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CROI 2023: Acute and Post-Acute COVID-19

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Abstract: *Studies of acute and post-acute COVID-19 were presented at the 2023 Conference on Retroviruses and Opportunistic Infections (CROI). Early treatment with ensitrelvir, a novel protease inhibitor, hastened viral clearance and symptom resolution during coronavirus disease 2019 (COVID-19) and appeared to reduce the prevalence of long COVID symptoms. The development of novel agents against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including those with broader sarbecovirus activity such as anti-angiotensin-converting enzyme 2 monoclonal antibodies, is underway. A growing understanding of the pathophysiology of long COVID has provided several potential therapeutic targets for individuals experiencing this condition. Efforts to understand COVID-19 in people with HIV have led to novel insights into the biology and natural history of SARS-CoV-2 coinfection in this vulnerable subpopulation. These and other studies are summarized herein.*

Keywords: coronavirus disease 2019, COVID-19, severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, post-acute sequelae of SARS-CoV-2, PASC, long COVID, HIV

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Introduction

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in early 2020 disrupted all aspects of life around the globe, had a major impact on preexisting research activities, and led to the rapid implementation of new scientific endeavors to understand the epidemiology, natural history, pathophysiology, and management of coronavirus disease 2019 (COVID-19) and, more recently, its post-acute consequences. Many HIV and infectious diseases scientists have led these efforts. This article highlights new research on acute and post-acute SARS-CoV-2 infection, including in people with HIV, presented at the 2023 Conference on Retroviruses and Opportunistic Infections (CROI).

Acute COVID-19

Epidemiology and Natural History of COVID-19

The risk factors for poor outcomes in acute COVID-19 have become clear. These include older age; medical comorbidities such as diabetes, obesity, and pregnancy; not being previously vaccinated against SARS-CoV-2; and lack of treatment during the acute phase of the infection. [1-3] However, questions remain about the risk of severe disease with recent and emerging Omicron subvariants. Cloherty and colleagues examined clinical outcomes by SARS-CoV-2 variant in Chicago and demonstrated attenuation of severity with Omicron subvariants compared with prior variants of concern (VOCs) (Abstract 889). Hospitalization and death rates were highest with the Delta variant, and those rates continued to decline when Omicron subvariants were prevalent.

Over the past 3 years, several groups developed in-hospital risk prediction algorithms to aid in clinician decision-making and counseling during hospitalization for COVID-19.[4,5] Parczewski and colleagues incorporated an artificial neural networks (ANN) analysis of computed tomography (CT) chest scans into a prediction model that also included laboratory and clinical variables, to identify the best predictors of poor hospital outcomes (Abstract 731). They identified an ANN-assigned percentage of lung involvement of greater than 50% and age over 80 years as the most significant risk factors predicting poor hospital outcomes from acute COVID-19. This suggests that the addition of ANN-analyzed chest CT scan scores could improve automated risk prediction models.

Two studies further validated the use of nucleocapsid (N)-antigen levels in the peripheral circulation as a biomarker predicting severe outcomes from COVID-19. Jain showed that higher N-antigen levels in blood during early hospitalization were associated with elevated risk for death within 90 days (hazard ratio [HR], 4.4; 95% confidence interval [CI], 3.2-5.9) and reduced incidence of sustained recovery through day 90 (Abstract 728). This suggests a pathogenic role for viremia, and importantly it identifies a group of hospitalized people who did not have high oxygen requirements but were still at high risk

for poor outcomes. Studying the placebo arm of the ACTIV-2 trial, Jilg and colleagues evaluated the association of anti-spike immunoglobulin G (IgG) and N-antigen in plasma with clinical outcomes in 229 nonhospitalized people with mild to moderate COVID-19 at risk for severe outcomes (Abstract 283). They found that absence of anti-spike antibody and higher levels of plasma N-antigen predicted hospitalization or death and delayed symptom improvement in COVID-19 outpatients. Taken together, these studies show a potential role for measurement of these biomarkers in some individuals during the acute phase of infection.

Pathogenesis and Immune Responses

Several groups described interactions between SARS-CoV-2 and the innate immune system. Bouhaddou and colleagues profiled mRNA, protein, phosphorylation, and virus–host protein–protein interactions in Calu-3 cells after infection with several VOCs (Abstract 108). VOCs alter viral RNA and protein production, evolve altered N phosphorylation, and differentially regulate host inflammatory responses. Most VOCs antagonize interferon-stimulated gene (ISG) induction, and the Omicron subvariant BA.5 showed a strengthened antagonism of innate immunity compared with subvariant BA.1. This may be the reason why Suryawanshi and colleagues found that subvariant BA.5 replicated to higher titers and more frequently led to lethal infection in keratin 18 (K18)-human (h) angiotensin-converting enzyme 2 (ACE2) mice (Abstract 275). Similarly, Shi and colleagues demonstrated that Omicron strains exhibited resistance to type I and type III interferons in primary nasal epithelial cells and provided evidence that this may be related to a novel route of cellular entry compared with older variants (Abstract 232). Puray-Chavez and colleagues showed that SARS-CoV-2 is restricted by basally active cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING), a DNA sensing pathway that underlies the basally high interferon pathway activity seen in airway-derived cells lines that do not support SARS-CoV-2 replication despite ACE2 cell surface expression (Abstract 224). Together, this work demonstrates that interferons, shown to be a determinant of acute COVID-19 disease severity,[1,6] are important for restriction of SARS-CoV-2 replication, and that new variants are evolving resistance to host interferon responses.

The role of natural killer (NK) and dendritic cells in SARS-CoV-2 pathogenesis is still being investigated. Balachandran and colleagues provided evidence with a nonhuman primate model that NK cells are important for clearance of virus from the pharynx and lung, with peak NK cell activity 10 days post infection (Abstract 340). Saini and colleagues extended this narrative into humans by demonstrating that COVID-19 hospitalization is associated with dysfunctional NK cells with low expression of CD16 (Abstract 341). They found that Siglec-9-defined NK cell subpopulations are highly cytotoxic against SARS-CoV-2.

Pickering and colleagues provided evidence to support the hypothesis that Fc-receptor–mediated infection of myeloid cells by SARS-CoV-2 may be responsible for the late production of

proinflammatory cytokines that characterizes severe COVID-19 (Abstract 231). They used a THP-1 human leukemia monocytic cell model system to show that spike receptor-binding domain (S-RBD)-specific monoclonal antibodies promoted infection of monocytes at subneutralizing concentrations and provided evidence for productive infection of primary macrophages via this mechanism. Cai and colleagues also used in vitro systems to demonstrate that plasmacytoid dendritic cells (pDCs) can sense SARS-CoV-2 infected cells, and that direct contact between infected cells and pDCs is required for type I interferon production (Abstract 342).

Many viruses evolve mechanisms to evade host restriction factors or to repurpose host cell machinery to support replication. Shi and colleagues demonstrated that subsequent SARS-CoV-2 variants become increasingly more efficient at downregulating BST2/tetherin, a transmembrane protein that prevents release of viruses after assembly in the host cell (Abstract 223). This downregulation of BST2 is due to mutations in spike that route BST2 for lysosomal degradation.

Two groups investigated the importance of SARS-CoV-2 non-structural protein 6 (NSP6) in viral pathogenesis. Chen and Serra-Moreno showed that SARS-CoV-2 uses NSP6 to remodel endosomal membranes, recruit them to perinuclear locations, and generate replication organelles required for efficient viral replication (Abstract 234). Taha and colleagues used a novel replicon system to characterize Omicron replication independent of spike and found that mutations in NSP6 lead to lower viral replication (Abstract 233).

Treatment Options

The development, testing, and authorization of therapeutics for COVID-19 proceeded at a rapid pace in the first years of the pandemic but have since slowed. Treatment guidelines have not substantially changed in the past year, with the exception of the removal of authorized monoclonal antibody products, which are predicted to have no activity against currently circulating Omicron subvariants. Excitingly, several abstracts reported on novel therapeutics in the pipeline, whereas other abstracts provided a deeper characterization of current therapeutics. Chew presented a detailed summary of the state of COVID-19 outpatient therapeutics (Abstract 31).

