

## Homo-harringtonine, highly effective against coronaviruses, is safe in treating COVID-19 by nebulization

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Dear Editor,

Coronaviruses have increasingly become the causal agent of epidemics in humans as well as in domesticated animals (Perlman and McIntosh, 2020; Wu et al., 2021). Since the beginning of this century, there have been 3 such epidemics, severe acute respiratory syndrome (SARS) (2003), Middle East respiratory syndrome (MERS) (2012) and coronavirus disease 2019 (COVID-19) (caused by SARS-CoV-2), and they may not be the last ones (Perlman and McIntosh, 2020). From its outbreak in 2019, the COVID-19 pandemic has led to a dramatic loss of human life and presented an unprecedented public health challenge worldwide (Ruan et al., 2022b).

Comprehensive vaccination by itself has not been able to eradicate COVID-19. At present, several antivirals against SARS-CoV-2 have been approved or are in advanced clinical trials. They can be divided into two classes, monoclonal antibodies (mAbs) directed against the Spike protein of SARS-CoV-2 and small molecules interfering with the viral replication machinery. The four FDA-approved mAbs (imdevimab, bamlanivimab, etesevimab, and casirivimab) are costly with recent data suggesting the loss of efficacy against

the Omicron variant (McCallum et al., 2022). Among the three FDA-approved antiviral small molecules, Remdesivir targets the conserved viral RNA-dependent RNA polymerase (RdRp) and was first approved by FDA in October of 2020. Its clinical benefit is still under debate (Consortium, 2021). Molnupiravir (also targeting RdRp) received emergency use authorization from FDA in December of 2021. With its lower efficacy (reducing hospitalization or death by 30%), the application potential is limited (Jayk Bernal et al., 2022). Nirmatrelvir (PF-07321332) targets the viral main protease M<sup>pro</sup> (or 3CL protease) (Hammond et al., 2022). When co-formulated with Ritonavir, Nirmatrelvir can reduce COVID-19 hospitalization by 90% (Hammond et al., 2022). The main limitations of Ritonavir-Boosted Nirmatrelvir (known as Paxlovid) include low supply and potential drug-to-drug interactions (see Table S1 in Supporting Information for a more comprehensive survey of other treatment schemes that have not progressed to, or beyond, advanced clinical trials). Therefore, developing antiviral drugs that can deal with the rapid evolution of SARS-CoV-2 is still urgently needed (Ruan et al., 2022a).

Coronaviruses possess the largest genomes (26.4–31.7 kb) among all known RNA viruses and use two thirds of their genomes to make a super-peptide comprising ~16 non-structural proteins (NSPs), synthesized in concatenation and later proteolyzed into its component proteins (Bai et al.,

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2022). In SARS-CoV-2 and its relatives, the super-protein is >700 kilodaltons and ~8,000 amino acids in size. Since human peptides are rarely larger than 5,000 amino-acids long and the few large ones are often highly tissue-specific with a relatively long half-life, the viral super-protein stands out among the host's proteins (Wu and Wen, 2020). This super-protein also sets coronaviruses apart from other viruses such as influenza or AIDS, thus explaining the lack of cross-reactivity among viruses to the same drugs (such as lopinavir, ritonavir, ribavirin, arbidol, favipiravir and penciclovir) (Simonis et al., 2021). In summary, the peculiar way of protein synthesis has been hypothesized to be the Achilles heel of SARS-CoV-2 (Wu and Wen, 2020).

In addition to the protein size, it has been pointed out that a common weakness of virus-infected cells is hyper-transcription or hyper-translation activities. After all, viruses often replicate at an exceedingly high rate. The heightened activities are true in cancer cells as well (Wu and Wen, 2020), and that may be why many anti-cancer and anti-virus drugs often target the transcription or translation machinery (Schneider-Poetsch and Yoshida, 2018). It is hence possible that some drugs may be effective against both cancers and viruses (Wu and Wen, 2020).

Homo-harringtonine (HHT), or omacetaxine mepesuccinate in its semi-synthetic form, is a cytotoxic plant alkaloid extracted from *Cephalotaxus* species. HHT is the strongest inhibitor of protein translation approved for clinical use and has been commonly used in China to treat cancer patients since the 1970s. It is the first agent approved by FDA (USA, in 2012) targeting the mRNA translation process (Alvandi et al., 2014). Given its wide use, the molecular mechanism of HHT has been well understood (Garreau de Loubresse et al., 2014).

After the SARS of 2003, there have been many efforts to identify drugs capable of repressing coronaviral replication. In each of these drug-screen studies (see details in Table S2 in Supporting Information), multiple drugs are reported. Curiously, there was little emphasis that HHT (and only HHT) appears in almost all lists. Before 2020, five coronaviruses have been documented to be repressed by HHT-MHV (murine coronavirus mouse hepatitis virus), Bovine coronavirus strain L9 (BCoV-L9), human enteric coronavirus strain 4408 (HECoV-4408), porcine epidemic diarrhea virus (PEDV) and MERS (Table S2 in Supporting Information).

