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Review Article

Lopinavir/ritonavir: Repurposing an old drug for HIV infection in COVID-19 treatment

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ABSTRACT

Currently, there is no specific antiviral treatment for COVID-19. However, drugs previously developed to treat other viral infections are being tested to verify if they might also be effective against SARS-CoV-2, the virus that causes COVID-19. Twenty years ago, the F.D.A. approved Lopinavir/ritonavir (LPV/r) to treat HIV infection. LPV and ritonavir were initially proposed to inhibit 3-chymotrypsin-like protease (3CL^{PRO}) of SARS-CoV and MERS-CoV and preliminary promising data on its efficacy for treating people infected with those viruses were available. Therefore, due to the high genetic similarities among those viruses and SARS-CoV-2, early during COVID-19 pandemic LPV/r was also proposed as one emergency treatment. We reviewed data from the literature about LPV/r treatment and SARS-CoV-2 infection, mainly focused on the efficacy and safety of this drugs for COVID-19 treatment. We can conclude that although up to date no clear benefit has been observed with the LPV/r treatment beyond standard care, its efficacy against SARS-COV-2 infection deserves further evaluations, particularly during the very early phase of the disease.

By the end of December 2019, the emergence of an outbreak of pneumonia of unknown etiology in Wuhan, Hubei Province of China, was communicated to the World Health Organization (WHO) China Country Office [1]. The RNA sequencing from the bronchoalveolar lavage fluid of an infected patient identified a new virus from the *Coronaviridae* family as the causative agent of this new disease [2]. The virus was successively named

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and the new pneumonia Coronavirus Disease (COVID-19) [3]. Similarly to other two coronaviruses which caused diseases outbreak in recent times [4,5], the Severe Acute Respiratory Syndrome (SARS-CoV) and the Middle-East Respiratory Syndrome Coronavirus (MERS-CoV), SARS-CoV-2 seems to have originated from bats [6], being mostly similar

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to the RaTG13 virus, isolated from *Rhinolophus affinis* [7]. It shares 79.6% sequence identity with SARS-CoV [7] and 50% with MERS-CoV [8]. SARS-CoV was the responsible for the Severe Acute Respiratory Syndrome (SARS) in the Guangdong Province of China in 2003, causing up to 813 deaths among 8437 cases reported [9]. The MERS-CoV outbreak occurred in 2012 in Saudi Arabia, reaching 2494 confirmed cases and 858 deaths worldwide [10]. In mid-February, SARS-CoV-2 spread in 188 countries, reaching 108,822,960 people and resulting in 2,403,641 deaths worldwide [11]. The spectrum of clinical manifestations of COVID-19 can vary from a mild respiratory syndrome to an Acute Respiratory Distress Syndrome (ARDS) requiring mechanical ventilation [12–15], where older age and comorbidities increase the risk of a more severe disease [16,17]. Case fatality rate has been reported to be around 4–4.5%, anyway varying widely among different countries and different testing strategies [18–20]. Up to date, only remdesivir and dexamethasone proved to be effective in clinical trials: remdesivir shortened the length of hospitalization, anyway without a meaningful impact on mortality [21], while dexamethasone significantly reduced mortality in patients requiring supplemental oxygen [22].

Lopinavir/ritonavir (LPV/r) is a fixed dose combination of two protease inhibitors, widely used as antiretroviral drug for Human Immunodeficiency Virus (HIV) second-line treatment [23]. Lopinavir is a potent inhibitor of the HIV-1 protease, thus producing immature, non-infectious virions. Anyway, lopinavir shows poor bioavailability, therefore it comes co-formulated with ritonavir, a potent inhibitor of the cytochrome P450 3A4 [24], which dramatically increase lopinavir blood levels.

