RESEARCH ARTICLE

REVISED Identification of potential inhibitors of SARS-CoV-2 S protein–ACE2 interaction by *in silico* drug repurposing [version 2; peer review: 2 approved]

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v2 **First published:** 07 May 2021, **10**:358
https://doi.org/10.12688/f1000research.52168.1
Latest published: 11 Nov 2021, **10**:358
https://doi.org/10.12688/f1000research.52168.2

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new coronavirus discovered that appeared in Wuhan, China, in December 2019, causes COVID-19 disease which have resulted in cases similar to SARS-atypical pneumonia. Worldwide, around 116 million cases and 2.57 million deaths are reported with new cases and increasing mortality every day. To date, there is no specific commercial treatment to control the infection. Repurpose drugs targeting the angiotensin-converting enzyme 2 (ACE2) receptor represents an alternative strategy to block the binding of SARS-CoV-2 protein S and forestall virus adhesion, internalization, and replication in the host cell.
Methods: We performed a rigid molecular docking using the receptor binding domain of the S1 subunit of S protein (RBD $_{S1}$)-ACE2 (PDB ID: 6VW1) interaction site and 1,283 drugs FDA approved. The docking score, frequency of the drug in receptor site, and interactions at the binding site residues were used as analyzing criteria.
Results: This research yielded 40 drugs identified as a potential inhibitor of RBD $_{S1}$-ACE2 interaction. Among the inhibitors, compounds such as ipratropium, formoterol, and fexofenadine can be found. Specialists employ these drugs as therapies to treat chronic obstructive pulmonary disease, asthma and virtually any respiratory infection.
Conclusions: Our results will serve as the basis for *in vitro* and *in vivo* studies to evaluate the potential use of those drugs to generate

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 2 (revision) 11 Nov 2021	✓ report	✓ report
version 1 07 May 2021	? report	? report

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affordable and convenient therapies to treat COVID-19.

Keywords
COVID-19, SARS-CoV-2, ACE2, Molecular Docking, Drug Repurposing

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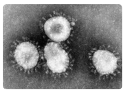

This article is included in the Coronavirus collection.

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Author roles: Tristán-Flores FE: Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Casique-Aguirre D**: Conceptualization, Formal Analysis, Investigation, Writing – Review & Editing; **Pliego-Arreaga R**: Investigation, Writing – Review & Editing; **Cervantes-Montelongo JA**: Investigation, Writing – Review & Editing; **García-Gutierrez P**: Conceptualization, Writing – Review & Editing; **Acosta-García G**: Resources, Writing – Review & Editing; **Silva-Martínez GA**: Conceptualization, Formal Analysis, Funding Acquisition, Project Administration, Resources, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Work supported by Consejo Nacional de Ciencia y Tecnología (CONACYT), "APOYO PARA PROYECTOS DE INVESTIGACIÓN CIENTÍFICA, DESARROLLO TECNOLÓGICO E INNOVACIÓN EN SALUD ANTE LA CONTINGENCIA POR COVID-19" grant no. C-295/2020 to GAS-M. DC-A is a post-doctoral researcher supported with a fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT), México (grant no. Estancias Posdoctorales por México – Estancia posdoctoral de incidencia 1er Año 2020 - 1)
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Tristán-Flores FE, Casique-Aguirre D, Pliego-Arreaga R *et al.* **Identification of potential inhibitors of SARS-CoV-2 S protein–ACE2 interaction by *in silico* drug repurposing [version 2; peer review: 2 approved]** F1000Research 2021, **10**:358 https://doi.org/10.12688/f1000research.52168.2

First published: 07 May 2021, **10**:358 https://doi.org/10.12688/f1000research.52168.1

REVISED **Amendments from Version 1**

Edits to the manuscript focus on an improved and better organization on the abstract, methods and discussion sections, accordingly to reviewers suggestions. New references were cited on manuscript to support Introduction and Discussion sections in order to addresses the reviewers comments.