If HHT acts against a general feature in the translation of coronaviruses, one would expect its effectiveness against SARS-CoV-2 and the effectiveness would be little affected by the evolution of SARS-CoV-2. *In vitro* data indeed show the effectiveness (Wen et al., 2021). In a set of confirmatory validations, we have further shown the same effectiveness against 3 recent SARS-CoV-2 strains in 3 mammalian cell lines with a mean  $EC_{50}$  value of  $<50 \text{ nmol L}^{-1}$  (unpublished data). In summary, six different coronaviruses have been

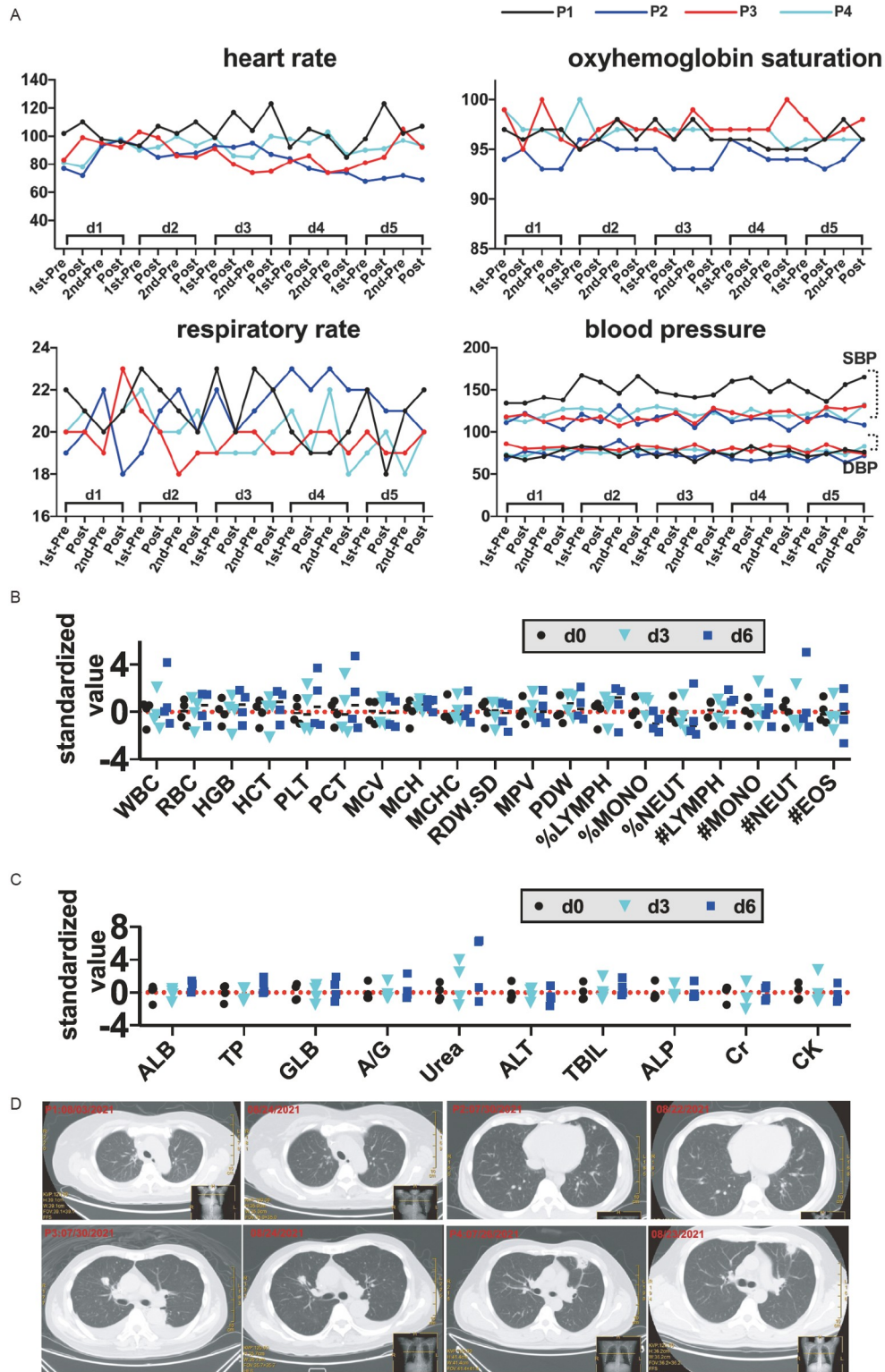
shown to be repressible by HHT at a comparable concentration, thus demonstrating HHT can repress multiple coronaviruses through a common mechanism.

Since HHT can repress multiple coronaviruses through a highly specific mechanism, the HHT scheme is expected to be effective in treating COVID-19 at a very low dose. Note that the safety of HHT by intravenous injection (IV) at a higher dose than recommended for treating COVID-19 has been well established. In a previous study (Wen et al., 2021), we proposed to target organs with the highest viral loads (say, the respiratory system) and deliver a low dose of HHT locally (by nebulization, for example). The remaining issue in clinical application is the safety of nebulization.