Both lopinavir and ritonavir were initially proposed to inhibit 3-chymotrypsin-like protease (3CL^{PRO}) of SARS-CoV and MERS-CoV [25]. In a study of 2004 from Chu et al. [26] in patients affected by SARS, the association of LPV/r with ribavirin showed a lower occurrence of adverse clinical outcomes (ARDS or death) in the treatment group in comparison with the historical controls treated with ribavirin only (2.4% v 28.8%, $p < 0.001$) at day 21 after the onset of symptoms. In the initial treatment group, a reduction in steroid usage and nosocomial infections, as well as a decreasing viral load and a rising peripheral lymphocyte count were observed. Similarly, the study from Chan et al. [27] found that the early addition of LPV/r to the standard therapy (broad spectrum antibiotics, ribavirin and corticosteroids) was associated with a significant reduction in the overall death and intubation rate (2.3% and 0%, respectively), when compared to standard treatment only (15.6% and 11.0% respectively, $p < 0.05$). Interestingly, in the subgroup who had received LPV/r later as rescue therapy, no differences in the overall death, intubation and oxygen desaturation rates were observed in comparison with the matched cohort. In MERS-CoV infection, a study on twelve common marmosets [28] showed that the LPV/r and the interferon-1 β (IFN-1 β)-treated animals had better outcomes than the untreated animals. Moreover, LPV/r and interferon-1 β -treatment groups showed lower mean viral loads in necropsied lung and in extrapulmonary tissues and lower mortality rates (0–33%) in comparison with the untreated and the mycophenolate mofetil-treated groups (67%). Based on those preliminary clinical evidences and on genetic similarities of these

two viruses with SARS-CoV-2, early during COVID-19 pandemic, LPV/r was also proposed as one emergency treatment.

Whether LPV/r may effectively target SARS-CoV-2 3CL^{PRO} is still under debate. Indeed, the HIV protease on which LPV/r is effective is from the aspartic protease family, whereas SARS-CoV-2 3CL^{PRO} from the cysteine protease family.

Drugs repositioning is an important strategy, especially in an emergency context such as a pandemic, because of lower costs of production and reduced times for drug availability and distribution [29]. Among those drugs which have been proposed in the therapy of COVID-19, the combination of lopinavir/ritonavir is still under evaluation.

Therefore, the aim of this review is to evaluate and make available the state of the art about the use of this drug in the treatment of COVID-19, in order to help clinicians in the management of affected patients.

Methodology and literature search strategy

Literature search

A comprehensive searching of PubMed, EMBASE and Cochrane Library was performed from June 1st up to September 1st 2020 with restriction to English, Italian, Spanish and Portuguese language papers. Unpublished trials were also identified from the clinical trial registry platforms (<http://clinicaltrials.gov>). Preprint articles were retrieved from the websites MedRxiv (<https://www.medrxiv.org>) and BioRxiv (<https://www.biorxiv.org>). Moreover, manual search was conducted by screening the reference lists of inclusive studies. Our search strategy included the following relevant terms: lopinavir AND COVID-19, lopinavir AND COVID 19, lopinavir AND coronavirus, protease inhibitors AND COVID-19, protease inhibitors AND COVID 19, protease inhibitors AND coronavirus, protease inhibitors AND covid-19 AND pre-clinic, animal model AND SARS-CoV-2 AND lopinavir, SARS-CoV-2 AND lopinavir AND ritonavir.

Studies selection

Three authors (P.M., E.Q.R. and M.P.) independently screened the titles, abstracts and full texts of retrieved articles to evaluate their eligibility [Fig. 1].

In vitro and *in vivo* pre-clinical studies, randomized controlled trials, prospective and retrospective cohort studies, case series and clinical cases performed among adults with COVID-19 were included in the current literature review. We reviewed studies from the literature about protease inhibitors use in COVID-19 mainly focusing on *in vitro* and *in vivo* efficacy, on clinical outcome, mortality rate, virological eradication, safety and tolerability.

Pre-clinical studies

Collected data are summarized in Table 1 and described more in detail below.

In vivo studies

We retrieved only one study that analyzed the efficacy of LPV/r in an animal model [30]. Park et al., inoculated ten ferrets with infective doses of a SARS-CoV-2 strain (NMC-nCoV02) through the intranasal route. At day-1 post-infection with SARS-CoV-2, ferrets were administered LPV/r, hydroxychloroquine sulfate or emtricitabine-tenofovir daily for 14 days. In addition, ten ferrets were treated with phosphate-buffered saline (PBS) or azathioprine for 7 days prior to SARS-CoV-2 infection. In this study, despite an overall reduction of clinical symptom intensity, treatment with LPV/r showed no improvement in disease duration in comparison with the control group. None of the antiviral candidates significantly diminished respiratory or gastrointestinal viral titers. Moreover, antiviral-treated groups had lower serum neutralization antibody titers than the control group, which was an interesting finding needing further studies.

