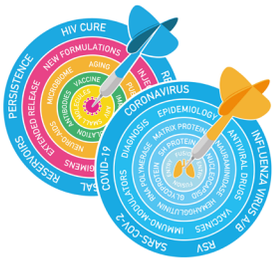


# HIV & Respi DART 2022

LOS CABOS, MEXICO • 4-8 DECEMBER 2022

# ABSTRACT BOOKLET

Global Antiviral Journal

ISSN (Online): 1556-9055

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## HIV DART

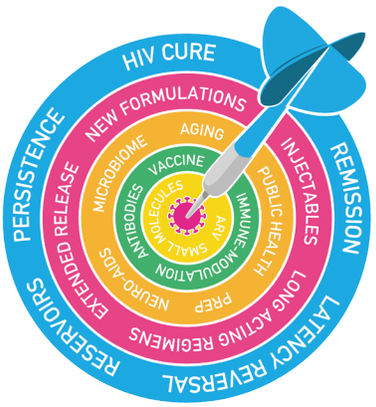


## RESPI DART

# INVITED SPEAKER ABSTRACTS

HIV DART 2022

LOS CABOS, MEXICO • 4-6 DECEMBER 2022



Session 1: HIV latency, reservoirs and advances in cure strategies
December 4th, 2022

**Interactions between HIV and COVID-19**

Matthew Spinelli MD, MS: Monica Gandhi MD, MPH[1]

Division of HIV, Infectious Diseases, and Global Medicine, University of California, San Francisco

**Introduction**

This talk will discuss the intersection of the HIV and COVID-19 epidemics with focus on COVID-19-related health outcomes and risk factors for SARS-CoV-2 among people living with HIV (PLWH), the effect of the COVID-19 epidemic on HIV prevention and treatment, the influence of the COVID-19 response on the mental health and substance use outcomes of PLWH, and COVID-19 immune responses and vaccine hesitancy among PLWH.

**Methods**

We performed a systematic review of papers (PubMed, Medline, etc.) and abstracts (CROI, International AIDS Conference, Vaccine Summit, etc.) discussing the overlap of HIV and COVID-19.

**Results**

Evidence to date do not suggest a higher incidence of SARS-CoV-2 infection among PLWH compared to the general population, although-once exposed-PLWH are at greater risk of severe COVID-19 outcomes. Key risk factors for severe COVID-19 include non-HIV comorbidities known to be associated with severe disease, as well as HIV-specific risk factors such as low CD4 + T-cell count, unsuppressed viral load, and tuberculosis co-infection. The disproportionate impact of the SARS-CoV-2 pandemic among Black, Latinx, and Native American/Alaskan Native PLWH could worsen pre-existing disparities in health outcomes among PLWH. Data on SARS-CoV-2 vaccine protection among PLWH needs additional study, although some studies suggest decreased humoral responses among those with low CD4 + T-cell counts, while there is a signal of increased vaccine breakthrough rates among PLWH in two large observational cohorts. Data on post-acute sequelae of SARS-CoV-2 (PASC) among PLWH is also limited. PLWH do not have a higher susceptibility to SARS-CoV-2, but once exposed, they are at higher risk of severe COVID-19 outcomes. Immune responses among PLWH to COVID-19 vaccination are generally the same as the general population but responses are reduced in those with low CD4 cell counts and high viral loads. Reasons for vaccine hesitancy include medical mistrust, fear of adverse effects, and overlap with non-virologic suppression. HIV outcomes regarding HIV testing, treatment and prevention have been severely compromised during the COVID-19 pandemic per UNAIDS and will require work to combat.

**Conclusion**

Additional resources will need to be dedicated to the development of interventions to improve health outcomes and address disparities among PLWH impacted by the COVID-19 pandemic.

Session 1: HIV latency, reservoirs and advances in cure strategies
December 4th, 2022

## Selection of HIV-1 reservoir cells during long-term ART

Mathias Lichterfeld[1,2,3]
[1]Brigham and Women's Hospital, [2]Ragon Institute of MGH, MIT and Harvard, [3]Harvard Medical School

### Background

Antiretroviral combination therapy is highly effective in suppressing HIV-1 replication and is recommended as the standard of care for all people living with HIV (PLHIV). Through the inhibition of HIV-1 reverse transcriptase, integrase or protease, antiretroviral drugs can effectively prevent infection of new viral target cells, however, they have no direct activity against already infected cells, some of which persist as a long-term viral reservoir and make HIV-1 infection an incurable disease. These cells have traditionally been considered as "latently-infected" lymphocytes that harbor "transcriptionally-silent" proviruses, but recent results have shown that a significant proportion of these cells can remain transcriptionally active, consistent with the fact that antiretroviral agents do not directly inhibit viral gene transcription from chromosomal DNA. Ongoing viral gene transcription and protein translation may make HIV-1 infected cells visible to immune cells and increase their vulnerability to host-dependent immune activity or viral cytopathic effects; in contrast, true viral latency, defined by extremely limited or no residual viral transcriptional activity, can be viewed as an effective strategy to escape from host immune surveillance. In the present work, we hypothesized that selection of viral reservoir cells with features of deep viral latency may longitudinally occur in ART-treated individuals. Reasoning that extended durations of suppressive antiretroviral therapy may allow such selection forces to become more visible, we here conducted a detailed analysis of the HIV-1 reservoir profile for a group of persons who remained on ART for approximately two decades.

### Methods

We used single-genome near full-length sequencing (FLIP-Seq) and matched integration site and proviral sequencing (MIP-Seq) to cross-sectionally and longitudinally evaluate the frequency and chromosomal positioning of intact proviruses in persons undergoing suppressive ART for approximately 20 years.

### Results

We demonstrate a markedly altered viral reservoir profile of long-term ART-treated individuals, characterized by large clones of intact proviruses preferentially integrated in heterochromatin locations, most prominently in centromeric satellite/micro-satellite DNA. Longitudinal evaluations suggested that this specific reservoir configuration results from selection processes that promote the persistence of intact proviruses in repressive chromatin positions, while proviruses in permissive chromosomal locations are more likely to be eliminated. A bias towards chromosomal integration sites in heterochromatin locations was also observed for intact proviruses in study participants who maintained viral control after discontinuation of antiretroviral therapy.

### Conclusions

Together, these results raise the possibility that antiviral selection mechanisms during long-term ART may induce an HIV-1 reservoir structure with features of deep latency and, possibly, more limited abilities to drive rebound viremia upon treatment interruptions.

Session 1: HIV latency, reservoirs and advances in cure strategies
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## When does the long-lived HIV-1 reservoir form?

Sarah B. Joseph[1], Melissa-Rose Abrahams[2], Lynn Tyers[2], Matthew Moeser[1], Olivia D. Council[1], Amy Sondgeroth[1], Ean Spielvogel[1], Ann Emery[1], Nancie Archin[1], Salim Abdool Karim[3], David M. Margolis[1], Sergei Kosakovsky Pond[4], Nigel Garrett[5], Ronald Swanstrom[1], Carolyn Williamson[2]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [2]University of Cape Town, Cape Town, South Africa, [3]CAPRISA, Durban, South Africa, [4]Temple University, Philadelphia, PA, USA, [5]University of KwaZulu-Natal, Durban, South Africa

**Background**
HIV-infected people on antiretroviral therapy (ART) are able to maintain undetectable levels of virus in their blood, yet a reservoir of HIV-infected cells persists that is capable of producing viral rebound. Previous studies observed that cells infected near the time of ART initiation, or their clones, make up the majority of both the replication competent reservoir identified by quantitative viral outgrowth (QVOA) and the largely defective proviral DNA reservoir. This suggests that initiation of ART increases the half-life of recently infected cells. We performed the first study comparing when the replication competent and defective reservoirs form in the same participants.

**Methods**
18 women from the CAPRISA-002 acute infection cohort donated blood every 6 months for an average of 4.5 years prior to initiating ART. vRNA was isolated from pre-ART blood plasma, and MiSeq with Primer ID was used to sequence 5 regions of the HIV-1 genome (1 in *gag*, 1 in *nef* and 3 in *env*). After an average of 5.1 yrs. of ART, end-point dilution PCR and PacBio sequencing with barcodes were used to generate 3' half genome sequences from the QVOA and DNA reservoirs. The DNA reservoir was screened for hypermutation (Los Alamos National Laboratories, Hypermut2) and hypermutated positions were masked to facilitate downstream analyses. Three computational methods were used to date reservoir entry. In addition, the coreceptor usage of QVOA virus was assessed based on sensitivity to coreceptor inhibitors and macrophage tropism was assessed based on sensitivity to sCD4.

**Results**
An average of 15 OGV and 31 DNA reservoir sequences were examined per participant. On average, 61% of the total reservoir was composed of variants circulating in the year before ART initiation (i.e. 'late' variants). Although not statistically significant, the percent of the reservoir that formed 'late' was higher for QVOA (69%) than for components of the DNA reservoir (total DNA 60%; hypermutated DNA 57%; non-hypermutated DNA 64%). Proviruses seeded 'early' (more than one year before ART initiation) and 'late' (within one year before ART) did not differ in their frequency of hypermutated sequences. Further, the cellular tropism of reservoir viruses was similar to that of variants circulating at the time of ART initiation and the majority of the reservoir was R5 T cell-tropic.

**Conclusions**
If selection removes proviruses expressing viral proteins, we predict that QVOA variants would be removed from the reservoir more readily than DNA variants and would on average seed the reservoir later. However, we observed that QVOA and DNA variants date to similar pre-ART timepoints (primarily near ART initiation). Further, we did not observe that variants that entered the reservoir 'early' were more often hypermutated than 'late' variants. These observations suggest that reservoir entry/persistence is not strongly impacted by viral expression potential.

Session 1: HIV latency, reservoirs and advances in cure strategies
December 4th, 2022

**SUMOylation by the SMC5/6 complex epigenetically silences unintegrated HIV-1 DNA leading to post-integration viral latency**

Bryan R. Cullen

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA

**Background**
The integration of the linear proviral DNA intermediate into the host cell genome is an essential step in the HIV-1 life cycle and IN inhibitors are therefore widely used to treat HIV-1 infected patients. In the absence of IN function, unintegrated retroviral DNA is efficiently epigenetically silenced, yet the mechanism underlying the silencing of unintegrated proviral DNA has remained unclear.

**Methods**
We performed an unbiased CRISPR/Cas genetic screen to define cellular factors required for silencing unintegrated HIV-1 DNA and then defined their role in regulating the expression of both unintegrated and integrated HIV-1 DNA in cultured T cells.

**Results**
The genetic screen identified all eight components of the host SMC5/6 complex as essential for unintegrated proviral DNA silencing. SMC5/6 binds to chromatinized, unintegrated HIV-1 DNA and then triggers epigenetic silencing by inducing its SUMOylation. Inhibition of this SUMOylation step, either by point mutagenesis of the SMC5/6 component NSMCE2, a SUMO E3 ligase, or using the SUMOylation inhibitor TAK-981, blocks epigenetic silencing and rescues transcription from unintegrated HIV-1 DNA. Remarkably, T cells lacking a functional SMC5/6 complex can support a spreading HIV-1 infection *in vitro* in the absence of integrase function. Unexpectedly, we also observed that loss of SUMOylation by SMC5/6 results in a >10-fold reduction in the number of latent HIV-1 infections in both CD4+ T cell lines and primary human T cells in culture.

**Conclusions**
These data demonstrate that epigenetic silencing of integration competent HIV-1 proviruses by the SMC5/6 complex prior to integration gives rise to latent HIV-1 infections and reveal that the human SMC5/6 complex is directly involved in promoting HIV-1 latency.

Session 2: Cutting edge developments in prevention and HIV therapeutics
December 5th, 2022

**Implementation and clinical management of injectable antiretrovirals for treatment**

Michael S. Saag, MD

University of Alabama at Birmingham

Over the last 25 years the successful use of antiretroviral (ARV) therapy has converted HIV from being a near-universally fatal infection to a chronic manageable condition. Improvements in therapy over the last decade has resulted in > 85% of patients establishing and maintaining suppression of viral replication, often with a single tablet of combination therapy administered once daily. Despite these remarkable achievements, a need exists to create longer-acting medications that reduce barriers to adherence and minimize potential stigma of daily medication administration.

Drug discovery programs had identified several lead compounds that possess potent antiretroviral activity along with long plasma and/or tissue $t_{1/2}$ pharmacokinetic profiles. Long-acting injectable formulations of cabotegravir and rilpivirine have demonstrated sustained clinical benefit for both treatment of PWH as well as prevention of acquisition of HIV infection among persons at risk via use of Pre-exposure prophylaxis (PrEP). Lenacapavir, a novel capsid inhibitor that has a long $t_{1/2}$ profile, has demonstrated clinical efficacy for use in highly treatment experienced PWH for whom limited options for antiretroviral therapy existed. It has strong potential as an initial antiretroviral treatment for HIV as well as PrEP. Islatravir, a novel nucleoside RT translocation inhibitor (NRTTI), has a long intracellular half-life of 78 -120 hr and is being evaluated as a long-acting ARV agent. Initial studies revealed unanticipated leukopenia, the mechanism of which has been evaluated and studies have resumed using lower doses of the drug. Broadly neutralizing antibodies (bNAbs) have demonstrated potent ARV activity and possess $t_{1/2}$ on the order of weeks, thereby allowing use as a potential component of long-acting ARV treatment regimens.

With the more wide-spread use of longer-acting ARV regimens some new challenges have emerged that may limit the ability to implement treatment to large populations of PWH or those in need of PrEP. These limitations include the need, in many cases, of parenteral administration, which often requires a visit to a health care facility, barriers to access to treatment (including transportation to the medical facility for injection), and cost of treatment. Another problem innate to the use of long-acting treatments is the potential for adverse effects and toxicities to be encountered and not have a mechanism to remove the drug once administered. Finally, a novel potential barrier is the perception of responsibility for the prescribing provider to track the administration of the injectable product and search for the patient to return to the medical facility when the next injection is due.

Future directions likely will focus on novel drug delivery systems that can prolong the half-life of products for up to a year, allowing annual administration of drug via injection or through implantable device technology. Slow-release technologies can be used as well. Regardless of the precise mechanism of drug delivery, long-acting ARV therapeutic approaches will gain more use over the next 10 years, in the realm of both treatment and prevention.

Session 2: Cutting edge developments in prevention and HIV therapeutics
December 5th, 2022

**Impact of subtype on antiretroviral drugs for treatment and prevention**

Rami Kantor[1]
[1] Division of Infectious Diseases, Department of Medicine, Alpert Medical school of Brown University, Providence, Rhode Island, USA

The extraordinary HIV diversity has resulted in different types, groups, subtypes, circulating recombinant forms and unique recombinant forms, which continue to evolve. People that harbor these diverse HIV variants are geographically and heterogeneously dispersed. Antiretroviral therapy to treat people living with HIV has been successfully developed and access to it is globally widespread. Despite the tremendous advantages of this welcomed key intervention and its ability to save lives, development of resistance to antiretroviral medications remains a major hurdle to sustainable antiretroviral success, still resulting in loss of treatment options, morbidity and mortality. Whether and to what extent HIV-1 subtypes matter to patient care has been an ongoing research focus, however these key questions still present a major gap in our understanding, and the impact of subtypes on the antiretroviral drugs is still unclear. This talk will introduce the paradox between globally circulating HIV-1 subtypes and our knowledge of their impact on antiretroviral therapy and patient care; review existing data on subtypes and ways to identify them; and discuss our current understanding of the relevance of these subtypes to different aspects of clinical care, with particular focus on the development of drug resistance.

Session 2: Cutting edge developments in prevention and HIV therapeutics
December 5th, 2022

**CCR5 in HIV prevention and cure**

Helen L. Wu[1], Gabriela M. Webb[1], Jonah B. Sacha[1]

[1]Vaccine & Gene Therapy Institute and Oregon National Primate Research Center, Oregon Health & Science University, Portland, OR, USA

**Background**

CCR5 plays a critical role in HIV infection as the major viral co-receptor. Individuals naturally lacking surface CCR5 expression through a homozygous 32 base pair deletion ($CCR5^{\Delta 32/\Delta 32}$) are highly resistant to HIV infection, and the only documented cases of HIV cure occurred following allogeneic stem cell transplantation from $CCR5^{\Delta 32/\Delta 32}$ donors. Mimicking the $CCR5^{\Delta 32/\Delta 32}$ phenotype is therefore an attractive avenue for both HIV prevention and cure.

**Methods**

Leronlimab is a CCR5-specific monoclonal antibody that binds to the extracellular loop-2 and N-terminus domains of CCR5, thereby directly outcompeting HIV for CCR5 engagement and blocking entry to CD4+ T cells. We generated long-acting leronlimab and administered it to rhesus macaques prior to weekly rectal challenge with CCR5-tropic SHIV. To test use of leronlimab in functional cure, an AAV vector expressing long-acting leronlimab was administered to macaques with established SHIV infection.

**Results**

A single dose of long-acting Leronlimab achieved full CCR5 receptor occupancy (RO) on CD4+ T cells isolated from both peripheral blood and rectal biopsies for >12 weeks. A single dose of long-acting leronlimab also significantly protected against acquisition following repeated, low dose rectal challenge with CCR5-tropic SHIV. In macaques with established CCR5-tropic SHIV infection, resolution of plasma viremia occurred following the emergence of full CCR5 RO on CD4+ T cells in peripheral blood. Of note, we observed viral blips concomitant with transient loss of full CCR5 RO on CD4+ T cells in blood.

**Conclusions**

Given the safety and protective efficacy of the naturally occurring $CCR5^{\Delta 32/\Delta 32}$ phenotype, leronlimab is an attractive addition to the growing arsenal of long-acting injectables for HIV prevention. AAV vectored delivery of leronlimab demonstrated proof of concept that the $CCR5^{\Delta 32/\Delta 32}$ phenotype can be phenocopied by gene therapy delivery of CCR5 blockade.

Session 4: Immunity, vaccines, inflammation, neuroAIDS, and aging
December 5th, 2022

**Understanding NeuroHIV in 2022: Neuroinflammation, viral persistence, and comorbidities**

Serena S. Spudich, MD

Department of Neurology & Center for Brain and Mind Health, Yale School of Medicine, New Haven, CT, USA

**Background**

The fact that HIV infection directly impacts the central nervous system (CNS) has been recognized since very early in the global HIV epidemic. However, the emergence of widespread antiretroviral therapy (ART) use has dramatically altered the clinical character of neurologic disease observed in HIV. The recognition that perturbations persist in the nervous system during ART has raised a host of questions related to the role of CNS injury prior to initiation of ART, ongoing inflammation and viral persistence the CNS during ART, and the role of comorbidities in brain dysfunction in people living with HIV.

**Methods**

This presentation will review key issues in the current landscape of clinical and scientific issues facing people living with HIV, providers, and investigators in the current era. Recent data related to the neuropathogenesis of HIV in people on ART will be summarized, and new tools and approaches to studying the CNS effects of HIV in humans will be highlighted.

**Results**

Potential mechanisms of HIV-related CNS dysfunction observed in patients treated with ART include persistent CNS infection, ongoing immune activation, effect of comorbidities and risk factors including vascular disease, metabolic syndrome, and coinfections, and neurologic injury accrued prior to initiation of antiretroviral treatment. Related to issues of persistent clinical CNS abnormality in the face of ART, an unexplored frontier of research has emerged due to isolated reports of successful HIV remission in the systemic field: whether the CNS may provide a reservoir for infection which may contribute to ongoing dysfunction during treatment. The pathobiology of this putative reservoir is under active investigation, including studies on the timing of establishment of CNS infection, the potential cells and tissues harboring HIV in the CNS, evidence of independent evolution of HIV in the CNS, and the possibility of autonomous CNS sources of HIV replication in the setting of suppressive therapy.

**Conclusions:**

Potent antiretroviral treatment for HIV has changed the spectrum of issues and questions related to HIV's effects on the nervous system. Although ART can almost always prevent or ameliorate the most severe form of HIV-associated dementia, numerous clinical and laboratory biomarkers suggest that the CNS is not always normal in ART-treated people with HIV. Whether this persistent perturbation of the CNS has deleterious effects on the brain is uncertain; whether it in part reflects a reservoir for HIV within cells of CNS in the setting of apparently successful ART is an essential question of relevance to both treatment and HIV remission strategies. Finally, many comorbidities in people living with HIV may be ameliorated by targeted treatments, and these may be important approaches to improving the neurologic and cognitive health of people living long term with treated HIV.

Session 4: Immunity, vaccines, inflammation, neuroAIDS, and aging
December 5th, 2022

**Insights from host epigenetic scars towards addressing HIV complications**

Lishomwa C. Ndhlovu[1] M.D., Ph.D.
[1] Weill Cornell Medicine, Department of Medicine, Division of Infectious Diseases.

**Overview:** Our HIV translational research program centers on understanding how HIV affects the immune system in blood and tissues with a focus on discoveries that identify novel applications in balancing strategies to prevent, slow or eliminate HIV infection while optimizing quality of life outcomes. A major focus covers many disciplines towards an HIV cure and also in identifying neuro-immune pathways that are associated with brain injury in people living with HIV to illuminate the complex pathology surrounding complications arising with HIV and aging. To study mechanism that underly such complicated processes, our program has employed a number of novel innovations including epigenetics and single cell approaches combined with machine learning analyses and this has opened the door to developing new approaches leading to a cure and ameliorating HIV- associated co-morbidities. Epigenetics is the study of how behaviors and environment can cause changes in how gene are expressed and work in cells, but do not change an individual's DNA. These carefully orchestrated chemical reactions that activate and deactivate parts of the genes at strategic areas have been shown to be critical in normal brain function and this presentation will reveal how epigenetic mechanisms may be key events in HIV associated brain injury with implications for HIV curative strategies.

Session 4: Immunity, vaccines, inflammation, neuroAIDS, and aging
December 5th, 2022

**HIV Cure : Bottlenecks and Hope**

Michaela Müller-Trutwin
[1] Institut Pasteur, Université Paris Cité, HIV, Inflammation and Persistence Unit, F-75015 Paris, France

We are soon facing 40 years of HIV research. Tremendous progress has been achieved at many levels in basic, translational and clinical research. The introduction of anti-retroviral treatment has been a game changer for the management of the disease and prevention of transmission. However, HIV is a complex virus raising major obstacles against its eradication. A vaccine and a cure are still missing.

A brief overview of current HIV cure research and concepts will be given. Whether full eradication will be achievable remains an open question. Recent advances in the field of post-treatment control will be presented. An overview and recent data on the impact of early versus late initiation of ART on the immune responses will be developed. A focus will be given on the role of NK cells in the control of viral reservoirs in nonhuman primate models and in people living with HIV. A potential role of HLA-E dependent responses in the regulation of inflammation will be discussed.

