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Supplementary appendix

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Protocol Title

An open-label randomized controlled trial on lopinavir/ ritonavir, ribavirin and interferon β-1b combination versus lopinavir/ ritonavir alone, as treatment for 2019 novel coronavirus infection

Principal Investigators
Prof Ivan Hung
Prof KY Yuen

Co-Investigators
Dr. Owen Tsang
Dr. Eugene Tso
Dr. Raymond Liu
Dr. Alan Wu
Dr. TC Wu
Dr. KM Sin
Dr. KC Lung
Dr. YY Ng
Dr. Anthony Tam
Dr. Tom Chung
Dr. Jenny Lo
Dr. WL Law
Dr. MY Chu
Dr Veronica Chan
Dr Alwin Yeung
Dr Simon Sin
Dr WM Chan
Dr WW Yan
Dr HP Shum
Dr. WS Leung
Dr. Jacky Chan
Dr. Jasper Chan
Dr. Kelvin To
Dr. Vincent Cheng
Dr. Johnny Chan
Dr. David Lung
Dr. Kitty Fung
Dr. Rodney Lee
Dr. KH Chan
Dr. TL Que

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1. PROTOCOL SYNOPSIS:

Protocol title	An open-label randomized controlled trial on lopinavir/ ritonavir, ribavirin and interferon β-1b combination versus lopinavir/ ritonavir alone, as treatment for 2019-novel-coronavirus (SARS-CoV-2) infection
Hypothesis	A combination of lopinavir/ ritonavir, ribavirin and interferon β-1b will expedite the recovery, suppress the viral load, shorten hospitalisation and reduce mortality in patients with SARS-CoV-2 infection compared with to lopinavir/ ritonavir alone
Primary objective	To evaluate the safety and efficacy in clinical improvement, viral load and mortality reduction with a combination of lopinavir/ ritonavir, ribavirin and interferon β-1b in the treatment of patient hospitalised for SARS-CoV-2 infection and compare this to lopinavir/ ritonavir alone
Subject/patient definition	Recruited subjects include adult patients ≥18 years of age, admitted to the Queen Mary Hospital, Princess Margaret Hospital, Pamela Youde Nethersole Eastern Hospital, Ruttonjee Hospital, United Christian Hospital, Tuen Mun Hospital, Queen Elizabeth Hospital, from February 2020 onwards, with laboratory confirmed SARS-CoV-2 infection. All subjects give written informed consent. Subjects must be available to complete the study and comply with study procedures.
Study design	This is a prospective open-label randomised controlled trial among adult patients hospitalised after February 2020 for virologically confirmed SARS-CoV-2 infection. Patients will be randomly assigned to either a 14-day course of lopinavir/ ritonavir 400mg/100mg twice daily, ribavirin 400mg bd and zero to three doses of subcutaneous injection of interferon β-1b1mL (0.25mg; 8 million IU) on day 1, 3 and 5 (depending on day of admission from symptoms onset) plus standard care, or a 14-day course of lopinavir/ ritonavir 400mg/100mg twice daily plus standard care alone (2:1).
Intervention/study article	lopinavir/ ritonavir, ribavirin and interferon β-1b
Primary outcome	Time to negative nasopharyngeal swab (NPS) SARS-CoV-2 viral RT-PCR

Secondary outcome	1. Time to complete allevation of symptoms as defined by NEWS of 0 maintained for 24 hours 2. Length of hospitalization 3. 30-day mortality 4. Time to negative SARS-CoV-2 RT-PCR for all samples including NPS, throat saliva, throat swab, urine and stool 5. All samples SARS-CoV-2 viral load changes post treatment 6. Cytokine/ chemokine changes 7. Adverse events during treatment
	Clinical and virological characteristics will be compared. $\chi 2$ test will be used for categorical variables where appropriate, whereas Kruskal-Wallis H test will be used for continuous variables. Significant factors by univariate analysis will be further assessed by the multivariate analysis by Cox-regression to identify the independent risk factors for day 7 negative NPS SARS-CoV-2 RT-PCR. SPSS 26.0 for Windows (SPSS Inc., IBM) will be used for statistical computation. P value <0.05 represents significant difference.

2. CLINICAL SECTION

2.1 Background and Rationale

2.1.1 Introduction

The novel coronavirus (SARS-CoV-2 named by ICTV), is a single-stranded RNA coronavirus. The virus was first isolated from patients presented with pneumonia in Wuhan in December 2019. It is believed that the virus first emerged from patients working in the Wuhan Seafood Market which also sold contaminated wild animals, consumed as a local delicacy. Sequences of the Wuhan betacoronavirus show similarities to betacoronaviruses found in bats, sharing a common ancestor with the 2003 SARS coronavirus (SARS-CoV) and the bat coronavirus HKU9-1, a virus found in fruit bats. Similar to SARS-CoV, it is a member of Beta-CoV lineage B. Five genomes of the novel coronavirus have been initially isolated and reported including BetaCoV/Wuhan/IVDC-HB-01/2019, BetaCoV/Wuhan/IVDC-HB-04/2020, BetaCoV/Wuhan/IVDC-HB-05/2019, BetaCoV/Wuhan/WIV04/2019, and BetaCoV/Wuhan/IPBCAMS-WH-01/2019 from the China CDC.

The SARS-CoV-2 has since spread to other parts of China including Beijing, Shanghai, Quanzhou, Shenzhen, Macau and to other parts of the world including Bangkok, Tokyo, Seoul, Taipei, and Washington DC. As of 22 January 2020, more than 400 cases have been confirmed with 9 fatalities. No specific antiviral treatment for the SARS-CoV-2 is currently available, but existing medication could be repurposed.