In vitro studies

Another important aspect to consider is whether a drug can reach the appropriate concentration *in vivo*. *In vitro* studies have showed that many putative agents, among those lopinavir and ritonavir, likely never reach the target concentrations necessary to adequately suppress SARS-CoV-2 in plasma. These findings were possible thanks to the prediction of the C_{max}/EC_{50} and C_{max}/EC_{90} [31]. Lopinavir, hydroxychloroquine, chloroquine, mefloquine, atazanavir (ritonavir boosted), tipranavir (ritonavir boosted), azithromycin and ivermectin achieved concentrations in the lungs 10-fold higher than their EC_{50} and exceeded by 3.4 fold their EC_{90} . In contrast, LPV/r, chloroquine and hydroxychloroquine reached a concentration in plasma lower than that required for antiviral activity [31]. Considering that the virus accumulates in the lung tissue of COVID-19 patients, the high concentration of antiviral drugs in the lung rather than plasma could be useful in pneumonia treatment during COVID-19.

The estimation of the protein-adjusted EC_{90} value of lopinavir revealed also that the dose required to provide optimal inhibition in plasma was unfeasible due to un-acceptable risk of toxicity. A recent study from Choy et al., tested the *in vitro* antiviral effect of lopinavir against SARS-CoV-2 which resulted in a half maximal inhibitory concentration (EC_{50}) of 26 μ M [32], 4000-to-8000 folds higher than that able to inhibit HIV (0.006 μ M) [33].

To bypass the high concentrations of lopinavir required in plasma, combinational therapy with other effective compounds against SARS-CoV-2 was considered [32,34]. When combinational drugs were used, the plasmatic viral load was lower than the single-drug treatment or placebo [34]. In this way, the formed complexes may increase synergy and reduce needed concentration of lopinavir under the maximal therapeutic plasma concentration [32].

Finally, another inhibitor of HIV-protease, darunavir, showed no *in vitro* antiviral activity against SARS-CoV-2 at clinical concentrations [35].

In silico studies

Data on molecular dynamic simulation available in the literature reported two contrasting results. On one hand, LPV/r had high stability in the binding domain of the SARS-CoV-2 protease in terms of electrostatic and van der Waals interactions [36–43]. On other hand, the binding affinity was found higher for drugs or natural compounds like ledipasvir, velpatasvir, carfilzomib, saquinavir and thymopentin, viomycin and lead phytochemicals than lopinavir and ritonavir [43–48].

Actually, the active site of SARS-CoV-2 protease is characterized by the presence of a cysteine residue, but lopinavir and ritonavir were designed to better interact with an aspartate belonging to the HIV-protease [47]. Using a drug-target interaction (DTI), lopinavir and ritonavir were predicted to have a potential affinity (Kd 78.49 and 41.60 nM, respectively) to SARS-CoV-2 helicase [44]. This evidence could explain why the inhibition efficiency of lopinavir and ritonavir is not favorable against the main protease of SARS-CoV-2.

According with the *in vitro* studies, the combination of drugs seems to be also more effective. In these terms, the binding energy of lopinavir, ritonavir and oseltamivir docked together against the SARS-CoV-2 protease improved over the binding energy of each drug separately [37].

Finally, darunavir was predicted to have the best docking score among some FDA approved antiviral drugs (e.g. lopinavir, ritonavir, remdesivir, saquinavir, amprenavir, rupintrivir) with higher binding affinity to the SARS-CoV-2 protease than the others antiviral drugs [39,40,49]. Nevertheless, De Meyer S. et al. suggested that such promising docking results were indicative of suboptimal binding to this protein and therefore darunavir was not to be considered the best candidate in the treatment of SARS-CoV-2 [35].

In conclusion, the diversity of outcomes from these *in silico* studies are probably due to different approaches applied to the molecular dynamic simulation.