The continuous technical developments and increasing insights into the virus-host interactions are a necessary step in the quest of a cure for HIV.

Session 5: Conceptual shifts to mitigate the HIV crisis
December 6th, 2022

**Role of macrophages in the context of HIV & the brain**

J. Victor Garcia[1]
[1]International Center for the Advancement of Translational Science, Division of Infectious Diseases, University of North Carolina School of Medicine.

Macrophages have long been considered to contribute to HIV infection of the CNS. We addressed the role of macrophages in HIV infection by first analyzing monocytes isolated from viremic patients and patients undergoing antiretroviral treatment. We were unable to find viral DNA or viral outgrowth in monocytes isolated from peripheral blood. To determine whether tissue macrophages are productively infected, we used 3 different but complementary humanized mouse models. Bone marrow/liver/thymus [BLT] mice, T cell–only mice [ToM], and myeloid-only mice [MoM] to specifically evaluate HIV replication in this population of cells and their ability to establish HIV infection in the brain. We demonstrated that macrophages could sustain HIV replication in the absence of T cells; HIV-infected macrophages are distributed in various tissues including the brain; replication-competent virus can be rescued ex vivo from infected macrophages; and infected macrophages can establish de novo infection. Together, these results demonstrate that macrophages represent a genuine target for HIV infection in vivo that can sustain and transmit infection. We then showed that HIV infection of tissue macrophages is rapidly suppressed by ART and that no viral rebound was observed in the plasma of 67% of the ART-treated animals at 7 weeks after ART interruption. Importantly, no replication-competent virus was rescued from the tissue macrophages obtained from these animals. In contrast, in a subset of animals (~33%), a delayed viral rebound was observed that is consistent with the establishment of persistent infection in tissue macrophages. These observations represent direct evidence of HIV persistence in tissue macrophages in vivo. Finally, we used BLT-hIL34 mice to establish the susceptibility of human microglia cells to HIV infection. In addition to being reconstituted with human hematopoietic cells, the brains of BLT-hIL-34 mice are also reconstituted with human microglia allowing for the direct analysis of HIV infection in these cells. Our results demonstrate the productive infection of microglia cells in the brain and that ART treatment results in suppression of HIV infection in the brains of BLT-hIL34 mice.

Session 5: Conceptual shifts to mitigate the HIV crisis
December 6th, 2022

**The HIV epidemic in Latin America: a time to reflect on the history of success and the challenges ahead.**

Alvaro López Iñiguez
HIV clinic, Infectious Diseases department. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran. Mexico city.

**Background**

In Latin America, according to UNAIDS, it is estimated that there are around 2,200,000 people with HIV infection (1,500,000-2,800,000) by 2021 and 110,000 new infections that happened that year. The pandemic caused by HIV in Latin American countries is heterogeneous and has experienced great advances, yet there are still challenges that need to be addressed and are different in each country.

There are social aspects such as stigma, discrimination, and inequity in health care that have impacted key populations' access to health promotion and sexual rights, STI and HIV prevention methods, identification and treatment of these infections, as well as barriers to access to the health system.

Meanwhile, the adoption of public policies such as "Treat-all", the implementation of national programs for free access to antiretroviral therapy, and the use of single-tablet regimens with drugs with a better tolerance profile have been elements of great progress in the care of HIV infection with an impact in the reduction of morbidity and mortality associated with HIV.

**Objective of the session**

Describe in Latin America, the epidemiological behavior of HIV infection, the cascade of care, the successes achieved in the diagnosis, treatment, and control of the infection, as well as the challenges that still lie ahead.

Session 5: Conceptual shifts to mitigate the HIV crisis
December 6th, 2022

**On the way to overcoming HIV/AIDS in Eastern Europe: challenges, scenarios and prospects**

Alexey Mazus
Moscow City Center for AIDS Prevention and Treatment of the Moscow Healthcare Department

**Goals and objectives.**

The epidemiological situation of HIV infection in the Member States of the WHO European Region is uneven. At the same time, the greatest incidence and morbidity are registered in the countries of Eastern Europe, actually determining epidemiological trends for the entire European region.
The goal of the work was to study the specifics of the spread of HIV infection in the countries of Eastern Europe and to develop general proposals on the regional features of the HIV response in these countries.

**Methods**

The HIV/AIDS surveillance in Europe 2021 – 2020 data report was analyzed. Stockholm: ECDC; 2021, including data on detected cases of HIV infection in the Member States of the WHO European Region, on the proportion of the population tested for HIV infection, on the proportion of people with low CD4+ lymphocytes among newly diagnosed patients, and on the ways of HIV transmission.

**Results and conclusions**

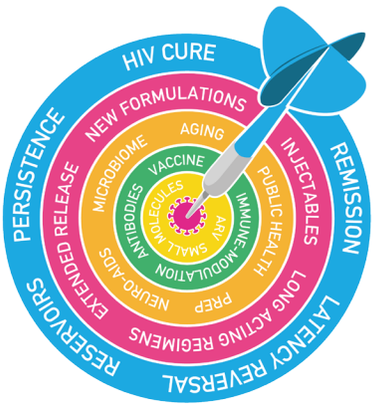
The situation of HIV infection in the Member States of the WHO European Region is heterogeneous. According to the WHO Regional Office for Europe, the entire European Region is conditionally divided into 3 parts – West, Center and East. The overwhelming number of new cases of HIV infection is registered in the East region, where the highest levels of morbidity and prevalence of HIV infection among the population occur.
At the same time, the epidemiological surveillance systems used in the countries of the region work according to different principles: in a number of countries informing about the detection of HIV infection is not mandatory, there are significant delays in the provision of complete data by countries. In this regard, when directly comparing epidemiological data by country, it is necessary to take into account, first of all, absolutely comparable parameters that allow objectively assessing the effectiveness of country measures to counter the spread of HIV infection.
The expansion of HIV testing is one of the principal areas of work supported and implemented by all countries of the region. At the same time, a comparison of available data on the coverage of the population of countries with HIV testing shows that most countries have population screening levels of about 3-8%. The highest levels of population coverage by testing were noted in some countries of the Eastern part of the region (13-25%).
A separate problem related to the effectiveness of the testing is the proportion of «late presenters». At the same time, some of the new patients are not examined at all for the level of immune status. Thus, among the Member States of the WHO European Region that provided information, only in some of them the level of coverage of new cases with CD4+ lymphocyte count testing was higher than 90%. Among the examined individuals, the proportion of patients with CD4+ lymphocyte levels below 350 cl/mcl ranged from 27.1% to 69.4%. At the same time, in the West and in the Center of the region there is a large proportion of late presenters. The smallest share of late presenters was noted in the Russian Federation, Montenegro and Cyprus.
When analyzing the structure of new cases of the disease in the countries of the region, in the East, attention is drawn to the fact that the predominant ways of HIV transmission, in contrast with the West and the Center, are intravenous drug use and heterosexual contacts.

To adequately counteract the epidemic process in Eastern Europe, it is necessary to introduce a special approach that takes into account both the epidemic situation and the cultural and social characteristics of the region.
The main directions of such a strategy should include maximum coverage of the population with HIV testing, early detection of new cases of HIV infection as well as extensive awareness-raising activities aimed at broad segments of the population in order to reduce the scale of risky and promote safer behavior.

# ORAL ABSTRACTS

HIV DART 2022

LOS CABOS, MEXICO • 4-6 DECEMBER 2022



## Abstract HO1

### Combating yellow fever virus with 2'-dihalo-uridine nucleoside analogs

Julia C. LeCher[1], Keivan Zandi[1] Vivian Vasconcelos Costa[2], Franck Amblard[1], Sijia Tao[1], Dharmeshkumar Patel[1], Sujin Lee[1], Felipe Rocha da Silva Santos[2], Matheus Rodrigues Goncalves[2], Celso Martins Queroz-Junior[2], Fernanda Martins Marim[3], Katie Musall[1], Shu Ling Goh[1], Tamara McBrayer[1], Jessica Downs-Bowen[1], Ramyani De[1], Niloufar Azadi[1], James Kohler[1], Mauro M. Teixeira[2], and Raymond F. Schinazi[1]

[1]Emory University School of Medicine, Atlanta, GA, [2]Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, 31270-901, Brazil.

**Background**: Yellow fever virus (YFV) is a deadly zoonotic flavivirus (33% mortality) transmitted to humans via mosquito vector endemic in tropical/sub-tropical Africa and South America. Following infection, macrophages serve as a viral reservoir as well as transfer virus to target organs such as the liver with over 50% of deadly cases having hepatic involvement. The WHO classifies YFV as a "high impact, high threat disease" with resurgent epidemic potential, however, there are no approved antiviral agents for YFV. This work investigated 2'-halogen-modified uridine nucleoside analogs as potential anti-YFV agents addressing the hypothesis that structural modification via isosteric replacement of functional groups on known anti-flavivirus nucleoside analogs could produce effective anti-YFV compounds.

**Methods**: A panel of ten 2'-dihalo-uridine nucleoside analogs and their respective prodrugs were screened by dose-response assay (0-10 μM) in human hepatoma cells for cytotoxicity via MTS assay and anti-YFV activity via qRT-PCR. Hits were selected and cellular metabolic profiles established by LC-MS. A 2'-fluoro,2'-bromouridine phosphoramidate prodrug (C9; Fig 1) was selected and predictive modeling performed to investigate binding of this compound with the YFV RdRp. C9 was further evaluated for anti-YFV activity in primary human macrophages and in a newly designed 3D spheroid human hepatoma model via RT-PCR as well as in the YFV murine A129 model. For mouse studies, equal numbers of male and female mice were infected with $10^6$ PFU/mL of YFV vaccine strain 17D, left uninfected, or mock infected via intravenous injection of the tail vein. At 1 hpi and every 24 hr for 2 days, 10 mg/kg of C9 was administered intraperitoneally (vehicle control group= saline). After 3 days, mice were sacrificed, and blood and liver obtained to test for serum alanine aminotransferase (ALT) levels via BioClin kinetic kit and viral burden via qRT-PCR. Histological analysis was performed on liver sections.

**Results**: From initial evaluations, 3 prodrugs exhibited sub-micromolar anti-YFV activity and low cytotoxicity. Metabolic assays revealed ready processing of these pro-drugs into active nucleotide triphosphate form in human hepatomas and primary hepatocytes. Of note, we identified a 2'-fluoro,2'-bromouridine phosphoramidate prodrug with low cytotoxicity ($CC_{50}$ 68.7 to >100 **μM in** multiple cell lines), favorable metabolism, and potent anti-YFV activity in hepatomas ($EC_{50}$ 0.9 ± 0.8; $EC_{90}$ 7.8 ± 3.6), primary macrophages ($EC_{50}$ 0.4 ± 0.1 μM; $EC_{90}$ 1.5 ± 0.6 μM) and 3D hepatoma spheroids ($EC_{50}$ 3.2 ± 1.4; $EC_{90}$ 11.7 ± 3.2). Molecular modeling predicted C9 binding to the active site of the YFV RdRp with a binding free energy of -54.11 kcal/mol. In A129 mice, C9 significantly reduced ALT levels (46% reduction, p = 0.014) and provided protection to the liver with a significant reduction in virus burden (~ 1 log reduction; p = 0.016), inflammatory infiltration (44% reduction, p = 0.0024), and steatosis (pathology score 25% reduction).

**Conclusion**: We demonstrated our 2'-fluoro,2'-bromouridine prodrug (C9) is an efficacious and effective anti-YFV agent in vitro and in vivo. Collectively, our data support the further pre-clinical development of C9 as an attractive new anti-YFV candidate.

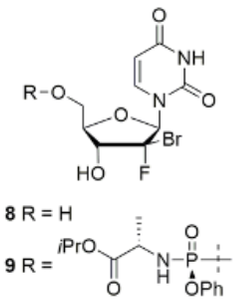



*Figure 1: Chemical structure of 2'-fluoro, 2'-bromouridine parent (8) and prodrug (9).*

## Abstract HO2

### The first-in-human clinical trial of STP0404, a novel potent HIV-1 allosteric integrase inhibitor

Xue Meng[1], Uk-Il Kim[1], Yves Donazzolo[2], Baek Kim[3,4], Kyungjin Kim[1]
[1] ST Pharm Co., Ltd., Seoul, Korea, The Republic of;
[2] Eurofins Optimed S.A.S, Grenoble, France;
[3] Emory University, School of Medicine, Department of Pediatrics, Atlanta, United States;
[4] Children's Healthcare of Atlanta, Center for Drug Discovery, Atlanta, United States

**Background**
STP0404 is a first-in-class HIV-1 allosteric integrase inhibitor (ALLINI) with a novel mechanism of action. It binds to the LEDGF/p75 binding site of integrase (IN) and inhibits viral maturation. STP0404 has shown potent *in vitro* anti-HIV-1 activities at low nanomolar level, an *in vitro* resistance profile different from those of other catalytic-site integrase inhibitors (CINIs), and favorable nonclinical safety and pharmacokinetics (PK) profiles.

**Method**
The first-in-human clinical study STP0404 was a double-blinded, placebo-controlled, randomized phase 1 trial in healthy male adult volunteers with once daily oral dose. This study is divided in 3 successive parts: Part A (Single Ascending Doses, SAD), evaluated safety and PK of STP0404 after a single dose of 200, 400, 600 and 800 mg; Part B (Multiple Ascending Doses, MAD) evaluated safety and PK of STP0404 after repeated doses of 200 and 400 mg for 10 days; Part C (Food Effect) evaluated effect of food intake on the safety and PK of STP0404 after a single dose of 200 mg.

**Results**
A total of 65 male subjects were enrolled (aged between 18 to 45 years old, inclusive). 32 for SAD, 21 for MAD and 12 for Food Effect. Two subjects withdrew consents due to personal reason.
Assessments on clinical examination, laboratory tests, vital signs and 12-ECGs were performed at both pre- and post-dose schedules, and found no clinically significant trends or abnormalities throughout the trial. 26 Adverse Events emerged post dosing and were mild (19) or moderate (7). There were no severe AE, SAE or withdrawn due to AE during the study, hence the maximum tolerated dose was not reached.
STP0404 demonstrated a less-proportional PK profile over the tested dose range except for 800 mg in SAD study. $C_{max}$ were reached at around 4.5 hours, and the elimination $t_{1/2}$ were ranged from 19 to 29 hours throughout the trial. Food-intake before dosing notably increased drug exposure and didn't affect $t_{1/2}$. After 10-day repeated dosing, steady state was reached between Day 3 and Day 6. Slight accumulation was observed for both 200 mg and 400 mg dose groups. The average $C_{trough}$ was 1.58 µg/mL for 200 mg dose group that gave out a minimum 700-fold therapeutic range when compare to the in vitro efficacy concentration.

**Conclusion**
STP0404 was well tolerated in this study and its PK profile indicated a once-daily regimen at a minimum dose of 200 mg given after meal is expected to achieve therapeutic concentration. A Phase 2 clinical trial of STP0404 is planned to start at 4Q, 2022.

## Abstract HO3

### GRL142 binds to and impairs HIV-1-Integrase-Nuclear localizing signal and exerts potent activity against INSTI-Resistant HIV-1

Manabu Aoki[1,2,3], Hiromi Aoki-Ogata[1,3], Haydar Bulut[1], Hironori Hayashi[4], Arun K. Ghosh[5], Alice K. Pau[6], and Hiroaki Mitsuya[1,3,7]

[1]National Cancer Institute, NIH, Bethesda, MD, USA; [2]Kumamoto Health Science University, Kumamoto, Japan; [3]National Center for Global Health & Medicine Research Institute, Tokyo, Japan; [4]Tohoku University Graduate School of Medicine, Miyagi, Japan; [5]Purdue University, West Lafayette, IN, USA; [6]National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD, USA; [11]Kumamoto University Hospital, Kumamoto, Japan

**Background**
Dolutegravir (DTG), one of the most used integrase strand transfer inhibitors (INSTIs), is currently recommended as first-line therapy in industrialized and resource-limited countries and is also often used as a part of salvage regimens. However, antiretroviral resistance to INSTIs often develops under suboptimal therapy with several characteristic mutation pathways. Development of agents with new HIV-1 inhibition modality is required.

**Methods**
We established a multi-class-drug-resistant HIV-1 variant ($HIV_{KGD}$) by consecutively exposing an HIV-1 variant with various antiretroviral agents including IN-strand-transfer-inhibitors (INSTI). A novel compound, GRL142, has an extremely potent HIV-1 protease inhibitor, which blocks the enzymatic activity and the dimerization process of HIV-1 protease. Virological, pharmacological and structural features of GRL142 in association with $HIV_{KGD}$ were determined.

**Results**
GRL142 exerted extremely potent antiviral activity against $HIV_{KGD}$, with $IC_{50}$ of 130 femtomolar. Amino acid substitutions in $HIV_{KGD}$'s IN-gene were associated with $HIV_{KGD}$'s extreme susceptibility to GRL142. When cells were infected with $HIV_{KGD}$'s IN-gene-containing recombinant-HIV and cultured in the presence of GRL142, a significant decrease of unintegrated 2-LTR-circular DNA was observed, suggesting that nuclear import of pre-integration complex (PIC) was severely compromised. X-ray crystallographic analyses revealed that GRL142 binds to and covers the critical sequence (DQAEHLK) of HIV-1-integrase-nuclear localizing signal (NLS) and was assumed to sterically block the transport of GRL-142-bound $HIV_{KGD}$'s PIC into the nucleus. INSTI-resistant variants isolated from patients with AIDS, who were heavily INSTI-experienced and had developed resistance to RAL and/or DTG, were susceptible to GRL142, suggesting that NLS-targeting agents could serve as agents for salvage therapy for highly INSTI-resistant-variant-harboring individuals.

**Conclusions**
GRL142's extremely potent activity against multi-drug-resistant HIV-1 variant ($HIV_{KGD}$ and other variants with RAL- and/or DTG-resistance) was associated three inhibition modalities (i) proteases' enzyme inhibition, (ii) protease's dimerization inhibition, and (iii) impairment of HIV-1-NLS. Further design and optimization based on GRL142 to potently block both IN and PR might lead to the development of promising treatment regimens with highly effective antiretroviral agents. The present study also sheds light on the design of inhibitors of other NLS-carrying viruses.

## Abstract HO4

### Identification of HLA-E-binding HIV-1 and HIV-2 Env-derived peptides and cytokine modulation of NKG2A/C$^+$ NK and NKG2A/C$^+$ CD8$^+$ T cells

Aurelio Orta Reséndiz[1], Caroline Petitdemange[1], Béatrice Jacquelin[1], Quentin Le Hingrat[2], Charlotte Charpentier[2], Florence Damond[2], Diane Descamps[2], Nicolas Huot[1], Michaela Müller-Trutwin[1]*, ANRS CO5 HIV-2 cohort study group

[1] Institut Pasteur, Université Paris Cité, HIV, Inflammation and Persistence Unit, F-75015 Paris, France

[2] Hôpital Bichat –Claude-Bernard, AP-HP Nord Université Paris Cité, INSERM UMR 1137 IAME, Paris, France

**Background**
HLA-E binds self-peptides derived from the leader-sequence of HLA-I. The peptide-binding stabilizes HLA-E at the cell surface. HLA-E can also bind viral peptides, i.e. from CMV and HIV-1 Gag. The HLA-E/peptide complex is recognized by NKG2A/C. NKG2A/C is expressed on unconventional CD8$^+$T and NK cells. Mature NKG2C$^+$ NK cells are associated with lower viremia levels in HIV primary infection. Mature highly cytotoxic NK cells also play an important role in the control of virus replication in lymph nodes during during $SIV_{agm}$ infection in African green monkeys. This control has been associated with IL-15 and IL-21. We have also shown that NKG2A/C$^+$CD8$^+$ T cells increase during $SIV_{agm}$ infection as well as in $SIV_{mac}$ controllers. Our objective was to identify HIV ENV-coding peptides that can bind to HLA-E and to test combinations of cytokines on the activity of NKG2A/C$^+$ NK and CD8$^+$ T cells.

**Methods**
We screened the leader sequence of HIV-1 and HIV-2 ENV for the presence of nonamers with the HLA-E canonical binding motif using a neural networks-based prediction method (NetMHCpan 4.1). The leader sequences were derived from HIV reference strains of public databases and sequences obtained from HIV viruses in blood of participants in the ANRS-CO5 cohort. The 9-mer candidates were tested for their binding capacity through analysis of HLA-E cell surface stabilization in vitro by flow-cytometry using an in-house assay with K562 cells expressing only HLA-E. Furthermore, we investigated the expression of GranzymeB, CD107a, IFN-g, TNF-α and Granulysin in NKG2A/C$^+$ CD8$^+$ T and NK cells before and after stimulation with IL-2 alone or in combination with IL-15, IL-12+IL-18, IL-21 and IL-23. Using a standardized assay with K562 target cells and polyclonal activation, we assessed the effector function of the NKG2A/C$^+$ CD8$^+$T and NK cells from HIV-negative human donors before and after cytokine stimulation.

**Results**
*In silico* analyses identified sixteen HIV-1/2 ENV-derived peptide candidates, from nine of which stabilized HLA-E at the cell surface. Combination of IL-2 with other cytokines enhanced GranzymeB production in NKG2A/C$^+$CD8$^+$T cells and increased their degranulation activity in response to target cells. The degranulation levels (CD107a) as well as the expression of IFN-g and TNF-α from NKG2A$^+$/C$^-$ and NKG2A$^-$/C$^+$ CD8$^+$ T cells were stimulated more strongly than that of NKG2A$^-$/C$^-$ CD8$^+$T cells. NKG2A$^+$/C$^-$ CD8$^+$ T cell degranulation activity against target cells were mostly stimulated by IL-15 and that of NKG2A$^-$/C$^+$ CD8$^+$ T cells by IL-21. NKG2A/C$^+$ NK cells were very sensitive to cytokines and increased their degranulation activity in particular in response to IFN-α.

**Conclusions**
This study describes for the first time HIV-2 ENV peptides binding to HLA-E. Env peptides might modulate the HLA-E-mediated response of NKG2A/C$^+$ CD8$^+$T and NK cells. NKG2A/C$^+$CD8$^+$ T cells are polyfunctional cells in human donors that could play a role in HIV acute and/or chronic phases through innate and adaptive responses. Cytokine combinations were strong inducers of human NKG2A/C$^+$ NK and CD8$^+$ T cell degranulation and immunomodulatory activities in response to target cells *in vitro*. These results highlight the importance of investigating NKG2A/C$^+$ NK and CD8$^+$ T-cell associated responses in HIV immunotherapy and adjuvant studies.

