), SN418 (IC₅₀ = 6.08 µM), and SN415 (IC₅₀ = 6.21 µM). Cytotoxicity characterization of these lead compounds is underway to determine CC₅₀ values and selectivity indices.

Conclusions: This study identified several type 2-pyridone derivatives with potent antiviral activity against PIV3, with SN121 emerging as the most promising candidate. Ongoing studies will complete cytotoxicity profiling and determine selectivity indices for lead compounds. The most active compounds will be evaluated in air-liquid interface cultures of human bronchial epithelial cells, investigated for their mechanisms of action, and assessed against all four PIV serotypes. These findings support further development of type 2-pyridone derivatives as promising antivirals against PIV infections and contribute to efforts to strengthen preparedness for future emerging respiratory virus threats.

Poster 14

Integrative Multiomics: Bridging Metabolomics and Proteomics for Systems-Level Insights

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COVID-19 displays substantial clinical heterogeneity, creating a need for biomarkers that support disease stratification and therapeutic development. We applied integrated metabolomic and proteomic analysis to plasma samples from a Mayo Clinic COVID-19 cohort to assess whether multiomic integration improves discrimination of disease status and severity.

The cohort comprised 666 samples, including healthy controls, pre-COVID-19 samples, post-COVID-19 samples, and individuals with outpatient, mild, severe, or critical COVID-19. Global metabolomic profiling was performed using complementary UHPLC-MS/MS methods covering hydrophobic, hydrophilic, basic, and small polar metabolites. Metabolomic and proteomic datasets, together with clinical severity data, were integrated using Metabolon's Integrated Bioinformatics Platform. Supervised multivariate analysis using DIABLO, logistic regression, random forest modelling, and pathway enrichment were used to compare outpatient versus healthy control samples, outpatient versus severe/critical disease, and pre- versus post-COVID-19 samples.

Integrated multiomic models improved classification performance compared with single-omic analyses and clearly separated participant groups across disease severity. The analyses identified biologically meaningful molecular signatures associated with COVID-19 progression, including eicosanoid-related pathways and altered oleamide and proline derivatives. Lower leukotriene-associated signals were observed in more severe disease, highlighting potential pathways for mechanistic follow-up and therapeutic target exploration.

These findings demonstrate how integrated metabolomics and proteomics can strengthen patient stratification, improve prediction of disease status and severity, and provide pathway-level insights into host responses to SARS-CoV-2 infection. This approach offers a scalable framework for translating multiomic data into actionable biomarker and drug-development hypotheses.

Dengue endotyping by systems level plasma proteomics- precision based severe infection prediction

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Background: In recent decades, dengue has emerged in newer geographical areas and has caused an increasing number of severe infections. Progression from warning signs to severity in a dengue patient remains a challenge. There is a significant overlap in clinical presentations that make categorization of patients, according to the WHO TDR 2009 classification, difficult due to biological heterogeneity. This study therefore used integrated clinical and proteomic endotyping to identify the molecular signatures associated with severe dengue by investigating the underlying pathobiology, thereby allowing the distinct triaging of patients in the appropriate severity groups.

Methods: Detailed clinical, biochemical, haematological, and prior immune status were collected from study participants, and were analysed for plasma proteomic profiles. High-throughput proteomic analysis was followed by unsupervised clustering, pathway enrichment and machine-learning based-classification to identify severe dengue, thus predicting endotype and its molecular features.

Results: Increasing dengue severity was associated with worsening liver function, coagulation and inflammatory mediators. This was also noted in the differential levels of IL-6, IL-15, HGF and MUC-16. Clustering as per WHO TDR 2009 classification revealed substantial overlap indicating that clinically observable signs do not clearly distinguish severe disease from dengue without or with warning signs. Integrated unsupervised clustering revealed various immuno-oncological markers were differentially expressed across primary and secondary infections as well as in across severity categories. The endotyping profiles revealed that protein signatures were vastly different between patients prone to high morbidity compared to those who developed mild to moderate warning signs and subsequently recovered.

Conclusion: The clinical challenges in the assessment of dengue severity is largely hindered due to the heterogeneity of symptom presentation. The WHO TDR 2009 classification has been essential in assessing patient health and appropriate triaging based on clinical signs and symptoms. Systems-level proteomics investigations unveil a new strategy in the understanding of the global host immune and inflammatory response at the protein level. This further enabled the integration of clinical and proteomic endotyping approaches to understand dengue progression, and to simplifying severity stratification thereby facilitating better patient management.

Iron homeostasis as a critical regulator of influenza virus replication and a target for host-directed therapy

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Background: Influenza is a major respiratory pathogen affecting humans and other vertebrates, relying extensively on host cellular machinery for replication. Iron, a critical cofactor for numerous host proteins, plays a central role in maintaining cellular homeostasis and may influence viral replication. However, the interplay between iron metabolism, redox balance, and influenza virus replication remains poorly understood.

Methods: We investigated the impact of IAV on iron concentration using ICP-MS. Its impact on host cellular mechanism was further investigated using proteomic analysis of IAV infected A549 cells. In this study, we investigated the role of iron in modulating influenza virus growth. Further impact of iron on virus replication was studied using iron chelator treatment in both in-vivo and in-vivo model system.

Results: IAV infection in A549 cells increased iron concentration in cells also disrupting the host iron homeostasis along with morphed mitochondrial dynamics. Treatment of infected cells with iron chelators, including Tiron and Deferoxamine (DFO), significantly reduced viral replication. This effect was associated with decreased reactive oxygen species generation and restoration of NRF2 mediated iron homeostasis following infection. Iron chelation restored mitochondrial integrity and function. In-vivo, DFO treatment in BALB/c mice challenged with a lethal dose of influenza virus resulted in reduced viral titers and improved survival.