Genetic sequencing demonstrated similarity of the SARS-CoV-2 to the SARS-CoV and MERS CoV. We expect patients infected with the SARS-CoV-2 will also present similarly with initial upper respiratory tract symptoms including fever, cough, sputum, myalgia and shortness or breath. More severe cases might complicate with pneumonia and required ventilatory or ECMO support. According to our previous studies in 2003 on patients hospitalized for severe SARS-CoV, the viral load peaked between day 7 from symptoms onset and coincided with clinical deterioration of pneumonia and respiratory failure, with majority of the patients required intensive care support. Higher viral load isolated from different human system also correlated with worsened SARS manifestation and complications.

Previously, we have demonstrated that treatment with a combination of protease inhibitor lopinavir/ ritonavir, an antiviral widely used in the treatment of HIV patients and ribavirin, was safe and had significantly clinical benefit in SARS patients in a pilot study. Patients treated with lopinavir/ ritonavir and ribavirin had significantly lower adverse clinical outcome of acute respiratory distress syndrome (ARDS) or death, when compared with historical ribavirin alone controls.

Another medication, interferon-β1b, commonly used in the treatment of multiple sclerosis and lopinavir/ ritonavir, also demonstrated to improve the outcome of MERS-CoV infection in a non-human primate model of common marmoset.

Therefore, we propose to conduct an open-label randomized controlled trial on lopinavir/ ritonavir, ribavirin and interferon β-1b combination treatment for patients hospitalized for 2019-n-CoV infection.

2.1.2 Background of the study articles

(1) Lopinavir/ ritonavir

It is a fixed dose combination medication for the treatment and prevention of HIV/ AIDS. It is a protease inhibitor (lopinavir) combined with a potent cytochrome P450 3A4 inhibitor.

Interactions

Ritonavir inhibits a liver enzyme, CYP3A4, involved in the metabolism of many other commonly prescribed drugs. Taking clarithromycin with other medications that are metabolized by CYP3A4 may lead to unexpected increases or decreases in drug levels. Common interactions include:

- Macrolide
- Statins
- Calcium channel blockers
- Carbamazepine
- Tenofovir

Enrolled patients taking the medications with potential drug-drug interaction with lopinavir/ ritonavir, these medications should be stopped or switched to other medications temporarily for 7 days during the treatment course. Medication will be resumed 24 hours after the treatment has completed.

Side effects

Lopinavir/ ritonavir is generally well tolerated with minimal adverse effects. However, the following serious complications may occur, although the incidence is extremely rare:

1. Anaphylaxis/hypersensitivity reactions: Hypersensitivity and anaphylactic reactions can occur, presented with difficulty in breathing, swelling of face, lips, tongue and throat.
2. Severe skin reaction presented with severe itching, burning and bleeding of the site of application.
3. Prolonged QTc interval.

Minor side effects:

1. Most common side effects are gastrointestinal including diarrhea (27%), nausea (16%), abdominal pain (3%) and vomiting.
2. Headache
3. Transient abnormal liver function tests

Contraindications

1. Hypersensitivity to lopinavir/ ritonavir
2. Known prolonged QTc syndrome, ventricular cardiac arrhythmias, including torsade de pointes, second or third degree heart block, QTc interval >500ms.

(2) Interferon β-1b

It is a cytokine in the interferon family, most commonly used to treat relapsing-remitting multiple sclerosis, via its immunomodulatory effects.

Interactions

It may interact with phenytoin and statins

Enrolled patients taking the medications with potential drug-drug interaction with Interferon β-1b, these medications should be stopped or switched to other medications temporarily for 14 days during the treatment course. Medication will be resumed 24 hours after the treatment has completed.

Side effects

Rare serious complications:

1. Anaphylaxis/hypersensitivity reactions: Hypersensitivity and anaphylactic reactions can occur, presented with difficulty in breathing, swelling of face, lips, tongue and throat.
2. Severe skin reaction presented with severe itching, and burning of the site of application.

Minor side effects:

1. Most common side effects are flu like symptoms after injection with mild fever, myalgia and headache which improves after 24 hours of injection
2. Transient mild derangement of liver function
3. Transient leucopenia

Contraindications

1. Severe depression

(3) Ribavirin

It is a guanosine analog used to stop viral RNA synthesis and viral mRNA capping, thus it is a nucleoside inhibitor.

Interactions

Common interactions include:

- Azathioprine
- Another nucleoside analogue

Enrolled patients taking the medications with potential drug-drug interaction with ribavirin, these medications should be stopped or switched to other medications temporarily for 14 days during the treatment course. Medication will be resumed 24 hours after the treatment has completed. All patients are advised to have

contraception during the 2 weeks' ribavirin treatment, and for 6 months afterwards.

Side effects

Ribavirin is generally well tolerated with minimal adverse effects. However, the following serious complications may occur, although the incidence is extremely rare:

1. Anaphylaxis/hypersensitivity reactions: Hypersensitivity and anaphylactic reactions can occur, presented with difficulty in breathing, swelling of face, lips, tongue and throat.
2. Severe skin reaction presented with severe itching, burning and bleeding of the site of application.

Minor side effects:

1.Anemia

2.Pancytopenia

Contraindications

1. Pregnancy
2. Lactation

3.1 Study Hypothesis and Objectives

3.1.1 Study hypothesis

A combination of lopinavir/ ritonavir, ribavirin and interferon β-1b will expedite clinical recovery, suppress the viral load, shorten hospitalisation and reduce mortality in patients with SARS-CoV-2 infection when compared with standard care alone.

