A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19

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BACKGROUND – THE STUDY QUESTION?	
Background	• During the 2003 Severe Acute Respiratory Syndrome (SARS) outbreak lopinavir, a protease inhibitor (PI) used in combination with the PI-booster ritonavir in the management of HIV-1, was identified as having *in vitro* inhibitory activity against SARS-CoV • Compared to a historical control arm that received ribavirin monotherapy (i.e., non-randomized and non-contemporary comparator group), combination lopinavir-ritonavir (LOPINAVIR-RITONAVIR) with ribavirin reduced the risk of acute respiratory distress syndrome (ARDS) or death in SARS-CoV • In vitro and in vivo animal studies support LOPINAVIR-RITONAVIR's potential activity against MERS-CoVand a clinical trial (in combination with recombinant interferon beta-1b) is currently enrolling participants (MIRACLE trial) • Lopinavir is thought to work via 3-chymotrypsin-like protease inhibition, which has been seen *in vitro* for other coronaviruses but there are no specific in vitro studies in SARS-CoV-2
Previous trials	• In a case series of 18 patients in Singapore, Young et al. described five hypoxemic patients treated with LOPINAVIR-RITONAVIR (200 mg/100 mg dose). Three patients experienced decreased oxygen requirements within 3 days, two patients cleared viral shedding within 2 days, and four patients experienced adverse events limiting treatment (nausea, vomiting and/or diarrhea). • Several retrospective cohort studies of patient with COVID-19 in Wuhan, China have also included patients treated with LOPINAVIR-RITONAVIR, however, these LOPINAVIR-RITONAVIR observational studies are limited by their inability to establish causation and a randomized controlled trial is needed
Why this study?	• Given the in vitro inhibitory activity and potential improvement in clinical outcomes with SARS-CoV and MERS-CoV, LOPINAVIR-RITONAVIR is an investigative treatment option for COVID-19 patients • The objective of the current study was to evaluate the efficacy and safety of oral LOPINAVIR-RITONAVIR for hospitalized adults with severe COVID-19
Null Hypothesis	• There is no difference in clinical improvement through 28 days post enrollment in patients who received LOPINAVIR-RITONAVIR versus standard of care (SOC)

GENERAL STUDY OVERVIEW		
	Summary	Critique
Funding	• Major Projects of National Science and Technology on New Drug Creation and Development, Chinese Academy of Medical Sciences, Emergency Project of CoVID-19, National Science Grant of Distinguished Young Scholars • LOPINAVIR-RITONAVIR freely provided by National Health Authority	
Trial design	• Single-center, open-label, randomized controlled trial, parallel-group superiority study • Patients randomized 1:1 via permuted blocks to receive either LOPINAVIR-RITONAVIR with SOC or SOC alone for 14 days	Pros: • RCTs with a control arm is gold-standard of evidence-based medicine • Randomization was stratified according to respiratory status at time of enrollment to ensure a balanced distribution of oxygen support between treatment groups • Allocation concealment through randomization minimized selection (allocation) bias Cons: • Not placebo-controlled because of the emergent nature of this study • Blinding only occurred during treatment allocation, which

