



Published in final edited form as:

Antiviral Res. 2024 December ; 232: 106037. doi:10.1016/j.antiviral.2024.106037.

Meeting report of the 37th International Conference on Antiviral Research in Gold Coast, Australia, May 20–24, 2024, organized by the International Society for Antiviral Research

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

The 37th International Conference on Antiviral Research (ICAR) was held in Gold Coast, Australia, May 20–24, 2024. ICAR 2024 featured over 75 presentations along with two poster sessions and special events, including those specifically tailored for trainees and early-career scientists. The meeting served as a platform for the exchange of cutting-edge research, with presentations and discussions covering novel antiviral compounds, vaccine development, clinical trials, and therapeutic advancements. A comprehensive array of topics in antiviral science was covered, from the latest breakthroughs in antiviral drug development to innovative strategies for combating emerging viral threats. The keynote presentations provided fascinating insight into two diverse areas fundamental to medical countermeasure development and use, including virus emergence at the human-animal interface and practical considerations for bringing antivirals to the clinic. Additional sessions addressed a variety of timely post-pandemic topics, such as the hunt for broad spectrum antivirals, combination therapy, pandemic preparedness, application of in silico tools and AI in drug discovery, the virosphere, and more. Here, we summarize all the presentations and special sessions of ICAR 2024 and introduce the 38th ICAR, which will be held in Las Vegas, USA, March 17–21, 2025.

1. Introduction

The International Society for Antiviral Research (ISAR) was formed in 1987 as a not-for-profit scientific organization for the purpose of advancing and disseminating knowledge in all areas of antiviral research. To help achieve this objective, ISAR organizes annual meetings, the International Conference on Antiviral Research (ICAR). Now in its 37th year, ICAR 2024 was held May 20–24 in Gold Coast, Australia, using a hybrid attendance model, with both in-person and on-demand virtual attendance options available. The ICAR meeting reports are intended to serve as an overview of these conferences, with session and event summaries contributed by a panel of society members (see, (Andrei et al., 2017; Brancale et al., 2022; Bray et al. ; Spengler et al., 2023, 2022; Tramontano et al. ; Vere Hodge, 2015, 2014, 2013, 2011, 2003a, 2003b, 2017). These include details of awardee lectures, invited keynote speakers, individual speaker's sessions, the posters and PechaKucha competitions, and the results of the Chu Family Foundation awards.

ICAR attendees were invited to attend the Australasian Virology Mini-Virology Symposium, "Advancing Knowledge, Protecting People," hosted at the Griffith University Gold Coast campus on May 19, 2024. ICAR 2024 proper began on May 20th with the Women in Science roundtable event, followed by the opening session featuring two plenary speakers and the Gertrude Elion Memorial Award Lecture, the first of the ICAR award lectures. The rest of the award lectures were on subsequent days at the beginning of the speaker sessions. The speaker sessions featured invited and selected talks from international participants and were organized in the following subject areas: broad-spectrum antiviral drugs, non-respiratory biothreat viruses, and pandemic preparedness; coronaviruses, influenza, respiratory syncytial virus (RSV), and other respiratory viruses; chronic, latent, and persistent viruses - retroviruses and herpesviruses; arboviruses; hepatotropic and gastrointestinal (GI) viruses; and machine learning and computational approaches for antiviral research. Two poster sessions were held in person, showcasing additional advances in antiviral research. The special sessions and events included the PechaKucha competition, a special virus late-breaking oral presentation session, the ISAR Annual Business Meeting, a career development interactive roundtable, and the evening closing award presentation event.

Overall, ICAR 2024 once again highlighted both the virological and chemical aspects of antiviral drug discovery, along with novel approaches to screening systems and disease modeling to facilitate these efforts. ICAR continues to showcase antiviral research efforts from public and private partners in academia, industry, health care, and other national, international, governmental, and not-for-profit organizations in preventing and mitigating viral threats.

2. ISAR awards

2.1. 2024 Gertrude Elion Memorial Award. My battle against viruses. Johan Neyts, Ph.D., Rega Institute, KU Leuven, Belgium

Johan Neyts was awarded the Gertrude Elion Memorial Award and delivered a lecture titled "My Battle Against Viruses," which summarized the prolific productivity and successes of his research group at KU Leuven, Belgium. Johan began his presentation by acknowledging

the massive impact of Nobel Laureate and award namesake, Trudy Elion, on the field of anti-infectives. He noted how she inspired a generation of scientists to push the field of antiviral research forward, including Erik De Clercq, Antonin Holý, and John Martin, who along with Trudy, motivated Johan to follow in their professional footsteps.



Johan's laboratory of virology and antiviral research (www.antivirals.be) is focused on a variety of emerging viruses. Hepatitis C virus (HCV) is a member of the Flaviviridae family but is genetically distinct from "classical" flaviviruses like dengue virus (DENV) and yellow fever virus (YFV). In the past 25 years, multiple antivirals have been developed that can cure humans of HCV infection. Johan and colleagues discovered the potent anti-HCV activity of tegobuvir and alisporovir and identified their mechanism of action; efficacy of both drugs was shown in phase 2 and phase 3 clinical trials, respectively. Yet during this same time, there was a dearth of small molecule development for the treatment of infections with flaviviruses such as DENV, even though an estimated ~100 million individuals develop dengue fever every year. Johan and his many collaborators have recently transformed the landscape of DENV antivirals, discovering a class of indole derivatives in a phenotypic antiviral screen against DENV2. Following an extensive optimization effort together with his colleagues at the Center for Drug Design and Development (Leuven) and later joined by Janssen Pharmaceutica, pan-serotype inhibitors with picomolar potency were obtained. Mechanism of action studies showed that these small molecules targeted DENV nonstructural protein 4B (NS4B), a transmembrane protein associated with viral replication complexes. Johan summarized the highly successful public-private partnership of KU Leuven, multiple other academic institutions, and Janssen Pharmaceuticals in the

development of JNJ-1802, a first-in-class pan-serotype DENV NS4B inhibitor. JNJ-1802 prevents the association of NS4B with the replication-complex enzyme NS3. DENV can acquire resistance to JNJ-1802, although only after many passages, indicating this molecule has a high barrier to resistance. Importantly, Johan and colleagues showed that JNJ-1802 was highly effective at reducing DENV replication in both mouse and non-human primate (NHP) models. Lastly, Johan presented a JNJ-1802 dose finding study in a provocative human challenge model, in which the highest dose of drug prevented infection in 6 of 10 people while individuals receiving lower doses became infected, demonstrating a dose-dependent antiviral effect of the drug in DENV-infected humans. Altogether, the landscape for DENV antivirals has changed dramatically over the past 10 years and the Neyts lab and their many collaborators have been major players in this transformation.

The COVID-19 pandemic revealed major lapses in global pandemic preparedness, especially in the area of broadly acting antivirals. Johan presented a strategy for increased preparedness involving the generation of vaccines, monoclonal antibodies, and broader-spectrum antivirals. The Belgian Federal Government and Johan's home institution, KU Leuven, have invested in research infrastructure to accelerate the generation of these medical countermeasures (MCM), including the creation of the VirusBankPlatform (www.virusbankplatform.be), a repository of viruses and model systems for testing MCM, and a fully contained and automated robotic virology lab capable of phenotypically screening small molecule libraries 24 hours a day at biosafety level (BSL) 3+.

Johan then described the discovery and mechanism of action of CIM-834, a small molecule inhibitor of the SARS-CoV-2 membrane (M) protein. The M protein is essential for infectious virus particle formation. CIM-834 is highly active both in cell lines ($EC_{50} = 0.07 \mu M$) and in human primary airway epithelial cells, and significantly reduces viral replication in mouse models. Resistance passaging identified a single mutation (P132S) in the C-terminal beta sheet of the SARS M protein. Virologic, biochemical, and ultrastructural studies indicated that CIM-834 blocks oligomerization of M protein and assembly of virus particles. Further structural studies indicated that CIM-834 blocks a conformational change in M, preventing the formation of the longer extended form of the protein needed for efficient viral particle formation. Johan then closed his talk with a series of slides on broadly acting drug development for a variety of emerging viral diseases caused by picornaviruses (rhinovirus, enterovirus, coxsackievirus, etc.), paramyxoviruses (human parainfluenza virus [PIV], RSV, etc.), hepatitis viruses (hepatitis B, D, and E), and rhabdoviruses (rabies). This part of the talk provided yet another example of how new technologies and assays can drive the discovery of novel antiviral molecules.

Altogether, Johan presented a compelling narrative for strategies that should increase pandemic preparedness for emerging viruses. In addition, he provided multiple examples of how academic drug discovery can be elevated through industry collaboration, resulting in the development of novel medicines to address unmet needs. The impact of Johan's work is obvious today, but like Trudy and others that came before him, the true impact of his work will be realized in the inspiration of future generations of antiviral researchers.

2.2. 2024 William Prusoff Memorial Award. Challenges and gains of pre-clinical screening models for vaccine and antiviral advancement. Jessica Spengler, D.V.M., Ph.D., M.P.H., Centers for Disease Control and Prevention, USA

Jessica Spengler provided an incredibly thoughtful overview of animal models for the advancement of vaccines and therapeutics for viral hemorrhagic fever (VHF) viruses. She began her lecture by acknowledging the significance of receiving this award named after William (Bill) Prusoff, who developed the first ever FDA approved antiviral, and the members of the Society who have received the award in the past. Jessica is an investigator in the Viral Special Pathogens Branch at the U.S. Center for Disease Control (CDC). She is an expert in the study of VHF viruses that are high-consequence, low-incidence pathogens. Except for Ebola virus (EBOV), there are no FDA approved vaccines or therapeutics for VHF viruses. Since infecting small animals with human VHF viruses typically does not recapitulate all aspects of human disease, viruses adapted to rodents or immune-compromised models are typically utilized for preclinical development of vaccines and therapeutics. Jessica stressed the importance of animal model development and the belief that if effort is put into developing high-quality, robust animal models, these models are, in turn, likely to generate high-quality, translatable data when utilized for MCM development.



Jessica then introduced Nipah virus (NiV), a bat-borne paramyxovirus whose spillover into humans causes a multiphasic disease with respiratory and neurological phases. Jessica and her team developed a hamster model for NiV along with new tools for clinical scoring, which has increased the translation of these models. These data showed that animals'

sex did not impact disease outcomes, clinical signs, or virus replication. Recent mouse model development by her team has focused on transient innate immune suppression. Rather than using whole-body knockouts of interferon receptor (IFNAR), a monoclonal antibody is delivered to wild-type mice to temporarily prevent IFNAR signaling. Using this strategy, Jessica demonstrated that both respiratory and neurological pathogenesis observed in humans was recapitulated in this model and the kinetics of viral replication were like those observed in IFNAR knockout mice.

Additional mouse work presented, included in vivo imaging of VHF viruses expressing fluorescent reporters to reveal new aspects of viral pathogenesis. In vivo imaging of Crimean-Congo hemorrhagic fever virus (CCHFV) indicated infection in sex organs of mice (Welch et al., 2019), including survivors of infection, a finding that supported limited reports of potential sexual transmission in humans (Pshenichnaya et al., 2016). In vivo imaging also identified EBOV replication in the reproductive tissues of surviving mice (Davies et al., 2023), once again demonstrating translatable findings to human disease patterns. Similarly, in vivo imaging of new Nipah reporter viruses in IFNAR knock out mice revealed long-term maintenance of viral replication, generating a new model for studying late-onset disease and recrudescence reported in humans.

In the last section of her talk, Jessica presented data on utilizing the above models to evaluate MCM. EIDD-2749, or 4'-fluoruridine (4'-FIU), is a broadly acting nucleoside analog. Jessica described the oral therapeutic administration of 4'-FIU in baby food to guinea pigs. When given beginning a week after infection with Lassa virus (LASV) Josiah strain, 4'-FIU provided complete protection from lethal disease. Delaying treatment to 12 days after infection, which is concurrent with peak body temperature elevation, resulted in fever resolution overnight after a single dose.

Outbreak responses to VHF viruses ideally would include a ring vaccination campaign to limit spread in the population, but there are limited options for approved countermeasures, with those for EBOV being the one current exception. The CDC Viral Special Pathogens Branch has developed multiple virus replicon particle- (VRP) based vaccine candidates for LASV, CCHFV, and NiV (Kainulainen et al., 2018; Scholte et al., 2019; Welch et al., 2023). Jessica presented data for in vivo safety testing these vaccines in multiple species and successful in vivo efficacy studies including those designed to assess short course vaccination and post-exposure prophylaxis. Immune responses to vaccination appear to be durable, with improved durability following a prime-boost vaccine approach. Finally, Jessica closed her talk emphasizing the need for continued development and reevaluation of animal models for VHF viruses, for maximizing the utility of all vertebrate animals for research, and for implementing alternative models to reduce animal use if appropriate.

2.3. 2024 Women in science and excellence award. Targets to treatment: antiviral research and precision medicine. Judith Breuer, M.D., FRCPATH, FMedSci, University College London and Great Ormond Street Hospitals, UK

Judith Breuer is a professor of virology at University College London and the director of the Pathogen Genomics Unit. She is a clinical virology specialist with a practice at the Great Ormond Street Hospital for Children (GOSH), which cares for many immunocompromised

children. This patient population was the focus of the first part of her presentation. Judith divided her talk into two stories: one about extended use and repurposed drugs for common RNA virus infections, and the second about identifying new targets for viruses that replicate in skin.



Antiviral therapy for common RNA viruses like norovirus (NoV), enteroviruses, and respiratory viruses is urgently needed for children at GOSH because these infections may be lethal to them or delay life-saving treatment for their underlying condition. For instance, immunodeficient children with chronic NoV cannot receive bone marrow transplants, and current evidence suggests that children are more likely to survive bone marrow transplants if they have no underlying gastrointestinal or respiratory virus infection. GOSH has an experimental treatment program to repurpose antiviral drugs for these children. There are multiple criteria for this program: life threatening infection; licensed drugs not available or not working; some evidence the experimental drug will work; manageable toxicity; drug availability for compassionate use; biomarkers that the drug is working; evidence for combination therapy. Some drugs have been tried, but with mixed efficacy. The RNA-dependent RNA polymerase (RdRp) inhibitors ribavirin, favipiravir, and molnupiravir are good candidates for repurposing. Nitazoxanide is a drug with broad-spectrum antiviral activity, although with limited clinical evidence. Conflicting reports in the literature make it difficult to know if a drug is effective against RNA viruses in children.

Judith presented a case study in which a child was treated with favipiravir for a chronic NoV infection. Favipiravir treatment reduced the need for antidiarrheal medications, and the child

gained some weight during treatment and felt better, but viral load in stools was unaffected. The viral genomes from stool samples were sequenced and showed evidence of viral mutagenesis during favipiravir treatment. Favipiravir is known to elicit viral mutagenesis by incorporating into the viral genome. In another case study, combination therapy for NoV was attempted in an 18-month-old child with Di George syndrome (no thymus). After a thymic transplant, while the immune system was reconstituting, the child was treated for NoV with a combination of nitazoxanide and favipiravir. The child improved during treatment and gained weight, but later succumbed to other complications. NoV samples were collected from this child and were studied in the zebrafish model in collaboration with Joana Rocha-Pereira at the Rega Institute in Leuven. The original NoV sample, taken before antiviral treatment, was sensitive to nitazoxanide and favipiravir. Viruses collected during treatment were much less infectious in zebrafish, and the 50% inhibitory dose (ID₅₀) of the compounds increased.

A key criterion of the GOSH program is determining whether the experimental drugs are effective. Some evidence from these cases suggested that favipiravir was working: clinical improvement, mutagenic signature, loss of infectivity, and dose-dependent effects. Genomic analysis of NoV showed that favipiravir selected for a viral subclone. Mutations in the RdRp gene rose to fixation when the favipiravir dose increased, conferring resistance to favipiravir. Interestingly, a homologous mutation in the influenza virus polymerase is known to confer favipiravir resistance. More children are being successfully treated with favipiravir so they are eligible to receive bone marrow transplants. The toxicity of this treatment is generally manageable. Following the GOSH protocol for experimental drugs, children with respiratory non-COVID-19 infections, even some patients on ECMO, were helped with treatment. In patients who took favipiravir long term, mortality dropped from >50% to 21%. At least 30 days of treatment were needed to see a benefit. Molnupiravir was tested in a clinical study and in an animal model for efficacy against SARS-CoV-2. In the Panoramic study, molnupiravir was evaluated in patients with comorbidities. However, molnupiravir is not being pursued in the UK due to low efficacy and concern that it may drive the emergence of new variants: treatment with molnupiravir for 5 days is a risk factor for persistence of SARS-CoV-2 beyond day 14. A 5-day course of molnupiravir is too short and treatment for 10 days may be needed to clear the virus. Another concern is that SARS-CoV-2 becomes highly mutated in the presence of molnupiravir, which could drive the emergence of new variants. In collaboration with Johan Neyts at the Rega Institute in Leuven, combination therapy with favipiravir and molnupiravir was tested in the hamster model of SARS-CoV-2 infection, leading to a significant decrease in viral loads in nasal washes and organs. Combination therapy was more effective than molnupiravir alone, indicating synergy. A possible mechanism is that many more mutations in the genome arose, which could have generated non-viable progeny. Coincidentally, both drugs cause SARS-CoV-2 to reach error catastrophe at 20 mutations per genome.

In the second part of her presentation, Judith described her work with her postdoctoral fellow, Cristina Tommasi (UCL), on viruses that cause skin lesions, particularly varicella zoster virus (VZV). VZV causes chicken pox and shingles, both of which cause skin blisters. This is critical for the virus because the blisters contain cell-free virus ready for transmission. Thus, skin anatomy is important for understanding virus tropism. Epidermal

keratinocytes differentiate from the basal layer through five strata, gaining and losing proteins such as cytokeratin 15 (KRT15) in basal stem cells and cytokeratins 1 and 10 (KRT1, KRT10) in the suprabasal layers. VZV alters keratinocyte gene expression to cause blisters. KRT10 and KRT1 in the spinous layer are decreased and tight junctions are lost, characteristics typical of any blistering disorder like epidermolysis bullosa. Concurrently, MDM2 and kallikreins 5, 6, and 7 are upregulated. Interestingly, the uncoupling of KRT1 and KRT10 with an increase in MDM2 is characteristic of ichthyosis. MDM2 binds p53 and promotes tumorigenesis. Nutlin 3, a small molecule inhibitor of MDM2, inhibits VZV replication. KRT15 is essential for VZV replication. Normally expressed in basal layers, VZV induces KRT15 in VZV lesions throughout the epidermis. Using organotypic raft cultures, overexpression of KRT15 increased VZV replication, while KRT15 knockdown abrogated VZV replication. KRT15's role in VZV infection is being studied. KRT15 binds to Telo2, a telomere maintenance protein and part of the DNA damage response (DDR); knockdown of KRT15 reduces DDR protein expression. Herpesviruses are known to use the DNA damage response for their own replication. One DDR protein, ATM, is needed for VZV replication. These patterns are found in other viruses that cause skin infections and blisters: herpes simplex type 1 (HSV-1), Mpox virus (MPXV), and human papilloma virus (HPV). They all suppress KRT10 and increase KLK6 and MDM2.

2.4. 2024 Diversity in Science Excellence Award. Chasing an HIV cure: the intersection of biological sex and latency reversal. Nancie Archin, Ph. D., University of North Carolina, USA

The Diversity in Science Excellence ISAR Award is the latest major award granted by ISAR. It highlights a scientist of any group historically underrepresented in our Society who has made outstanding contributions to the field of antiviral research. The 2024 most deserving Awardee was **Nancie Archin**. Nancie started by describing her life in the remote mountains of Haiti before moving to New York, where she attended high school and college, in which she first tried to major in music and then astronomy. She found the first not to be her calling and the second too boring, so she eventually “settled” for biology. In her career, she went on to study Alzheimer disease, HSV, and finally HIV persistence and sex differences, which was the topic of her talk.



Nancie first described the general replication cycle of HIV and the generation of long-term reservoirs. Anti-retroviral therapy (ART) suppresses replication and controls disease, but the long-term reservoirs in memory CD4 T cells and perhaps macrophages are not eliminated and viremia rebounds if the person stops therapy. Most people living with HIV are women and girls and most new infections are in women, yet women are underrepresented in HIV cure studies. Most data are from studies in males, with only 11% of all cure studies in women. There are sex-related differences in responses, however: women are more likely to spontaneously control infection or to control infection after interruption of treatment. Viremia levels are lower and CD4 counts are higher in women, but they are more likely to promptly progress to AIDS without therapy. Type I IFN responses are also higher in women.

Sex hormones affect HIV reservoirs and one report indicated that viral loads vary with the menstrual cycle. Nancie's first question was to address whether sex affects long-term HIV reservoirs in ART-suppressed women and men matched by age and race. No major differences were observed in the frequency of total or replication-competent long-term reservoirs between sexes. Another study additionally matched by CD4 counts and other factors, also identified no significant differences in reservoir DNA levels, although women had lower residual viremia and lower multi-spliced cell-associated HIV RNA (Scully et al., 2019). A study with a larger cohort in Uganda found no differences in total proviral DNA but did find a two-fold lower inducible replication-competent reservoir in women (Prodger et al., 2020). In summary, all these studies suggest that existing differences between HIV reservoirs in men and women may be subtle, and larger cohorts may be needed to understand the biological significance of these differences.

Nancie then moved on to discuss the use of latency-reversal agents (LRA), an approach that basically consists of reactivating HIV reservoirs while using ART to inhibit infection of other cells. Nancie considered the effects of estrogen changes during the menstrual cycle on viremia. An estrogenic environment is not supportive of reactivation from latency and can also be somewhat immunomodulatory. Furthermore, estrogen receptor suppresses HIV transcription. Nancie focused on the effects of LRAs on estrogen receptor alpha (ER- α) expression in CD4 T cells, cells most relevant for HIV, to understand the influence of LRAs on ER- α -dependent gene transcription. As expected, ER- α mRNA levels did not differ between men and women. Various classes of LRAs downregulated ER- α expression in CD4 T cells but did so equally in males and females. Nancie's group then used pharmacological approaches to modulate ER expression, using anticancer drugs such as tamoxifen and fulvestrant to better understand the relationship between downregulating ER- α and reactivation of HIV by LRAs. However, none of the agents affected ER- α expression in CD4 T cells, in contrast to cancer cells, in which, for instance, fulvestrant induced the expected degradation. The group then used proteolysis targeting chimeras (PROTACs) to induce ER- α ubiquitination and degradation, but once again, while it induced ER- α degradation in cancer cells, it did not do so in CD4 T cells. Moreover, ER did not translocate to the nucleus in CD4 T cells exposed to estrogen, whereas it did so in cancer cells. This suggests that ER regulation in immune cells may be different than in cancer cells.

In the final part of her talk, Nancie focused on using the HIV trans-activator Tat as an LRA. Many clinical trials of LRAs have not been successful, possibly because of insufficient reactivation, LRA toxicity, or failure of the immune system to properly recognize and kill cells containing the reactivated virus. Blocking HIV transcription in the reservoirs mostly occurs at proximal elongation, then polyadenylation, and then poly-splicing. Nancie summarized the role of Tat binding to Tar in releasing the proximal stalling of RNA polymerase II on the HIV proviral genome. In collaboration with Janssen pharmaceuticals, her group has encapsulated Tat-encoding mRNA (T66) in lipid nanoparticles. These nanoparticles activated HIV provirus transcription in wild-type virus but not in virus mutant lacking the Tar RNA loop recognized by Tat. They then evaluated the synergy between Tat and traditional LRA in culture and primary CD4 T cells from ART-treated infected individuals. Some combinations of these factors robustly induced LRA without major cytotoxicity, with certain combinations appearing at least additive if not synergistic.

Nancie concluded that sex differences in HIV reservoirs between men and women are subtle and large clinical trials may be needed to evaluate their biological significance, but they should nonetheless be taken into consideration in HIV treatment campaigns and studies. She concluded that ER- α works by unique mechanisms in CD4 T cells, which deserve further studies, and that the T66 approach to reverse latency is worth pursuing. She finished her talk by thanking collaborators, her laboratory, and particularly the people living with HIV who participated in the studies she presented. During the question period, she indicated that estrogen treatment would effectively prevent virus rebound because of all the other roles estrogen plays, including immunomodulatory functions.

3. ISAR plenary speakers

Chaired by Kathie Seley-Radtke and Luis Schang.

3.1. Virus emergence at the human-animal interface. Edward C. Holmes, FAA, FRS, University of Sydney, Australia

The first plenary talk was delivered by Professor **Edward (Eddie) Holmes**. His lecture framed the global scope of the meeting by presenting the global diversity of the world virosphere and the increasingly porous human-animal interface. He precluded his talk with a brief comment about the ongoing HPAI H5N1 influenza outbreak in cattle, which went undetected for seven months and had just produced the first human case (fortunately very mild) at the time of the meeting, only 4 years after the start of the COVID-19 pandemic. There is obviously still much to learn from past and recent experiences!

Eddie began by presenting a brief history of virology to bring attention to the inherent bias for focusing on viruses affecting humans and important crops or farm animals. However, there are some 8.7 million animal species, affected by roughly 10 viruses per species, which means that there are ~87 million viruses (within a factor of ~10), of which only about a few hundred affect humans. The bias is still towards viruses affecting species of main interest, and until recently most of the viruses characterized were mammalian. The rise of virogenomics has changed this balance. Eddie briefly described his group's approach, which is basically shotgun sequencing of total RNA after depleting ribosomal RNA to analyze the sequences.