3.1.2 Primary objectives

Time to negative nasopharyngeal swab (NPS) SARS-CoV-2 RT-PCR

3.1.3 Secondary objectives

1. Time to negative SARS-CoV-2 RT-PCR for all samples including NPS, saliva, urine and stool
2. Nasopharyngeal aspirate 2019-n-CoV viral load changes post treatment
3. Saliva SARS-CoV-2 viral load changes post treatment
4. National Early Warning Score (NEWS) changes post treatment
5. Length of hospitalization
6. Adverse events during treatment
7. 30-day mortality
8. Cytokine/ chemokine changes

3.2 Selection of Study population

3.2.1 Inclusion criteria

1. Recruited subjects include all adult patients ≥18 years hospitalised for virologically confirmed SARS-CoV-2 infection.
2. NEWS of ≥1 upon recruitment
3. Auditory temperature ≥38°C or other symptoms including cough, sputum

production, sore-throat, nasal discharge, myalgia, headache, fatigue or diarrhoea upon admission

4. Symptom duration ≤14 days
5. All subjects give written informed consent. For patients who are critically ill, requiring ICU, ventilation or confused, informed consent will be obtained from spouse, next-of-kin or legal guardians.
6. Subjects must be available to complete the study and comply with study procedures. Willingness to allow for serum samples to be stored beyond the study period, for potential additional future testing to better characterize immune response.

3.2.2 Exclusion criteria

1. Inability to comprehend and to follow all required study procedures.
2. Allergy or severe reactions to the study drugs
3. Patients with known prolonged QTc syndrome, ventricular cardiac arrhythmias, including torsade de pointes, second or third degree heart block, QTc interval ≥480ms
4. Patients taking medication that will potentially interact with lopinavir/ ritonavir, ribavirin or interferon β-1b
5. Patients with known history of severe depression
6. Pregnant or lactating women
7. Received an experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month prior to recruitment in this study or expect to receive an experimental agent during this study.
8. To participate in an unrelated trial during the current clinical trial. Nevertheless, the patients have the right to withdraw from the current clinical trial to join another clinical trial.
9. Have a history of alcohol or drug abuse in the last 5 years.
10. Have any condition that the investigator believes may interfere with successful completion of the study.

3.3 Study Design and organization

3.3.1 Overall study design

This is a phase 2, multiple centers open-label randomized controlled clinical trial. Recruited subjects include all adult patients ≥18 years hospitalized for virologic confirmed SARS-CoV-2.

Recruited patients will be randomly assigned into one of two groups by simple randomisation with no stratification. In the study group, for those who presented <7 days from symptoms onset, a combination of lopinavir/ ritonavir, ribavirin for 14 days and interferon β-1b alternate day for 3 days (1, 3, 5), depending on the day of admission, and standard care or those presented between 7 and 14 days from symptoms onset, interferon β-1b will not be given, and lopinavir/ ritonavir alone (ratio 2:1). The control group will receive lopinavir/ ritonavir for 14 days.

3.3.2 Intervention

Patients will be randomised to one of the following study or control groups in the ratio of 2:1 upon qualifying for the study:

The study group: for those presented ≤7 days from symptoms onset, the patients will receive standard care, plus a combination of 14-day course of oral lopinavir/ ritonavir 400mg/100mg capsule twice daily, ribavirin 400mg bd and subcutaneous injection of interferon β-1b 1mL (0.25mg; 8 million IU) for 3 alternative days (1, 3, 5) depending on the day of commencement of the trial. That is, if the patient commences the trial on day 1 to day 2 from symptom-onset, the patient will receive all 3 doses. If commences on day 3 to day 4, the patient will receive 2 doses, and if commences on day 5 to day 6, the patient will receive 1 dose only. For those commence between day 7 and 14, a combination of 14-day course of oral lopinavir/ ritonavir 400mg/100mg capsule twice daily, and ribavirin 400mg bd will be given without the interferon β-1b.

The control group: a combination of 14-day course of oral lopinavir/ ritonavir 400mg/100mg capsule twice daily and standard care.

The ribavirin dosage will be reduced to 600mg daily at any time if the haemoglobin dropped below 10g/dL.

Patients with no history of prolonged QTc syndrome but found to have prolonged QTc interval (male >440ms; female >460ms; but <480ms both sex), bundle branch block, sinus bradycardia upon ECG examination, and those who developed raised ALT of 3x ULN, lopinavir/ ritonavir will be reduced to 400mg/100mg daily. Lopinavir/ ritonavir will be stopped if ALT > 6x upper limit of normal (>270 U/L) or QTc interval >500ms.

Patients with no history of prolonged QTc syndrome but found to have prolonged QTc interval (male ≥440ms; female ≥460ms; but <480ms both sex), bundle branch block, sinus bradycardia upon ECG examination, and those with underlying cardiac condition will be put on cardiac monitor.

Initiation of the interventional treatment has to be commenced within 48 hours after hospital admission. Standard of care includes oxygen, non-invasive and invasive ventilatory support, ECMO support, dialysis support and antimicrobial treatment for secondary bacterial infection as indicated clinically. Stress dose of corticosteroids (hydrocortisone 50mg q8h IV, taper over 7 days) could be given for any patients, who develop oxygen desaturation required non-invasive or invasive ventilatory support, beyond day 6 from symptoms onset.

3.3.3 Randomisation & Blinding

Randomized treatment will be open-label. Patients will be assigned to serial number by the study-coordinator. Each serial number will be linked to a computer-generated randomization list assigning the antiviral treatment regimens. The study medications will be dispensed by the hospital pharmacy and then to the patients by the medical ward nurses.

3.3.4 Withdrawal

Subjects may withdraw at anytime without necessarily giving a reason and without prejudice. The investigator can also withdraw the subject for the following reasons:

1. Severe adverse event occurs
2. Protocol violation
3. The attending physician considers that this is not in the best interest for the subjects to continue the study
4. For patients who withdraw consent because of their own reasons, and not of protocol violations by the investigators or toxicity or physician choice, they will be able to finish the course of treatment.