Any further responses from the reviewers can be found at the end of the article

Introduction

Emerging viruses can be defined as those whose incidence has increased in the last twenty years or whose presence has a high probability of increasing in the near future. Diseases caused by emerging viruses are one of the biggest public health threats globally[1]. Some of the viruses that fall within this catalog are the avian influenza virus subtype H5N1, severe acute respiratory syndrome (SARS), Ebola, Zika, and MERS-CoV, to name a few[2]. Coronaviruses (CoVs) are classified into four genera, α-CoV, β-CoV, γ-CoV, and δ-CoV2. α and β infect mammals, γ birds and δ birds and mammals, respectively[3]. These viruses are of public health importance because they cause enteric, renal, and neurological respiratory diseases that range from asymptomatic to fatal[4,5].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appeared in Wuhan, China, in December 2019, causing cases of SARS-like atypical pneumonia[6,7], with a clinical picture of fever, general malaise, dry cough, shortness of breath and was called the coronavirus disease 2019 (COVID-19)[8]. It can be asymptomatic, develop mild-to-severe symptoms, or may cause death in patients with chronic diseases, such as hypertension, diabetes, and obesity[9]. On January 31st 2020, the World Health Organization (WHO) declared COVID-19 a public health emergency of international concern and, on March 12th, it was declared a global pandemic[10]. In Mexico, local transmission (phase 2 of transmission) was declared on March 24th 2020, which resulted in the suspension of non-essential activities in the country, generating economic losses in addition to public health problems and deaths associated with the disease. As of March 1st 2021, Mexico had reached 2.1 million cases of COVID-19 and 186 thousand deaths; around 116 million cases and 2.57 million deaths have been reported worldwide.

To date, there is no specific commercial treatment to control the infection[11–13]. Measures such as early detection, blocking the route of transmission through social isolation, isolation of suspected cases, disinfection of objects, as well as frequent hand washing with soap, in addition to the use of biosafety equipment such as surgical masks for health personnel, may reduce the transmission of COVID-19 among population.

Coronaviruses, such as SARS-CoV-2, are positive-stranded RNA viruses enveloped on a membrane. The coronaviral genome is composed of approximately 30,000 nucleotides containing the envelope (E), membrane (M), spike (S), nucleocapsid (N) and ORFs, that encode non-structural proteins, including enzymes that appear during their in-host reproductive cycle-genes[14].

This virus measures 70 to 100 nm and belongs to the genus β-CoV[15] and it has been proposed that any of the aforementioned proteins that make up CoVs may be targets for the development of vaccines or drugs[4]. Protein S plays an essential role on COVID-19 infection as it mediates the internalization on host cell and for the spread of the virus in the infected host[16,17]. This starts when the receptor binding domain of the S1 subunit (RBD_{S1}) of S protein binds to the peptidase domain of angiotensin-converting enzyme 2 receptor (ACE2)[18] and it is know that disrupting the binding of S protein to ACE2 prevents the attaching an the later internalization of the virus to the host cell[19].

This protein interaction has recently been crystallized and deposited in the Protein Data Bank database[20], allowing us to use it as a model of study to test different strategies to counter SARS-CoV-2 infection, like blocking S glycoprotein-ACE2 interaction through the discovery of sites of potential pharmaceutical interest.

In 2019, Research and Development (R&D) spending in the pharmaceutical industry totaled 186 billion U.S. dollars globally and its projected to reach 233 billion U.S. dollars to 2026. Unfortunately, drug development takes large time and financial resources that not all countries possess, especially developing countries, like Mexico.

In this sense, drug repurposing or repositioning allow us to integrate all evidence, pharmacodynamics/kinetics, bioavailability, among other important parameters, from an existing and approved drug in order to manage emerging diseases, like COVID-19. All this translates into a considerable decrease in research time and investment of resources in R&D[21].

Different approaches have been taken in order to disrupt SARS-CoV-2 protein S-ACE2 interaction, as an example, many works has focus on finding potential biding sites on protein S structure, however, new variant strains has been detected worldwide, like B117 in UK, P1351 in South Africa, P1 and P2 in Brazil. All variant strains display the N501Y mutation, which is located on the RBD of the S protein, making the interaction more effective. In this sense, targeting RBD may be a transitory approach, therefore, an alternative strategy would be aiming at the ACE2 receptor. Some authors have pointed out some concerns about using drugs that targeting the renin–angiotensin signaling (RAS) pathway[22–24], but Jia and collaborators[25] highlight current efforts of exploiting ACE2 as therapeutic target, like the use of pseudo-ligands to dominate the binding site for SARS-CoV-2 as an example. Therefore, inhibition of the SARS-CoV-2 protein S-ACE2 interaction trough aiming ACE2 receptor it is a plausible strategy. In this study, we screened a library consisting of 1,283 FDA-approved drugs and acquired by Ministry of Health of Mexico in order to identify potential inhibitors of SARS-CoV-2–ACE2 interaction.