Eddie focused on bat viruses first, emphasizing that, contrary to popular belief, bats do get sick and do not carry a particularly large number of viruses. He then compared the state-of-the-art knowledge of Orthomyxoviridae in 2015 to current knowledge. Just in the last 8 years, our knowledge of the virome of invertebrates has expanded drastically, moving from traditionally studied species like mosquitoes, flies, and ticks into a wider range of species like mollusks. The Orthomyxoviridae family now includes many viruses affecting invertebrates and previously understudied chordates, mostly fish, which were found to carry many viruses like hantaviruses and filoviruses, previously thought to exclusively affect mammals. The textbook description of influenza has aquatic birds as the center of epidemiology and evolution. However, Eddie showed an unpublished phylogenetic tree of influenza that includes viruses infecting salamanders, toads, hagfish, and even tunicates. His group has continued to delve further and further into the virosphere. He showed a (weak) phylogenetic tree of the most conserved RNA virus gene, RdRp, to illustrate that the virome is about 20% more diverse than the full tree of life. Eddie then acknowledged the intrinsic limitation to using virogenomics in its current form, which can only detect sequences that are related to known viruses, precluding the identification of other viruses that have more diverse RdRp. To solve this limitation, his group is now using AI to expand the virosphere. So far, they have discovered about 160,000 additional viruses in 180 virus phyla or classes, including many in aquatic systems.

Eddie chose to focus on Nidovirales, which includes the coronaviruses. Coronaviruses had been known to affect mammals and birds, but his group has found many coronaviruses in

fish, even primitive fish including lamprey, suggesting a long evolutionary history. They also found that another group of Nidovirales, the arteriviruses, track the evolution of their hosts for millions of years.

Eddie expanded their analyses to aquatic invertebrates, such as jelly fish and hydra, which are also infected by viruses related to influenza and flaviviruses, indicating that these, too, are very ancient. Excitingly, his group is now sampling stromatolites, ancient microbial reefs created by cyanobacteria dating back 3.5 billion years. He also described how they sample the whale's blow or spout, and that they are also collecting samples from crocodiles. Not surprisingly, not one member of the audience volunteered for the latter!

Eddie then focused on the origins of SARS-CoV-2, highlighting the risk posed by the game and fur animal trade. He presented his own pictures from the wild-life farms he had taken before the pandemic of 2020 sampling the animals. His group had identified 21 high-risk viruses, including BathKU8 coronavirus in a civet and H9N2 avian influenza in an Asian badger, and showed videos of many sick animals on these farms, highlighting the poor sanitary conditions in which the animals are held. They also sampled some 500 animals that had succumbed to diseases and identified very rich viromes, including 39 high-risk viruses. They found Japanese encephalitis virus in guinea pigs, rotavirus in deer, influenza A (IAV) viruses in many species, and seven coronaviruses including a bat merbecoronavirus, which likely uses ACE2, in racoons. Concerningly, the same merbecoronavirus that had been identified in a racoon in a fur trade farm has been identified in the virome of companion animals. Additionally, highly pathogenic avian influenza (HPAI) H5N1 has been identified in mink farms in Finland. Eddie also displayed pictures of the Huanan market in Wuhan taken in 2014, before the start of the COVID-19 pandemic, showing the animals held in the market and the conditions in which they were held.

Eddie then described the epidemiological evidence from Wuhan and the market itself. All animals had been removed from the market before it could be sampled, but most positive swabs were collected from the areas of the market in which live animals were sold. The stalls from which the most positive swabs were collected were those containing the most animals, although it is not technically possible to determine from nucleic acid samples whether the virus came from infected animals or not. These swabs also contained nucleic acids from many other viruses, highlighting the overall risk of cross-species transmission. Eddie also discussed that the first 118 cases of COVID-19 from December 2019 clustered around the market, including patients who had had no direct contact with the animals. Moreover, the molecular clock data give the same starting date for the epidemic as the clinical data and identify two different sub-strains from early in the outbreak. However, determining how the virus got to the market is most difficult. The reservoirs of the sarbecoronavirus are mostly in Southern China, Cambodia, Vietnam, and Thailand area. The SARS-CoV-1-like viruses are predominantly found in bats from Yunnan and northwards, and the SARS-CoV-2-like viruses are predominantly seen in bats southward of Yunnan.

During the question period, Eddie stated that the fish viruses his group identified were mostly collected from the oropharyngeal samples because fish viruses mostly spread by ingestion. This general rule holds for viruses that are related to mammalian respiratory

viruses, so the tropism of the related viruses in different species differs. Answering another question, he stated that it is unclear whether the viruses they identify are acute or chronic, but they mostly appear not to produce major disease in the wild populations, with the caveat that diagnosing a sick hydra, for example, would be quite difficult. Also, during the questions period, Eddie stated that, in general, invertebrate viruses tend to be more complex than vertebrate ones in the same families, suggesting negative selection against multiple viral proteins in the latter.

In summary, Eddie delivered a scintillating dissertation about the magnitude of the virome of the planet and the relationships between viruses affecting most diverse species with the corollary that fur and game animal trading and farming pose a continuous high risk of introducing new viruses into the human population. One could say that Eddie presented an extensive menu of future antiviral needs. It must be highlighted that although the basis for this work has been published through the years (e.g., He et al., 2022; Shi et al., 2018), most of Eddie's talk focused on unpublished data.

3.2. Bringing antivirals to the clinic: challenges and opportunities. Jenny Low, M.B.B.S., M.P.H., Singapore General Hospital, Duke NUS Medical School, Singapore

Jenny Low delivered a wide-ranging presentation focused on three main scientific areas: (1) why antiviral drugs are needed; (2) new direct acting antivirals (DAA) and biologics on the horizon against DENV; and (3) strategies to accelerate drug development. Jenny began with an introduction summarizing her journey as a physician scientist focused on acute viral infections. She was born and raised in Singapore, a locale with a tropical climate and year-round, endemic DENV transmission. Moreover, as a busy transport hub, Singapore is constantly exposed to new and emerging pathogens. This motivated Jenny to specialize in infectious diseases during her medical training. During this time and shortly thereafter, Jenny saw the impact of emerging viral disease on her community during the SARS-CoV-1 epidemic in 2003, when many people, including those in her own hospital, were infected. Of note, this epidemic happened at a time before vaccines or therapeutics to treat or prevent any human coronavirus infection. In 2005, Singapore experienced a large DENV outbreak, which triggered the establishment of clinical research endeavors to better understand dengue disease, identify risk factors associated with increased disease severity in adults, and evaluate the therapeutic potential of repurposed antivirals. While the antiviral trial Jenny led at the time showed that celtigosevir did not impact viral replication or disease outcome, the experiences were invaluable for improving the design of her future studies. She then posed the question, "Do we need drugs against acute viral diseases?" She posited that we may not need them for endemic viruses for which we have some level of baseline immunity, but newly emerging viruses introduced into naïve populations can have catastrophic effects, as we saw with SARS-CoV-2, the cause of COVID-19. While COVID-19 vaccines eventually reached much of the global population, it was not before millions of people had died from the disease. Although we now have COVID-19 vaccines that can prevent severe disease, breakthrough infections fueled by perpetual variant emergence are still causing infections with significant morbidity and mortality. In addition, diseases like yellow fever continue to cause high morbidity and mortality in Africa because the yellow fever vaccine is not easily accessible. Lastly, in this section, Jenny spoke about long-COVID-like sequelae

associated with DENV infection, in which some survivors experience depression, fatigue, and neurological symptoms like brain fog. She posited that antivirals may prevent the development of these post-acute syndromes. Thus, antivirals for acute viral diseases still have the potential to greatly benefit public health even when effective vaccines are available.

The second section of Jenny's presentation provided an update on several promising DAAs and biologics to treat flavivirus infections. First, she presented data on JNJ-1802, a very potent pan-serotype inhibitor of DENV NS4B, which has a 50% effective dose (EC₅₀) in sub-nanomolar levels. A phase 2 trial was initiated in 2021 but recruiting patients was challenging due to the ongoing COVID-19 pandemic. Jenny described NITD-688, another potent pan-serotype inhibitor of DENV NS4B, which is now in clinical phase 2A testing. Jenny then talked about two monoclonal antibodies (mAb) targeting flaviviruses. The DENV envelope guides binding, fusion, and entry into the host cell and can be divided into three functional domains. VIS513 is a pan-DENV humanized IgG1 targeting envelope domain 3, which contains the receptor binding domain. While clinical development was delayed because of the COVID-19 pandemic, a phase 2 trial has been initiated in India by Serum Institute India and the study has recently completed recruitment. Jenny then described her efforts in the development of Tyzivumab, an mAb targeting Zika virus (ZIKV), which was taken through phase 1 trials before development ceased due to a rapid reduction in case numbers. The explosive YFV outbreak in South America and Africa once again fueled the discovery and development of TY014, a fully human IgG1 anti-YFV monoclonal antibody, which progressed from discovery to the end of phase 2 in 12 months, a clear demonstration that therapeutics can be developed on an accelerated timeline to respond to a public health emergency.

The last section of Jenny's presentation focused on strategies to accelerate drug development. Multiple times during her presentation, Jenny touched upon the theme of molecular endpoints, or measuring the host response using multi-omics technologies to better define disease metrics that can be leveraged to complement traditional ways of measuring antiviral safety and efficacy in human trials. In addition, learning about the early host response to acute viral infection may lead to discovery of new targets for host-directed therapies that can alter the trajectory of disease. The COVID-19 pandemic resulted in the implementation of adaptive trial designs for vaccines and therapeutics, which contributed to the rapid assessment of new products in humans. Since human trials often require thousands of participants for adequate statistical analyses, Jenny spoke about the potential power of human challenge models for dengue (dengue human infection models [DHIM]). Depending on the input virus, DHIM models can range from replication models with viremia but without fever to mild disease models with viremia, headache, myalgia, fatigue, and fever. DHIM requires few patients (20–30) to demonstrate antiviral effects with adequate statistical power. For example, JNJ-1802 was first demonstrated to have antiviral activity in a prophylaxis setting using such DHIM. Jenny then posed the question of whether more robust models can be developed to replace traditional human trials, which will further accelerate the antiviral and vaccine developmental timeline. She closed out her presentation by reiterating the need for therapies to treat acute viral diseases and stressing that this endeavor must be fueled by public-private partnerships, as development is unlikely to be undertaken by the pharmaceutical industry alone. Nevertheless, Jenny clearly demonstrated

the power of compressed antiviral drug development timelines and the acceleration of antiviral agents towards human clinical testing in response to public health emergencies.

4. Broad-spectrum antiviral drugs, non-respiratory biothreat viruses, and pandemic preparedness

Chaired by John Bilello and Subash Vasudevan.

4.1. Oral pharmacokinetics and efficacy of modified oral lipid RVn prodrugs against SARS-CoV-2 in Mice. Aaron Carlin, M.D., Ph.D., Department of Pathology and Medicine, University of California San Diego, USA

Remdesivir (GS-5734) was approved for emergency use to treat COVID-19 in many countries, but its major drawback is its intravenous (IV) administration. **Aaron F. Carlin** reported an attempt to develop potent orally bioavailable antivirals to treat RNA virus infections. His group synthesized a nucleoside phospholipid analog ODBG-P-RVn (V2043), a lipid prodrug of GS-441524 monophosphate, which demonstrated in vivo efficacy in a SARS-CoV-2 mouse model (Carlin et al., 2023; Schooley et al., 2021). A structure-activity relationship study of the phospholipid side chain identified two additional drug candidates: V2053 (bearing 3-fluoro-4-methoxy-benzyl) and V2067 (bearing 4-cyano-benzyl). These modifications significantly increased antiviral potency (compared to V2043) against SARS-CoV-2 and many other clinically important RNA viruses such as EBOV, NiV, DENV, ZIKV, and RSV in various cell lines (McMillan et al., 2023). The group also evaluated the in vivo efficacy of oral, once-daily administration of V2043, V2053, and V2067 as prophylaxis (first dose 12 hours before infection) and treatment (first dose 12 hours after infection) in a murine SARS-CoV-2 infection model. The compounds significantly reduced lung viral titers on day 2 and led to undetectable virus in the lung by 4 days post-infection (dpi). Oral and IV single-dose pharmacokinetics studies determined the oral bioavailability of V2043, V2053, and V2067 to be between 51% and 74%. The compounds are stable in plasma, active as a single oral daily dose, and are at least 5 times more active than obeldesivir on a molar basis. The prodrugs represent promising leads for the development of potent broad-spectrum antivirals.

4.2. Towards a novel host-targeted anti-infective strategy against COVID-19 and other acute respiratory viral diseases. Stephan Ludwig, Ph.D., Institute of Virology, Germany

Severe influenza and COVID-19 are hyper-inflammatory viral diseases that progress in subsequent stages. The first stage is dominated by damage caused by the virus itself, while the second stage occurs due to hyper-induction of cytokines. DAA are only effective in the early stages of the disease and not during the hyper-inflammatory phase. To alleviate hyper-inflammatory responses, novel agents should be developed that target host cell signaling pathways needed for virus replication and involved in pro-inflammatory cytokine expression. In his talk, **Stephan Ludwig** discussed the identification of mitogen-activated protein kinase (MAPK) signaling cascade (Raf/MEK/ERK kinase pathway), on which the replication of IAV was strongly dependent (Schreiber et al., 2020). Other viruses like RSV, Borna disease virus (BDV), and hantaviruses were also sensitive to MEK inhibition, suggesting that MEK inhibitors could act as novel broadly active antivirals with a dual

benefit: acting as direct antivirals and balancing immune responses (Ludwig et al., 2023). The MEK inhibitor zapnometinib (ATR-002) resulted in sustained inhibition of SARS-CoV-2 propagation, led to reduced expression of virus-induced pro-inflammatory cytokines (Schreiber et al., 2022b), acted synergistically with other DAA (Schreiber et al., 2022a), and was highly resistant to emergence of escape mutants (Schreiber et al., 2024). Results of a phase II clinical trial with Zapnometinib in hospitalized COVID-19 patients (RESPIRE, [NCT04776044](#)) indicated its clinically relevant efficacy with a favorable safety profile (Rohde et al., 2023). Zapnometinib reduced viral titers and lowered cytokine responses in hospitalized patients with moderate or severe COVID-19. Thus, zapnometinib represents a clinical proof-of-concept for targeting the Raf/MEK/ERK kinase pathway as a promising anti-infective strategy.

4.3. Exploring viral RNA methyltransferases for antiviral drug design. Radim Nencka, Ph.D., Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Czech Republic

Radim Nencka provided background on viral RNA methyltransferases, pivotal enzymes responsible for methylating viral RNA 5'-cap structures (Nencka et al., 2022). Radim's talk focused on two methyltransferases: nsp14 and nsp16/nsp10 from the SARS-CoV-2 virus. Using structural models, a series of nanomolar inhibitors of SARS-CoV-2 nsp14 N-7 methyltransferase (e.g., compound TO507 (Otava et al., 2021)) were designed and further refined through rational design (Štefek et al., 2023). Furthermore, a crystal structure was obtained of the optimized inhibitor in complex with the nsp14 methyltransferase domain. Although the best compounds were single-digit nM inhibitors and were able to efficiently cross cell membranes and accumulate in cells, none of them exhibited any significant activity against SARS-CoV-2 in cell culture. Interestingly, the crystal structure of 2'-O-RNA methyltransferase VP39 from MPXV (a double-stranded DNA virus from the *Poxviridae* family) with several inhibitors (e.g., sinefungin and TO507) were obtained, and several potent inhibitors of VP39 were identified through the established high-throughput screens (Silhan et al., 2023). These compounds were able to enter cells and inhibit MPXV replication in cell culture (Zgarbová et al., 2023). Next, Radim's group obtained the first crystal structure of the SARS-CoV-2 nsp16/nsp10 complex with a non-selective pan-methyltransferase inhibitor. The image revealed an allosteric cavity in nsp16/nsp10 that was capable of binding both reversible covalent (e.g., compound JK1197) and non-covalent (e.g., JK1361) inhibitors. This allosteric binding site of SARS-CoV-2 nsp16/nsp10 complex can be exploited for future drug design and development (Inniss et al., 2023).

4.4. Targeting host kinases to identify novel broad-spectrum antiviral strategies. Valeria Cagno, Ph.D., Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Switzerland

Valeria Cagno reminded the audience that targeting the host is a strategy for overcoming the development of resistance to antivirals and for identifying broad-spectrum antivirals. The ultimate goal is to find a cellular protein redundant for the cell but essential for the virus, in order to limit cytotoxicity while maintaining antiviral activity. Host kinases are druggable proteins largely studied to fight cancer but not yet exploited as antivirals. Thus, a series of bithiazole derivatives were identified as potent inhibitors of phosphatidylinositol 4-kinase

III β (PI4KIII β), which is required in the life cycle of various viruses. These compounds were active against several members of *Picornaviridae*, *Flaviviridae*, and *Coronaviridae* viral families. The next goal is converting them into PROTACs to achieve even more potent inhibition. In parallel, the group identified several Src (non-receptor tyrosine kinase) inhibitors; these showed activity against ZIKV, MPXV, HIV, and herpes simplex virus 2 (HSV-2). Interestingly, the lead compound also exhibited activity against intracellular bacteria *Chlamydia trachomatis*, suggesting the possible use of these compounds as ingredients of vaginal/anal microbicides. The obtained data suggest that targeting these host kinases may result in broad-spectrum antiviral treatment.

4.5. Non-structural protein 4B: jewelry or rock for flavivirus drug development. Xuping Xie, Ph.D., University of Texas Medical Branch, USA

Xuping Xie presented on the overall drug discovery and development progress at the AViDD award recipient University of Texas's Medical Branch (UTMB)-Novartis Alliance for Pandemic Preparedness (UNAPP) Center, a unique iterative academic-industry partnership for antiviral drug discovery. It involves UTMB as the primary academic institute, along with Emory University and Uniformed Services University, partnered with Novartis Global Health Disease area from the industry side. UNAPP was established during the COVID-19 pandemic, aiming to create a collaborative environment focused on preparing antiviral drug candidates for potential future pandemics. This partnership leverages the strengths of academic research and industry resources to accelerate the development of effective antiviral treatments and preventive measures.

UNAPP aims to deliver clinical candidates for pandemic preparedness, advance antiviral research, and train the next generation of drug hunters. The diverse team acts on lessons learned from the COVID-19 pandemic, acknowledging how broad-spectrum antivirals in late-stage preclinical or clinical development expedited treatment options for COVID-19. UNAPP is composed of four core centers: 1) Virology Core for testing antiviral activities of compounds at BSL2-to BSL-4 facilities, profiling antiviral mechanisms of action, and creating tools to facilitate antiviral screening, thus advancing basic knowledge of viral proteins and pathology; 2) High-Throughput Screening (HTS) core for large target-based and cell-based antiviral screening without high containment; 3) Translational Research Core for determining compound pharmacokinetics, preclinical safety, and IND-enabling studies; and 4) Drug Discovery Core with a team of medicinal chemists, data scientists, structural chemists and protein scientists for target deconvolution, compound prioritization, hit-to-lead optimization, and chemical synthesis. Through these cores, UNAPP is targeting coronaviruses, henipavirus, and flaviviruses, employing target- and cell-based screening approaches to develop broad-spectrum inhibitors. The collaborative center is pursuing main protease (M^{pro}) inhibitors for coronaviruses, L protein (RdRp) inhibitors against NiV, nucleoside and non-nucleoside polymerase inhibitors, protease inhibitors, and NS4B inhibitors against flaviviruses.

Xuping highlighted his laboratory's experience with COVID-19 to develop a live-attenuated SARS-CoV-2 tool for facilitating high-throughput screening of compounds under BSL-2 conditions at UNAPP. This live-attenuated SARS-CoV-2 was built from the SARS-CoV-2

infectious cDNA clone that was created by his team early in the pandemic (Xie et al., 2020). His team took two approaches: 1) deleting open reading frames (ORFs) 3, 6, 7, and 8, whose functions are to antagonize host responses to the virus; and 2) mutating transcription regulatory sequences to prevent reinsertion of these genes into the attenuated virus by homologous recombination when co-infection with a wild-type virus occur (Adam et al., 2023; Liu et al., 2022). The resulting Δ 3678 SARS-CoV-2 virus was significantly attenuated in cell culture with severely impeded ability to spread in primary human airway cells. Additionally, it was attenuated in hamsters, resulting in no body weight loss or disease in the infected animals. Δ 3678 SARS-CoV-2-infected hamsters had substantially lower virus titers in nasal washes, oral swabs, and tracheal and lung tissues than did those infected with wild-type virus. Similar results were observed in the highly susceptible K18-hACE2 mice: a high inoculation titer of the Δ 3678 virus was not lethal and viral titers in the lungs were 100-fold lower than in mice infected with wild-type virus. The Δ 3678 virus was approved for BSL-2 use by UTMB and NIH OSP. This system was advanced further to include an NLuc reporter gene for high-throughput screening and to evaluate antiviral resistance phenotypes, as utilized in the UNAPP antiviral discovery programs.

In particular, UNAPP is addressing the medical need for dengue fever countermeasures by advancing the DENV NS4B inhibitor NITD-688, which was discovered following a phenotypic screening and lead optimization campaign (Moquin et al., 2021). Xuping summarized UNAPP's efforts to determine NITD-688's distinct mechanism of action. Prolonged NITD-688 treatment of DENV-infected cells selected for mutations within NS4B that conferred resistance to NITD-688. Subsequent evaluation of NS4B recombinant viruses bearing these mutations confirmed that they were responsible for reduced susceptibility to NITD-688. Additionally, direct binding of NITD-688 to a recombinant NS4B protein was shown by isothermal titration calorimetry (ITC), with affinity reduced in proteins harboring the selected resistance sub-stitutions. Cellular immunoprecipitation assays showed that NITD-688 specifically inhibited the NS3/NS4B interaction when treatment was initiated before and after NS4B/NS3 complexes formed in the cells. ITC and a biolayer interferometry assay further demonstrated that NITD-688 inhibited both the *de novo* interaction of NS4B with NS3 and disrupted the already-formed NS3/NS4B complexes. Furthermore, imaging DENV-infected cells with specific NS4B monoclonal antibodies demonstrated that NITD-688 could disrupt NS4B/NS3 complexes in infected cells. NITD-688 has progressed into phase 2 clinical trials for treating DENV infection (<https://classic.clinicaltrials.gov/ct2/show/NCT06006559>).

4.6. Molnupiravir extends its broader spectrum of activity to human norovirus and rotavirus in 3D human intestinal enteroids. Nanci Santos-Ferreira, Ph.D. Candidate, KU Leuven, Rega Institute, laboratory of virology and Chemotherapy, Belgium

The broad-spectrum inhibitor molnupiravir has received emergency use authorization as an oral treatment for COVID-19. Molnupiravir is metabolized to N4-hydroxycytidine (NHC), which is a substrate for viral polymerases. Incorporation of NHC by viral polymerases causes base-pair mismatching during viral genomic replication and transcription, leading to lethal mutagenesis and inhibition of viral replication. Because the viral polymerase active site region is highly conserved among RNA viruses, **Nanci Santos-Ferreira** and her

group evaluated molnupiravir effectiveness against human norovirus (hNoV) and human rotavirus, which are leading causes of gastrointestinal diarrhea (Santos-Ferreira et al., 2024). No antivirals are approved for either virus and the rotavirus vaccine provides insufficient protection in certain populations, including immunocompromised individuals. The recent development of human intestinal enteroids (HIEs) provided a model to evaluate antivirals in a physiologically relevant model susceptible to infection and replication of multiple hNoV and rotavirus genotypes isolated from clinical samples. Tissue-derived HIEs self-assemble to recapitulate the 3-dimensional structure and cellular diversity of the human small intestine. Replication of hNoV and rotavirus can be measured by determining viral RNA levels by RT-qPCR or by calculating the percentage of cells with positive staining for viral capsid proteins. The HIE hNoV and rotavirus infection models were validated by confirming that the antiviral activity of known inhibitors of these viruses in this model was similar to prior results in immortalized cellular infection models with lab-adapted viruses. Specifically, molnupiravir and NHC were comparably potent against all hNoV and rotavirus strains tested in the HIE model. Molnupiravir reduced the number of rotavirus viroplasms (compartments for replication and assembly) that could be visualized in cells. Further evaluation of molnupiravir mechanism of action against hNoV and rotavirus replication is underway.

4.7. Targeting Mpox virus resolvase (Mpr): in vitro assay development and inhibitors.
Zhengqiang (ZQ) Wang, Ph.D., Center for drug design, college of Pharmacy, University of Minnesota, USA

Mpox virus (MPXV) is endemic to Central (clade I) and Western (clade II) Africa; however, the recent outbreak of clade IIb variants is associated with significant mortality, especially in immunocompromised individuals. The smallpox drug tecovirimat has been deployed against MPXV, but its barrier for resistance emergence is low, highlighting the need for additional antiviral drugs. One target for novel antiviral drugs is the poxvirus resolvase, which resolves genomic DNA concatemers that are separated by Holiday junctions and are formed during poxvirus replication. The poxvirus resolvase (Mpr) is an RNaseH-like endonuclease required for concatemer resolution (Li et al., 2016). Deletion of an orthologous vaccinia virus resolvase results in the accumulation of concatemer junctions and reduced virus replication (Garcia and Moss, 2001). Inhibitors of resolvase activity targeting the divalent metal cation binding the DEDD motif common among RNaseH-like enzymes may be potent antivirals for poxviruses, including MPXV. **ZQ Wang** and his laboratory developed a FRET-based assay to measure MPXV resolvase activity and validated it using an initial HIV-1 integrase inhibitor previously observed to inhibit fowlpox resolvase (Culyba et al., 2012) in a fluorescence polarization assay, and to inhibit vaccinia virus in a cellular assay. Screening of a University of Minnesota metal-binding compound library in this assay yielded 38 compounds with Mpr enzymatic potencies and IC₅₀ values < 10 µM, with 2 compounds having IC₅₀ values ≤ 10 nM. FRET-based Mpr results were confirmed using an in-gel fluorescence assay, demonstrating compound inhibition of Mpr substrate cleavage. Most of the Mpr compound hits also inhibited fowlpox resolvase in the FRET assay, highlighting their potential as broad-acting poxvirus inhibitors. Representative compounds were potent in a cellular antiviral assay using vaccinia virus as a surrogate for MPXV, with sub-micromolar EC₅₀ values and high selectivity indices.