3.4 Outcome measurements

3.4.1 Primary outcome measurement

Time to negative nasopharyngeal swab (NPS) SARS-CoV-2 viral RT-PCR

3.4.2 Secondary outcome measurements

1. Time to resolution of symptoms as defined by NEWS of 0 maintained for 24 hours
2. Length of hospitalization
3. 30-day mortality
4. Time to negative SARS-CoV-2 RT-PCR for all samples including NPS, throat saliva, throat swab, urine and stool
5. All samples SARS-CoV-2 viral load changes post treatment
6. Cytokine/ chemokine changes
7. Adverse events during treatment

3.4.3 Clinical diagnosis and assessments

Initial diagnosis of SARS-CoV-2 infection will be made upon admission. All recruited patients must be laboratory confirmed to have SARS-CoV-2 infection by RT-PCR in the NPS specimens (detail below). All recruited patients must have a NEWS ≥1 upon admission.

Admission to intensive care unit, requirement of oxygen, mechanical ventilatory, bilevel positive airway pressure and continuous positive airway pressure support will be documented. Arterial blood gas will be measured in patients who required respiratory support. We will investigate blood, sputum or endotracheal aspirates, and urine bacteriologically, as clinically indicated. The NPS upon admission will also be assessed by BioFire® FilmArray® Respiratory Panel 2 *plus* (bioMérieux, Marcy l'Etoile, France) for 18 respiratory virus targets and 4 bacteria, including adenovirus, coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, and MERS-CoV), human metapneumovirus, respiratory syncytial virus, human rhinovirus/enterovirus, influenza A viruses (H1, H1-2009 and H3), influenza B virus, parainfluenza viruses (types 1-4), *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

Baseline ECG/ CXR; clinical data, NPS, saliva and blood specimens will be collected daily from admission till discharge, transfer to convalescent hospitals or death.

3.4.4 Laboratory diagnosis of SARS-CoV-2

3.4.4.1 Reverse transcription-polymerase chain reaction (RT-PCR) for case identification of SARS-CoV-2

Total nucleic acid will be extracted from patient's specimens using MagNA Pure 96 extraction system (Roche, Basel, Switzerland). Real-time RT-PCR assay targeting the RdRp/Hel gene will be performed using QuantiNova Probe RT-PCR Kit (QIAGEN, Hilden, Germany) in a LightCycler 480 II Real-Time PCR System (Roche, Basel, Switzerland).

3.4.4.2 The Filmarray Panel and Cytokine assays

BioFire® FilmArray® Respiratory Panel 2 *plus* (bioMérieux, Marcy l'Etoile, France) will be performed according to the manufacturer's instructions to detect 18 respiratory virus targets and 4 bacteria, including adenovirus, coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, and MERS-CoV), human metapneumovirus, respiratory syncytial virus, human rhinovirus/enterovirus, influenza A viruses (H1, H1- 2009 and H3), influenza B virus, parainfluenza viruses (types 1-4), *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

The cytokine assays of human IL-6 , human IL-10 and human TNF-α will be mesured in patient serum samples using ELISA kits from Invitrogen (Thermo Fisher Scientific, USA). The ELISA protocol will be performed according to the manufacturer's instruction. Briefly, 100 µl of diluted serum or standard and 100 µl of Human IL-6 Biotin conjugate simultaneously were added to the appropriate wells and incubated for 2 hours at room temperature. For Human IL-10 and Human TNF-α, 100 µl of corresponding Biotin conjugate was added to wells after washing 4times with wash buffer and incubated for 1 hour at room temperature. The wells were then washed again for 4 times with wash buffer and followed by addition of 100 µl ofstreptavidin-HPR, and further incubated for 30 minutes. The wells were washed for 4 timeswith wash buffer and added with 100 µl of stabilized chromogen, and incubated for 30minutes at room temperature in the dark. One hundred µl of stop solution was added to eachwell. The absorbance was read at 450 nm. The concentration of each serum sample wasdetermined from the standard curve.

3.5 Adverse events

3.5.1 Evaluation of adverse events

Adverse events may occur in the course of the use of lopinavir/ ritonavir, ribavirin and interferon β-1b specified by the protocol. Investigators will monitor each subject for clinical and laboratory evidence of adverse events till discharge from the acute hospital for the current infection or death. All subjects will be follow-up throughout the study period up to 30 days post treatment initiation. The investigator will assess and record any adverse event in detail on the adverse event case report forms (CRF) including the date of onset, description, severity, duration and outcome, relationship of the adverse event to the study articles, an alternate aetiology for events not considered "probably related" to study product, final diagnosis, and any action(s) taken. For adverse events

to be considered sporadic, the events must be of similar nature and severity. Adverse events, whether in response to a query, observed by site personnel, or reported spontaneously by the subject will be reported on the appropriate CRF.

Investigators will report all the adverse events and serious adverse events to the Study Monitor and serious adverse events to the IRB within 24 hours. Each study site will appoint its own study monitor, who is one of the investigators. He/ she will be responsible for reporting the AEs or SAEs to the cluster IRB.

A Drug Safety Monitoring Board, including the investigators, pharmacists, and physicians of the IRB independent of the study will provide regular monitoring for all safety aspects of the study. Regular interim reports will be sent to the IRB for monitoring of the study. The DSMC will meet every 3 months. The DSMC will include all the principal and co-investigators from all study sites.

3.5.2 Definition of adverse events

An adverse event (AE) is any untoward medical occurrence, all episodes of abnormal findings, subjective and objective symptoms of disease, intercurrent illness and accidents in a subject during the study, which does not necessarily have a causal relationship with the investigational product, and they can also occur outside the time that the study articles were given. AE includes the following:

- Any clinically significant worsening of a preexisting condition;
- Any re-occurrence of a pre-existing condition;
- An AE occurring from overdose of a study drug whether accidental or intentional
- An AE occurring from abuse of a study drug
- An AE that has been associated with the discontinuation of the use of a study drug.