Methods

Molecular modeling, electric partial-charge assignation, ligand conformer, searching of potential binding sites, energy minimizations, visualization and docking were performed with Molecular Operating Environment package[26].

Ligand preparation

The chemical structure of 1,283 drugs that comprises the updated list of reference drugs, as well as the National Compendium of Health Supplies of Mexico (June 2020 update) were obtained from the DrugBank, ZINC15 and PubChem database in September 2020. In order to simulate ligand flexibility for our rigid docking simulations, we generated a set of low-energy conformer for each drug with *Conformer Import* tool, with an imposed conformational cut-off energy of 3 kcal/mol from minimum energy structure of each compound, calculated with the AMBER10-EHT force field. The resulted *in-house* molecular data base (mdb) contain multiple conformers for each molecule and were used for rigid docking simulation.

Protein selection for ligand docking

The X-ray crystal structure of SARS-CoV-2 RBD_{S1} in a complex with the ACE2 (PDB ID: 6VW1, resolution of 2.68 Å) was selected as a protein target for docking simulations. Importantly, this engineered structure is the first to presents all the functionally important epitopes in the SARS-CoV-2 receptor binding motif[20]. Potential binding sites in ACE2 near the interface region between the SARS-CoV-2 RBD_{S1} and ACE2 proteins, were identified with *Site Finder* tool. All crystallographic water and ligands molecules were removed from the system (chains B, E, F). Hydrogen atoms (*Protonate 3D* tool) and partial charges (*Potential Setup* tool) were added to ACE2 assuming pH equal to 7.0 and using the AMBER10-EHT force field, respectively. Before docking, the ACE2 protein structure was subjected to energy minimization using the same forcefield, in order to optimize atomic contacts. Docking simulations between the optimized ACE2 structure and each of the conformers contained in the *in-house* database, was carried out under the rigid-docking protocol. The docking parameters were set to take each ligand conformation as unique molecule, using the Alpha Triangle algorithm as placement method (at least 100 different orientations or poses on potential binding site) and further evaluation keeping the thirty best poses accordingly the London scoring function for binding affinity with a second refinement as a Rigid Receptor using Affinity dG algorithm keeping the ten best poses. The results were analyzed by docking score, frequency of the chemical compound as a stable conformation and the types of interactions at the binding site residues.

Results

Structural analysis of SARS-CoV-2 – ACE2 interaction

The structural analysis for the SARS-CoV-2 RBD_{S1} of the spike protein in a complex with the ACE2 (PDB ID: 6VW1; Figure 1A) revealing a potential site for ligand binding inside ACE2 structure (Table 1). The identified receptor site (Figure 1B) is proximal to the binding site of RBD_{S1} with a size of 86, therefore it can be used for simulating rigid molecular docking since receptor atoms are in an exposed region of the structure, which could be in favor of drug binding.

Virtual screening and molecular docking

An average of 78 conformations were generated for each ligand by Conformation Import MOE, generating 100,450 ligand conformations of the FDA approved and prescript drugs by the Mexican Public Health System.

The docking results were sorted and analyzed based on their S score, binding frequency which the drug binds to the receptor

