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Early combination treatment with existing HIV antivirals: an effective treatment for COVID-19?

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Abstract. – OBJECTIVE: Since no effective therapy exists, we aimed to test existing HIV antivirals for combination treatment of Coronavirus disease 19 (COVID-19).

MATERIALS AND METHODS: The crystal structures of SARS-CoV-2 main protein (Mpro) (PDB ID: 6Y2F) and SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) (PDB ID: 7BV2) both available from Protein Data Bank were used in the study. Automated Docking by using blind and standard method both on Mpro and RdRp bound to the modified template-primer RNA was performed with AutoDock 4.2.6 program suite. Lamarckian genetic algorithm (LGA) was used for structures docking. All inhibitors were docked with all bonds completely free to rotate.

RESULTS: Our molecular docking findings suggest that lopinavir, ritonavir, darunavir, and atazanavir activated interactions with the key binding sites of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) protease with a better inhibition constant (Ki) for lopinavir, ritonavir, and darunavir. Furthermore, we evidenced the ability of remdesivir, tenofovir, emtricitabine, and lamivudine to be incorporated in SARS-CoV-2 RdRp in the same protein pocket where poses the corresponding natural nucleoside substrates with comparable Ki and activating similar interactions. In principle, the four antiviral nucleotides might be used effectively against SARS-CoV-2.

CONCLUSIONS: The combination of a protease inhibitor and two nucleoside analogues, drugs widely used to treat HIV infection, could be evaluated in clinical trials for the treatment of COVID-19.

Key Words:
SARS-CoV-2, Docking, Antiretroviral, Nucleos(t)ide analogues, Protease inhibitor, COVID-19.

Introduction

At the end of 2019, several severe acute respiratory syndrome cases have been reported in Wuhan, China. On January 7, 2020, a new Coronavirus was detected[1] and called SARS-CoV-2. The World Health Organization (WHO) declared Coronavirus disease 19 (COVID-19) a public health emergency of international concern and declared the pandemic on March 12, 2020. Since the start of the pandemic, the number of reported cases worldwide is 53,766,728, with 1,308,975 deaths as of November 15, 2020[2,3], plus hundred thousand deaths due to the delay of treatment for other diseases[4-7].

The replicase complex is believed to be comprised of up to 16 viral subunits and several cellular proteins. Besides RNA-dependent RNA polymerase (RdRp), RNA helicase, and protease activities based on their physiological role, coronavirus proteases can be classified into the accessory and main proteases. The first group is responsible for cleaving the more divergent N-proximal pp1a/pp1ab regions at two or three sites. Instead, the main proteases (M^{pro}) cleave the major part of the polyproteins at 11 conserved sites and release the conserved key replicative functions, such as RdRp, helicase, and three of the RNA processing domains[8,9]. The structural conformation of SARS-CoV-2 M^{pro} obtained by resolving the crystal structure has been recently described by Zhang et al[10]. The SARS-CoV-2 M^{pro} is a homodimer whose each monomer is tightly joined and comprises Glu166 and Thr285 residues. Each monomer possesses three domains that are highly similar

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to those represented in the crystal structure of SARS-CoV. The substrate-binding sites of SARS-CoV-2 M^{pro} were identified after lead optimization and according to the favored interactions of an *in vitro* (mice and human) effective peptidomimetic inhibitor co-crystallized which SARS- CoV-2 M^{pro}. Information on active sites identified Cys145 and His41 as amino acids that were likely involved in the catalytic site, whereas Thr190 and Gln189 as amino acids were characterizing a bulky and small hydrophilic pocket, respectively. Glu166 and Thr285 residues represent the interaction site of the enzyme's dimerization necessary for catalytic activity.

The RdRp, also known as non-structural protein 12 (nsp12), catalyzes the synthesis of viral RNA and thus plays a central role in the replication and transcription cycle of the COVID-19 virus, possibly with the assistance of nsp7 and nsp8 as co-factors. Two structures of SARS-CoV-2 RdRp has recently appeared in the literature. Gao et al[11] describes interactions with conventional antiviral nucleos(t)ides co-crystallized with RdRp (resolution 2.9 Å) where, unfortunately, metal atoms and RNA template, both responsible for virus replication, are missing in the protein. Immediately after, Yin et al[12] published the crystal structure of RdRp protein bounded to the template-primer RNA with the active form of remdesivir, a known coronavirus inhibitor. In virtue of viral RNA primer presence, the latter crystal structure (resolution 2.5 Å) provides more reliable information at the atomic level on drug design and repositioning of conventional drugs targeting viral RdRp.

There is high sequence conservation in the RdRp across Coronaviruses (CoVs). For example, the RdRp of SARS-CoV-2 has 99.1% similarity and 96% amino acid identity to SARS-CoV[13]. The SARS-CoV-2 domain adopts the conserved architecture of the viral polymerase family and is featured by three subdomains containing catalytic cations. In the central cavity of a polymerase, three elements occur in viral replication reaction; the nucleos(t)ide triphosphate interacts with the template-primer RNA providing the final nascent strand.

Challenges to CoV antivirals development are multiple. The replication of positive-sense RNA virus genomes is generally characterized by high error rates, high viral yields, short replication times, and abundant homologous and non-homologous recombination[14]. As a result, genome mutants with varying degrees of fitness are generated.

The clinical picture of COVID-19 is characterized by a broad spectrum of presentations ranging from asymptomatic to life-threatening disease[15]. Common symptoms of SARS-CoV-2 infection are fever, dyspnea, cough, anosmia, and ageusia, while less common symptoms are represented by gastrointestinal disorders, dizziness, headache, and cutaneous manifestation[16-25]. The clinical course has been divided into three stages: the first is the early (mild) infection with minor symptoms, the second (moderate) grade of severity characterized by pulmonary involvement with or without hypoxia, and a third stage is characterized by extra-pulmonary systemic hyper-inflammatory syndrome[26]. The first stage is characterized by the replication of SARS-CoV-2, mainly in the upper respiratory tract, and represents the best moment to start an antiviral treatment to avoid the progression of COVID-19.

Different approaches have been tried so far, but no definitive treatment still exists and COVID-19 vaccines have been only recently introduced in some prioritized populations including healthcare staff, patients with comorbidities and elderly people. Thanks to the common features shared between different coronavirus, the repositioning of conventional antivirals effective on SARS-CoV and MERS-CoV could be a valuable pharmacological approach in finding antiviral agents selective SARS-CoV-2 infection. Very recently, some articles on this topic using *in silico* studies have been published; nevertheless, the resolution of the crystal protein and the computational approach plays a crucial role in finding reliable molecular docking and thus the best ligand[27-29].

Our research aimed to perform molecular docking studies on the high-resolved crystal structure of SARS-CoV-2 M^{pro} and SARS-CoV-2 RdRp to investigate potential combination antiviral treatment (cART) for COVID-19 using widely available drugs used to treat HIV infection. Many studies showed how cART has excellent tolerability, even elderly and in patients with several comorbidities[30-36]. For this purpose, we decided to test four HIV protease inhibitors (lopinavir, ritonavir, darunavir, and atazanavir) and to compare remdesivir, an established drug for COVID-19 treatment available only for intravenous use, with other orally administered nucleos(t)ide analogues (tenofovir, emtricitabine, and lamivudine).