Note: A procedure is not an AE, but the reason for a procedure may be an AE.
A preexisting condition is a clinical condition (including a condition being treated) that is diagnosed before the subject signs the informed consent and is documented as part of the subject's medical history.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or in frequency will be used to determine whether an event is a treatment-emergent adverse event. An AE is considered to be treatment emergent if: (1) it was not present when the active phase of the study began and is not a chronic condition that is part of the subject's medical history; or (2) it was present at the start of the active phase of the study or as part of the subject's medical history, but the severity or frequency increased during the active phase.

The active phase of the study begins at the time of the first dose of study medication and concludes with the end of the study.

3.5.3 Definition of serious adverse events

If an adverse event meets any of the following criteria, it is to be reported to the Study Monitor and respective CREC as a serious adverse event (SAE) within 24 hours of occurrence or notification of the site:

- **Death**: An event, which results in the death of a subject.
- **Life-threatening**: An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
- **Prolong hospitalisation**: An event, which occurs while the study subject, is hospitalized and prolongs the subject's hospital stay.
- **Persistent or Significant Disability/Incapacity**: An event, which results in a condition that substantially, interferes with the activities of daily living of a study subject. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g., sprained ankle).
- **Important Medical Event Requiring Medical Intervention to Prevent Serious Outcome:** An important medical event that may not be immediately life-threatening or result in death, but based on medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death of subject, life-threatening, hospitalisation, prolongation of hospitalisation, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasia or convulsion that does not result in inpatient hospitalisation, or the development of drug dependency or drug abuse.

3.5.4 Definition of intensity of AE

- **Mild**: The AE is transient and easily tolerated by the subject.
- **Moderate**: The AE causes the subject discomfort and interrupts the subject's usual activities.

Severe: The AE causes considerable interference with the subject's usual activities and may be incapacitating or life threatening.

3.5.5 Definition of relationship to investigational drug

The relationship of the adverse event with the investigational product using a modified version of that of Karch and Lasagna (1975) as below:

- **Definite:** A reaction that follows a reasonable temporal sequence from administration of the investigational product or in which the level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected investigational product, and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, with reappearance of the reaction on repeated exposure (re-challenge)
- **Probable:** A reaction that follows a reasonable temporal sequence from administration of the investigational product, that follows a known or expected response pattern to the suspected investigational product, that is confirmed by stopping or reducing the dosage of the investigational product, and that could not be

reasonably explained by the known characteristics of the subject's clinical state

- **Possible:** A reaction that follows a reasonable temporal sequence from administration of the investigational product, that follows a known or expected response pattern to the suspected investigational product, but that could readily have been produced by a number of other factors
- **Not Assessable:** A relationship for which no evaluation can be made
- **Not Related:** A reaction for which sufficient information exists to indicate that the aetiology is unrelated to the investigational product

3.5.6 Documentation and reporting of adverse events

3.5.6.1 Non-serious adverse events

Adverse events will be continually monitored for, or asked about at all visits. The occurrence of all adverse events will be documented in the CRF with the following type of information where appropriate:

- Nature of adverse event
- When the adverse event first occurred
- Intensity of the adverse event
- Relationship to investigational product
- How long the adverse event persisted
- Whether the event was once or intermittent (Ideally each occurrence of an adverse event should be reported. However, certain adverse events may occur frequently, such as vomiting or diarrhea, and it is more sensible to record these as a single event with an intermittent periodicity if the intervals are less than 24 hours)
- Countermeasures

3.5.6.2 Serious adverse events

All serious adverse events, the onset of which occur during the course of the study with the study treatment, since signing the informed consent form until the end of the study, regardless of study drug or protocol relationship, must be reported by the investigator to the IRB by fax within 24 hours of his/her becoming aware of the event. The investigator will document all available information regarding the serious adverse event on the Serious Adverse Event pages contained in the individual Case Report Form and fax to the IRB for serious adverse event reporting. The investigator should both wait to receive additional information to fully document the event before notifying IRB of a serious adverse event. The initial notification should include, as a minimum, sufficient information to permit identification of:

- Subject's study number
- Subject's initials (initial from family name last; subject's name is not to be communicated for reasons of confidentiality)
- Time and date of receiving the study articles
- Time and date of occurrence of the event
- A brief description of the event and countermeasures taken
- Investigator's opinion of the relationship with the study articles

The initial fax report should be followed by a full summary using the Serious Adverse Event pages in the individual Case Report Form detailing relevant aspects of the

adverse events in question. Where applicable, information form relevant hospital case records and autopsy reports should be obtained.

The investigator is obliged to comply with applicable regulatory requirement(s) related to the reporting of serious adverse events to the IRB.

3.5.6.3 Follow-up of subjects with non-serious or serious adverse events

All subjects experiencing non-serious or serious adverse events, whether considered associated with the use of the study articles or not, must be monitored until symptoms subside and any clinically relevant changes of laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings must be reported in the subject's file.

3.6 Statistical Methods

3.6.1 Sample size

As SARS-CoV-2 is a novel virus, the sample size calculation is based on the findings from our pilot study performed in 2003 on SARS-CoV infection.[6] The current study is designed an estimated difference of 26.4% in the 21-day mortality/ ARDS rate in patients with severe SARS-CoV-2 infection, when treated with lopinavir/ritonavir (2.4%) vs. historical controls without antiviral treatment (28.8%). The necessary sample size has been calculated to be 30 patients per group to detect such a difference at a two-sided alpha level of 0.05, with 80 percent power. The protocol proposed recruiting at least 35 subjects per group to allow for a 17% drop out rate, due to adverse effects or premature termination of randomised treatment.