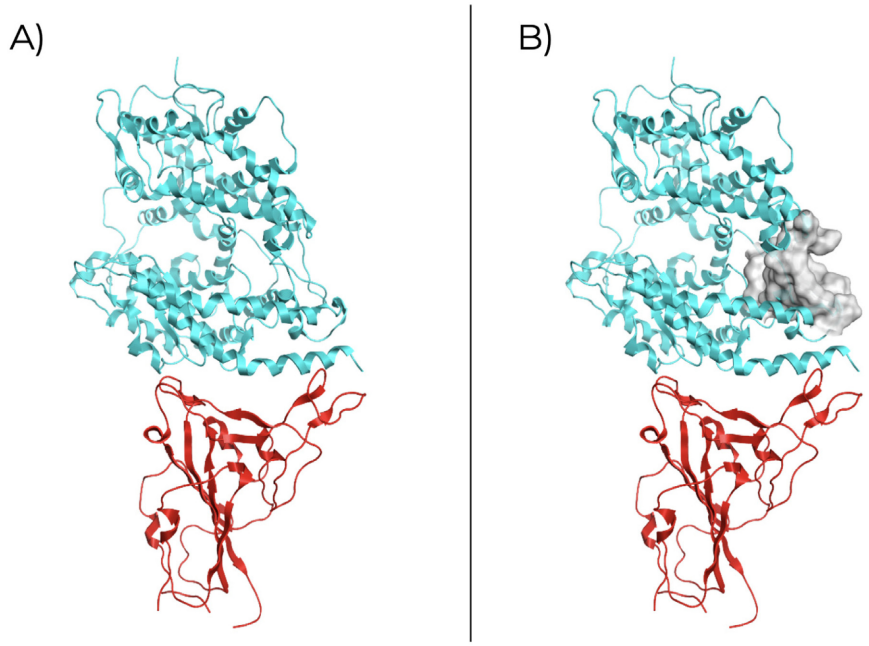

Figure 1. SARS-CoV-2 RBD_{S1} interaction with human ACE2 receptor. A) Crystallographic structure (PDB ID: 6VW1) of RBD_{S1}(red) and ACE2 receptor (blue); **B**) Molecular surface of the selected binding site in ACE2.

site and type of interactions, preferably, hydrogen bond, of the ligand with the selected site. We selected 38 drugs (Table 2) that presents the best docking score between −10.04 and −4.04.

Subsequently, we shortlisted nine drugs based on their risk of teratogenicity, route of administration, interaction with other drugs, side effects and by their background as pharmacological therapy for the treatment of respiratory diseases[27,28].

Table 1. General characteristics of RBD_{S1}-ACE2 receptor site.

Size.	PLB	Hyd.	Side	Residues
86	0.84	20	55	Gln81 Tyr83 Pro84 Leu85 Gln86 Leu95 Gln98 Ala99 Gln101 Gln102 Asn103 Ala193 Asn194 His195 Tyr196 Gly205 Asp206 Tyr207 Glu208 Asn210 Arg219 Lys562

Table 2. List of potential inhibitors of the RBD_{S1}-ACE2 interaction.

Drug name	S-Score	Interaction type (number)		
		Hydrogen bond	Ionic bond	Pi-bond
Uridine, trisodium salt	-9.53	10	3	0
Methotrexate sodium	-10.04	8	2	1
Raltritedex	-8.93	8	2	0
Folotyn	-8.19	8	2	0
CDP-choline(1-)	-8.10	8	0	0
Cefuroxime	-8.06	7	2	0
Fexofenadine	-7.89	7	0	0
Fludarabine phosphate	-7.99	6	0	1
Cefixime	-9.02	5	3	0
Aloin	-8.16	5	0	0
Domperidone	-6.63	5	1	0
Tamsulosin	-6.62	5	2	0
Cromoglycic acid	-8.63	4	0	1
Macitentan	-8.06	4	0	2
Tafluprost -Taflutan	-7.94	4	0	1
Thiopental(1-)	-5.42	4	0	0
Metoprolol	-5.10	4	0	0
Irinotecan	-8.72	3	0	1
Pitavastatin(1-)	-8.40	3	0	1
Amlodipine	-5.94	3	0	0
Verapamil	-5.85	3	1	0
Tolterodine	-4.77	3	0	0
Lopinavir	-8.62	2	0	0
Glimepiride	-8.47	2	1	0
Arformoterol	-6.85	2	0	0

Drug name	S-Score	Interaction type (number)		
		Hydrogen bond	Ionic bond	Pi-bond
Formoterol	-6.15	2	2	1
Ipratropium	-5.38	2	0	1
Pargeverine	-4.53	2	2	0
Pyrilamine	-4.26	2	1	0
Biperiden	-4.23	2	0	1
Orlistat	-8.16	1	0	0
Glyburide	-8.10	1	0	0
Ribociclib	-7.96	1	0	1
Ibesartan	-7.17	1	0	3
Cholecalciferol	-5.81	1	0	0
Testosterone enanthate and estradiol valerate	-5.39	1	0	0
Disopyramide phosphate	-4.22	1	0	2
Primaquine	-4.04	1	1	2