4.8. Potential genetic determinants affecting virulence of Heartland bandavirus infection in mice and therapeutic intervention with the ribonucleoside analog EIDD-2749. **Jonna Westover, Ph.D., Institute for Antiviral Research, Utah State University, USA**

Heartland virus (HRTV) is an emerging bandavirus, transmitted by the Lone Star tick, that causes febrile illness in humans with a 5–10% fatality rate. Based on wildlife seroprevalence data, HRTV is prevalent in the eastern, midwestern, and southern United States. No vaccines or approved antivirals are available to address the health needs of HRTV infection. A robust mouse fatality model of HRTV infection was established by passaging the MO-4 virus strain in AG129 mice 5 times and harvesting passaged virus from clarified liver homogenates for the next round of infection. While the lethality of the initial MO-4 passage 0 strain (P0) was approximately 10%, the 5th passage of the mouse-adapted virus (P5; MA-HRTV) was 100% lethal by 7 dpi. Sequencing the negative-sense, single-stranded RNA HRTV genomes of the initial P0 and P1–P5 virus stocks revealed multiple mutations in the L, M, and S segments of the virus. **Jonna Westover** highlighted that the I221V substitution outside the interferon-antagonizing region of NSs (the non-structural protein of the S segment encoded in ambisense orientation) in the P5 virus, as well as a G32D substitution in the L RNA-dependent RNA polymerase segment within the endonuclease region, is predicted to enhance the structural stability of this protein subunit. Infecting naïve AG129 mice with the MA-HRTV stock resulted in severe viral-induced lesions within the liver, spleen, and lung.

This enhanced HRTV lethality mouse model was used to evaluate the efficacy of EIDD-2749 (4'-fluorouridine) (Westover et al., 2024). Prophylactic oral doses of 3 mg/kg EIDD-2749 once-daily for 7 days were 100% protective, accompanied by no weight loss and an absence of viral titers by 5 dpi. Therapeutic doses of 3 mg/kg EIDD-2749 starting 2 or 3 dpi prevented mortality and resulted in minimal weight loss and a significant reduction in viral titers. Delaying EIDD-2749 treatment until 4 dpi protected 90% of mice from death with more pronounced weight loss than earlier treatment initiation times; however, surviving mice had significantly lower HRTV titers in liver and spleen than did vehicle-treated animals and regained weight at a rate similar to mock-infected mice. When EIDD-2749 treatment initiation was delayed until 5 dpi, surviving animals had lost 10% weight prior to the first dose; however, 20% of the mice were rescued and recovered weight to the level of sham-infected controls. Delaying EIDD-2749 treatment until 6 dpi resulted in no protection.

4.9. Understanding mechanisms of infection and immunity of viral diseases for therapeutics. **Lisa Ng, Ph.D., A*STAR Infectious Diseases Labs, Singapore**

Lisa Ng presented a synopsis of immune responses to arboviruses with a particular focus on how these might be targeted therapeutically to limit severity of disease. She first provided an overview of the Agency for Science, Technology and Research (A*STAR) Infectious Diseases Labs (A*IDL) in Singapore. A*IDL was established in 2021 to provide a comprehensive, multi-disciplinary framework for researching vector-borne diseases, antimicrobial resistance, and respiratory diseases. The translational mission of A*IDL is to enhance pandemic responses and improve diagnostics and therapeutics in this context. Viruses of interest include alphaviruses, flaviviruses, enteroviruses, influenza, and others. A*IDL has multiple available in vitro therapeutics platforms, mouse models and immunoprofiling assays to study each infection in depth. Next, Lisa provided an overview

of chikungunya virus (CHIKV) animal infection models, which have resulted in numerous publications and discoveries. These models recapitulate viral and joint inflammation kinetics observed in humans. One area of focus is on mosquito salivary immune modulators, which may enhance infection in human hosts by suppressing host local immunity and recruiting virus-susceptible cells. Other studies showed that CHIKV targets human blood cells, particularly circulating monocytes. A*IDL investigators discovered that *Aedes aegypti* salivary peptide X reduces monocyte activation and viral infection of these cells. Peptide X may therefore have therapeutic potential, as it reduces joint inflammation in infected mice without impacting viremia. CHIKV-induced joint swelling has been demonstrated to be driven by CD4⁺ T cell activity and is abrogated by fingolimod in mice. Specifically, CD4⁺ T cells drive differentiation of macrophages, which are inflammatory mediators in infected footpads. Therapies that disrupt this interaction have the potential to limit CHIKV-associated joint immunopathogenesis.

4.10. Development and mechanism of novel diphyllin derivatives against Ebola virus infection. Patrick Keiser, Ph.D. Candidate, NEIDL, Boston University, USA

Patrick Keiser presented novel derivatives of diphyllin as potent inhibitors of EBOV, a filovirus associated with high mortality that continues to cause outbreaks in Africa. Currently, no small molecule inhibitors have been approved for treatment of filovirus disease. The novel diphyllin derivatives described in this talk demonstrate low nanomolar potency against EBOV infection with improved, lower cytotoxicity in both stable cell lines and primary human macrophages, a major target cell type for EBOV infection. To gain a better understanding of target specificity and mechanism, cells were generated lacking expression of ATP6V0A isoforms, transmembrane components of the V-ATPase multi-subunit enzyme that was previously reported to be a target of diphyllin and is necessary for acidification of endosomes (Wang et al., 2019). Patrick and colleagues found that isoform ATP6V0A2 is a key contributor to diphyllin activity against EBOV. Furthermore, they showed that by transiently expressing mutant ATP6V0A2, cells lacking native ATP6V0A2 expression can be used to probe for novel residues of interaction. Finally, they demonstrated that these novel derivatives of diphyllin may be promising therapeutics for EBOV disease, potentially addressing the gap in treatment options for this severe illness.

4.11. Identification and evaluation of novel Lassa virus entry inhibitors using computational counterscreening and chemical informatics. Brianna Close, Ph.D. Candidate, NEIDL, Boston University, USA

Brianna Close presented a novel approach to identifying viral entry inhibitors for LASV, a hemorrhagic fever-causing arenavirus of significant public health concern. LASV enters host cells via binding of the virus glycoprotein (GP) to matriglycan-modified α -dystroglycan at the cell surface, followed by a receptor switch to LAMP1 within the endolysosome. Despite numerous efforts to identify therapeutics that inhibit cell entry, a clinically approved small molecule LASV inhibitor has not been reported (Iyer et al., 2024). To address this gap, Brianna and her group developed a computational strategy to identify specific inhibitors of GP-mediated virus entry based on a previous screen of over 350,000 small molecules performed by the group, comprising the NIH MLPCN library, with data deposited into PubChem. They developed datamining techniques to select small molecules based on

potency and to remove promiscuous compounds with widespread activity in other assays in the database, allowing prioritization of hits by potency and mechanism specificity. Cheminformatics was then applied to select compounds with diverse chemical scaffolds not previously reported. They then tested the ability of each compound to inhibit pseudotyped viruses bearing GP from LASV, Junín, and vesicular stomatitis virus GPs, as well as wild-type LASV at BSL4. This approach identified candidates displaying high selectivity and sub-micromolar potency for LASV entry and infection. Preliminary mechanistic evaluation suggests disruption of GP attachment to α -dystroglycan and prevention of glycosylation of host receptors critical for binding. The development of this computational approach aims to accelerate identification and evaluation of potent antivirals by prioritizing candidates with minimal off-target effects, expediting their progression toward therapeutic development.

4.12. Identification of a macrocyclic compound targeting the Lassa virus polymerase. **Mike Flint, Ph.D., Centers for Disease Control and Prevention, USA**

Mike Flint began his presentation by introducing his work at the U.S. Centers for Disease Control and Prevention, where his group performs high-throughput compound screens against several highly pathogenic viruses, including LASV. To identify compounds with anti-LASV activity, his group conducted a cell-based compound screening campaign of almost 60,000 compounds for their ability to inhibit the growth of an infectious, recombinant reporter LASV based on a clade IV prototypic sequence (Welch et al., 2016). Mike explained that this library contained nearly 10,000 macrocycles, cyclic compounds containing a large ring structure with at least 12 atoms. He explained that this class of compound occupies a different chemical space from more traditional compounds, and in theory may address difficult targets unperturbed by traditional small molecules. The most potent macrocycle hit was Mac128, which had an EC₅₀ of 0.57 μ M (SI > 175) against the reporter LASV, and also inhibited the wild-type virus, reducing titers by over 4 logs (Aida-Ficken et al., 2024). Mike described further time-of-addition studies and assays recapitulating individual steps in the viral life cycle, which suggested that Mac128 acted at the level of viral replication: LASV glycoprotein-dependent entry and Z-protein budding were both unaffected, but replication of the LASV minigenome assay system was blocked. Mike then went on to describe the large genetic diversity of LASV, grouped into 7 recognized geographically and genetically distinct clades with up to 32% nucleotide diversity between these clades. Dose-response experiments with other LASV strains showed that Mac128 strongly inhibited clades III, IV, and V, and moderately inhibited clades I and VI, but was ineffective against a clade II virus. Further investigation of these data used the minigenome assay. By switching the clade IV and clade II support plasmids driving the minigenome, Mike's group showed that Mac128's mechanism of inhibition occurred primarily at the level of the polymerase (Aida-Ficken et al., 2024). Mike concluded his presentation by describing Mac128 as a tool compound for studying LASV replication, and a novel starting point for an optimization campaign with the aim of obtaining a candidate LASV therapeutic.

4.13. Tribbles pseudokinase 3 promotes enterovirus A71 infection via dual mechanisms. Hui-Qiang Wang, Ph.D., Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, China

Enterovirus A71 (EV-A71) is the main pathogen causing hand, foot, and mouth disease (HFMD) in children, and is occasionally associated with neurological diseases such as aseptic meningitis, brainstem encephalitis (BE), and acute flaccid paralysis. Severe infections can lead to death, and there are no approved drugs for the prevention and treatment of EV-A71 infection. In his presentation, **Hui-Qiang Wang** described the dual roles that cellular pseudokinase tribbles 3 (TRIB3) play in facilitating EV-A71 infection. He introduced pseudokinase TRIBs, discussing how they are considered to be biomarkers and potential therapeutic targets for many diseases, though their role in viral replication has rarely been reported. He presented data showing that EV-A71 infection upregulated TRIB3 expression in culture; TRIB3 overexpression promoted viral replication both in several EV-A71 strains and in other human enteroviruses (coxsackievirus B3 [CVB3] and coxsackievirus A16 [CVA16]). He also showed that viral replication was inhibited in TRIB3 knockout cell lines. These data revealed TRIB3 as a proviral factor facilitating the replication of EV-A71 and other related human enteroviruses. Further data were presented showing that TRIB3 did not directly interact with any EV-A71 proteins but affected viral propagation via indirect interactions. TRIB3 maintains the metabolic stability of scavenger receptor class B member 2 (SCARB2, the receptor of EV-A71) by repressing SCARB2 k48 ubiquitylation and degradation, which in turn enhances infectious entry and spread of the virus. TRIB3 also facilitates the replication of EV-A71 viral RNA in a SCARB2-independent manner. Finally, Hui-Qiang presented in vivo data showing that, in comparison to wild-type C57BL/6 mice, EV-A71 infection in TRIB3 knockdown mice (Trib3^{+/-}) resulted in significantly lower viral loads in muscular tissues and reduced lethality and severity of clinical scores and tissue pathology (Wang et al., 2024). Hui-Qiang concluded his presentation by proposing that TRIB3 is a key host cellular protein required for the infection and pathogenesis of EV-A71 and some other human enteroviruses, and may thus be a therapeutic target for combating the infection of those viruses.

4.14. Generation and optimization of Bangladesh and Malaysian recombinant reporter Nipah viruses for antiviral screening in vitro and disease modeling in vivo. Michael Lo, Ph.D., Centers for Disease Control and Prevention, USA

Michael Lo began by giving some background on NiV, a highly pathogenic paramyxovirus responsible for regular outbreaks of disease in India and Bangladesh with a case fatality rate of ~75%. A zoonotic pathogen, NiV is carried by fruit bats, and human cases arise from either direct human-to-human transmission or indirect contact with infected animals through ingestion of contaminated date palm sap. Since there are no approved therapeutics for NiV disease in humans, the WHO has designated NiV and henipaviral diseases a priority pathogen for research and development. Michael described the two recognized genotypes of NiV, strain Malaysia (NiV-M) and strain Bangladesh (NiV-B), and discussed his group's recent development of a reporter NiV-B virus termed rNiV-B-ZsG, in which the coding sequence (CDS) for the fluorescent protein ZsGreen1 was linked to the M protein CDS via a self-cleaving P2A sequence (Lo et al., 2024). He presented data showing

that, similarly to the existing reporter Malaysia virus (rNiV-M-ZsG), this modification did not affect in vitro growth kinetics, nor alter susceptibility to neutralization by anti-fusion protein or anti-glycoprotein antibodies. He followed up with screening assay optimization data showing the importance of cell type when developing these screens: MRC-5 cells and HSAEC1-KT cells were shown to be optimal when using rNiV-M-ZsG and rNiV-B-ZsG, respectively, based on reporter protein expression levels and assay Z' scores. Screening rNiV-B-ZsG with a synthetic compound library directed against viral RdRp identified a hit compound, OTA-4. Confirmatory screening with wild-type NiV, rNiV-B-ZsG, and a newly constructed, bioluminescent red fluorescent double reporter NiV (rNiV-B-BREP) confirmed the sub-micromolar activity ($EC_{50} \sim 0.02 \mu\text{M}$; selective index >600) of OTA-4. Michael then described how, by conducting time of addition, NiV minigenome, and NiV glycoprotein-mediated cell-cell fusion assays, his group determined that OTA-4 is possibly a non-nucleoside replication inhibitor of NiV. He concluded his presentation by describing in vivo work carried out using both Bangladesh NiV reporters (rNiV-B-ZsG and rNiV-B-BREP) in the Syrian hamster model of NiV disease, showing that not only do the reporter viruses retain comparable pathogenicity to wild-type viruses, but their expression levels in infected animals also support their use as tools for both pathogenesis and advanced pre-clinical in vivo studies (Lo et al., 2024).

4.15. Intranasal route to immunity: single-dose mucosal delivery of viral replicon particle vaccine protects uniformly against lethal Nipah virus challenge in African green monkeys. Stephen Welch, Ph.D., Centers for Disease Control and Prevention, USA

Following up from the previous presentation was another talk involving NiV, with **Stephen Welch** describing the development of a novel vaccine candidate for this pathogen. He began by introducing the vaccine itself, a non-spreading, non-infectious, single-cycle viral replicon particle (VRP) called NiV Δ F. He described how his group first engineered the viral genome of NiV strain Malaysia to completely remove the fusion protein (F) gene to generate the NiV Δ F genome, and then secondly engineered cell lines to constitutively express the NiV F protein. With the established NiV rescue system (Lo et al., 2012), they were able to generate the vaccine by using a plasmid expressing the NiV Δ F genome and rescuing the VRP in a cell line expressing F (Welch et al., 2023). Safety studies in 3 small animal models of NiV disease (Syrian hamsters, IFNAR knockout mice, and suckling mice) demonstrated an excellent safety profile for the vaccine, with no observed clinical signs of disease or lethality. Stephen then discussed how, in previous work in Syrian hamsters and IFNAR knockout mice, both intranasal (IN) and intraperitoneal vaccination routes were fully protective against NiV clinical disease or lethality in as little as 3 days (Welch et al., 2023). Next, he introduced the NHP (African green monkey [AGM]) disease model of NiV (Cong et al., 2017), and described how his group evaluated the NiV Δ F vaccine using both the standard intramuscular (IM) route and an IN inoculation route. Similar to the small animal data, vaccination in AGMs was not associated with any clinical disease. After challenge, all mock-vaccinated animals ($n = 4$) succumbed to NiV infection by 9 dpi, but none of the IM-vaccinated animals ($n = 6$) displayed any clinical signs of disease and all survived until end of study at 56 dpi. In the IN-vaccinated ($n = 6$) group, 2 animals succumbed to infection, with the 4 surviving animals exhibiting mild clinical disease 6–9 dpi. Stephen then discussed that because AGMs, which are prey animals, are prone to hiding overt disease

presentation, imaging techniques like chest CT scans and brain MRI were used scans to assess respiratory and neurological disease, respectively. Encouragingly, in IM-vaccinated animals, no lesions were visible with either imaging technique throughout the study, and only mild lung congestion was seen in the surviving IN-vaccinated cohort 6–14 dpi. Stephen finished his presentation by discussing basic immunology in the vaccinated animals, and how, protection was seen in the absence of a neutralizing antibody response similar to findings in earlier small animal experiments. He concluded his presentation discussing the advances made with the NiVΔF vaccine, and suggesting future studies that will investigate shorter vaccine periods to determine the time course of protection. He also commented that the NiVΔF system, which has been removed from the US Select Agent list and downgraded by the NIH to BSL-2, can also be a useful tool to use in high-throughput compound screens for NiV inhibitors, since it fully recapitulates the viral lifecycle without the need for specialized containment facilities.

4.16. Evaluation of small molecules as promising broad-spectrum anti-filoviral agents. **Jazmín Galván Achi, Ph.D. Candidate, University of Illinois, USA**

Jazmín Galván Achi began her presentation by introducing the filoviruses EBOV and Marburg virus, infection with which can cause VHF with mortality rates of 23–90%. Currently, available options for anti-filovirus medical countermeasures are limited: two FDA-approved monoclonal antibodies (Inmazeb and Ebanga) and one vaccine (rVSV-EBOV; brand name Erbevo). However, these therapeutics only display efficacy against Zaire EBOV, while recent EBOV outbreaks have been caused by Sudan EBOV. This has emphasized the need for more broad-spectrum vaccines and antivirals. Jazmín began by describing previous work using a cell-based high-throughput screen for EBOV entry inhibitors that identified CBS1118 as a hit. This compound had an $EC_{50} < 10 \mu M$ against both EBOV and MARV, and was further improved by optimization studies to generate the product ING-15–89. She then introduced her current project, using structure-activity relationship (SAR) studies of ING-15–89 to broaden its spectrum, which led to the identification of two novel derivatives designated MWAC-0001911 and MWAC-0002606. Both compounds were shown to have nanomolar potency against pseudotyped MARV and multiple EBOV strains, including Sudan, with high specificity over unrelated pseudotyped viruses, including influenza H5N1, used in the counter-screen. Furthermore, testing with infectious Zaire EBOV confirmed this antiviral activity, again with nanomolar EC_{50} values for both compounds. Drug-induced phospholipidosis (DIPL) assays showed that DIPL was not observed with MWAC-0002606 at compound concentrations known to have antiviral effects. Next, Jazmín described mechanism of action data from biophysical and molecular studies, which indicated that these compounds bind to a region close to the toremifene-binding site in the EBOV glycoprotein (GP1/GP2 region), providing structure-based optimization strategies these inhibitors. Jazmín finished by describing drug metabolism and pharmacokinetic (DMPK) studies that showed that both compounds had good metabolic stability in humans and mice and low toxicity at both concentrations administered, although MWAC-0002606 displayed 6-fold lower inhibition of human ether-à-go-go-related gene (hERG) ($IC_{50} = 9.4 \mu M$) than that of MWAC-0001911 ($IC_{50} = 1.5 \mu M$). She concluded her presentation by describing how broad-spectrum anti-filoviruses compounds discovered

through these studies could be used in combination with vaccines to aid in the treatment and prevention of future deadly filovirus outbreaks.

4.17. Small molecule antiviral candidates for Rift Valley fever. Wenjun Ma, Ph.D., University of Missouri, USA

Wenjun Ma presented on his group's Rift Valley fever virus (RVFV) antiviral screening efforts. He emphasized the critical impact of this mosquito-borne virus on both agricultural and human health. Wenjun provided an update on his group's vaccine development efforts, which include a formalin-inactivated vaccine; the live attenuated Smithburn vaccine; Clone 13, a naturally attenuated virus isolate; MP12, an attenuated virus derived from serial passages of ZH548 in the presence of 5-fluorouracil; and work on subunit vaccines based on viral glycoproteins Gn and Gc. However, to date, there are no commercial, fully licensed RVFV vaccines for human or animal use in the U.S., Europe, or Asia. Similarly, no antivirals are licensed to treat Rift Valley fever, but ribavirin and favipiravir (T-705) have both been pursued as candidates, with limitations for clinical application. Wenjun detailed his group's approach to identify effective antivirals for RVFV. Current efforts have screened libraries containing approximately 26,136 chemical compounds that are predicted to cross the blood-brain barrier. The group's primary screening system, based on a high-throughput assay using the MP12 vaccine strain expressing *Renilla* luciferase, identified 280 compounds of interest. After reconfirmation, 239 compounds were included in subsequent cheminformatics analysis. Then, using rMP12-GFP and wild-type M12 virus-based assays, 35 compounds were selected for full analysis that identified 8 compounds very effective against RVFV. The group then examined the compounds' mechanisms of action and found that 2 of the 8 inhibited polymerase activity, and a subset exhibited broad-spectrum antiviral activity (effective against Andes virus and Cache Valley virus). Future work will include in vivo efficacy evaluation and further mechanism studies.

4.18. In silico tools for antiviral research and future pandemic forecasting. Eugene Muratov, Ph.D., University of North Carolina, USA

Eugene Muratov provided an overview of key computational methods and their applications for antiviral research. He covered over 10 years of research, summarizing what modelers could do for virology by presenting key examples of a wide variety of approaches and specifically developed tools. Examples of modeling contribution included generating hypotheses, developing medicinal chemistry tools for drug repurposing and drug discovery (virtual screening, targeted design, med-chem optimization), drug matching for combination therapy, future outbreak forecasting, and novel alternative methods (NAMs) to reduce animal research. Eugene then asked the audience to consider what else could be done with a computer to support these efforts and encouraged later discussions. He emphasized that a modeler could do nothing without collaborators who are doing the experimental research; while modeling's potential for positive impact is high, modelers must collaborate with virologists, chemists, and other researchers.

Eugene continued his talk by presenting a series of examples of his group's work to illustrate the contributions of modeling to antiviral research. These included important analyses that concluded that we must not "flatten the curve of antiviral research" by

demonstrating that the only virus successfully battled has been HIV (Bobrowski et al., 2020), research for which has been consistently funded. He emphasized that it is better to have continued funding than large amounts of funding over a short period. He presented the group's (i) Reasoning Over Biomedical Objects linked in Knowledge Oriented Pathways (ROBOKOP), an abstraction layer and user interface to more easily query knowledge graphs and store, rank, and explore query results (Beasley et al., 2022; Cox et al., 2020); (ii) liability predictor, a tool to reduce false positives in HTS (e.g., thiol reactivity, redox activity, luciferase firefly, luciferase nano) (Alves et al., 2023); (iii) PhaKinPro, a protein kinase (PK) profiler tool made considering the 12 major PK properties (e.g., bioavailability, CACO-2, blood-brain barrier permeability, etc.) to triage compounds with undesired PK profiles (Rath et al., 2024); (iv) work on drug repurposing and drug discovery during the COVID pandemic (Alves et al., 2021); (v) investigation of host targets for SARS-CoV-2 (ACE-2 allosteric binders), showing collaboration between modeling and virology (Hochuli et al., 2022); (vi) small molecule antiviral compounds collection (SMACC), a database to support the discovery of broad-spectrum antiviral drug molecules (Martin et al., 2023); (vii) modeling approaches to develop broad-spectrum therapeutics by looking for conserved proteins (Melo-Filho et al., 2022); (viii) combination therapy work for SARS-CoV-2 (Bobrowski et al., 2021); (ix) outbreak prediction that, in 2021, successfully predicted the current dengue outbreak in Brazil (Sessions et al., 2023); and finally, (x) substituting animal testing with models using a StopTox-NAM tool for "six-pack" toxicity tests (Borba et al., 2022) and PRES/MD to predict risk related to medical devices (Alves et al., 2022).

5. Coronaviruses, influenza, RSV, and other respiratory viruses

Chaired by Rhonda Cardin, Tim Sheahan, Larissa Dirr, and Mark von Itzstein.

5.1. Drug discovery efforts towards human metapneumovirus. Larissa Dirr, Ph.D., Institute for Glycomics, Griffith University, Australia

Larissa Dirr started her talk describing the global health burden caused by human metapneumovirus (HMPV) infections, particularly in paediatric and immunocompromised individuals. HMPV has a trimeric type I fusion protein (F) that is essential for target-cell recognition, attachment, and entry. To fulfill its dual role of host recognition and fusion, the F protein undergoes a large conformational change from the pre-fusion to the post-fusion conformation. In its prefusion conformation (pre-F), F recognizes and binds to heparan sulfate proteoglycans, DC-SIGN, L-SIGN, and RGD-binding integrins (Cox et al., 2012; Gillespie et al., 2016). While there have been several strategies developing HMPV F and HMPV replication inhibitors described in literature (Van Den Bergh et al., 2022a), an effective drug candidate or approved vaccine therapy are missing to date. Therefore, Larissa's team conducted an in vitro medium-throughput screening assay using the Compound Australia Open Access Drug library, identifying 6 hits with an IC₅₀ value < 10 µM and selective index >20 (Van Den Bergh et al., 2022b). Through mode of action studies, the hit candidates were further classified into entry or replication inhibitors. Among the inhibitors, the approved immunosuppressant drug mycophenolic acid (MPA) showed the strongest anti-HMPV potency. Drug combinations of the HMPV replication inhibitor MPA

with entry inhibitors aurointricarboxylic acid or Evans blue presented an additive antiviral effect.