		could negatively impact internal validity
Objectives	• The primary objective was to LOPINAVIR-RITONAVIREvaluate the efficacy and safety of oral LOPINAVIR-RITONAVIR for SARS-CoV2 infection	This was the first published RCT on LOPINAVIR-RITONAVIR for COVID-19 treatment.
Enrollment	• Patients were enrolled from 01/18/2020, through 02/03/2020 (the date of enrollment of the last patient), at Jin Yin-Tan Hospital, Wuhan, Hubei Province, China	• Single-centered, very short study enrollment period of approximately two weeks • Study suspended enrollment after remdesivir became available for investigational use
METHODS		
Inclusion criteria	• Adults 18 years or older • Diagnostic specimen positive for SARS-CoV2 • Pneumonia confirmed by chest imaging • Oxygen saturation ≤94% on room air or PaO2:FiO2 ratio ≤300 mm Hg	• Inclusion criteria of respiratory requirements are consistent with hospitalized COVID-19 patients in the United States (i.e., external validity) • All patients had confirmed SARS-CoV2 detected by RT-PCR
Exclusion criteria	• Pregnant or breast-feeding • HIV infection • Physician decision not to enroll patient in trial • Condition that would not allow study protocol to be followed safely • Known allergy or hypersensitivity to LOPINAVIR-RITONAVIR • Severe liver disease (e.g., cirrhosis with ALT/AST >5x upper limit of normal) • Use of medications contraindicated with LOPINAVIR-RITONAVIR that could not be replaced or discontinued during trial period • LOPINAVIR-RITONAVIR	• Exclusion criteria were appropriate but does limit results to non-pregnant females and those without HIV infection, which are two overlooked populations in current COVID-19 studies • Unclear what conditions would preclude safe following of the protocol because the full study protocol (supplemental file) was not translated to English
Interventions	• LOPINAVIR-RITONAVIR (400mg/100mg) orally twice daily plus standard care for 14 days; patients who were unable to swallow received LOPINAVIR-RITONAVIR via nasogastric tube • Comparator group was standard care only which included supplemental oxygen, non-invasive and invasive ventilation, antibiotics, vasopressors, renal replacement therapy, and extracorporeal membrane oxygenation (ECMO) as needed	• Standard care could include concurrent antibiotics, and while not expected to be differentially distributed between the two comparator groups, these pharmacologic interventions could diminish LOPINAVIR-RITONAVIR's treatment effect
Primary Endpoints	• Time to clinical improvement, defined as the time from randomization to an improvement of two points on a seven-category ordinal scale or alive at discharge, whichever occurred first • Assessments occurred once daily by trained nurses	• Investigators have used a similar end point in a previous influenza study and also cited the WHO R&D Blueprint expert group recommendations for COVID-19 R&D • Ordinal scale allows for comparison of patients who have vastly different baseline clinical presentations
Secondary Endpoints	• Clinical improvement on days 7 and 14 • Mortality at day 28 • Duration of mechanical ventilation • Duration of hospitalization • Time (in days) from treatment initiation to death • Proportion of patients with viral RNA detection over time (days 5, 10, 14, 21, and 28) from oropharyngeal swabs • Viral RNA titer area-under-the-curve (AUC) measurements	• The US CDC recommends obtaining nasopharyngeal over oropharyngeal (OP) swabs, both detect SARS-CoV2 in the upper respiratory tract, not the lower respiratory tract, which may be a more important virologic measure for hospitalized patients • Adverse events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events

	• Safety outcomes included adverse events that occurred during treatment, serious adverse events, and premature treatment discontinuation	
Statistical analyses	• Total sample size calculated to be 160 patients(power of 80%, two-sided alpha of 0.05) to detect a difference of 8 days in median time to clinical improvement assuming median time to outcome in control was 20 days and that 75% of patients reached clinical outcome • Assessment at 160 patients determined trial was underpowered, enrollment continued • Intention-to-treat (ITT) population to assess primary endpoint • Kaplan-Meier plot for time to clinical improvement and compared with log-rank test • Right-censored for those who did not reach primary endpoint at day 28 (i.e., failure to reach clinical improvement or death) • Cox proportional-hazards model with hazard ratios (HR) and 95% confidence intervals (CIs) • Modified intention-to-treat (mITT) analysis that excluded three early deaths in the LOPINAVIR-RITONAVIR group (randomized but died before 1st dose) • Two post-hoc analyses were also completed: National Early Warning Score 2 (NEWS2) $\leq$ 5 vs > 5, time to lopinavir–ritonavir treatment $\leq$ 12 days vs > 12 days after illness onset	Pros: • Use of ITT instead of per-protocol improved external generalizability of study results • Right censoring also accounts for potential competing risks Cons: • Unclear why 8 days median difference was chosen, does not seem to be based on previous studies of influenza or SARS-CoV-1 • Large effect size likely resulted in calculated sample size small enough to seem attainable in a short time period • Data was analyzed, determined to be underpowered, enrollment continued, and more collection and re-analysis occurred – multiplicity and inflation of Type I error rate likely and not accounted for • Five patients (5%) assigned to LOPINAVIR-RITONAVIR did not receive any doses (excluding three patients who died within 24 hours comprised the mITT population) • Post-hoc analyses were not powered to allow for detection of clinically relevant differences in sub-groups, and the time-to-initiation analysis is highly important because antiviral treatment effects are best measured during the first 1-2 days following illness onset (as demonstrated with influenza and SARS) • Cox proportional-hazards model without adjustment for other variables, which may still be necessary in an RCT
RESULTS		
Enrollment	• 199 patients underwent randomization; 99 to lopinavir–ritonavir plus standard care, 100 to standard care alone • In the LOPINAVIR-RITONAVIR arm five patients did not receive any study drug: 3 deaths occurred within 24 hours of randomization and 2 because physician refused to prescribe LOPINAVIR-RITONAVIR • One patient randomized to the standard care group received LOPINAVIR-RITONAVIR on day 10 but remained in the standard care group for all analyses except the safety analysis	• Bias introduced by lack of blinding to treatment
Baseline characteristics	• Median age: 58 years (IQR 49, 68), 60% male • Median time from symptom onset to randomization: 13 days (IQR 11, 16) • No significant differences in baseline characteristics between arms • Systemic glucocorticoids were administered to 33% LOPINAVIR-RITONAVIR and 35.7% standard care	• Delayed time to enrollment and randomization from symptom onset inconsistent with findings of benefit if initiated early in SARS-CoV-1 • No significant differences in glucocorticoid administration between the two arms • Approximately 11% were on interferon at enrollment (9.1% vs 13% in LOPINAVIR-RITONAVIR and standard care