Conclusion: These findings demonstrate that iron availability is a critical determinant of influenza virus replication and pathogenesis. Targeting iron metabolism may therefore represent a promising host-directed therapeutic strategy for the treatment of influenza virus infections.

Flaviviruses hijack host E3-ligases to promote infectious-particle production

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Flaviviruses, including Zika virus (ZIKV) and dengue virus (DENV), extensively reprogram the host cellular machinery to establish successful infection. Both replication-organelle biogenesis and particle formation depend on host ubiquitylation. However, how infection controls the ER ubiquitin ligases that mediate this requirement is largely undefined.

We mapped the post-translational modifications landscape during ZIKV infection and identified an interconnected set of host ubiquitin ligases hijacked by flaviviruses. Within this module, G8, a host E3 ligase was required for ZIKV and DENV infectivity.

G8 abundance was tightly controlled: genetic deletion of G8 markedly reduced extracellular infectious titres, with a less pronounced reduction in viral RNA, whereas dose-dependent overexpression suppressed ZIKV and DENV infection. During late-stage infection with multiple ZIKV strains and DENV serotypes, G8 was depleted despite increased G8 transcript abundance. This turnover appeared to require G8 catalytic activity.

G8 associated with several ZIKV proteins, most prominently capsid. Initially, G8 localized to envelope- and dsRNA-positive ER-associated membranes, consistent with viral replication and assembly compartment. At later stages, G8 redistributed into plasma membrane-tethered punctate structures that co-localized with both capsid and envelope proteins.

AP-MS revealed an infection-dependent shift in the G8 interactome: basal G8 associates primarily with RNA-surveillance and stress-granule complexes, whereas ZIKV infection redirects G8 towards networks regulating membranous organelle assembly, macroautophagy, and ER-associated degradation. We captured proteins receiving ubiquitin from catalytically active and inactive G8 using E3-substrate tagging by ubiquitin biotinylation (E-STUB). The identified G8 substrates comprised autophagy receptors and the ubiquitin-p97 machinery, the same processes recovered by AP-MS. G8 also co-precipitated with its homologue and an ERAD-associated chaperone, linking G8 to host protein quality control, and a broader E3-ligase family network.

Together, we propose a conserved mechanism in which flaviviruses recruit catalytically active G8 to ER-associated replication and assembly membranes to coordinate viral structural-protein trafficking, infectious-particle formation, and egress.

Development and qualification of a lentiviral-based pseudovirus neutralisation assay for highly pathogenic avian influenza H5 clade 2.3.4.4b viruses

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Highly pathogenic avian influenza (HPAI) H5 clade 2.3.4.4b viruses have caused extensive outbreaks in birds and mammals, with an increasing number of zoonotic infections in humans. These events highlight the urgent need for safe, high-throughput assays to evaluate immune responses and antiviral efficacy.

We developed and qualified a lentiviral-based pseudovirus neutralisation assay (PNA) for HPAI H5 viruses that can be conducted under Containment Level 2 (CL-2). Four H5 pseudoviruses (H5N1-AIV09, H5N1-AIV48, H5N1-2.3.4.4b B3.13 and H5N8-Astrakhan) were generated by co-transfecting 293FT cells with the gag-pol plasmid p8.91, the firefly-luciferase reporter pCSFLW, and HA/NA expression plasmids (pcDNA3.1-derived) at optimised ratios. Comparative analyses demonstrated that 293FT cells produced signal intensities up to one log higher than MDCK cells, identifying the 293FT settle assay format as optimal. Implementation of the settle assay reduced operator time, reagent use, and enabled throughput scaling from 96- to 384-well plate formats. Using DOE, the assay was optimised and robustness testing confirmed assay stability when using cell suspensions within 1 h of preparation, and acceptable performance across neutralisation (45–60 min) and incubation (46–48 h) time windows. The qualified assay met predefined acceptance criteria for precision, accuracy, linearity and specificity, and showed strong correlation with live-virus and reverse-genetic influenza neutralisation assays as well as correlation with alternative influenza immunological assays; HAI and ELISA.

This qualified HPAI H5 PNA provides a rapid, reliable, and CL-2 compatible alternative to live-virus neutralisation, supporting serological evaluation of vaccine candidates, therapeutic antibodies, and surveillance studies essential for pandemic preparedness. By increasing accessibility and throughput, the HPAI PNA strengthens outbreak preparedness and accelerates efforts to reduce the high mortality associated with avian influenza.

Targeted lipid nanoparticle-mediated in vivo reprogramming of CD8 T cells for antiviral immunotherapy against hepatitis C virus

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Chronic hepatitis C virus (HCV) infection remains a global health challenge, highlighting the need for innovative antiviral immunotherapies. This project aims to develop a nucleic acid-based strategy for direct in vivo reprogramming of CD8 T cells using targeted lipid nanoparticle (LNP) delivery of mRNA encoding HCV-specific T cell receptors (TCRs). These engineered TCRs recognize conserved viral antigens and enable antigen-specific cytotoxic killing of infected hepatocytes. A critical component of this strategy is the efficient delivery of nucleic acids to resting immune cells. Therefore, we are optimizing LNP formulations to enhance transfection of primary T cells. We have generated LNPs and obtained preliminary data demonstrating their ability to transfect immune cells, along with characterization of their physicochemical properties. Current efforts focus on improving specificity by conjugating targeting ligands to the LNP surface to enable selective delivery to CD8 T cells. To evaluate therapeutic efficacy, both *in vitro* and *in vivo* models will be employed. TCR potency will first be assessed using in vitro HCV replicon systems in which subgenomic HCV RNA is actively replicating, enabling evaluation of antigen-specific recognition and cytotoxic activity. For *in vivo* validation, humanized NOD SCID gamma mice reconstituted with human peripheral blood mononuclear cells will be used. In parallel, a transgenic mouse model expressing HCV antigens in the context of human HLA-A2 will allow assessment of HLA-restricted TCR recognition and effector function. This work establishes a platform for targeted in vivo immune cell engineering and scalable antiviral immunotherapy for HCV and other chronic viral infections.