Clinical studies

Efficacy

Clinical trials

An exploratory randomized controlled trial aimed to study efficacy and safety of LPV/r or arbidol in adult patients with mild/moderate COVID-19 (included 86 patients: 34 received LPV/r, 35 arbidol, and 17 with no antiviral medication) found that both monotherapies present little benefit in improving the clinical outcome compared with control group [50].

Hung et al. [51] performed a multicenter, prospective, open-label, randomized, phase 2 trial comparing monotherapy with LPV/r with a combination treatment including LPV/r, ribavirin and IFN-1 β (the latter was administered only if treatment was started < 7 days after symptoms onset). Eighty-six (86) patients were randomly assigned to the combination group and 41 were assigned to LPV/r monotherapy. Overall, the analysis reported a better performance of the combination therapy, that showed shorter times to negative nasopharyngeal swab and clinical

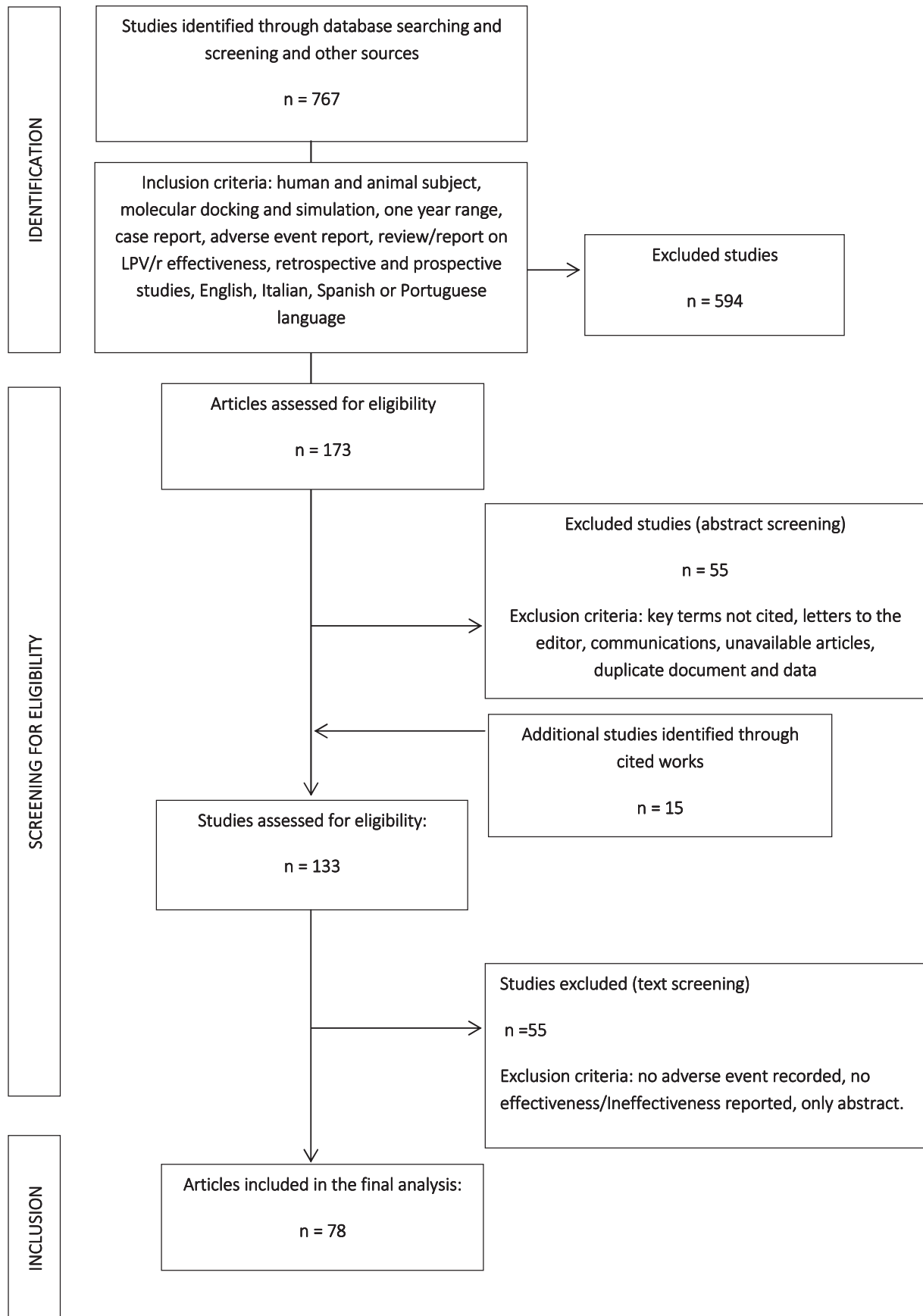


Fig. 1 Flow chart of database searching and screening of studies for the systematic review.