		groups, respectively) and this was not addressed in the text despite preliminary evidence of LOPINAVIR-RITONAVIR and interferon combination against MERS-CoV
Monitoring	• Patients assessed once daily by trained nurses for efficacy and safety from days 0 to 28 after enrollment • Other clinical data monitored according to WHO-ISARIC (World Health Organization–International Severe Acute Respiratory and Emerging Infections Consortium) • Serial oropharyngeal swabs obtained on days 5, 10, 14, 21, and 28 until discharge or death	• Monitoring only through 28 days which may be discordant with above stated methods of time to clinical improvement or alive at discharge, both of which could have occurred prior to 28 days • Sampling did not stop when a negative result was obtained at a given time-point
Primary Outcome	• No difference in median time to clinical improvement in L–R vs standard care (16 vs 16 days, HR = 1.31, 95% CI 0.95to 1.8) in ITT population • Median time to clinical improvement in mITT population was 15 days in the LOPINAVIR-RITONAVIR group vs 16 days in the standard care group (HR = 1.39, 95% CI 1 to 1.9) • No differences in primary outcome in post-hoc subgroup analyses • No difference in clinical deterioration (one-category increase on the seven-category scale) between the two groups (HR = 1.01, 95% CI 0.76 to 1.34)	• Other clinically-relevant measures such as C-reactive protein were not included • Although not significantly different between groups there was no attempt to adjust HR in multivariable model based on clinical *a priori* knowledge • Study was not powered for subgroup analysis, in particular in the post-hoc analysis the subgroup $\leq$ 12 days was 42 patients in the LOPINAVIR-RITONAVIR group versus 48 patients in the standard care group (HR = 1.25, 95% CI 0.77 to 2.05)
Secondary Outcomes	• Numerically lower 28-day mortality; in ITT was 19.2% LOPINAVIR-RITONAVIR vs 25% standard care (difference, -5.8%, 95% CI, -17.3 to 5.7); and in mITT was 16.7% LOPINAVIR-RITONAVIR vs 25% standard care (difference, -8.3%, 95% CI, -19.6 to 3) • LOPINAVIR-RITONAVIR arm had shorter median hospital LOS after randomization (12 vs 14 days, difference 1 day, 95% CI 0 to 3 days) • LOPINAVIR-RITONAVIR arm had higher day 14 clinical improvement LOPINAVIR-RITONAVIR(45.5% vs. 30%; difference, 15.5%; 95% CI, 2.2 to 28.8) • LOPINAVIR-RITONAVIRMean viral RNA load among those in the LOPINAVIR-RITONAVIR was higher than standard care at baseline (4.4 $\pm$ 2.2 vs 3.7 $\pm$ 2.1 $\log_{10}$ copies/mL) however viral loads did not differ over time between two groups • Total of 69 patients (35%) had a negative RT-PCR result on a subsequent throat swab	• No other differences in secondary outcomes between groups, all deaths in safety cohort secondary to respiratory failure • Body of text is misleading with respect to difference in ICU LOS, which reported the difference of 5 days among all patients; however, among *survivors* the median ICU LOS was 9 days (5, 44) vs 11 days (9,14) • Among those enrolled $\leq$ 12 day from symptom onset 28-day mortality was 19% vs 27.1% in the LOPINAVIR-RITONAVIR and standard care groups, respectively • The clinical relevance of the difference in viral RNA load from OP swabs at enrollment is unknown, especially given potential pre-analytic limitations (of note sampling time violations occurred in 35 patients)
Other Clinical events	• A total of 46 (48.4%) of LOPINAVIR-RITONAVIR patients and 49 (49.5%) standard care patients reported adverse events, with GI-related being the more common in LOPINAVIR-RITONAVIR • Four serious GI adverse events (acute gastritis and lower digestive tract hemorrhage) occurred in the LOPINAVIR-RITONAVIR group (all determined to be related to study drug) and none in the standard care group	• Nearly 14% of patients in the lopinavir–ritonavir arm was unable to complete their 14-day course, primarily secondary to GI adverse events (e.g., anorexia, nausea, abdominal discomfort, diarrhea) • There were numerically higher proportions of patients in the standard care group who were on vasopressors, renal replacement therapy, and mechanical ventilation; this was not adjusted for in the Cox proportional-hazards model