Every cell is covered in a dense array of sugars, known as glycans, and several viruses utilise these glycans as host cell receptors to initiate infection. Larissa's group studied the interactions of the trimeric F protein (stabilised in its pre-fusion conformation) and host cell glycan receptors using glycan array (Van Den Bergh et al., 2024). The minimal glycan motif recognized by HMPV F was determined to be Gal β 1–3/4GlcNAc and consequently the binding affinities of 30 glycan hits harbouring this motif were determined by surface plasmon resonance. The biological relevance of the glycans with the strongest binding affinities were validated using in vitro infection assays. The tetrasaccharide lacto-*N*-neotetraose (LNnT) and its sialylated pentasaccharide sialyllacto-*N*-neotetraose both clearly inhibited HMPV binding, thereby competing with the host cellular receptors. Finally, saturation-transfer-difference NMR spectroscopy revealed that LNnT binds in a way highly similar to whole purified viral particles as well as recombinant F protein, suggesting that the LNnT binding site is in a region accessible when F is presented on the viral surface. The identified glycan motifs are currently being used as templates for designing new glycomimetics with therapeutic potential.

5.2. Nanobodies against COVID-19 and other emerging viruses. Fang Li, Ph.D., Institute of Virology, Germany

Nanobodies are single-domain antibodies that are a fraction of the size of conventional antibodies, which drives interest in their therapeutic potential. Because of their size, nanobodies have exceptional tissue permeability and access to epitopes, along with the potential to be administered into the lungs via intranasal drug delivery routes. These favorable properties make nanobodies ideal for treating respiratory diseases such as COVID-19. In his presentation, **Fang Li** reviewed advances made by his group toward developing nanobodies with high affinity for the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Nanobodies that can bind the hidden conformation of the RBD to limit viral evasion would be even more desirable. Li and his team have identified nanobodies from alpacas immunized with the prototypical spike protein that potently bind non-overlapping regions of the RBD. Nanosota-2 has remarkably high potency in vitro ($IC_{50} = 2$ pM) and in vivo (~10,000-fold reduction in lung viral titers). Although slightly less potent, Nanosota-3 has been engineered to inhibit infection by contemporary omicron variants, and Nanosota-4 can also block SARS-CoV-1 infection. A newer generation nanobody, Nanosota-9, is currently the lead therapeutic candidate, with high potency and an expanded capacity to bind both configurations of the RBD of SARS-CoV-2 omicron variant. Finally, Li also spoke about Nanosota-5, a nanobody that inhibits SARS-CoV-2 infection by binding to a non-RBD region and stabilizing the pre-fusion spike. The nanobody approach is also being applied to other emerging viruses like EBOV. Li's work highlights the potential of nanobodies as a therapeutic strategy to combat current and emerging viral diseases.

5.3. Activating the RIG-I pathway by RNA agonists or small molecule drugs triggers innate immune programming to control infection by influenza A virus and SARS-CoV-2.

Amina Negashi, Ph.D., Center for Innate Immunity and Immune Disease, Department of Immunology, University of Washington, USA

Pathogenic RNA viruses like SARS-CoV-2, which continuously evolves as part of its normal replication cycle, can emerge with mutations that confer resistance to current antivirals, evade natural and vaccine-induced immunity, and pose a threat of causing a new global pandemic. Therefore, novel and effective treatment options are always needed. Typically, RNA viruses are recognized by intracellular pathogen-recognition receptors (PRRs) such as the RIG-I-like receptors (RLRs) (Saito et al., 2008; Schnell et al., 2012; Thompson et al., 2011). At steady state, RIG-I remains in a closed conformation; upon recognizing its cognate ligand, a short RNA molecule containing a 5'-ppp motif, it undergoes a conformational change to expose the CARD domains. This open conformation enables RIG-I and MAVS to interact and triggers the activation of key transcription factors like NF κ B and IRF3. Translocation of NF κ B and IRF3 into the nucleus drives the expression of proinflammatory factors and interferons (IFNs), respectively, which are important for establishing the antiviral state for virus control.

Amina Negishi asked the question: can we harness this key innate signaling pathway to design host-directed antivirals? To answer this question, direct agonists that can bind and activate RIG-I were developed, and small-molecule therapeutic (SMT) drugs that can modulate key components of this pathway. RIG-I-activating RNA (RAR) is a short RNA (~100 nucleotides) with a uridine tract that defines its PAMP activity. To test the antiviral activity of RAR both in vitro and in vivo, a good RNA formulation platform is needed that protects the RNA from degradation and preserves its immunogenic activity. To this end, a novel nucleic acid delivery platform was utilized, Lipid InOrganic Nanoparticle (LION) (Erasmus et al., 2020), which is a proprietary nucleic acid delivery technology. The ultimate goal was to use the LION carrier to formulate RAR for intranasal delivery to treat acute viral infections. When RAR is formulated with LION, it can trigger innate immune signaling, as marked by the induction of IRF3 activation, IFN expression, and upregulation of several interferon-stimulated genes (ISGs). The group then asked if RAR's ability to stimulate signaling was restricted only to IFN and ISG induction. To assess this, they performed an RNA-seq analysis, which revealed that RAR induces diverse cellular programming. Given its immunogenic activity, does RAR exhibit antiviral activity against SARS-CoV-2? In vitro, both in pre-treatment and post-treatment settings, RAR-treated cells controlled infection efficiently as compared to untreated cells. The group then tested RAR in vivo, pretreating mice with RAR intranasally and then challenging them with SARS-CoV-2. All RAR-treated mice survived with a clear decline in viral load over time and reduced tissue inflammation. Second, when mice were challenged with SARS-CoV2 and then intranasally treated with RAR, both RAR-treated and control groups developed disease. Eventually, however, only the treated mice started recovering and showed a good infection outcome. In conclusion, this work shows that the novel RNA delivery technology LION can be used to deliver immunogenic RNA (RAR), prophylactically or therapeutically, as a host-directed antiviral to control a viral infection.

5.4. Understanding and inhibiting SARS-CoV-2 NiRAN domain catalytic activities through structural studies and large-scale docking. Gabriel Small, Ph.D., Laboratory of Molecular Biophysics at The Rockefeller University, USA

The nidoviral RdRp-associated nucleotidyltransferase (NiRAN) domain is conserved across all families of the order *Nidovirales* and is encoded N-terminal to the RdRp as part of the same nonstructural protein (nsp) (Lehmann et al., 2015). The enzyme was first identified in equine viral arteritis with self-NMPylation activity, but in coronaviruses (CoVs), the NiRAN domain is encoded in nsp12 and modifies a separate protein, nsp9. The three catalytic activities identified in vitro for this domain are (1) NMPylation – the modification of nsp9's N-terminal amine with an NMP moiety (Park et al., 2022; Slanina et al., 2021; Small et al., 2023); (2) RNAYlation – the modification of nsp9's N-terminal amine with a 5'-monophosphorylated RNA moiety; and (3) deRNAYlation/mRNA capping the removal of a 5'-monophosphorylated RNA from Nsp9 with a GDP and the concurrent covalent linkage between the GDP and the 5'-monophosphorylated RNA, resulting in the production of a 5'-GpppA cap structure (Park et al., 2022; Small et al., 2023). This final activity implicates the NiRAN domain as the capping enzyme in CoVs and explains why mutational analysis has identified the domain to be essential for viral replication and, thus, a target for therapeutic intervention.

Gabriel Small reported the structural analysis of catalytic intermediates of the NiRAN domain via single-particle cryo-electron microscopy that revealed the structural basis for substrate recognition and catalysis of CoV mRNA capping (Small et al., 2023). Importantly, these data can be used to provide insights into designing docking models that require high-resolution information on critical interactors, catalytic metals, and active site waters. An in silico docking pipeline was then used to screen 185 million anions against the NiRAN domain. After scoring the top compounds, hits were filtered to only pull out novel chemotypes unlike biomolecules, filtered for interactions with important active site residues, clustered for similar hits, and finally manually curated to ensure that the docked compounds were valid. These were then ordered and synthesized for in vitro screening.

Prior to this study, no high-throughput assay existed for the NiRAN domain's capping activity. Thus, the NiRAN domain was initially incubated with nsp9 and a cy5-labeled ATP. This produced a cy5-AMP-nsp9 compound that was purified to serve as the starting molecule for a fluorescence polarization-based assay. The cy5-AMP-nsp9 was incubated with the NiRAN domain and GDP in a 384-well format; GDP served as the nucleophile, substituting nsp9 and producing a GpppA-cy5 molecule with a lower mass and higher tumbling of the fluorophore, leading to lower polarization. This reaction can be disrupted with an inhibitor, and the compounds from the in silico docking campaign were evaluated using this assay. Of the 71 synthesized compounds, a single compound with a novel chemotype dissimilar to GDP demonstrated significant and reproducible inhibitory activity against the NiRAN domain's deRNAYlation/mRNA capping activity. This compound is still in its early stages and must be tested with a full dose-response curve, as well as optimized to improve its inhibitory activity. This compound represents a promising lead towards designing a specific NiRAN domain inhibitor without relying on nucleoside scaffolds.

5.5. Combating emerging henipaviruses, Daniel Watterson, Ph.D., University of Queensland, Australia

Daniel Watterson presented on developing vaccines and therapeutics against henipaviruses (HNVs). He emphasized the role of understanding structure and function of viral proteins as key to developing effective vaccines and therapies, and introduced the molecular clamp platform that holds viral proteins in their original form to produce advanced subunit vaccine candidates. These vaccines offer several advantages, including prefusion conformation stabilization, high manufacturing yield, universal purification, and improved stability. The molecular clamp approach has been used to develop vaccines for a variety of pathogens, with rapid development of one for SARS-CoV-2 through phase 1 trials (Chappell et al., 2023). Additionally, phase 1 trials for an RSV/hMPV vaccine are beginning this year. Several vaccine candidates were presented in more detail to demonstrate how the molecular clamp approach confers improved antigen quality, stability, and immunogenicity. Dan then transitioned to discuss his group's vaccine and therapeutic work on henipaviruses, with a focus on developing broad-spectrum medical countermeasures to protect against divergent HNVs. Given the recent identification of Langya (LayV) virus in China in August 2022 and its association with clinical disease in over 25 patients (Zhang et al., 2022), the lab began developing a next-generation molecular clamp2 candidate for prefusion stabilization of LayV & Mòjiāng virus (MojV) F glycoproteins. Both LayV and MojV have highly similar structural architecture to NiV F despite sequence divergence. However, these viruses have a distinct antigenic profile from that of NiV (Isaacs et al., 2023), suggesting that a broad-spectrum vaccine against diverse henipaviruses may require a multivalent approach.

5.6. Design of SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model. Jun Wang, Ph.D., Rutgers, The State University of New Jersey, USA

SARS-CoV-2 continues to present challenges as it evolves and drug resistance to first-line antivirals inevitably emerges. **Jun Wang** presented on the discovery of a new SARS-CoV-2 papain-like protease (PLpro) binding site, Val70Ub, from its crystal structure with a designed covalent inhibitor Jun 11313 (Tan et al., 2024). His team then designed, synthesized, and tested a library of biarylphenyl amide compounds, identifying candidates with nM inhibition constant (K_i) values. Co-crystal structures with the lead compounds, including Jun 12682, revealed the expected binding pose, with inhibitors targeting both the Val70Ub site and the BL2 groove. Jun 12682 showed consistent antiviral activity against SARS-CoV-2 variants and, more importantly, nirmatrelvir-resistant strains. Jun 12682 was then evaluated in the mouse-adapted SARS-CoV-2 infection model. A 3 day oral treatment starting 1 hour post viral challenge resulted in complete protection against mortality and significantly reduced lung viral burden. Consistent with its antiviral activity, treatment with Jun 12682 effectively reduced lung pathology and proinflammatory cytokine levels in the mice. These findings validate PLpro as a promising antiviral target and justify PLpro inhibitors as promising oral antiviral candidates to complement current RdRp and M^{pro} inhibitors (Tan et al., 2022) (2). Further studies will include lead optimization to increase potency and improve pharmacokinetics, demonstrate efficacy delaying treatments until 24 or 48 hours post infection, explore combination therapies with approved SARS-CoV-2 antivirals, and investigate the emergence of drug resistance to the lead PLpro inhibitor.

5.7. Identification of adenosine analogues that inhibit the N7 methyltransferase activity of SARS-CoV-2. **Adrien Delpal, Ph.D., Laboratoire Architecture et Fonction des Macromolécules Biologiques (AFMB), France**

Methylation of the SARS-CoV-2 viral RNA cap protects it from degradation and facilitates the initiation of translation while dampening the host cell's ability to detect the viral RNA and initiate the antiviral response to infection. The SARS-CoV-2 nsp14 is one of the key enzymes engaged in the synthesis of capped viral RNA for transcription by methylating the N7 position of guanine from S-adenosylmethionine (SAM). **Adrien Delpal** described efforts to exploit this potential vulnerability by synthesizing adenosine analogs to SAM, with modifications allowing their interaction with both the viral RNA cap and SAM binding site. Initial evaluation of SAM analogs shows that MH44 is most potent against the methyltransferase (MTase) domain of nsp14 with an IC₅₀ of 19 nM (Ahmed-Belkacem et al., 2022). Additional compounds were synthesized based on the MH44 scaffold, and more potent and selective compounds were identified with high specificity for coronavirus N7-MTase. Data for this new compound series demonstrated sub-nM activity against the SARS-CoVs (Hausdorff et al., 2023) and low nM activity against MERS-CoV, with almost no inhibition of the human MTases and no activity against DENV MTase. Docking studies revealed that compound 26 (harboring C7-substituted 7-deaza-adenine) and related analogs fit into the SAM entry channel. Despite encouraging inhibitory activity in the enzymatic biochemical assay, compound 26 and others did not significantly inhibit SARS-CoV-2 replication in infected cells. Nevertheless, this work lays the foundation for further medicinal chemistry to improve anchoring of lead compounds in the nsp14 MTase domain and increase the bioavailability of the compounds to the cytosolic compartment where they can act to selectively inhibit nsp14-mediated methylation and thereby disrupt viral RNA translation while also promoting innate immune system recognition of the unmethylated cap structure.

5.8. Identification of novel small-molecule inhibitors of SARS-CoV-2 by chemical genetics. **Shuofeng Yuan, Ph.D., The University of Hong Kong, SAR China**

Although the world is moving towards a post-COVID phase, it is crucial to be prepared for the emergence of new SARS-CoV-2 variants or another zoonotic spillover of coronaviruses from wildlife reservoirs. One crucial measure to control emerging coronavirus outbreaks is to develop effective antivirals with broad-spectrum activity. **Shuofeng Yuan** reported a 3-tier, cell-based screen that was utilized to identify novel compounds with anti-SARS-CoV-2 activity. One compound demonstrated broad-spectrum antiviral activity against multiple human pathogenic coronaviruses and different SARS-CoV-2 variants of concern. Mechanistic studies validated by reverse genetics showed that the compound inhibits the 3-chymotrypsin-like protease (3CLpro) by binding to an allosteric site and reduces 3CLpro dimerization. A drug synergistic checkerboard assay demonstrated that the compound synergizes with nirmatrelvir in vitro, which could lower the risk of antiviral resistance. Safety panel assays suggested a low risk of off-target effects of the compound. Pharmacokinetic analysis in a BALB/c mouse model determined the C_{max} of the identified compound as 6.89 µmol/L, ~3.8-fold higher than its antiviral IC₅₀ of 1.82 µmol/L. In vivo studies confirmed its antiviral activity in both golden Syrian hamsters and K18 humanized

ACE2 mice. Overall, this study identified an alternative druggable site on the SARS-CoV-2 3CLpro, proposed a potential combination therapy with nirmatrelvir to reduce the risk of antiviral resistance, and shed light on the development of allosteric protease inhibitors for treating a range of coronavirus diseases.

5.9. Intranasal administration of a live attenuated vaccine derived from nsp16-deficient SARS-CoV-2 confers sterilizing immunity in rodent models. Zi-Wei Ye, Ph.D., The University of Hong Kong, SAR China

Current COVID-19 vaccines do not elicit mucosal immunity and require repeated boosters due to the short duration of immunity. To address these shortcomings, **Zi-Wei Ye** and colleagues have developed an attenuated SARS-CoV-2 vaccine candidate (called d16) with a point mutation that renders the 2'-O-methyltransferase activity associated with the nsp16 protein nonfunctional (Ye et al., 2022). Zi-Wei presented data from hamster and mouse models of SARS-CoV-2 infection, demonstrating the safety, immunogenicity, and efficacy of the d16 virus. Single-dose intranasal administration of d16 was well tolerated in both species and elicited strong neutralizing antibody responses. In the non-lethal hamster challenge model, sterilizing immunity was observed in lung samples devoid of the high viral loads seen in unvaccinated animals. In co-housing studies in hamsters, d16 vaccination prevented transmission of SARS-CoV-2 to naïve animals, in contrast to the efficient spread of the virus observed among unvaccinated, virus-infected animals. In the lethal K18-hACE2 mouse challenge model, immunization with d16 afforded complete protection against mortality. Cell-mediated immunity was also assessed in the mice, and strong activation of T cell responses to SARS-CoV-2 epitopes was observed. As with any attenuated virus vaccine, concerns about reversion exist. Future optimization of the d16 vaccine virus is underway and will include the deletion of ORF3a to reduce pulmonary inflammation and updating the spike protein to the more contemporary omicron version.

5.10. Click chemistry-based rapid identification and crystallographic studies of novel 1,2,3-triazole-bearing diazabicyclooctane derivatives as non-covalent SARS-CoV-2 M^{pro} inhibitors with potent antiviral activity and improved drug-resistance profile. Peng Zhan, Ph.D., Shandong University, China

Peng Zhan described his general research direction of targeted library design and screening to discover antivirals, where innovations in chemistry enabled the rapid synthesis of diverse molecules in high throughput. A key objective of his work focuses on strategies to combat drug resistance through medicinal chemistry (Du et al., 2024; Ma et al., 2021). Peng outlined his work on SARS-CoV-2 main protease inhibitors, focusing on novel scaffolds that have a higher barrier to resistance and avoid co-dosing with PK enhancers.

The medicinal chemistry started from a piperazine scaffold (Gao et al., 2022, 2023). This scaffold is non-peptidic, and piperazine can be further functionalized to include a covalent warhead. The leads have high target specificity and low cytotoxicity, and further crystallographic studies were performed on these inhibitors.

Peng then described the use of microscale chemistry and modular reactions to accelerate drug discovery. In particular, clean modular reactions can facilitate direct screening without

purification, as well as enable efficient construction of focused compound libraries. The co-crystal structures of the piperazine series of M^{Pro} inhibitors revealed unoccupied space around the S2 and S4 pockets of the active site, and a triazole was tolerated. To probe this space, a focused library was constructed using the CuAAC click chemistry with alkyne templates and azide building blocks. Triazole libraries were assayed without purification, and active compounds were purified. Liquid chromatography-mass spectrometry (LCMS) showed that the click chemistry reaction proceeded to completion with high yield.

Potent compounds from the library had low nM biochemical IC₅₀ against the main protease, and potent activity in cell antiviral assays in Calu-3 and VeroE6 cells. In particular, compound C5N17B had EC₅₀ = 0.08 μM in Calu-3 cells, and further characterizations confirmed that it blocks the accumulation of viral protein and viral RNA. C5N17B exhibited broad-spectrum anti-coronavirus properties, potently inhibiting SARS-CoV-2 variants (EC₅₀ = 0.13–0.26 μM), HCoV-OC43, and nirmatrelvir-resistant mutants T21I/E166V and L50F/E166V. In rodent pharmacokinetic studies, C5N17B has modest metabolic stability and oral bioavailability, but acute and sub-acute preclinical toxicity studies revealed no adverse effects. Structural biology studies showed that C5N17B occupies a new binding site exploring the S2 pocket.

5.11. Identification of small-molecule inhibitors of coronaviruses by targeting protein-protein interactions in RNA-dependent RNA polymerase complex. Jeremy Blavier, M.S., Viral Interactomes Laboratory, GIGA Institute, University of Liege, Belgium

The presentation by **Jeremy Blavier** highlighted ongoing efforts at the GIGA Institute to target protein-protein interactions (PPI) as a complementary approach to antivirals that target enzymatic activity (Trepte et al., 2024). The interaction between the nsp12 RdRp of SARS-CoV-2 and the nsp7 and nsp8 core replication machinery cofactors was targeted because of the high degree of conservation across residues at these protein-protein interfaces. Using bioluminescence assays that measure nsp12 interactions with nsp7 or nsp8 (Choi et al., 2019), the nsp12-nsp8 interface was identified as a superior target for direct PPI inhibition. The nsp12-nsp8 interaction was then used to screen a library of approximately 18,000 small molecules, identifying several attractive candidates for further investigation. Of these, compound JB12 inhibited infection in a cell-culture assay using a SARS-CoV-2 fluorescent reporter virus; JB12 had an IC₉₀ of 12.5 μM and produced no cytotoxicity in cells. The compound was subsequently evaluated for pan-coronavirus activity against multiple SARS-CoV-2 virus variants (including omicron), SARS-CoV, and HCoV-229E, and was found to inhibit all. Jeremy then discussed the group's findings when combining suboptimal concentrations of JB12 (3.13 μM) and remdesivir (0.12 μM) in the fluorescent SARS-CoV-2 cell culture infection assay. Alone, each compound minimally inhibited infection. In combination, however, the compounds acted synergistically to nearly eliminate viral replication. Further medicinal chemistry is underway to generate new analogs with nM potency and favorable pharmacokinetics for in vivo proof-of-concept efficacy studies.

5.12. A CRISPR/Cas9 genetically engineered organoid biobank to study coronavirus host factors. **Mart Lamers, Ph.D., Duke-NUS, Singapore**

Mart Lamers started his talk with the different entry mechanisms used by coronaviruses. He then highlighted that overexpression of type II transmembrane serine proteases (TTSP), such as TMPRSS2, TMPRSS4, or TMPRSS11D (Hoffmann et al., 2020, 2021), mediates SARS CoV-2 infection, and that SARS-CoV-2 relies on TTSPs rather than cathepsins for entry into airway organoids (Mykytyn et al., 2021). To test which protease is specifically required for viral entry, intestinal organoids (IOs) were chosen as the model system; these organoids are increasingly used to study human disease and are amenable to genetic engineering. To study this, a CRISPR/Cas9 knockout biobank was created, containing mutant clonal IOs for 19 host genes involved in coronavirus biology, focusing on host proteases involved in mediating coronavirus entry (Beumer et al., 2021).

Interestingly, the team found that entry of the early 614G SARS-CoV-2 strain does not require the endosomal cathepsin B/L proteases, but specifically depends on the cell surface protease TMPRSS2 (Beumer et al., 2021). Similarly, SARS-CoV-1 and MERS-CoV depended on TMPRSS2. Next, Mart highlighted that it is now widely accepted that omicron variants use TMPRSS2 less efficiently than earlier variants (Meng et al., 2022; Willett et al., 2022), and instead use cathepsins, based on experiments in cell lines; however, these findings have yet to be verified in more relevant cell models. Using IOs, the team found that the entry of omicron variants still relies on TMPRSS2 and not on cathepsin B/L (Mykytyn et al., 2023). These findings were verified in airway organoids using protease inhibitors, and are line with findings obtained by others using mouse models (Iwata-Yoshikawa et al., 2022). Overall, he concluded on the importance of TMPRSS2 as an attractive pan-coronavirus therapeutic target and emphasized that the clonal organoid biobank as a useful tool to rapidly phenotype emerging viruses.

5.13. Identification of novel host proteins that are associated with macrophage control of influenza A virus replication. **Sarah Longdrigan, Ph.D., The University of Melbourne, Peter Doherty Institute, Australia**

At the start of her talk, **Sarah Longdrigan** gave an overview of her lab's focus on systematically investigating host responses that limit respiratory virus replication. Influenza virus productively infects epithelial cells, and key host ISGs like IFTIM3 can limit viral infection (Longdrigan et al., 2020; Meischel et al., 2021). However, less is known of IAV replication in airway macrophages, particularly in the late stages of viral infection (reviewed in Meischel et al., 2020). Airway macrophages are key components of the innate immune system and depleting them in animal models is associated with enhanced disease severity. Interestingly, seasonal IAV infection of airway macrophages is abortive, in contrast to productive replication of IAV in epithelial cells (Longdrigan et al., 2015). They found that no virus assembly can be seen in airway macrophages, and that the transcriptional profile of IFN-dependent host factors in macrophages is remarkably different from that in epithelial cells (Ma et al., 2019).

Next, Sarah highlighted that the overarching aim of their work is to identify novel host factors that could limit viral replication in macrophages as a novel, broad-acting,

host-directed antiviral strategy. To avoid donor variability, she collaborated with Professor Daniel Steinfort (Royal Melbourne Hospital and University of Melbourne) to source airway macrophages, monocyte-derived macrophages, and airway epithelial cells from the same patient. Previous studies have shown that some highly pathogenic avian influenza virus strains replicate productively in airway macrophages, an ability that has been mapped to the viral hemagglutinin (HA) protein (Cline et al., 2013). Therefore, Sarah's group used proteomic analysis to investigate associations between host and viral proteins during productive and abortive replication in airway macrophages.

The group has developed and published several studies as a pipeline for overexpression and/or knockdown of host genes to investigate the modulation of viral replication (Meischel et al., 2021). As an example, Sarah presented their findings that overexpressing ISG proteins, which are more highly expressed in macrophages than in epithelial cells, in HEK293 cells can inhibit viral replication of IAV strains. Overall, she concluded that these ongoing approaches give biological insights into host factors that block viral replication at distinct stages of the viral life cycle, and emphasized that macrophage-specific responses in airway epithelium have the potential to limit replication of many respiratory viruses.