The impact of the virus-producing cell type on the spread and neutralization sensitivity of HIV swarms

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The human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), remains a global health concern despite effective antiretroviral therapy. HIV originated from simian immunodeficiency virus (SIV) via the transmission from non-human primates to humans. Studies with SIV show that depending on the virus producing host cell type the infectivity, transmissibility and neutralization sensitivity of the virus differs. Whether this also holds true for HIV remains insufficiently explored. Here, we aim to provide the first comprehensive analysis of how the virus-producing cell type and specific host factors shape HIV functions and modulate antibody and lectin interactions.

To achieve this, we engineered reporter viruses based on HIV-1 89.6 strain which is capable of infecting both, CD4⁺T cells and macrophages. First, enhanced green fluorescent protein (eGFP), flanked by viral protease cleavage sites, was inserted into *gag* to enable its incorporation into virions and subsequent release during viral maturation. Further, we cloned monomeric cherry red (mCherry) or true anthozoan blue fluorescent protein (TagBFP) into *nef* to serve as a read-out for infection. Currently, we are producing virus in HEK 293T cells and will confirm its functionality before infecting primary human CD4 T cells (T-HIV) and macrophages (M-HIV) to produce T-HIV, M-HIV, and mixed virus stocks.

In the future, we will characterize T-HIV and M-HIV populations by glyco-lipidomics and glyco-proteomics. LASSO regression and Random Forest models will be applied to integrate molecular and functional datasets and identify molecular features predictive of viral infectivity, transmissibility, and neutralization sensitivity. Through this approach, we aim to define specific host cell-derived features of HIV which are associated with differences in viral functions and might represent molecular drivers of swarm behaviour. Thereby we intend to identify combinations of lectins and neutralizing antibodies that confer optimal protection against HIV infection, independent of the virus-producing cell type.

Recurring preparedness deficiencies across successive WHO reviews of H1N1, Ebola and COVID-19: a comparative document analysis

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Background: Major viral epidemics repeatedly prompt formal assessments of preparedness, yet it remains unclear to what extent comparable deficiencies in pathogen detection and outbreak response recur across successive reviews. We examined recurrence across three biologically distinct viral emergencies evaluated through the World Health Organization (WHO) International Health Regulations (IHR) review process.

Methods: We conducted a structured longitudinal comparative document analysis of the 2011 IHR Review Committee report on pandemic influenza A(H1N1), the 2016 IHR Review Committee report following the West African Ebola epidemic, and the 2021 IHR Review Committee report on COVID-19. Four preparedness domains were predefined: surveillance and notification, diagnostic and laboratory capacity, health-system response capacity, and international coordination/IHR implementation. Recurrence of comparable deficiencies was distinguished from explicit evidence concerning implementation of earlier recommendations.

Results: Comparable preparedness deficiencies were documented across successive reviews in all four domains. The H1N1 review identified uneven surveillance capacity, reporting delays and laboratory constraints, including systems overwhelmed by diagnostic demand. The Ebola review described continuing weaknesses in surveillance, health systems and international response, and explicitly linked failures during Ebola to those documented during the H1N1 response. The COVID-19 review identified critical gaps including diagnosis/testing and preparedness capacity and concluded that implementation of recommendations from previous IHR Review Committees had been uneven and too slow. Recurrence therefore did not necessarily indicate absence of implementation; later reviews documented both continuing deficiencies and partial progress.

Conclusions: Across influenza virus, Ebola virus and SARS-CoV-2 emergencies, successive WHO reviews documented recurring deficiencies spanning viral detection, laboratory preparedness, healthcare response and international coordination. Distinguishing documented recurrence from implementation status provides a framework for identifying preparedness vulnerabilities while recognizing areas of progress.

Synthetic virology for immunodominance mapping: rapid prototyping of antigenic-site mutants of influenza A virus

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Background: Understanding how human antibody responses are distributed across hemagglutinin (HA) antigenic sites is important for defining influenza immunodominance and interpreting antigenic evolution.

Methods: We developed a synthetic virology platform based on a modified ambisense pDZ reverse-genetics plasmid and exploited conserved restriction sites within HA to enable computer-assisted design and modular replacement of HA sequences. This allows synthetic influenza A viruses to be generated directly from viral genome sequence information without requiring a corresponding physical virus isolate or DNA template.

Results: Using this platform, we generated antigenically modified influenza A viruses, including A/California/07/2009 (pdmH1N1)-derived mutants targeting the five canonical HA antigenic sites. In addition to individual antigenic-site mutants and a quintuple $\Delta 5$ mutant carrying combined mutations across all five sites, we successfully generated two novel $\Delta 4$ antigenic-site mutant viruses, expanding the combinatorial antigenic landscape that can be experimentally interrogated.