Protease inhibitors. Uehara and colleagues reported phase III results from SCORPIO-SR (Efficacy and Safety of Ensitrelvir in Patients With Mild-to-Moderate COVID-19: A Protocol for a Multicenter, Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study), a multicenter, randomized, double-blinded, placebo-controlled trial of ensitrelvir in people with mild-to-moderate COVID-19 within 5 days of symptom onset (Abstract 166). Ensitrelvir is a novel oral SARS-CoV-2 3C-like (3CL) protease inhibitor that does not require ritonavir boosting and has an active emergency use authorization (EUA) in Japan. In this population of mostly vaccinated people infected with Omicron subvariants, ensitrelvir was safe and well tolerated. Compared with those receiving placebo, participants receiving ensitrelvir within 72 hours of symptom onset experienced shortened duration of symptoms and shortened

duration of infectious viral shedding from the upper respiratory tract by approximately 1 day each. Ensitrelvir treatment during acute COVID-19 was also associated with decreased incidence of long COVID, as discussed below.

Tong and colleagues demonstrated that pomotrelvir, another investigational protease inhibitor, retains broad in vitro activity against all SARS-CoV-2 isolates to date, including 5 Omicron subvariants, and it has a favorable resistance profile (Abstract 551). They additionally showed in vitro additivity when pomotrelvir is combined with remdesivir or molnupiravir, but not nirmatrelvir, likely because they share the same binding site. This observation suggests that combination therapy with antivirals may be worthy of consideration in the future.

Nirmatrelvir/ritonavir remains the first-line agent for outpatient COVID-19 therapy due to its effectiveness and oral formulation. Henderson and colleagues used data from a large academic health system to demonstrate that the use of nirmatrelvir/ritonavir was associated with a 98% relative reduction in age-adjusted risk for hospitalization within 14 days of diagnosis, compared with no therapy in outpatients diagnosed with COVID-19 (Abstract 172). This is even greater than the 89% relative risk reduction observed in the clinical trial.[7] In a multivariate regression model predicting COVID-19 hospitalization, the effect of treatment with nirmatrelvir/ritonavir was similar to that conferred by young age (approximately 20 years), demonstrating the remarkable impact of this agent. Butt and colleagues investigated approximately 8000 propensity-matched people from the US Veterans Affairs (VA) health system and found that nirmatrelvir/ritonavir use was associated with a modest reduction in hospitalization and death among nonhospitalized people at high risk of progression to severe disease (Abstract 569). The clearest benefit was seen in those who were older, unvaccinated, or asymptomatic at baseline. Despite these data establishing the clear benefits of this agent, Shen and colleagues showed that, although it is increasing, the uptake of nirmatrelvir/ritonavir in people diagnosed with COVID-19 in the outpatient setting remains low (Abstract 567).

One reason clinicians and patients remain wary of nirmatrelvir/ritonavir is reports of symptom and virus rebound after completion of the 5-day course of treatment. Li presented a detailed summary of what is known about symptom and viral rebound with and without antiviral therapy (Abstract 32). He highlighted that rebound phenomena are common in untreated patients and that the field currently lacks a direct comparison of rebound in people who have and have not received nirmatrelvir/ritonavir. Perelson and colleagues expanded on a previous viral dynamics model and predicted that extending the treatment duration of nirmatrelvir/ritonavir to 10 days or initiating a second 5-day treatment course 1 day after symptoms reappear would not prevent rebound (Abstract 568). Deo and colleagues characterized symptom and viral rebound in the untreated and placebo arms of the ACTIV-2 trial, enrolling primarily unvaccinated people in the pre-Omicron era (Abstract 171). They found that 1 in 4 participants had symptom rebound, and 1 in 8 had high-level viral rebound, though having both simultaneously was uncommon, providing

strong evidence that symptom and viral rebound are common without treatment.

Nucleoside and nucleotide analogues. Remdesivir, a nucleotide prodrug of an adenosine analogue, was one of the first antiviral therapies to enter phase III clinical trials for SARS-CoV-2 infection. It remains the only antiviral drug approved by the US Food and Drug Administration (FDA) for the treatment of COVID-19. However, because remdesivir must be administered intravenously or intramuscularly, its use in the outpatient setting has been limited. Using a mouse model, Carlin and colleagues presented promising data on the therapeutic effectiveness of 1-O-octadecyl-2-O-benzyl-sn-glyceryl-3-phospho-RVn (V2043), an oral prodrug of remdesivir, and identified modifications to V2043 that improved its potency (Abstract 543). Hedskog and colleagues showed that remdesivir retains excellent potency against all recent Omicron subvariants (Abstract 562). Using a novel replicon system, Han and colleagues demonstrated that common RNA-dependent RNA polymerase (Nsp12) mutations in VOCs do not decrease the potency of remdesivir (Abstract 962). Work from Mozaffari and colleagues used data from more than 700 hospitals to show that early hospital use of remdesivir is associated with significant reductions in mortality in all people, including immunocompromised people at 14 and 28 days across all levels of severity and all VOCs (Abstracts 556-557).

Molnupiravir, an oral prodrug of β-D-N^4-hydroxycytidine, is less effective in reducing hospitalization in at-risk outpatients with COVID-19 than nirmatrelvir/ritonavir or remdesivir, and in the US it is currently an alternate therapy used only if nirmatrelvir/ritonavir or remdesivir are not available or appropriate.[8] Using data from the VA health system, Butt and colleagues found that molnupiravir was not associated with significant reduction in hospitalization or death within 30 days of diagnosis compared with no therapy (Abstract 570). Efforts are needed to further define the role this agent should play in the management of COVID-19.

Monoclonal antibody therapy. Although monoclonal antibody (mAb) products have been removed from the arsenal of treatments for COVID-19 due to loss of efficacy against currently circulating Omicron subvariants,[8] efforts are underway to develop products that target invariant regions of SARS-CoV-2. Ruiz and colleagues isolated 2 novel pan-sarbecovirus mAbs that potently bind highly divergent SARS-related coronaviruses, including sarbecoviruses that do not use ACE2 as a receptor (Abstract 309). One of these displays broad and potent neutralizing activity. Guenthoer and colleagues isolated 2 further novel spike-specific mAbs that target a functionally constrained region of RBD and a conserved region in spike subdomain 1 (SD1) that show breadth and potency across VOCs (Abstract 310). Bieniasz and colleagues identified anti-ACE2 antibodies that represent a promising, broadly potent, pan-sarbecovirus therapeutic (Abstract 107, discussed further below). Using data from an ACTIV-2 clinical trial of amubarvimab and romlusevimab, Choudhary and colleagues provided evidence that dual active mAbs resulted in faster viral clearance and lower rates of resistance than single active mAbs, lending support to

the development and use of dual active mAb therapeutics (Abstract 168). Bender Ignacio and colleagues presented results from ACTIV-2 demonstrating safety and tolerability of intramuscular administration of combination mAbs in the thigh, which could lower barriers to outpatient implementation of mAb treatment in the future (Abstract 571).

Interferon therapy. Glenn and colleagues presented data on the use of pegylated interferon lambda from a phase III study of more than 1900 mostly vaccinated but high-risk, nonhospitalized participants in TOGETHER, an adaptive, multicenter, randomized, placebo-controlled trial (Abstract 167). A single subcutaneous injection of peginterferon lambda was associated with a 58% risk reduction of hospitalization or emergency department visit if administered within 3 days of symptom onset. More rapid viral clearance was seen in those treated with peginterferon lambda, and adverse effects were similar to placebo. Fischer and colleagues presented phase II inhaled interferon-β1A (SNG001) results from approximately 220 participants in ACTIV-2 (Abstract 169). This agent was shown to be safe, but it did not accelerate the clearance of nasopharyngeal SARS-CoV-2 RNA over 2 weeks and was not associated with more rapid symptom recovery. There was a nonsignificant trend toward fewer hospitalizations in the treatment group, but the trial was not powered to detect a difference in risk of hospitalization.

Miscellaneous approved drugs. Provocative data from the COVID-OUT phase III, blinded, placebo-controlled trial suggested that metformin, a widely available and commonly used oral agent for prediabetes and diabetes, may be of benefit in acute COVID-19. Having already demonstrated a modest reduction in risk of emergency department visits, hospitalization, and death with metformin,[9] Bramante and colleagues presented self-collected nasal viral RNA data from days 1, 5, and 10 of the trial (Abstract 170). Trial participants taking metformin were more likely to have undetectable viral RNA at days 5 and 10 than those taking placebo (odds ratio [OR], 1.355; 95% CI, 1.054-1.742). Metformin is thought to work by inhibiting mammalian target of rapamycin (mTOR)-dependent viral translation and inhibiting Nod-like receptor family pyrin domain containing 3(NLRP3) inflammasome activation.[10] Naggie and colleagues from ACTIV-6 presented results from a randomized trial comparing ivermectin and placebo in mild-to-moderate COVID-19, demonstrating no difference in time to recovery (Abstract 572).