## Abstract HO5

### The pDC/IFN-I inflammatory pathway in HIV-1 pathogenesis and metabolic dysregulation

Guangming Li[1,2], Liang Cheng[2], Haitao Guo[2], Qi Wang[2], James Ahodantin[1,2], Yu Lin[1], Jenny Ting[2], and Lishan Su[1,2]

[1]Institute of Human Virology, Department of Pharmacology, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201; [2]Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Plasmacytoid dendritic cells (pDC) are the major type I interferon (IFN-I) producing cells and play important roles in antiviral immune responses during acute virus infection. However, sustained pDC activation and IFN-I induction has been correlated with disease progression in chronic virus infection. We have recently functionally defined the HIV-pDC-IFN axis in HIV-1 immuno-pathogenesis and studied the mechanisms of pDC/IFN-induced immune suppression, and its role in HIV reservoir persistence. We further show that low levels of pDC/IFN-I signaling contribute to the immune dysfunction and foster HIV-1 persistence in cART-treated hosts. Transient depletion of pDC or blocking IFNAR during suppressive ART has been shown to enhance immune recovery and to control HIV-1 reservoirs. Mechanistically, persistent HIV-1 infection and IFN-I signaling lead to distinct metabolic alterations of CD4 and CD8 T cells. Modulation of OXPHOS and glycolysis pathways can suppress HIV-1 replication and immune-pathogenesis in humanized mice or in human PBMC from HIV-infected patients. Our findings thus functionally reveal the role of pDC/IFN-I in HIV-induced inflammatory diseases. The pDC/IFN-I inflammation and reprogramed glycolysis pathways will also be targeted with novel therapeutics to i) resolve inflammation-associated diseases in HIV-infected hosts under ART, ii) recover anti-HIV immunity and iii) reduce or control HIV-1 reservoirs.

## Abstract HO6

### Selection of HIV-1 for resistance to fifth generation protease inhibitors reveals two independent pathways to high-level resistance

Ean Spielvogel[1,2], Sook-Kyung Lee[2], Shuntai Zhou[2], Gordon J. Lockbaum[3+], Mina Henes[3#], Amy Sondegroth[1,2], Klajdi Kosovrasti[3], Ellen A. Nalivaika[3], Akbar Ali[3], Nese Kurt Yilmaz[3], Celia A. Schiffer[3*], Ronald Swanstrom[2,4,*]

[1]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA; [2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA; [3]Department of Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, MA 01605, USA; [4]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA

**Background**
Antiviral drugs can be evaluated for their potency (the ability to inhibit viral replication) and the size of the genetic barrier to resistance (the number of mutations that must evolve to overcome the potency of the drug). HIV-1 protease inhibitors are unique among anti-HIV-1 drugs in that they have very high potency and they also have a very high genetic barrier to resistance. The current most potent protease inhibitor is darunavir (DRV), which also reaches levels in the blood that are nearly 1000-fold higher than needed to inhibit virus in cell culture.

**Methods**
We have examined the pathway to resistance to DRV in cell culture and to a series of 10 chemical variants of DRV (UMASS 1-10). Resistance was achieved by passaging HIV-1 in the presence of escalating drug concentration until virus replication occurred in the presence of drug concentrations 1000-fold or more above the EC50. Deep sequencing was used to follow the longitudinal evolution in each culture. Viral sequences, the selected virus pools, and the encoded viral proteases were all characterized for fitness and resistance. In addition, selected inhibitors were examined by X-ray crystallography.

**Results**
We selected for resistance to high drug concentrations against ten PIs and their structural precursor DRV. Mutations accumulated through two pathways (anchored by protease mutations I50V or I84V). Small chemical changes in the inhibitor P1' side chain led to preferential use of one pathway over the other, with each pathway having unique and shared compensatory mutation and with the I50V pathway being less fit. Changes in the inhibitor P2' side chain determined differences in potency that were retained in the resistant viruses. The difference in residual potency with the P2' side chain correlated with the ability to form a main chain interaction with the protease rather than through water molecules. Viral variants from the two pathways showed differential selection of compensatory mutations in Gag cleavage sites.

**Conclusions**
These results show that small chemical changes to DRV can drive the resistance pathway to a less fit starting mutation. In addition, the ability to create a main chain interaction avoided specific mutation escape and resulted in residual potency against the resistant virus. These compounds should be considered in therapeutic strategies designed to reduce drug burden.

## Abstract HO7

### Development of an immunomodulatory AAV viral vector to minimizing adaptive immune responses to AAV-delivered broadly neutralizing antibodies

[1]James M. Termini, [1]Sebastian P. Fuchs, [1]José M. Martinez-Navio, [2]Eva G. Rakasz, [1]Desiree E. Mendes, [1]Claudia P Muniz, [1]Ronald C. Desrosiers

[1]Department of Pathology, Miller School of Medicine, University of Miami, Miami, Florida, USA
[2] Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, USA

**Background**
Given the vast assortment of human monoclonal antibodies with potent broadly neutralizing activity, one can envision long-term delivery of a combination of neutralizing antibodies to achieve sterilizing immunity against the majority of circulating HIV strains. Adeno-associated virus (AAV) vectors are ideally suited for such long-term delivery and have proven safe in numerous human trials. Unfortunately, in many preclinical and clinical studies, host immune responses to the delivered antibody have severely limited the efficacy and long-term expression of the antibody. The TLR9-MyD88-type I interferon pathway has been identified as playing an important role in initiating adaptive immune responses against both AAV and the delivered IgG.

**Methods**
In order to dampen the crippling effects of anti-antibody humoral response as well as potential transgene-specific CTL responses, we developed two novel immunomodulatory AAV vectors (IM-AAV). shRNA constructs capable of knocking down the expression of TLR9/MyD88 and IFN-alpha/IFN-beta were designed and cloned into recombinant AAV vectors encoding the broadly neutralizing antibody 3BNC117. Due to the packaging limitations of the AAV vector, two vectors were cloned: one targeting TLR9/MyD88 and one targeting IFN-alpha/IFN-beta. Knockdown of TLR9, MyD88, IFN-alpha, and IFN-beta was validated by real-time PCR following transfection and IM-AAV transduction. shRNA constructs were also validated in HEK-Blue hTLR9 reporter cells following CpG stimulation and AAV transduction. IM-AAV vectors were validated in a 9-macaque trial: 4 control macaques received wildtype AAV9-3BNC117 and 5 macaques received our two immunomodulatory AAV vectors pooled.

**Results**
shRNA constructs were capable of knocking down expression levels of both TLR9 and MyD88 by ~50-60% following transfection into HEK-Blue hTLR9 cells. A549 cells stably expressing our IFN-alpha/IFN-beta shRNA constructs exhibited a similar ~60% decrease in IFN-alpha and IFN-beta mRNA levels following stimulation. When tested in a HEK-Blue TLR9 signaling assay, TLR9/MyD88 construct #13 was capable of reducing TLR9 signaling (measured by SEAP secretion) by 2.8-fold following CpG stimulation and 2.5-fold following AAV transduction. When macaques were transduced with IM-AAV9-3BNC117 vectors, peak antibody levels were significantly lower than control animals which received wildtype AAV9-3BNC117: 1-3ug/ml vs 18-30ug/ml, respectively. Interestingly, macaques that received IM-AAV exhibited significantly decreased levels of anti-3BNC117 antibody responses. And although 3BNC117 serum levels dropped to undetectable for all animals around 30 days post-AAV-inoculation, 3 of the 5 IM-AAV animals recovered low levels of 3BNC117 expression starting at day 78 which continued until the end of the study. 3BNC117 serum levels remained undetectable for the control animals.

**Conclusions**
Although IM-AAV vectors performed well *in vitro,* demonstrating the ability to knock down TLR9/MyD88 and IFN-alpha/IFN-beta, as well as reducing CpG and AAV-induced SEAP secretion in a TLR9 reporter cell line, expression levels of delivered 3BNC117 *in vivo* were disappointing. Although no difference in 3BNC117 antibody production can be observed following plasmid transfection of AAV-3BNC117 and IM-AAV-3BNC117 vectors *in vitro*, IM-AAV-3BNC117 demonstrated a ~10-fold decrease in 3BNC117 serum concentrations in macaques. We are currently investigating the exact cause of these decreased levels. It is important to note that a significant decrease in anti-3BNC117 antibody levels was observed in the IM-AAV group as well as a low-level rebound in 3BNC117 serum concentration 78 days post-AAV inoculation. As rebound of AAV-delivered bNAbs is rarely seen once anti-antibody responses are established, the fact that 3 of 5 macaques demonstrated rebound is encouraging. We hope in the future that targeting the TLR9-Type I interferon pathway can enhance the long-term expression of AAV-delivered bNAbs.

## Abstract HO8

### Therapeutic neutralizing monoclonal antibody administration protects against lethal yellow fever infection

Lauren Rust[1], Michael J. Ricciardi[2,3], Nuria Pedreño-Lopez[3,4], Sofiya Yusova[1], Sreya Biswas[1], Miranda Fischer[5], **Gabriela M. Webb**[1], Lucas Gonzalez-Nieto[6], Thomas B. Voigt[2,3], Johan J. Louw[3], Fernanda D. Laurino[2], John R. DiBello[2], Hans-Peter Raué[5], Lidiane M.S. Raphael[7], Aaron Yrizarry-Medina[3], Brandon C. Rosen[6], Rebecca Agnor[5], Lina Gao[5], Caralyn Labriola[1,5], Michael Axthelm[5], Jeremy Smedley[5], Justin G. Julander[8], Myrna C. Bonaldo[7], Laura M. Walker[9], Ilhem Messaoudi[10], Mark K. Slifka[5], Dennis R. Burton[11], Esper G. Kallas[2,12], Jonah B. Sacha[1,2], David I. Watkins[2,3] and Benjamin J. Burwitz[1,5]

[1]Vaccine and Gene Therapy Institute, Oregon Health and Science University, [2]Mabloc, LLC, [3]George Washington University, [4]Irsicaixa Institut De Recerca De La Sida, [5]Oregon National Primate Research Center, Oregon Health and Science University, [6]University of Miami, Miller School of Medicine, [7]Laboratório De Biologia Molecular De Flavivírus, Instituto Oswaldo Cruz, [8]Institute for Antiviral Research, Utah State University, [9]Adagio Therapeutics, Inc., [10]Microbiology, Immunology, and Molecular Genetics, University of Kentucky, [11]Immunology and Microbiology, The Scripps Research Institute, [12]Infectious and Parasitic Diseases, University of Sao Paulo

**Background**
Despite availability of a Yellow Fever virus (YFV) vaccine, supply deficits, vaccine hesitancy, and waning immunity have resulted in significant number of at-risk individuals as evidenced by recent outbreaks in South America. Thus, there is an urgent need for YFV treatment strategies. Here, we explored two potent YFV-specific neutralizing monoclonal antibodies (mAb) as a treatment strategy in highly pathogenic YFV rodent and non-human primate models.

**Methods**
We identified two YFV-specific mAbs, MBL-YFV-01 and MBL-YFV-02, that neutralize pathogenic lab strains and primary isolates of YFV isolates *in vitro*. Efficacy of mAb administration was tested *in vivo* in both Syrian golden hamsters (SGH) and rhesus macaques (RM). YFV-infected SGH received a single dose of either YFV-specific mAb or control mAb (20mg/kg) three days post-infection. RM were also administered a single dose of either YFV-specific mAb (50mg/kg) two days post-infection or left untreated. Disease severity was assessed in all animals measured by elevated serum alanine transaminase (ALT) levels as well as viral loads.

**Results**
Treatment of YFV-infected SGH with either MBL-YFV-01 or MBL-YFV-02 significantly increased survival as compared to animals that received isotype control mAb. In RMs, all mAb-treated animals survived through 21 days post-infection. Notably, RM treated with either mAb showed no clinical signs of severe disease, maintained low serum ALT levels and had no detectable YFV in serum. In contrast, control animals exhibited viral loads above $1x10^{11}$ copies/mL and high serum ALT levels, and met clinical endpoints requiring humane euthanasia at day 5 post-infection.

**Conclusion**
Our data demonstrate that a single dose of a YFV-specific mAb delivered within days of YFV infection was sufficient to prevent severe disease and death in a relevant, preclinical, non-human primate model of pathogenic YFV infection. These results provide strong rationale for expedited clinical development of this therapeutic intervention.

## Abstract HO9

### Long-term ART-free SIV remission following allogeneic hematopoietic cell transplantation in Mauritian cynomolgus macaques

Wu H[1], Weber W[1], Waytashek C[1], Boyle C[1], Bateman K[1], Reed J[1], Hwang J[1], Shriver-Munsch C[1], Swanson T[1], Northrup M[1], Armantrout K[1], Price H[1], Robertson-LeVay M[1], Uttke S[1], Kumar M[2], Fray E[2], Junell S[1], Moats C[1], Bochart R[1], Sciurba J[1], Bimber B[1], Sullivan M[1], Dozier B[1], MacAllister R[1], Hobbs T[1], Martin L[1], Siliciano R[2], Siliciano J[2], Smedley J[1], Axthelm M[1], Meyers G[1], Maziarz R[1], Burwitz B[1], Stanton J[1], Sacha J[1]
[1]Oregon Health and Science University, Portland, Oregon, USA,
[2]Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background**
Two patients achieved ART-free HIV remission following allogeneic hematopoietic cell transplant (HCT) from CCR5-deficient donors, but the mechanisms responsible remain unknown. Here, we examined the impact of CCR5-wildtype allogeneic HCT on the SIV reservoir in ART-suppressed Mauritian cynomolgus macaque recipients.

**Methods**
Grafts collected from MHC-matched, SIV-naïve donors were transplanted into four SIV+, ARTsuppressed recipients following reduced intensity conditioning (RIC). Donor chimerism was measured by sequencing SNPs. Total and intact SIV DNA levels were measured by SIVgag qPCR and intact proviral DNA assays, respectively. Recipients were maintained on ART until analytic treatment interruption (ATI) at 700-900 days post-HCT.

**Results**
Following RIC and HCT, lymph node CD4+ T cell-associated SIV DNA levels decreased ~10-fold within 30 days in all four recipients. Recipient 1 reached 100% donor chimerism without further intervention. Recipients 2, 3, and 4 presented with mixed T cell chimerism and received donor lymphocyte infusions (DLIs), which successfully induced full donor chimerism in recipients 3 and 4, but not recipient 2. Graftversus-host disease (GVHD) manifested in recipients 3 and 4, but not recipients 1 or 2. In all recipients and across tissue reservoirs, we observed a strong inverse correlation between cell-associated SIV DNA and donor chimerism in CD4+ T cells. Prior to ATI, intact SIV was undetectable in blood, lymph node, spleen, and bone marrow in GVHD-experiencing recipients 3 and 4, but persisted in recipients 1 and 2. Upon ATI, recipient 2 rebounded with SIV viremia within 11 days while recipients 1, 3, and 4 remained aviremic for >17 weeks. Recipient 1 experienced SIV rebound at week 18, while GVHD-experiencing recipients 3 and 4 remain in SIV remission >2 years post-ATI despite CD8-depletion. SIV Env-binding antibody titers fell below 15 ug/mL in all four recipients post-HCT, increased above 100 ug/mL in recipients 1 and 2 upon rebound of SIV plasma viremia post-ATI, but remain below 15 ug/mL in aviremic recipients 3 and 4 post-ATI.

**Conclusions**
These data demonstrate that alloHCT-mediated viral reservoir clearance occurs concomitantly with full CD4+ T cell donor chimerism and is associated with GVHD, suggesting that allogeneic immunity drives viral reservoir clearance in alloHCT-mediated HIV cure.

## Abstract HO10

**HIV-1 Infection potentiates Alzheimer's disease pathology in a novel humanized APP knock-in mouse**

Pravin Yeapuri[1], Jatin Machhi[1], Yaman Lu[1], Rana Kadry[1], Mai Mostafa[1], Prasanta Dash[1], Krista L Namminga[1], Emiko M Waight[1], Chen Zhang[1], Shaurav Bhattarai[1], Eugene Lu[1], Santhi Gorantla[1], R. Lee Mosley[1], Howard E. Gendelman[1]

[1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198, USA

**Background**

Antiretroviral therapy (ART) has increased the life spans of people living with HIV (PLWH). Those over 50 years of age represent the fastest growing PLWH population. Evidence suggests that the incidence and progression of Alzheimer's disease (AD) is increased in PLWH, however, no reliable animal models are available to study relationships between virus and AD progression. The interplay between HIV infection, CD4+ T cell subset numbers and function, innate microglial immunity, ART, and the role of each in neurodegenerative disease are poorly understood. While transgenic mouse models of human mutations associated with AD exist, these permit only the study of overexpression of AD mutant genes and murine immune interactions, but preclude the putative additive effects of human immunodeficiency virus (HIV) infection due to species restriction of productive infection. Moreover, arguments have been presented that expression and involvement of endogenous murine homologs to those mutatnt genes may confound AD pathological processes in those models. To address this problem, we created a "third generation" AD mouse with human $APP^{KM670}$,671NL, $PS1^{M146V}$, or $MAPT^{P301S}$ mutations that are knocked-in (KI) using CRISPR technology that eliminates expression of endogenous murine homologs. These mice are crossed with immunodeficient NOD/Shi-scid/IL-2R$\gamma^{null}$Tg(CMV-IL34) (NOG/hIL34) mice that are permissive for 1) adaptive and innate immune reconstitution, 2) development of human microglia in the brain, and 3) productive HIV infection. Thus these mice provide a novel platform from which to study the effects of HIV infection on AD pathogenesis and progression.

**Methods**

NOD/IL34 mice were reconstituted with human hematopoietic stem cells (HSCs) from cord blood to produce humanized APP-KI mice with functional human adaptive and innate immune systems. Three-month-old, immune-reconstituted APP-KI mice were infected with the macrophage-tropic $HIV\text{-}1_{ADA}$. Four weeks after infection, plasma viral load confirmed productive HIV infection, the mice were sacrificed, and evaluated for HIV infection and AD associated pathology.

**Results**

Expression of HIV-1gag and p24 in microglia and spleen cells confirmed viral infection in brain and in the peripheral immune system, respectively. Notably, amyloid burden from $HIV\text{-}1_{ADA}$ infected mice was increased compared to uninfected controls as determined by amyloid beta peptide 1-42 ($A\beta_{42}$) ELISA. The co-localization of Aβ fibrils (monoclonal antibody clone OC immunoreactive, $OC^{+}$) and human HLA-$DR^{+}$ microglia was observed by immunofluorescence (IF) staining.

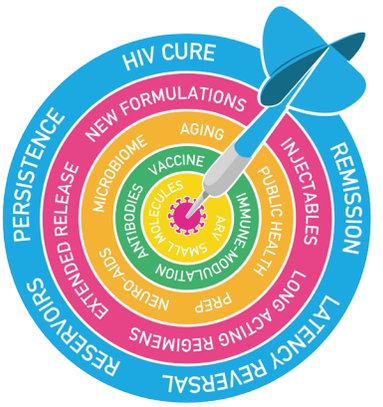
**Conclusions**

These results highlight the biological features of this novel model of Alzheimer's disease and the effects of HIV infection on AD pathology. Continued evaluation of immune and biochemical mechanisms will assess convergence of acute and chronic HIV infection, combined antiretroviral therapy (cART), and pathobiological neurodegenerative processes that potentiate AD progression in PLWH.

# POSTER ABSTRACTS

HIV DART 2022

LOS CABOS, MEXICO • 4-6 DECEMBER 2022



## Poster Abstract HP1

### Modelling therapeutic vaccination targeting the functions of HIV-1 accessory protein, Nef

Sateesh Apte[1], David Miller[2], Roscoe Moore[3]

[1] Immgenuity, Inc. Dover, Delaware, [2] Immgenuity, Inc. Dover, Delaware, [3] Former Assistant US Surgeon General

**Background**
Epidemiologically significant increases in drug resistance demands that new antiretroviral (ARV) targets be sought against HIV-1 accessory proteins essential for viral proliferation and immune escape. Because life spans of patient populations have substantially increased, studies have shown that chronic antiretroviral therapy gives inadequate protection from HIV associated neurological impairment. It is estimated that 57% of HIV patients on ARVs and 100% of the patients not on ARVs develop Central Nervous System (CNS) inflammation and HIV Associated Neurocognitive Disorder (HAND) as well as 5-10% who develop HIV Associated Dementia (HAD.) Because of the partial ineffectiveness of ARVs and a rise of drug resistant strains, an urgent need exists for the discovery and development of new therapeutic approaches that can address neurological manifestations of HIV infection.

**Methods**
We reviewed the literature focusing on HIV-1 Nef, a viral accessory protein essential for HIV pathogenesis and responsible for disrupting immune signaling.

**Findings**
A review of studies demonstrates immune escape, immune disruption and increased viral pathogenicity attributed to Nef. A large body of research strongly identifies Nef in HIV-1 immune signaling disruption, immune escape and AIDS progression. HIV-1 Nef is a small, polymorphic protein expressed early in the viral life cycle. Nef is shown to attach to cellular membranes and functioning through protein-protein interactions with a range of host cell proteins. These interactions result in downregulation of cell-surface MHC-I and viral receptors (CD4/CXCR4/CCR5), remodeling of the actin cytoskeleton, and disruption of host cell signaling pathways. These functions of Nef allow HIV-infected cells to avoid immune surveillance by the host, making Nef a primary determinant of viral pathogenesis. Nef reroutes a variety of cell surface proteins to disrupt the host's adaptive and in turn, innate immunity, promoting viral replication and immune escape. Addressing this role of Nef in blocking immune signaling creates a target for therapeutic intervention which has the potential to re-enable immune detection and clearance of reservoirs. A pathophysiological understanding of Nef's ability to command protein trafficking and its hijacking of the immune signaling has started to coalesce. Evidence has established that most HIV non-progressors and elite controllers of HIV are infected with a *nef* deleted HIV.

**Conclusions**
Using a decades long scientific approach of a live attenuated vaccine and the findings above creates a strong case for a trial of a *nef* deleted, live attenuated therapeutic vaccine. This is likely to restore immune signaling and establish control by □□cytotoxic T cells. This subsequent modulation is very likely to decrease levels of circulating inflammatory cytokines by favoring the establishment of a viable immune response to HIV infection and be an emerging therapeutic model to reduce inflammation in the CNS and progression of HAND. Facilitating further research by publicly sponsored research networks of a live attenuated *nef* deleted HIV therapeutic vaccine holds the promise to advance critical research in instituting immune signaling and establishing host control of reservoirs. This would allow for a potential remission.