Conclusions: These engineered viruses can serve as differential serological probes to dissect antibody reactivity against individual and combined HA antigenic sites in human sera. By integrating programmable synthetic virology with human serological profiling, this platform provides a framework for experimentally mapping antibody immunodominance hierarchies and investigating how population-level immune responses may shape influenza antigenic evolution.

Single-cell transcriptomic landscape of avian influenza H9N2 virus infection in human and chicken cells

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Background: Influenza A viruses have demonstrated the capacity to cross species barriers, spreading from their natural reservoirs to infect poultry and a broad range of mammals, including humans. While highly pathogenic avian influenza viruses (AIVs) are known to infect humans, low pathogenic AIVs, particularly H7N9 and H9N2 subtypes, have also emerged as significant zoonotic threats.

Methods and Results: In this study, we applied single-cell transcriptomics to profile the cellular response of human (A549) and avian (DF-1) cells at 24 hours post-infection with zoonotic H9N2 virus. Our findings revealed distinct differences in the transcriptional responses between the two cell lines. Notably, Dipeptidyl peptidase-4 (DPP4), and Carboxypeptidase Q (CPQ) exhibited significantly higher transcription levels in infected DF-1 cells than in A549 cells, suggesting their potential involvement in viral replication. Overexpression of CPQ and DPP4 gene in A549 cells revealed more significant virus replication compared to mock transfected wild type cells infected by avian influenza H9N2 virus. Furthermore, Gene Set Enrichment Analysis of differentially expressed genes identified the downregulation of lipid metabolism pathways in cell clusters highly expressing H9N2 virus genes in both the DF-1 cells and A549 cells, implicating metabolic remodelling as a potential mechanism supporting viral replication.

Conclusions: These results provide insights into the transcriptional responses of DF-1 and A549 cells to H9N2 infection and identify candidate host factors and pathways associated with viral replication. These findings provide a foundation for future studies using physiologically relevant models to investigate the cellular determinants of H9N2 infection.

New genetic Crimean-Congo hemorrhagic fever virus vaccines

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Crimean-Congo Hemorrhagic fever (CCHF) is a viral hemorrhagic fever transmitted by hyalomma ticks. It has a fatality ratio of 10-40% according to the WHO and no vaccine is available on a global scale. This study aims to develop vaccine candidates against CCHF. We generated both DNA and mRNA/LNP based vaccines and evaluated these in mice. Groups of mice were vaccinated on weeks 0 and 3 and venous blood was obtained at 2 and 5 weeks. Antibodies to the CCHF C-terminal glycoprotein (Gc) and nucleoprotein (NP) was determined by enzyme-linked immunosorbent assay (ELISA) and T cells by interferon gamma (IFN- γ) enzyme-linked immunosorbent spot (ELISpot). The vaccines containing Gc and NP induced strong antibody and T cell responses. Importantly, the vaccines induced neutralizing antibodies to CCHF as determined by a pseudovirus neutralization assay. The vaccines also provided protection during a challenge study in mice. These findings support clinical development of Gc and NP containing vaccine candidates.

Risk map of dengue transmission in the metropolitan area of Mexico City

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Background: During the 2015-2025 decade, *Aedes aegypti* has been documented in the Metropolitan Area of Mexico City (ZMCM), coinciding with an increase in travel-related imported dengue cases and the confirmation of the first confirmed locally acquired dengue case in 2023. This study aims to identify potential high-risk areas for active dengue transmission within the ZMCM.

Methods: We integrated *Aedes aegypti* occurrence data, records of imported dengue cases and local climatic variables to develop epidemiological risk prediction models using machine learning algorithms (LightGBM and XGBoost). Principal component and cluster analyses were applied to identify spatial patterns and high-risk clusters.

Results: The models identified several municipalities as potential high-risk zones for active dengue transmission due to persistence of positive ovitraps and increasing number of imported cases.

Conclusion: These findings provide a basis for strengthening preventive and vector control strategies in a metropolitan area with approximately 21 million persons at risk.

Dynamics of SARS-CoV-2-specific cellular immunity and its association with breakthrough infection and disease severity (SARSSURV study)

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Background: Repeated SARS-CoV-2 infections and immunizations generate heterogeneous cellular and humoral immune responses shaped by individual exposure history. We investigated the longitudinal dynamics of SARS-CoV-2-specific cellular immunity and its relationship with subsequent infection and disease severity alongside humoral immunity.

Methods: Longitudinal T cell response to SARS-CoV-2 was analyzed according to the number, type, and chronological sequence of infections and immunizations using mixed-effects models. Exposure history was encoded chronologically as infection (I), primary vaccination (P), and booster (B). Associations of cellular and humoral responses with breakthrough infection (N=966; 347 events) and reinfection (N=600; 104 events) were assessed using Cox proportional-hazards and joint models, incorporating baseline measurements and longitudinal trajectories, respectively. Logistic and ordinal regression models evaluated associations between pre-infection immune responses and symptom occurrence and severity at infection day (N=485), one week post-infection (N=477), and two weeks post-infection (N=471).

Results: Cellular immunity was strongly shaped by exposure history. Repeated infections (II) generated lower T cell response than infection followed by vaccination (IP), while infection followed by primary vaccination and boosting (IPB) generated higher responses than repeated vaccination (PBB). Cellular responses declined over time. Higher humoral responses were associated with lower breakthrough infection risk in Cox and joint models, while T cell response was not. Reinfection analyses showed a similar, although weaker, pattern. In contrast, higher pre-infection T cell response was associated with lower symptom occurrence and severity in logistic and ordinal regression analyses, including after adjustment for humoral responses.