Materials and Methods

In Silico Docking Study, Computational Equipment and Software

The crystal structures of SARS-CoV-2 M^{pro} (PDB ID: 6Y2F, in the monoclinic crystal form co-crystallized with peptidomimetic inhibitor O6K, resolution: 1.95 Å)[10] and SARS-CoV-2 RdRp (PDB ID: 7BV2, co-crystalized with remdesivir monophosphate, resolution: 2.50 Å)[37] were released from the RCSB protein databank (www.rcsb.org). SARS-CoV-2 RdRp was crystallized with remdesivir trisphosphate, experimentally recognized as the active form of remdesivir. In the crystal structure, remdesivir trisphosphate appears co-crystallized as monophosphate with the diphosphate residue, bonded to magnesium of the protein Mg1004 being positioned close to remdesivir monophosphate with which activate interaction POP1003.

The starting proteins were prepared, stripping the crystallographic water molecules and ligands. Hydrogen atoms were added to the structures using ADT of the MGLTools 1.5.7rc1 module[38].

All the ligands were constructed by using the standard molecule deposited in PubChem. Gasteiger-Marsili charges method was applied to assign all ligands and proteins[39].

Computational modeling experiments were carried out on an HP8100 Workstation and EXXACT Tensor Workstation TWS-1686525-AMB with the Cuda platform.

Binding of the compounds was performed using AutoDock 4.2.6 docking programs[40,41]. Lamarckian genetic algorithm (LGA) was used for structures docking. In the case of the blind docking procedure of protease, the LGA was defined through a centered grid, with coordinates: x = -4.876, y = -0.822, z = 11.992, of 126 × 126 × 126 grid points in x, y, z dimensions, respectively, spacing 0.555 Å. The LGA protocol was set to 100 runs, with a population size of 150 individuals and the maximum number of generations and energy evaluations of 27,000 and 25,000,000, respectively.

For the standard docking focused on the binding site of the protein, a grid with 60 × 60 × 60 points in x, y, z dimensions were selected for SARS-CoV-2 M^{pro}, and a grid size of 50 x 50 x 50 points in x,y,z dimension was selected for SARS-CoV-2 RdRp, respectively. For both proteins, the grid spacing was set to 0.375 Å.

All inhibitors were docked with all bonds completely free to rotate.

Free energy of ligand binding (EFEB, ΔG), the estimated inhibition constant (E.I.C., K_i) for each ligand was calculated according to equation (1):

$$K_i = \exp [(\Delta G \times 1000)/(R \times T)] \quad (1)$$

Where ΔG is the docking estimated free energy, R (gas constant) = 1.98719 cal/(K × mol), and T = 298.15 K.

Graphical representation of the poses derived from the docking calculation and hydrophobic interactions were obtained using MGLTools 1.5.7rc1, Chimera 1.13.1, and LigPlot+ software[42,43].

Results

Computational Studies

The reliability of the docking approach was verified on SARS-CoV-2 M^{pro} by stripping the peptidomimetic inhibitor O6K from 6Y2F.pdb crystal structure and by considering O6K as a normal ligand. Two experiments were carried out for the validation of the protocol. O6K was forced into the conformation of the crystal by blocking rotatable bonds for two different dockings, the first on the site with the standard grid used for this series of experiments (60 x 60 x 60, spacing 0.375 Å) the second with the maximum grid and spacing such as to cover the entire macromolecule (126 x 126 x 126, spacing 0.555 Å). After repositioning of O6K into the protein, the new ligand location was in accord with the original X-ray structure, with only minimal conformational changes, therefore confirming the reliability of the system (100% overlaid on the site, 50% in the Blind Docking) as depicted in **Supplementary Figure 1.**

From docking studies on O6K ligand and SARS-CoV-2 M^{pro}, a set of amino acid residues able to activate the best interactions with the pharmacophore lead was identified (Table I).

Cys145, Ser144, Gly143, and His41 point to the catalytic site; a restricted lipophilic pocket characterizes the last amino acid residue. Both Thr190 and Gln189 occupy a large hydrophobic site, whereas Glu166 and Thr285 residues are located on the interaction site of enzyme dimerization necessary for catalytic activity. Four conventional antiviral drugs, namely lopinavir, ritonavir, darunavir, and atazanavir, were selected for docking intro 6Y2F crystal structure.

Structurally, all of them are stereocontrolled peptidomimetics because they mimic a natural peptide. They are characterized by substituents with different lipophilicity and structural flexibility that influence protein-ligand interactions and the conformational state of the molecule. To a different extent, all conventional antiviral drugs activated interactions with the protease active sites that, theoretically, would interfere with the function of SARS-CoV-2 M^{pro}. Ritonavir, lopinavir, and darunavir had the best Ki estimated 15.13, 37.44, and 56.76 nM for each of them, whereas atazanavir Ki was 1.10 µM, identifying a minor virtual interaction with SARS-CoV-2 M^{pro}.

Supplementary Figure 2 and Table II represent hydrophobic and H-bond interactions, respectively, predicted for the four antiviral drugs activated with SARS-CoV-2 M^{pro}. At least five H-bonds were estimated for each drug, evidencing the high affinity with the protein. Lopinavir interacted with more key amino acids with both hydrophobic and H-bond interactions, such as Cys145, Ser144, and Gly143, involved in the catalytic site, with Thr190 and Gln192, located on the bulky hydrophilic site and with Glu166, representative of the interaction site of protein dimerization. Unlike the other drugs, atazanavir activates H-bond with oxygen and nitrogen of Thr26 and hydrophobic interaction with Thr25, two amino acids far from the theoretical active pocket site. In Figure 1, an image of each drug's best poses in the active sites of SARS- CoV-2 M^{pro} is being represented.

In SARS-CoV-2 RdRp (PDB ID: 7BV2) crystal structure, remdesivir trisphosphate (remdesivir-3P) appears co-crystallized as monophosphate partially bonded to U20 and U10 of uracil base and A11 of adenine base[12]. The crystal sets the situation when diphosphate residue has just been released; it is being bonded to magnesium atom M1004 of the protein and activates interaction POP1003 with remdesivir monophosphate. The ligand engages interactions with Thr688 and Asp761 of the protein and with A11, U10, and U20. In our docking approach, remdesivir monophosphate and the diphosphate residues were extracted from the crystal structure. After, remdesivir, tenofovir, lamivudine, and emtricitabine, all of them as triphosphate (3P), were launched for molecular docking to find the right pose among the generated conformations. Nearly complete superimposed poses were reached with co-crystallized remdesivir monophosphate and remdesivir-3P with the contemporary alignment of the nucleos(t)ide bases (Figure 2).

Table I. Estimated interactions of antiviral drugs with the amino acids residues of SARS-CoV-2 M^{pro}.

Active metabolite	N° run	Active torsion	M.B.E.[a]	E.F.E.B.[b]	E.I.C., Ki[c]	Interactions
Atazanavir	100	19	-8.13	-8.13	1.10, µM	Thr24, Thr25, Thr26, Leu27, His41, Thr45, Ser46, Met49, Leu141, Gly143, Cys145, His163, His164, Met165, Glu166, Leu167, Pro168, Gln189, Thr190, Gln192
Darunavir	100	14	-9.89	-9.89	56.76, nM	His41, Met49, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, His172, Asp187, Arg188, Gln189, Thr190, Gln192
Ritonavir	100	19	-10.13	-10.13	37.44, nM	His41, Met49, Phe140, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, Leu167, Pro168, His172, Asp187, Arg188, Gln189, Thr190,Gln192
Lopinavir	100	16	-9.34	-10.67	15.13, nM	His41, Met49, Leu141, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, Leu167, Pro168, Asp187, Arg188, Gln189, Thr190, Ala191, Gln192

[a]M.B.E.: Mean Binding Energy, [b]E.F.E.B.: Estimated Free Energy of Binding, [c]E.I.C., Ki: Estimated Inhibition Constant, Ki.