## Poster Abstract HP2

### HIV-1 productively infects and replicates in human lung-like macrophages

Sujin Lee[1], Vincent D Giacalone[2], Tamara McBrayer[1], Jessica Downs-Bowen[1], Nina Azadi[1], Milagros Aldeco[2], Ramyany De[1], Rabindra Tirouvanziam[2], and Raymond F Schinazi[1]

[1] Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, and [2]Division of Pulmonary, Asthma, Cystic Fibrosis & Sleep, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA

**Background**

Macrophages are susceptible to HIV infection and contribute to the establishment of viral reservoir in various tissues and organs. Macrophage phenotypes and functions differ starkly between tissues and organs, as exemplified by lung alveolar macrophages and brain microglia. Since the most widely used *in vitro* models of HIV-1 macrophage infection are based on blood-derived monocyte-derived macrophage (MDM), we attempted to re-evaluate HIV-1 macrophage infection utilizing more tissue-relevant, organotypic models. Compared to blood brain barrier models, lung models have received less attention in the HIV field. Thus, we developed a novel model to obtain human lung-like macrophages by inducing transmigration (TM) of primary human blood monocytes through a well-differentiated airway epithelium mimicking *in vivo* recruitment into the lungs.

**Methods**

Purified $CD14^+$ monocytes from peripheral blood mononuclear (PBM) cells were infected with $HIV_{BaL}$ after in vitro differentiation into macrophages using two methods (Table 1): (a) in conventional MDM culture conditions, in the presence or absence of granulocyte-macrophage colony-stimulating factor (GM-CSF, #1 and 2), and (b) by TM through airway epithelium in the presence or absence of GM-CSF (#3 and 4). Next, we investigated cell morphology, and compared HIV infectivity from cells by qRT-PCR and from supernatants by gag p24 ELISA. Cell phenotypes and secreted immune mediators were determined by flow cytometry and multiplexed protein array (Mesoscale), respectively.

**Table 1. Macrophage differentiation conditions tested in this study**

| # | *A. Initial cell type* | *B. GM-CSF* | *C. Transmigration (TM)* | *D. Model* |
|---|---|---|---|---|
| 1 | Monocyte | - | - | Monocyte derived macrophage (MDM) |
| 2 | Monocyte | + | - | GM-CSF Monocyte derived macrophage (MDM) |
| 3 | Monocyte | - | + | TM Monocyte derived macrophage (TM MDM) |
| 4 | Monocyte | + | + | GM-CSF TM Monocyte derived macrophage (TM MDM) |

**Results**

Infection with HIV-1 of human lung-like macrophages obtained after TM through airway epithelial cells (#3 and 4) was successful. Infectivity in TM-generated lung-like macrophages was very similar to cells obtained via the conventional MDM model (#1 and 2). Interestingly, infectivity was significantly increased in cells differentiated in the presence of GM-CSF (#2 and 4) compared to those without (#1 and 3). Next, we observed more stretched and elongated morpholvogy in GM-CSF treated macrophages obtained after TM (#4) than those obtained in MDM conditions (#2). Moreover, flow cytometry confirmed expression of the chemokine coreceptors for HIV entry, CXCR4 and CCR5, in > 60 % of both macrophages differentiated in TM and MDM conditions. Finally, while conventional MDMs produced high levels of IL-1b and TNF-a, human lung-like macrophages generated by TM produced high levels of vascular endothelial growth factor **(**VEGF).

**Conclusions**

Lung macrophages are susceptible to HIV infection due to the expression of CXCR4 and CCR5 and thus should also be targeted in comprehensive manner to achieve HIV eradication. We determined for the first time that human lung-like macrophages produced via TM in vitro are susceptible to HIV-1 infection and differ in their cytokine secretion profile from MDMs. Since HIV productively replicates in our novel *in vitro* model, these human lung-like macrophages may be used to develop strategies to prevent infection and eliminate HIV-1 in the lungs. We also anticipate that this novel model will facilitate to understand the contribution of lung macrophages to HIV pathogenesis and serve as a powerful tool to reveal details of macrophage infection and its outcome in the lungs and other organs.

## Poster Abstract HP3

### Interest of HIV-1 Next-Generation Sequencing in a diagnosis laboratory

Justine Hamel[1], Caroline Deguet[1], Sofiane Mohamed[2], Justine Adnet[1], Amira Doudou[2], Rezak Drali[2], Matthieu Barralon[3], Chalom Sayada[3], Astrid Vabret[1,4], Julia Dina[1,4]
[1] Virology Department, University Hospital of Caen ; [2]Advanced Biological Laboratories Diagnostics (ABL), France; [3]ABL SA, Luxembourg ; [4]CHU Caen, France ; [4]INSERM U1311 DYNAMICURE

**Background**
The identification of the HIV subtype and the detection of resistance mutations are routinely sought in infected patients, before starting treatment as well as in the context of treatment modifications for escape or switch. The scientific mobilization due to the emergence of SARS-CoV-2 has brought the rapid development and access of many laboratories to NGS sequencing solutions. In the context of the HIV activity, Sanger sequencing methods are preferentially used routinely in France. The objective of this study consists in the analysis of the interest of HIV sequencing, targeted , protease (PR), reverse transcriptase (RT), integrase (INT) and V3 loop or of the complete genome at the virology department of the University Hospital of Caen.

**Methods**
A total of 94 samples, plasma or whole blood, were included to be analysed after nucleic acids extraction using EZ1 Qiagen automate. Viral loads were quantified before sequencing by Aptima HIV-1 Hologic kit for RNA and by Generic HIV DNA Cell [RUO] Biocentric for DNA. The inclusions were carried out prospectively from 05/01/2021 to 03/31/2022. Samples with low viral load (VL), <1000 cp/ml were concentrated using the Amicon Ultra 0.5 kit. Three HIV-1 genomic targets were amplified, RT-PCR and Nested, using the CEIVD DeepChek® Assays PR, RT and INT regions (Advanced Biological Laboratories, ABL) and were sequenced using both, the Sanger method and NGS. Libraries were prepared using DeepChek® Assays HIV-1 Library prep (ABL) kit and analysed on the iSeq100 Illumina genetic Analyser. NGS consensus (20%) sequences were compared to those obtained by Sanger sequencing. The whole genome HIV-1 was analysed in ten patients under HAART. The genome amplification was fulfilled using the DeepChek® Assays Whole Genome HIV-1 (ABL). DeepChek® and Viroscore HIV-1, target specific, RT/PR/INT, and Whole Genome software from ABL were used to analyse the FastQ files sequences obtained with Illumina iSeq100 and ABI files from Sanger sequencing.

**Results**
Of the 94 samples, 78 were plasmas and 16 were whole bloods. RNA VL were between 100 and 4 656 805 copies/ml (range 2 to 6.6 log) and DNA VLs between 50 and 3227 copies/million cells (range 1.7 to 3.5 log). Among the 78 plasmas, 68 samples were analyzed by NGS for the 3 targets. Sanger sequencing allowed to successfully analyzed the target genes for 55 samples. For whole blood, 12/16 samples were successfully analysed by NGS sequencing and 10/16 by Sanger. The failures were mostly linked to a low viral load.
The concordance of the virus subtype was 100% and perfect consistency was identified between the reports of resistance mutations from the sequences obtained by the 2 methods (Sanger and NGS ABL). The sensitivity of NGS sequencing made possible the identification of minority populations with mutations having an impact on the treatment for 8 samples.
Among the 10 samples analyzed in complete genome from 5 amplified fragments, only 1 sample with a low VL (50 cp/ml) failed amplification for the whole genome. For the remaining 9 samples, amplification and whole genome sequencing was performed for 3 samples. The subtypes identified for these samples are subtype B, C and CRF-02 respectively. For 2 samples, the amplification of 3/5 of the fragments was possible and for 4 samples 4/5 fragments were analyzed. For these samples the HIV-1 subtype were non-B non-typable, G and A-like.
From the extraction of the nucleic acids to the rendering of the results (12 samples/run), the technical time for the Sanger sequencing was 10 days with the intervention of 2 technicians for the proofreading of the chromatograms and of a biologist for the interpretation of the results. NGS sequencing makes possible to carry out the analysis in 4 days with 1 technician for the technical realization and 1 biologist for the analysis and interpretation of the results.

**Conclusions**
NGS should occupy a more prominent place in HIV resistance surveillance and clinical care. The decreasing costs, due to the COVID-19 pandemic, and its ability to reveal resistant minority variants of the virus and the study of their impact. The ability to reveal resistant minority variants and the study of their impact allow to quickly adapt the treatment. The complete genome analysis provides information on the new HIV targets, capsid/maturation inhibitors, and the detection of compensatory mutations that could explain certain escapes without the detection of resistance mutations in the targeted genes.

## Poster Abstract HP4

### Efficacy and Safety of Bulevirtide Monotherapy Given at 2 mg or 10 mg Dose Level Once Daily for Treatment of Chronic Hepatitis Delta: Week 48 Primary Endpoint Results from a Phase 3 Randomized, Multicenter, Parallel Design Study

H. Wedemeyer[1], S. Aleman[2], M. Brunetto[3], A. Blank[4], P. Andreone[5], P. Bogomolov[6], V. Chulanov[7], N. Mamonova[7], N. Geyvandova[8], V. Morozov[9], O. Sagalova[10], T. Stepanova[11], D. Manuilov[12], V. Suri[12], Q. An[12], J.F. Flaherty[12], A.Osinusi[12], J. Schulze zur Wiesch[13], M. Cornberg[1], S. Zeuzem[14], P. Lampertico[15,16]

[1]Klinik für Gastroenterologie, Hannover, Germany; [2]Karolinska University Hospital/Karolinska Institutet, Stockholm, Sweden; [3]University Hospital of Pisa and Department of Clinical and Experimental Medicine, Pisa, Italy; [4]Heidelberg University Hospital, Heidelberg, Germany; [5]University of Modena and Reggio Emilia, Italy; [6]State budgetary institution of health care of Moscow region, Moscow, Russian Federation; [7]FSBI National Research Medical Center for Phthisiopulmonology and Infectious Diseases of the Ministry of Health of the Russian Federation, Moscow, Russian Federation; [8]Stavropol Regional Hospital, Stavropol, Russian Federation; [9]Hepatolog, Samara, Russian Federation; [10]Ural State Medical University of Ministry of Health of the Russian Federation of Ministry of Health Chelyabinsk, Russian Federation; [11]Clinic of Modern Medicine, Moscow , Russian Federation; [12]Gilead Sciences, Foster City, USA; [13]Universitätsklinikum Hamburg, Hamburg, Germany; [14]University Hospital Frankfurt, Frankfurt am Main; [15]Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [16]CRC "A. M. and A. Migliavacca" Center for Liver Disease, Milan, Italy

**Background**
Bulevirtide (BLV) is a novel first-in-class entry inhibitor for the treatment of chronic hepatitis D virus (HDV) infection (CHD) that was conditionally approved in the European Union. In an interim 24-week (W) analysis of a Phase 3 study (MYR301; NCT03852719), BLV monotherapy at 2 or 10 mg once daily demonstrated significantly greater combined virologic/biochemical response vs control and favorable safety.

**Methods**
Patients with CHD (N=150) were randomized 1:1:1 and stratified by compensated cirrhosis status: Arm A (control), no active anti-HDV treatment for 48W followed by BLV 10 mg/d for 96W (n=51); Arms B and C, BLV 2 (n=49) or 10 mg/d (n=50), respectively, for 144W. All arms then entered a 96W treatment-free follow-up. The primary endpoint was combined response (undetectable HDV RNA or decrease by ≥2 $\log_{10}$ IU/mL from baseline and alanine aminotransferase [ALT] normalization) at 48W. Other endpoints included viral response (undetectable HDV RNA or decrease by ≥2 $\log_{10}$ IU/mL from baseline), biochemical response (ALT normalization), change in HDV RNA, and change in liver stiffness measured by elastography.

**Results**
Baseline characteristics: mean (SD) age, 41.8 (8.4) years; 57.3% males; 82.7% White; 47.3% with compensated cirrhosis; 60% on nucleos(t)ide analogues; mean (SD) HDV RNA, 5.05 (1.35) $\log_{10}$ IU/mL; mean (SD) ALT, 110.9 (69.0) U/L. Combined response was achieved by 22 (44.9%) and 24 (48.0%) patients in Arms B and C vs 1 (2.0%) in Arm A ($P < .0001$ for both). Viral and biochemical response rates were similar in both BLV arms and significantly greater than control at W48 (both $P < .0001$). No adverse events (AEs) led to BLV discontinuation; no serious AEs were attributed to BLV. Asymptomatic total serum bile salt elevations and injection-site reactions occurred more frequently with BLV 10 mg.

**Conclusions**
BLV treatment resulted in a significantly greater combined response vs control and was well-tolerated at 48W.

## Poster Abstract HP5

### Healthcare staff perspectives of HIV prevention injectable: early results from the PrEP Implementation Study for Cabotegravir Long Acting for Men in the Real World (PILLAR)

Nanlesta Pilgrim[1], Amber Haley[1], Alison Gaudion[2], Deanna Merrill[1], Bo Li[3], Roberto Ortiz[4], Rupa Patel[5], David Andrae[6], Ken Chow[7], Damon Warren[3], Jean van Wyk[2], Maggie Czarnogorski[1]

*[1]ViiV Healthcare, Durham, NC, United States; [2]ViiV Healthcare, Brentford, United Kingdom [3]GlaxoSmithKline, Collegeville, PA, United States;[4]Bliss Health, Orlando, FL, United States;[5]Whitman Walker, Washington, DC, United States;[6]Evidera, Bethesda, MD, United States; [7]GlaxoSmithKline, Mississauga, Canada*

**Background:** PILLAR is a Phase IV implementation science study that evaluates the feasibility and acceptability of implementation strategies for the delivery of long-acting cabotegravir for PrEP (APRETUDE) in low and high-volume PrEP sites across the United States. APRETUDE is indicated in at-risk adults and adolescents weighing at least 35kg to reduce the risk of sexually acquired HIV-1 infection. We report emerging data from clinic staff regarding implementing APRETUDE.

**Methods:** To date, study-staff participants (SSPs) from 9 of 17 clinics across the United States completed baseline questionnaires prior to using implementation strategies and enrolling participants into PILLAR. Clinical sites included research institutions, private clinics, federally qualified health centers, and non-profit organizations. SSPs demographics, implementation perceptions, optional oral lead-in (OOLI) perspectives, and appropriateness of APRETUDE by patient characteristics were collected.

**Results:** 43 SSPs completed baseline questionnaires. SSPs' mean age was 40.0 (SD: 13.9) and majority identified as cis-gender female (56%). A majority was White (58%); 21% identified as Hispanic/Latino. An equal proportion (12%) were nurse practitioners, physician assistant and nurses, 21% were physicians and less than 10% for other role categories.

To date, a majority of SSPs (77%) reported feeling extremely positive or positive about implementing APRETUDE. A smaller percentage (47%) perceived implementation would be very easy or somewhat easy in their clinic.

Only 5% of 20 SSPs who are prescribers were planning to prescribe OOLI to all their patients; others would prescribe to some (40%), to none (20%) or were unsure (35%). The main factors that would drive OOLI use decisions were patient request (80%), ability to assess APRETUDE's tolerability/safety (50%), being cautious with a new medication (40%), and providing patients with protection against HIV until the injection arrives (40%). For planned missed injections, providers would offer TDF/FTC (80%), TAF/FTC (55%), or cabotegravir oral tablets (45%).

Ninety-three percent of SSPs (n=40/43) reported that patients at their clinics asked for (or about) APRETUDE. Over 90% reported APRETUDE was appropriate for individuals who have multiple sexual partners, condomless sex, engage in anal sex, have a partner living with HIV, have had a recent STI, live in areas with high HIV prevalence, could benefit from PrEP but do not want to use daily PrEP, and feel stigmatized about oral PrEP. 40% reported APRETUDE was appropriate for individuals in monogamous relationships. Forty-four percent of SSPs also reported individuals with specific demographics were more appropriate for APRETUDE. Of these, SSPs identified Black and Latinx transgender men and women (range: 84%-90%) as well as MSM (range: 84%-95%) as appropriate while cis-gender Black men and women, and cis-gender White women along with White transgender men were reported as appropriate by 74%-79% of SSPs.

**Conclusions:** At baseline, a high percentage of SSPs reported APRETUDE was appropriate for their patients. Additionally, patient request was the highest determinant of the OOLI decision for providers, with very few intending to prescribe it to all patients. Exploring implementation supports, including how to address perceptions of patient appropriateness, for APRETUDE integration into clinical settings is needed. Updated data will be presented at the conference.

## Poster Abstract HP7

**Probing cell death pathways in response to NNRTI treatment using a THP-1 infection model**

Zhiyu (Neil) Fang, U-Ming Lim, Bonnie J. Howell, Paul Zuck, and Carl J. Balibar
Merck & Co., Inc., Rahway, NJ, USA

**Background**
Certain non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as Efavirenz, have been reported to induce HIV-1 infected cell death through CARD8-mediated pyroptosis. We refer to this activity as a targeted activator of cell killing (TACK). Probing the pathway which leads to the TACK effect can be challenging due to the difficulty in infecting primary cells at high rates as well as challenging with the effective CRISPR editing of primary T-cells and making clonal populations. A model which readily allows editing of the host cell's genome as well as higher infection rates can help aid in the pathway's study.

**Methods**
We have developed an infection model using THP-1 cells to further interrogate the TACK effect. This monocyte-like cell line can be readily infected by VSV-G pseudotyped HIV-1 with high efficiency and HIV-1 infected cells from this line are sensitive to the TACK effect of NNRTIs. Using CRISPR knockout methods, clonal lines were prepared with knockouts of various members of the apoptotic and pyroptotic pathways. The KO cells were then used to test the ability of TACK-active NNRTIs to selectively induce cell death of HIV-1 infected cells.

**Results**
Using these tools, we evaluated NNRTIs for their ability to specifically induce death in HIV-1 infected wild type cells as well as cells knocked out for members of the apoptotic and pyroptotic pathways. By identification of factors which are involved in NNRTI-induced cell death as well as factors which are not, we're able to gain a more complete mechanistic understanding of the TACK effect.

**Conclusions**
We describe here a THP-1 based model for studying TACK-active NNRTIs to further mechanistic understanding of NNRTI-mediated killing of HIV-1 infected cells. Since THP-1 cells are readily manipulated genetically, this allows for effective knock out and clonal selection of members of pyroptotic and apoptotic pathways to better tease out the exact mechanism of action of these molecules' infected cell killing ability. Additionally, this model could serve as an important screening tool to identify and unravel mechanism of action of novel agents capable of inducing HIV-1 infected cell death.

## Poster Abstract HP11

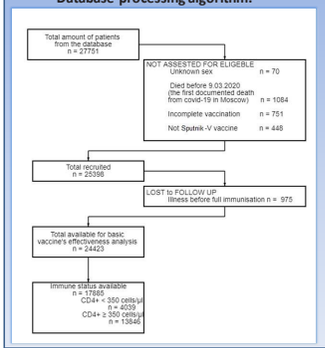
# Sputnik V protection from COVID-19 in people living with HIV under antiretroviral therapy

Vladimir A. Gushchin[1], Elena V. Tsyganova[2], Darya A. Ogarkova[1], Ruslan R. Adgamov[1], Dmitry V. Shcheblyakov[1], Nataliia V. Glukhoedova[2], Aleksandra S. Zhilenkova[2], Alexey G. Kolotii[3], Roman D. Zaitsev[3], Denis Y. Logunov[1], Alexander L. Gintsburg[1], and Alexey I. Mazus[2]

[1]Federal State Budget Institution "National Research Centre for Epidemiology and Microbiology named after Honorary Academician N F Gamaleya" of the Ministry of Health of the Russian Federation, Moscow, Russia, [2]Moscow City Center for AIDS Prevention and Control, Moscow, Russia, [3]Autonomous Noncommercial Organization, Moscow Center for Innovative Technology in Health Care, Moscow, Russia

## Methods

**Database-processing algorithm.**




We performed a retrospective cohort study to assess the effectiveness of the standard Sputnik V vaccination regimen in 24,423 HIV+ Moscow residents during spring - summer 2021, that included dominance of delta variant, with estimation of hospitalization and severe illness rates in vaccinated and unvaccinated patients. Data were extracted from the Moscow anti-COVID-19 vaccination and COVID-19 incidence Registries.

**General characteristics of HIV+ individuals from the Moscow COVID-19 Vaccination and COVID-19 incidence Registries**

| Sample characteristics | Vaccinated, n=2543 (10·4%) | Unvaccinated, n=17592 (72·0%) | Uncompleted vaccination *, n=4288 (17·5%) | p |
|---|---|---|---|---|
| Mean age (M±SD, 95% CI) | 44·69±10·12 (44·30 – 45·09) | 41·50±10·11 (41·35 – 41·65) | 41·65±8·28 (41·40 - 41·90) | <0·001 Welch's T-test) |
| Females | 762 (30·0%) | 7 883 (44·8%) | 1 798 (41·9%) | <0·001 (chi square) |
| Males | 1 781(70·0%) | 9 709 (55·2%) | 2 490 (58·1%) | |
| CD4+ (Me[IQR]) cells/µl (n=17 885, 73·2%) | n=2 198 (86·4%) 639[484 - 821] | n=12 134 (69·0%) 526[334 - 730] | n=3 553 (82·9%) 586[424 - 774] | <0·001 (Mann - Whitney test) |
| CD4+ ≥350 cells/µl (n=13 846) | 1 967 (89·5%) | 8 883 (73·2%) | 2 996 (84·3%) | <0·001 (chi square) |
| CD4+ <350 cells/µl (n=4039) | 231 (10·5%) | 3 251 (26·8%) | 557 (15·7%) | |

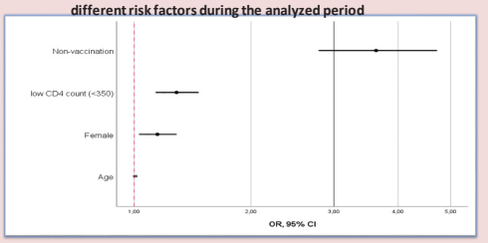
## Results

**Overall vaccine effectiveness in the entire group of HIV+, receiving ART**

| Patient cohorts | Vaccinated | Unvaccinated |
|---|---|---|
| COVID-19 illness | 71 (2·8%) | 1354 (8·2%) |
| No Covid-19 illness | 2472 (97·2%) | 15252 (91·8%) |
| Prior history of Covid-19 | 0 | 986 |
| Excluded from calculation due to incomplete immunization | 4288 | |
| Epidemiological effectiveness | 76·33% (95% CI: 69·84% - 81·43%) p<0·001 (chi square test) | |

The analysis shows that the overall epidemiological effectiveness of vaccination with Sputnik V in HIV+ patients undergoing ART included in our study was 76¢33% (95% CI 69¢84–81¢43%).