Conclusions: The type and sequence of SARS-CoV-2 antigenic exposures shape subsequent cellular immunity and its persistence. Cellular and humoral responses showed distinct but complementary associations with clinical outcomes: humoral immunity was more closely associated with protection against subsequent infection, while cellular immunity was associated with reduced symptom burden and disease severity following infection.

Batai virus restriction in *Aedes albopictus*: spatio-temporal dynamics of segment-specific vDNA biogenesis for pandemic preparedness

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Background: The threat posed by emerging arboviruses underscores the need for novel transmission-blocking strategies within global pandemic preparedness frameworks. In mosquitoes, the balance between vector survival and viral replication is regulated by RNA interference (RNAi), which is bolstered by virus-derived DNA (vDNA) biogenesis. However, vDNA biogenesis remains poorly understood. This study aims to identify the Batai virus (BATV) genomic regions used as templates for vDNA production in *Aedes albopictus* and to characterise how this biogenesis shifts across infection.

Methods: Female mosquitoes were microinjected with BATV and fed sucrose with or without a reverse transcriptase inhibitor (vDNA-deficient vs. vDNA-producers; n=40/group). For vDNA detection, we used PCR targeting 26 regions across genomic segments in mosquito bodies; and viral loads in legs and wings at 7 and 14 days post-infection (dpi) by RT-PCR. We used Mann-Whitney U and direct pairwise Fisher's exact tests to compare groups.

Results: The vDNA-deficient group had a viral load similar to the vDNA-producers at 7 dpi, which increased 3.5-fold at 14 dpi ($p<0.001$). At 7 and 14 dpi, vDNA production frequency differed significantly between genomic segments S and L ($p<0.01$), whereas genomic segment M differed significantly from segment L only at 7 dpi ($p=0.0313$). At 7- and 14-dpi, S-segment vDNA production concentrated within central coding regions overlapping reading frames of the nucleocapsid and non-structural NSs proteins. At 7 dpi, M-segment vDNA production mapped to internal structural domains of glycoproteins Gn and Gc, as well as the non-structural NSm protein. By 14 dpi, this production shifted to the amino-terminal ectodomain and terminal regions preceding the 3' UTR in the BATV genome.

Conclusion: The segment-specific vDNA biogenesis acts as a critical molecular checkpoint restricting BATV replication, providing a blueprint for programmable vector immunisation platforms.

The VirusBank platform: a unique research infrastructure promoting enhanced preparedness against future epi- and pandemics

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The global impact of the SARS-CoV-2 pandemic has revealed deficiencies in health surveillance, disease prevention, and societal readiness against pathogens with epi- and pandemic potential. To promote better pandemic preparedness, the Belgian Federal Government has invested in establishing a “VirusBank Platform” at the University of Leuven (KU Leuven). The VirusBank Platform (VBP) brings together leading expertise in virology with the mission to develop state-of-the-art assay toolboxes for relevant epidemic and pandemic virus families (www.virusbankplatform.be). VBP spans three distinct locations, including a state-of-the-art BSL-2 facility equipped with fully automated, high-throughput systems as well as close affiliations with the Neyts Lab (www.antivirals.be) at the Rega Institute and the Center for Drug Design and Discovery (CD3; www.cd3.be). These affiliations provide additional access to BSL-2/3 animal laboratories and a BSL-3/3+ facility with integrated Caps-It core, an automated platform for high-content imaging of live or unknown-risk pathogens, as well as a fully automated compound handling system at CD3. VBP maintains a strategically curated virus collection representing eight priority virus families, with ongoing expansion, and supports a wide range of advanced tools and experimental models aimed at expediting the development of biopharmaceuticals (vaccines, antibodies, and antiviral drugs) for the treatment or prophylaxis of viral infections. These include a large bank of well-characterized cell lines representing different organ systems, reverse genetics capabilities for each prioritized virus family, nanopore sequencing expertise, and in vivo infection models in zebrafish and small mammals. Moreover, VBP possesses a chemical library of 350,000 small drug-like molecules and a drug repurposing library comprising 7,000 compounds, which can be screened in custom-developed high-throughput phenotypic antiviral screening assays. VBP goes beyond being just a virus collection and strives to offer an end-to-end platform for researching virus families of epidemic or pandemic concern, thereby ultimately facilitating the development of new preventive or therapeutic strategies against these viruses.

Dengue virus envelope protein induces B-cell activation through NF- κ B-mediated POU2AF1 transcriptional regulation