Table II. Estimated H-bond of antiviral drugs with the amino acids residues of SARS-CoV-2 M^{pro}.

Hydrogen bond interactions			
Ligands	**N° H-bond**	**Protein atom**	**Ligand atom**
Atazanavir	5	Thr26:O (OA)[a] Thr26:HN (HD) Glu166:HN (HD) Thr190:HN (HD) Gln192:HE22 (HD)	H13 (HD)[b] O14 (OA) O42 (OA) N56 (NA)[c] N56 (NA)
Darunavir	7	Phe140:O (OA) Gly143:HN (HD) Glu166:OE2 (OA) Glu166:HN (HD) Glu166:HN (HD) Gln192:HE22 (HD) Gln192:HE22 (HD)	H15 (HD) O6 (OA) H14 (HD) O20 (OA) O26 (OA) O30 (OA) O32 (OA)
Ritonavir	5	Glu166:HN (HD) Glu166:O (OA) Thr190:HN (HD) Gln192:HE22 (HD) Gln192:HE22 (HD)	O23 (OA) H24 (HD) O9 (OA) O7 (OA) O9 (OA)
Lopinavir	5	Ser144:OG (OA) His163:HE2 (HD) Glu166:HN (HD) Glu166:O (OA) Gln192:HE22 (HD)	H14 (HD) O15 (OA) O5 (OA) H29 (HD) O31 (OA)

[a]Oxygen acceptor; [b]Hdrogen donor; [c]Nitrogen acceptor.

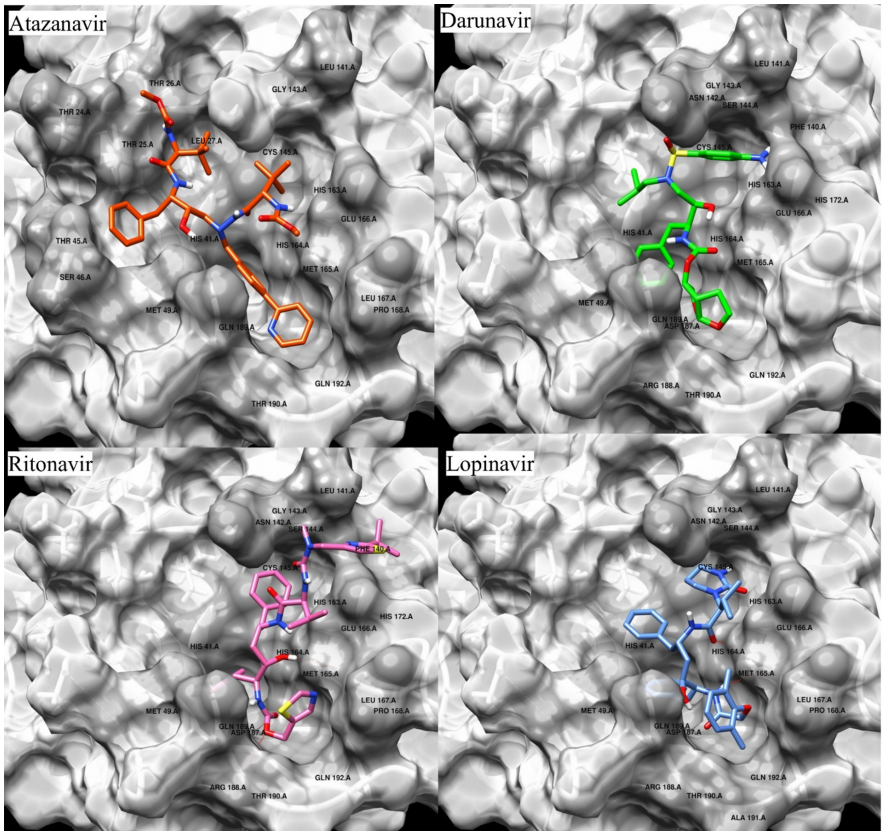

Figure 1. The best fit of atazanavir, darunavir, lopinavir, and ritonavir to the active sites of SARS-CoV-2 M^{pro} after molecular docking. The dark grey spots represent lipophilic pockets.

Adenosine triphosphate (adenosine-3P) and cytosine triphosphate (cytosine-3P) were also added to the simulation study because they represent the natural substrate used by RdRp to replicate the viral RNA-based genome. 7BV2 RNA crystal possesses uracil base alignment suitable for the complementary adenosine nucleos(t)ide base; thus, when cytosine-3P and cytosine nucleos(t)ide antivirals were docked, uracil was replaced with guanine base, the complementary base of cytosine.

The best interactions of adenosine-3P, tenofovir-3P, and remdesivir-3P with the amino acids residue of SARS-CoV-2 RdRp have been represented in Table III, where Ki reached a magnitude of 10^{-18}M. Remdesivir-3P and tenofovir-3P activate similar interactions with the polymerase and comparable to those of remdesivir monophosphate co-crystallized with SARS-CoV-2 RdRp (7BV2 crystal). Conversely to the crystal where remdesivir monophosphate is covalently bonded to the primer strand of RNA, in our study, remdesivir-3P appeared perfectly aligned with the nucleos(t)ide base with which activated strong interactions. Remdesivir monophosphate Tenofovir-3P and remdesivir-3P interact with the same viral RNA bases even though remdesivir-3P provides additional interactions with Asp623, Ser682, Thr687, and Asn691 of the protein and more lipophilic and H-bond interactions (**Supplementary Figure 3A** and Table IV). Figure 3 represents the best poses of adenosine-3P, tenofovir-3P, and remdesivir-3P in the viral polymerase's active pocket.

As estimated for adenosine-analogues antivirals, lamivudine-3P and emtricitabine-3P, two cytosine-analogues antivirals, target the active pocket of the polymerase and with the same pose as cytosine-3P does (Figure 4). Except for Asp760, lamivudine-3P interacts with the same set of amino acids, RNA bases, and magnesium cations of emtricitabine-3P and cytosine-3P, all of them with significantly low Ki (Table III). Conversely, lamivudine-3P activates more H-bond with the polymerase than emtricitabine-3P, whereas both activate analogues lipophilic interactions with the protein (Table IV and **Supplementary Figure 3B**).

Discussion

The active form of remdesivir, a nucleotide clinically evaluated against Ebola virus disease, and COVID-19[44,45], have been compared *in silico* with four HIV protease inhibitors (lopinavir, ritonavir, darunavir, and atazanavir) by binding with SARS-CoV-2 RdRp. In order to enhance the effect of HIV protease inhibitors, three orally administered nucleos(t)ide analogues (tenofovir, emtricitabine, and lamivudine) have been evaluated and compared *in silico* with the protease binding of remdesivir.