Table 1 Details of revised studies.

References	Method	Drugs	Results
Park S.J. et al., 2020 [30]	<i>In vivo</i> (animal model)	Lopinavir/ritonavir, Hydroxychloroquine sulfate, Emtricitabine-Tenofovir	Reduced overall clinical symptoms and not significantly diminished respiratory or gastrointestinal SARS-CoV-2 titers
Arshad U. et al., 2020 [31]	<i>In vitro</i> Vero cells in available literature	Lopinavir, Ritonavir	<i>In lung tissue:</i> C _{max} /EC ₅₀ > 10: Hydroxychloroquine, Atazanavir, Chloroquine, Tipranavir, Mefloquine, Ivermectin, Azithromycin, Lopinavir; <i>In plasma:</i> C _{max} /EC ₅₀ > 1: Nelfinavir, Chloroquine, Remdesivir, Lopinavir (Ritonavir boosted), Eltrombopag, Hydroxychloroquine, Atazanavir (Ritonavir boosted), Indomethacin, Favipiravir, Sulfadoxine, Niclosamide, Mefloquine, Tipranavir (Ritonavir boosted), Ritonavir, Merimepodib, Anidulafungin, Nitazoxanide 1 < C _{max} /EC ₉₀ < 2: Anidulafungin, Lopinavir, Chloroquine, Ritonavir
Cattaneo D. et al., 2020 [33]	<i>In vitro</i> IC90 estimation	Lopinavir	Lopinavir IC ₅₀ : 26 μM Lopinavir IC ₉₀ : 234 μM Lopinavir Protein-adjust IC ₉₀ in plasma: 4680 μM Lopinavir Protein-adjust IC ₉₀ in epithelial lining fluid: 393 μM Lopinavir Protein-adjust IC ₉₀ in cerebrospinal fluid: 58,500 μM To the reduction in viral RNA copy: EC ₅₀ Lopinavir: 26.1 μM
Choy K.T. et al., 2020 [32]	<i>In vitro</i> Vero E6 cells	Remdesivir, Lopinavir	
Kang C.K. et al., 2020 [34]	<i>In vitro</i> Kidney Vero cells	Lopinavir, Ritonavir	Concentration groups tested: - Kaletra (7 μg/mL of Lopinavir and 1.75 μg/mL of Ritonavir); - Hydroxychloroquine 1 μg/mL; - Hydroxychloroquine 2 μg/mL; - Kaletra + Hydroxychloroquine 1 μg/mL; - Kaletra + Hydroxychloroquine 2 μg/mL
De Meyer S. et al., 2020 [35]	<i>In vitro</i> Caco-2 cells	Darunavir	Visual Cytopathogenic Effect-read out EC ₅₀ : Darunavir: > 100 μM 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method EC ₅₀ : Darunavir: > 100 μM Cytotoxic concentration causing death to 50% (CC50): Darunavir: >100 μM
Das S. et al., 2020 [36]	<i>In silico</i> Blind molecular docking analyses with Mpro	Commercially available compounds	Free binding energy for SARS-CoV-2 protease: Lopinavir ΔG: - 9.00 kcal/mol Ritonavir ΔG: - 9.52 kcal/mol
Beck B.R. et al., 2020 [44]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Drug-target interaction (DTI) prediction results against COVID-19's helicase: Lopinavir: 78.49 nM Ritonavir: 41.60 nM Darunavir: 90.38 nM
Khan S.A. et al., 2020 [49]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Free binding energy for SARS-CoV-2 protease by MM (GB/PB)SA method: Darunavir: - 48.1041 kcal/mol

(continued on next page)

Table 1 – (continued)