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Severe dengue remains a significant global health burden affecting millions annually. Antibody-dependent enhancement, cytokine dysregulation, and endothelial dysfunction exacerbate disease severity. Dengue progresses unpredictably from asymptomatic or mild infection to life-threatening haemorrhagic disease, with severe manifestations paradoxically associated with vigorous immune responses. The molecular mechanisms by which dengue envelope protein (antigenic determinant), reprograms B cells toward pathogenic immunity remain uncharacterized, representing a critical knowledge gap for therapeutic intervention. This study investigates how dengue envelope protein activates B cells and drives pathological immune responses through NF- κ B mediated POU2AF1 dependent signaling pathways. Recombinant DENV envelope protein was purified, biochemically characterized, and immunologically validated. B-cell internalization was confirmed by immunofluorescence microscopy under envelope protein stimulated and transfected pcDNA 3.1-Envelope conditions. B-cell activation markers (CD69), inflammatory cytokine production (IL-6, IFN γ), and relative mRNA expression (POU2AF1) were quantified using flow cytometry and real-time quantitative PCR respectively. Transcriptomic analysis of dengue patient cohorts stratified by disease severity (DF, DHF, DSS) from public databases (GEO) examined POU2AF1 expression patterns. Immune cell composition analysis was performed using CIBERSORT computational deconvolution. POU2AF1 promoter architecture was analyzed bioinformatically to identify conserved transcription factor binding elements. NF- κ B pathway activation was validated by Western blot analysis of phosphorylated p65 and p50. DENV-1 envelope protein efficiently translocated into B cells and induced elevated CD69 expression, increased IL-6 production, and suppressed IFN γ secretion. Transcriptomic analysis demonstrated sequential POU2AF1 upregulation correlating with disease severity progression (DF < DHF < DSS). Bioinformatic analysis identified conserved NF- κ B binding motifs (NF- κ B1, RelA) upstream of POU2AF1. Envelope protein stimulation induced dose-dependent POU2AF1 upregulation accompanied by p65/p50 phosphorylation. These findings establish DENV-1 envelope protein as a direct B-cell activator through NF- κ B-mediated POU2AF1 upregulation. Progressive POU2AF1 expression correlation with disease severity identifies this signaling axis as a potential diagnostic biomarker and therapeutic target for severe dengue intervention.

Synthetic virology for immunodominance mapping: rapid prototyping of antigenic site mutants of influenza A virus

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Human antibody responses to influenza A virus are shaped by immunodominance, whereby antibodies preferentially target a restricted subset of epitopes within the viral hemagglutinin (HA). Defining these immunodominance hierarchies is important for understanding immune history, antigenic evolution, and variability in vaccine-induced protection, but is limited by the availability of suitable antigenically defined viral probes.

Here, we developed a synthetic virology platform for the rapid generation of influenza A viruses carrying rationally designed mutations in discrete HA antigenic sites. Using A/California/07/2009 (H1N1pdm09) as a model, we adapted an ambisense pDZ reverse-genetics system to enable sequence-guided, computer-assisted construction of viral genome segments without requiring a physical virus isolate or pre-existing viral DNA template. We generated a panel of recombinant viruses containing escape mutations targeting each of the five canonical H1 HA antigenic sites, together with a combined five-site mutant designed to broadly disrupt antibody recognition of the HA head.

These antigenically defined viruses serve as differential serological probes, enabling changes in antibody recognition between wild-type and mutant viruses to resolve the relative contribution of individual HA antigenic sites to polyclonal human serum responses.

By integrating synthetic genome engineering with human serology, this platform provides a modular framework for mapping influenza immunodominance and can be adapted to emerging or historical viral sequences. This approach may facilitate studies of immune imprinting, antigenic drift, population-level antibody landscapes, and the evaluation of next-generation influenza vaccines.

Analysis of role of dengue virus capsid on host transcriptome and identification potential capsid inhibitors

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Dengue is a viral disease caused by the dengue virus (DENV), with an alarming global prevalence. The DENV capsid protein is critical for encapsulating the viral RNA genome to form the nucleocapsid. Although viral particle assembly takes place on the endoplasmic reticulum membranes, the capsid localizes in nucleoli. The significance of capsid nuclear localization during infection and its influences on host nuclear processes remains poorly understood. Moreover, the capsid, being a multifunctional protein, is a promising target as a single drug targeting the capsid can disrupt several pathogenic processes at once. Our primary aim is to elucidate the impact of the DENV capsid on host nuclear transcriptome regulation and to identify potential capsid inhibitors. To address this, we performed RNA sequencing (RNA-Seq) on the *HEK293T* cell line transfected with a capsid-expressing construct to identify differentially expressed genes (DEGs). Further, selected DEGs identified by RNA-Seq were validated using quantitative Real time PCR (qRT-PCR). The most significantly upregulated and downregulated genes identified by qRT-PCR are known to be associated with nuclear processes. Subsequently, western blot and pathway analysis will confirm their differential expression at the protein level. To identify potential capsid inhibitors via *in-silico* studies, a library (ChEMBL) of 1900 molecules was subjected to molecular docking against DENV1 capsid. The top five compounds with the lowest (most favorable) binding energies were further subjected to molecular dynamics (MD) simulations to evaluate the stability of the docked complexes. We found a promising candidate for further *in vitro* experimental validation. Overall, our findings suggest that the capsid orchestrates host transcriptional reprogramming by modulating pathways involved in cellular stress, ribogenesis and antiviral responses and will facilitate the development of antiviral strategies against DENV.

A Biophysical Toolbox for Pandemic Preparedness

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Background: Rapid identification and characterization of highly pathogenic viruses is hampered by elaborate safety measures and time-consuming experimental procedures. We develop fast, robust, and innovative synthetic virology tools to identify molecular key players and intervention strategies for improved pandemic preparedness.

Methods: To reduce biological complexity, we employ bottom-up synthetic biology by functionalizing microparticles with lipids, proteins, and/or glycans as host-cell mimics to study viral attachment. This system quantifies interactions between viral particles expressing Marburg virus glycoprotein (MARV-GP) and lipid, glycan, and protein receptors. We complement this with top-down synthetic biology using giant plasma membrane vesicles (GPMVs) to study Influenza A hemagglutinin–sialic acid receptor interactions. Giant virological synapses (GVSs) enable spatiotemporal analysis of receptor clustering and cofactor engagement. Additionally, we establish cost-efficient procedures to prepare and store native membrane libraries from different cell lines for screening unknown host-pathogen interactions.