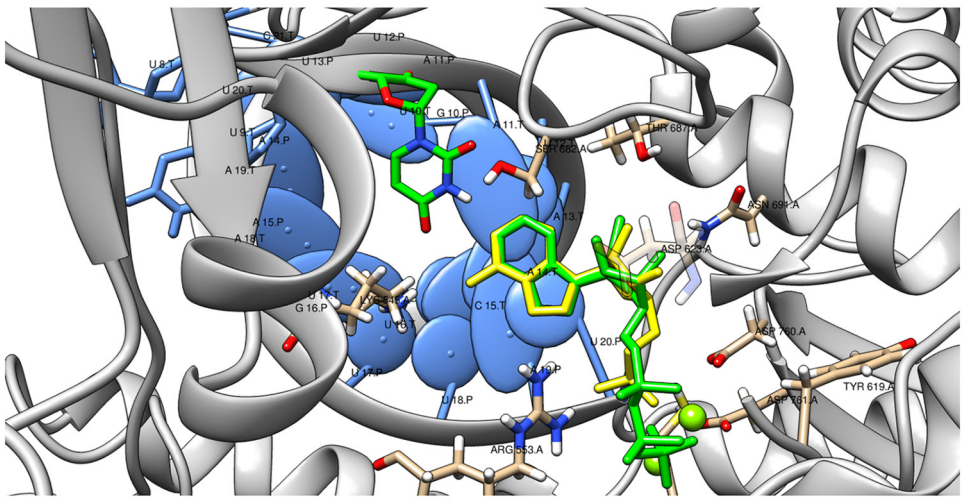

Figure 2. Remdesivir monophosphate from 7BV2 (*yellow*) and molecular docking of remdesivir-3P (*light green*).

Table III. Estimated interactions of adenosine-3P (ATP), tenofovir-3P and remdesivir-3P, cytosine-3P (CTP), emtricitabine-3P and lamivudine-3P with the amino acids residues of SARS-CoV-2 RdRp[a].

Active metabolite	% cluster	M.B.E.[b]	E.F.E.B.[c]	E.I.C., Ki[d]	Interactions
ATP	19	-19.42	-20.89	483.59, aM	MG1, MG2, U10, A11, U20, Lys545, Arg553, Asp618, Tyr619
Tenofovir-3P	11	-20.04	-21.86	95.15, aM	MG1, MG2, U10, A11,U20, Asp760, Asp761
Remdesivir-3P	8	-21.91	-24.01	2.51, aM	MG1, MG2, U10, A11,U20, Asp623, Ser682, Thr687, Asn691, Asp760, Asp761
CTP	14	-20.16	-21.44	192.32, aM	MG1,MG2, G10,U20, Ser682, Thr687, Asp760, Asp761
Emtricitabine-3P	15	-21.33	-22.86	17.51, aM	MG1, MG2, G10, U20, Arg553, Ser682, Thr687, Asn691
Lamivudine-3P	9	-21.34	-23.00	13.83, aM	MG1, MG2, G10, U20, Ser682, Thr687, Asp760, Asp761

[a]In 7BV2 crystal of SARS-CoV-2 RdRp, uracil was replaced with guanine base for interactions with CTP, emtricitabine-3P, and lamivudine-3P; [b]M.B.E.: Mean Binding Energy; [c]E.F.E.B.: Estimated Free Energy of Binding.

Our *in silico* results suggest that lopinavir, ritonavir, darunavir, and atazanavir activated interactions with the key binding sites of SARS-CoV-2 protease with a better Ki for lopinavir, ritonavir, and darunavir. Although atazanavir appears to be less effective in silico, it would not rule out pharmacokinetics and the pharmacodynamics of the molecule under human trials. These four drugs have been extensively used as part of combination antiretroviral treatment of HIV infection for more than a decade[46-48], and very recently, a *in silico* study on interactions between SARS-CoV-2 protease and the lopinavir-ritonavir combination has been published[29]. In the clinical use in HIV-infected patients, ritonavir is currently used only at a low dose as a pharmacokinetic booster in combination with lopinavir, darunavir, and atazanavir.

A randomized, controlled, open-label study reported the efficacy and safety of lopinavir/ritonavir for treating hospitalized adults with severe COVID-19[49]. In this trial, 99 patients received lopinavir/ritonavir and 100 standard care for 14 days. Of note, the median interval time between symptoms onset and randomization was 13 days. No statistically significant difference in time to clinical improvement was observed between arms. However, 28 days mortality was lower in the lopinavir/ritonavir group (19.2%) than the control group (25%).

Furthermore, patients on lopinavir/ritonavir had significantly shorter stay (6 *vs.* 11 days) in the intensive care unit (ICU) regarding the control group. Overall, faster clinical recovery and reduced mortality were observed in those treated within 12 days of symptom onset. Gastrointestinal complaints were more common in the lopinavir/ritonavir group, whereas serious adverse events did not differ between the two arms. These results suggest that lopinavir/ritonavir was associated with a faster clinical recovery in those who started early and with a shorter duration of ICU stay.

The efficacy of lopinavir/ritonavir was also tested in the "Recovery" trial against three other different single drug approaches (dexamethasone, hydroxychloroquine, or azithromycin). No differences were found in mortality and duration of hospitalization[50]. However, the median time between the appearance of the symptoms and treatment start was 8 (IQR 4-14) days.

The combination of darunavir/cobicistat has been studied both *in vitro* and *in vivo*. De Meyer et al[51] demonstrated that darunavir/cobicistat has low antiviral activity against SARS-CoV-2. This combination as monotherapy compared to conventional treatment for COVID-19 was studied in a small open-label randomized clinical trial (30 participants in the two arms, NCT04252274). The outcome was the virological clearance rate of oropharyngeal swabs. No difference was found between the two arms[52]. Unfortunately, no real-life data are available for atazanavir in the treatment of COVID-19.

Table IV. Estimated interactions of H-bonds of adenosine-3P (ATP), tenofovir-3P and remdesivir-3P, cytosine-3P (CTP), emtricitabine-3P and lamivudine-3P with the amino acids residues of SARS-CoV-2 RdRp[a].

Hydrogen bond interactions			
Active metabolite	**N° H-bond**	**Protein atom**	**Ligand atom**
ATP	4	Arg553:HH12 (HD) U20:HO3 (HD) U20:O3' (OA) U10:O4 (OA)	O18 (OA)[b] O20 (OA) H21 (HD)c H34 (HD)
Tenofovir-3P	2	U20:O4 (OA) U10:O4 (OA)	H28 (HD) H29 (HD)
Remdevisir-3P	4	Asn691:HD22 (HD) U10:H3 (HD) U10:O4 (OA) U20:O4 (OA)	O22 (OA) N14 (NA)[d] H18 (HD) H19 (HD)
CTP	4	Thr687:OG1 (OA) Ser682:O (OA) U20:'HO3 (HD) G10:H21 (HD)	H32 (HD) H33 (HD) O9 (OA) N28 (NA)
Emtricitabine-3P	3	Ser682:O (OA) Thr687:OG1 (OA) G10:H21 (HD)	H30 (HD) H29 (HD) N25 (NA)
Lamivudine-3P	4	Thr687:OG1 (OA) Thr687:OG1 (OA) G10:H21 (HD) G10:H1 (HD)	H28 (HD) H29 (HD) N24 (NA) O26 (OA)

[a]In 7BV2 crystal of SARS-CoV-2 RdRp, uracil was replaced with guanine base for interactions with CTP, emtricitabine-3P and lamivudine-3P; [b]Oxygen acceptor; [c]Hydrogen donor; [d]Nitrogen acceptor.