**The OR of COVID-19 infection probability associated with modification of different risk factors during the analyzed period**




The resulting regression model is statistically significant (p < 0·001). A 1-year increase in the age variable increases the chance of contracting COVID-19 by a factor of 1·007 (95% CI: 1.001 – 1.014). Being vaccinated decreases the probability of contracting COVID-19 by 3·631 times (95% CI: 2.794 – 4.720) as compared with being unvaccinated. A CD4+ T-cell count ≥ 350 decreased the probability of contracting COVID-19 by 1·317 times (95% CI: 1·155 – 1·502). Being a woman increases the probability of COVID-19 by a factor of 1·168 (95% CI: 1.035 – 1.317) as compared with men. There is a positive correlation between the probability of contracting COVID-19 and age, and a negative correlation with CD4+ T-cell count and being vaccinated. Women have a higher risk of contracting COVID-19 than men.

**Impact of patient's immune status on vaccine effectiveness in terms of protection from hospitalization during two time periods**

| Time period | 15 March - 15 May | | 1 June – 31 July | |
|---|---|---|---|---|
| Immune status of HIV+ on ART | CD4+ <350 cells/µl | CD4+≥350 cells/µl | CD4+ <350 cells/µl | CD4+≥350 cells/µl |
| Number of vaccinated with no documented COVID-19 illness | 111 | 961 | 227 | 1961 |
| Number of unvaccinated with no documented COVID-19 illness (including immune stratum) | 2201 | 7051 | 1755 | 4796 |
| Number of hospitalized among vaccinated, n | 1 | 0 | 3 | 6 |
| Number of hospitalized among unvaccinated, n | 58 | 54 | 62 | 74 |
| VE, % (95% CI) | 64·82% (-156·32% – 95·17%) | 100% | 59·92% (-28·74% – 87·52%) | 75·77% (44·25% – 89·47%) |

Analysis of vaccine effectiveness in terms of prevented hospital admissions and protection against disease progression to moderate or severe forms of COVID-19 shows that in patients with CD4+ T-cell counts ≥ 350 cells/µl the vaccine averted hospitalization in 100% of the group during the first period and in 75.77 % (95% CI 44.25% – 89.47%) during the second (with predominant occurrence of the delta variant). In immunodeficient patients, these percentages were 64.82% (95% CI -156.32% – 95.17%) and 59.92% (95% CI -28.74% – 87.52%), respectively.

**Impact of patient's immune status on vaccine effectiveness against severe disease during two time periods**

| Time period | 15 March - 15 May | | 1 June – 31 July | |
|---|---|---|---|---|
| Immune status of HIV+ on ART | CD4+ <350 cells/µl | CD4+≥350 cells/µl | CD4+ <350 cells/µl | CD4+≥350 cells/µl |
| Number of vaccinated with no documented COVID-19 illness | 111 | 961 | 227 | 1961 |
| Number of unvaccinated with no documented COVID-19 illness (including immune stratum) | 2201 | 7051 | 1755 | 4796 |
| Number of hospitalized among vaccinated, n | 1 | 0 | 2 | 1 |
| Number of hospitalized among unvaccinated, n | 28 | 32 | 27 | 43 |
| VE, % (95% CI) | 27·14% (-440·48% – 90·18%) | 100% | 38·64% (-159·75% – 85·51%) | 93·05% (49·51% – 99·04%) |

Vaccine effectiveness in preventing moderate or severe infection in patients with relatively preserved immune function was 100% in March–May 2021 and 93.05% (95% CI 49.51% – 99.04%) in summer 2021. The corresponding numbers in patients with CD4+ T-cell counts < 350 cells/µl were 27.14% (95% CI -440.48% – 90.18%) and 38.64% (95% CI -159.75% – 85.51%), respectively, which probably reflects insufficient information because of the width of the confidence interval.

**Vaccine effectiveness among HIV+ in subgroups by CD4+ counts**

| CD4+ count | CD4+ < 350, n=4039 | | | CD4+ >=350, n=13846 | | |
|---|---|---|---|---|---|---|
| Patient cohorts | Vaccinated documented (n=231) | Unvaccinated documented (n= 2997) | P (chi square test) | Vaccinated documented (n=1967) | Unvaccinated documented (n= 8352) | P (chi square test |
| COVID-19 illness | 11 (4·8%) | 352 (11·7%) | 0·002 | 51 (2·6%) | 779 (9·3%) | <0·001 |
| No Covid-19 illness | 220 (95·2%) | 2645 (88·3%) | | 1916 (97·4%) | 7573 (90·7%) | |
| Prior history of Covid-19 | 0 | 254 | | 0 | 531 | |
| Excluded from calculation due to incomplete immunization | 352 | | | 779 | | |
| Epidemiological effectiveness | 73·15% (50·27% – 85·50%) | | | 79·42% (72·54% – 84·57%) | | |

The effectiveness of the vaccine depended on the immune status of the individual: In the group of patients with CD4+ T-cell counts ≥ 350 cells/µl, the effectiveness was higher and constituted on average 79.42% (95% CI 72.54% – 84.57%), while in the group with CD4+ T-cell counts < 350 cells/µl it was lower, on average 73.15% (95% CI 50.27% – 85.50%). Therefore, vaccine effectiveness in HIV+ on ART with preserved immune status (CD4+ T-cell counts ≥ 350 cells/µl) was not different from the 80% level in the general population.

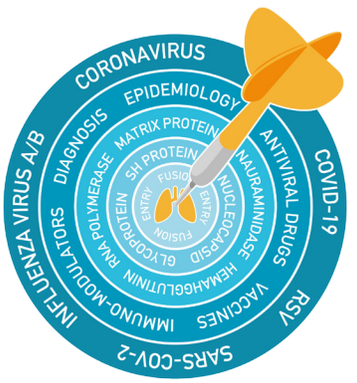
## Conclusion

The results obtained strongly suggest that Sputnik V vaccine is highly efficient in protection of HIV+ Moscow residents under ART from SARS-CoV-2 infection, especially from the most severe effects of COVID-19: the need for hospitalization, and death. However, in immunocompromised HIV+ individuals vaccine effectiveness was lower than in non-immunocompromised HIV+ patients. The effectiveness of this vaccine against the delta variant of SARS-CoV-2 was only slightly lower than against the original variant in patients with CD4+ T-cell counts > 350 cells/µl. In summary, despite decreased epidemiological effectiveness against the delta variant especially in immunocompromised HIV+ individuals undergoing ART, Sputnik V vaccine protection against moderate or severe disease remains sufficient for it to be recommended it for all HIV+ ART-treated individuals. A similar analysis should be now performed for the upcoming omicron SARS-CoV-2 variant.

# INVITED SPEAKER ABSTRACTS

Respi DART 2022

LOS CABOS, MEXICO • 6-8 DECEMBER 2022



Session 1: Advances in SARS-CoV-2 antiviral modalities
December 6th, 2022

**Nirmatrelvir resistance in Severe Acute Respiratory Syndrome Coronavirus 2**

Shuiyun Lan[1,2*], Grace Neilsen[1,2*], Ryan L. Slack[1,2], William A. Cantara[1,2], Andres Emanuelli[1,2], Zachary C. Lorson[1,2], Huanchun Zhang[1,2], Jasper Lee[1,2], Maria E. Cilento[1,2], Philip R. Tedbury[1,2], Stefan G. Sarafianos[1,2,#]
[1] Division of Laboratory of Biochemical Pharmacology, Division of Infectious Diseases, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA,
[2] Children's Healthcare of Atlanta, Atlanta, GA 30322, USA

**Background**
One of the leading antivirals against SARS-CoV-2 is paxlovid (PAX), which consists of nirmatrelvir (NIR, PF-07321332) that targets the main protease of SARS-CoV-2, nsp5 (also referred to as Mpro or 3CLpro) and ritonavir, which slows down nirmatrelvir's catabolism. NIR forms a covalent bond with the nsp5 active site residue Cys145.

**Methods**
To explore the potential for resistance to nirmatrelvir and other nsp5 inhibitors, we analyzed the structure of nsp5 complexes with inhibitors and peptide substrates, to guide design of mutations that may selectively impair binding of inhibitor over substrate. A combination of reverse genetics studies with SARS-CoV-2 replicon supplemented by cell-based complementation and *in vitro* enzymatic assays were used to assess the predicted putative resistance mutations.

**Results**
We show that the E166V nsp5 mutation enhanced up to 60-fold NIR resistance in the Washington WA1 and Omicron BA.1 strains. Consistent with its low prevalence in viral isolates from untreated people, this mutation affects replication fitness due to its effect on the nsp5 enzymatic activity. Importantly, we identified protease inhibitors that maintain their potency against the E166V mutant.

**Conclusion**
These data provide possible mechanisms of SARS-CoV-2 resistance to nirmatrelvir/paxlovid and offer the first insights into the design of second-generation nsp5-targeting antivirals efficient against nirmatrelvir-resistant viruses.

Session 1: Advances in SARS-CoV-2 antiviral modalities
December 6th, 2022

**Pre-clinical development of a broad-spectrum antiviral agent against SARS-CoV-2 3CL protease**

Ladislau C. Kovari[1], Franck Amblard[3], Dharmeshkumar Patel[3], Peng Liu[3], Shaoman Zhou[3], Michael Muczynski[1], Abdullah Al-Homoudi[1], Iulia A. Kovari[1], Tami McBrayer[3], Navnath Gavande[2], Raymond F. Schinazi[3]

[1]Department of Biochemistry, Microbiology, and Immunology, School of Medicine, Wayne State University, Detroit, MI 48201, USA
[2]Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI 48201, USA
[3]Laboratory of Biochemical Pharmacology, Department of Pediatrics, School of Medicine, Emory University and Children's Healthcare of Atlanta, Atlanta, GA 30322, USA

**Background**
We aim to design and develop a safe and effective antiviral agent targeting the SARS-CoV-2 3CL protease (3CLpro) for treating coronavirus disease 2019 (COVID-19) and human coronavirus infections in general, including nirmatrelvir-resistant variants. The central hypothesis is that inhibition of SARS-CoV-2 polyprotein cleavage results in the inhibition of viral replication and that the drug can be used to prevent or treat COVID-19. Our research targets the 3CLpro, a key enzyme for SARS-CoV-2 polyprotein cleavage and viral replication, using our established and proven drug discovery expertise.

**Methods**
Our drug discovery platform includes (1) our proprietary libraries and medicinal chemistry expertise of viral protease inhibitors; (2) molecular modeling and virtual screening capabilities of millions of compounds using the Schrödinger programs and other drug discovery suites; (3) cell-based coronavirus assays in our in house BSL-2* and BSL-3 facility; (4) recombinant protein-based enzyme assays and X-ray crystallographic studies of SARS-CoV-2 3CLpro, and; (5) the capability of evaluating candidate inhibitors for bioavailability and ADME-T characteristics, and efficacy through animal models of COVID-19 such as golden Syrian hamsters and non-human primates.

**Results**
We selected the nsp7/nsp8 cleavage site as representative for the SARS-CoV-2 3CLpro cleavage employing a covalent inhibitor design strategy. We decided to proceed with a P-side (P4-P1) inhibitor design. To further enhance inhibitor binding, we added an electrophilic warhead designed to react with the nucleophilic thiol group of Cys145 of the SARS-CoV-2 3CLpro. To develop highly potent and specific lead molecules, we targeted the catalytic Cys145-His41 dyad and other essential residues within the binding pocket, which are critical in the proteolytic process of SARS-CoV-2. We identified compound 3150 (and several backup compounds) with enzyme $IC_{50}$ (57 nM for 3294 and 210 nM for 3150, nirmatrelvir $IC_{50}$ reference 40 nM). Our compounds are unique, non-toxic, small peptidomimetic molecule that inhibits structurally related viral 3CL proteases (e.g., norovirus and enterovirus) with an $EC_{50}$ of 1 to 20 nM in cell culture, and SARS-CoV-2 replication (in Vero cells $EC_{50}$ = 0.6 μM), with no apparent cytotoxicity up to 100 μM. Co-crystal structures of the 3150 and 3240 demonstrate a covalent mechanism of action and provide a platform for further enhancing the drug-like properties of these compounds.

**Conclusions**
Using multiple approaches, we designed, synthesized, and characterized a series of compounds, including leads 3150 and 3240. Both compounds showed excellent inhibitory activity against the 3CL protease in a biochemical assay and a potent anti-SARS-CoV-2 activity in a cell-based assay. The X-ray crystal structures of SARS-CoV-2 3CLpro in complex with 3150 and 3240, determined at less than 1.9 Å resolution, showed that the aldehyde group is covalently bound to Cys145 of 3CLpro. Both inhibitors showed low nanomolar activity and low toxicity, suggesting that these compounds are promising drug candidates with the potential to be developed further as antiviral drugs against human coronaviruses.

**Acknowledgments**
An NIH R01 grant AI161570 provides generous support for this research to RFS and LCK.

Session 1: Advances in SARS-CoV-2 antiviral modalities
December 6th, 2022

## Lipid-oxidizing B cells in successful vaccine responses despite immunosuppression

Elizabeth A. Thompson[1,2], Katerina Roznik[1,3], Andrew H. Karaba[1], Katherine Cascino[1], Santosh Dhakal[3], Wabathi Ngecu[1], Sabra L. Klein[1,3], Daniel S. Warren[5], Caroline X. Qin[1,5], Jonathan Mitchell[5], Allan B. Massie[5], Jacqueline M. Garonzik-Wang[7], Brian J. Boyarsky[5], Joel N. Blankson[1], Michael J. Wolfgang[1,9], Erika L. Pearce[2,3], Edward J. Pearce[2,3], Christine M. Durand[1], Dorry L. Segev[4], Aaron A.R. Tobian[1,5], William A. Werbel[1], Justin R. Bailey[1], Andrea L. Cox[1-3]

[1]Department of Medicine, Johns Hopkins University School of Medicine, [2]Bloomberg~Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University School of Medicine, [3]Department of Molecular Microbiology and Immunology, Johns Hopkins University Bloomberg School of Public Health, [4]Department of Surgery, Johns Hopkins University School of Medicine, [5]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

**Background**
People who require immunosuppression due to organ transplantation or autoimmune disease respond less robustly to vaccines that protect other populations, including COVID-19 vaccines. Immunocompromised individuals have higher COVID-19 mortality rates, diminished antibody titers, and higher rates of breakthrough infections following vaccination, with the lowest seroconversion rates occurring in solid organ transplant recipients (SOTRs). We and others have previously demonstrated the benefits of a third vaccine dose in SOTRs with negative or low anti-spike (S) IgG titers after two vaccine doses. However, even following a third dose of vaccine, a subset of SOTRs fail to develop SARS-CoV-2 spike-specific antibodies at titers thought to be protective. Understanding what drives successful vaccine responses in immunosuppressed individuals, including SOTRs, is critical to prepare for future pandemics. Due to limited cross-reactivity with seasonal coronaviruses, the novel SARS-CoV-2 vaccine provides a unique opportunity to evaluate naïve B cell priming in immunosuppressed people. Moreover, little is known about the metabolic landscape of successful or impaired human B cell responses.

**Methods**
To gain additional insight into factors associated with vaccine response or nonresponse in immunocompromised people, we assessed clinical parameters and characterized global and antigen-specific B cell responses following second and third dose COVID-19 vaccination in SOTRs utilizing a high dimensional flow cytometry panel designed to evaluate immunologic and metabolic phenotypes at the single-cell level. We compared the in-depth immunologic and metabolic profiling of B cells from vaccine responder and non-responder SOTRs on a variety of immunosuppression regimens following COVID-19 vaccination.

**Results**
There were no significant differences between responders and non-responders in the vaccine received, organ transplanted, age, or time since transplant. However, we found that all participants who failed to respond to a third dose received mycophenolate mofetil (MMF) or its active metabolite mycophenolic acid (MPA). In-depth immunologic and metabolic profiling of B cells demonstrated that the dominant B cell population in SOTRs who responded to vaccine were lipid-oxidizing alternative lineage CD11c+ B cells with high expression of carnitine palmitotransferase 1A (CPT1A), the rate limiting enzyme for long chain fatty acid oxidation (FAO). This is a minor B cell population in vaccinated healthy controls. We identified a dose-dependent effect of MPA, the immunosuppressive agent associated with non-response, on FAO. Low dose MPA induced CPT1a-dependent FAO in B cells, but not T cells, in which elevated CPT1A expression was not detected. In contrast, high dose MPA inhibited FAO.

**Conclusions**
These data highlight a novel pathway to vaccine responsiveness in immunosuppressed individuals and a mechanism for vaccine failure in SOTRs receiving high dose MMA.

Session 2: Respiratory viruses in children and inflammation
December 6th, 2022

**Successful research takes a village: Insight gained from the longitudinal surveillance of SARS-CoV-2 IgG antibodies in pediatric healthcare workers**

Claudia R. Morris[1-3], Sneh Lata Gupta[1,2], Dunia Hatabah[1]; Grace Mantus[1], Srikant Iyer[1,2], Stacy Heilman[1], Andres Camacho-Gonzalez[1,2,5], De Leake[2], Mimi Le[1], Mark Griffiths[1,2], Tiffany Hughes[1], Carson Norwood[1,5], Samuel Shih[1,5], Wendalyn K. Little[1,2], Chris Rees[1,2], Laura Benedit[2], Mehul Suthar[1,4], Jens Wrammert[1,5], and Miriam B. Vos[1-3]
[1]Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia; [2]Children's Healthcare of Atlanta, Atlanta, Georgia;[3]Center for Clinical and Translational Research, of Children's Healthcare of Atlanta and Emory University, Atlanta, GA; [4]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta GA; [5]Center for Childhood Infections and Vaccines of Children's Healthcare of Atlanta and Emory University, Atlanta, GA

**Background**
Healthcare workers (HCW) work in high-risk exposure setting while treating patients with COVID-19 infection in hospitals. The effectiveness of both mRNA vaccines such as BNT162b2 vaccine (Pfizer–BioNTech) and mRNA-1273 vaccine (Moderna) is more than 88%, tested in HCWs irrespective of age (adult or aged), ethnicity and race. The temporality of humoral immune response between SARS-Cov-2 recovered and naïve individuals is different. The vaccine against SARS-Cov-2 targets the spike glycoprotein that is shared by several of the coronaviruses, imparting partial immunity. The cross reactivity of vaccine generated antibodies between SARS-Cov-2 recovered and naïve individuals is unknown. There is minimal information on longitudinal immune profiling of different subgroups including SARS-CoV-2 recovered vs. naïve individuals and vaccinated vs. non-vaccinated in pediatric HCWs (pHCW). In response to the pandemic, a multidisciplinary team rapidly partnered without an external funding source to initiate time-sensitive research on the prevalence and incidence of this new virus in pHCW.

**Objective**
To determine the prevalence and incidence of SARS-CoV-2 IgG antibodies, and to explore the impact of SARS-CoV-2 vaccination on IgG antibody titers over time in a longitudinal cohort of pHCW .

**Methods**
pHCW were enrolled in this prospective longitudinal cohort to determine SARS-CoV-2 IgG antibody prevalence, incidence and vaccine triggered antibody titers over time. Serum samples were analyzed using a multiplex electro-chemiluminescent-based multiplex immunoassay provided by Mesoscale Discovery (MSD). The V-PLEX COVID-19 Coronavirus Panel 1 plates was used to assess wild-type (Wuhan strain) binding titers to RBD and Spike along with other beta coronaviruses such as SARS-CoV1, MERS-CoV, HCoV-OC43 and HCoV-HKU1 spike protein.
**Results:** From April through August 2020, a total of 642 pHCW were enrolled from Emory University and Children's Healthcare of Atlanta. Anti-SARS-CoV-2-IgG prevalence was 4.1%; 8.1% among Emergency Department (ED) versus 2.1% among non-ED pHCW, p=0.001. Incidence of new SARS-CoV-2 infection from September 2020 to January 2021 was 8.2% with no difference based on work location. SARS-CoV-2-IgG antibody titers post-infection waned within 3 months with mean decrease of 48%. Of the 642 HCW, 354 participants returned for testing for post Covid-19 vaccine titers. Of those 354, there were SARS-CoV-2 naïve individuals who were vaccinated, SARs-CoV-2 recovered and vaccinated, SARS-CoV-2 naïve and non-vaccinated, and SARS-CoV-2 recovered and non-vaccinated. Results showed robust antibody response against RBD, spike and NTD in vaccinated individuals as compared to non-vaccinated. SARS-CoV-2 recovered participants showed higher titers than naïve individuals. Antibodies to SARS-CoV-2 Nucleocapsid antigen were higher in recovered individuals regardless of vaccination status. Vaccinated individuals showed a more robust antibody binding for SARS-CoV-1 than non-vaccinated, and recovered participants showed higher SARS-CoV-1 antibodies titers than naïve individuals. Recovered participants also showed higher antibody titers for other beta-corona variants irrespective of vaccination. Single dose of vaccine was sufficient to attain maximum titer in Covid-19 recovered participants compared to naïve that required both doses of vaccine. RBD and Spike antibody titers higher and more durable after booster as compared to primary series of vaccination.

**Conclusion**
Our results show that ED-based pHCWs had more than a 4-fold increased risk of SARS-CoV-2 IgG antibody prevalence early in the pandemic compared to non-ED-based HCWs, suggesting early Covid-19 exposure prior to universal use of personal protective equipment. No work location-based differences were found with the incidence of new SARS-CoV-2 IgG antibodies beyond August 2020. Covid-19 vaccination caused a robust increase in antibody titers against SARS-CoV-2 in all vaccine-recipients, with the highest titers present in vaccinated-recovered individuals. In addition, our results also show that both SARS-CoV-2 infection and vaccination yield antibodies that cross react to other beta-corona viruses, imparting additional immunity against different strains and genera of the subfamily Coronaviridae. The Emory+Children's Center for Clinical & Translational Research leadership rapidly assimilated specialists in emergency medicine, infectious disease, epidemiology, vaccine specialists, and the biorepository core team to perform this study early in the pandemic, emphasizing the point that successful research takes a village.