References	Method	Drugs	Results
Muralidharan N. et al., 2020 [37]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Free binding energy for SARS-CoV-2 protease: Lopinavir: - 4.1 kcal/mol Oseltamivir: - 4.65 kcal/mol Ritonavir: - 5.11 kcal/mol Lopinavir/Oseltamivir: - 5.4 kcal/mol Lopinavir/Oseltamivir/Ritonavir: - 8.32 kcal/mol
Nutho B. et al., 2020 [38]	<i>In silico</i> - Molecular dynamic simulation	Lopinavir and Ritonavir	Free binding free energy (ΔG_{bind}) for SARS-CoV-2 protease by MM/PBSA: Lopinavir: - 10.89 \pm 1.89 kcal/mol Ritonavir: - 14.93 \pm 1.83 kcal/mol Binding free energy (ΔG_{bind}) for SARS-CoV-2 protease by MM/GBSA: Lopinavir: - 13.83 \pm 1.91 kcal/mol Ritonavir: - 27.78 \pm 1.82 kcal/mol
Ortega J.T. et al., 2020 [45]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Free binding energy for SARS-CoV-2 protease: Lopinavir: -9.1 kcal/mol
Pant S. et al., 2020 [39]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Binding free energy (ΔG_{bind}) for SARS-CoV-2 protease by MM/GBSA: Ritonavir: -87.24 kcal/mol Lopinavir: -73.33 kcal/mol Darunavir: -69.20 kcal/mol Docking Score: Ritonavir: -8.878 Lopinavir: -8.358 Darunavir: -7.208
Peele K.A. et al., 2020 [40]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Docking score using GLIDE module: Lopinavir: -9.918 Darunavir: -8.843
Mahanta S. et al., 2020 [48]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	-CDocker Energy for SARS-CoV-2 protease: Lopinavir -62.07 kcal/mol Ritonavir -65.73 kcal/mol
Shah B. et al., 2020 [41]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Docking score from interaction with different COVID-19 structures as 5R7Y, 5R7Z, 5R80, 5R81 5R82: Lopinavir: - 6.834, -6.968, -7.331, -8.44, -7.58 Ritonavir: -7.621, -, -6.736, -6.764, -7.316
Chen Y.W. et al., 2020 [46]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Binding energy for chain A active site of SARS-CoV-2 protease: Mean score: -8.2 kcal/mol Ritonavir: -7.9 kcal/mol Lopinavir: -8.0 kcal/mol Binding energy for chain B active site of SARS-CoV-2 protease: of SARS-CoV-2 protease: Mean score: -7.1 kcal/mol Ritonavir: -6.9 kcal/mol Lopinavir: -6.8 kcal/mol
Wang Q. et al., 2020 [47]	<i>In silico</i> Molecular dynamic simulation	Commercially available compounds	Free binding energy for SARS-CoV-2 protease: Darunavir: - 11.1 kcal/mol Ritonavir: -11.8 kcal/mol Lopinavir: -11.9 kcal/mol

Mamidalal E. et al., 2020 [42]	In silico Molecular dynamic simulation	Commercially available compounds	Free binding energy for SARS-CoV-2 protease: Lopinavir: -6.11 kcal/mol Ritonavir: -8.25 kcal/mol Binding energy raging: Lopinavir: > - 5.70 kcal/mol
Gupta S. et al., 2020 [43]	In silico Molecular dynamic simulation	Literature available compounds	Range of predicted IC50 values: Standard compound(Lopinavir): 15.7–21.3 μM The total interaction energy of protein-ligand complex: Lopinavir- Mpro: -181.795 ± 13.5 kJ/mol Toxicological profile (PLD ₅₀): Lopinavir: 5000 mg/kg body weight Docking score range of Darunavir: - 8.6 to - 8.2 Very few interactions of Darunavir with the active site of the protease
	In silico Molecular dynamic simulation	Darunavir	

improvement and shorter hospital stays. It is interesting to notice that in the post-hoc analysis, there were no differences in the outcomes between the two groups.

In the open-label individually randomized controlled study from Cao et al. [52], 99 patients were treated with LPV/r for 14 days and 100 patients received standard of care (SOC). Median time between symptoms onset and treatment was 13 days. There was no improvement in time to clinical improvement in the LPV/r group, where the 28-day mortality was numerically lower in the LPV/r group (14/99) in comparison with the SOC group (25/100), anyway this difference was not statistically significant in the intention-to-treat analysis. In those treated within 12 days from symptom onset mortality was numerically lower.