Perez-Zsolt and colleagues showed that plitidepsin, a clinically approved antitumor agent that blocks eukaryotic translation elongation factor 1A (eEF1A), inhibits the synthesis of all SARS-CoV-2 proteins and the formation of viral particles (Abstract 548). Plitidepsin has a less potent effect on the cellular proteome, likely because of compensatory upregulation of eukaryotic initiation factor 4A2 (eIF4A2) and eIF2S3. They also demonstrated that plitidepsin inhibits replication of other RNA-dependent, nonintegrated DNA viruses such as Zika, hepatitis C virus, and herpes simplex virus, suggesting that it could be developed and evaluated for a variety of viral indications.

Miscellaneous drugs in development. Several new compounds are in development as COVID-19 therapeutics. Miller and colleagues reported that BIT225, an investigational HIV-1 compound that targets SARS-CoV-2 envelope protein, protected against death and reduced inflammatory and viral markers in a mouse model of COVID-19 (Abstract 552). Xing and colleagues showed that spike ACE2 inhibitor (SAI)4, a promising small molecule inhibitor of spike-RBD-ACE2 binding, inhibited cell entry, remained potent against several VOCs, and inhibited SARS-CoV-2 replication in lung tissue in a mouse model of COVID-19 (Abstract 547).

Two studies evaluated zotatifin, an inhibitor of eIF4A, which is a host RNA helicase required for SARS-CoV-2 replication. Zotatifin can be administered intravenously or subcutaneously. Using this agent, Patick and colleagues demonstrated in vitro inhibition of translation and replication in SARS-CoV-2 variants, Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), and human coronavirus 229E (HCoV-229E). They also demonstrated its synergy with other therapeutics in cell culture (Abstract 544). Warner and colleagues demonstrated safety and tolerability of zotatifin in PROPEL, a phase Ib clinical trial on people with mild-to-moderate COVID-19 (Abstract 545). Du and colleagues demonstrated that PAV-104, a pan-viral inhibitor of a subset of host protein assembly machinery, inhibits replication of several SARS-CoV-2 variants in primary airway epithelial cells at a step post entry by interfering with N oligomerization and blocking viral assembly and release (Abstract 549). Taken together, these studies emphasize the ongoing development of treatments for SARS-CoV-2 that target novel pathways and may ultimately broaden the armamentarium of available treatments for COVID-19.

Vaccines and Prevention

The first SARS-CoV-2 vaccines outside of a clinical trial were administered in December 2020 as a result of a breathtakingly rapid global effort. COVID-19 vaccinations were estimated to prevent more than 14 million deaths globally in the first year of the vaccination campaign.[11] However, much work remains, including distributing vaccines equitably, developing a new generation of vaccines for future VOCs, developing on-demand, self-administered prophylactic agents, understanding the correlates of sterilizing immunity, developing evidence-based vaccination schedules, combating misinformation, understanding vaccine effectiveness in special populations, and determining which nonpharmaceutical interventions are most effective and when best to implement them. A variety of research presented at CROI 2023 addressed these issues.

Prophylactics under development. Although the SARS-CoV-2 spike protein evolves in response to selective pressure, the human SARS-CoV-2 receptor ACE2 cannot evolve rapidly and thus represents an attractive target for antibody and drug development.

Bieniasz and colleagues identified high-affinity anti-ACE2 antibodies with potent pan-sarbecovirus activity, presented the mechanism for their effectiveness (steric clash at the SARS-CoV-2 spike-RBD-ACE2 binding site), demonstrated their effectiveness against several human polymorphisms of ACE2, and showed almost sterile protection of the lung when used for prophylaxis in a mouse model (Abstract 107). Importantly, they showed that endogenous ACE2 activity is not affected by these antibodies and that antibody binding does not trigger ACE2 internalization. Resistance to these antibodies was rare and had only subtle effects on potency. This work complemented other efforts described above to develop pan-sarbecovirus prophylactic or therapeutic agents for future SARS-CoV-2 variants or future new emerging sarbecoviruses (Abstracts 309-310).

Monoclonal antibodies have been the mainstay for those who need prophylactic agents in addition to or instead of vaccination, especially in populations with immune compromise or dysfunction, yet neither mAbs nor vaccines deliver sterilizing immunity, and both require administration in a health care setting. An on-demand, self-administered prophylactic with sterilizing immunity would be a practical and beneficial agent for people at elevated risk of severe COVID-19 to take prior to entering a high-risk setting. Neary and colleagues reported that intranasal nafamostat was protective against airborne transmission of SARS-CoV-2 in a Syrian golden hamster model (Abstract 553). Nafamostat inhibits transmembrane serine protease 2 (TMPRSS2), the host cell surface serine protease that mediates the cleavage of spike that is required for virus–host cell fusion and entry. Although intravenous (IV) nafamostat did not show efficacy in a phase IIa clinical trial of people with moderate-to-severe COVID-19 pneumonia, its half-life is short after IV administration and, like other therapeutics, it may have more beneficial effects when given early or prophylactically. Nabeta and colleagues demonstrated the safety of intranasal Q-griffithsin (Q-GRFT) in a phase I clinical trial (Abstract 554). Q-GRFT is an engineered oxidation-resistant variant of griffithsin, an algal antiviral that binds oligomannose residues in glycoproteins on viral envelopes. The researchers showed that nasopharyngeal fluids from people treated with intranasal Q-GRFT neutralized the Omicron BA.5 subvariant, as well as MERS-CoV.

Vaccine effectiveness, uptake, and equity. People with hybrid immunity (induced by a combination of infection and vaccination) have the highest protection against severe acute COVID-19 outcomes.[12] Mayer-Blackwell and colleagues tracked SARS-CoV-2 spike-specific T cells over time in people recovering from COVID-19 who were subsequently vaccinated and found that mRNA vaccination broadened the postinfection memory response by expanding low-abundance clonotypes (Abstract 360). However, the recalled memory clonotypes from infection predominated. People who were hospitalized with COVID-19 developed greater spike-reactive CD4+ T cell diversity that persisted after vaccines than people who had mild-to-moderate COVID-19. The investigators also identified vaccine-reactive CD8+ T cell clonotypes present in nasal mucosa that were also present in the blood after booster dosing. Pérez-Caballero and colleagues showed that, among people with hybrid

immunity, those who had infection prior to vaccination had broader and higher magnitude T cell responses to numerous SARS-CoV-2 peptides than people who had vaccination prior to infection (Abstract 345).

Henderson and colleagues used data from an academic health system during the Delta and Omicron waves, and found that people with 3 vaccine doses compared with those with none had a 71% relative risk reduction for hospitalization within 14 days of diagnosis, with risk higher in the Delta era and in older people (Abstract 172). There was a 28% relative increase in risk after 180 days since the last vaccine dose; those most at increased risk were aged 75 years or older. Older age was the most influential overall predictor of hospitalization.

Although the rapid development of COVID-19 vaccines was a success story, the distribution of vaccines left much to be desired. As of March 2023, only 28% of people in low-income countries have received at least 1 dose of a COVID-19 vaccine.[13] One of the many reasons for this inequity is the restricted access to intellectual property for mRNA vaccines.[14] In a meta-analysis of 35 clinical trials in healthy nonpregnant adults, Venkatesh and colleagues demonstrated that protein subunit vaccines, many of which are patent-free and could be mass produced, have similar neutralizing antibody titers to mRNA vaccines (Abstract 356). Lee and colleagues presented data from nearly 24 million Taiwanese people demonstrating that a protein subunit vaccine (MVC-COV1901; Medigen) was similarly effective at preventing severe COVID-19 and death as the BNT162b2 mRNA vaccine (Pfizer-BioNTech) (Abstract 355). People who received AZD1222 (AstraZeneca, a replication-deficient chimpanzee adenovirus vectored vaccine) as a primary series, regardless of which vaccine they received as a booster (mRNA, AZD1222, or protein subunit), had significantly lower protection against severe COVID-19 and death than those who received an mRNA vaccine or the protein subunit primary series.