Session 2: Respiratory viruses in children and inflammation
December 6th, 2022

**Inflammatory response to SARS-CoV-2 infection**

Brian S Dobosh, Diego Moncada Giraldo, Keivan Zandi, Shu Lin Goh, Kathryn Musall, Milagros Aldeco, Julia LeCher,Vincent D Giacalone,Junkai Yang, Devon Eddins, Eliver Ghosn, Vikas Sukhatme, Raymond F Schinazi, Rabindra Tirouvanziam

Emory University School of Medicine, Atlanta, GA, USA

**Background**
Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the agent of the recent coronavirus disease or 2019 (COVID-19) pandemic, is a betacoronavirus with tropism for the airway epithelium. The course of human infection by SARS-CoV-2 is highly variable, ranging from asymptomatic in the majority of children, to mild in the majority of adults, to severe and possibly fatal in some adults. Moreover, a subset of infected hosts develop lingering symptoms weeks to months after the initial infection. Host inflammatory responses mediated by the airway epithelium and by recruited monocytes, neutrophils and other leukocytes are recognized as key toboth immediate and prolonged responses to SARS-CoV-2, controlling host symptoms and paving the way for T-and B-cell responses. Because of evolutionary divergence in airway defense and leukocyte function, human inflammatory responses to SARS-CoV-2 infection cannot be readily extrapolated from animal models, highlighting the need for biomimetic models recapitulating the human airway microenvironment.

**Methods**
We adapted an transmigrationmodel validated by our group in priorstudies of neutrophilic and monocyticrecruitment to the airway lumen in response to viral and bacterial infection occurring acutely (acute lung injury –Grunwell et al., Sci Rep 2019) or chronically (cystic fibrosis –Forrest et al. JLB 2018and 2022; Margaroli et al., Cell Rep Med 2021; Laucirica et al., Imm Cell Biol 2022). In brief, human club cells (H441 line) were differentiated for two weeks at air-liquid interface, and then infected with SARS-CoV-2 (Washington strain) or control viruses (OC43 betacoronavirus and PR8H1N1 influenzaA virus)to assess epithelial responses across viral doses (MOI of 0.01, 0.1 and 1) and time (24, 48 and 72 h). Next, to mimic the monocyte-dominated inflammatory phase that is a hallmark of human hosts progressing from asymptomatic infection to the symptomatic, COVID-19 phase, primary human blood monocytes were transmigrated through the infected epithelium. The course of infection and inflammation was followed over 72 h in the absence or presence of the antiviral drug remdesivir and/or immunomodulatory drug baricitinib, both clinically approved forCOVID-19.

**Results**
Similarly to OC43, SARS-CoV-2 was able to inhibit antiviral signaling and promote survival in epithelial cells, while inducingmonocytic rather than neutrophilic recruitment. Recruited monocytes acquired SARS-CoV-2 from the infected epithelium and replicated its genome but did not produce infective virions. Infected monocytes were able to overcome the blockade to antiviral signaling and neutrophil recruitment mediated by SARS-CoV-2 in the epithelium. Remdesivir decreased viral burden in the epithelium and monocytes while baricitinib primarily impacted monocyte transmigration and downstream inflammatory signaling, without compromizing their antiviral response. Overall,these findingsconcur withthe limited in vivo data from airway samples of COVID-19 patientsavailable to date.

**Conclusions**
Our model mimics key virus/host interactions driving early (epithelial) and late (monocytic) phases of SARS-CoV-2 infection in humans(see more in Dobosh et al. Cell Rep 2022). Future studies will incorporate neutrophils and macrophages and SARS-CoV-2variants which have evolved new ways to modulate human inflammatory responses, and determine whetherapproved and novel antiviral and immunomodulatory treatment may help contain complications of COVID-19.

Session 3: Animal disease models and long COVID-19
December 7th, 2022

**Therapeutic approaches to control acute and chronic emerging sarbecovirus pathogenesis**
Ralph S. Baric, University of North Carolina at Chapel Hill

**Background**
The ongoing coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). New antivirals, antibody therapies, and small molecule inhibitors have diminished acute fatality rates. However, about 40% of symptomatic and asymptomatic COVID-19 survivors develop post-acute sequelae, termed PASC or “long COVID,” with features that include dyspnea, fatigue, chest pain, cognitive decline, and multiorgan damage, especially chronic lung disease . Direct acting antivirals and host based therapies are desperately needed to treat and control acute and chronic COVID19 disease outcomes.

**Methods**
The mouse-adapted SARS-CoV-2 strain MA10 produces an acute respiratory distress syndrome in mice similar to humans. To investigate PASC pathogenesis, studies of MA10-infected mice were extended from acute to clinical recovery phases through 120 days postinfection.

**Results**
At 15 to 120 days after virus clearance, pulmonary histologic findings included subpleural lesions composed of collagen, proliferative fibroblasts, and chronic inflammation, including tertiary lymphoid structures. Longitudinal spatial transcriptional profiling identified global reparative and fibrotic pathways dysregulated in diseased regions, similar to disease phenotypes noted in human COVID-19. Populations of alveolar intermediate cells, coupled with focal up-regulation of profibrotic markers, were identified in persistently diseased regions. Early intervention with antiviral EIDD-2801 and other small molecule inhibitors reduced acute as well as chronic disease outcomes.  Early treatment with host based therapeutics, like the antifibrotic agent (nintedanib), modified early disease severity, yet animals eventually progressed to chronic outcomes that were not distinguishable from untreated controls.

**Conclusions**
Our data suggests that COVID19 infection in humans and mice progress to a chronic organizing pneumonia with fibrotic lesions, heralding the potential for late stage end stage lung disease outcomes.  Pathway analyses identifies likely targets for the development of host based therapies to attenuate chronic disease outcomes. The MA10 murine model provides new opportunities to identify pathways associated with persistent SARS-CoV-2 pulmonary disease and to test countermeasures to ameliorate SARS-CoV2-instigated PASC.

Session 3: Animal disease models and long COVID-19
December 7th, 2022

**Coronavirus animal models and pathogenesis**

Stanley Perlman, M.D., Ph.D.
Department of Microbiology and Immunology
University of Iowa, Iowa City, IA 52242

As the COVID-19 pandemic continues around the world, greater understanding of the immune response to the virus and pathogenesis are required. Experimentally infected animal models of COVID-19 are required for these purposes. Over the past few months, we have developed several mouse models for COVID-19. Mice are naturally resistant to the original strains of SARS-CoV-2, so either the mouse needs to be sensitized for SARS-CoV-2 infection or the virus needs to be modified to use the mouse host cell receptor. To obtain mice useful for studying COVID-19, we used several approaches. In a first approach, we sensitized mice for infection by transduction with an adenovirus vector expressing human ACE2, the virus receptor. We also used K18-hACE2 mice that we developed during the SARS epidemic. Most recently, we isolated a mouse-adapted SARS-CoV-2 that causes severe disease in young and old BALB/c mice, and in aged C57BL/6 mice. Disease is confined to the lungs but inflammatory changes are widespread, even in organs that are not infected. These mice develop anosmia and also develop long term neurological disease. Mice infected with this mouse-adapted virus will useful for studies of pathogenesis of COVID-19 in the lungs, but also for manifestations of disease in other organs.

Session 3: Animal disease models and long COVID-19
December 7th, 2022

**Long COVID pathobiology and therapeutics: Where we are and where we need to go**

Michael Peluso
Division of HIV, Infectious Diseases, and Global Medicine
University of California, San Francisco

**Background**
It is now widely accepted that SARS-CoV-2 infection can affect long-term health and quality of life. Long COVID, a type of post-acute sequelae of COVID-19 (PASC) characterized by persistent unexplained symptoms, has a major impact on the health of many COVID survivors. However, the mechanisms driving Long COVID remain incompletely understood and there are no accepted therapies. The implementation of clinical trials for Long COVID has been delayed by skepticism about the condition, uncertainty regarding measurement and outcomes, and limited investment to date by key stakeholders.

**Methods**
This presentation will provide an overview of the proposed biological mechanisms contributing to Long COVID, with a focus on the developing therapeutic agenda. Studies from the UCSF Long-term Impact of Infection with Novel Coronavirus (LIINC) cohort, which opened in April 2020 and has followed many individuals with Long COVID for over two years, will be discussed in the context of the larger literature on Long COVID pathophysiology.

**Results**
Long COVID syndromes are variable and include general (e.g., fatigue) and organ-system specific symptoms (e.g., shortness of breath, palpitations, neurocognitive symptoms), as well as symptoms resembling other medically unexplained syndromes (e.g., myalgic encephalomyelitis/chronic fatigue syndrome, dysautonomia, post-exertional malaise). Mechanisms that might plausibly contribute to Long COVID include irreversible tissue damage associated with acute infection, persistence of SARS-CoV-2 antigen or a viral reservoir, residual or ongoing immune activation and inflammation, reactivation of other latent human viruses, microvascular dysregulation and thrombotic events, microbial translocation, dysbiosis, and autoimmune phenomena. These mechanisms may act in isolation or in combination to drive Long COVID symptoms. While potential therapeutics targeting each mechanism are available, few have been studied systematically to date.

**Conclusions**
Many individuals experiencing Long COVID are debilitated and desperate to return to their pre-COVID state of health. As a result, there is an urgent need to conduct clinical trials ranging from small, proof-of-concept studies that elucidate the underlying biology to large randomized, controlled trials focused on clinical outcomes. Such studies will be critical to advance the field from management approaches focused on symptom alleviation toward the disruption of the underlying pathobiology. If they are to succeed, these efforts will require substantial investment on the part of regulators, funders, and industry partners.

Session 5: Host-virus interactions and basic science discoveries
December 7th, 2022

**Infection of primary nasal epithelial cells differentiates among human coronaviruses**

Clayton J. Otter[a,c], Alejandra Fausto[a,c], Nikki Tanneti[a,c], Li Hui Tan[b,d], Anant Patel[a,c], Noam A. Cohen[b,d], Susan R. Weiss[a,c]

Departments of [a]Microbiology and [b]Otorhinolaryngology-Head and Neck Surgery, [c]Penn Center for Research on Coronaviruses and Other Emerging Pathogens, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [d]Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA, USA

SARS-COV, SARS-CoV-2 (SARS-2) and MERS-CoV (MERS) are pathogenic coronaviruses (CoVs) that have caused public health emergencies in the past 20 years. HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1 are “common” or “seasonal” coronaviruses causing primarily nonlethal upper respiratory infections. The nasal epithelium is the initial portal of entry and primary barrier to infection by all human coronaviruses (HCoVs). Thus, studies of viral replication, host cell tropism, and innate immune responses in the nasal airway are essential to understanding pathogenesis and transmission. We have utilized primary nasal epithelial cells grown at air-liquid interface (ALI), which recapitulate the heterogeneous cellular population as well as mucociliary clearance functions of the *in vivo* nasal epithelium once differentiated, to compare lethal SARS-2 and MERS and seasonal NL63. While these HCoVs all replicate productively in these cultures, SARS-2 replication surpasses that of MERS and NL63 by ~2 $\log_{10}$ PFU/ml. We compared viral replication at 33ºC vs 37ºC reflecting upper and lower respiratory tract temperatures respectively. We found that MERS replicates to similar titers at both temperatures. SARS-2 replicates at both temperatures as well but at later time points optimal replication is at 33ºC. NL63 requires 33ºC for replication with almost no detectable infectious virus produced at 37ºC, reflecting it propensity for upper respiratory infections. The three HCoVs diverge significantly in terms of cytotoxicity induction following infection. Using transepithelial electrical resistance (TEER) as a measure of barrier functional integrity, we found that NL63 causes the most disruption while SARS-CoV-2 causes reduction in TEER and MERS-CoV infection produces no detectable change indicating that the epithelial barrier function remains intact. Consistent with the TEER measurements, NL63 and SARS-CoV-2 induce significant cellular cytotoxicity as assessed by lactate dehydrogenase (LDH) release, while MERS infection causes little if any cytotoxic effects. Treatment of nasal ALI cultures with IL-13 to induce goblet cell hyperplasia, (which reflects the asthmatic or allergic airway) highlights further difference among the HCoVs in the nasal epithelium. MERS DPP4 receptor expression and replication is increased following IL-13 treatment while ACE2 receptor and SARS-2 and NL-63 replication is decreased significantly. We then compared infections of the nasal ALI cultures with SARS-2 wild type and several variants of concern. We found that the delta variant replicates to highest titiers in nasal ALI cultures and causes deciliation as well as loss of TEER. Further understanding of host-virus interactions at this initial site of viral replication is critical, as they likely determine infection outcomes such as clinical disease severity, propensity for spread to the lower airway, and eventual transmissibility.

## Abstract

**Preclinical antiviral characterization of the novel SARS-CoV-2 3CL protease inhibitor ALG097558**

Andreas Jekle[1], Koen Vandyck[2], Rana Abdelnabi[3], Dirk Jochmans[3], Dorothée Bardiot[4], Sandro Boland[4], Patrick Chaltin[4,5], Laura Vangeel[3], Sandra Chang[3], Kusum Gupta[1], Steven De Jonghe[4], Kha Le[1], Cheng Liu[1], Antitsa Stoycheva[1], Sarah K Stevens[1], Arnaud Marchand[4], Vladimir Serebryany[1], Jerome Deval[1], Sushmita Chanda[1], Lawrence M Blatt[1], Leonid Beigelman[1], Johan Neyts[2] and Julian A Symons[1]

*[1]Aligos Therapeutics Inc., South San Francisco, CA; [2]Aligos Belgium BV, Leuven, Belgium, [3]Rega Institute for Medical Research, KU Leuven, Belgium, [4]Cistim Leuven, Belgium, [5]Centre for Drug Design and Discovery (CD3), KU Leuven, Belgium.*

**Background:**
There is an urgent need for novel antiviral drugs for the treatment of Covid-19, especially in unvaccinated individuals and at-risk patients. In 2021, the FDA granted emergency use authorization for the SARS-CoV-2 3CL protease (3CLpro) inhibitor Paxlovid (nirmatrelvir/ritonavir). Here, we describe ALG-097558, a novel 3CLpro inhibitor with pan-coronavirus antiviral activity and a favorable ADME profile without the need of a pharmacoenhancer such as ritonavir.

**Methods:**
Biochemical and cell-based assays, and a hamster SARS-CoV-2 infection model were used to assess the antiviral activity. Pharmacokinetic studies were conducted in rat, dog and monkey following single intravenous and oral doses.

**Results:**
Lead optimization of different series of SARS-CoV-2 3CLpro inhibitors led to the discovery of ALG097558, a potent and selective inhibitor exhibiting picomolar activity in a 3CLpro enzymatic assay ($K_i$ = 74 pM). ALG097558 has pan-coronavirus activity against various SARS-CoV-2 variants (including Omicron and its subvariant BA.2 and BA.5) with $EC_{50}$ values ranging from 3-13 nM. ALG-097558 exhibits potent activity against other human coronaviruses (HCoVs) such as SARS-CoV-1, MERS, a-hCoV 229E and NL63, as well as b-hCoV OC43 and HKU-1. No cytotoxicity was observed when tested up to 100 µM in multiple cell lines. ALG-097558 also exhibits a high level of potency against SARS-CoV-2 replicating in a 3D human airway epithelial cell culture model ($EC_{99}$ and $EC_{99.9}$ of 44 and 54 nM, respectively, in presence of 40% human serum). In a biochemical assay, ALG-097558 retains antiviral activity (≤ 3-fold shift) against a 3CLpro L50F/E166A/L167F resistance mutant significantly affecting nirmatrelvir, ensitrelvir and PBI-0451 (>65-fold loss in activity). Using a therapeutic dosing regimen, where drug administration was initiated 8 h or 24 h post-infection, ALG097558 potently reduced viral RNA and infectious virus titers to the lower limit of quantification in the lungs of SARS-CoV-2 infected hamsters when assessed at 96 h post infection. ALG-097558 exhibits a favorable ADME and toxicological profile, with no significant inhibition of CYP450 at relevant compound concentration. Human PK projections based on PK parameters in preclinical species led to a projected efficacious dose of 370-600 mg administered twice a day without the need for ritonavir boosting.

**Conclusions:**
ALG-097558 is a highly potent pan-coronavirus 3CLpro inhibitor with a favorable resistance profile and in vivo efficacy upon therapeutic dosing. ALG097558 is anticipated to demonstrate efficacy in patients without the need of a pharmacokinetic enhancer such as ritonavir. First-in-human clinical trials are expected to start in early 2023.

Session 5: Host-virus interactions and basic science discoveries
December 7th, 2022

**Interferons and SARS-CoV-2: Role in pathogenesis and therapeutics**
Jordan J. Feld MD MPH

**Background**
Interferons are a key component of the innate antiviral immune response. Early studies in SARS-CoV-2 infection showed that impairment of the interferon (IFN) response was associated with more severe disease. This combined with the broad activity of IFNs led to their investigation early in the pandemic as a potential therapeutic strategy.

**Methods**
To clarify the role of IFNs in the pathogenesis of SARS-CoV-2 infection, studies have evaluated the impact of impaired IFN-$\alpha$ production (or enhanced destruction) on disease outcome and have explored how the virus evades IFN responses. Early studies showed activity of Type I and Type III IFNs against SARS-CoV-2 in cell culture and animal models, leading to rapid evaluation in clinical trials, initially with virological endpoints and subsequently exploring clinically relevant outcomes.

**Results**
Early studies confirmed that induction of Type I and Type III IFNs was limited by SARS-CoV-2 and that patients with circulating IFN-$\alpha$ antibodies were more likely to have a severe course of disease. Polymorphisms in important IFN-stimulated genes (ISGs) were shown to be associated with outcome of COVID-19 as well. Animal studies had shown that Type I IFNs could be potentially harmful in severe respiratory viral infections due to their pro-inflammatory activity and indeed, although initial studies of IFN-beta for COVID-19 showed promising results, later studies were stopped early due to concerns for worse outcomes in the IFN-beta-treated patients. Type III IFNs (IFN lambdas) induce a similar profile of ISGs as Type I IFNs, however, because the IFN lambda receptor has a limited tissue distribution and very limited expression on immune cell populations, they are associated with less systemic side effects and potentially with a lower risk of pro-inflammatory complications. This led to investigation of IFN-lambda for COVID-19. A Phase II trial showed that a single dose of peg-IFN-lambda accelerated viral clearance in outpatients with mild-moderate COVID-19, with the greatest effects seen in those with high baseline viral loads. A similar study found less promising results but enrolled patients later in the disease course with lower viral loads. Both studies found minimal toxicity with a similar adverse event profile to placebo. A Phase III study of a single dose of Peg-IFN-lambda vs placebo enrolled over 1900 patients at high risk of severe COVID-19 in Brazil and Canada and showed a 51% reduction in hospitalization or death. The beneficial effects of therapy were more pronounced when treatment was given within the first 3 days of symptoms and the treatment was active in both vaccinated and unvaccinated participants, as well as across a number of SARS-CoV-2 variants of concern. Ancillary studies have shown that the antiviral effects of IFN-lambda are due to direct ISG activation rather than immune stimulation and that treatment overcomes delayed T cell responses, as well as impaired endogenous IFN responses.

**Conclusions**
As with most viral infections, IFNs play an important role in the natural history of SARS-CoV-2 infection. The broad activity of IFNs makes them appealing therapeutic agents, particularly for emerging pathogens, however the use of Type I IFNs has been limited by their pro-inflammatory risks and side effect profile. With its limited receptor distribution, IFN-lambda offers a potential alternative for COVID-19 as well as for future respiratory viral pathogens. Recent data show a promising therapeutic effect of Peg-IFN-lambda, which may serve an important role as the pandemic continues to evolve.

Session 6: Immunology and advances in vaccine development
December 8th, 2022

**Vaccines and therapeutics for monkeypox virus infections**

Rachel L Roper, Professor Microbiology & Immunology, Brody Medical School, East Carolina University

**Background**
Vaccines and therapeutics have been developed for smallpox virus infection, a major biowarfare/bioterrorism concern. These countermeasures are now being applied in the human monkeypox outbreak worldwide because smallpox, monkeypox, and the vaccinia virus vaccine strains are closely related (all are Orthopoxvirus genus).

**Methods**
Because smallpox virus is the most restricted virus in the world and is officially only held in two locations (the US CDC and the former Soviet Biowarfare Unit, VECTOR, in Novosibirsk, Russia), and human smallpox infections are not occurring naturally nor possible experimentally, most of the countermeasures developed in the past 5 decades for smallpox have been tested in primate models using monkeypox virus.

**Results**
The drugs and vaccines approved for smallpox have shown safety and efficacy against monkeypox virus in monkeys. The history of smallpox vaccination dates back hundreds of years, and several versions have been employed. Use, route, dose, efficacy, side effects, and safety data of variolation and vaccinations with cowpox, Dryvax, ACAM 2000, and Jynneos (Imvamune) will be described for smallpox and monkeypox in animal models and humans where available. Antiviral drug safety and efficacy data will be described for: Cidofovir (Vistide), Brincidofovir (CMX001, Tembexa), ST246 (Tecovirimat or Tpoxx, FDA approved 2018), and intravenous antibodies - VIG vaccinia immune globulin.

**Conclusions**
Safe and efficacious vaccines and anti-viral therapeutics exist for the prevention and treatment of monkey poxvirus, however more data in human monkeypox is needed. New strategies for vaccines and antiviral drugs will be discussed.

Session 6: Immunology and advances in vaccine development
December 8th, 2022

**Sex differences in immune responses to influenza infection and vaccination**

**Sabra L. Klein, PhD**
W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland USA

**Background**
Strong evidence of sex differences in the pathogenesis of influenza virus infections suggest that in addition to pregnancy, being a female of reproductive ages is a predictor of severe outcomes due to immunopathology. Females also demonstrate greater vaccine-induced immunity and adverse events in clinical studies and preclinical animal models.

**Methods**
Cellular, serological, symptomatology, and outcome data from international epidemiological, regional clinical, and preclinical studies, data pertaining to influenza virus infection and vaccination will be presented.

**Results**
Females possess immunological features that contribute to greater immunopathology following infection with pandemic strains of influenza A viruses but also greater protection following vaccination. Molecular mechanisms including X-linked genes (e.g., *TLR7*) as well as sex steroids, including estrogens and androgens, play important roles in mediating the development of sex differences in cellular and serological immunity to infection and vaccination. Aging can impact the expression of sex differences in influenza virus pathogenesis vaccine-induced immunity and protection, which will be presented.

**Conclusion**
Sex is a biological variable should be considered in the analyses of infectious diseases and vaccination data.