Fexofenadine showed interactions of hydrogen bond with Lys 74, Ala 99, Ser 105, Ser 106, Trp 203 and Asp 509 (Figure 2A) with a docking score of −7.89. Pitavastatin displays hydrogen bond interaction with Gln 102, Tyr 196, Asp 206 and pi-H stacking with Leu 73 (Figure 2B) and a docking score of −8.40. Arformoterol showed hydrogen bond interactions with Tyr 202 and Asp 206 (Figure 2C) with a docking score of −6.85. Formoterol presented hydrogen bond interactions with Gln 98, Asn 194, ionic interaction with Glu 208 and pi-H interaction with Leu 85 (Figure 2D) and presents a docking score of −6.15. Ipratropium exhibited hydrogen bond interaction with Gln 98, Gln 208 and pi-H stacking with Asp 206 (Figure 2E) and a docking score of −5.38. Pargeverine shows hydrogen bond interactions with Gln 98, Gly 205, Glu 208 and ionic interaction with Glu 208 (Figure 2F) and presents a docking score of −4.53. Cholecalciferol presented hydrogen bond interaction with Gln 102 (Figure 2G) and had a docking score of −5.81. Lopinavir displays hydrogen bond interaction with Gln 102, Tyr 196 (Figure 2H) and a docking score of −8.62. Cefixime showed hydrogen bond interaction with Gln 98, Tyr 202, Glu 208, Arg 219, Lys 562 and ionic interaction with Arg 219, Lys 562 (Figure 2I) and a docking score of −9.02.

Some pharmacokinetics characteristics[27,28] of the shortlisted potential inhibitors of the RBD_{S1}–ACE2 interaction are summarized in Table 3.

Discussion

It has been established that S protein of SARS-CoV-2 virus plays a major role during viral infection. The S protein mediates receptor recognition, cell attachment and fusion of viral membrane with host cell membrane[29–34]. The S protein binds to ACE2 receptor through the RBD_{S1}, mediating viral attachment to host cell[35]. Expression of ACE2 is ubiquitous in lung, intestine, heart and kidney, also alveolar epithelial type II cells had higher expression levels[23]. SARS-CoV-2, as one RNA viruses, has shown a high mutation rate as a result of lack of proofreading mechanisms, which leads to gain the ability to rapidly adapt to changes in their environment, which in turn leads to a great challenge for treating and preventing infections[36]. In this sense, the RBD region is a critical therapeutic target (vaccines and drugs) due to its indispensable function; however, it is suggested that mutations in this region may render pharmacological or immunological therapies ineffective[37,38], therefore, it is needed to search and design alternative treatments.

In order to block this event, we propose an *in silico* approach to identify potential inhibitors of the SARS-CoV-2 –ACE2 interaction aiming at the ACE2 receptor, blocking the virus accessibility to the membrane-bound ACE2. In this sense, Jia and collaborators[25] present an extensive review for this underexplored approach to treat COVID-19, pointing that it is imperative to determine, by clinicians, the stage of the disease and comorbidities that could prove consequential for an ACE2-targeting regimen. Here, we screened a drug library consisting of 1,283 drugs, FDA approved and prescribed by the Mexican Public Health System, for potential SARS-CoV-2–ACE2 inhibitors, using a rigid receptor docking approach. Utilization of an FDA-approved drug library is an effective and ideal tool for drug repurposing in antiviral research[39,40], such as zika virus[41], human rhinovirus[42] and hepatitis B virus[43]. We identify 38