Remdesivir is reported to act via chain termination of nascent viral RNA in Ebola virus infection[53] and is also effective against a broad spectrum of human and pre-epidemic zoonotic CoVs potently inhibits replication of SARS-CoV and MERS- CoV in primary human airway epithelial cultures[54,55].

One potential drawback of remdesivir monotherapy is represented by the possible selection of resistance, mediated by RdRp residues F480L and V557L in SARS-CoV, resulting in a 5-fold shift half-maximal inhibitory concentration (IC_{50})[55]. Importantly, the two remdesivir resistance mutations, alone or together, conferred increased sensitivity to inhibition by β-D-N4-hydroxycytidine (NHC), a novel potential broad-spectrum antiviral, suggesting unique patterns of resistance for single drugs[13].

Since naturally occurring mutations in RdRp could lead to drug resistance phenomena[26], in our study, residues F480 and V557 are not involved in the set of residues with the highest frequency identified in the RdRp of the HIV protease inhibitors investigated.

A more studied antiretroviral, remdesivir, has been shown to effectively inhibit SARS-CoV-2 replication in Vero E6 cells[56] and interact with RdRp by forming two H-bonds Trp509 and His381, π-cation contacts with Phe504, and hydrophobic interactions with Lys508, Leu401, Asn386, and Ser384[27]. Williamson et al[57] showed in the animal model that very early administration of remdesivir to rhesus macaques reduced virus titers in bronco-alveolar lavage significantly. The viral load was significantly lower in the lungs of remdesivir-treated animals than in controls. However, the authors admit that the results were difficult to translate in real life of patients' management because remdesivir was administered 12 hours after virus inoculation that in human disease is coincident with an asymptomatic incubation period. It is currently under evaluation as a potential monotherapy treatment for COVID-19 in at least nine randomized clinical trials.

The main issue concerning remdesivir is that it is available only for intravenous administration that is logistically impossible to implement for home treatment, especially on a large scale.

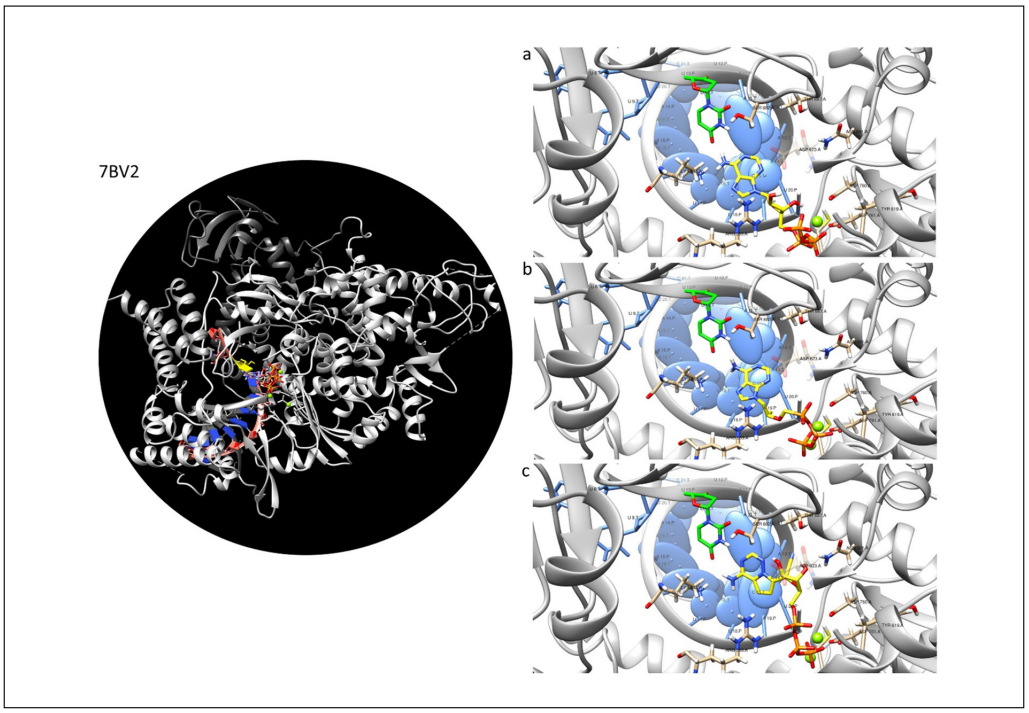

Figure 3. The best fit for the active sites of SARS CoV-2 RdRb after molecular docking with adenosine-3P and nucleoside antivirals (*yellow*). Left: 7BV2 crystal where uracil (*green*) points to the active sites for interactions with adenosine-3P and antiviral adenosine analogues, right: adenosine-3P **(a)**, tenofovir-3P **(b)**, remdesivir-3P **(c)** poses in the active sites of the polymerase.

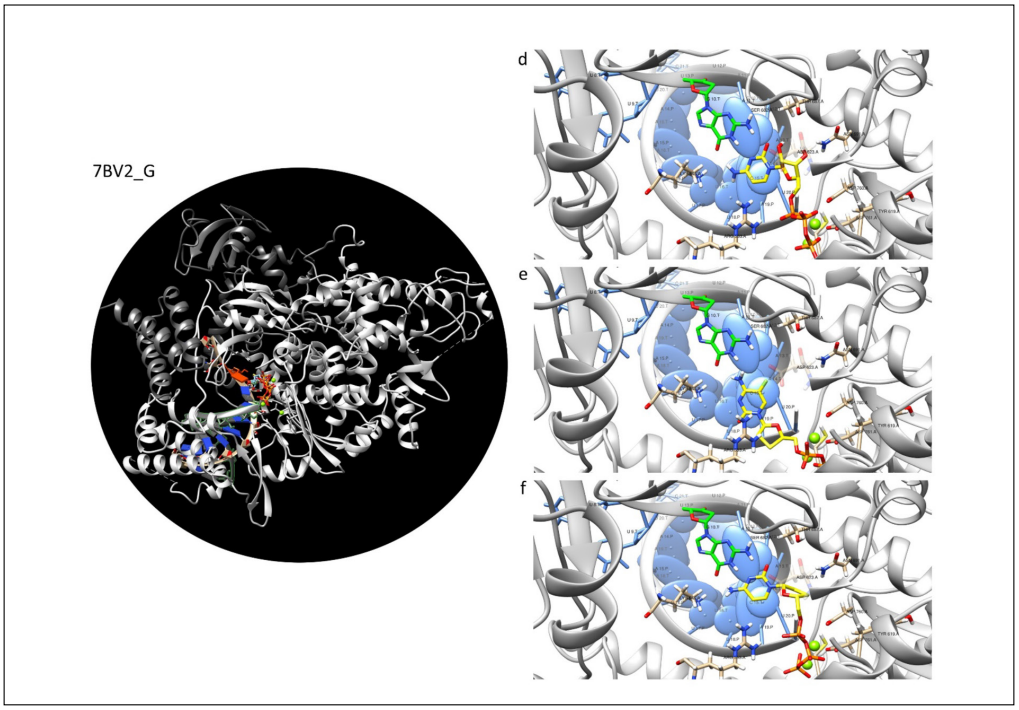

Figure 4. The best fit for the active sites of SARS CoV-2 RdRb after molecular docking with cytosine-3P and nucleoside antivirals (*yellow*). Left: 7BV2_G, from crystal 7BV2 where uracil was replaced with guanine (*green*) (G) for interactions with cytosine-3P and antiviral cytosine analogues, right: cytosine-3P **(d)**, lamivudine-3P **(e)**, emtricitabine-3P **(f)** poses in the active sites of the polymerase.