5.14. A mouse model of human parainfluenza virus type 3 infection to study prophylactic and therapeutic modalities. Yuxia Lin, Ph.D., KU Leuven, Belgium

Yuxia Lin started her presentation by highlighting that human parainfluenza virus type 3 (HPIV-3), the most virulent serotype of parainfluenza virus, causes upper and lower respiratory tract infections, resulting in acute lower respiratory tract illnesses like bronchiolitis and pneumonia (Ben-Shimol et al., 2013; Maeda et al., 2017). The mortality rate associated with HPIV-3 in transplant patients can reach 75% (Branche and Falsey, 2016), and is particularly concerning because there are currently no approved vaccines or antiviral drugs. The lack of robust and convenient small animal models for HPIV-3 has likely hindered the development of antiviral candidates. Considering HPIV-3's sensitivity to IFNs (Rabbani et al., 2016), the team explored viral replication in various mouse strains (BALB/c, SCID, C57BL/6, *Ifnar*^{-/-}, and AG129 [double IFN α / β and IFN γ receptor knockout mice]) and found that AG129 mice support HPIV-3 replication most efficiently. Intranasal inoculation of AG129 mice with HPIV-3 resulted in viral replication in both the upper and lower airways, with peak viral loads seen 1 dpi in lung samples and 1–5 dpi in nasal-pharyngeal-laryngeal-tracheal (NPLT) washes. In collaboration with Professor Peter Mombaerts (Max Planck Research Unit, Germany), the group used multiplex fluorescence RNAscope and immunohisto-chemistry to demonstrate that HPIV-3 infects and replicates in the club cells and ciliated cells of the lung epithelium. However, no colocalization of HPIV-3 particles was observed in pneumocytes. To validate the model for antiviral studies, GS-441524 (Cox et al., 2021), the parent nucleoside of remdesivir, was used. Oral treatment with GS-441524 (50 mg/kg), starting one day prior to infection and continuing until study endpoints, significantly reduced infectious virus titers to undetectable levels with relatively normal lung physiology. In conclusion, AG129 mice serve as a robust small animal model for developing antiviral strategies against HPIV-3. This model may also be suitable for assessing the efficacy of neutralizing antibodies and of vaccine candidates. Additionally, the

group suggested further investigation of GS-441524 and its prodrug forms to treat HPIV-3 infection in humans.

5.15. Engineering protease-resistant peptides to inhibit human parainfluenza viral respiratory infection. Anne Moscona, M.D., Columbia University Vagelos College of Physicians and Surgeons, USA

Anne Moscona began her talk by describing how paramyxoviruses enter host cells via membrane fusion after engaging with their host cell receptor. HPIV-3, an important paramyxovirus, uses its hemagglutinin-neuraminidase (HN) protein to bind to sialylated glycoconjugates on the target cell. Dimeric HN is present on the virus in a complex with the trimeric fusion protein, and upon receptor binding, HN triggers the fusion protein to activate the fusion/entry process (Marcink et al., 2023). The fusion protein extends into a transient intermediate, with the hydrophobic fusion peptides inserted into the target cell membrane, thereby exposing C- and N-terminal complementary heptad repeat (HR) domains. These HR domains drive the refolding process of the F protein to its final stable state with the HR domains forming a 6-helix bundle that is required for fusion of the viral and cell membranes. Anne's group (in collaboration with Matteo Porotto) designed HRC-based peptide inhibitors that disrupt the interaction between the HRN and HRC domains of the fusion protein and prevent formation of the 6-helix bundle, blocking fusion. Anne explained that the antiviral effect of these lipopeptides is based more on their structure than on the exact sequence. Therefore, the peptides derived from the HPIV3 HRC also inhibit entry of other paramyxoviruses such as henipaviruses (Porotto et al., 2010).

To increase the potential clinical utility of these antiviral peptides, they were conjugated with a lipid moiety that anchors the peptides at the site of action in the target cell membrane (Mathieu et al., 2017). This approach increased efficacy against HPIV-3 infection in an ex vivo human airway epithelium model and an in vivo cotton rat model, and permitted the peptide antivirals to be administered via the airway (by inhalation or intranasally). Protease degradation can lead to unfavorable pharmacokinetics and is a concern for peptide development. Lipid conjugation improved half-life markedly. To further address this issue, in collaboration with Samuel H. Gellman, Anne's group selectively replaced α -amino acid residues with β -amino acid residues to generate α/β -peptides that retain antiviral activity but are poor protease substrates (Outlaw et al., 2020). α/β -HRC peptide-mimetics showed improved antiviral activity compared to all α -peptides in an in vivo pre-treatment regimen in cotton rats, likely due to their longer persistence and higher concentration in the lung and serum (Outlaw et al., 2021). These lipopeptides are promising entry inhibitors that can be delivered via the airway, are unlikely to elicit resistance, and have the potential for broad-spectrum activity.

5.16. Aptamer-based glycoprotein broad-spectrum blocking strategy inhibits respiratory syncytial virus infection. Ge Yang, Ph.D., Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical, China

Ge Yang introduced the versatility of aptamers as a technology platform. Aptamers are 8–200 nt single-strand oligonucleotides that can be designed to recognize diverse classes of targets with high specificity, yet have low immunogenicity and toxicity. Ge's lab has

built a screening platform for aptamer-based antivirals directed at proteins of SARS-CoV-2 (S1, RBD, 3CLpro, RdRp, ACE2, TMPRSS2, and OAS3), MPOX virus (A29L, M1R), RSV (G protein, F protein, N protein, IGF1R), HBV (capsid), H1N1 (NA, NP), HPIV-3 (hemagglutinin, neuraminidase), DENV, and adenovirus.

Ge specifically related his lab's work screening aptamers against RSV. RSV attaches to cells by binding glycoprotein G to host receptors, so an aptamer screening campaign was directed at the RSV G protein to inhibit cell entry. Six aptamers were discovered through four rounds of CE-SELEX. These aptamers demonstrated potency and specificity against RSV G protein, and surface plasmon resonance studies revealed a $K_D = 0.02\text{--}100$ nM. Moreover, the various aptamers showed different binding kinetics, suggesting diverse building sites.

Gold nanoparticle colorimetry was used to verify the affinity and specificity of the aptamers, suggesting their suitability as biosensors. Further, the six aptamers can inhibit RSV in cytopathic effect (CPE) assays, and inhibit RSV cell absorption in A549, H460, and Hep2 cells. In addition, 2 aptamers have inhibitory effects on RSV A2 strain. Further characterization on the 2 most potent aptamers shows that they can inhibit RSV genome and protein expression. Molecular docking predicted the binding site of the aptamers, and aptamers can be truncated based on the predicted structures.

5.17. Inhibition of rhinovirus infection in differentiated primary human bronchial epithelial cells by nanoparticle-encapsulated small interfering RNA. Nathan Bryant, Ph.D., University of Newcastle, Australia

Nathan Bryant presented his work on siRNA directed against rhinovirus (RV). RVs account for ~50% of yearly common cold infections. Although most infections are benign, infants, immunocompromised patients, and patients with chronic airway diseases are at risk of severe disease. There are no antivirals or vaccines for RV infections; the 174 genetically distinct RV subtypes show significant genetic diversity across structural and non-structural proteins. The 5'-untranslated region (UTR) is the most conserved region in RVs. Nathan's group aimed to target conserved regions of 5'-UTR of the RV genome with siRNAs, delivered via lipid nanoparticles (LNP). To validate this hypothesis, they first designed and tested RV 5'-UTR targeting siRNA in monolayer cell lines, then formulated LNP-siRNAs, and finally assessed antiviral activity of LNP-siRNAs in differentiated bronchial epithelial cells.

To design siRNA, 136 RV sequences with complete genomes were first aligned. Criteria for siRNA were 19–25 nt length (+dTdT over-hang), 35–60% GC content, no palindromes longer than 4 nt to avoid hairpin risk, A/U at position 19 (3'-end of sense strand), G/C at first position (5'-end of sense strand), >3 A/U at positions 15–19, and absence of GC stretch >9 nt in length. These constraints led to a promising siRNA that was tested in a monolayer system. Cells were seeded 48 hours before infection, and siRNA was administered 24 hours before infection with MOI = 0.5 of RV. Apical media and RNA were harvested 48 hours post infection. Antiviral effect was quantified using infection dose assay (TCID₅₀) and qPCR for viral RNA. siRNA reduced loads of RVA1 in BCI-NS1.1 (basal epithelial) cell system by ~90%. In MRC-5 (lung fibroblasts), the effect was less pronounced, with only ~48%

reduction in viral RNA. This is potentially because the virus replicates better in the MRC-5 system.

To formulate the siRNA, an Ignite NanoAssemblr system was used, with lipids in ethanol and siRNA in an acidic aqueous buffer. The siRNA was encapsulated in LNPs with over 96% encapsulation efficiency, Z-average of 95 nm, and polydispersity index of 0.13, parameters within the ideal range for airway delivery. These LNP-siRNAs were then tested in a differentiated bronchial epithelial cell system. LNP-siRNAs were treated 6 hours before infection with RVA1 (MOI = 0.1), and apical wash and RNA were harvested 48 hours after infection. LNP-siRNA reduced viral load by 74%.

5.18. Development of small molecule entry inhibitors as novel therapeutics against influenza viruses. Lijun Rong, Ph.D., University of Illinois, USA

Lijun Rong introduced the role of HA for influenza entry. HA can be clustered into two groups: group 1, which includes H1, H5, and H9; and group 2, which includes H3 and H7. A screening campaign was directed at each group. A 10,000 compound HTS was performed against H7N1 (Group 2) and Lassa pseudovirus, leading to the identification of several compounds with SI > 10 and specificity against H7N1. The lead compound, CBS1194, had $IC_{50} = 0.25 \mu M$ (Alqarni et al., 2022; Du et al., 2021). CBS1194 was further optimized to SA-67 and ING-16–36 by optimizing the substituents around the imidazo[1,2-a]pyrimidine core. SA-67 inhibits H7N1 pseudovirus with $IC_{50} = 0.02 \mu M$, and H3N2 infectious virus with $IC_{50} = 0.42 \mu M$. CryoEM structures of SA-67 and ING-16–36 bound to H7 HA protein reveal that they share the same binding site. Separately, an HTS was directed at group 1, screening 19, 200 compounds against H5N1, EBOV, and MARV pseudovirus (Hussein et al., 2020). Seven H5N1-specific hits with SI > 10 were identified. The lead compound, CBS1116, had $IC_{50} = 0.4 \mu M$ against H1N1 PR8 infectious virus and $3.9 \mu M$ against H5N1 pseudovirus. Optimizing the hit whilst fixing the N-phenylpiperidin-4-amine core led to CBS1117, with $IC_{50} = 0.27 \mu M$ against H1N1 PR8 infectious virus and $3.04 \mu M$ against H5N1 pseudovirus. X-ray crystallography of CBS1117 bound to HA revealed the interactions underlying group 1 specificity, leading to an improved inhibitor ING-14–66 ($IC_{50} = 0.07$ and $0.24 \mu M$ against H1N1 PR8 infectious virus and H5N1 pseudovirus, respectively). Comparing inhibitor-bound group 1 (CBS1117) and group 2 (ING-16–36) HA structures showed that the binding sites are separated by 24 Å, perhaps suggesting why a broad-spectrum small molecule inhibitor spanning both binding sites would be challenging to achieve. Finally, in vivo imaging of a mouse model of IAV infection was presented to show that the viral infection occurs in the lung, and that viral replication can be suppressed with oseltamivir. Oral gavage of ING-16–36 resulted in a reduction in H1N1 PR8 lung viral titers in vivo (Gaisina et al., 2020), and ING14–66 protects mice from lethal IAV infection, either as a single agent or synergistically in combination with oseltamivir (Gaisina et al., 2024).

6. Chronic, latent, and persistent viruses - retroviruses and herpesviruses

Chaired by Joy Feng, Zlatko Janeba, Jennifer Moffat, and Gerald Kleymann.

6.1. Development of broad-spectrum antiviral drugs. Hong Lui, Ph.D., Shanghai Institute of Materia medica, Chinese Academy of Sciences, China

Viral diseases have a significant impact on human society, and the development of broad-spectrum antivirals is a crucial goal for current research. **Hong Liu** introduced the construction and development of a multidimensional, multi-target, structurally diverse library of compounds, which has greatly accelerated the development of antiviral drug candidates (two antiviral drug candidates are currently undergoing clinical trials). At the onset of the COVID-19 pandemic, FB2001 (also named DC402234) was designed and synthesized based on the structure of SARS-CoV M^{pro}. FB2001 exhibited potent and broad-spectrum antiviral activity and has a higher resistance barrier than nirmatrelvir (the active ingredient of Paxlovid[®]) and ensitrelvir. Notably, FB2001 did not require a pharmacokinetic booster like ritonavir, implying that it has a low risk of drug-drug interaction when co-administered with other compounds. Injectable and nebulized forms of FB2001 are currently undergoing international multicenter phase II/III trials (Dai et al., 2020). Meanwhile, FB2001 also displayed broad-spectrum antiviral activity against enteroviruses, noroviruses, and coronaviruses, and represents an important reference for the future design and development of anti-COVID-19 drugs.

A structure-based design of peptidomimetic α -ketoamides was exploited to discover potent inhibitors of enterovirus 3C^{pro} and coronavirus M^{pro}. Further structural optimization led to compounds with broad-spectrum antiviral activity and the most potent compounds inhibited MERS-CoV in Huh7 cells with EC₅₀ of 0.4 nM (Zhang et al., 2020). Using a scaffold hopping strategy, a novel series of peptidomimetic aldehydes was designed and synthesized (e.g., DC402209), with broad-spectrum activity against a variety of enteroviruses (EV71, EV68, coxsackievirus, and rhinovirus) and SARS-CoV-2. The crystal structure of EV71 3C^{pro} in complex with DC402209 (PDB code: 7DNC) revealed that the compound formed a covalent bond via an aldehyde group with C147, and the NH of the indole moiety formed a hydrogen bond with the S4 pocket of 3C^{pro}. The compound also exhibited good pharmacokinetic properties (Dai et al., 2022). Further modifications of the P2 and P3 fragments of DC402209 yielded compounds with improved anti-norovirus activity: DC402267 inhibited mouse (MNV; EC₅₀ = 40 nM) and human norovirus (GI.1; EC₅₀ = 12 nM) 100-fold more efficiently than did DC402209 (Van Dycke et al., 2021).

Hong further discussed the design and development of multi-target inhibitors targeting coronaviral and/or host proteases as a new generation of anti-coronavirus drugs. Novel dual-target inhibitors have been designed targeting host cathepsin L and calpain-1; they are highly effective against coronaviruses, including SARS-CoV-2 and its variants (Xie et al., 2024). The inhibitors are again based on α -ketoamides and form covalent bonds with Cys25 in the catalytic active site of human cathepsin L, and with Cys115 in the catalytic active center of calpain-1, as well as numerous hydrogen bonds. The compounds exhibited significant anti-inflammatory effects in the LPS-induced mouse model of acute inflammation. Based on these compounds, a triple-target inhibitor DC406068 was designed to inhibit M^{pro} and host cathepsin L/calpain-1 with high inhibitory activity. DC406068 also exhibited high inhibitory activity against a variety of coronaviruses in different cells. The crystal structures confirmed that DC406068 covalently interacts with Cys145, Cys25, and Cys115 of SARS-CoV-2

M^{Pro} and human cathepsin L/calpain-1, respectively. The compound seems to block both viral entry (by inhibiting human proteases) and viral replication (by inhibiting M^{Pro}) and has entered preclinical studies. In summary, these advancements may prove beneficial for preventing and treating other viral diseases.

6.2. Some capsid/core assembly modulators (CAMs) can induce an inhibition of HBV RNA biogenesis. David Durantel, Ph.D., Equipe Hepvir, CIRI, ENS-Lyon, France

Hepatitis B core protein (HBc) is involved in the formation of the viral capsid, which plays a key role in HBV life cycle and is important for virion production, since reverse transcription happens within the capsid. Since HBc is mainly localized in the nucleus in non-dividing infected cells, it also interacts with many nuclear replication intermediates and has regulatory functions through these interactions. For example, HBc interacts with covalently closed circular DNA (cccDNA), regulating binding of HBc from incoming virions to cccDNA, as well as HBc-HBV RNA interactions in nucleus and cytosol. **David Durantel** introduced two classes of core assembly modulators (CAM) currently in clinical evaluation: (1) CAM-Ab, where Ab stands for aberrant capsid structures that do not incorporate the pregenomic RNA (pgRNA)-polymerase complex; and (2) CAM-Em, where Em stands for empty. These molecules stabilize dimer-dimer interactions, consequently increasing the speed of nucleocapsid assembly, thereby promoting the formation of normal nucleocapsids that are devoid of pgRNA-polymerase complex. Both classes of compounds showed IC₅₀ in nM ranges for inhibiting HBV replication in cell-based assays. David explained the four existing known mechanisms of action (MoA) for these CAMs and offered his working hypothesis on the 5th one, as summarized below. (1) CAMs (both types) primarily inhibit nucleocapsid assembly and, subsequently, inhibit reverse transcription of pgRNA to relaxed circular DNA (rcDNA) along with virion formation. (2) CAMs (both types) can prevent cccDNA establishment when administrated before infection; however, higher concentrations of CAMs (10 × EC₅₀) are needed. (3) High concentrations of CAMs (mainly CAM-Em; 5–10 μM) can impair HBeAg biogenesis. (4) CAM-Ab can induce cell death in cells expressing very high levels of HBc. (5) CAMs can potentially inhibit cccDNA transcription, promote vRNA elongation, enable RNA escaping RNA splicing, and reduce RNA stability. Using a HepaRG model, the group showed that high concentrations (5–10 μM) of CAM-Ab compounds decreased HBV total RNA and pgRNA biogenesis. The readouts included chemiluminescent immuno-assay (CLIA) and Northern blot analysis of the specific RNAs and expression of downstream genes like hepatitis B surface antigen (HBsAg) and HBeAg using CLIA. However, the effect of CAM on RNA accumulation is not associated with recovery of the innate immune response, which was monitored by the expression of two cytokines, RSAD2 and IL6, measured by CLIA. David's group also tested CAM compounds in primary human hepatocytes (PHH), the gold standard study model. They observed the following: (1) at 5–10 μM, CAM-Ab/JnJ-890 also decreased HBV RNA biogenesis but had no effect on cccDNA levels. In contrast, a compound from the other class, CAM-Em/JnJ-827, did not inhibit cccDNA transcription; (2) neither type of CAM affected HBV RNA degradation kinetics in HepaRG or PHH, while CAM-Ab/JnJ-890 induced a decrease in expression of HNF4a, a transcription factor. The authors tested more CAM-Ab with various levels of activity, but CAM-Ab/JnJ-890 remained the most potent. The compound effect on decreasing HBV RNA was concomitant with decreased HNF4a

expression. The team then tested the long-term effects of up to 56 days of administering 5 μ M CAM-Ab/JnJ-890 in HepaRG cells. Time-dependent decreases of viremia, HBeAg, and HBsAg were observed, with viremia decreasing first, followed by HBeAg, and finally HBsAg. The decrease in HBsAg was concomitant with the loss of cccDNA coprecipitated with anti-HBc. David concluded that more potent CAMs are needed and nuclear functions of HBc can be targeted by CAMs, offering a new class of HBV antivirals.

6.3. The B cell repertoire in multiple sclerosis reveals molecular mimicry between EBV EBNA1 and GlialCAM. Tobias Lanz, MD., Stanford University, USA

Tobias Lanz introduced the link between Epstein-Barr virus (EBV) and autoimmunity to the audience. EBV is associated with several highly prevalent autoimmune diseases that cause significant levels of disability, including multiple sclerosis (MS), systemic lupus erythematosus, and Sjögren's syndrome. The evidence for EBV's role in autoimmunity is best established for MS, as large epidemiological studies showed that nearly 100% of MS patients contract EBV before the onset of their clinical symptoms. Tobias discussed several mechanisms that could explain this association. He pointed out that B cells orchestrate the adaptive immune response in MS, and that EBV, as a B cell-tropic virus, has the potential to drive B cells towards more inflammatory phenotypes, which might contribute to autoimmunity in susceptible individuals. He went on to present his own data, which provided evidence for molecular mimicry between EBV and self antigens in the central nervous system. Using single-cell sequencing of the B cell receptor repertoire in the spinal fluid of MS patients, Tobias identified antibodies that bind the EBV transcription factor EBNA1 and cross-react with the glial cellular adhesion molecule GlialCAM. He showed that serine phosphorylation on GlialCAM facilitates the high-affinity interaction between the antibody and GlialCAM. Interestingly, the unmutated germline antibody, expressed by naïve B cell precursors before affinity maturation, already bound EBNA1 with high affinity. However, somatic hypermutation and affinity maturation were necessary for the antibody to increase binding affinity to the self antigen GlialCAM. Tobias therefore proposed a disease model in which a naïve unmutated B cell becomes activated by EBV EBNA1 in the periphery, undergoes affinity maturation, and, at some point, traffics into the central nervous system. There, the B cell encounters GlialCAM and continues to mutate its B cell receptor towards a high-affinity binder of this autoantigen. Tobias showed that B cell reactivity against EBNA1 and GlialCAM aggravates the mouse model of MS, and that EBNA1/GlialCAM cross-reactive antibodies can be detected in the sera of approximately 20–25% of MS patients. In his closing remarks, he discussed currently approved and experimental treatments for MS. He pointed out that B cell depletion is a highly effective MS treatment, and that one of its under-appreciated mechanisms of action is likely suppression of EBV. Newer, more targeted approaches include allogenic EBV-specific T cell therapies and EBV vaccines, the latter of which are in development as both preventative and therapeutic approaches. Small-molecule therapeutics for EBV are long-needed, and the most promising target in the context of autoimmunity is EBNA1, the only protein expressed during low latency. A potent inhibitor of EBNA1 has the potential to become a fundamentally novel therapy for MS as it would target a major causal factor of the disease.

6.4. Towards an HIV cure – the role of immunotherapy and gene therapy. Sharon Lewin, FRACP, Ph.D., Doherty Institute, The University of Melbourne, Australia

Sharon Lewin spoke on novel approaches to reduce the HIV reservoir in people with HIV on effective antiretroviral therapy (ART). Over the past four decades, management of HIV with ART has changed dramatically, resulting in declines in mortality and improving life expectancy of people with HIV to that similar of people without HIV. However, ART must be taken for life because of the persistence of latent virus in resting CD4⁺ T-cells. HIV rapidly rebounds within 2–3 weeks following ART cessation. There is now a global scientific effort to find a way to allow people with HIV to safely stop ART through either eliminating all persistently infected cells (eradication) or reducing the pool of infected cells and increasing immune control of any residual virus (ART-free viral control or remission).

Sharon spoke about one approach that her team has pioneered to reduce the pool of infected cells through activating HIV transcription in latently infected cells. This would lead to expression of viral proteins not usually expressed in latently infected cells, allowing for immune-mediated clearance or virus-induced cytolysis. This approach, called “shock and kill,” has not been successful to date, largely because currently available latency reversing agents (LRAs) lack sufficient potency to express viral proteins, only activate a small proportion of infected cells, have off-target effects and therefore dose-limiting toxicities, and are unable to induce death of infected cells, potentially because of overexpression of pro-survival proteins such as B cell lymphoma 2 (BCL-2) (reviewed in Tanaka et al., 2022). Sharon discussed recent work using mRNA delivered by a unique T cell-tropic lipid nanoparticle (LNP) that can potently activate virus transcription in both latently infected cell lines and CD4⁺ T cells from people with HIV on ART. Her group has developed two approaches. One used mRNA to deliver exon 1 of the Tat protein, a viral protein and potent trans-activator of the HIV long terminal repeat (LTR). The other approach used clustered regularly interspaced short palindromic repeats (CRISPR) activation, which contains a catalytically inactive dead Cas9 protein together with two proteins that are potent transcriptional activators, along with guide RNAs designed to bind to HIV LTR. CRISPRa components were delivered as three separate mRNAs, co-packaged in LNP, and resulted in potent and highly specific activation of HIV LTR.

Sharon also discussed other approaches that aim to promote specific death of latently infected cells by using a licensed BCL-2 antagonist, venetoclax. Prior work has shown that latently infected cells express pro-survival proteins and antagonism sensitizes the reservoir to elimination (Ren et al., 2020), so her team tested the effects of venetoclax given to humanized mice infected with HIV and on suppressive ART (Arandjelovic et al., 2023). Following ART cessation, mice that received venetoclax had significantly longer time to viral rebound than vehicle-treated controls, consistent with reduction of the viral reservoir. The delay in viral rebound was even further prolonged when venetoclax was given together with an inhibitor of another pro-survival protein, MCL-1. The team also evaluated the effects of increasing doses of venetoclax ex vivo on CD4⁺ T-cells collected from people with HIV on ART who underwent leukapheresis. Venetoclax led to a dose-dependent reduction in both HIV DNA and levels of intact virus (measured by PCR). Bulk RNA sequencing using venetoclax-treated cells from people with HIV on ART demonstrated enhanced death

of naïve and effector memory T cells as well as dose-dependent depletion of cells that expressed pro-death transcripts. Given that venetoclax is licensed for the treatment of chronic lymphocytic leukemia and is a well-tolerated oral drug, a clinical trial of venetoclax in people with HIV on ART is now enrolling participants in both Denmark and Australia ([NCT05668026](#)).