To test the safety of HHT nebulization, we carried out an investigator-initiated phase I/II trial for treating advanced tumors with lung metastasis. In the literature, the efficacy of HHT against several solid tumors has been reported (Alvandi et al., 2014), besides the efficacy against hematological malignancies. The study was approved by the Institutional Review Board (IRB) of Guangdong Clifford Hospital and registered in Chinese Clinical Trial Registry (ChiCTR) with the accession number ChiCTR2100049182. In this trial, the therapeutic regimen in a 3-week cycle is as follows: 0.5 mg HHT per inhalation, twice a day in Day 1–5 of the first week, and two days of treatment each week in the next two weeks (see details of this clinical trial in <https://www.chictr.org.cn/index.aspx>).

Below, we present the safety data while the clinical benefits on lung metastases are pending the completion of the trial. The safety of HHT nebulization in humans is most relevant to the proposed treatment of COVID-19. At the time of this writing, four patients had completed the first cycle of treatment (Table S3 in Supporting Information). During nebulization and up to six weeks after the completion of the first cycle, treatment-related adverse events (such as cough, wheezing, difficulty in breathing, dizziness, throat irritation) were not observed. We investigated their blood pressure, heart rate, degree of blood oxygen saturation, and respiratory rate, all of which are stable during each inhalation (Figure 1A). Patients had no significant changes in blood-routine-examination (Figure 1B) or serum biochemistry test during treatment (Figure 1C). The patients had a chest CT scan after the first treatment cycle (Figure 1D). CT results did not show ground glass opacities, consolidation, reticular patterns, linear opacities, air bronchograms, thick small vessel shadows, pleural hypertrophy, or fibrosis etc. Careful exploration of these results did not find any added respiratory signs apart from their primary pathology. Collectively, the safety profile in ChiCTR2100049182 suggested that HHT inhalation at the proposed dose for COVID-19 treatment (0.5 mg HHT per inhalation, twice a day over five consecutive days) is well-tolerated in humans.

In summary, HHT is effective in repressing multiple



**Figure 1** Safety evaluation of HHT administration by nebulization. A, Physical examination (blood pressure, heart rate, degree of blood oxygen saturation, and respiratory rate) of each patient participated in the clinical trial. Data was collected before and after each inhalation. B and C, Blood-routine examination (B) and serum biochemistry test (C) after HHT nebulization. Blood samples were taken at d0 (before the treatment), d3, and d6 of the first week for blood-routine-examination and serum biochemistry test. Values were standardized by  $z_{i,j}=(x_{i,j}-\mu_j)/\sigma_j$  of the normal distribution. Each dot is a sample, taken at the indicated day, from each patient. For each test, data in d3 or d6 were compared with d0 separately using the Mann-Whitney *U* test and Bonferroni correction for multiple comparisons. No significant different value was observed between d3 and d0, or between d6 and d0. D, Chest CT scan to assess the safety of HHT nebulization. Chest CT scan was performed both before the treatment (left part of each patient's CT scan results) and when the first cycle of treatment was finished (right part of each patient's CT scan results). Potential pathological changes (including ground glass opacities, consolidation, reticular patterns, linear opacities, air bronchograms, thick small vessel shadows, pleural hypertrophy, or fibrosis) were carefully examined by a panel of CT imaging specialists.

coronaviruses tested *in vitro* (Table S2 in Supporting Information and Wen et al. (2021)). The drug concentration necessary for viral clearance *in vitro* is consistently under  $1 \mu\text{mol L}^{-1}$ . As stated above, the specific mechanism of HHT repression of viral protein translation may be the key. While IV of HHT is the delivery method to treat leukemia (Alvandi et al., 2014), this systemic administration may not be the optimal delivery method in treating SARS-CoV-2. Alternatively, by nebulization, the drug is concentrated in the respiratory tract, where the viral load is the highest. While the virus is known to invade other organs, the clearance from the respiratory tract may allow the immune system to cope with the remaining viral load. With a portable device (like the one used for asthma), nebulization can be used on a large number of infected patients without hospital stay. Most crucially, no adverse effects by HHT nebulization at the proposed dose against SARS-CoV-2 could be observed in lung cancer patients (Figure 1A–D).

A protocol for clinical trials of HHT nebulization on COVID-19 patients have been registered (ChiCTR-2100045993) by the Ditan Hospital, which is solely responsible for all COVID-19 patients in the Beijing area. However, since the protocol was approved by the IRB of Ditan Hospital, there have been no COVID-19 patients in or near Beijing. We propose that, if a small-scale Phase II trial works as well as all available data have indicated, a large-scale trial may be done in facilities that have patients for such trials. Finally, we would like to emphasize that rapid clearance of the viral load from the respiratory tract of infected patients may be crucial for the dual objectives of preventing disease progression and arresting viral spread.

**Compliance and ethics** The author(s) declare that they have no conflict of interest.

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## SUPPORTING INFORMATION

The supporting information is available online at <https://doi.org/10.1007/s11427-021-2093-2>. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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