Furthermore, it appears difficult to treat patients in the first days of infection in the asymptomatic stage. Furthermore, its effectiveness as monotherapy is debated in the literature. Many trials have been conducted, and others are still ongoing.

Beigel et al[58] demonstrated that patients treated with remdesivir, compared with those who assumed placebo, had a shorter time to recovery and a low 14-days mortality, while no significant difference was present ad 29 days. Serious adverse events were less frequently reported in the remdesivir group compared to placebo.

WHO conducted a trial called "SOLIDARITY trial", which results are not yet published. They studied, with five arms, four different drugs: Remdesivir, Hydroxychloroquine, Lopinavir/ritonavir, and Interferon-β1a. No single study drug showed to reduce mortality, initiation of ventilation, or recovery time[59].

The combination of tenofovir/emtricitabine has been evaluated by Ayerdi et al[60] in a cohort of people assuming tenofovir/emtricitabine as pre-exposure prophylaxis, which had a higher SARS-CoV-2 seroprevalence than the control group, showing a non-efficacy of tenofovir/emtricitabine to prevent the infection.

Therefore, no nucleos(t)ide analogues combinations are under evaluation in clinical trials to treat COVID-19. Del Amo et al[61] conducted a cohort study on 236 people living with HIV that had SARS-CoV-2 infection. In this cohort, people receiving TDF/FTC (tenofovir disoproxil fumarate/emtricitabine), compared with those receiving other regimens, had a lower risk of developing COVID-19 and being hospitalized.

Our *in silico* studies evidenced the ability of remdesivir, tenofovir, lamivudine, and emtricitabine to be incorporated, as active forms, in SARS-CoV-2 RdRp in the same protein pocket where poses the corresponding natural nucleos (t)ide substrates with comparable Ki value and activating similar interactions. In principle, the four antiviral nucleos(t)ides might be used effectively against SARS-CoV-2. To maximize the antiviral effect on SARS-CoV-2 RdRp, a cytosine-based antiviral nucleos(t)ide could be associated with an adenosine-based one.

Tenofovir, emtricitabine, and lamivudine are nucleos(t)ide analogues already widely available in the market as antiretroviral drugs. These medications have been extensively used in HIV-infected patients showing a favorable safety profile, especially when considering the limited duration of COVID-19 treatment[62-64]. Furthermore, these drugs are available for oral administration and show minimal drug-drug interactions, thus representing ideal candidates for both out and inpatients treatment in the early stage COVID-19. The possibility to treat patients with effective combinations at home is of crucial importance given the possible further waves of the pandemic in the upcoming months while waiting for a protective vaccine.

From lessons learned from other viral infections, including HIV and HCV, the combination of drugs with different targets may reduce more rapidly SARS-CoV-2 viral load, the probability of interhuman transmission, and selection of resistance to single medications. Furthermore, early antiviral treatment has been associated with better clinical outcomes in COVID-19 patients in a large observational study conducted in China[65].

Conclusions

Based on the available literature and our experimental results, we propose that a combination of a boosted protease inhibitor (lopinavir/r, darunavir/r, or atazanavir/r) and two nucleos(t)ide analogues (tenofovir disoproxil or tenofovir alafenamide + emtricitabine or lamivudine) started early in the course of the disease (ideally within the first seven days) and administered for a short time (5-10 days depending on the severity of the disease) could be evaluated as soon as possible in clinical trials for early treatment of outpatients with COVID-19.

Conflict of Interest

GM has been advisor for Gilead Sciences, Janssen and Merck Sharp and Dohme and ViiV Healthcare and has received speakers' honoraria from Gilead Sciences, Merck Sharp and Dohme, Janssen and ViiV Healthcare; all other authors declare they have no potential conflict of interest to disclose.

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Our thoughts go to all COVID-19 patients' and their families and to all healthcare workers who risk their life everyday hiding smiles, tears and sweat under their protective suites, helmets and respirators.

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Authors' Contribution

GM, PAS, ADV, GD, AD, RND participated in the formulation or evolution of research goals and aims of the study; GD, AD, RND were responsible for data curation; GM, PAS, ADV, RND, AD, GD analyzed the data; RND, AD performed the experiments and created the models; GM, PAS and GD were responsible for the research activity and execution; GD provided the resourches for the conduction of the experiments including the computational study and molecular modelling; GM, PAS, GD were responsible for the overall supervision of the research; RND, AD, GD were responsible for verification of the overall replication/reproducibility of experiments; GM and GD were responsible for visualization and data presentation; GM and GD drafted the initial manuscript; GM, PAS, ADV, GD, AD, RND critical reviewed, revised and approved the final manuscript.

Data and Materials Availability

All data is available in the main text or in the **Supplementary Materials**. All data used in the analysis are available to any researcher for purposes of reproducing or extending the analysis upon request.

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References

1) New-type coronavirus causes pneumonia in Wuhan: expert - Xinhua | Access at: http://www.xinhuanet.com/english/2020-01/09/c_138690570.htm.
2) World Health Organization. Weekly Epidemiological Update on COVID-19 [Internet]. WHO. 2020. Access at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20201012-weekly-epi-update-9.pdf
3) Perrella A, Carannante N, Berretta M, Rinaldi M, Maturo N, Rinaldi L. Editorial - Novel Coronavirus 2019 (SARS-CoV2): a global emergency that needs new approaches? Eur Rev Med Pharmacol Sci 2020; 24: 2162-2164.
4) Hartman HE, Sun Y, Devasia TP, Chase EC, Jairath NK, Dess RT, Jackson WC, Morris E, Li P, Hochstedler KA, Abbott MR, Kidwell KM, Walter V, Wang M, Wang X, Zaorsky NG, Schipper MJ, Spratt DE. Integrated survival estimates for cancer treatment delay among adults with cancer during the COVID-19 pandemic. JAMA Oncol 2020; 6: 1881-1889.
5) Bersano A, Kraemer M, Touzé E, Weber R, Alamowitch S, Sibon I, Pantoni L. Stroke care during the COVID-19 pandemic: experience from three large European countries. Eur J Neurol 2020; 27: 1794-1800.
6) Jalaeefar A, Eslami B, Omranipour R. Letter to the Editor – The impact of COVID-19 on cancer multidisciplinary teams' meetings and tumor boards: what should we think about to overcome and amend? WCRJ 2020; 7: e1584.
7) De Vito A, Madeddu G. Editorial - HIV and cancer during COVID-19 pandemic: sailing through the perfect storm. Eur Rev Med Pharmacol Sci 2020; 24: 13103-13104.
8) Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 2000; 81: 853-879.
9) Snijder EJ, Bredenbeek PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LLM, Guan Y, Rozanov M, Spaan WJM, Gorbalenya AE. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol 2003; 331: 991-1004.
10) Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 2020; 368: 409-412.
11) Gao Y, Yan L, Huang Y, Liu F, Zhao Y, Cao L, Wang T, Sun Q, Ming Z, Zhang L, Ge J, Zheng L, Zhang Y, Wang H, Zhu Y, Zhu C, Hu T, Hua T, Zhang B, Yang X, Li J, Yang H, Liu Z, Xu W, Guddat LW, Wang Q, Lou Z, Rao Z. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science 2020; 368: 779-782.
12) Yin W, Mao C, Luan X, Shen D-D, Shen Q, Su H, Wang X, Zhou F, Zhao W, Gao M, Chang S, Xie Y-C, Tian G, Jiang H-W, Tao S-C, Shen J, Jiang Y, Jiang H, Xu Y, Zhang S, Zhang Y, Xu HE. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science 2020; 368: 1499-1504.
13) Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, Leist SR, Schäfer A, Dinnon KH, Stevens LJ, Chappell JD, Lu X, Hughes TM, George AS, Hill CS, Montgomery SA, Brown AJ, Bluemling GR, Natchus MG, Saindane M, Kolykhalov AA, Painter G, Harcourt J, Tamin A, Thornburg NJ, Swanstrom R, Denison MR, Baric RS. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020; 12: eabb5883.
14) Perales C, Domingo E. Antiviral strategies based on lethal mutagenesis and error threshold. Curr Top Microbiol Immunol 2016; 392: 323-339.
15) Niuz Morshed Khan M., Sharif Sarker M. A review of Coronavirus 2019 (COVID-19), a life threating disease all over the world. WCRJ 2020; 7: e1586.
16) De Vito A, Geremia N, Fiore V, Princic E, Babudieri S, Madeddu G. Clinical features, laboratory