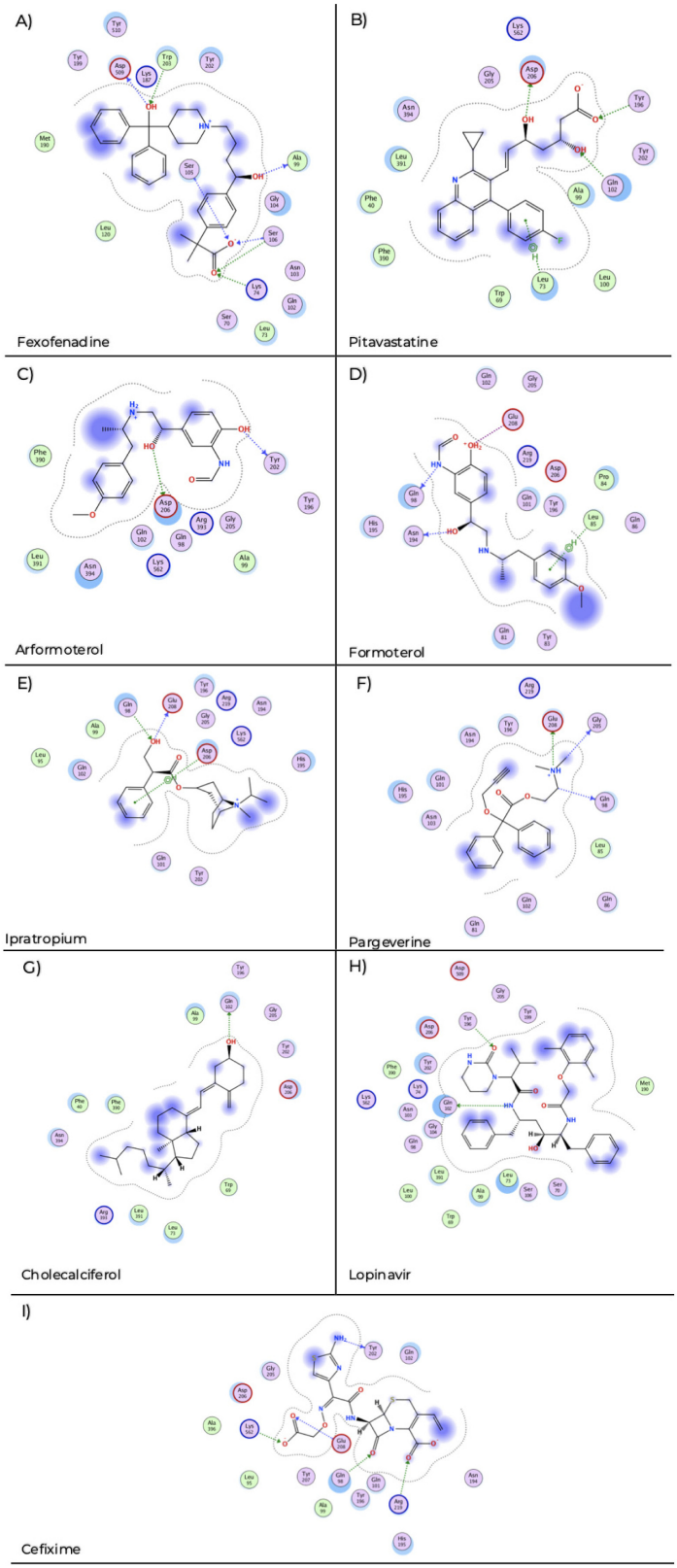

Figure 2. Two-dimensional representation of the interactions of the selected drugs with the ACE2 receptor binding site. The blue arrows indicate the structural hydrogen bridge bonds, and the green arrows are the hydrogen bridge bonds with the side chain. **A**) Fexofenadine, **B**) pitavastatine, **C**) aformoterol, **D**) formoterol, **E**) ipatropium, **F**) pargeverine, **G**) cholecalciferol, **H**) lopinavir and **I**) cefixime.

Table 3. Potential inhibitors of RBD_{S1}-ACE2 interaction selected according to desired characteristics.

Drug	Pharmacokinetics					Route of administration	Drug Type/ Teratogenic risk
	Bioavailability (%)	Protein binding (%)	Metabolism	Half-life (hours)	Excretion		
Fexofenadine	30-41	60-70	Hepatic	14.4	Urine and feces	Oral	Antihistaminic (third generation) /B
Cefixime	30-50	60	Hepatic	3-4	Urine and bile	Oral	Antibiotic (Cephalosporin; third generation)/ B
Pitavastatine	60	96	Hepatic	11	Feces	Oral	Statin /X
Lopinavir	25	98-99	Hepatic	2-3	Urine and feces	Oral	Antiviral -Protease inhibitor / C
Arformoterol	21-37	61-64	Hepatic	26	Urine	Inhaled	Long lasting β agonist/ C
Formoterol	61	50	Hepatic	17	Urine	Inhaled	Long lasting β agonist/ C
Ipratropium	2	0-9	Gastrointestinal	1.6	Urine	Inhaled	Anticholinergic bronchodilators / B
Pargeverine	80	90	Hepatic	1.5-2	Urine	Oral, intravenous, intramuscular, rectal	Opium alkaloid antispasmodic / D
Cholecalciferol	NA	NA	Hepatic	NA	Urine	Oral, intramuscular	Vitamin

potentially inhibitor drugs of SARS-CoV-2–ACE2 interaction and these are listed on Table 2. Several of those drugs were previously reported to be used for the treatment of respiratory diseases.

Within this list of potential inhibitors of the SARS-CoV-2–ACE2 interaction, is fexofenadine, a third generation antihistamine whose therapeutic indication is the treatment of symptoms of stationary allergies through the selective blockade of H1 receptors[44]. It possesses direct effect on combating the cytokine storm caused by SARS-CoV-2 through inhibition of histamine and interleukin-6 (IL-6) release. *In silico* evidence[45,46] suggest that it may interact with the SARS-CoV-2 main protease enzyme M^{Pro}, a key enzyme in viral replication[47], acting as a potential inhibitor.