# ORAL ABSTRACTS

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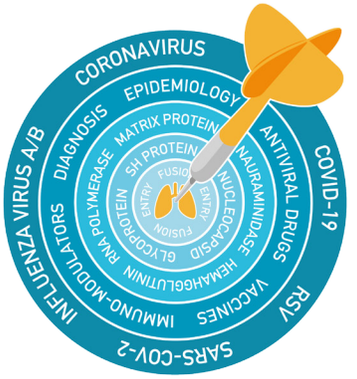

## Abstract RO1

### Recovery of infectious SARS-CoV-2 from fecal specimens

Leda Bassit[1,2,3], Mohammad Salman[1], Jessica M Ingersoll[2,4], Nils Schoof[2,4], Heather B Bowers[1,2,3], Ethan Wang[2,4], Anne L Piantadosi[2,4], Anuradha Rao[2,3], Ben Lepene[5], Liu Pengbo[6], Christine L Moe[6], Raymond F Schinazi[1,2,3]

[1]Laboratory of Biochemical Pharmacology, Department of Pediatrics, Children's Healthcare of Atlanta, Atlanta, GA, USA; [2]The Atlanta Center for Microsystems-Engineered Point-of-Care Technologies, Atlanta, GA, USA; [3]Center for Childhood Infections and Vaccines of Children's Healthcare of Atlanta and Emory University, Atlanta, GA, USA; [4]Department of Pathology and Laboratory Medicine; Emory University School of Medicine, Department of Pediatrics, Atlanta, GA, USA; [5]Ceres Nanosciences, Inc, Manassas VA, USA. [6]Emory University Rollins School of Public Health.

**Background**
SARS-CoV-2 RNA has been found in the stools of patients with COVID-19 who have had gastrointestinal symptoms, including nausea, vomiting, and diarrhea, which are common in up to 15% of the cases. We aimed to investigate whether SARS-CoV-2-positive stools and wastewater are viable and can be cultured in Vero or Caco-2 cells. Aim 1. We tested eight clinical stool samples and four positive wastewater samples for SARS-CoV-2 for their potential to have infectious virions. Aim 2. We spiked lab-propagated SARS-CoV-2 (USA-WA-1, Delta, or Omicron) into negative human stools to test the recovery of the virions from the stool samples to infect permissive cells after three different incubation temperatures.

**Methods**
Samples were filtered using 0.22 µm filter tubes to remove debris. RT-PCR for N1 and N2 genes, ddPCR, and sequencing were performed. Infectivity foci forming assays and ELISpot readout were performed in three cell types.

**Results**
None of the wastewater samples were culturable. One of seven clinical stool samples was sequenced of Delta sub-lineage AY.25 ($1.25 \times 10^6$ GE/ml) post-filtration, with a Ct of ~24. However, no increase in SARS-CoV-2 RNA levels was observed in the infectivity assays. In contrast, infectious virus was recovered from stools spiked with SARS-CoV-2, including wild type (WT), Delta, and Omicron. WT and Delta were contagious to susceptible cell types under different incubations. In contrast, infectious Omicron was present only in Vero cells after being recovered from stool kept at -80°C, immediately after spiking, or in Vero-TMPRSS2 cells after being held at 4°C.

**Conclusions**
Complete SARS-CoV-2 genome sequencing from a positive clinical stool indicates the existence of the intact virus in this sample. Infectious SARS-CoV-2 was recovered from spiked stool samples, suggesting the possibility of viable virions in the wastewater or stool specimens.

## Abstract RO2

**Development of human lung-like macrophages from primary blood monocytes: A new platform for experimental studies of respiratory virus infection**

Justin Hosten[1,2], Sujin Lee[1], Diego Moncada Giraldo[1], Brian S Dobosh[1], Vincent D Giacalone[1], Milagros Aldeco[1], Krishnendu Roy[2], Raymond F Schinazi[1], Rabindra Tirouvanziam[1]
[1]Emory University School of Medicine; [2]Georgia Institute of Technology, Atlanta, GA, USA

**Background**
Airway macrophages are a prime target for infection by respiratory viruses, either via direct interaction with surface receptors, or scavenging-dependent mechanisms of virus pinocytosis or infected cell efferocytosis. Airway macrophages derive from fetal progenitors but can also arise from postnatal differentiation of blood monocytes recruited to the airway upon stress. Because of constant evolutionary pressure from pathogens, airway macrophage function differs between humans and rodent models that are often used to model respiratory virus infections. Thus, there is a need for novel experimental platforms enabling dynamic infections of human lung macrophages by viruses in vitro.

**Methods**
We extended prior studies from our group showing transcriptional plasticity and reprogramming of primary human leukocytes upon transmigration (TM) through a differentiated airway epithelium (Margaroli et al. Cell Rep Med 2021; Ford et al. Int J Mol Sci 2021) by combining monocyte TM with subsequent 4-day exposure to macrophage-colony stimulating factor (MCSF) to induce differentiation. We compared these in vitro transmigrated and MCSF-differentiated macrophages to monocytes pre-TM, monocytes pre-TM differentiated with MCSF, and monocytes post-TM without MCSF-induced differentiation by cytometry (for size, granularity, differentiation and scavenger receptor expression), RNASeq (for transcriptomic profiles), and a multiplexed protein array (for immune mediator secretion).

**Results**
We observed significantly increased size, granularity and expression of differentiation markers (CD14: lipopolysaccharide receptor: CD16: low affinity IgG receptor; HLA-DR: class II major histocompatibility complex) and scavenger receptors (CD163; CD206; and MARCO) in TM and MCSF-differentiated cells compared to other culture conditions, mimicking patterns observed in human lung macrophages in vivo. Transcriptional and secretory profiles highlighted distinct effects of TM combined with MCSF-induced differentiation in the functional properties of these cells. We also confirmed that these in vitro-produced lung-like human macrophages had active metabolism, long-term viability, and abilities to proliferate and interact with influenza and coronaviruses.

**Conclusions**
Compared to MCSF-treated blood monocytes traditionally used as human macrophage surrogates in virology and immunology experiments, blood monocytes treated with MCSF after TM exhibited enhanced differentiation and closer properties to human lung macrophages. Because these cells can be mass-produced from any primary blood donor and banked for later use, this new method opens broad avenues not only for generic studies of respiratory virus tropism and pathogenesis in human lung-like macrophages but also for personalized assays of infectivity and drug targeting.

## Abstract RO3

**3D models of the human airway for rapid drug development**

Julia C. LeCher[1], Jihoon Lee[2], Raymond F. Schinazi[1], and Shuichui Takayama[1]

[1]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, [2] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, 30332, USA

**Background**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel zoonotic human respiratory virus and the causative agent of the ongoing worldwide CoVID-19 pandemic. The rapid spread of SARS-CoV-2 through populations with no existing immunity as well as the rise of variants with heightened levels of infectivity, spread, and immune evasion highlight the vital need for platforms for rapid drug development. Our work has centered on the development of 3D primary human respiratory cell culture models as high-throughput screening platforms. 3D models are biomimetic tissues that inform on in vivo responses. Inclusion in the drug development pipeline allows us to discover effective drugs faster in the pre-clinical stage which may serve to reduce costly failures later on. Lung models on the market are low-throughput, costly, and time consuming. To address this, stably-inverted, apical-out organoids were engineered from primary human bronchial and tracheal cells grown in high-throughput screening capacity with a 1-per well manufacture for rapid antiviral compound screening.

**Methods**

Organoids were generated with stable inversion in 386-well hanging drop plates. After organoid formation, basal epithelial cells were induced to differentiate into airway epithelium. Single-cell RNA seq and immunofluorescence (IF) confirmed expression of representative cells of the airway epithelium as well as receptors for SARS-CoV-2. For SARS-CoV-2 studies, organoids were transferred into 96-well plates and infected with SARS-CoV-2 Washington strain. Kinetics studies were performed using 3 MOIs (0.1, 1.0, 10) over 1-3 days. Post infection, organoids were evaluated for SARS-CoV-2 entry via IF and replication via qRT-PCR. For drug development, known compounds (Remdesivir, GS-441524, AT-527, and nirmatrelvir) were screened by dose-response assay (0-20 μM) over 3 days infection with SARS-CoV-2 at an optimized MOI 1.0. As a control, well characterized ALI cultures of human bronchial/tracheal epithelial cells were used. Supernatant and cells were pooled, viral RNA extracted and qRT-PCR with standard curve performed to detect viral genomes and quantify viral load.

**Results**

Organoids reached maturity (full differentiation) after 2 weeks and were viable over 2 months with an average diameter of 477 ± 148 **μM**. From a single donor, ~144K organoids could be formed. RNAseq and IF revealed genes and markers for basal, ciliated, goblet, club, and tuft cells as well as IF identified expression of the SARS-CoV-2 receptors ACE-2 and TMPRSS2. Of note, ACE-2 displayed an in vivo-like restricted expression pattern to the apical face while TMPRSS2 expression was more diffuse. SARS-CoV-2 readily infected organoids and segregated to ACE-2:TMPRSS2 co-expressing cells. Replication over 3 days yielded a significant increase in virus yield that was ~0.5 log less than control ALI cultures. For all compounds screened, calculated effective concentrations were similar to what was obtained in ALI. Remdesivir - $EC_{50}$ 0.3 organoid vs 0.7 ALI; GS-441524 - $EC_{50}$ 1.1 organoid vs 1.0 ALI; AT-527 - $EC_{50}$ 1.3 organoid vs 0.6 ALI; Nirmatrelvir- $EC_{50}$ 0.07 organoid vs 0.04 ALI.

**Conclusion**

Collectively, our data validate these apical-out lung organoids from primary human tissues as a high-throughput platform for rapid and reliable evaluation of new compounds in development.

## Abstract RO4

**Design of a Ligand-Targeted Immunotherapy for Treatment of Influenza Virus Infections**

Imrul Shahriar,[1] Mohini Kamra,[1] Charity Lynn Campbell,[1] Ananda K Kanduluru,[2] Madduri Srinivasarao,[1,2]and Philip S Low[1]*

[1]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA.

[2]Eradivir, Inc., 1281 Win Hentschel Blvd, West Lafayette IN 47906, USA.

**Background**

Nearly 10% of world's population is affected by influenza virus infections. Based on the most recent report from Centers for Disease Control and Prevention, more than 35 million Americans were infected during the 2019-2020 flu season, with 380,000 patients requiring hospitalization. While flu vaccines are widely available, they were only 39% effective during the 2019-2020 season. Approved drugs such as Tamiflu and Xofluza are only effective when taken in the early stages of infection. Therefore, there is an unmet need for developing novel flu therapeutics, especially for treating later stages of infection.

**Methods**

Herein we report a targeted therapy with a dual mechanism of action that elicits a host immune response against both the virus and virus-infected cells. Because neuraminidase is expressed on both the viral envelope and infected host cell surface, we repurposed the neuraminidase inhibitor zanamivir for use as the targeting ligand. We then linked zanamivir to two distinct haptens that bind to two different naturally occurring antibodies in humans. Once recruited, these anti-hapten antibodies recruit the innate immune system to kill both virus and virus-infected cells. Even though we recently showed remarkable antiviral activity with a zanamivir-mono-hapten conjugate, more recently we investigated whether a dual-hapten conjugate might exhibit greater antiviral potency.

**Results**

When tested in BALB/c mice supplemented with physiological levels of human IgG (IVIG) and infected with 10x$LD_{50}$ of influenza A virus (H1N1, A/Puerto Rico/8/1934), our zanamivir-dual hapten conjugate demonstrated superior antiviral activity than the mono-hapten conjugates at both early and late-stage infection. More importantly, a single dose of our dual hapten conjugate showed much better activity in late-stage infections than daily doses of the standard-of-care drugs, Tamiflu and Xofluza. In addition, the dual hapten conjugate also caused a significantly faster reduction of viral titer in the lungs of infected mice.

**Conclusions**

Our zanamivir-targeted dual hapten immunotherapy has the potential to treat both early and late-stage influenza infections more effectively and rapidly than current standard-of-care drugs. Further testing in more clinically relevant ferret models, however, will be necessary to confirm the above benefits.

## Abstract RO5

### Development of highly potent SARS-CoV-2 $M^{pro}$ Inhibitors Containing P1' Fluorobenzothiazole Moiety

Nobuyo Higashi-Kuwata[1], Kohei Tsuji[2], Hironori Hayashi[3], Haydar Bulut[4], Masaki Imai[5], Shogo Misumi[6], Yoshihiro Kawaoka[5,7], Hirokazu Tamamura[2], and Hiroaki Mitsuya[1,4,8]

[1]National Center for Global Health and Medicine Research Institute (NCGMRI), Tokyo 162-8655, Japan; [2]Tokyo Medical and Dental Univ, Tokyo 101-0062, Japan; [3]Tohoku Univ, Sendai 980-8572, Japan; [4]National Cancer Institute, NIH, Bethesda, MD 20892, USA; [5]Research Center for Global Viral Diseases, NCGMRI, Tokyo 162-8655, Japan; [6]Kumamoto Univ, Kumamoto 862-0973, Japan; [7]Univ of Wisconsin-Madison, Madison, WI 53711, USA; [8]Kumamoto Univ Hospital, Kumamoto, 860-8556, Japan

**Background**
Halogenation of certain compounds often enhances their lipophilicity, potency, cell membrane penetration, and oral bioavailability by forming strong directional interactions. SARS-CoV-2's main protease ($M^{pro}$) has proven to be an attractive target for the development of therapeutics for COVID-19.

**Methods**
Based on the antiviral and structural features of a benzothiazole-containing $M^{pro}$ inhibitor 5h (Hattori & Mitsuya, *Nat Commun* 2021), we designed and synthesized a variety of halogenated analogs and determined their virological, enzymological, and pharmacological features. Structural analyses of such compounds with their target $M^{pro}$ were also conducted.

**Results**
We identified TKB-245 and TKB272, both of which contain P1' fluorobenzothiazole with difference in positioning fluorines, specifically inhibit the enzymatic activity of $M^{pro}$ of SARS-CoV-2, potently block the infectivity and replication of various SARS-CoV-2 strains with the $EC_{50}$ values of 30 and 7 nM, respectively, and significantly block the replication of the Delta and Omicron variants without recognizable adverse effects in the lung of human-ACE2 receptor-knocked-in mice. As determined in human liver-chimeric (PXB) mice without ritonavir, oral bioavailability values of TKB245 and nirmatrelvir were 48 and 56%, and their $T_{1/2}$ values were 3.8 and 1.0 hr, respectively. Native mass spectrometric analysis revealed that most $M^{pro}$ molecules exist in the form of single protomer and the addition of TKB245 and TKB272 promoted $M^{pro}$ protomer dimerization with one or two such molecules bound per dimer. X-ray crystallographic analysis showed that both TKB245 and TKB272 covalent bond with the catalytic amino acid Cys-145, form additional interactions inside the active-site $M^{pro}$'s compared to nirmatrelvir, with the fluorine atom of the benzothiazole moiety pointed to solvent or toward inside the active site cavity.

**Conclusions**
The general order of anti-SARS-CoV-2 potency against SARS-CoV-2 strains was: TKB272 >> TKB245 > nirmatrelvir > molnupiravir. The present data warrant that TKB245 and TKB272 be considered as potential therapeutics for COVID-19 and shed light upon further optimization to develop more potent and safer therapeutics for treating COVID-19.

## Abstract RO6

**Performance of serum liver biomarkers and algorithm combining plasma nuclear magnetic resonance (NMR) biomarkers as indicators of risk for future severe SARS-CoV-2 infection disease in the UK Biobank cohort (UKB).**

Thierry Poynard[1,2,3], Olivier Deckmyn[3], Valentina Peta[3], Fabienne Drane[3], Jean Marie Castille[3], Chantal Housset[4], Dominique Thabut[5] and Patrice Cacoub[6]

[1]Hepato-Gastroenterology, APHP Pitié-Salpêtrière, [2]Sorbonne University, Inserm UMR S938, [3]Research, Biopredictive, [4]Hôpital Pitié Salpêtrière, APHP Paris, France & Inserm UMR S938, CDR Saint-Antoine & Institute Cardiometabolism Nutrition (ICAN), [5]Sorbonne University, Brain-Liver Pitié-Salpêtrière Study Group (BLIPS), [6] Internal Medicine and Clinical Immunology, Internal Medicine APHP, Pitié Salpêtrière, Paris France.

**Background**

Recently the low serum level of several liver biomarkers, apolipoprotein-A1 (ApoA1) (Poynard 2020), total bilirubin (Nocentini 2022), ALT (Wang 2021), and albumin, have been associated with the susceptibility to future severe SARS-CoV-2 infection. No study has assessed if these factors were cumulative. The release of NMR biomarkers data at scale in the UKB permitted to assess if metabolic parameters could permit to construct new algorithms for personal assessment of risk.

**Methods**

Between 2006 and 2010, the UK Biobank recruited a total of 502,411 participants (Pts) who were between 40 and 70 years (yrs) of age. Subsequent to the COVID-19 (C19) pandemic in 2020, the UKB began releasing the results of PCR-based tests for SARS-CoV-2 infection, primarily restricted to symptomatic Pts admitted to one of the National Health Service hospitals and interpreted as a surrogate for a severe case of COVID-19 in a subset of 168,350 Pts. Characteristics and biomarkers were measured at enrollment. Here we used the most studied cutoffs in the recent publications. A subset of 39,388 Pts had plasma NMR. Also a total of 30,553 Pts at least once PCR-positive were included, median (Interquartile range) age 53 (46-61), 47% males, 29% BMI>=30kg/m2, 3.8% glucose >=7mmol/l. AUROC, Kaplan-Meier survival, regression analysis and Cox models were used, the main endpoint being first PCR positive or death from C19, adjusted on age separately for females and males and at 15 yrs (PCR-C19). 321 serum biomarkers and plasma NMR components were tested in combinations, k-fold randomized construction permitted internal validation of algorithms.

**Results**

Statistics were detailed in Table 1. At least one biomarker with low-level was present at inclusion for 56% of females and 55% of males. In 84,959 females 15,153 PCR-C19 (20%) occurred. Pts with the previously recommended ApoA1 cutoff >=1.25g/L had a 6% higher survival without PCR-C19, than Pts <1.25g/L. Similarly Pts with the recommended cutoff for Albumin >=38g/L had a 10% differences of survival without PCR-C19. Differences were lower but significant for low-ALT cutoff (2%) and not significant for low-Bilirubin in females, the 5 umol/L cutoff being inappropriate due to lower Bilirubin production. In multivariate analysis only albumin added clinically significant prognostic value to the age at inclusion. Among the 253 NMR components the following increased significantly: HDL-C .53 (.39-.71;P<.001), ApoA1 2.42(1.60-3.65;P=<.001), DHA .09(.03-.26;P<0.001), Lactate 1.06(1.03-1.09;P<.001), and Omega3% 1.06(1.00-1.11;P=.04). In 72,465 males 15,854 PCR-C19 (22%) occurred. The differences were more clinically relevant for low-bilirubin at the 5 umol/L cutoff with 8% lower survival, and for low ApoA1 (-6%) and added significant prognostic value to age in multivariate analysis. Among the 253 NMR components the following increased significantly: Albumin .98(.97-.99;P<.001), Lactate 1.06(1.03-1.10;P<.001) and GlycA 1.57(1.17-2.10;P=.002). Main limitations are the Caucasian origin and the absence of external validation.

**Conclusions**

Apparently healthy subjects with at least one low-level value of ApoA1, Bilirubin, Albumin or ALT at inclusion had a 5% higher risk of severe Covid-19 15 years later. Algorithms combining NMR components with simple liver biomarkers could improve the identification of such subjects prior to future pandemic. A consensus on the low-level of Bilirubin is needed.

## Abstract RO6

**Table 1.**

| | Cutoff | 15-yrs % survival (95%CI) without PCR+ or C19 death | P-value | Cox Risk Ratio age adjusted | P-value |
|---|---|---|---|---|---|
| **84,959 Females** | | | | **95% CI** | |
| ApoA1 g/L | <1.25 | 42 (36-47) | .0001 | 1.1(1.1-1.1) | .02 |
| | >= 1.25 | 50 (49-51) | | | |
| Albumin g/L | <38 | 44 (34-53) | .0001 | 1.4 (1.2-1.7) | **.0001** |
| | >= 38 | 54 (53-55) | | | |
| Bilirubin umol/L | <5 | 53 (52-54) | .002 | 1.0 (1.0-1.0) | .44 |
| | >= 5 | 55 (54-56) | | | |
| ALT IU/L | <18 | 53 (52-55) | .002 | 1.0 (1.0-1.0) | .84 |
| | >= 18 | 55 (54-56) | | | |
| **Sum Low levels** | >0 | **53 (52-54)** | **.00001** | **1.1 (1.0-1.1)** | **<.0001** |
| | 0 | 58 (57-59) | | | |
| **72,204 Males** | | | | | |
| ApoA1 g/L | <1.25 | 45 (43-47) | .0001 | 1.1 (1.1-1.1 | **.0001** |
| | >= 1.25 | 52 (50-53) | | | |
| Albumin g/L | <38 | 42 (28-55) | .005 | 1.4 (1.1-1.8) | .01 |
| | >= 38 | 50 (49-51) | | | |
| Bilirubin umol/L | <5 | 42 (36-47) | .0001 | 1.2 (1.1-1.3) | **.0006** |
| | >= 5 | 50 (49-51) | | | |
| ALT IU/L | <23 | 52 (50-53) | .63 | 1.0 (1.0-1.1) | .03 |
| | >=23 | 49 (47-50) | | | |
| **Sum Low levels** | >0 | **49(48-51)** | **.00001** | **1.1 (1.1-1.1)** | **<.0001** |
| | 0 | 54 (52-55) | | | |

## Abstract RO7

**Development of small molecule entry inhibitors of influenza A viruses**

Lijun Rong
Department of Microbiology and Immunology, College of Medicine,
University of Illinois at Chicago, Chicago, IL 60612, USA

**Background**
Seasonal or pandemic flu caused by influenza A viruses (IAV) is a major public health concern due to the high morbidity and significant mortality, especially in high-risk population groups. Although there are several classes of drugs targeting different viral proteins, emergence of drug resistance strains calls for a continuous search for new drug candidates that can be used alone or in combinations.

**Methods**
We are developing small molecule inhibitors targeting IAV hemagglutin (HA) proteins. Since viral HA proteins are classified into two disitinctive groups (group 1 and group 2), and small molecule inhibitors generally block either group 1 or group 2, but not both, we are developing two classes of inhibitors specifically tageting either group 1 or group 2 HA proteins. The initial hit compounds were idnetified by high-throuput screens, and the lead inhibitors were chemically optimized and evaluated both *in vitro* and *in vivo*.

**Results**
We have developed a series of lead compounds which are highly potent against IAV infection *in vitro*. Further, we have developed an effacious lead inhibitor that can be orally delivered and it works syngistically with the clinical drugs targeting other viral proteins. These lead inhibitors are promising drug candidates against IAV infections.

**Conclusions**
These lead candidates have potential to be developed as novel antivirals against IAV inffection and diseases.