findings and predictors of death in hospitalized patients with COVID-19 in Sardinia, Italy. Eur Rev Med Pharmacol Sci 2020; 24: 7861-7868.

17) Zinellu A, Arru F, De Vito A, Sassu A, Valdes G, Scano V, Zinellu E, Perra R, Madeddu G, Carru C, Pirina P, Mangoni AA, Babudieri S, Fois AG. The De Ritis ratio as prognostic biomarker of in-hospital mortality in COVID-19 patients. Eur J Clin Invest 2021; 51: e13427.

18) Vaira LA, Deiana G, Fois AG, Pirina P, Madeddu G, De Vito A, Babudieri S, Petrocelli M, Serra A, Bussu F, Ligas E, Salzano G, Riu G De. Objective evaluation of anosmia and ageusia in COVID-19 patients: a single-center experience on 72 cases. Head Neck 2020; 42: 1252-1258.

19) Baratella E, Crivelli P, Marrocchio C, Bozzato AIM, De Vito A, Madeddu G, Saderi L, Confalonieri M, Tenaglia L, Cova MA. Severity of lung involvement on chest X-rays in SARS-coronavirus-2 infected patients as a possible tool to predict clinical progression: an observational retrospective analysis of the relationship between radiological, clinical, and laboratory data - PubMed. J Bras Pneumol 2020; 46: e20200226.

20) Geremia N, De Vito A, Gunnella S, Fiore V, Princic E, Panu Napodano C, Madeddu G, Babudieri S. A case of vasculitis-like skin eruption associated with COVID-19. Infect Dis Clin Pract 2020; 28: e30-e31.

21) Guarneri C, Rullo EV, Pavone P, Berretta M, Ceccarelli M, Natale A, Nunnari G. Silent COVID-19: what your skin can reveal. Lancet Infect Dis 2021; 21: p24-p25.

22) Guarneri C, Venanzi Rullo E, Gallizzi R, Ceccarelli M, Cannavò SP, Nunnari G. Diversity of clinical appearance of cutaneous manifestations in the course of COVID-19. J Eur Acad Dermatology Venereol 2020; 34: e449-450.

23) Vaira LA, Hopkins C, Salzano G, Petrocelli M, Melis A, Cucurullo M, Ferrari M, Gagliardini L, Pipolo C, Deiana G, Fiore V, De Vito A, Turra N, Canu S, Maglio A, Serra A, Bussu F, Madeddu G, Babudieri S, Giuseppe Fois A, Pirina P, Salzano FA, De Riu P, Biglioli F, De Riu G. Olfactory and gustatory function impairment in <scp>COVID</scp> -19 patients: Italian objective multicenter-study. Head Neck 2020; 42: 1560-1569.

24) Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. Zhonghua Liu Xing Bing Xue Za Zhi 2020; 41: 145-151.

25) Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-513.

26) Siddiqi HK, Mehra MR. COVID-19 Illness in Native and Immunosuppressed States: a clinical-therapeutic staging proposal. J Hear Lung Transplant 2020; 39: 405-407.

27) Yu R, Chen L, Lan R, Shen R, Li P. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. Int J Antimicrob Agents 2020; 56: 106012.

28) Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S. Potential Inhibitor of COVID-19 main protease (M pro) from several medicinal plant compounds by molecular docking study. Preprints 2020: 1-14.

29) Elfiky AA. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study. Life Sci 2020; 253: 117592.

30) Zanet E, Berretta M, Benedetto F Di, Talamini R, Ballarin R, Nunnari G, Berretta S, Ridolfo A, Lleshi A, Zanghì A, Cappellani A, Tirelli U. Pancreatic cancer in HIV-positive patients: A clinical case-control study. Pancreas 2012; 41: 1331-1335.

31) Di Benedetto F, De Ruvo N, Berretta M, Masetti M, Montalti R, Di Sandro S, Quintini C, Codeluppi M, Tirelli U, Gerunda GE. Don't deny liver transplantation to HIV patients with hepatocellular carcinoma in the highly active antiretroviral therapy era. J Clin Oncol 2006; 24: e26-27.

32) Rossetti B, Baldin G, Sterrantino G, Rusconi S, De Vito A, Giacometti A, Gagliardini R, Colafigli M, Capetti A, d'Ettorre G, Celani L, Lagi F, Ciccullo A, De Luca A, Di Giambenedetto S, Madeddu G. Efficacy and safety of dolutegravir-based regimens in advanced HIV-infected naïve patients: results from a multicenter cohort study. Antiviral Res 2019; 169: 104552.

33) Bonfanti P, De Vito A, Ricci E, Menzaghi B, Orofino G, Squillace N, Molteni C, De Socio GV, Salomoni E, Celesia BM, Dentone C, Colombo V, Madeddu G. Bone Safety of dolutegravir-containing regimens in people living with HIV: results from a real-world cohort. Infect Drug Resist 2020; 13: 2291-2300.

34) Giuntini R, Martinelli C, Ricci E, Vichi F, Gianelli E, Madeddu G, Abeli C, Palvarini L, Penco G, Marconi P, Grosso C, Pellicano G, Bonfanti P, Quirino T. Efficacy and safety of boosted and unboosted atazanavir-containing antiretroviral regimens in real life: results from a multicentre cohort study. HIV Med 2010; 11: 40-45.

35) Madeddu G, Bonfanti P, De Socio G V., Carradori S, Grosso C, Marconi P, Penco G, Rosella E, Miccolis S, Melzi S, Mura MS, Landonio S, Ricci E, Quirino T, for the CISAI Group. Tenofovir renal safety in HIV-infected patients: results from the SCOLTA project. Biomed Pharmacother 2008; 62: 6-11.