Results: Bottom-up studies identified ASGR1 as a host cell factor interacting with MARV-GP glycans. We also demonstrated that T-cell immunoglobulin and mucin domain-containing proteins (TIMs) bind phosphatidylserine on viral particles independent of MARV-GP. GVS-based analysis revealed that degree of GPMV-clustering can serve as a sensitive & label-free indicator for screening potential therapeutic interventions, overcoming classical limitations of conventional virus-host interaction assays.

Conclusions: Our integrated biophysical toolbox accelerates identification of viral receptors and host factors while reducing biosafety constraints and experimental complexity. These approaches offer innovative platforms for rapid drug screening and pandemic preparedness.

Positive selection in SARS-CoV-2: beyond receptor affinity

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Elucidating the evolutionary mechanisms underlying the epidemiological and pathological characteristics of SARS-CoV-2 is crucial for predicting disease trends and informing effective strategies for prevention, control, and treatment. Sites under positive selection (PSS) in SARS-CoV-2 have been identified, and because these shape viral survival and adaptation, mutations occurring at these sites should be of concern. Here, we address the evolutionary forces acting on SARS-CoV-2 Spike receptor binding domain (RBD; residues 331–531) PSS by combining available experimental binding affinity data from deep mutational scanning (DMS) with EVEscape predictions of immune escape, and overall fitness.

Our results show that, on average, the 20 identified PSS differ from Non-PSS, with significantly higher binding affinity, fitness and immune escape capabilities when mutated. These results are independent of the variant background or the specific amino acid substitution. Experimental binding affinity data could explain 63% of the mutations recorded for our list of PSS. Twenty Non-PSS have mutations that, if observed, could confer increased binding affinity. Nevertheless, seven of these Non-PSS do not show amino acid variation, and thus could never be identified as PSS. Moreover, another five only have mutations emerged in nature that decreased receptor affinity according to DMS, and thus have also not been identified as PSS. Conversely, for immune escape and overall fitness metrics, Non-PSS never behave as PSS when exhibiting the mutations observed in nature. These last two metrics further support the previously determined PSS. Hence, although affinity to the host receptor is a major evolutionary driver, integrating immune escape and fitness with affinity provides a more comprehensive view of the evolutionary pathways available to SARS-CoV-2.

High throughput, multiparametric system for screening of cell surface receptors in Marburg virus attachment

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Viruses can adopt different host attachment pathways depending on the targeted cell types and environmental conditions and the repertoire of available receptors may vary between tissues or even within individual cells. To this day, the attachment of Marburg virus (MARV) glycoprotein (GP) remain incompletely defined. Based on prior literature, we identified nine candidate host proteins that may play a part in MARV attachment to the host cell. The aim of this study is to investigate the affinity of binding of the GP to these candidates. The proteins are expressed on various human cells targeted by the virus, especially hepatocytes. To investigate their contribution to viral attachment, we employed a high-throughput platform that mimics cell-virus interactions. It is a modular synthetic cell system composed of bead-supported lipid bilayers functionalized with recombinant host proteins. These artificial cells were incubated with fluorescent virus-like particles presenting either the wild type MARV GP or a GP Δ MLD variant lacking the mucin-like domain MLD. Virus-host interactions are then quantified using microscopy and flow cytometry. By investigating the binding affinity of host proteins to MARV GPs, this study aims to understand which factors are responsible for the cell entry and could serve as potential drug targets.

LRP8-dependent trafficking of TBEV to early endosomes: implications for antiviral strategy

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Introduction: Tick-borne encephalitis virus (TBEV) is a flavivirus responsible for severe neurological disease in humans. During cellular infection, TBEV uncoating occurs in acidic endosomal compartments. Although TBEV entry via binding to the receptor LRP8 has been described, the subsequent intracellular trafficking of the virus through endosomal compartments remains poorly characterized. A detailed understanding of the cellular mechanisms governing viral uncoating is essential for the development of effective antiviral strategies. Here, we investigate the role of endosomal transport during TBEV entry.

Methods: We employed immunofluorescence staining of viral and endosomal markers to track TBEV trafficking in A549 cells overexpressing LRP8. Cell surface expression of LRP8 was assessed using a plasma membrane biotinylation assay. To investigate the role of endosomal transport, we utilized transient siRNA-mediated gene silencing in combination with the pharmacological inhibitor EGA to disrupt endosomal function and trafficking. Viral infectivity was quantified using either quantitative PCR or immunofluorescence. Additionally, we generated neurons from human induced pluripotent stem cells as a physiologically relevant model of TBEV infection to assess the antiviral effects of EGA.

Results: Our findings demonstrate that TBEV and LRP8 predominantly traffics to early endosomes upon entry into cells. Significant colocalization was observed with EEA1 (an early endosome marker), whereas only limited colocalization was detected with Rab7 and LAMP1 (markers of late endosomes and lysosomes, respectively), suggesting that the virus–receptor complex dissociates within early endosomes, followed by viral uncoating. Consistently, genetic ablation of Rab5a, an essential regulator of early endosomes, but not Rab7a, significantly reduced viral infection at early time points, underscoring the critical role of early endosomes. Pharmacological inhibition of endosomal transport using EGA resulted in disruption of early endosomal integrity and a marked reduction in viral infectivity, indicating that proper trafficking to early endosomes is essential for efficient TBEV infection. Finally, we demonstrated that EGA treatment protected differentiated neurons against TBEV infection.