3.6.2 Analysis of the Study

All patients recruited will undergo intention to treat analysis. Clinical and virologic characteristics will be compared. $\chi 2$ test will be used for categorical variables where appropriate, whereas Kruskal-Wallis H test will be used for continuous variables. Significant factors by univariate analysis will be further assessed by the multivariate analysis by binary logistic regression to identify the independent factors for day 7 negative NPS SARS-CoV-2 RT-PCR. SPSS 26.0 for Windows (SPSS Inc., IBM) will be used for statistical computation. P value <0.05 represents significant difference.

3.6.3 Recruitment period and follow-up

All patients will be follow-up till discharge from the acute hospital for the current infection or death. All patients will be follow-up for at least 30 days post treatment. Discharge patients will also be assessed at the outpatient clinic within 30 days after discharge.

4.1 Informed Consent

The investigator or his/her representative will explain the nature of the study to the subjects, and answer all questions by the subjects regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subjects and the person who administered the informed consent. All subjects will be given one hour to consider

whether he or she would like to join the study.

4.2 Confidentiality of data

4.2.1 Confidentiality of Study Data

The aims and content of this study, and the results thereof are confidential and are not to be transmitted to any third party in any form or fashion. All persons involved in the study are bound by this confidentiality clause.

4.2.2 Confidentiality of Subject Data

Permission for direct access to a subject's data will be sought in writing by the investigator and from the subject as part of the informed consent procedure. This gives permission to examine, analyse, verify and reproduce any records and reports that are important to the evaluation of the study. Any party (e.g., domestic and foreign regulatory authorities, monitors and auditors) with direct access must take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of the subjects' identities and the Hospital Authority's proprietary information. It is the monitor's responsibility to verify that each subject has consented, in writing, to direct access.

4.2.3 Archive of data

The investigator must retain all study documentation pertaining to the conduct of the study at the study site for a period of 5 years.

4.3 Ethical and Administrative endorsement

4.3.1 Administrative and ethical approval

The study will be approved by the Institutional Review Board (IRB) of the University of Hong Kong and Hospital Authority

4.3.2 Study Team

Principal investigators: design of the study, coordination and assessment of subjects for study enrollment, report and documentation of adverse events of study articles, data collection, analysis and writing up. Co-investigators: coordination and assessment of subjects for study enrollment, report and documentation of adverse events of study articles, data collection and analysis. Study nurse: randomisation of subjects.

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Appendix Text

Nucleic acid extraction and real-time reverse transcription-polymerase chain reaction (RT-PCR) for SARS-CoV-2

Nasopharyngeal swab and saliva specimens were subjected to total nucleic acid (TNA) extraction by MagNA Pure 96 extraction system (Roche, Basel, Switzerland). 250 µl of each specimen was mixed with external lysis buffer. After extraction, the TNA was recovered in 50µl elution buffer. In-house one-step real-time RT-PCR assay for SARS-CoV-2 was performed using QuantiNova Probe RT-PCR Kit (QIAGEN, Hilden, Germany). The forward primer (5'- CGCATACAGTCTTRCAGGCT -3'), reverse primer (5'- GTGTGATGTTGAWATGACATGGTC -3') and probe (5' FAM- TTAAGATGTGGTGCTTGCATACGTAGAC -IABkFQ 3') targeting RdRp/Hel region of SARS-CoV-2 were used.[1] The reagent mixture (20 µl) contained 10 µl of 2x QuantiNova Probe RT-PCR Master Mix, 0.2 µl of QN Probe RT-Mix, 1.6 µl of each 10 µM forward and reverse primer, 0.4 µl of 10 µM probe, 1.2 µl of RNase-free water and 5 µl of TNA as the template. The thermal cycling condition was 10 min at 45 °C for reverse transcription and 5 min at 95 °C for PCR initial activation, followed by 45 cycles of 5 s at 95 °C and 30 s at 55 °C. Ten-fold serial dilutions of *in vitro* RNA transcript were used to generate a standard curve for viral load measurement. All reactions were performed using the LightCycler 480 II Real-Time PCR System (Roche).

BioFire® FilmArray® Respiratory Panel 2 *plus*

The NPS upon admission was also assessed by BioFire® FilmArray® Respiratory Panel 2 *plus* (bioMérieux, Marcy l'Etoile, France) for 18 respiratory virus targets and 4 bacteria including adenovirus, coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-

HKU1, and MERS-CoV), human metapneumovirus, respiratory syncytial virus, human rhinovirus/enterovirus, influenza A viruses (H1, H1-2009 and H3), influenza B virus, parainfluenza viruses (types 1-4), *Bordetella pertussis*, *Bordetella parapertussis,* and *Mycoplasma pneumoniae.*

Cytokine assays

Human IL-6, Human IL-10 and Human TNF-α were measured in patient serum samples using ELISA kits from Invitrogen (Thermo Fisher Scientific, USA). The ELISA protocol was performed according to the manufacturer's instruction. Briefly, 100 µl of diluted serum or standard and 100 µl of Human IL-6 Biotin conjugate simultaneously were added to the appropriate wells and incubated for 2 hours at room temperature. For Human IL-10 and Human TNF-α, 100 µl of corresponding Biotin conjugate was added to wells after washing 4 times with wash buffer and incubated for 1 hour at room temperature. The wells were then washed again for 4 times with wash buffer and followed by addition of 100 µl of streptavidin-HPR, and further incubated for 30 minutes. The wells were washed for 4 times with wash buffer and added with 100 µl of stabilized chromogen, and incubated for 30 minutes at room temperature in the dark. One hundred µl of stop solution was added to each well. The absorbance was read at 450 nm. The concentration of each serum sample was determined from the standard curve.[2-4]