36) Facciolà A, Ceccarelli M, Venanzi Rullo E, d'Aleo F, Condorelli F, Visalli G, Cacopardo B, Pinzone MR, di Rosa M, Nunnari G, Pellicanò GF. Prostate cancer in HIV-positive patients: a review of the literature. WCRJ 2018; 5: e1136.

37) Yin W, Mao C, Luan X, Shen DD, Shen Q, Su H, Wang X, Zhou F, Zhao W, Gao M, Chang S, Xie YC, Tian G, Jiang HW, Tao SC, Shen J, Jiang Y, Jiang H, Xu Y, Zhang S, Zhang Y, Xu HE. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science 2020; 368: 1499-1504.

38) Sanner MF. PYTHON: A PROGRAMMING LANGUAGE FOR SOFTWARE INTEGRATION AND DEVELOPMENT. J Mol Graph Mod 1999; 17: 57-61.

39) Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity-a rapid access to atomic charges. Tetrahedron 1980; 36: 3219-3228.

40) Huey R, Morris GM, Olson AJ, Goodsell DS. A semiempirical free energy force field with charge-based desolvation. J Comput Chem 2007; 28: 1145-1152.

41) Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998; 19: 1639-1662.

42) Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera? A visualization system for exploratory research and analysis. J Comput Chem 2004; 25: 1605-1612.

43) Laskowski RA, Swindells MB. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011; 51: 2778-2786.

44) Tchesnokov EP, Feng JY, Porter DP, Götte M. Mechanism of inhibition of ebola virus RNA-dependent RNA polymerase by remdesivir. Viruses 2019; 11: 326.

45) Mulangu S, Dodd LE, Davey RT, Tshiani Mbaya O, Proschan M, Mukadi D, Lusakibanza Manzo M, Nzolo D, Tshomba Oloma A, Ibanda A, Ali R, Coulibaly S, Levine AC, Grais R, Diaz J, Lane HC, Muyembe-Tamfum J-J, the PALM Writing Group. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med 2019; 381: 2293-2303.

46) Qazi NA, Morlese JF, Pozniak AL. Lopinavir/ritonavir (ABT-378/r). Expert Opin Pharmacother 2002 3: 315-327.

47) McCoy C. Darunavir: a nonpeptidic antiretroviral protease inhibitor. Clin Ther 2007; 29: 1559-1576.

48) Piliero PJ. Atazanavir: a novel HIV-1 protease inhibitor. Expert Opin Investig Drugs 2002; 11: 1295-1301.

49) Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020; 382: 1787-1799.

50) Horby PW, Mafham M, Bell JL, Linsell L, Staplin N, Emberson J, Palfreeman A, Raw J, Elmahi E, Prudon B, Green C, Carley S, Chadwick D, Davies M, Wise MP, Baillie JK, Chappell LC, Faust SN, Jaki T, Jefferey K, Lim WS, Montgomery A, Rowan K, Juszczak E, Haynes R, Landray MJ. Lopinavir-ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet 2020; 396: 1345-1352.

51) De Meyer S, Bojkova D, Cinatl J, Van Damme E, Buyck C, Van Loock M, Woodfall B, Ciesek S. Lack of antiviral activity of darunavir against SARS-CoV-2. Int J Infect Dis 2020; 97: 7-10.

52) Chen J, Xia L, Liu L, Xu Q, Ling Y, Huang D, Huang W, Song S, Xu S, Shen Y, Lu H. Antiviral activity and safety of darunavir/cobicistat for the treatment of COVID-19. Open Forum Infect Dis 2020; 7: ofaa241.

53) Tchesnokov EP, Feng JY, Porter DP, Götte M. Mechanism of inhibition of Ebola virus RNA-dependent RNA polymerase by remdesivir. Viruses 2019; 11: 326.

54) Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, MacKman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 2017; 9: eaal3653.

55) Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Smith EC, Case JB, Feng JY, Jordan R, Ray AS, Cihlar T, Siegel D, Mackman RL, Clarke MO, Baric RS, Denison MR. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. MBio 2018; 9: e00221-18.

56) Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020; 30: 269-271.

57) Williamson BN, Feldmann F, Schwarz B, Meade-White K, Porter DP, Schulz J, Doremalen N van, Leighton I, Yinda CK, Pérez-Pérez L, Okumura A, Lovaglio J, Hanley PW, Saturday G, Bosio CM, Anzick S, Barbian K, Cihlar T, Martens C, Scott DP, Munster VJ, Wit E de. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 2020; 585: 273-276.

58) Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD,

Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the treatment of Covid-19 - Final Report. N Engl J Med 2020; 383: 1813-1826.

59) WHO Solidarity Trial Consortium, Pan H, Peto R, Henao-Restrepo AM, Preziosi MP, Sathiyamoorthy V, Abdool Karim Q, Alejandria MM, Hernández García C, Kieny MP, Malekzadeh R, Murthy S, Reddy KS, Roses Periago M, Abi Hanna P, Ader F, Al-Bader AM, Alhasawi A, Allum E, Alotaibi A, Alvarez-Moreno CA, Appadoo S, Asiri A, Aukrust P, Barratt-Due A, Bellani S, Branca M, Cappel-Porter HBC, Cerrato N, Chow TS, Como N, Eustace J, García PJ, Godbole S, Gotuzzo E, Griskevicius L, Hamra R, Hassan M, Hassany M, Hutton D, Irmansyah I, Jancoriene L, Kirwan J, Kumar S, Lennon P, Lopardo G, Lydon P, Magrini N, Maguire T, Manevska S, Manuel O, McGinty S, Medina MT, Mesa Rubio ML, Miranda-Montoya MC, Nel J, Nunes EP, Perola M, Portolés A, Rasmin MR, Raza A, Rees H, Reges PPS, Rogers CA, Salami K, Salvadori MI, Sinani N, Sterne JAC, Stevanovikj M, Tacconelli E, Tikkinen KAO, Trelle S, Zaid H, Røttingen JA, Swaminathan S. Repurposed antiviral drugs for Covid-19 - Interim WHO solidarity trial results N Engl J Med 2021; 384: 497-511.

60) Ayerdi O, Puerta T, Clavo P, Vera M, Ballesteros J, Fuentes ME, Estrada V, Rodríguez C, Del Romero J. Preventive efficacy of tenofovir/emtricitabine against SARS-CoV-2 among PREP users. Open Forum Infect Dis 2020; 7: ofaa455.

61) Del Amo J, Polo R, Moreno S, Díaz A, Martínez E, Arribas JR, Jarrín I, Hernán MA. Incidence and Severity of COVID-19 in HIV-Positive persons receiving antiretroviral therapy : a cohort study. Ann Intern Med 2020; 173: 536-541.

62) Sampath R, Zeuli J, Rizza S, Temesgen Z. Tenofovir alafenamide fumarate for the treatment of HIV infection. Drugs of Today 2016; 52: 617-625.

63) Saravolatz LD, Saag MS. Emtricitabine, a new antiretroviral agent with activity against HIV and Hepatitis B Virus. Clin Infect Dis 2006; 42: 126-131.

64) Perry CM, Faulds D. Lamivudine: A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection. Drugs 1997; 53: 657-680.

65) Wu J, Li W, Shi X, Chen Z, Jiang B, Liu J, Wang D, Liu C, Meng Y, Cui L, Yu J, Cao H, Li L. Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). J Intern Med 2020; 288: 128-138.