6.5. Antivirals targeting the conserved HIV-1 TIM-TAM riboswitch specifically reactivate HIV-1 from latency through modulating viral RNA biology. Damian Purcell, Ph.D., Doherty Institute, The University of Melbourne, Australia

In a search for new therapeutic agents that can reactivate latent HIV in a way that exposes it to immune detection, **Damian Purcell** sought to target highly conserved functional RNA structures that serve as riboswitches for important roles in viral replication and latency. He showed that much of the residual latent HIV reservoir exists as provirus integrated within transcriptionally active cellular DNA. From this location, HIV provirus may be transcribed as pre-mRNA incorporated within introns of cellular genes that are removed during mRNA splicing. He proposed that evolutionary plasticity of HIV might reveal highly conserved, structured HIV RNA riboswitches that exist within these non-coding regions of transcribed cellular pre-mRNA. He suggested that these have crucial roles in HIV reactivation and serve as targets for HIV latency reversing agents. Following this rationale, he identified an RNA structure overlapping the HIV Tat coding sequence that is highly conserved relative to the protein coding sequence. The element was shown to play a role in mediating an internal ribosome entry site (IRES) mechanism for the translation of Tat protein from otherwise non-coding RNA, such as viral genome within cellular intronic lariats. This riboswitch was called the Tat IRES modulator of tat mRNA (TIM-TAM). In addition to the IRES function, the TIM-TAM riboswitch also incorporated control elements for alternative mRNA splicing of Tat mRNA that plays a role in the transition between latent and productive infection. Expression of Tat protein serves as the master regulator for HIV replication and latency by co-opting cellular proteins that control histone architecture, RNA splicing, RNA capping, and microRNA biogenesis, which all intimately connect to the control of HIV gene expression during productive and latent infection. The TIM-TAM riboswitch controls these activities and was shown in RNA-pulldown experiments to bind to cellular proteins SRP14 and HMGB3, which, respectively, increase or diminish the expression of Tat and consequent productive HIV replication.

The TIM-TAM riboswitch was incorporated into a novel cell-based screening assay of 114,000 curated drug-like compounds that identified 7 leads, 5 of which were BRD-4 bromodomain inhibitors, an amidothiazole, and a 2-amidopyridine. The hit amidothiazole compound had an EC_{50} of 16.2 μ M and progressed to further development through medicinal chemistry to an EC_{50} < 80 nM but with increasing toxicity. This compound family could reactivate dormant virus from latency, leading to expression of HIV mRNA in primary patient cells, and synergized with the JQ1(+) bromodomain inhibitor in achieving potent reactivation of latent HIV from patient leukaphoresis cells. No convincing protein target was identified for the amidothiazole family of compounds, but in silico docking predicted that the amidothiazoles bind directly to a conserved structured pocket within TIM-TAM RNA with thermodynamic reactivities that matched the potency and structure-activity relationship

of the series. The RNA binding site for these compounds overlapped the AUG start codon for the Tat protein, the exon-splice enhancer and silencer motifs for Tat mRNA-splice control, and a DRACH sequence loop that contains a site for m6A modification of this RNA motif. Using STM2457, a potent catalytic inhibitor of the METTL3 writer protein for m6A modification of cytoplasmic cellular mRNAs, the m6A modification at the DRACH motif (AAACU) was confirmed to modulate the function of the TIM-TAM motif. The DRACH motif in TIM-TAM also overlaps the binding site for HMGB3, showing that control through this riboswitch involves both RNA methylation and structure modification, impacting cellular protein binding and viral Tat protein expression. The 2-amidopyridine family of compounds still requires optimization through medicinal chemistry, but can nonetheless dock into the interior of the TIM-TAM helix adjacent to the DRACH site. Overall, these studies reveal an important multiparameter RNA switch for HIV Tat and control of HIV latency that serves as a target for HIV latency-reversing agents.

6.6. Mechanisms of HIV-1 hypersensitivity to islatravir (4'-ethynyl-2-fluoro-2'-deoxyadeosine (EFdA)). Alexa Snyder, Ph.D. Candidate, Emory University School of Medicine, USA

Alexa Snyder presented her research on the nucleoside reverse transcriptase translocation inhibitor (NRTTI) islatravir (ISL). Her objective was to elucidate the mechanism behind ISL hypersensitivity in HIV-1 variants that emerge during antiviral therapy, focusing particularly on the NNRTI-resistant F227C mutation in reverse transcriptase (RT). Understanding hypersensitivity mutations is important because the HIV epidemic persists and current therapies have several limitations. Leveraging these mutations could be advantageous. Furthermore, there is a pressing need for new ARTs that are long-acting and possess novel mechanisms of action. ISL, an analog of 2'-deoxyadenosine with 4'-ethynyl and 2-fluoro modifications (also known as EFdA), is currently in phase 3 clinical trials and exhibits a novel mechanism of action. Alexa investigated three potential mechanisms of RT F227C hypersensitivity to EFdA: incorporation, excision, and translocation. Through structural and biochemical approaches, she determined the primary mechanisms contributing to this increased susceptibility. Based on the hypersensitivity profile of ISL, it was proposed that ISL might be most effective when combined with NNRTIs that select for the F227C mutation, such as doravirine, elvitegravir, rilpivirine, etravirine, and others. Her concluding remark was, "When it comes to HIV combination therapy, why not take the weakness of one drug and make it the strength of another?"

6.7. HSV-1 latency is established in human neurons in which viral genes are expressed and viral DNA is replicated during acute infection. Arryn Owens, B.Sc., Cornell University, USA

Arryn Owens presented her project about HSV-1 infection and latency in neurons derived from inducible human progenitor cells. This work is important because while HSV-1 disease is common, no treatment strategies target latent genomes. HSV-1 latency is currently studied in models that employ small animals, tissue explants, and cell culture. The main drawback of these systems is that antivirals are required to suppress lytic HSV-1 replication during the establishment of latency. Therefore, Arryn and her co-authors developed a novel system using neuronal progenitor cells that are differentiated into neurons for 5 weeks before HSV-1

infection. This system uses HSV-1 strain KOS without addition of antivirals to the culture. Virion production proceeds for about 15 days before declining without intervention, and virus remains undetectable for more than a month.

The group's first question was whether HSV-1 genomes are maintained or cleared from the neurons, so they subjected the cells to heat stress to trigger reactivation. Infectious HSV-1 virions were evident within 2 days, indicating that genomes were not cleared from the neurons and remained biologically active. Their second question was whether the newly made HSV-1 genomes were from cells in which virus never replicated or from cells in which the virus initially replicated before the genomes were silenced. To address this, they labeled viral genomes with EdU and EdC and visualized them with click chemistry. This approach creates a "time stamp" on replicating DNA when the label is present in the medium. HSV-1 genomes were also specifically labeled with antibodies against the ssDNA binding protein ICP8. The micro-graphs showed replicating HSV-1 genomes in distinctive replication compartments through colocalization of the click chemistry label and the ssDNA binding protein. Widespread HSV-1 DNA replication was evident in neuronal cultures 4 and 10 dpi, but undetectable 19 and 35 dpi. Replicating genomes were visible again 2 days after heat stress reactivated the virus. The researchers next labeled replicating DNA at 4 dpi and examined its localization 15, 25, and 35 dpi. The genomes labeled at 4 dpi appeared compacted into progressively smaller and compacted foci at later time points, consistent with models proposing that chromatin dynamics regulate lytic and latent infections (Flores Cortes et al., 2024; Hu et al., 2019; Schang et al., 2021). After heat stress, these foci of replicated viral genomes decompacted into new, smaller foci that colocalized with the HSV-1 ssDNA binding protein, indicating that the genomes were replicating again.

In summary, Arryn showed that 1) HSV-1 genomes are latent in neurons and can be reactivated; 2) the genomes are compacted during latency; 3) no antivirals are required to establish latency in the group's model, unlike other tissue culture latency models; and 4) this model permits screening of potential antivirals that affect latency.

6.8. Evaluation and pharmacokinetics of the POM-L-BH DU-MP prodrug against varicella zoster virus and herpes simplex virus 1 in vivo. Jennifer Moffat, Ph.D., SUNY Upstate Medical University, USA

Jennifer Moffat presented the results of a collaboration between Uma Singh and David Chu at the University of Georgia and Mihyung Kim of Anterogen (South Korea). This project aimed to evaluate and develop a novel prodrug nucleotide analog, POM-L-BH DU-MP, for treating VZV and HSV-1 infections. Topical therapies are especially desired for these herpesvirus infections that cause skin rashes. The prodrug strategy was recently published in the *Journal of Medicinal Chemistry* and describes adding a bis(pivaloyloxymethyl) group to the bromovinyl-L-dioxolane uridine nucleoside analog L-BH DU. This prodrug has potent activity against VZV and HSV-1 in cells, though it is about 100 × less potent against HSV-2. Jennifer used a human skin explant model to evaluate topical drugs, and found that POM-L-BH DU-MP was effective against VZV and HSV-1. Moreover, in nude mice implanted with human skin, POM-L-BH DU-MP was effective orally against VZV. In the BALB/c mouse cutaneous flank model of HSV-1 infection, subcutaneously administered

POM-L-BH DU-MP was compared to valaciclovir given in drinking water. Both treatments prevented HSV-1 skin lesions and mortality, but only POM-L-BH DU-MP prevented weight loss. Combined treatment was no better than either drug alone. PK/PD studies were performed using nude mice with VZV-infected skin xenografts because human skin contains the viral TK enzyme that activates the compound. POM-L-BH DU-MP was converted to L-BH DU, and its oral bioavailability was high; its half-life was 5–6 hours. L-BH DU was distributed in mouse organs, including the brain. The di- and triphosphate forms of L-BH DU were in the micromolar range in VZV-infected human skin xenografts, which exceeded the EC₉₀. Jennifer concluded that POM-L-BH DU-MP was highly active against VZV and HSV-1, effective orally, and well tolerated, and is therefore a promising nucleotide analog prodrug.

7. Arboviruses

Chaired by Lara Herrero and Dahai Luo.

7.1. Beyond retroviruses: restriction of flavivirus replication by TRIM5 α . Sonja Best, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

Certain TRIPartite motif (TRIM) proteins can be induced by type I IFN and have been shown to serve as restriction factors of certain viruses by directly interacting with viral proteins. TRIM5 α is a high-consequence restriction factor for primate susceptibility to retroviruses, which has been thought to be highly virus-specific. TRIM5 α recognizes HIV after cell entry and stimulates premature virion uncoating, resulting in activation of the IFN response. In her talk, **Sonja Best** discussed whether TRIM5 α might also have an antiviral effect against orthoflaviviruses. Several orthoflaviviruses have emerged into human populations from primates, having a global impact on human health. These include mosquito-borne orthoflaviviruses, such as DENV and ZIKV, but also tick-borne orthoflaviviruses. For instance, the tick-borne encephalitis virus (TBEV) annually causes thousands of infections in different regions of the world. In addition, emerging tick-borne viruses like Powassan virus (POWV) can result in severe neurological disease in humans.

To study the potential impact of TRIM5 α on orthoflaviviruses, primate TRIM5 α was stably expressed in cell culture. This resulted in significant restriction of TBEV replication. Conversely, CRISPR knockout of TRIM5 α in a HAP-1 cell line led to a 10-fold increase in TBEV secretion in the supernatant. When IFN- β was added, the virus was still able to replicate well in TRIM5 α knockout (KO) cells, although it was inhibited in wild-type cells. A panel of orthoflaviviruses was evaluated, showing that most tick-borne orthoflaviviruses were sensitive to TRIM5 α expression (except for the laboratory strain of POWV), whereas the mosquito-borne viruses were not. Immunofluorescence assays showed that TRIM5 α colocalized with NS2B/3, but not with NS5, suggesting that NS3 is the target for this restrictive action. To confirm the interaction between TRIM5 α and tick-borne orthoflaviviruses, stable cell lines were engineered expressing variants of TRIM5 α . When the ring domain of TRIM5 α was mutated, most, though not all, of its inhibitory activity on Langat virus (LGTV) infection was lost. When the SPRY domain of TRIM5 α (responsible

for binding to NS3) was deleted, the inhibitory effect was totally absent. This work has been previously published (Chiramel et al., 2019).

The next part of the talk more closely examined TRIM5 α mechanism of action, which suggested that sequence differences in NS3 are responsible for different sensitivities of orthoflaviviruses to TRIM5 α -mediated restriction. Sonja ended her talk by concluding that TRIM5 α potentially restricts the replication of specific orthoflaviviruses and can both access replication organelles and recognize diverse molecular patterns. It could therefore be of interest for antiviral drug design against the orthoflavivirus protease to consider long-range molecular dynamics as well.

7.2. Liver-targeted therapeutic siRNAs against highly conserved yellow fever virus genomic sequences effectively limit infection and mortality in a hamster model. Justin Julander, Ph.D., Institute for Antiviral Research, Utah State University, USA

YFV causes an estimated 60,000 deaths per year despite the availability of safe and effective vaccines. Although most YFV infections are asymptomatic, some people develop a hemorrhagic fever that typically involves extensive infection of the liver, resulting in severe liver damage, steatosis, and necrosis. Antiviral drugs are thus urgently needed to treat severe cases of yellow fever. In this talk, **Justin Julander** presented a new strategy to reduce YFV replication and associated liver injuries by silencing highly conserved regions of the YFV genome using siRNAs. Collaborators at Arrowhead Pharmaceuticals developed a targeted RNAi molecule platform that targets siRNAs to hepatocytes to specifically silence genes that cause disease. A few of these hepatocyte-targeted siRNA conjugates are currently in late-stage clinical trials. The platform specifically uses an N-acetyl galactosamine sugar for liver targeting. First, an siRNA panel targeting conserved genome regions of YFV was evaluated against YFV 17D in a cell-based assay, with siRNAs TA4 and TA7 showing the most potent efficacy. Consequently, both siRNAs were tested in a yellow fever hamster model. In this model, hamsters are infected with an adapted Jimenez YFV strain, resulting in manifestations that are similar to human disease. TA4 and TA7 were administered as a single dose of 6 mg/kg subcutaneously, resulting in complete survival of infected hamsters. Next, lead-optimized versions of these siRNAs were evaluated as a single dose in vivo, resulting in significant viral load reduction in serum compared to vehicle-treated controls. Serum alanine transaminase levels were also reduced, indicating that liver damage was limited. Finally, the siRNA treatment was also evaluated in a prophylactic setting in which a single treatment was initiated 28 or 14 days prior to viral challenge. Both siRNAs showed significant antiviral activity and protection against liver injury. In conclusion, this presentation highlighted liver-targeted, pan-genotype siRNAs as a promising strategy for the development of urgently needed therapies for severe YFV infections.

7.3. Ingestion of the antiviral drug JNJ-A07 by mosquitoes during blood-feeding significantly reduced dengue virus transmission. Ana Lucia Rosales-Rosas, Ph.D. Candidate, KU Leuven, Belgium

Ana Rosas discussed her work on examining the efficacy of the pan-DENV antiviral JNJ-A07 in *Aedes aegypti* mosquitoes (Kaptein et al., 2021). When ingested in the blood meal prophylactically, JNJ-A07 was antiviral against DENV in 100% of JNJ-A07-treated, DENV-

challenged mosquitoes. When JNJ-A07 was then used as a treatment strategy administered 6 days post DENV infection, mosquitoes showed a reduced infection and dissemination rate compared to mock (DMSO-only) treated controls. In addition to reducing viral infection and dissemination, treatment also significantly decreased viral loads within infected mosquitoes. Assessing the kinetics of JNJ-A07 in mosquitoes with liquid chromatography with tandem mass spectrometry (LC-MS-MS) showed detectable compound levels in mosquitoes higher than the EC₅₀ up to 7 days post ingestion. To conclude, Ana presented some interesting modeling data simulating the use of JNJ-A07 in the field. Using a logistic model followed by an agent-based model, the group showed that using JNJ-A07 has the potential to significantly reduce the number of DENV outbreaks in endemic areas. One question asked after the presentation was the sensitivity in the detection of JNJ-A07 using LC-MS-MS. Ana explained that the group's methods were able to detect compounds in individual mosquitoes, with no pooling of mosquitoes required. Overall, this interesting work showed that JNJ-A07 has the potential to limit the infection and spread of DENV when ingested in the blood meal of *Ae. aegypti* mosquitoes.

7.4. Structural basis of dengue and zika virus NS1 multimerization and antibody recognition. Alvin Bing Liang Chew, Ph.D., Nanyang Technological University, Singapore

Alvin Chew presented his work on the NS1 protein of flaviviruses DENV and ZIKV. NS1 has many known roles in the DENV life cycle: secreted NS1 is highly immunogenic and a target for diagnostic tests. However, there is still uncertainty surrounding the physiological structure of the NS1 protein itself. Wanting to solve the native form of infection-derived, secreted NS1 (isNS1), Alvin focused on a DENV2 strain EDEN2 T164S, known to produce high levels of NS1 (Chan et al., 2019). Using immunoaffinity purification of DENV-infected Vero cells, the group showed that isNS1 appears as a 250 kDa complex of NS1 and apolipoprotein A1 (ApoA1) on Western blot, confirmed by mass spectrometry. Using similar methods with ZIKV, they confirmed that isNS1 of ZIKV is also complexed with ApoA1. Using cryo-electron microscopy, they then showed that NS1 forms spherical structures associated with high-density lipoprotein (HDL). Cross-linking mass spectrometry then confirmed that the isNS1 interaction with the HDL component ApoA1 occurs via the NS1 wing and hydrophobic domains (Chew et al., 2024). This same complex was found in both infected mouse serum and the serum of a DENV patient. Switching back to ZIKV, the group showed that recombinant ZIKV NS1 forms monofilaments, which had not been previously reported. Overall, the study redefines the landscape of what is known about flavivirus NS1 and provides a basis for the development of NS1-directed therapeutics.

7.5. Identification of mRNA processing machinery as druggable host factor targets for dengue virus infection. Min Jie Alvin Tan, Ph.D., Duke-NUS Medical School, Singapore

Min Jie Alvin Tan described his current work on identifying new host targets to treat DENV infection. He first explained that viral diseases, including DENV-induced disease, are driven by a combination of virus-derived and host-derived factors and therefore both are suitable targets for antiviral drug development. Using an affinity-purification mass spectrometry approach followed by comparative analysis to identify prospective interacting proteins (PIPs), he created a comprehensive DENV-human protein interactome, which was then used to identify host factors impacted by DENV infection. Top hits included CCT

complex, asp-N-linked glycosylation pathway, and metabolism of the RNA pathway. The identification of the metabolism RNA pathway led the group to focus on NS3, which is known to be involved in mRNA processing. Using NS3 in a targeted host screen identified interaction with cleavage and polyadenylation-specific factor (CPSF), a protein critical for cleaving the 3'-region of newly synthesized pre-mRNA in cellular transcription. To provide functional validation of the CPSF target in the context of DENV infection, a pre-mRNA cleavage assay was performed, showing that NS3 did not inhibit pre-mRNA cleavage. However, it did affect the cellular localization of CPSF, moving the complex from a predominately nuclear localization to a more distributed cellular pattern. Further examination of the functional significance of CPSF using siRNA knockdown showed that CPSF is critical for DENV replication, with reduction of CPSF3 abrogating DENV replication in a dose-dependent manner. However, further analysis using JTE-607, an inhibitor of CPSF3 endonuclease activity, showed that whereas CPSF complex is critical for DENV infection, this is not due to its endonuclease activity. Overall, these studies have the potential to inform strategies for disrupting DENV replication using RNA interference and small molecules.

7.6. Discovery of pan-flavivirus protease inhibitors. Christoph Nitsche, Ph.D., Australian National University, Australia

Christoph Nitsche described his work on the development of pan-flavivirus antivirals. First, homology analysis of the NS2B-NS3 protease regions of seven mosquito-borne (ZIKV, DENV, YFV, West Nile virus, Murray Valley encephalitis virus, Japanese encephalitis virus, and Usutu virus) and three tick-borne viruses (TBEV, Kyasanur Forest disease virus, and POWV) was used to identify regions of sequence and structural similarities, which may serve as targets for the development of a pan-flavivirus therapeutic. Studying the substrate specificity of the proteases identified that P1 and P2 have strong preferences for basic residues, specifically arginine in P1 for all ten viral proteases and a strong preference for a second basic residue, commonly lysine or arginine, in P2 (Voss et al., 2024). Optimizing the development of covalent peptide-based inhibitors, Christoph and the team developed highly constrained and highly charged bicyclic peptides which were able to enter cells in the 5 nmol range. Functional validation of these peptides showed that although they readily entered cells, they only inhibited flavivirus replication in the 20 μ M range, at which point the peptides start to cause cellular toxicity. Further analysis showed that the peptides become sequestered in the endosome and therefore cannot reach their targets. A key message from this work is that the NS2B-NS3 region is a highly robust target for the development of a pan-flavivirus inhibitor, and small molecules will be required to functionally reach this target in vivo. Fragment-based screens are ongoing to identify a pan-flavivirus antiviral that targets the NS3 and the NS2B-NS3 region with high affinity.

7.7. Combination therapy of approved drugs potentiates broad-spectrum antiviral activity against alphaviruses in human skin fibroblasts and mice. Leen Delang, Ph.D., KU Leuven, Belgium

Leen Delang presented work on alphavirus antivirals focusing on the globally prevalent chikungunya virus (CHIKV). Emphasizing that alphaviruses can cause both acute and chronic disease that significantly impacts quality of life, Leen reiterated the importance

of developing antivirals against them. Using a drug repurposing approach focusing on using antivirals already licensed against other viruses, the team developed an antiviral screening assay against CHIKV in human dermal fibroblasts, and screened sofosbuvir (SOF), molnupiravir (MPV), and favipiravir (FAV) in a range of combinations. They then used a 'synergy finder' tool developed at the University of Helsinki (Ianevski et al., 2020) to analyze the results, finding that the combination of SOF and FAV had the highest synergy score of almost 29. Similar screens with other alphaviruses, Semliki Forest and Sindbis viruses, showed similar results in drug combinations demonstrating a broad-spectrum effect. To validate these findings in vivo using a mouse model of disease, the group showed that MPV monotherapy reduced CHIKV-induced disease at the early stages, with reduced footpad swelling and viremia 2 and 3 dpi. Combination studies with SOF (80 mg/kg) and MPV (10 mg/kg) showed that the combination reduced viremia and moderately reduced footpad swelling. Studies are ongoing with these drug combinations, expanding to other alphaviruses and to preclinical modeling studies. The question was raised whether any analysis was performed on the host response, including the production of proinflammatory cytokines. Leen answered that this work will be included in future studies.

7.8. Medicinal chemistry optimization and therapeutic efficacy of 2-pyrrolidinoquinazolinones in lethal murine models of venezuelan and eastern equine encephalitis viruses. Jennifer Golden, Ph.D., University of Wisconsin-Madison, USA

Encephalitic alphaviruses, including Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), and Eastern equine encephalitis virus (EEEV), belong to the *Togaviridae* family and are spread by mosquito bites. VEEV and EEEV are recognized as bio-terrorism threats and as pathogens of concern and pandemic potential. In 2019, an EEEV outbreak occurred, with 38 confirmed human cases resulting in 19 deaths. However, there are currently no approved countermeasures available to treat infections with these encephalitic viruses. In her talk, **Jennifer Golden** presented work performed by the Center of Excellence for Encephalitic Alphavirus Therapeutics. This research was initiated years ago with phenotypic screening using the vaccine strain VEEV TC83, during which 348,140 compounds were evaluated in a cytopathic effect inhibition assay. This screen resulted in the identification of hits including a quinazoline compound with a potent antiviral profile. This quinazoline was further studied in structure-activity relationship (SAR) studies, which resulted in new chemistry and led to a new amidine scaffold. The amidine compounds turned out to have more potent antiviral activity. The amidines were further optimized in another SAR to improve the metabolic stability of the amidine. This resulted in new analogs with potent in vivo activity in a VEEV TrD lethal mouse model, as well as in an EEEV mouse model. Further amidine optimization revealed adaptations that could be made on a separate quinazolinone scaffold, imparting greater development flexibility and resulting in BDGR-164, an analog in which the nitro-aryl toxophore was replaced. This molecule had improved microsomal stability and maximum tolerated dose along with suitable bioavailability and brain exposure. BDGR-164 was evaluated in an in vivo delayed treatment study with the VEEV TrD strain in a lethal mouse model. Mice treated prophylactically (2 hours prior to viral challenge) and therapeutically (treatment +1, +2, and +4 dpi) were completely protected when 24 mg/kg of BDGR-164 was administered twice daily for 8 days. The viral brain titers were also significantly reduced. In a lethal EEEV

infection mouse model, >90% of the mice that were given 24 mg/kg of BDGR-164 twice daily for 8 days were protected, even when treatment started as late as 3 dpi. In addition, the R-isomer of BDGR-164 was shown to perform better in in vitro antiviral assays and mouse pharmacokinetic parameters than the S-isomer. This presentation thus summarized the structural evolution of this promising scaffold and showed an important step forward in the development of an effective inhibitor of VEEV and EEEV.