Nanopore sequencing of nsp5 gene (encoding the protease 3CLpro)

Nsp5 gene was amplified from TNA by SuperScript™ III one-step RT-PCR system with Platinum™ *Taq* High Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) using the primers ONT-COVID19-10022F (5'-

TTTCTGTTGGTGCTGATATTGCCCACAAACCTCTATCACCTCAG-3') and ONT-COVID19-11132R (5'-ACTTGCCTGTCGCTCTATCTTCCAGACATAGCAATAATACCCATAGC-3'). The underlined bases represent 5' universal tails that are complementary to the barcode primers of PCR Barcoding kit [EXP-PBC096] manufactured by Oxford Nanopore Technologies. RT-PCR conditions were 50°C for 30 min, 94°C for 2 min, then 40 cycles of 94°C for 30 s, 55°C for 30 s and 68°C for 1 min 10 s. Subsequent library preparation was performed according to manufacturer's instructions as we described previously.[5] Briefly, amplified PCR products were purified by 0.8x AMPure XP bead (Beckman Coulter, California, CA, USA) and were then subjected to PCR barcoding (EXP-PBC096). Barcoded PCR products were purified by 0.8x AMPure XP bead before pooled together in equal molar for end repair and sequencing adaptor ligation (SQK-LSK109). Finally, sequencing was performed on Oxford Nanopore MinION platform using R9.4.1 flow cell for 12 hours.

After sequencing, the raw signal data was basecalled and demultiplexed into FASTQ files using Guppy v3.4.1 with the accurate basecalling mode and an extra of 60 bp was trimmed at both ends to remove low quality bases. The QC passing score was set to 10 to ensure high quality reads were obtained. Next, the DNA sequences were mapped on the reference genome Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 (NCBI accession number MN908947.3) to generate an alignment file using Minimap2 with the default settings.[6] BCFtools mpileup was used in creating a variant file.[7] While BCFtools call, vcfutils.pl[8] and Seqtk seq were used in generating the FASTA consensus sequences. A mutation is considered to be true if it exists in >20% of the reads at a particular nucleotide

position. The consensus sequences and raw reads have been deposited into GenBank (Accession number: pending) and Bioproject (Accession number: PRJNA615965).

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Appendix Table 1– Univariable analysis for baseline factors associated with negative day 7 nasopharyngeal swab viral load

	Negative NPS (n=51)	**Positive NPS** (n=76)	p value
Age; median (range)	46 (31-62)	55 (34-62)	0.17
Sex (male) %	27 (53)	41 (53)	0.91
Days of starting treatment from symptoms onset; median (IQR)	4.0 (3.0-7.0)	6.5 (4.0-8.0)	0.07
Treatment-group	45 (88)	41 (54)	<0.0001
Underlying diseases %			
Diabetes mellitus	2 (4)	15 (20)	0.010
Hypertension	12 (24)	24 (32)	0.32
Coronary artery disease	3 (6)	7 (9)	0.50
Cerebrovascular disease	1 (2)	1 (1)	0.78
Hyperlipidemia	11 (22)	18 (24)	0.78
Thyroid disease	2 (4)	2 (3)	0.68
Obstructive sleep apnoea	1 (2)	1 (1)	0.78
Crohn’s disease	0 (0)	1 (1)	0.41

Epilepsy	0 (0)	1 (1)	0.41
Old pulmonary tuberculosis	2 (4)	0 (0)	0.080
Chronic hepatitis B	2 (4)	1 (1)	0.34
Chronic hepatitis C	0 (0)	1 (1)	0.41
Malignancy	0 (0)	2 (3)	0.24
Smoker	3 (6)	4 (5)	0.88
Symptoms			
Fever	38 (75)	64 (84)	0.18
Chills	4 (78)	15 (20)	0.070
Cough	20 (39)	48 (63)	0.0080
Sputum	15 (29)	27 (36)	0.47
Shortness of breath	2 (4)	12 (16)	0.036
Sore throat	13 (25)	13 (17)	0.25
Myalgia	3 (6)	15 (20)	0.028
Malaise	7 (14)	17 (22)	0.22
Nausea or vomiting	1 (2)	0 (0)	0.22
Diarrhoea	7 (14)	17 (22)	0.22

Rhinorrhoea	9 (19)	15 (19)	0.77
Anosmia	3 (6)	2 (3)	0.36
Headache	3 (6)	3 (4)	0.61
Chest tightness	0 (0)	2 (3)	0.24
Anorexia	0 (0)	1 (1)	0.41
Initial laboratory findings (normal range)			
Hemoglobin (11.5-14.8 g/dL)	13.1 (12.7-14.9)	13.5 (12.6-14.8)	0.91
White cell count (3.89-9.93 x 10^9/L)	5.1 (3.7-6.6)	5.3 (4.4-6.2)	0.60
Platelet (154-371 x 10^9/L)	194 (171-264)	194 (169-252)	0.47
Neutrophil (2.01-7.42 x 10^9/L)	3.4 (2.2-4.3)	3.3 (2.6-4.4)	0.69
Lymphocyte (1.06-3.61 x 10^9/L)	1.1 (0.8-1.6)	1.0 (0.8-1.5)	0.59
ALT (8-45 U/L)	22 (14-32)	26 (16-40)	0.06
ALP (42-110 U/L)	56 (46-73)	65 (52-76)	0.111
LDH (143-280 U/L)	177 (146-209)	213 (170-289)	0.0010
Bilirubin (4-23 μmol/L)	6 (5-9)	7 (6-10)	0.22