## Abstract RO8

**Molnupiravir exhibits a high barrier to the development of SARS-CoV-2 resistance in vitro**

Julie M. Strizki[1], John Gaspar[1], Nick Murgolo[1], John Howe[1], Beth Hutchins[1], Hiroshi Mohri[2], David Ho[2], Daria J. Hazuda[1], Jay A. Grobler[1]
[1]Merck & Co., Inc., Rahway, NJ, USA, [2]Aaron Diamond AIDS Research Center, Columbia University Medical Center, New York, NY, USA

**Background**
Molnupiravir is an orally available prodrug of the antiviral nucleoside analog N-Hydroxycytidine (NHC). In preclinical studies NHC has shown broad-spectrum antiviral activity against multiple RNA viruses including SARS-CoV-2. Incorporation of NHC by viral polymerases impairs replication by introducing errors into the viral genome. NHC has been shown to have a high barrier to the development of resistance in vitro with RSV, Influenza and Venezualen Equine Encephalitis viruses. In these studies, we have explored the potential for SARS-CoV-2 to develop resistance to NHC in cell culture.

**Methods**
Vero E6 cells were infected with SARS-CoV-2 (WA-1) in triplicate in the presence of NHC or a C3L-protease inhibitor (MRK-A). Culture supernatants from wells with the highest drug concentration exhibiting a cytopathic effect (CPE) score of ≥ 2+ were repassaged and at each passage, IC50 values were estimated based on CPE scoring. At each passage, full genome next generation sequencing (NGS) was performed on the viral RNA.

**Results**
No change in susceptibility to NHC (EC50 fold change ≤ 1.1) was noted in 2 of 3 cultures and a 2-fold change was observed in one culture after 30 passages. In contrast, a 3- to 4-fold decreases in susceptibility to the 3CL protease inhibitor were seen by passage by 12, with increasing resistance of 4.6- to 15.7-fold observed by passage 30. NHC passaged viruses exhibited 53 to 99 amino acid changes, including substitutions and deletions (both in-frame and frameshift), across 25 different viral proteins as compared with 10 to 13 changes in 13 proteins in the MRK-A cultures. With NHC, 3 to 4 changes were observed in the viral polymerase; however, these were randomly distributed, and none were observed more than once. In contrast, the 3CL protease passaged virus had a nsp5 T21I substitution detected in all 3 cultures.

**Conclusions**
No evidence of SARS-CoV-2 phenotypic or genotypic resistance was observed following 30 passages with NHC. A random pattern of protein changes were observed across multiple proteins consistent with the mechanism of action of NHC. In the same study, resistance was readily selected to a control 3CL protease inhibitor. Together these data support previous reports demonstrating the high barrier to resistance of NHC.

## Abstract RO10

### Differential antibody kinetics following SARS-CoV-2 infection, primary vaccination, and booster vaccination in an at-risk, longitudinal cohort

Erin Williams[1,3], Alexander Kizhner[2], Juan Manuel Carreño[6,7], Michael Hoffer[1,5], Florian Krammer[6,7], Suresh Pallikkuth[2], Savita Pahwa[2]

[1]Department of Otolaryngology, University of Miami Miller School of Medicine, [2]Department of Microbiology and Immunology, University of Miami Miller School of Medicine, [3]Department of Biomedical Engineering, University of Miami, [4]University of Miami Miller School of Medicine, [5]Department of Neurological Surgery, University of Miami, Miller School of Medicine, [6]Department of Microbiology, Icahn School of Medicine at Mount Sinai, [7]Department of Pathology, Molecular and Cell-based Medicine, Icahn School of Medicine at Mount Sinai

**Background**
Understanding the SARS-CoV-2 infection-induced and vaccine-induced humoral immune response is critically important in guiding future vaccine development and ensuring vaccine uptake. In this work, our primary aim was to characterize differential antibody kinetics in response to natural infection, primary vaccination, and booster vaccination in a high-risk, ethnically diverse, longitudinal cohort. We also investigated relationships between antibody breadth and durability in the context of breakthrough infection.

**Methods**
Following written informed consent (IRB #20201026), healthcare workers and other individuals (n=228) at high-risk for adverse SARS-CoV-2 outcomes participated in study-specific blood draws at regular intervals. Demographics were collected at baseline. Additional visits were scheduled following antigenic challenges, to include COVID vaccine receipt and/or breakthrough infection. Linear mixed effects models (LMM) were generated to compare differential antibody responses following natural infection, primary vaccination with or without a history of SARS-CoV-2 prior to vaccine receipt and following additional doses. To examine the efficacy of a third, so-called "booster" dose, we constructed a linear model comparing antibody titers at the nearest timepoint before booster dose administration and ≥14 days after the third mRNA vaccine dose. Logistic regression was used to predict booster dose responders (≥2-fold change increase) by modelling fixed effects of SARS-CoV-2 infection prior to vaccination, vaccination manufacturer combinations across doses, and demographic factors.

**Results**
Following primary vaccination, participants with a prior history of COVID-19 (n=33) had a higher peak antibody response relative to those who were COVID-19 naive (n=52). Moderna recipients had higher antibodies compared to Pfizer recipients, but no other demographic factors significantly affected the peak antibody response. Following the 3$^{rd}$ dose, the antibody response observed was more robust than that following primary vaccination in any group examined. Antibody decay following the 3$^{rd}$ dose was comparable to the rate observed following primary vaccination, with the exception of the previously infected, unvaccinated participants wherein antibodies deteriorated more readily. Notably, individuals who suffered from breakthrough infection following booster vaccination had -0.622 log lower antibody titers compared to participants who did not, with a median 216 days (range: 61-326) from booster to self-reported breakthrough. Fold-change comparisons following 3$^{rd}$ (n=106) and 4$^{th}$ booster doses (n=26) were also conducted. The median log fold-change following the 3$^{rd}$ and 4$^{th}$ booster dose was 4.00 (SD: 2.14) and 1.58 (SD: 2.08), respectively. Participants were considered booster responders if the vaccine induced a ≥2-fold increase in antibody titers; accordingly, a majority of participants were responders following the 3$^{rd}$ dose (88.7% [94/106]). Interestingly, less than half of those who received a 4$^{th}$ dose (46.2% [12/26]) were deemed responders, suggestive of a possible ceiling effect in individuals with robust circulating antibodies at the time of booster receipt.

**Conclusions**
Booster doses to wild type vaccine antigens induce vigorous antibody responses superior to those following primary vaccination, though these may not be sufficiently protective against mutant variants. Future work is needed to establish the relationship between the humoral and cellular immune responses following vaccination and booster doses on the acquisition of breakthrough infection to best guide future vaccine strategies.

# POSTER ABSTRACTS

Respi DART 2022

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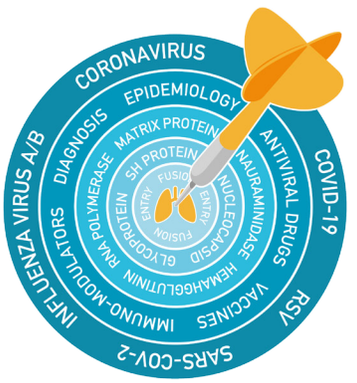

## Abstract RP1

### Systems analysis of lung autopsy tissue reveals role of lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19

Kathie-Anne Walters[1] and Kelsey Scherler[1]
[1]Institute for Systems Biology, Seattle, WA, USA.

**Background**
Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is characterized by respiratory distress, multiorgan dysfunction, and, in some cases, death. The pathological mechanisms underlying COVID-19 respiratory failure and the interplay with aggravating risk factors have not been fully defined.

**Methods**
Post-mortem lung and plasma specimens from eighteen COVID-19 patients, with symptom onset-to-death times (SOTDs) ranging from 3 days up to 47 days, were evaluated using multiplex plasma protein measurements, pulmonary gene expression and fluorescent imaging analyses.

**Results**
Prominent histopathological features in this case series included progressive diffuse alveolar damage with excessive thrombosis and late-onset pulmonary tissue and vascular remodeling. Acute damage at the alveolar-capillary barrier was characterized by significant loss of surfactant protein expression with injury to alveolar epithelial cells, endothelial cells, and respiratory epithelial basal cells. and defective tissue repair processes. Other key findings included i) defective tissue repair processes due to loss of basal cells, ii) impaired clot fibrinolysis with increased concentrations of plasma and lung plasminogen activator inhibitor-1 and iii) modulation of cellular senescence markers, including p21 and sirtuin-1, in both lung epithelial and endothelial cells.

**Conclusions**
These findings further define the molecular pathological features underlying the pulmonary response to SARS-CoV-2 infection and provide important insights into signaling pathways that may be amenable to therapeutic intervention to ameliorate pulmonary damage and/or promote more effective repair of lung epithelium.

## Abstract RP2

**The rise of the variants: Mechanisms of SARS-CoV-2 host immune avoidance**

Michael A Joyce[1,2*], Holly A. Bandi[1,2] Karyn Berry-Wynne[1,2], Justin Shields[1,2], William Addison[1,2], Jamil N. Kanji[3,4,5], Matthew Croxen[3,4,5], John Law[1,2], Gregory J Tyrrell[2,5], Graham Tipples[2,3,5], D. Lorne Tyrrell[1,2*]

[1] Department of Medical Microbiology and Immunology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton T6G 2R3, Alberta, Canada
[2] Li Ka Shing Institute of Virology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton T6G 2E1, Alberta, Canada
[3] Public Health Laboratory, Alberta Precision Laboratories, Foothills Medical Centre, 3330 Hospital Drive NW, Calgary, AB, T2N 4W4
[4] Division of Infectious Diseases, Department of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, T2N 4W4
[5] Division of Diagnostic and Applied Microbiology, Department of Laboratory Medicine and Pathology, University of Alberta, Alberta Precision Laboratories-Public Health, Edmonton, Alberta, Canada, T6G 2J2.

COVID-19 has exacted a huge toll on the world both in morbidity/mortality as well as societal disruption. An enormous scientific and medical response to the pandemic has provided unprecedented access to viral variants and the opportunity to evaluate factors involved in the emergence of successive variants. We investigated the ability of successive variants to avoid the innate and adaptive immune response. Variant growth kinetics, specific infectivity, cytopathic effect, interferon resistance, neutralization by patient antibodies were assessed. We found that all variants replicated best in Vero TMPRSS2 cells and despite the presence of the growth promoting D614G mutation in all of the variants except WT, no variants grew more quickly than the WT Hu-1 strain. The Alpha and Omicron variants grew significantly more slowly and had higher numbers of inactive particles in preparations. Remarkably the ability of variants to avoid the effects of both interferon-$\alpha$ and patient antibodies changed in a progressive manner through the pandemic. Variants that arose early in the pandemic were more resistant to the effects of IFN-a and neutralizing antibodies due to syncytia formation. However, variants that arose later in the pandemic had acquired traits that resulted in the ability to prevent STAT-1 nuclear localization and to genuinely avoid the effects of patient antibodies as is evident on the Omicron and its sub-variants. Additionally, the variants demonstrated progressively less cytopathic effects than the WT strain. Taken together, these data demonstrate the somewhat boring conclusion that pandemic SARS-CoV-2 acquires new traits in the human population at the cost of replicative fitness.

## Abstract RP3

# Identification of inhibitors against the main protease of SARS-CoV-2 using DNA Encoded Chemical Libraries (DECL) technology

Dario Akaberi[1,5], Alexander Craig[2], Lindon Moodie[2], Anja Sandström[2], Oscar Verho[2], Monireh Lati[2], Martin Moche[3], Tomas Nyman[3], Kristian Sandberg[4], Åke Lundkvist[1], Johan Lennerstrand[5]

[1]Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, Uppsala University, Sweden, [2]Department of Medicinal Chemistry, Uppsala University; [3]Protein Science Facility (PSF), Karolinska Institute Sweden, [4]SciLifeLab Drug Discovery & Development platform, Uppsala University; [5]Department of Medical Sciences, Uppsala University, Sweden

**Background and methods:** The SARS-CoV-2 3CLpro (Main protease) is a chymotrypsin-like cysteine protease that is essential for viral replication and is a proven drug target for the treatment of SARS-CoV-2 and possibly other coronavirus infections. To identify 3CLPro inhibitors, we employed DNA Encoded Chemical Libraries (DECL), a unique technology that allows simultaneous screening of large compound libraries against an immobilized drug target. SciLifeLab Drug Discovery & Development platform provided access to DECL selection of binders in the DELopen library provided by WuXiApptec (Shanghai) (> 4 billion compounds), as well as for developing FRET and SPR validation assays. In a traditional medicinal chemistry approach, we also employed peptidomimetic SARS-CoV 3CLPro inhibitors (X77 analogues) as molecular starting points to explore structure activity relationships and guide inhibitor development. We soon expect to identify optimized hit compounds with sub micromolar antiviral activity in cell-based infection model. Promising leads will be developed towards SARS-CoV-2 therapeutics and will be tested against other relevant pathogenic coronaviruses to identify broad-spectrum protease inhibitors.

**Preliminary results:** SARS-CoV-2 protease expressed and purified by Protein Science Facility (PSF, Karolinska Institute), has been successfully used to select six high affinity binders from the DELopen library (>4 billion compounds). Binding and inhibitory activity of selected compounds against SARS-CoV-2 3CLpro was validated using FRET-based fluorogenic protease enzymatic assay. Five out of six compounds were potent inhibitors of SARS-CoV-2 3CLpro with the two best compounds having $IC_{50}$ values of 35 and 50 nM. Both compounds were Co-crystallized in complex with SARS-COV-2 3CLpro to guide further structure-based development. Twenty variants of the most potent compound were synthetized and tested by enzymatic assay providing valuable structure activity relationship (SAR) information. The antiviral activity and cytotoxicity of the compounds is currently under evaluation with cell-based assays at our BSL3 lab (Zoonosis Science Center). In our second approach, over 100 analogues (X77) have been synthesized and evaluated in FRET assays. Starting from an initial hit $IC_{50}$ of 8.2 μM (original X77), our most recent analogue has a sub μM $IC_{50}$, and compounds with low μM $EC_{50}$ values have been validated in cell-based infection assays. The structure activity relationships revealed from these studies, along with a 2.0 Å resolution crystal structure of one X77 analogue in complex with SARS-CoV-2 3CLpro (PSF), are currently guiding the design of a second-generation inhibitor series.

**Conclusion:** We have successfully identified potent sub micromolar inhibitors of SARS-CoV-2 3CLpro using DECL and antiviral activity is currently being evaluated with our cell-based infection model. A second research line focuses on exploring X77 analogues as inhibitors. Our approach has identified hit compounds and structure-activity relationships, important for the development of therapeutics against SARS-CoV-2 and other Coronaviruses.

## Abstract RP4

### Utility of 1% astodrimer sodium nasal spray for pandemic preparedness demonstrated by broad-spectrum antiviral effects and protection against SARS-CoV-2 Omicron, and Influenza A and B virus infection

Philippe Gallay[1], Carolyn A. Luscombe[2], Alex Castellarnau[2], Graham P. Heery[2], Aynaz Seta[2], Michael Bobardt[1], Jeremy R.A. Paull[2]

[1]The Scripps Research Institute, La Jolla, CA, USA, 92307; [2]Starpharma Pty Ltd, Abbotsford, VIC, Australia.

**Background**
The continued spread of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted the need for preventative strategies alongside vaccines and for future pandemic preparedness for the protection of vulnerable populations. Astodrimer sodium is a broad-spectrum antiviral dendrimer that has been developed as a topical nasal spray to protect from infection and reduce transmission of SARS-CoV-2 Variants of Concern and other pandemic-causing viruses, including influenza viruses.

**Methods**
Astodrimer sodium has been evaluated in dose-response antiviral and virucidal studies in Vero E6, Vero E6-TMPRSS2-ACE2 and Calu-3 cells against SARS-CoV-2 Omicron BA.1 (hCoV-19/USA/MD-HP20874/2021), and in MDCK cells against influenza A and B viruses (IAV & IBV). For virucidal evaluations, virus was mixed with astodrimer sodium, incubated for 0.5, 1, 15, and 30 minutes, and then neutralized. Infectious progeny virus was quantitated by plaque assay. Three groups of K18-hACE2 mice (N=12) were inoculated intranasally (IN) with $10^3$ SARS-CoV-2 Omicron PFU on Day 0. Two groups were administered astodrimer sodium nasal spray and the third group received PBS IN. On Day 0, one astodrimer group and the PBS group received the allocated treatment 5 minutes before and after IN virus challenge (pre-/post-exposure); the second astodrimer group was treated only 5 minutes after IN virus challenge (post-exposure). All groups received their respective treatment once daily IN for 6 additional days. Four mice of each group were euthanized on Day 2, 4, and 7 for assessment of viral load by qPCR in blood, nasal secretion, and lung and tracheal tissue homogenates. Inflammatory status was assessed by an ELISA cytokine panel.

**Results**
Astodrimer sodium demonstrated potent antiviral and virucidal activity against Omicron BA.1, IAV and IBV *in vitro*. All animals treated with astodrimer sodium nasal spray before and after Omicron virus challenge had no detectable virus in lung, trachea or nasal cavity up to 4 days after exposure to virus. Viral load in lung and trachea of these astodrimer sodium-treated animals was reduced by >99.999% compared with the virus levels in PBS-treated animals when assessed 7 days after viral challenge. Astodrimer sodium nasal spray treatment pre- and post-infection resulted in 10/12 animals having no evidence of virus replication in the lung, trachea, nasal secretion, and serum at Day 7. Animals treated with astodrimer sodium only after IN viral Omicron challenge also exhibited >99.999% reduction of virus in lung and trachea 7 days after infection compared with PBS control. All astodrimer sodium-treated animals also exhibited a significant reduction in proinflammatory cytokines compared with PBS, indicating reduced severity of disease.

**Conclusions**
Astodrimer sodium was active against SARS-CoV-2 Omicron, and IAV and IBV *in vitro*. The broad-spectrum antiviral nasal spray protected against infection with the highly transmissible SARS-CoV-2 Omicron variant in a viral challenge model, even when used only after exposure to virus. The broad-spectrum activity and ability to protect against infection *in vivo* suggest astodrimer sodium nasal spray has a potential role for pandemic preparedness strategies.

## Abstract RP5

### Design of solid materials with anti-SARS-CoV-2 activity

Victoria G. Snider[1], Julia C. LeCher[2], Fahad Salman[1], Jessica Downs-Bowen[2], Tamara McBrayer[2], Raymond F. Schinazi[2], and Craig L. Hill[1]
[1]Department of Chemistry and [2]Pediatrics, Emory University, Atlanta, GA 30322, USA

**Background**
To prevent the spread of COVID-19, virucidal thin-film coatings containing polyoxometalates (POMs) have been fabricated. POMs are a class of oxygen-rich inorganic compounds with potent antiviral activity against certain viruses (DOI: 10.1021/cr960396q) .

**Methods**
The three POMs chosen for this study were selected due to high anti-CoV-2 activity and simultaneous low cytotoxicity in Vero cells: $K_8[\beta_2\text{-}SiW_{11}O_{39}]$ (**POM 1**), $K_{11}H[(VO)_3(SbW_9O_{33})_2]$ (**POM 2**), and $K_5[Co(III)W_{12}O_{40}]$ (**POM 3**).

**Results**
To fabricate the reactive solid polymeric coatings, anionic POMs were combined with cationic *N*-methylated polyethyleneimine (m-PEI) and polyvinylidene fluoride (PVDF) via non-solvent induced phase separation (NIPS). PVDF thin-films are highly attractive due to good thermal and chemical stability and excellent mechanical strength. The films were characterized via scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX), thermal gravimetric analysis (TGA), and Fourier Transform Infrared spectroscopy (FTIR).
**Conclusions:** Cytotoxicity and antiviral evaluation of the POM compounds against SARS-CoV-2 in Vero Cells will be reported in addition to the antiviral activity of the POM/polymer composite thin-films.

## Abstract RP6

**Saliva COVID-19 PCR testing for asymptomatic individuals in Japan, and the viral genome analysis**

Authors
Junko S. Takeuchi[1], Yukumasa Kazuyama[2], Teiichiro Shiino[1], Hajime Ohyanagi[1], Shigeru Yoshida[3], Kiyomi Sugawara[2], Minoru Kato[2], Kei Sato[4], Moto Kimura[1], Masato Ikeda[2], Wataru Sugiura[1]
Affiliations
[1] Center for Clinical Sciences, National Center for Global Health and Medicine
[2] SB Coronavirus Inspection Center Corp.
[3] Health Sciences University of Hokkaido
[4] The Institute of Medical Science, The University of Tokyo

**Background**
Since the beginning of the COVID-19 pandemic in January 2020, Japan has experienced seven epidemic waves, with 21 million infected and 45 thousand death cases. The difficulties in coping with this pandemic are those SARS-CoV-2 acquires mutations and changes its clinical features drastically, and emerging variants can drive new waves of infection. Secondly, many asymptomatic cases remain uncaptured, and their role in the transmission network is uncleared. However, there are few reports of longitudinal analysis of SARS-CoV-2 variants derived from asymptomatic individuals. To understand the complete picture of the SARS-CoV-2 epidemic and the virological features of asymptomatic cases, we performed viral full genome analysis using the saliva samples from asymptomatic COVID-19 individuals in Japan.

**Methods**
Total RNA was extracted from saliva samples, followed by followed by cDNA synthesis, target amplification, library preparation using Illumina COVIDseq, and SARS-CoV-2 genome sequencing on Illumina NextSeq 2000. The resulting sequences were applied to the Nextstrain program for the phylogenetic analysis. Worldwide viral genome sequences (n=973) were obtained from GISAID database as control derived from symptomatic individuals.

**Results**
Of the 4,035,524 asymptomatic cases examined between July 2020 and February 2022, 4,297 (0.11%) cases were positive for COVID-19 PCR testing (Ct values < 40), including 169 vaccine breakthrough infections. All positive samples (n=4,297) were sequenced on Illumina COVIDseq, of which 905 (21.1 %) full-genome sequences (>29,000 bp) were determined. Samples with lower Ct values were more likely to determine full-genome sequences (mean Ct values for “full-genome sequenced” and “not determined” were 28.9 and 33.9, respectively [$p<0.0001$]). The 20B, 20I/Alpha, 21J/Delta, and 21K/Omicron were dominant in the 3$^{rd}$, 4$^{th}$, 5$^{th}$, and 6$^{th}$ waves in Japanese asymptomatic cases, consistent with data derived from Japanese symptomatic cases registered in GISAID. Interestingly, 20A was the second dominant strain in the 3$^{rd}$ wave of asymptomatic cases, which is rarely observed in the Japanese GISAID sequences.

**Conclusions**
We demonstrated that non-invasive saliva sample is useful for epidemiological and viral evolutionary studies, even though asymptomatic cases had low Ct values (mean value is 33.0). Further analyses are warranted to determine epidemiological significance of the asymptomatic individuals.