Cefixime is a third-generation antibiotic derived from cephalosporin whose use is indicated for the treatment of infections in the upper and lower respiratory tract, otorhinolaryngological[48] and urinary tract[49], inhibiting the synthesis of the bacterial wall by binding to specific binding proteins for penicillin and is currently used as a secondary therapy to prevent opportunistic infections during the development of COVID-19[50].

Pitavastatin is a statin indicated for lowering blood cholesterol levels by inhibiting HMG-CoA reductase, preventing cholesterol synthesis[51], and it has also been observed that statin treatments can interfere with viral infectivity through inhibition of glycoprotein processing[52] also, they modulates the inflammatory process at cellular level[53], which is a remarkable characteristic of the SARS-CoV-2 infection. Additionally, *in silico* findings suggest that could be an efficient inhibitor of SARS-CoV-2 M^{Pro}[54] and SARS-CoV-2 RNA-dependent RNA polymerase (RdRp)[55] thru active site binding.

Lopinavir is a protease inhibitor indicated as first barrier therapy, in conjunction with Ritonavir, to treat infection caused by the HIV virus by inhibiting the HIV-1 protease[56] in addition, studies in cell cultures have shown its effectiveness as an inhibitor of the replication of the MERS-CoV virus[57] and SARS-CoV-1[58], while in severe cases of SARS-CoV-2 infection, the results of clinical trials indicate that it is not useful[59].

Formoterol and aformoterol, anenantiomer of formoterol, are long-lasting selective β agonists indicated for the treatment of chronic obstructive pulmonary disease (COPD) and bronchospasms[60], in the same way there is evidence of the

use of these drugs as a partial inhibitor of viral replication in primary epithelial cells cultures[61] and *in silico* data suggest their binding to the papain-like protease PL_{pro}, a coronavirus enzyme essential for viral spread[62].

Ipratropium is a bronchodilator anticholinergic indicated for the treatment of asthma, shortness of breath, cough and tightness in the chest in patients with COPD[63,64]. Inhalation therapy with ipratropium is currently in use to dilate bronchioles in COVID-19 patients to increase oxygen saturation levels (from <80% to 94%)[65].

Pargeverine is an antispasmodic opioid alkaloid whose therapeutic indication is aimed at the treatment of painful spasms[66], also, acts as anticholinergic and has a moderate and non-selective blockade of muscarinic cholinergic fibers[67]. Since cholinergic activity contribute to airway narrowing, this might be a potential agent to open airway obstruction.

Cholecalciferol, is a form of vitamin D (vitamin D3) that can be synthesized naturally in the skin and acts as a hormonal precursor, being converted into calcitriol, and therapeutically is used as a vitamin supplement to treat deficiencies of this vitamin[68]. In addition, it has been observed that vitamin D supplementation is favorable to reduce viral infections such as influenza[69,70] or more aggressive cases such as HIV[71] and it has recently been suggested that it also presents favorable effects before and during the infection caused by SARS-CoV-2[72].

Likewise, it is important to take into account that ACE2 plays an important biological role since regulates cardiovascular functions and innate immune system[73] and, therefore cautions must be taken. Another point to consider is the delivery method of the drug, since the primary target must be smooth muscle, like the one surrounding the bronchioles, and lung epithelial cells in the airway and airspace compartments, hence, inhalable delivery would be the acceptable choice to deliver the drug in a selectively and localized manner.

Given these characteristics, the results obtained through our *in silico* approach, we consider that the aforementioned drugs are outlined as possible inhibitors of the RBD_{S1}-ACE2 interaction. These drugs are well tolerated, commonly used and affordable, hence, most of the drugs on this list can be tested *in vitro*, and even *in vivo* and, consequently, in clinical trials for the development of adjuvant therapies to treat COVID-19.