Several groups examined factors associated with low vaccine uptake. Liang and colleagues examined county-level COVID-19 booster coverage by age group in southeastern US states and found that counties with higher racial housing segregation had lower percentages of booster coverage across age groups (Abstract 1006). Hoffman and colleagues examined correlates of COVID-19 vaccine update in Malawian adults and found that older age, having children, greater educational attainment, confidence in vaccine safety, and belief that its benefits outweighed its risks positively correlated with up-to-date COVID-19 vaccination status (Abstract 1018).

Nonpharmaceutical interventions. Numerous studies have demonstrated that lockdowns with high adherence reduced SARS-CoV-2 transmission early in the COVID-19 pandemic. However, it remains difficult to study the comparative effectiveness of different nonpharmaceutical interventions, given that these were often implemented simultaneously and without control groups. Thiebaut and colleagues used 3 models to examine interventions in France and demonstrated that all nonpharmaceutical interventions studied effectively reduced viral transmission, but the effectiveness of lockdown interventions decreased with time, potentially due to decreased adherence or enforcement (Abstract 1008). Nonpharmaceutical interventions are

needed to contain deadly airborne respiratory pathogen epidemics if vaccine coverage is low, and rapid vaccine rollout is essential. Stuart and colleagues used the open-source COVID-19 Agent-based Simulator (Covasim) to define time intervals for ideal deployment of variant-chasing vaccines (Abstract 1015). They described an ideal variant-containing strategy of global monitoring for highly immune-evading virulent variants paired with temporary nonpharmaceutical interventions, to buy time during rapid rollout of variant-specific or broad and potent vaccines.

Special Populations of Interest

Acute SARS-CoV-2 infection and vaccination in people with HIV. Recent work from the UK Biobank demonstrated differences in regional brain volume in the thalamus, caudate, putamen, ventral striatum, and hippocampus between people who went on to acquire SARS-CoV-2 infection and people who did not acquire SARS-CoV-2 infection,[15] Paul and colleagues evaluated brain volumes using 3 Tesla magnetic resonance imaging (MRI) and neurobehavioral characteristics among 112 Thai men who have sex with men with HIV enrolled in the RV254/SEARCH010 cohort (Abstract 188). Using a machine learning algorithm, they found that a collection of volumetric features, particularly in right hemisphere regions that are implicated in impulsivity and risk-taking behavior, were associated with subsequent SARS-CoV-2 infection. Their findings were generally consistent with those of the UK Biobank study.

Several studies addressed the pathophysiology of acute COVID-19 in people with HIV. Augello and colleagues evaluated 18 HIV seropositive and 18 HIV seronegative individuals hospitalized with COVID-19 pneumonia a median of 10 days after symptom onset (Abstract 344). They found that people with HIV were more likely to have SARS-CoV-2 RNAemia, greater systemic inflammation, and worse disease severity, and they concluded that the data showed a link between HIV-related T cell dysfunction and poor control over circulating SARS-CoV-2. These observations are potentially important as evidence that additional measures to reduce the viral burden or improve immune control during early infection might be warranted in people with HIV. There may also be implications for the post-acute phase of COVID-19, because some early factors can predict longer-term clinical outcomes.[16] Kolossváry and colleagues studied participants in the REPRIEVE (Randomized Trial to Prevent Vascular Events in HIV) trial and identified certain proteins in the granzyme family that predicted the development of moderate to severe COVID-19 (Abstract 274). They suggested that baseline immune dysregulation may relate to the severity of acute infection through these physiologic mechanisms. In another REPRIEVE analysis, Schnittman and colleagues compared 2181 donors who were SARS-CoV-2 negative with 283 individuals who were SARS-CoV-2 positive (most of whom had asymptomatic infection) and determined that among people with HIV, high body mass index (BMI) and low CD4 nadir were associated with unique SARS-CoV-2 humoral signatures. In this analysis, high BMI was associated with a hyperinflammatory response and low CD4 nadir was associated with dysfunctional antibody class switching. (Abstract 348). The authors

suggested that these observations could explain more severe COVID-19 among people with HIV. Abela and colleagues leveraged the Swiss HIV Cohort Study to identify that certain preexisting immunity to human coronaviruses was associated with reduced susceptibility to SARS-CoV-2 infection (Abstract 352). They also identified a weaker overall SARS-CoV-2 antibody response in those with HIV infection.

Several studies addressed issues related to SARS-CoV-2 vaccination in people with HIV. MatveevL and colleagues evaluated immune responses to booster doses in people with HIV on ART who were older than 55 years of age (Abstract 369). They found that although vaccines elicited equally strong anti-spike IgG in people with HIV compared with people who were HIV seronegative, the median neutralizing titers after the second dose were lower among people with HIV. However, these differences resolved following a third dose. This study, as well as another study by Duncan and colleagues, did not find an impact of SARS-CoV-2 mRNA vaccination on the HIV reservoir (Abstract 370). Nowak and colleagues showed that responses to BNT162b2 vaccination in people with HIV correlated with the presence of certain gut microbiota populations, providing evidence that characteristics of the microbiome may predict the strength of vaccine responses (Abstract 372).

Liu and colleagues examined the effectiveness of different COVID-19 vaccines and the evolution of antibody responses in 1496 adult Taiwanese people with HIV who had received a third dose of a SARS-CoV-2vaccine (Abstract 1020). They found similar effectiveness of a third dose of mRNA-1273 (Moderna) 100 µg or 50 µg, BNT162b2, and MVC-COV1901 (Medigen protein subunit vaccine) in preventing SARS-CoV-2 infection or seroconversion of anti-N IgG. People with HIV with CD4+ T cell counts less than 200/µL and plasma viral load greater than 200 copies/µL had reduced antibody responses. Matusali and colleagues analyzed live-virus neutralizing activity against Omicron subvariants BA.5, BQ.1.1, and XBB.1 together with T cell responses after the bivalent third booster shot—that is, a fifth vaccine—in 48 people with HIV with CD4+ T cell count nadirs more than 200 cells/µL with and without hybrid immunity stratified by CD4+ T cell count (Abstract 364). They found that hybrid immunity was associated with greater neutralizing responses against BA.5 but not against BQ.1.1 and XBB.1, which currently predominate. This fifth shot elicited strong neutralization against BA.5 and retained cross-neutralization against BQ.1.1 and XBB.1, although levels were 3-fold to 4-fold lower. There was no effect on T-cell mediated responses.

Many people wonder whether they will have a reaction to a COVID-19 vaccine after infection. Tapley and colleagues examined a safety subset of 1267 unvaccinated people with HIV from the COVID-19 Prevention Network (CoVPN) 3008 (UBUNTU) phase III efficacy trial of the mRNA-1273 vaccine, of whom 73% had evidence of prior SARS-CoV-2 infection (Abstract 1013). Overall, 43% of people with HIV reported local or systemic reactions in the first 7 days after the first vaccination, with maximum severity of mild (62%), moderate (36%), or severe (3%). Women and people with CD4+ counts above 500 cells/µL had increased odds of moderate or severe reactogenicity. Spinelli and colleagues found that 7% of surveyed people with HIV in the US in the

Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort from February 2021 to April 2022 were not vaccinated and probably or definitely had no desire to receive one (Abstract 1011). Factors associated with vaccine hesitancy included age less than 30 years old, viral load greater than 200 copies/µL, female sex at birth, and Black race rather than White race, with vaccine hesitancy decreasing with time over the observation period.

Acute SARS-CoV-2 infection and vaccination in children and adolescents. Several abstracts addressed SARS-CoV-2 infection in children and adolescents. Tagarro and colleagues studied more than 1700 children from hospitals in Spain and Colombia (Abstract 835). They identified that comorbidities such as asthma and chronic neurologic and cardiac conditions, but not diabetes or cancer, were associated with more severe outcomes from COVID-19 in this pediatric population. Such individuals were at higher risk of death, mechanical ventilation, and pediatric intensive care unit admission.

Antiviral treatment options for children with COVID-19 remain limited. Bernardi and colleagues studied a high-risk pediatric population in Rome who had received nirmatrelvir/ritonavir, and found that this therapy was safe and effective (Abstract 834). Among 40 children treated, there were only 5 adverse events (nausea, increased creatine phosphokinase level, and metallic taste). The mean time of viral shedding was 13 days, and 1 patient was persistently positive for 56 days. Notably, 4 children received a longer (10-day) course due to viral persistence and severe comorbidities.

