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Supplementary appendix

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Table S1. Summary of studies on the persistence of SARS-CoV-2 in post COVID-19 patients

Author	Country	Specimen	Testing Method	Major founding
Stein SR, et al.[1]	USA	Autopsied tissues, perimortem plasma, other body fluids	ddPCR, RT-qPCR, sequencing, ISH,etc	SARS-CoV-2 replication is present in multiple respiratory and non-respiratory tissues, including the brain, early in infection. Persistent virus RNA was detected in multiple anatomic sites as late as 230 days following symptom onset.
Bussani R, et al.[2]	Italy	Autopsied Tissues, BAL	RT-PCR, IHC, etc	Recovered COVID-19 patients had progressively worsened in their clinical conditions despite repeated viral negativity on nasopharyngeal swabs or bronchoalveolar lavage for 11-300 consecutive days. Positive SARS-CoV-2 RNA and virus-infected cells were detected in autopsied lung tissue.
Caniego-Casas T, et al.[3]	Spain	Autopsied Tissues	RT-PCR, IHC, ISH	Long-term presence of genomic SARS-CoV-2 RNA (gRNA) and subgenomic RNA (sgRNA) in the persistence of severe lung lesions in COVID-19 patients and the role of host immunity in the clearance of SARS-CoV-2 infections.
Goh D, et al.[4]	Singapore	Surgical tissues	RNAscope, mIHC,	Persistent SARS-CoV-2 RNA and/or viral antigen in the appendix, skin, and breast tissues of 2 patients who exhibited LC symptoms 163 and 426 days after symptom onset. Before the surgery, patients underwent PCR testing for SARS-CoV-2 and received negative results.
Martin-Cardona A, et al.[5]	Spain	Surgical tissues	IHC,TEM	Persistence of SARS-CoV-2 demonstrated by TEM in intestinal tissue after COVID-19 recovery and SARS-CoV-2 nasopharyngeal clearance.
de Melo GD, et al.[6]	France	Human nasal cytobrush	IF, SEM, RNA sequencing, RT-qPCR	Long-term persistence of anosmia from COVID-19-associated patients in olfactory mucosa sampling revealed the presence of viral transcripts and SARS-CoV-2 infected cells.
Zollner A, et al.[7]	Austria	Intestinal Biopsies	ELISA, qPCR, IF	SARS-CoV-2 RNA, viral nucleocapsid protein and SARS-CoV-2 antigen were detected in the gut mucosa ~7 months after mild acute COVID-19 patients.
Jin JC, et al.[8]	USA	Stool from newborns	ELISA, qRT-PCR	Although all neonates were negative for SARS CoV-2 nasal PCR, viral RNA and Spike protein were detected in the stool of neonates.
Swank Z, et al.[9]	USA	Plasma	Simoa antigen assays	Circulating SARS-CoV-2 spike protein was detected in the majority of long COVID patients.
Craddock V, et al.[10]	USA	Plasma	ddPCR, ELISA	Spike protein and/or viral RNA fragments persist in long COVID patients for up to 1 year or longer regardless of their presence in the acute COVID-19 phase.

Files JK, et al.[11]	USA	Plasma	Flow cytometric, ELISA	The prolonged post-COVID-19 symptoms in long COVID patients are associated with the persistence of viral antigens in the blood.
Tejerina F, et al.[12]	Spain	Plasma, urine, or stool	RT-PCR	Half of patients with persistent symptoms presented SARS-CoV-2 RNA in extra-respiratory samples. Systemic viral persistence in patients with post-COVID-19 syndrome may benefit of antiviral treatment.

BAL, bronchoalveolar lavage; ddPCR, droplet digital polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IHC, immunohistochemistry; ISH, in situ hybridization; mIHC, multiplex immunohistochemistry; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

Table S2. Approved nirmatrelvir-ritonavir clinical trials for long COVID treatment

Clinical trial	1	2	3
Trial number	NCT05576662	NCT05595369	NCT05668091
First submitted date	October 10, 2022	October 11, 2022	December 24, 2022
Countries	USA	USA	USA
Responsible party	Stanford University	Duke University	Yale University
Recruitment status	Recruiting	Not yet recruiting	Not yet recruiting
Randomized prospective	Yes	Yes	Yes
Double blinded	Yes	Yes	Yes
Phase of clinical trial	II	III	II
Placebo-controlled	Yes	Yes	Yes
Number of participants	200	1700	100
Intervention Model	Parallel assignment	Parallel assignment	Parallel assignment
Nirmatrelvir-ritonavir dosage	300 mg nirmatrelvir, 100 mg ritonavir , twice daily for 15 days	300 mg nirmatrelvir, 100 mg ritonavir , twice daily for 15 days	300 mg nirmatrelvir, 100 mg ritonavir , twice daily for 15 days
Control	Placebo to match nirmatrelvir plus ritonavir	Placebo to match nirmatrelvir plus ritonavir	Placebo to match nirmatrelvir plus ritonavir
Route of administration	Oral	Oral	Oral
Primary measures	The difference between treatment with nirmatrelvir-ritonavir versus placebo on any of the 6 core symptoms of PASC at week 10	The change in exercise intolerance symptom cluster, cognitive dysfunction symptom cluster, autonomic dysfunction symptom cluster	Physical Health Summary Score at Day 28 between nirmatrelvir-ritonavir and placebo/ritonavir treatment estimated