Cai et al. [53] performed an open-label, non-randomized, before-after controlled study. Patients were treated with oral LPV/r or favipiravir plus IFN-1β by aerosol inhalation for 14 days (or less if viral clearance occurred). Overall, 35 patients were treated with favipiravir and 45 with LPV/r. The favipiravir group showed shorter time of viral clearance (4 d vs 11 d, $p < 0.001$) and better results at chest computed tomography (CT) at day 14 (91.4% vs 62.2%, $p = 0.004$).

On March 2020, WHO launched the SOLIDARITY study, a multinational randomized trial to facilitate the rapid worldwide comparison of COVID-19 unproved treatment (four existing antivirals and anti-inflammatory drugs including LPV/r, are compared with standard of care) [54]. A European satellite of SOLIDARITY trial program, DisCoVeRy intended to analyze the efficacy and safety of treatment options for patients in a limited time frame [55].

On July 4th, WHO discontinued the LPV/r treatment arm from the SOLIDARITY trial after an interim results analysis and after a review of the evidences from all trials presented at the July 1st-2nd WHO Summit on “COVID-19 research and innovation” showing how LPV/r produces little or no reduction in the mortality of hospitalized COVID-19 patients [56]. Also data from DisCoVeRy trial (NCT04315948) [55], announced at the beginning of July, showed that, at 28 days, the death rate was not significantly different in patients randomly allocated to receive LPV/r compared with those randomly allocated to usual hospital care only (22.1% versus 21.3%, 95% confidence interval 0.98 to 1.26, $p = 0.10$). There was also no evidence of beneficial effects on risk of progression to mechanical ventilation or length of hospital stay [57].

Retrospective studies

In a small retrospective study from Deng et al., the combination of arbidol plus LPV/r was found to be more effective in viral clearance at day 7 and 14, and in chest CT scans at day 7, in comparison with LPV/r monotherapy [58]. Interestingly, 41% of patients in the LPV/r versus 6% in the combination group received corticosteroids. In the preliminary results from the RECOVERY study, dexamethasone administration showed a 20% reduction of 28-day mortality in patients receiving oxygen supplementation [22]. Therefore, whether the combination of antiviral and steroids, or whether the time of administration of one or both agents may have a role in these results, needs further studies.

The study from Jiang et al. reported a median of 14 days to viral clearance in a cohort of 60 patients treated with three

different combinations of antiviral therapies (IFN-1 β + LPV/r, IFN-1 β + LPV/r + arbidol and IFN-1 β + LPV/r + oseltamivir) [59]. Anyway, no analysis of differences among the three treatment groups or severity of disease was performed and the number of days between symptoms onset and start of therapy was not reported.

Hraiech et al. [60] reported the findings of a cohort of severe patients admitted in four different Intensive Care Units (ICUs) in France. Seventeen patients were treated with azithromycin plus hydroxychloroquine, 13 with LPV/r and 15 received SOC only. No differences among the three different groups were observed at 6 days post-treatment in viral clearance at the nasopharyngeal swab. Interestingly, in the subgroup of patients that started antiviral treatment <5 days from symptoms onset, the LPV/r group had higher viral clearance than the azithromycin + hydroxychloroquine group (3/7 vs 0/7, $p = 0.05$). Data from a retrospective study that included 122 patients [61] showed that median duration of viral shedding was longer in patients who did not receive LPV/r (28.5 days) in comparison with LPV/r treatment group (22 days) (log-rank $p = 0.009$), where the difference was bigger for those who started LPV/r early during COVID-19 (19 days, log-rank $p < 0.001$) while no difference at all was observed in those who started LPV/r late (27.5 days, log-rank $p = 0.51$). Conversely, length of hospitalization was higher for patients in patients treated with LPV/r, thus probably reflecting the higher rates of severe disease in this group.