Several studies addressed SARS-CoV-2 vaccination in children. Milligan and colleagues reported on a study of vaccines in infant rhesus macaques and found that mRNA- and protein-based vaccines induced antibody responses and protected against severe lung disease on SARS-CoV-2 challenge at 1 year (Abstract 840). The protein-based vaccine induced higher titers of neutralizing antibodies, but the mRNA vaccines induced greater spike-specific T-cell responses. The authors concluded that either approach is likely to be efficacious in children. If so, this would mirror efficacy data in adults discussed previously. Di Chiara and colleagues conducted a multicenter, prospective, observational study evaluating immune responses to mRNA vaccines in 82 Italian children, 60 of whom had confirmed COVID-19 before vaccination (Abstract 841). The magnitude of the antibody response was higher in children with prior SARS-CoV-2 infection than in those without preexisting immunity, and levels of antibodies decayed between 1 and 6 months post vaccination. Chemaitelly and colleagues evaluated the BNT162b2 vaccine in adolescents and found that compared with a 10 µg dose, a 30 µg dose was associated with 23% higher effectiveness against infection with Omicron subvariants in adolescents who were infection naive (Abstract 842). They noted that this higher dose of BNT162b2 conferred similar improvement in protection as the mRNA-1273 vaccine, which is also a 3-fold higher dose.

Acute SARS-CoV-2 infection and vaccination in pregnant individuals. Two groups reported SARS-CoV-2 antibody responses in pregnancy.

Lacourse and colleagues examined 71 people with SARS-CoV-2 infection during pregnancy and found that maternal and cord blood SARS-CoV-2 antibody binding and neutralizing responses were higher among those who had been vaccinated prior to infection than among those who were unvaccinated prior to infection (Abstract 794). Approximately 18% of people who had been unvaccinated prior to SARS-CoV-2 infection during pregnancy did not have sustained neutralizing antibodies by the time of delivery, and 100% of those with hybrid immunity had neutralizing antibodies. Govindaraj and colleagues examined people with an mRNA vaccine primary series during pregnancy and found that neutralizing antibody titers to Omicron subvariants were lower than those to pre-Omicron variants (Abstract 796). Pregnant individuals remain an important subpopulation for further study of COVID-19 pathogenesis, and efforts are underway to continue to characterize the impact of SARS-CoV-2 infection and vaccination in pregnant individuals and their offspring.

Acute SARS-CoV-2 infection and vaccination in people who are immunocompromised. It is postulated that VOCs arise in immunocompromised hosts who inadvertently maintain prolonged replication of SARS-CoV-2 in the presence of the selective pressure of exogenous or endogenous SARS-CoV-2–specific antibodies. Kim and colleagues investigated viral kinetics with weekly saliva testing in an immunocompromised cohort (70% in active chemotherapy and 30% with solid organ transplant) (Abstract 726). The median duration of shedding of culture-positive virus was 4 weeks. Having received 3 or more vaccinations was associated with shorter shedding duration, and people receiving B-cell depleting therapy generally shed viable virus for longer. Ferré and colleagues sequenced the viral genomes of more than 700 people and reported higher frequency of minority nonsynonymous mutations in most genes of all variants studied in people who were immunocompromised (Abstract 354). This provides additional support for the hypothesis that viral evolution can occur in the immunocompromised population.

Several groups reported vaccine effectiveness in populations with and without immune dysfunction. Sun and colleagues presented data on COVID-19 bivalent booster effectiveness in people with immune dysfunction in the N3C (National COVID Cohort Collaborative). (Abstract 214). The bivalent booster was negatively associated with breakthrough infection and hospitalization in immune competent populations and in those with mild immune dysfunction, but its effectiveness was reduced in people with moderate-to-severe immune dysfunction. Liu and colleagues examined longitudinal anti-spike IgG titers in people on anti-CD20 (B-cell depleting) therapy and found that the likelihood of mounting antibody responses increased with a third primary dose or with time after anti-CD20 administration (Abstract 368). Rocco and colleagues found that people with idiopathic CD4+ lymphopenia and absolute CD4+ counts above 100 cells/μL mounted similar humoral and cellular immune responses to healthy controls, and people with idiopathic CD4+ lymphopenia and CD4+ counts below 100 cells/μL had impaired vaccine immunity (Abstract 367). Taken together, these data will help people with immune dysfunction and their

clinicians make informed decisions about best strategies for prevention of severe COVID-19.

Post-Acute COVID-19

There is growing recognition that SARS-CoV-2 infection can affect long-term health.[17] Major efforts are now underway to understand the post-acute sequelae of SARS-CoV-2 infection (PASC, or long COVID), which include incident medical diagnoses potentially caused by SARS-CoV-2 infection, as well as the persistent, unexplained, and sometimes debilitating symptoms.[18] Peluso provided an overview of the current state of knowledge on long COVID syndromes, including epidemiology, natural history, biology, and the potential for therapeutics (Abstract 33). Several studies presented at CROI 2023 provided additional insight into these topics.

Epidemiology and Natural History

Adding to observations that long COVID affects a substantial proportion of individuals recovering from SARS-CoV-2 infection,[19,20] Berry and colleagues studied the incidence of long COVID in nearly 45,000 patients previously hospitalized for COVID-19 (Abstract 718). More than 27,000 patients experienced 1 or more long COVID symptoms between 90 and 270 days post hospitalization. Electronic health records most commonly captured neuropsychiatric symptoms, dyspnea, fatigue, and joint pain in this population. However, other important symptoms of long COVID such as dysautonomia and protracted disturbances in taste and smell were infrequently captured by the International Classification of Diseases (ICD)-10 codes. This led the authors to conclude that this incidence is likely to be an underestimate in addition to the fact that individuals may be unlikely to seek care for all relevant symptoms. Malambo and colleagues described long COVID in post-COVID-19 clinics in Zambia, demonstrating that this condition continues to be observed in many populations, including those outside the high-resource settings where it was initially described (Abstract 719).

Identification of objective biomarkers of long COVID symptoms remains a priority for the field.[18] McAlpine and colleagues correlated symptoms of long COVID with objectively measurable defects (Abstract 493). The authors applied the Research Domain Criteria (RDoC) to better understand neuropsychiatric alterations in people with neurologic long COVID symptoms. In addition to providing support for the observation that these individuals exhibit impairment in executive functioning, processing speed, attention, and verbal fluency, among other symptoms, they observed novel alterations in motor and negative valence systems that warrant further investigation. Dziarski and colleagues also observed impairment in processing speed at 1 month post infection, which improved by 4 months in a highly vaccinated cohort (Abstract 495). Brew and colleagues assessed individuals for up to 2 years following initial SARS-CoV-2 infection and found that olfactory performance declined over time, especially among those

exhibiting initial impairment (Abstract 715). In another study, Brew and colleagues identified several potential MRI-based biomarkers of blood–brain barrier impairment, neuronal and axonal injury, and excitotoxicity in individuals with post-COVID-19 neurocognitive symptoms (Abstract 492). They saw variable improvement in these parameters over 10 months of follow-up and suggested that these markers might be useful in future studies. De Bree and colleagues identified early elevations in plasma levels of IL-1β and sCD14 measured within the first 4 weeks as potential biomarkers for long COVID at 6 months (Abstract 710). Taken together, these studies contribute to a growing literature tying subjective symptoms of long COVID to objective biomarker or physiologic measurements.

Efforts are now underway to confirm the clinical observation that there are different syndromic phenotypes of long COVID,[21] which are possibly driven by different biologic processes.[18] Mateu and colleagues performed a hierarchical cluster analysis and identified 3 clusters of increasing severity in the Spanish King cohort study (Abstract 723). These included a milder cluster in which fatigue and dyspnea were dominant, followed by a more moderate cluster that also included headache, arthralgia, chest pain, and neurocognitive symptoms, followed by the most symptomatic cluster, which also included tachycardia, neurosensitive symptoms, and cough. Importantly, only a small proportion (7.6%) of individuals achieved recovery at 2 years of follow-up. An additional analysis of the same cohort presented by Nevot and colleagues identified 5 clusters to guide biologic analyses (discussed further below) (Abstract 711). These observations are generally consistent with symptom-based phenotypes described from smaller cohort studies.[22,23]

Pathogenesis and Immune Responses

The pathophysiology of long COVID remains incompletely understood. Potential mechanisms include persistence of SARS-CoV-2 viral antigens (including subgenomic RNA and protein), immune dysregulation, reactivation of latent herpesviruses (eg, Epstein-Barr virus), microbial translocation, autoimmunity, microvascular dysfunction, and mitochondrial dysfunction, among others.[18,24] A number of studies advanced our understanding of the potential contribution of each of these mechanisms.