Creatinine (49-82 µmol/L)	80 (65-95)	74 (63-93)	0.49
Urea (2.9-8 mmol/L)	3.9 (2.9-4.7)	3.9 (2.9-4.7)	0.89
Creatine kinase (22-198 U/L)	68 (44-117)	99 (67-158)	0.023
CRP (<0.76 mg/dL)	3 (1-6)	3 (2-9)	0.18
ESR (<12 mm/hr)	23 (10-38)	22 (21-48)	0.45
Normal CXR (baseline)	18 (35)	13 (17)	0.019
Oxygen therapy (baseline)	0 (0)	1 (1)	0.41
NEWS 2 (baseline)	2 (2-2)	2 (2-2)	0.54
SOFA (baseline)	0 (0-1)	0 (0-1)	0.38
Virologic findings [RT-PCR (log_{10} copies/ml)] median (IQR)			
NPS VL (baseline)	6.0 (4.4-7.6)	6.6 (4.3-8.4)	0.30
POS VL (baseline) *	5.2 (3.6-7.1)	5.3 (4.3-7.0)	0.42
TS VL (baseline)	4.3 (2.3-5.6)	4.6 (3.4-6.3)	0.22
Stool VL (baseline)	2.9 (1.3-3.3)	4.1 (3.0-7.0)	0.017
Cytokine concentration (log_{10} pg/mL); median (IQR)			

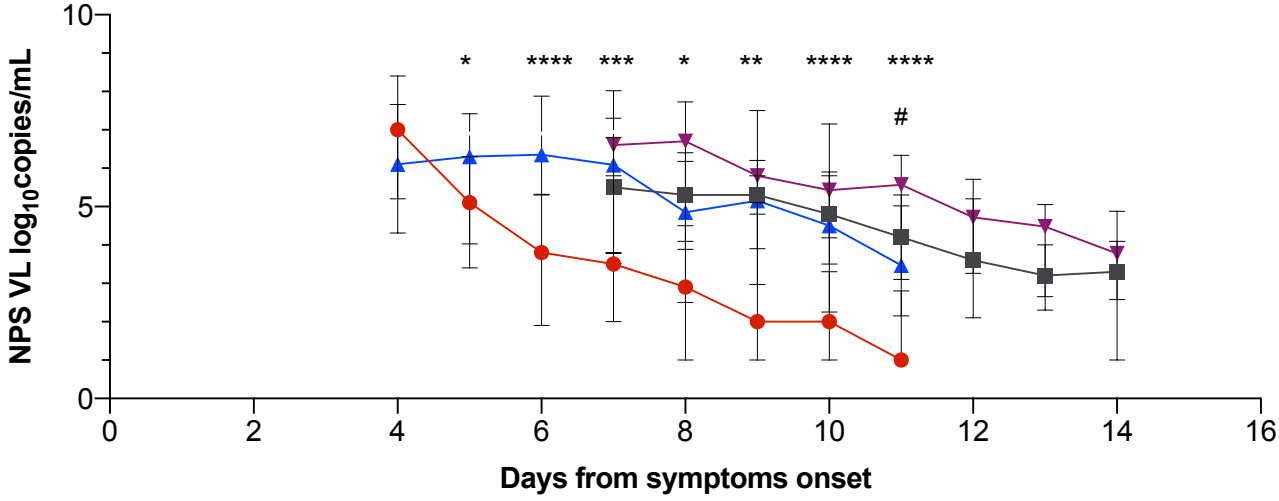
IL-6 (baseline)	1.4 (1.3-1.5)	1.4 (1-1.4)	0.26
TNF-α (baseline)	1 (1-1)	1 (1-1)	0.94

Treatment group: 52 cases treated by triple combination of IFN beta-1b, lopinavir/ritonavir and ribavirin;
34 cases treated by lopinavir/ritonavir and ribavirin
NEWS 2: National Early Warning Score 2; SOFA score: Sequential Organ Failure Assessment Score
NPS: nasopharyngeal swab; POS: posterior oropharyngeal saliva; TS: throat swab
RT-PCR: reverse transcription polymerase chain reaction; VL: viral load
IL-6: Interleukin 6; TNF- α: tumour necrosis factor α; IQR: interquartile range
ALT: alanine transaminase; ALP: alkaline phosphatase; LDH lactate dehydrogenase; CRP: C reactive protein;
ESR: erythrocyte sedimentation rate
IQR : interquartile range

Appendix Table 2. Multivariable analysis of independent factors associated with negative day 7 nasopharyngeal swab viral load

Factors	HR (95% CI)	p-value
Treatment-group	4.27 (1.82-10.02)	0.0010
Normal CXR	1.97 (1.11-3.50)	0.021

Treatment group: 52 cases treated by triple combination of IFN beta-1b, lopinavir/ritonavir and ribavirin;
34 cases treated by lopinavir/ritonavir and ribavirin
CXR: chest radiograph
HR: hazard ratio; CI: confidence interval



Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
IFN beta-1b + lopinavir/ritonavir + ribavirin (<7 days symptoms-onset) (number of samples):					52	52	52	52	52	49	45	45			
lopinavir/ritonavir (<7 days symptoms-onset) (number of samples):					24	24	24	24	24	23	22	21			
lopinavir/ritonavir + ribavirin (≥7 days symptoms-onset) (number of samples):								34	34	34	34	33	33	31	30
lopinavir/ritonavir (≥7 days symptoms-onset) (number of samples):								17	17	17	17	17	17	17	16

RT-PCR; Median (IQR)
*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001 (treatment vs. control <7 days from symptoms-onset)
#P<0.05 (treatment vs. control ≥7 days from symptoms-onset)
Baseline 100% positive
NPS: nasopharyngeal swab; VL: viral load
IFN beta-1b: Interferon beta-1b