In the study from Ye et al. [62], patients treated with LPV/r in combination therapy showed lower times to negative swabs results in comparison with those not treated with LPV/r. Wang et al. found a median duration of viral spread of 10 days in patients treated with LPV/r diagnosed with COVID-19, while asymptomatic [63]. No difference was found in length of hospital stay and time to negative PCR in the study from Yuan et al. in patients treated with interferon- α (IFN- α) + LPV/r \pm ribavirin [64]. In the study from Ding et al. [65], median time to RNA negative result was 11 days with no difference between the two antiviral regimens (LPV/r + IFN- α \pm arbidol). Interestingly, the only factor that was independently associated with a rapid viral clearance was a high lymphocyte count ($\geq 1.3 \times 10^9/l$). A similar median time to negative RNA results was found in the study from Zhang et al. [66]. The study of Zuo et al. [67] found that the association of LPV/r + IFN- α was associated with a short viral shedding duration (defined as <20 days) ($p = 0.007$), in comparison with LPV/r + IFN- α + arbidol and LPV/r alone. Interestingly, patients who started treatment <5 days since symptoms onset displayed a faster RNA clearance in comparison with those who started >5 days after symptoms appearance ($p = 0.001$).

Case series

Cheng et al. [68] found no difference in the duration of viral shedding in patients with mild pneumonia who were treated with LPV/r. Anyway, the study population was very small (2 patients treated with LPV/r and three with SOC only) and one of the two patients treated with LPV/r interrupted treatment after four days.

Liu et al. [69] reported a median of 13 days of hospital stay in patients treated with LPV/r alone or in combination with

interferon- $\alpha 2b$ (IFN- $\alpha 2b$) in a cohort of 10 patients that started therapy 5 days after symptoms onset on average. Anyway, sample size was very small and four out of 10 patients changed their therapies during the observation period for adverse events and/or clinical deterioration. In another case serie, two patients started treatment two days after symptoms onset (one was asymptomatic) with a combination of LPV/r, arbidol, IFN- $\alpha 2b$ and lianhuaqingwen granules. Negative swabs were obtained after 8 days for patient 1 and after 15 days for patient 2. Both had a mild pneumonia that improved at chest XR at day 8 of therapy [70].

Finally, several case series describing outcomes of SARS-CoV-2 and HIV co-infected patients showed as HIV-patients have been infected with SARS-Cov-2 spite of efficient antiretroviral therapy including LPV/r (data not shown).

Case report

One case report showed no detectable or low SARS-CoV-2 titers in a patient treated with LPV/r since the first day after LPV/r administration. Anyway, treatment was started 10 days after symptoms onset and the finding may be due to the natural course of the healing process from COVID-19 [71]. In another case report, where LPV/r was started at day 9 from symptoms onset, RNA titers at nasopharyngeal swabs decreased gradually until resulting negative in day 15. Anyway, a rebound in viral titers was observed at day 11 and 12, despite LPV/r administration [72].

Adverse events (AE)

As far as is known, the most common adverse events (AE) observed with LPV/r combination in HIV-infected patients have been diarrhoea, nausea, vomiting, hypertriglyceridaemia and hypercholesterolemia. Cases of pancreatitis and rare increases in the PR interval on electrocardiogram (ECG) have been reported in patients receiving LPV/r [73].

In the report from Cao et al., almost half of patients with COVID-19 in the LPV/r and in the control group (48.4% vs. 46.7%, respectively) reported one or more adverse events: gastrointestinal-related complaints including nausea, vomiting and diarrhea were more common in the LPV/r group [52]. In the study of Ly et al. [50] the percentage of patients with adverse events were lower but higher than in the arbidol group (35.3% patients in the LPV/r group experienced adverse events vs 14.3% patients in the arbidol group).

Another cases serie collected in China confirm that half of patients suffered digestive adverse effect and 7/10 patients showed hypokalemia [68,69]. Two recent meta-analyses [74,75] evaluating efficacy and safety of the most frequently used therapies for COVID-19 show as most of the AEs rate were comparable across the intervention and control groups. Conversely, LPV/r alone showed trend to more diarrhea events, while the combination of LPV/r, ribavirin and corticosteroids reduced incidence of diarrhea significantly.

Cheng et al. reported psychiatric symptoms such as depression, insomnia and suicidal thoughts in one patient treated with LPV/r, anyway isolation and anxiety for the disease may have played an important role in this case [68].

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