One of the most pressing questions for the field is whether persistence of SARS-CoV-2 antigen contributes to long COVID. Peluso and colleagues presented a study using the single molecule array (Simoa) platform to investigate persistence of SARS-CoV-2 proteins in the plasma of individuals during the post-acute phase (Abstract 282). They found that 24% of individuals studied had at least 1 antigen detected during at least 1 time point up to 14 months after infection, but antigen was detected only sporadically in most individuals. Antigen persistence was strongly associated with hospitalization during acute infection, and appeared more prevalent among those consistently reporting high numbers (>8) symptoms. In complementary work, Eden and colleagues reported on the lack of persistent antigen in the cerebrospinal fluid following SARS-CoV-2 infection; though this

does not rule out viral persistence in central nervous system tissue, it suggests that viral persistence may not be easily measurable in cerebrospinal fluid (CSF) (Abstract 189). Although the prevalence of antigen detection was much lower than previously reported,[25] this phenomenon may drive at least some cases of long COVID and is likely to remain an area of intense investigation.

Inflammation has been consistently identified in individuals with long COVID compared with those who have fully recovered.[18,24] Several studies evaluated immune responses and potential dysregulation in the post-acute phase. De Bree and colleagues assessed inflammatory markers during the early post-acute phase (4 weeks) and at 24 weeks post-COVID-19. Compared with healthy controls, they found that people with prior SARS-CoV-2 infection had ongoing elevations in IL-6, IL-10, IL-17, and IL-1B (Abstract 710). They further observed that early immune dysregulation was an important determinant of long COVID, and that C-reactive protein level elevations at week 24 were associated with ongoing symptoms. Nevot and colleagues identified differential expression of 14 cytokines when comparing 5 symptomatic clusters of long COVID with individuals who had fully recovered and uninfected controls (Abstract 711). This study provided an initial approach by which cluster analyses might be paired with biomarker analyses to better understand the biology underlying certain phenotypes of long COVID. Mouchati and colleagues demonstrated that PASC is associated with increased zonulin, a marker of gut permeability, consistent with prior observations suggesting that microbial translocation could be an important driver of post-COVID-19 inflammation among those with long COVID symptoms (Abstract 288).[26] McAlpine and colleagues presented data from a study of neurologic aspects of long COVID (Abstract 190). Although they did not identify significant differences in the CSF of those with PASC compared with prepandemic controls, there were clinical and demographic differences between the 2 groups that may have biased against identification of an effect. Furthermore, they identified elevations in certain soluble markers of inflammation and glial fibrillary acid protein (GFAP) in plasma, consistent with prior observations in other cohorts.[27]

Several studies further explored long-term humoral and cellular immune responses following SARS-CoV-2 infection. Yin and colleagues presented new cytometry by time-of-flight (CyTOF) data comparing 27 individuals who consistently met the case definition of long COVID with 16 individuals who reported complete recovery over an 8-month period prior to receipt of any SARS-CoV-2 vaccine (Abstract 346). Among those with long COVID, the researchers found higher levels of markers of tissue homing on CD4+ T cells and immune exhaustion on CD8+ T cells, which they suggested might represent indirect evidence of tissue antigen persistence. They also found a dissociation between the humoral and cellular immune responses in these individuals. For example, SARS-CoV-2–specific CD4+ and CD8+ T-cell responses directly correlated with anti-RBD antibodies in those reporting full recovery, but not in those with long COVID. The authors suggested that discoordination between the 2 arms of the adaptive immune system might drive long COVID.

Building on prior work demonstrating the potential role of human herpesviruses in long COVID,[16,28,29] Peluso and colleagues identified cytomegalovirus (CMV) serostatus as an important protective factor with regard to post-COVID-19 neurocognitive symptoms (Abstract 273). This observation stands in contrast to prior findings that high-level immune responses to and serologic evidence suggesting reactivation of Epstein Barr virus, another human herpesvirus, are associated with increased odds of long COVID. The reason for this surprising observation was unclear, but the authors suggested that it could relate to CMV-specific immunoregulatory cytokines (eg, virus-specific IL-10), differential anatomic compartmentalization of these viruses in relation to SARS-CoV-2, or enhancement of immune responses in those who are CMV seropositive. Further work will be needed to determine if this observation can be confirmed in other cohorts, including in those with prepandemic serologies that can be assessed.

Acute COVID-19 is known to be associated with the generation of autoantibodies, especially in those with more severe acute illness.[30] Using electronic health record data from a large health network, Hileman and colleagues compared individuals with COVID-19 with propensity score-matched controls (Abstract 712). They observed that autoimmune diseases, although rare in both groups, were more likely to be diagnosed in the first year after COVID-19 than in age- and sex-matched comparators. Although conditions like rheumatoid arthritis, psoriasis, and type 1 diabetes mellitus had the highest incidence after COVID-19, conditions such as polyarteritis nodosa, reactive arthritis, and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides had the highest risk ratios. This adds to the growing literature suggesting an increased risk of autoimmune conditions in the post-acute period.[31] However, whether autoimmunity is a driver of unexplained symptoms (ie, long COVID) remains unclear.

There has been much recent attention on the role of platelet dysregulation, clotting dysfunction, and endothelial dysfunction driving microvascular abnormalities in long COVID.[32] Zisis and colleagues used Endopat testing to show that long COVID is associated with arterial stiffness, and that the lowest arterial elasticity scores were more strongly associated with cardiopulmonary symptoms than neurocognitive or other systemic symptoms (Abstract 714). Durieux and colleagues extended this observation and showed that sex was an important modifier of this relationship, demonstrating that women were disproportionately affected (Abstract 717).

Dirajlal-Fargo and colleagues showed differences in oxygen consumption and oxidative stress in individuals with long COVID compared with those without COVID-19 and those who fully recovered (Abstract 285). This observation builds on prior work that showed an association between decreased mitochondrial health and neuropsychiatric symptoms in the post-acute phase.[33]

Treatment and Prevention

Acute-phase treatment and long COVID outcomes. An important unanswered question is whether treatment during the acute phase of COVID-19 has an impact on long COVID outcomes. Uehara and colleagues presented data

from a phase III, double-blind, randomized trial of ensitrelvir, a SARS-CoV-2 3CL protease inhibitor approved in Japan (also discussed above) (Abstract 166). The study is notable because it is one of the first clinical trials to prospectively assess symptomatology during the acute phase of COVID-19 and in the post-acute phase, at 3 and 6 months post infection. In addition to reducing the duration of acute symptoms by approximately 1 day and accelerating viral clearance, the authors observed a 25% reduction in the presence of any long COVID symptoms and 26% reduction in neurologic symptoms at 6 months among those who received ensitrelvir compared with those receiving placebo. This finding was more dramatic when the population was restricted to individuals who had the highest baseline symptom scores, in whom the researchers identified a 45% reduction in any long COVID symptoms and 33% reduction in neurologic symptoms. Most symptoms appear to have been reduced by 20% to 70% compared with placebo, with reduction in smell disorder, difficulty concentrating, and insomnia achieving statistical significance. In support of this observation, a retrospective analysis of data from a single center presented by Antoni found that early outpatient treatment with antivirals or monoclonal antibodies was associated with 50% to 60% lower odds of symptoms at 3 months, although the data were potentially biased by a low survey response rate and potentially important clinical differences between groups (Abstract 733).

In contrast, related work presented by Evering and colleagues from the ACTIV-2 study did not identify an effect of early therapy with amubarvimab and romlusevimab on long COVID symptoms or on quality of life at 36 weeks in high-risk outpatients with mild-to-moderate COVID-19, despite an improvement in early differences in death and hospitalization (Abstract 721).

As an extension of their analysis showing benefit of metformin treatment during the acute phase, Bramante and colleagues showed a potential benefit over the long term (Abstract 170). The same trial participants were followed up with surveys every 30 days through 10 months. The team described a 42% reduction in long COVID through month 10 among participants taking metformin titrated over 14 days during acute infection compared with those receiving placebo. This provocative observation may be related to inhibition of viral translation, or to other metabolic effects of metformin that warrant further investigation. Ultimately, more data will be needed to answer the important question of whether early treatment improves long COVID clinical outcomes.