Table S3. Approved clinical trials of the persistence of SARS-CoV-2 in post-COVID-19 patients

Clinical trial	1	2	3
Trial number	NCT04448145	NCT04546776	NCT05220241
First posted date	June 25, 2020	September 14, 2020	February 2, 2022
Countries	USA	UK	France
Responsible party	Columbia University	Quadram Institute Bioscience	Hôpital Fondation A. de Rothschild
Recruitment status	Recruiting	Active, not recruiting	Recruiting
Study type	Prospective, observational, cohort	Prospective, observational, cohort	Prospective, cross-sectional
Official title	Characterizing SARS-CoV-2 persistence in host reservoirs, post-viral sequelae, and associations with host and viral determinants in a cohort of convalescent COVID-19 cases	Prevalence and persistence of SARS-CoV-2 in stool of COVID-19 positive subjects	Investigation of the persistence of SARS-CoV-2 in the olfactory mucosa of patients with prolonged symptoms of COVID-19 with neurocognitive complaints: a prospective cross-sectional multicenter case/control study
Number of participants	325	105	50
Study groups	Participants who have been diagnosed with COVID-19 or experienced COVID-19 symptoms. Participants who have never tested positive for COVID-19.	Participants who have been diagnosed with COVID-19	Participants with neurocognitive complaint. Participants with COVID-19 cured and without neurocognitive complaint.
Biospecimen	Saliva, Nasal Swab, Blood, Stool, Semen, Breastmilk	Faecal and saliva	Micro-brushing the mucous membrane of the olfactory cracks under local anesthesia
Primary measures	Duration of SARS-CoV-2 viral persistence in naso/oropharyngeal, stool or rectal swab, semen, breast milk samples; prevalence of cell immune responses, post-viral sequelae and SARS-CoV-2 persistence and bacterial/viral community structures; duration of COVID-19 Symptoms	Prevalence and persistence of the SARS-CoV-2 virus in the faeces, and change in associated health status	Presence of SARS-CoV-2 RNA in the olfactory mucosa

Supplementary Panel: Recommendations for long COVID research, clinical practice and future directions

1. SARS-CoV-2 is capable of widespread infection and persistent replication at sites in the human body and brain for several months. More autopsy studies (post-COVID> 1 year) are needed to understand the pathogenesis of long COVID.
2. Some clinical trials have shown that the incidence of rebound in the Paxlovid group is about 2% to 6%[13,14]. A large observational study showed that Nirmatrelvir-Ritonavir (Paxlovid) reduced the risk of long COVID-19 by 26% over a six-month period[15]. Well-designed further investigations are needed for informed decision-making regarding antiviral drugs in long COVID patients.
3. For long COVID patients, ddPCR for detection and quantification of SARS-CoV-2 or more sensitive testing techniques are required for tissue biopsy (bronchoscopy, colonoscopy, etc) or stool samples when upper respiratory samples are negative.
4. Mild COVID-19 patients are at risk for a small number of health outcomes, most of which are resolved within 1 year from diagnosis[16]. In hospitalized patients with COVID-19, the burden of symptomatic sequelae remained fairly high. COVID-19 survivors had a remarkably lower health status than the general population at 2 years[17-19]. Hence, use of ddPCR to determine the complete clearance of viral infection would be one of the appropriate interventions for long COVID, especially in patients with severe COVID-19 and immunocompromised hosts.
5. Persistent RNA virus infection may not be apparent, but persistent infection can sometimes lead to chronic disease or recurrence of acute disease. Long-recognized examples include liver fibrosis, cirrhosis and hepatocellular carcinoma as a result of patients persistently infected with hepatitis C virus (HCV)[20]. Future studies should investigate the mechanisms by which SARS-CoV-2 evades immune detection, which will be critical in guiding future therapeutic approaches to facilitate viral clearance.
6. The prolonged persistence of SARS-CoV-2 virus-infected cells in COVID-19 patients can induce pathology itself, as well as prolong abnormal stimulation of immune reactivity, and maintain a chronic inflammatory and pro-thrombogenic status. If the persistent virus is not effectively suppressed, this may lead to progressive deterioration of multi-organ function and death. Hence, anti-SARS-CoV-2 and other applicable therapies may contribute to viral clearance or reduction of inflammation and thus improve long COVID symptoms. We believe that a comprehensive understanding of the pathogenesis of long COVID will provide an opportunity to pave the way for better outcomes for all patients experiencing similar conditions.

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