AUTHORS' CONCLUSIONS

- The addition of lopinavir–ritonavir to standard of care was not associated with clinical improvement or mortality in patients with severe COVID-19
- Overall mortality (22%) in this study was higher than previously reported (11% to 14.5%) in the literature for COVID-19
- In a post-hoc subgroup analysis, the difference in mortality was numerically higher in patients randomized within 12 days of enrollment with findings consistent that SARS-CoV-2 viral pneumonia patients experience clinical deterioration within the second week of disease course
- The number of patients in the LOPINAVIR-RITONAVIR group who had serious complications or requiring mechanical ventilation were fewer than the standard care group, and this finding requires additional studies to determine whether LOPINAVIR-RITONAVIR treatment at a certain illness stage can reduce COVID-19 complications
- The addition of lopinavir–ritonavir did not impact RNA viral loads or duration of viral detection, a potential reason for lack of effect may have been due to intermittent sampling and lower viral yields from oropharyngeal swabs
- Since there is no *in vitro* data for lopinavir–ritonavir in SARS-CoV-2 and there was no pharmacokinetic sampling, the 50% effective concentrations (EC_{50}) and whether it is being obtained with studied dose is unknown
- There were notable limitations to the current trial, including the lack of blinding

GENERALIZABILITY/CRITIQUE/DISCUSSION

- Cao B et al. are commended for organizing a RCT with a control arm in a very short period of time in an emergent attempt to answer key clinical questions surrounding novel COVID-19 treatments but there are notable limitations to the current study as documented throughout the critique above
- Major limitations are the lack of blinding and placebo control, which can lead to observer and detection bias; however, most of the categories (with perhaps the exception of the first two that assesses resumption of normal activities) on the seven-category scale were objective outcomes (e.g., oxygen requirement) that are less at risk of bias from lack of blinding and placebo control
- The sample size initially calculated was based on an aggressive difference between treatment groups that is likely unattainable leading to being significantly under-powered to find true differences between groups
- More than half of patents were enrolled more than 12 days after symptom onset, likely mitigating any potential benefits of LOPINAVIR-RITONAVIR, which was shown in SARS-CoV-1 to be beneficial early in treatment
- Virologic measures (e.g., viral RNA assessment) have several flaws based on sampling technique and timing, and remains less clinically meaningful until it is confirmed whether detection of viral RNA is indicative of an infectious process
- The benefit of a protease inhibitor in SARS-CoV-2 treatment remains unknown, although toxicity with LOPINAVIR-RITONAVIR is a valid concern limiting treatment (e.g., hepatic injury, pancreatitis, severe cutaneous reactions, QT prolongation, and drug interactions). Of note, other HIV-1 protease inhibitors are understudy
- The current IDSA guidelines recommend use of LOPINAVIR-RITONAVIR only in the context of a clinical trial and additional studies are warranted to understand any potential role of protease inhibitors in COVID-19 treatment