Treatment of established long COVID. There is no standard of care for established long COVID, and most treatment is focused on ruling out conditions that might mimic long COVID and on trying to optimize symptoms. Importantly, each of the mechanisms that has been proposed as a potential contributor to long COVID is potentially targetable using antivirals, monoclonal antibodies, and various forms of immunotherapy. Limited data were presented on the treatment of established long COVID, except for a study by Augustin and colleagues that did not find an effect of therapeutic SARS-CoV-2 vaccination in those with established symptoms (Abstract 720). Further work focused

on therapeutics in the post-acute phase is urgently needed to address long COVID in those who already have it.

Special Populations of Interest

PASC in people with HIV. Preexisting HIV infection could potentially alter the risk of developing long COVID.[34] Two large studies leveraging electronic health records showed concerning trends among people with HIV recovering from SARS-CoV-2 infection. Yendewa and colleagues presented an analysis from the TriNetX health research database, which includes 69 health care organizations within the US (Abstract 724). They found that people with HIV had significantly higher odds of incident diabetes, heart disease, malignancy, thrombosis, and mental health disorders than HIV-seronegative people at least 28 days post COVID. They also observed that among people with HIV, vaccination was protective. In a complementary study working with data from the N3C cohort, Liang and colleagues compared people with HIV who had a history of SARS-CoV-2 infection with contemporary controls who were HIV seropositive who did not have SARS-CoV-2 infection during the study period (Abstract 884). They found a higher risk of pulmonary, renal, neuropsychiatric, and cardiovascular complications among people with HIV following SARS-CoV-2 infection. This observation addressed the criticism of prior studies that compared people with HIV with people who were HIV seronegative without accounting for the fact that people with HIV may have a higher likelihood of developing complications attributed to PASC independent of SARS-CoV-2 infection. Taken together, these studies suggest that there is additional risk of SARS-CoV-2 coinfection beyond the risks of HIV alone.

Other, smaller cohort studies did not clearly identify longer-term complications of SARS-CoV-2 infection among people with HIV evaluated prospectively. For example, Ocampo and colleagues evaluated young people with HIV in Thailand who were mostly vaccinated, who were on stable ART, and who had few comorbidities (Abstract 494). They found no major clinical adverse events following COVID-19 and observed that cognitive and mood parameters, which are sometimes part of long COVID syndromes, remained stable after COVID-19. Antar and colleagues conducted a US-based, nationwide, fully remote, prospective observational cohort study to compare HIV seropositive and seronegative people with and without SARS-CoV-2 infection (Abstract 722). They found that although people with HIV were more likely to report long COVID at 2 months following infection, this was not the case at 4 to 6 months. They identified a negative correlation between cortisol and post-acute memory problems, and positive correlations between C5a, TIM-3, and TGF-beta levels, and pain, anxiety, and muscle aches, respectively. Dziarski and colleagues performed detailed neurocognitive testing in the same cohort and observed that differences in neurocognitive scores between people with HIV and people who were HIV seronegative post COVID appeared to be primarily attributable to HIV status (Abstract 495). Similarly, Durstenfeld and colleagues found that exercise capacity measured on cardiopulmonary exercise testing was reduced among people with HIV independent of SARS-CoV-2 infection status or subjective long COVID symptoms

(Abstract 666). Taken together, the data suggest that people with HIV may be at higher risk of post-COVID complications and face at least equivalent risks of developing long COVID, but they are not necessarily at higher risk of persistent long COVID symptoms. Further work will be needed to understand long COVID epidemiology, natural history, and biology in this subpopulation.

PASC in children and adolescents. Several studies addressed manifestations of long COVID in children and adolescents, a population in which this condition has received relatively little attention. Moraleda and colleagues evaluated children at 1 year post hospitalization with a history of multisystem inflammatory syndrome in children (MIS-C), comparing those hospitalized with COVID-19 with those with surgical peritonitis (Abstract 837). They found that symptom frequency was highest in those with MIS-C, and that 88% of children studied following this condition had symptoms at 1 year. The most common symptoms were headache, fatigue, insomnia, and concentration problems. Fatigue and concentration problems were also common among those hospitalized for COVID-19 who did not meet criteria for MIS-C. In contrast, Tagarro and colleagues did not find that symptoms were significantly different in children hospitalized for COVID compared with those hospitalized for other reasons (Abstract 836). The most common symptoms in both groups were fatigue, headache, poor appetite, abdominal pain, and heart rate variability. Longer hospital admission was generally associated with persistent symptoms.

Izquierdo-Pujol and colleagues performed immunophenotyping of peripheral blood mononuclear cells in children with long COVID and compared them with 23 controls who did not experience long COVID symptoms (Abstract 838). They identified differences in memory B-cell populations that suggested viral antigen persistence and differences in CD4+ effector memory T cells reexpressing CD45RA (TEMRA) that could be related to autoimmune phenomena, but they did not find significant differences in levels of 42 biomarkers between groups. Maddaloni and colleagues found potential dysregulation of immune responses in children for up to 6 months post COVID, regardless of long COVID symptoms (Abstract 839). Specifically, they identified overexpression of factors in the NLRP3 inflammasome pathway and suggested that prolonged activation of this pathway might be a driver of long COVID symptoms.

Conclusion

Work presented at CROI 2023 was at the cutting edge of our understanding of SARS-CoV-2, COVID-19, and long COVID. The studies described herein advanced our knowledge of the epidemiology, natural history, pathophysiology, and management of the acute and post-acute phases of SARS-CoV-2 infection and are expected to shape the field over the next several years.

Abstracts cited in the text appear in the virtual CROI 2023 Abstract eBook, available online at www.CROIconference.org.

The IAS–USA identifies and resolves ahead of time any possible conflicts of interest that may influence CME activities with regard to exposition or conclusion. All financial relationships with ineligible companies for the authors and reviewers are below.

Financial affiliations in the past 24 months: Dr Antar has no financial relationships with ineligible companies to disclose (Updated March 21, 2023). Dr Peluso has received consulting fees from AstraZeneca and Gilead Sciences, Inc. (Updated March 29, 2023).

All relevant financial relationships with ineligible companies have been mitigated.

Top Antivir Med. 2023;31(3).

Additional References

1. Gao YD, Ding M, Dong X, et al. Risk factors for severe and critically ill COVID-19 patients: A review. *Allergy.* 2021;76(2):428-455. 10.1111/all.14657 [doi].
Ref ID: 17576

2. Tenforde MW, Self WH, Gaglani M, et al. Effectiveness of mRNA vaccination in preventing COVID-19-associated invasive mechanical ventilation and death - United States, March 2021-January 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(12):459-465. mm7112e1 [pii];10.15585/mmwr.mm7112e1 [doi].
Ref ID: 17577

3. Lewnard JA, McLaughlin JM, Malden D, et al. Effectiveness of nirmatrelvir-ritonavir in preventing hospital admissions and deaths in people with COVID-19: a cohort study in a large US health-care system. *Lancet Infect Dis.* 2023; S1473-3099(23)00118-4 [pii];10.1016/S1473-3099(23)00118-4 [doi].
Ref ID: 17578

4. Wongvibulsin S, Garibaldi BT, Antar AAR, et al. Development of evere COVID-19 adaptive risk predictor (SCARP), a calculator to predict severe disease or death in hospitalized patients with COVID-19. *Ann Intern Med.* 2021;174(6):777-785. aim-olf-M206754 [pii];10.7326/M20-6754 [doi].
Ref ID: 17579

5. Buttia C, Llanaj E, Raeisi-Dehkordi H, et al. Prognostic models in COVID-19 infection that predict severity: a systematic review. *Eur J Epidemiol.* 2023;1-18. 10.1007/s10654-023-00973-x [pii];973 [pii];10.1007/s10654-023-00973-x [doi].
Ref ID: 17580

6. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science.* 2020;370(6515) science.abd4585 [pii];10.1126/science.abd4585 [doi].
Ref ID: 17130

7. Hammond J, Leister-Tebbe H, Gardner A, et al. Oral mirmatrelvir for high-risk, nonhospitalized adults with COVID-19. *N Engl J Med.* 2022;386(15):1397-1408. NJ202202163861504 [pii];10.1056/NEJMoa2118542 [doi].
Ref ID: 17581