7.9. Suicidal capsid protease from O'nyong'nyong virus: Unveiling the inhibitory potential of indole derivatives. Yuliya Chykunova, Ph.D., Virogenetics Laboratory of Virology, Malopolska Center of Biotechnology, Jagiellonian University, Poland

The O'nyong'nyong virus (ONNV) is a re-emerging arthritogenic alphavirus causing fever, myalgia, and incapacitating polyarthralgia. This virus was first isolated in Uganda in 1959 and has caused four outbreaks so far. The alphavirus polypeptide is processed by two viral proteases: the non-structural protein 2 and the capsid. The capsid has a C-terminal protease domain and only performs one cleavage, after which the capsid is auto-inhibited ("suicidal" protease). Since the capsid protease has an important role in the life cycle of alphaviruses, it could be a promising antiviral target. **Yuliya Chykunova** presented the development of serine capsid protease (CP) inhibitors. First, the expression and purification of the ONNV CP was optimized and assessed for enzymatic activity. Next, a high-resolution crystal structure of the active form of the ONNV CP was solved. Indole inhibited ONNV CP in a FRET-based proteolytic assay, indicating that indole derivatives could constitute a starting point for designing the inhibitor. Commercially available indole derivatives and serine protease inhibitors were evaluated in the enzymatic assay, but none were able to inhibit CP activity. Consequently, screening of approximately 500 novel compounds was performed, resulting in the identification of a hit indole compound with micromolar activity against the enzyme activity of ONNV CP (IC₅₀ value of 86 µM) and with a high binding affinity compared to indole. In conclusion, this study showed that the CP of ONNV could be a new antiviral target for the development of anti-alphavirus therapies and highlighted the potential of indole derivatives as promising CP inhibitors.

7.10. Treatment with 6MMPr potentiates the activity of favipiravir in a hamster model of yellow fever. Justin Julander, Ph.D., Institute for Antiviral Research, Utah State University, USA

Despite the availability of an effective vaccine, YFV still poses a significant threat to human health, resulting in severe morbidity and mortality in regions where it is endemic. Therefore, antiviral drugs for YFV are needed. Favipiravir is a broad-spectrum antiviral drug with activity against diverse viruses, including YFV. The compound 6-methylmercaptapurine riboside (6MMPr), on the other hand, has only low antiviral activity against YFV and DENV in cell culture. Favipiravir needs to be converted by a phosphoribosyltransferase in its ribosylated triphosphate form to be active. However, the *de novo* pathway for purine biosynthesis is favored during virus infection, making the conversion of favipiravir slower. 6MMPr blocks the PRPP amido transferase, which results in increased levels of PRPP and therefore also higher levels of ribosylated favipiravir triphosphate. In this talk, **Justin Julander** demonstrated treating cells with 6MMPr resulted in higher PRPP levels and nucleobase conversion. Interestingly, he showed that the combination of favipiravir with

6MMPr resulted in synergistic broad-spectrum antiviral activity in cell culture against flaviviruses (DENV, ZIKV), coronaviruses (OC43 and SARS-CoV-2), and IAV. In a hamster model of yellow fever, suboptimal doses of favipiravir were combined with 6MMPr to evaluate the effect on survival, weight change, viremia, and alanine transaminase levels in serum. When administered just prior to viral challenge, disease parameters were significantly improved for the combination therapies, whereas monotherapy with favipiravir or 6MMPr did not differ significantly from placebo. Finally, the effect of the combination therapy was evaluated when treatment was initiated 4 hours prior to virus challenge or 2 dpi. In both conditions, a significant improvement in survival was noticed. This study thus demonstrated that combination treatment with 6MMPr would be able to lower the effective dose of favipiravir that is needed to treat viral infections.

8. Hepatotropic and GI viruses

Chaired by Kara Carter and David Durantel.

8.1. Advances towards HBV cure. Margaret Littlejohn, Ph.D., Peter Doherty Institute for Infection and Immunity, Australia

Chronic infection with HBV is a major global public health problem. Of the estimated 254 million people chronically infected worldwide, nearly three-quarters live in the Asian region (WHO, 2024). Despite an effective preventative vaccine, there is no cure for those chronically infected with the virus. Without appropriate management, 15–25% of people with chronic hepatitis B (CHB) will develop advanced liver disease or hepatocellular carcinoma. HBV causes 40% of liver cancers, and currently available treatments reduce, but do not eliminate, the risk of liver cancer. Available antiviral therapy suppresses HBV replication but does not target the viral nuclear reservoir of episomal cccDNA. It is this nuclear reservoir that makes HBV elimination so difficult, serving as the source of viral mRNAs and proteins that are associated with disease progression. Eliminating or silencing cccDNA is the major challenge in the field. HBV has a complex replication cycle, involving both DNA and RNA forms and nuclear and cytoplasmic phases. This complex HBV lifecycle also creates multiple targets and opportunities for new antiviral therapies. There are now almost 50 new anti-HBV treatments being openly investigated, and many of these are already undergoing phase I and II clinical trials (Hepatitis B Foundation, 2024). These therapies have three main goals: A) inhibit viral replication; B) lower viral antigen burden; or C) boost the immune response.

Margaret Littlejohn began by discussing current strategies to inhibit viral replication. The viral entry inhibitor bulevirtide competes with HBV for binding to the NTCP receptor on hepatocytes. Although its clinical trials as monotherapy in HBV patients have been disappointing, this therapy is showing great promise against hepatitis delta virus (HDV), a satellite virus of HBV that shares the HBV envelope proteins, causes the most severe form of liver disease, and has very limited treatment options (Asselah et al., 2024). Currently, the most exciting approach for targeting the HBV cccDNA reservoir largely responsible for virus persistence is CRISPR/Cas, a gene editing system adapted from bacterial immune systems. Newer forms of Cas include base editors, which introduce targeted mutations into

the HBV genome, but these approaches remain in preclinical studies at this stage. One of the first published studies utilized the base editing properties of Cas9 endonuclease variants to create point mutations, introducing premature stop codons into the target sequence to inhibit protein expression (Yang et al., 2020). This approach can target both HBV cccDNA and integrated DNA. Another study showing promising early results investigated LNP delivery in an in vivo mouse model (Smekalova et al., 2024).

Margaret then moved onto strategies to lower the viral antigen burden. All HBV RNA transcripts overlap, ending with the same polyA tail, representing a potential target for new novel treatments. This has been highlighted by pre-clinical and clinical studies using siRNAs and antisense oligonucleotides. Targeting and degrading HBV RNAs not only decreases HBV replication but, more importantly, can also reduce HBsAg levels. Studies have also shown that reducing HBsAg result in a more effective immune response. Antisense oligos, siRNA, or RNA interference and CRISPR/Cas13 cleave HBV RNA molecules, reducing the levels of viral proteins and replication products. A number of these compounds are in phase II trials, with some moving to phase III. These trials generally use a platform approach, with many incorporating combinations with immunotherapeutic agents. Nucleic acid polymer molecules that block assembly and secretion of HBV subviral particles are also in early phase clinical trials with promising results: some patients achieve HBs loss/anti-HBs seroconversion.

Targeting the HBV nucleocapsid can affect multiple steps of the viral replication cycle. Capsid assembly modulators are small molecule inhibitors that can result in defective capsid assembly. This can block pgRNA encapsidation, stopping production of viral particles and release. An important secondary mechanism is interfering with the reverse transcription step, which can prevent viral replication and recycling of the rcDNA to the nucleus to replenish the cccDNA pool. A number of these compounds are in clinical trials, including in combination with siRNAs, although apparent antagonism with siRNA in some combination trials has lessened enthusiasm for this approach.

Margaret concluded her presentation by focusing on strategies targeting the immune response. The importance of the immune system in HBV infection has been most clearly demonstrated by HBV reactivation even after HBsAg loss in patients undergoing potent immunosuppression. Exposure to the virus in adults with a robust immune system generally resolves after an acute infection. However, the immune response in chronic infection is weak or dysfunctional. Different strategies are being explored to restore antiviral adaptive immunity, including blocking inhibitory receptors on T cells, developing therapeutic vaccines, and engineering T cells. Some strategies are targeting the innate immune response, including TLR agonists. Attempts to enhance HBV-specific T cell responses have focused on therapeutic vaccines and interruption of the PD1 and PD1 ligand axis. A pre-clinical study has shown proof of principle that combination therapy, in which the viral antigen load of HBsAg was knocked down prior to administration of a therapeutic vaccine, can be successfully curative (Michler et al., 2020).

In summary, though many challenges remain, considerable advances have been made in the development of new HBV therapies, with multiple classes of antiviral and immunomodulatory therapies under investigation.

8.2. Hepatitis B virus cccDNA biosynthesis, epigenetics, and antiviral development.

Haitao Guo, Ph.D., University of Pittsburgh, USA

Chronic HBV infection remains a significant public health burden worldwide. HBV NA cccDNA is essential to the viral life cycle by serving as the persistent form of viral genome and transcription template; its complete elimination, degradation or loss, or inactivation/transcriptional silencing during chronic infection, is considered critical for an HBV cure but has not yet been achieved by current and investigational antivirals.

Upon initial HBV entry into hepatocytes, cccDNA is formed from incoming viral genomic rcDNA (contained in the viral particles) by hijacking host DNA repair machinery. Once formed, cccDNA exists in a stable, nuclear, episomal mini-chromosome (1–50 copies per cell), decorated with host histones and non-histone host and viral proteins. As it does not replicate, cccDNA is only maintained and replenished by further repair of rcDNA upon hepatocyte super-infection or by recycling of mature rcDNA-containing nucleocapsids to the nucleus (instead of budding out). Theoretically, cccDNA should not be preserved through cell mitosis, but ongoing investigations challenge this model. Accumulating evidence suggests that epigenetic modifications of cccDNA contribute to viral replication and the outcome of chronic HBV infection. Worth noting, HBV X protein (HBx) is known as a cccDNA transcription activator and is essential for maintenance of cccDNA at transcriptionally active epigenetic configuration.

In his presentation, mainly based on recent and some unpublished work performed in his lab, **Haitao Guo** described recent advancements in HBV cccDNA formation and epigenetics research, emphasizing the virus-host interplay between these processes. Furthermore, he discussed investigational antiviral strategies and approaches aiming at eradicating and/or inactivating cccDNA, therefore holding promise for achieving a cure of chronic HBV infection, particularly when integrated into combination therapy regimens.

Regarding the various biochemical processes involved in converting rcDNA into cccDNA by leveraging host DNA repair machinery, Haitao related that his team has identified a cccDNA precursor form in whole-cell Hirt DNA extract called deproteinated-rcDNA (DP-rcDNA), which is depleted from covalently-linked HBV polymerase in the cytoplasm. The group mapped out the nature of the extremities of both strands using T5 and ExoI/III exonucleases, 5'/3' RACE, and 5' radiolabeling (Cai et al., 2020; Guo et al., 2007, 2010). He then listed the factors that his team and others have found to be involved in the transition/conversion from rcDNA or DP-rcDNA into bona fide cccDNA; these include ATR-Chk1, Pol- α , Pol-k, Pol-I, Pol-d, PARP1, FEN-1, Top1, Top2, and ligases I, III, and IV (Chen et al., 2022; Kitamura et al., 2018; Long et al., 2017; Luo et al., 2020; Qi et al., 2016; Sheraz et al., 2019; Tang et al., 2019). He also recognized work from Dr. Alexander Ploss's team, which identified 5 factors as necessary and sufficient to allow conversion of rcDNA to cccDNA in a test tube: Pol-d, FEN-1, Lig-I, PCNA, and RFC (Wei and Ploss, 2020). To identify other host factors involved in this process, Haitao's team engineered a biotinylated rcDNA that

was incubated to nuclear fraction and purified by affinity to identify the associated proteins by mass spectrometry. They identified other factors, including several topoisomerases and DNA damage binding proteins 1 and 2. The latter were functionally validated by loss-of-function approaches in HBV cell culture models, and an updated model for rcDNA to cccDNA formation was presented (Marchetti et al., 2022).

On the epigenetic regulation side, the group has recently aimed at identifying host factors associated with inactive cccDNA. To achieve this, his team has established a dual system of inducible reporter cell lines modeling infection with wild-type and HBx-null HBV, both secreting HA-tagged HBeAg as a semi-quantitative marker for cccDNA transcription. Using these cell lines and HBc associated with cccDNA as bait, they pulled down cccDNA and performed mass spectrometry analysis to identify the associated host proteins. HMGB1 was identified as associated to silenced cccDNA in absence of HBx and was shown responsible for inactivity by a loss-of-function approach (Kim et al., 2022). Mechanistically, the group found that HBx interacts with HMGB1 and prevents its binding to cccDNA without affecting the steady-state level of HMGB1. Taken together, the results suggested that HMGB1 is a novel host restriction factor of HBV cccDNA with epigenetic silencing mechanisms that can be counteracted by viral transcription activator HBx.

Haitao stressed that HBx is an excellent target for therapeutic development, because cccDNA is silenced in its absence. This is due to several functions of HBx, which counteract the action of host restriction systems (e.g., SMC5/6, HMGB1) and positively transactivate transcription. There is currently no drug directly targeting HBx (type degraders/PROTACS), but HBx expression can be reduced by siRNA and RNA degraders. Current DAAs (NUC and CAMs) can theoretically prevent cccDNA maintenance by inhibiting both recycling of mature nucleocapsids and superinfection (via their effect on viremia). To identify novel therapeutic concepts, an innovative cell-based cccDNA assay, in which the secreted HBeAg is used as a cccDNA-dependent surrogate, was established by Haitao's team. Using this model, they identified CCC-0975, a disubstituted sulfonamide, which inhibits cccDNA production at low micromolar EC₅₀ in cell culture (Cai et al., 2012). Using a similar model expressing tagged HBeAg in a cccDNA-dependent manner, they also identified a bromodomain-containing protein 4 (BRD4) inhibitor MS436, which markedly inhibited cccDNA transcription. Interestingly, MS436-mediated inhibition of cccDNA transcription was accompanied by cccDNA destabilization in HBV infection and a recombinant cccDNA system, indicating that BRD4 activity may also play a role in cccDNA maintenance, and more generally suggesting that stability and transcriptional activity of cccDNA could be intimately linked (Yu et al., 2023). Most recently, they have established a third-generation reporter system producing cccDNA-dependent HiBiT luciferase signal, which is currently being developed into a high-throughput platform for antiviral screening.

To conclude, there is still much to understand regarding cccDNA formation and maintenance, as well as the epigenetic mechanisms regulating cccDNA transcriptional activity. This knowledge will be invaluable for targeting cccDNA by improved antiviral strategies.

8.3. Discovery of a pan-genotype hepatitis E virus replication inhibitor exerting potent in vivo efficacy. Suzanne Kaptein, Ph.D., Rega Institute for Medical Research, KU Leuven, Belgium

Hepatitis E virus (HEV) constitutes a substantial public health burden with ~20 million annual human infections, including >3 million symptomatic cases and ~50,000 deaths/year. It accounts for 3–5% of deadly viral hepatitis worldwide (WHO, 2024). HEV causes mainly acute illness but can also lead to chronic viral hepatitis in humans. Depending on genotype and geographical areas, it is fecal-orally transmitted through contaminated drinking water or undercooked meat. The only available vaccine (Hecolin®) is exclusively available in China and Pakistan and is not effective against all HEV genotypes (Nimgaonkar et al., 2018). Treatment options are limited to the off-label use of ribavirin; PEGylated IFN can also be used in some circumstances, and the use of sofosbuvir was inconclusive in clinical trials (Nimgaonkar et al., 2018). Appropriate treatment options for HEV-infected pregnant women and immunocompromised patients are currently lacking, underscoring the pressing requirement for safe and efficacious pan-genotype therapies to combat HEV infections.

Suzanne Kaptein and colleagues reported on the identification of JNJ-9117, a sugar-modified guanosine analog initially developed against HCV (Wang et al., 2020). It exerts nanomolar, pan-genotype antiviral activity against HEV in many different cell types used to replicate HEV post transfection or infection, including the gold standard PHH model. Being a nucleoside analog, JNJ-9117 is assumed to target the viral polymerase in its 5'-triphosphate form; it was found to be similarly (or only moderately less) effective on ribavirin- and sofosbuvir-associated mutants. JNJ-9117 had a promising pharmacokinetic and safety profile in rats and dogs. In addition, it exhibited strong antiviral activity in the nude rat HEV infection model when administered by gavage twice daily at a dose of at least 70 mg/kg/day. Remarkably, starting treatment 5 or 10 dpi led to a rapid and significant reduction in viral loads within 4 days. Moreover, at the highest dose (200 mg/kg/day), suppression of viral RNA levels in feces and liver tissue was effectively maintained until the end of the study 17 or 22 days after treatment discontinuation. An additive effect was observed in vitro when combining JNJ-9117 with ribavirin. Altogether, these results illustrate the potential of JNJ-9117 as a promising candidate for treating HEV infections.

8.4. Functional evaluation and mode of action of a novel non-nucleoside drug inhibiting the replication of hepatitis delta virus. David Durantel, Ph. D., Equipe Hepvir, CIRI, France

Co-infection with HDV along with HBV significantly increases the risk of hepatitis progression to cirrhosis and liver cancer. Since the HDV genome does not encode a specific enzyme, it is impossible to develop a conventional direct-acting antiviral, and there is still no satisfactory treatment. HDV is the smallest human pathogenic virus and a satellite of HBV. Indeed, HDV hijacks HBV's envelope glycoprotein (HBsAg) to produce its own virions.

David Durantel spoke about a screening program initiated to isolate small molecules capable of inhibiting the replication and propagation of HDV. These efforts have led to the identification of a new non-nucleoside inhibitor of both HBV and HDV. This molecule, called APY1, belongs to a chemical series based on an alkoxy pyrazole central core. The antiviral activity of APY1 has been established in cultures of HepaRG cells and PHH co-infected with HBV and HDV. While APY1 reduced all virological parameters of HBV

in vitro (secretion of HBsAg/HBeAg, intracellular viral RNA, and virion secretion), this molecule most efficiently inhibited the production of infectious HDV particles with an IC_{50} in the micromolar range. The authors showed that while the secretion of HBsAg is not quantitatively inhibited by APY1, this molecule disrupts the glycosylation pattern and folding of HBsAg so that its antigenic loop is no longer detected by conformation-specific antibodies. This explains the loss of infectivity of the produced HDV particles. To optimize its properties, more than 350 analogs of APY1 have been synthesized and their evaluation showed a strong correlation between the ability of the molecules to alter HBsAg folding and the loss of infectivity of the HDV particles.

The team is currently seeking to further elucidate the mode of action of APY1. Preliminary data showed that APY1 induces ER stress as evidenced by the induction of CHOP, and modifies glutathione levels, which control the cellular redox balance. Since HBsAg protein is particularly rich in disulfide bonds, the working hypothesis is that the APY1 modifies the redox balance in the ER, thus altering the formation of the S-S bonds necessary for the proper folding of the HBV envelope. These results suggest that the network of disulfide bonds, which is particularly dense in HBsAg, is a vulnerability of both HBV and HDV and a potential therapeutic target.

8.5. Discovery of first-in-class hydrophobic tagging (HyT)-based degraders of HBV core protein. Shujing Xu, Ph.D., School of Pharmaceutical Sciences, Shandong University, China

Interfering with the assembly of the HBV capsid is a promising approach for treating CHB (Diab et al., 2018). Direct anti-HBV agents with novel mechanisms of action are urgently required to increase the functional cure (i.e., virosuppression and long-term HBsAg sero-depletion, associated or not with anti-HBs antibody sero-conversion) rate, which is very low with the currently used nucleos (t)ide analogs in clinical practice. Core protein allosteric modulators (CpAMs) are being extensively developed, with several generations of drugs already investigated in clinical trials. As mentioned in the presentation by Durantel and colleagues, one unmet challenge is targeting the core/capsid protein dimers, which are thought to have important regulatory functions in the HBV life cycle. Besides CpAMs, targeted protein degradation (TPD) constitutes an excellent candidate for this purpose.

Shujing Xu and colleagues have reported the first induced degradation of the HBV core protein (HBc) using small-molecule degraders developed by hydrophobic tag (HyT) technology (Xie et al., 2023); this has led to a paradigm shift in therapeutic strategies and offer innovative avenues for drug design. HyTs are bifunctional TPD molecules consisting of lipophilic small-molecule tags and a small-molecule ligand for the target protein. Leveraging their relatively low molecular weight and reduced numbers of hydrogen bond donors/acceptors in comparison with PROTACs, HyTs represent a compelling opportunity for enhancing the druglike properties of such molecules.

Using SAR studies, Shujing and colleagues revealed that compound S7, which contains an adamantyl group, displayed improved inhibitory activity ($EC_{50} = 0.46 \mu M$) and substantial degradation ability against HBc in a time- and dose-dependent manner. Mechanistic studies demonstrated that autophagic lysosome pathways were potential drivers of S7-induced HBc

degradation. Remarkably, S7 effectively degraded 11 drug-resistant mutants, including HBc from viral strains P25G and T33N, which are highly resistant to the phase III drug GLS4. Furthermore, molecular dynamics simulation results showed that the adamantyl group extended on the surface of the protein, which may help S7 to interact with specific molecular chaperones to achieve HBc degradation. This first proof-of-concept study underscores the potential of HyT-mediated targeted HBc protein degradation, presenting an innovative and highly promising avenue for discovering novel HBV and other antiviral agents with favorable drug resistance profiles.

8.6. Proof of concept: Exploring the therapeutic potential of G3BP1 targeted degradation against norovirus infection. Liliana Echavarria-Consuegra, Ph.D., University of Cambridge, UK

hNoV is the most common cause of acute gastroenteritis. Only 18 viral particles are needed to infect a person, but an infected individual can shed billions of viral particles over the course of illness. This is why hNoV is often a major issue in hospital wards, cruise ships, and seafood restaurants. There are no vaccines or antivirals approved for hNoV. Since there are no robust cell culture systems to study hNoV, other caliciviruses are used as surrogate models, such as murine norovirus (MNV1). Ras-GTPase-activating protein binding protein 1 (G3BP1) is a cellular protein that regulates RNA stability in response to stress and is required for hNoV VPg-dependent translation (Hosmillo et al., 2019). **Liliana Echavarria-Consuegra** showed a side-by-side comparison of wild-type (WT) and G3BP1-KO cells, both infected with MNV1. Only the WT cells produced viral proteins such as NS7. Similarly, viral replication in KO cells was approximately 5000-fold lower than in WT. All these findings suggest that G3BP1 could be used as a target to inhibit viral replication, so Liliana's group targeted G3BP1 for proteasomal degradation through the E3 ligase pathways using PROTACs technology. Since currently there are no G3BP1-targeting PROTACs, her team chose to use HaloPROTAC3 and G3BP1-KO cells stably expressing G3BP1 fused to a Halotag. In MNV1-infected cells, virus-induced CPE and replication was only observed in WT cells and not in the KO cells, while MNV1 replication was restored in G3BP1-KO cells by expression of Halo-G3BP1. In a proof-of-concept study, the authors first titrated HaloPROTAC3 (Buckley et al., 2015) and showed that cells treated with <2 μ M of it remained >90% viable. They then used FACS to study Halo-G3BP1 degradation after 48 hours of treatment with a range of HaloPROTAC3 concentrations. The concentration needed to degrade 50% of Halo-G3BP1 (DC_{50}) was ~600 nM. Furthermore, HaloPROTAC3 was studied for its inhibitory effect on MNV1 viral replication in a prophylactic setting. First, G3BP1-KO cells stably expressing Halo-G3BP1 were treated with HaloPROTAC3 for 48 h, followed by MNV1 infection for 1 hour and addition of HaloPROTAC3. Viral replication was monitored at different time points including 10, 18, 24, and 27 hours post infection (hpi). The %-infection was monitored by quantifying dsRNA + cells using FACS and viral RNA (RT-qPCR), which showed dose-dependent decreases. At 1.2 μ M of HaloPROTAC3, a maximal 80% decrease in viral RNA was observed. Reduction in virus infectious titers was observed 18, 24, and 27 hpi, with a maximal 92% decrease when using 1.2 μ M of HaloPROTAC3. In conclusion, PROTAC-mediated degradation of key host factors involved in the viral life cycles is a potential antiviral approach.

8.7. Protease inhibitor activity varies between genogroup I and genogroup II noroviruses.

Alice McSweeney, Ph.D., University of Otago, New Zealand

hNoV causes ~600 million cases of gastroenteritis globally every year, and no treatments are available. The viral genus is diverse, consisting of ten genogroups termed genogroup I (GI) to GX. Of these, GI and GII viruses, particularly GII.4, cause the most human infections. hNoV 3CL protease is critical for viral replication because it processes the viral polyprotein, and is therefore an attractive antiviral target. Most antiviral development and structural characterization has focused on GI NoV 3CLPro, resulting in 51 published structures, but only 2 structures of GII 3CL Pro have been resolved. As part of the Ward lab, **Alice McSweeney** is interested in finding broad-spectrum 3CLPro antivirals against GI and GII hNoV. Design of peptidomimetic protease inhibitors targeting GI and GII hNoV is feasible since a protein sequence alignment showed that the scissile bonds (P1/P1') are identical. In terms of inhibitor design, the team used a peptide backbone with a glutamine surrogate at P1, a lipophilic group at P2, a heterocycle at P3, and an electrophile that reacts with the active site Cys139. Alice shared the chemical structures and biochemical IC₅₀ values of four representative compounds and pointed out that these compounds were 2–25 fold less potent against GII.4 3CLPro than against GI 3CLPro. To understand the structural basis for this discrepancy, the team solved the X-ray crystal structure of GII.4 Sydney 3CLPro in the absence and presence of inhibitor NV004. The ligand-free crystal structure was resolved at 2.79 Å and showed conserved architecture with the previously reported 3CL proteases. In this structure, there are two conformations of Arg112: the closed conformation, with Arg112 facing in toward the active site, and the open conformation, in which Arg112 faces away from the active site. In contrast, in GI 3CLPro Arg112 is in the open conformation. This difference raises the possibility that a closed Arg112 may hinder access of inhibitors to the active site of GII, resulting in higher IC₅₀ values. Indeed, another group showed that mutating Arg112 in GII could change the protease inhibitor IC₅₀ values to be more like those against GI 3CLPro. The Ward lab then resolved the first GII 3CLPro crystal structure with an inhibitor, NV004, in the active site at 1.83 Å. The team also identified a second partial NV004 (P2 and P3 groups) in the S4 binding pocket, but whether this binding affects compound activity is unclear. When comparing the structures of GII.4 3CLPro with and without NV004, the team found structural changes in three regions of domain II close to the active site, and the binding of NV004 left Arg112 in the open conformation. These structures will aid in structure-guided antiviral development targeting both GI and GII hNoV 3CLPro.