Conclusion: This study identifies early endosomal trafficking as a critical step in the TBEV infection process. These findings highlight the importance of early endosomes in facilitating viral entry and uncoating, and suggest that components of the endosomal pathway represent targets for antiviral intervention.

Comparative immunogenicity of DNA and mRNA–LNP vaccines targeting hepatitis E virus

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Background: Hepatitis E virus (HEV) is a major global cause of acute hepatitis, transmitted via contaminated water and zoonotic food sources. Despite substantial disease burden, only one licensed vaccine is available and remains geographically limited. Vaccine development has therefore focused on the capsid protein, which contains neutralizing epitopes and forms highly immunogenic virus-like particles.

Methods: This study evaluated the immunogenicity of capsid-based vaccines delivered via DNA and mRNA platforms in BALB/c mice. Animals received two doses of either 50 µg capsid-expressing DNA administered intramuscularly with electroporation or 2 µg nucleoside-modified capsid mRNA formulated in lipid nanoparticles. Serum and splenocytes were collected following booster immunization.

Results and Conclusions: Both platforms induced strong capsid-specific IgG responses in all mice. IFN-γ ELISpot analysis revealed robust T cell responses to multiple capsid peptide pools in using both DNA and mRNA, with partially overlapping but distinct epitope recognition profiles between DNA and mRNA vaccination. mRNA–LNP immunization resulted in IgG titers comparable to those observed with DNA vaccination, despite the lower antigen dose used. These findings suggest that the mRNA–LNP platform may achieve efficient immunogenicity under the conditions tested.

Characterization of BK polyomavirus serologic responses in kidney transplant recipients and healthy subjects

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Background: Kidney transplant recipients (KTRs) frequently experience persisting BK polyomavirus (BKPyV) replication and a subsequent decrease in allograft function, leading to BKPyV-associated nephropathy. In this study, we aimed to assess the antibody-mediated immune response to BKPyV genetically similar genotypes using a viral-like particle-based multiplex serology. The analyzed immunosuppressed patient groups had varied BKPyV replication kinetics based on the duration of virus replication and viral load in serum or urine.

Methods: Anti-BKPyV IgG and IgM response to serotypes I and IV in patients and healthy controls was determined using a Luminex Multi-Analyte-Profilig (xMAP) assay. The sensitivity of the xMAP IgG assay was validated using two methods, a commercial qualitative and semiquantitative ELISA assay (VIDITEST, VIDIA; n=70) and a quantitative pseudovirus-based neutralization assay (n=50). The thresholds indicating viral reactivation based on IgM and IgG levels were calculated using results from BKPyV DNA-negative KTRs. The seronegative threshold was determined using 18-24-month-old sera.

Results: Luminex xMAP IgG assay showed good correlation with ELISA ($r^2=0.96$) and neutralization assay ($r^2=0.88$). KTRs were classified into four groups based on their viral kinetics: BKPyV DNA detected only in urine (n=28), low-level DNAemia (in blood; n=18), and high-level DNAemia with $>10^4$ copies/ml (transient-resolved, n=26, or persisting DNAemia, n=7). All groups with BKPyV⁺ DNA significantly increased IgG and IgM antibody levels compared to BKPyV DNA-negative controls. At the end of the study period, IgG and IgM levels exceeded the viral reactivation threshold in 66 out of 79 and 42 out of 78 BKPyV DNA⁺ patients, respectively.

Conclusion: In this study, we developed and validated a quantitative IgG/IgM assay against two BKPyV serotypes. We demonstrated that levels of anti-BKPyV IgG and IgM were significantly increased in all KTRs with detectable levels of BKPyV DNA in plasma or urine, and that the serologic response was proportional to the level of virus replication.

DENV-3 predominance and increasing dengue activity in northern Petén, Guatemala: evidence from integrated clinical and laboratory surveillance

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Background: Dengue transmission in Guatemala shows substantial temporal and geographical heterogeneity, creating challenges for timely case detection and surveillance. Northern Petén is a geographically dispersed region where integration of clinical surveillance with laboratory confirmation can support the identification of changes in arbovirus circulation. We characterized dengue activity and virological findings among samples referred from Northern Petén during the 2025–2026 transmission period.

Methods: We conducted a descriptive analysis of 215 serum samples collected in Northern Petén between 28 December 2025 and 19 August 2026 and referred for laboratory investigation through the national surveillance system. Demographic, temporal and laboratory data were analyzed to describe dengue positivity, age distribution, temporal trends and DENV serotypes. Available diagnostic results for other arboviruses were also reviewed.

Results: Overall, 93/215 samples (43.3%) were dengue-positive. Positivity increased markedly during mid-2026, from 21.1% in May to 65.0% in June, 59.5% in July and 69.0% through 19 August. The highest positivity was observed among children aged 10–14 years (28/37; 75.7%), followed by children aged 5–9 years (35/66; 53.0%). Among 63 dengue-positive samples with an explicitly reported serotype, DENV-3 accounted for 61 (96.8%), while DENV-1 and DENV-2 were each identified once. One chikungunya IgM-positive result was recorded. No conclusions regarding the prevalence of other arboviruses were made because testing was not uniform across all samples.

Conclusions: Integrated clinical and laboratory surveillance identified a mid-2026 increase in dengue activity, marked DENV-3 predominance, and particularly high dengue positivity among children in Northern Petén, Guatemala. Strengthening decentralized case detection, laboratory confirmation and molecular characterization may improve recognition of changing arbovirus transmission patterns and support preparedness in geographically dispersed endemic settings.