8. COVID-19 Treatment Guidelines. Antiviral agents, including antibody products summary recommendations. https://www.covid19treatmentguidelines.nih.gov/therapies/antivirals-including-antibody-products/summary-recommendations/. Updated March 6, 2023. Accessed March 30, 2023.
Ref ID: 17582

9. Bramante CT, Huling JD, Tignanelli CJ, et al. Randomized trial of metformin, ivermectin, and fluvoxamine for COVID-19. *N Engl J Med.* 2022;387(7):599-610. 10.1056/NEJMoa2201662 [doi].
Ref ID: 17583

10. Xian H, Liu Y, Rundberg NA, et al. Metformin inhibition of mitochondrial ATP and DNA synthesis abrogates NLRP3 inflammasome activation and pulmonary inflammation. *Immunity.* 2021;54(7):1463-1477. S1074-7613(21)00210-7 [pii];10.1016/j.immuni.2021.05.004 [doi].
Ref ID: 17629

11. Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. *Lancet Infect Dis.* 2022;22(9):1293-1302. S1473-3099(22)00320-6 [pii];10.1016/S1473-3099(22)00320-6 [doi].
Ref ID: 17584

12. Bobrovitz N, Ware H, Ma X, et al. Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. *Lancet Infect Dis.* 2023; S1473-3099(22)00801-5 [pii];10.1016/S1473-3099(22)00801-5 [doi].
Ref ID: 17585

13. Mathieu E, Ritchie H, Ortiz-Ospina E, et al. Coronavirus (COVID-19) vaccinations. Our World in Data. https://ourworldindata.org/covid-vaccinations?country=OWID_WRL. Updated March 30, 2023. Accessed March 30, 2023.
Ref ID: 17586

14. Kunyenje CA, Chirwa GC, Mboma SM, et al. COVID-19 vaccine inequity in African low-income countries. *Front Public Health.* 2023;11:1087662. 10.3389/fpubh.2023.1087662 [doi].
Ref ID: 17587

15. Douaud G, Lee S, Alfaro-Almagro F, et al. SARS-CoV-2 is associated with changes in brain structure in UK Biobank
1. *Nature.* 2022;604(7907):697-707. 10.1038/s41586-022-04569-5 [pii];4569 [pii];10.1038/s41586-022-04569-5 [doi].
Ref ID: 17588

16. Su Y, Yuan D, Chen DG, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell.* 2022;185(5):881-895. S0092-8674(22)00072-1 [pii];10.1016/j.cell.2022.01.014 [doi].
Ref ID: 17589

17. Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. *Nat Med.* 2021;27(4):601-615. 10.1038/s41591-021-01283-z [pii];10.1038/s41591-021-01283-z [doi].
Ref ID: 17590

18. Peluso MJ, Deeks SG. Early clues regarding the pathogenesis of long-COVID. *Trends Immunol.* 2022;43(4):268-270. S1471-4906(22)00047-3 [pii];10.1016/j.it.2022.02.008 [doi].
Ref ID: 17591

19. Wulf-Hanson S, Abbafati C, Aerts JG, et al. Estimated global proportions of individuals with persistent fatigue, cognitive, and respiratory symptom clusters following symptomatic COVID-19 in 2020 and 2021. *JAMA.* 2022;328(16):1604-1615. 2797443 [pii];joi220113 [pii];10.1001/jama.2022.18931 [doi].
Ref ID: 17592

20. Perlis RH, Santillana M, Ognyanova K, et al. Prevalence and correlates of long COVID symptoms among US adults. *JAMA Netw Open.* 2022;5(10):e2238804. 2797782 [pii];zoi221101 [pii];10.1001/jamanetworkopen.2022.38804 [doi].
Ref ID: 17593

21. Zhang H, Zang C, Xu Z, et al. Data-driven identification of post-acute SARS-CoV-2 infection subphenotypes. *Nat Med.* 2023;29(1):226-235. 10.1038/s41591-022-02116-3 [pii];2116 [pii];10.1038/s41591-022-02116-3 [doi].
Ref ID: 17594

22. Peluso MJ, Kelly JD, Lu S, et al. Persistence, magnitude, and patterns of postacute symptoms and quality of life following onset of SARS-CoV-2 infection: cohort description and approaches for measurement. *Open Forum Infect Dis.* 2022;9(2):ofab640. ofab640 [pii];10.1093/ofid/ofab640 [doi].
Ref ID: 17595

23. Kenny G, McCann K, O'Brien C, et al. Identification of distinct long COVID clinical phenotypes through cluster analysis of self-reported symptoms. *Open Forum Infect Dis.* 2022;9(4):ofac060. ofac060 [pii];10.1093/ofid/ofac060 [doi].
Ref ID: 17596

24. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol.* 2023;21(3):133-146. 10.1038/s41579-022-00846-2 [pii];846 [pii];10.1038/s41579-022-00846-2 [doi].
Ref ID: 17597

25. Swank Z, Senussi Y, Manickas-Hill Z, et al. Persistent circulating severe acute respiratory syndrome coronavirus 2 spike Is associated with post-acute coronavirus disease 2019 sequelae. *Clin Infect Dis.* 2023;76(3):e487-e490. 6686531 [pii];10.1093/cid/ciac722 [doi].
Ref ID: 17598

26. Giron LB, Peluso MJ, Ding J, et al. Markers of fungal translocation are elevated during post-acute sequelae of SARS-CoV-

2 and induce NF-kappaB signaling. *JCI Insight.* 2022;7(15) 160989 [pii];10.1172/jci.insight.160989 [doi].
Ref ID: 17599

27. Peluso MJ, Sans HM, Forman CA, et al. Plasma markers of neurologic injury and inflammation in people with self-reported neurologic postacute sequelae of SARS-CoV-2 infection. *Neurol Neuroimmunol Neuroinflamm.* 2022;9(5) 9/5/e200003 [pii];NXI-2022-200003 [pii];10.1212/NXI.0000000000200003 [doi].
Ref ID: 17600

28. Gold JE, Okyay RA, Licht WE, Hurley DJ. Investigation of long COVID prevalence and its relationship to Epstein-Barr virus reactivation. *Pathogens.* 2021;10(6) pathogens10060763 [pii];pathogens-10-00763 [pii];10.3390/pathogens10060763 [doi].
Ref ID: 17601

29. Peluso MJ, Deveau TM, Munter SE, et al. Chronic viral coinfections differentially affect the likelihood of developing long COVID. *J Clin Invest.* 2023;133(3) 163669 [pii];10.1172/JCI163669 [doi].
Ref ID: 17602

30. Chang SE, Feng A, Meng W, et al. New-onset IgG autoantibodies in hospitalized patients with COVID-19. *Nat Commun.* 2021;12(1):5417. 10.1038/s41467-021-25509-3 [pii];25509 [pii];10.1038/s41467-021-25509-3 [doi].
Ref ID: 17603

31. Roessler M, Tesch F, Batram M, et al. Post-COVID-19-associated morbidity in children, adolescents, and adults: A matched cohort study including more than 157,000 individuals with COVID-19 in Germany. *PLoS Med.* 2022;19(11):e1004122. PMEDICINE-D-22-00600 [pii];10.1371/journal.pmed.1004122 [doi].
Ref ID: 17604

32. Pretorius E, Venter C, Laubscher GJ, et al. Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with Long COVID/Post-Acute Sequelae of COVID-19 (PASC). *Cardiovasc Diabetol.* 2022;21(1):148. 10.1186/s12933-022-01579-5 [pii];1579 [pii];10.1186/s12933-022-01579-5 [doi].
Ref ID: 17605

33. Peluso MJ, Deeks SG, Mustapic M, et al. SARS-CoV-2 and mitochondrial proteins in neural-derived exosomes of COVID-19. *Ann Neurol.* 2022;91(6):772-781. ANA26350 [pii];10.1002/ana.26350 [doi].
Ref ID: 17606

34. Peluso MJ, Spinelli MA, Deveau TM, et al. Postacute sequelae and adaptive immune responses in people with HIV recovering from SARS-

COV-2 infection. *AIDS.* 2022;36(12):F7-F16. 00002030-202210010-00001 [pii];10.1097/QAD.0000000000003338 [doi].
Ref ID: 17607