9. Machine learning and computational approaches for antiviral research

Chaired by Alpha Lee and Joshua Schiffer.

9.1. Accelerating antiviral discovery with artificial intelligence. Alpha Lee, Ph.D., PostEra Inc., USA

Alpha Lee started by summarizing how PostEra's (<https://postera.ai/>) technology platform accelerates the key stages of medicinal chemistry - hit identification, hit-to-lead, and lead optimization - and its application to antiviral drug discovery.

In hit identification, machine learning algorithms are used to generate locations of likely pharmacophores given the structure of the binding site. Virtual libraries of molecules are searched to find molecules that fit the pharmacophores, and these molecules are then aligned to the binding site and scored. By coarse-graining to pharmacophores, billion-scale chemical spaces can be efficiently searched. In the hit-to-lead stage, a machine learning-guided parallel chemistry approach is often used. Machine learning algorithms are used to identify key disconnections that can be rapidly diversified using robust chemistry, as well as to select building blocks to provide the most information about that vector. In lead optimization, scaffold changes are often required to satisfy sets of often competing properties. To this end, Alpha's group developed generative machine learning tools that extract scaffold transformations from the literature and apply them to leads. This approach is akin to brainstorming with all possible historic colleagues in the room – rather than a black box, the model is interpretable with references from historic campaigns. Finally, the overarching arc of medicinal chemistry is synthesis, and Alpha's group has developed machine learning models based on natural language processing, which can accurately predict outcomes of chemical reactions, plan retrosynthesis, and quantitatively estimate synthetic accessibility.

Alpha then discussed examples of how machine learning methods are used to accelerate antiviral discovery, drawing from his work during the COVID-19 pandemic and for pandemic preparedness. During the pandemic, he co-founded the COVID Moonshot initiative, which aimed to discover inhibitors of SARS-CoV-2 M^{Pro}. The campaign started as a fragment screen directed at M^{Pro} in March 2020. A crowdsourcing campaign was then kickstarted on Twitter to find ways to merge fragments into inhibitors, and the team rapidly identified ligand-efficient starting points for non-covalent inhibitors. Combining high-throughput libraries with machine learning led to identification of potent non-covalent M^{Pro} inhibitors with in vivo efficacy. The project nominated a development candidate, which is currently undergoing preclinical development led by the Drugs for Neglected Diseases Initiative (Boby et al., 2023).

Alpha also disclosed a drug discovery campaign, in collaboration with Pfizer, directed at the SARS-CoV-2 PL^{pro}. Starting from GRL0617, a chemical probe developed for SARS-CoV PL^{pro}, and using rounds of iterative high-throughput library chemistry, the team discovered inhibitors with nM biochemical and cellular potency that maintains high metabolic stability. The lead compound demonstrated in vivo efficacy in a murine infection model and validates PL^{pro} as an antiviral target (Garnsey et al., 2024).

Beyond pandemic response, Alpha is co-leading the AI-driven Structure-Enabled Antiviral Platform (ASAP), a NIAID-funded Antiviral Drug Discovery Center (AViDD). ASAP focuses on coronaviruses, picornavirus, and flaviviruses, and aims to discover broad-spectrum antivirals and drugs targeting hitherto underexplored targets. ASAP's key underlying approach is the combination of crystallographic fragment screening, structural biology, and machine learning. Alpha showcased results from SARS-CoV-2 macrodomain, a viral ADP-ribosyl hydrolase hypothesized to modulate host immune response. Starting from a fragment screen, the team discovered potent cell-permeable inhibitors. These inhibitors surprisingly showed that macrodomain inhibition does not translate to antiviral activity in cell lines and primary cell systems, either as a single agent or in combination

with nirmatrelvir (an M^{pro} inhibitor), GRL0617 (a PL^{pro} inhibitor), or IFN- γ . Alpha also summarized promising fragment-based drug discovery efforts against enterovirus 3C proteases and the ZIKV ns2b/3 protease.

9.2. Atomistic model of the coronavirus nsp3/nsp4 double membrane vesicle pore. Jason Perry, Ph.D., Gilead Sciences, USA

Jason Perry from Gilead Sciences presented an atomistic model of the coronavirus double membrane vesicle (DMV) pore. The DMV is a result of coronavirus-induced remodeling of host cell membranes to sequester viral replication away from innate immune responses. Non-structural protein 3 (nsp3) and nsp4 form hexameric DMV pores that allow export of nascent viral RNA. Nsp3 is the largest coronavirus protein; its full length has been predicted using AlphaFold, though the highly variable region and linker between NAB and β SM domains are disordered. SARS-CoV-2 nsp3 hexamers were fit domain by domain to published cryo-ET density, which demonstrated that the protein spans the outer DMV membrane, the gap between membranes, and the extra-membrane crown, but not the inner membrane. Twelve nsp4 subunits form a ring around the nsp3 hexamer, bridging the inner and outer membranes, a result predicted by AlphaFold and confirmed by cryo-ET. The structure is predicted to form prior to polyprotein cleavage by nsp5. A model of the pore precursor is formed from six proteins spanning nsp4 to nsp10, and six spanning nsp4 to nsp16. This complex activates the nsp5 protease, cleaving nsp4 to form the pore with nsp3 and trapping the remaining proteins within the vesicle. The stoichiometry of the cleaved polyproteins is consistent with a previously proposed full hexameric replication complex.

9.3. Antiviral therapy optimization for SARS-CoV2: A mathematical modeling approach. Shadi Esmaeili-Wellman, Ph.D., Fred Hutch Cancer Center, USA

Shadi Esmaeili-Wellman highlighted the important role of clinical trial simulation in developing antivirals. She is part of a team at Fred Hutchinson Cancer Center that also includes Dr. Joshua Schiffer and Dr. Katherine Owens, who have accurately recapitulated the results of antiviral clinical trials with mathematical models that incorporate drug pharmacokinetics and pharmacodynamics, as well as viral-immune dynamics governing infection in the absence of treatment. The latter component is critically important because drug effectiveness may depend on timing of treatment, as immune responses intensify over time to synergize with drugs to eliminate virus. On the other hand, immune responses may become harmful if too intense or prolonged, which is why early treatment is favored for most human viral pathogens. Shadi demonstrated that her models recapitulated the amount of viral reduction during nirmatrelvir-ritonavir treatment of SARS-CoV-2 in the EPIC-HR and PLATCOV trials (Esmaeili et al., 2024). She used pre-existing viral load data from over 1500 infected people to generate representative simulated cohorts (Owens et al., 2024). Using this approach, she accurately reproduced primary viral load reduction endpoints from the trials. She also applied the model directly to viral load trajectories from individuals in PLATCOV participants as a model validation step. Key outcomes of the model were that: 1) 40–60 times more drug is needed in human plasma than in vitro to inhibit viral replication; 2) early therapy nevertheless prevents hundreds of thousands of cells from becoming infected; 3) short half-life and reduced in vivo potency suggest that greater viral load reductions are possible with second generation protease inhibitors (a result that has

since been verified in trials) (Cao et al., 2024; Yotsuyanagi et al., 2024); 4) earlier treatment is counterintuitively associated with greater frequency of viral rebound due to blunting of innate immune responses, preservation of target cells, and incomplete elimination of infected cells; and 5) these results explain the higher rate of viral rebound in community settings relative to the EPIC-HR trial. Shadi concluded by highlighting other work suggesting that further mechanistic insights can be made by modeling trials of other SARS-CoV-2-targeting agents, including molnupiravir, ensitrelvir, and remdesivir.

10. Late-breaking oral presentations

Chaired by Kara Carter and David Durantel.

10.1. Design and synthesis of clickable photoaffinity probes for binding site identification on yellow fever virus NS4B target built upon a benzodiazepine antiviral. Yanming Du, Ph.D., Baruch S. Blumberg Institute, USA

Yanming Du presented work on photoaffinity identification of the binding site of BDAA to NS4B of YFV. Previously, Jinhong Chang' lab had performed a cell-based assay using an IFN- β reporter readout to identify compounds with antiviral activity against YFV and identified BDAA as an active compound. Further studies revealed that mutation P219S conferred resistance to BDAA (Guo et al., 2014, 2016). Yanming presented the generation of a novel analog of BDAA that introduces a photoaffinity probe (PAP) as well as a biotin binding moiety while maintaining the antiviral activity of BDAA. This compound was used to test the hypothesis that BDAA interacts with NS4B to alter its topology and thus induce antiviral activity, while the NS4B P219S mutant no longer supports BDAA interaction. Following UV exposure to cross-link the PAP probe on BDAA to bound protein, streptavidin pull-down demonstrated binding to NS4B, but not NS1, NS3, or NS5. Current work is underway to map the site of the NS4B PAP-compound binding. Yanming was asked whether he had performed time-of-addition experiments to determine whether the compound bound ahead of NS4B complex formation or after complex formation with other viral proteins. Such work has not been done yet, but this was noted to be a very good suggestion.

10.2. Establishment of the first high-throughput screening assay for rhinovirus C antiviral drug discovery. Erion Lyoo, Ph.D., KU Leuven, Belgium

Erion Lyoo presented the establishment and validation of a robust rhinovirus C (RV-C) HTS. RV-C causes wheezing and bronchiolitis in infants, pneumonia in immunocompromised patients, and severe exacerbations of asthma and COPD. RV-C does not replicate in standard cell lines, though it can be cultured in primary human epithelial cells systems. To date, screening has relied on replicon systems (Mello et al., 2014), but this does not allow for screening of inhibitors of the entire viral lifecycle. Erion generated a stable HeLa cell line expressing a C529Y mutant of the RV-C receptor CDHR3, which increases the infectivity of the cell line (Bochkov et al., 2015). In addition, a viral strain of RV-C15 that incorporates two adaptive mutations for replication in cell culture and GFP to track viral infection was generated (Bochkov et al., 2016). Using these cells and virus, 70–80% of the cells can be infected. Through rigorous assay development and validation, the group first developed a 96-well format HTS followed by a 384-well format. These robust

assays have Z' scores of >0.8 . Erion showed late-breaking data of assays developed with RV-C11 and RV-C41 and discussed plans for expanding the assay with more C serotypes. A question was asked about any issues with the cell line showing variability in the surface expression of CDHR3, as this is seen in vivo. Erion reported that cell line stability is being evaluated and that only low-passage cells are used in the assay in the meantime. Additionally, another person asked whether transcriptomics were monitored to investigate asthma-associated transcriptional signatures, but this has not been done.

10.3. Suppression of hepatitis B virus replication and protein expression using CRISPR-Cas13b – pre-clinical investigations of a new antiviral approach. Margaret Littlejohn, Ph.D., Peter Doherty Institute for Infection and Immunity, Australia

Margaret Littlejohn presented the work of Laura McCoullough that used the CRISPR-Cas13 system to knock down HBV RNA as a novel therapeutic approach to treat CHB. Noting that global prevalence of CHB leads to significant morbidity and mortality, Margaret introduced the need for additional therapeutics with novel mechanisms of action. The CRISPR-Cas13 system targets RNA directly for cleavage using a highly sequence-specific gRNA. She presented data for CRISPR-Cas13 constructs targeting both the 5'-ends of the longest HBV RNAs and the 3'-end of all HBV RNAs that are coterminal. In an HBV-transfected HepG2 cells system, the constructs targeting the 5'-region knocked down all viral replication intermediates, as did the constructs targeting the 3'-region, with the latter knocking down all viral RNAs as well. No cytotoxicity was observed for any of the constructs. The constructs were active against multiple HBV genotypes. Additionally, Margaret showed that in both the AD38 and HepG2-NTCP infection system, transfection of these constructs reduced HBeAg and HBsAg by 40% and 50%, respectively, despite a poor transfection efficiency of 10%. When using a lipid nanoparticle formulation to increase transfection, HBsAg was reduced by 80% in a cell line expressing HBsAg. Importantly, in an in vivo study using the hydrodynamic injection model for introducing an HBV plasmid to livers of mice, the constructs were able to reduce HBsAg by 50% one week post transduction (CRISPR-Cas13b mediated suppression of hepatitis B virus replication and protein expression [McCoullough et al., 2024]). Margaret was asked whether these impressive results had been directly compared to activity with siRNA, but that has yet to be ascertained.

10.4. The CD8⁺T cells response is sufficient for protection with a CCHFV M segment-based DNA vaccine and GP38 enhances vaccine immunogenicity. Aura Garrison, Ph.D., USAMRIID, USA

Aura Garrison presented work on development of a CCHFV DNA vaccine based on the M segment of the virus as part of an MCM program. Aura began her talk by commenting on the challenges in developing vaccines for MCMs, including lack of knowledge of immune correlates of protection, testing vaccine candidates against limited strains, and the need for refining NHP models to establish clear disease markers beyond viremia. The CCHFV DNA vaccine candidate in these studies, CCHV-MA, was tested in mice susceptible to CCHFV either due to IFNAR KO or transient immune suppression with mAb 5A3 (Suschak et al., 2021). Upon challenge, 100% of the mice were protected from disease and T cell response to GP38 (the N-terminus of Gc and NSM) was detected, but there was

no saturating mAb response. Suboptimal vaccination to determine correlates of protection showed no correlation to antibody levels, including total mAb levels, mAb affinity, or mAbs neutralizing activity (Garrison et al., 2017). Dissecting the vaccine construct demonstrated that GP38 appears to be the key viral protein driving the protective effect (Suschak et al., 2021). Follow-up studies with mice with either B or T cell KO confirmed the role of T cells for protection, and further evaluation of CD8⁺ T cell-specific knock-out mice highlighted the critical role of CD8⁺ T cells in protection (Golden et al., 2023). Additional studies in NHPs, a model in need of further refinement with viremia currently as the only marker, demonstrated that after IV-administered viral challenge, only 1 of the 6 vaccinated animals developed viremia, whereas 6/6 sham-vaccinated animals did. In these studies, the vaccinated animals did not show significant induction of neutralizing antibodies, and their T cell responses were more divergent than in the mice. No significant differences were seen between the vaccinated and unvaccinated animals regarding hematology, though vaccinated animals had lower ALT/AST levels. Notably, virus was found in the testes of these animals, suggesting the need to examine vaccine effectiveness against persistence in future vaccine studies. Aura noted that there are a number of preclinical CCHFV vaccines and the NHP challenge model must be refined to help characterize and prioritize these vaccines as well as understand the translational value of the murine models.

10.5. Unveiling host-cell glycosylation changes upon parainfluenza virus infection.

Plabon Kumar Das, M.S., Institute for Glycomics, Griffith University, Australia

Plabon Kumar Das presented work examining host cell glycosylation changes following infection with PIV. He noted that PIV causes significant disease world-wide, particularly affecting young, elderly, and immunocompromised individuals. There are no therapeutics approved for PIV infection. Glycans are diverse molecules that can be found on their own or modifying other molecules and provide integrity to cells, help in protein folding, and help to maintain cell-cell interactions. Plabon examined glycans on human airway epithelial (HAE) cells as well as in cell lines post PIV infection. For N-linked glycans, he found 90 different species that were grouped by biosynthetic pathways and structures. These included oligomannose, complex neutral glycans, paucimannose, and hybrid glycans, levels of all of which increased, and complex sialylated glycans, levels of which dropped 24 hpi. For O-linked glycans, he found 60 different species that were also grouped by biosynthetic pathway and structures. Core 1 and unidentified core glycans increased while core 2 and core 4 glycans decreased.

11. Shotgun presentations

For the second-to-last session of the meeting, four presentations were selected from the poster abstracts as 5 minute “Shotgun” presentations. The session was chaired by Laura Sanford and Brianna Close, following their outstanding presentations in the PechaKucha Competition. Shotgun presentations included.

- Sophie van Leur, Ph.D. candidate (University of Oxford, Oxford, United Kingdom), “Unlocking the Flaviviral Blueprint: Leveraging Flaviviral Regulation of Selective Autophagy for Therapeutic Intervention” (Abstract #105)

- Flavio Alvarez, Ph.D. candidate (Institut Pasteur, France), “By Interacting with Cell Junction and Polarity Proteins, the PDZ Binding Motif of SARS-CoV-2 Envelope Protein Constitutes a Major Determinant of Pathogenicity and Appears as an Innovative Therapeutic Target” (Abstract #319)
- Calvin Gordon, Ph.D. candidate (University of Alberta, Canada), “Mechanism of Action of a 4'-Cyano Modified Nucleotide Analog Against a Platform of Diverse Polymerases of Respiratory RNA Viruses” (Abstract #321)
- Chaker El Kalamouni, Ph.D. (Université de La Réunion, France), “Evaluating SARS-CoV-2 Antiviral Activity Using the Drosophila Midgut Model” (Abstract #324)

12. Women in science forum

The annual Women in Science forum was held prior to the opening session of ICAR 2024. This year's event was led by Rhonda Cardin, chair of the ISAR Women in Science Committee. The forum featured a roundtable event with 8 moderated tables, each discussing a different topic pertaining to women in science. The topics and corresponding moderators included: networking (Jessica Spengler, US CDC), negotiation (Rhonda Cardin, Pfizer), mentoring (Zlatko Janeba, IOCB Prague), gender inequality (Jennifer Moffat, SUNY Upstate Medical University), women in leadership roles (Kara Carter, Evotec), “being the only” (Kathie Seley-Radtke, University of Maryland, Baltimore County), dealing with microaggressions (Luis Schang, Cornell University), and life-work balance (Leen Delang, KU Leuven). Participants joined a roundtable for the entirety of the session, while moderators rotated at 20 min intervals, allowing the participants to discuss three of the eight topics during the event. Overall, there was a total of 61 participants, including those serving as moderators. The event successfully facilitated numerous thoughtful and engaging discussions on key topics for women in science, served as an important opportunity for all attendees, including women and men from all career stages, to share their thoughts and experiences in a welcoming and open forum, and allowed new and returning ICAR attendees to meet and reconnect facilitating continued discussions and interactions throughout the meeting. ISAR appreciates the support of Pfizer for this important workshop.

13. PechaKucha competition

This year's PechaKucha contest was, as always, one of the highlights of the ICAR program. Seven brave contestants each took the stage to impress us with their ability to convey key aspects about their research projects, as well as personal information about themselves, all while using humor and adhering to the strict PechaKucha rules. For those not familiar with the format, it was invented by two architects in Japan in 2003 and requires the speaker to present using 20 slides at 20 seconds each. The speaker has no control over the movement of the slides, so they must practice and time themselves perfectly. We have adapted the format slightly due to time constraints, so graduate students and post-docs only have 15 slides at 20 seconds each to tell their stories. Just as important as timing is the use of humor or some sort of theme - another critical aspect of the format is entertaining the audience while still getting across the information.

As many previous ICAR attendees remember, we have had numerous themes over the past few years ranging from James Bond, Lord of the Rings, Star Wars, Star Trek, Game of Thrones, Dune, wizards, to a jungle explorer, and so it was no surprise that this year we saw a number of very clever themes for the talks. **Alvin Chew** from Nanyang Technological University in Singapore told us of his “Top-Gun”-inspired mission, while our second **Alvin – Min Jie “Alvin” Tan** – from Duke-NUS Medical School, a medal winning athlete, tied in the parallels between his research project and running marathons. **Maria Nalewaj** from the Institute of Bioorganic Chemistry at the Polish Academy of Science tied in the idea of a “quadruplex contest” to Eurovision, while dressed as a music star performer. Not to be outdone, **Destiny Durante** from the University of Illinois, Chicago, pulled out all the stops as “Drug Discovery” Barbie, complete with an appropriate “hair toss,” head-to-toe Barbie outfit (of course!), and pink-themed slides! Well done, Destiny!

And if that weren’t enough – this year’s three finalists made it incredibly hard for the judges – all three slammed it, and truly made this aspect of the conference so much fun! Coming in third (\$75), **Brianna Close** from Boston University literally took us to online dating and made her science funny! Her presentation’s theme was “My Chemical Romance,” complete with great pictures, song titles for her biological activity slides, and a “love connection” with her compounds and their targets. Second place (\$125) went to **Myra Hosmillo** from the University of Cambridge, who wowed us with her “rap” – including music – presentation!! Not only did she show some beautiful slides of the Philippines, but her entire presentation rhymed perfectly – and the “mic drop” at the end sealed the deal! Finally, our winner (\$250) was absolutely spot on – dressed as Dorothy from *The Wizard of Oz* in braids, gingham-check dress, and RED SHOES - only missing Toto!! **Laura Sanford** from Purdue University really took us down the Yellow Brick Road! She even had tornados on her slides, not to mention, the “poison poppy field of solubility.” Truly epic!

Unfortunately, our two runners up, Destiny Durante and Alvin Tan, who were given the honor of being the chairs for the shotgun talks during the closing session of ICAR 2024, both had to leave early on Friday morning. Laura and Brianna filled in for this role and did a great job of chairing the session. Congratulations again to all of our winners and contestants, and we look forward to seeing what creative talks will dazzle us next year in Las Vegas in March 2025!

14. Career development interactive roundtable

This year, the ICAR career development event was again an interactive Career Roundtable. The Career Roundtable aims to give ICAR attendees the opportunity to meet with established researchers and talk to them about career development, professional opportunities and potential pitfalls for early career scientists.

The Career Roundtable event took place on Tuesday during lunch time in the Phoenix room. The session started with a light lunch, followed by a short introduction of the senior researchers at the tables and an explanation of the concept. Ten round tables were set up, with one or two senior researchers at each table, organized by different career paths. Attendees were free to choose a table of their interest and interact with the senior scientist(s)

at this table in a comfortable, small group setting. After 15 min, attendees could change tables to interact with other scientists having a different career path.

The Career Development committee was very grateful to have a group of excellent senior scientists representing a variety of career experiences (including academia, industry, publishing, funding bodies, and others) who were so kind to participate in the roundtable: Chaker El Kalamouni, Brian Gentry, Luis Schang, Nesya Goris, Katherine Squires, Simon Fletcher, Eric Stavale, Dipanwita Basu, Scarlet Sett, and Nicolette Van Dijk. We would like to thank them for willing to share their advice and experiences. We also thank all participants for their attendance!

15. Poster award

At ICAR 2024, 43 posters were competing to win a poster prize in one of the following categories: (1) graduate student, (2) post-doctoral researcher, and (3) young investigator (junior faculty member or the equivalent). The posters in the competition were visited by at least 2 poster judges during two in-person poster sessions. Both sessions were well attended, with a lot of interaction and interesting discussions between poster presenters and attendees. More than 15 senior scientists volunteered to judge posters.

In the graduate student category, the following prizes were awarded: the first prize was given to Flavio Alvarez for “By interacting with cell junction and polarity proteins, the PDZ Binding Motif of the SARS-CoV-2 Envelope protein constitutes a major determinant of pathogenicity and appears as an innovative therapeutic target”; the second prize to Laura Sanford for “Evaluation of Potency and Metabolic Stability of Diphyllin-Derived Vacuolar-ATPase Inhibitors”; and two third prizes were awarded to Gabriel Small for “Understanding and Inhibiting SARS-CoV-2 NiRAN Domain Catalytic Activities Through Structural Studies and Large-Scale Docking” and to Ana Lucia Rosales Rosas for “Ingestion of the antiviral drug JNJ-A07 by mosquitoes during blood-feeding significantly reduced dengue virus transmission”.

In the post-doctoral researcher category, the first prize was awarded to Dr. Alvin Chew for “Structural basis of dengue and Zika virus NS1 multimerization and antibody recognition”. The second prize was awarded to Dr. Daniel Rawle for “Preclinical evaluation of insect-specific virus platform vaccines (ISVac) for Japanese encephalitis virus and chikungunya virus in PC3 mouse models.” The third prize was given to Dr. Shadi Sadat Esmaeili-Wellman for “Antiviral therapy optimization for SARS-CoV2: a mathematical modeling approach”.

Finally, in the young investigator category, one first-prize was awarded to Dr. Myra Hosmillo for “Development and establishment of reporter replicons for antiviral drug discovery against human Noroviruses”.

16. Chu Family Foundation awards

This year’s winners of the Chu Family Foundation fellowships were Ms. **Sofia Maldonado** and Ms. **Agostina Belen Marquez**. Both are graduate students at the University of Buenos

Aires in Argentina. Sofia plans to use the funds to support a six-month stay with her collaborator, Dr. Karine Alvarez, in Marseille, France at AFMB. She hopes to be able to elucidate the mechanism of action of the new nanoformulation of her drug. Agostina will use her funds for a stay at the laboratory of her collaborator, Dr. David Jans at Monash University in Australia. She plans to elucidate the mechanism of action of CBD as it relates to nuclear transport. And of course, as always, ISAR greatly appreciates the generosity of Dr. David and Mrs. Jane Chu and the Chu Family Foundation for their continued support of this impactful program.

17. Invitation to the 38th ICAR – Las Vegas, United States, May 2025

David Durantel, the new Chair of the Program Committee and president Elect, together with the Society's President and the Program Committee and the Board of Directors have already started organizing ICAR2025, to be held March 17–21, 2025 in Las Vegas, Nevada, USA. After the first in person meeting following the pandemic in Seattle and two years abroad, in Lyon and Gold Coast, ICAR returns to the USA for another exciting meeting for all those interested in antiviral research and development in academia, industry (startup, small, medium and large pharma), and governmental and non-governmental organizations. Join us next year in Las Vegas!

Acknowledgements

We thank all the speakers, presenters, participants, organizers, and sponsors of the 37th ICAR for their key contributions to the success of the meeting. We also thank Joy Feng for all the wonderful photos of the event and Tatyana Klimova for assistance with editing the manuscript.

Data availability

No data was used for the research described in the article.

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