Conclusion

In the absence of approved therapies for treatment or prevention, drug repurposing has provided fast and valuable insight into the treatment of COVID-19. Targeting ACE2 receptor as a COVID-19 therapy it is a conceivable approach since it is essential for the viral internalization. However, this approach requires an integrative evaluation of the pros and cons by a clinical context since ACE2 is a multifunctional protein. Several drugs are currently investigated by clinical trials or are already in use to treat COVID-19 patients, like lopinavir or ipratropium. In this *in silico* study using structure-bases virtual screening, we identified potential inhibitors of SARS-CoV-2–ACE2 by their interaction with ACE2 receptor. Based on desired characteristics like pharmacokinetics, route of administration or by their background as pharmacological therapy, we propose a shortlist of drugs suitable for testing their potential RBD_{S1} –ACE2 inhibitory activity: fexofenadine, cefixime, pitavastatine, lopinavir, arformoterol, formoterol, ipratropium, pargeverine and cholecalciferol. Our identification of potential inhibitors of the SARS-CoV-2–ACE2 interaction among commonly use drugs highlights their potential use for treating COVID-19. Further *in vitro, in vivo* or clinical trial are needed to validate their potential use as inhibitors of SARS-CoV-2–ACE2 interaction.

Data availability

Source data

Protein Data Bank: Crystal structure of SARS-CoV-2 receptor binding domain (RBD_{S1}) of the spike protein in a complex with the ACE2 receptor. https://identifiers.org/pdb:6vw1.

Ministry of Health of Mexico: Drug list used by the Ministry of Health of Mexico (June 2020 version). http://www.csg.gob.mx/Compendio/CNIS/cnis.html.

PubChem: Ligands. https://pubchem.ncbi.nlm.nih.gov[74].

Drugbank: Ligands. https://go.drugbank.com[75].

ZINC database: Ligands. http://zinc.docking.org[76].

Extended data

Figshare: Identification of potential inhibitors of SARS-CoV-2 S protein–ACE2 interaction by in silico drug repurposing, https://doi.org/10.6084/m9.figshare.14466333[77]

This project contains the underlying data file:

- Table_E1_DrugsAccessionNumber.xlsx (Accession numbers of drugs used for docking simulations)

Author contributions

FET-F and DC-A performed most of the bioinformatics work, shared first authorship, equal contribution to this work; FET-F wrote the initial manuscript draft; JAC-M and RP-A generated the ligand data base and assisted bioinformatic analysis; GA-G revising critically the initial manuscript draft and provision facility resources; P.G.-G, DC-A and GAS-M contributed intellectually to the project conceptualization and participate in the initial manuscript draft; DC-A and G.A.S.-M designed and supervised the project. G.A.S.-M conceptualize, acquire the financial support for the project leading to this publication, supervised the bioinformatic work and wrote the final version of the manuscript; all authors read and approved the final manuscript version.

Acknowledgements

The authors would like to thank Ruy E. Pineda-Silva for the supporting work in data acquisition; Martha C. Silva-Martinez, MD. and Omar Pineda-Gama, MD. for advice, assistance, and their valuable feedback.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 26 November 2021

https://doi.org/10.5256/f1000research.78910.r99909

✔ **Fernando Yepes-Calderon**
GYM Group SA, Cali, Colombia

No further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical devices development, Medical Imaging, Methods development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 November 2021

https://doi.org/10.5256/f1000research.78910.r99910

✔ **Yogendra Nayak**
Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, India

The revision of the manuscript by the authors looks satisfactory to index the article.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacy and Pharmacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 21 September 2021

https://doi.org/10.5256/f1000research.55408.r93444

?

Yogendra Nayak
Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, India
Krishnaprasad Baby
Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, India

I am pleased to write the review for this manuscript. In this manuscript, authors have used computational tools to predict the activity of a suitable drug to treat/ prevent COVID-19. The specific target can prevent the entry of SARS-CoV-2 in human body. Thus keeping the receptor for virus entry ACE2, the available drugs are screened for their binding efficacy, which indirectly correlates the biological activity. Though the computational predictions are widely accepted, this manuscript has few limitations in its current form. Hence, I would suggest minor changes or amendments might be necessary before acceptance. Followings are a few of them:

Abstract:

- As the pandemic is dynamically changing the numbers, giving a country specific might not hold good. I suggest to add the data related to the world-wide data can be put.
  An important observation is the number of drugs from US-FDA can be written the same number as it was mentioned later in the manuscript (1,283 may not be correct).

Introduction:

- I feel many places few more additional support from the literature is required. Which can be strengthened by citing references.

Methods:

- Molecule selection for docking appears incorrect. It is protein selection for ligand docking. Moreover, authors have optimised the protein structure, which should have been more appropriate. The resolution of 6VW1 is missing. The rationale for taking 6VW1 appears missing in methodology.
  Database preparation appears incorrect. It can be dataset/ or ligand preparation. When DrugBank/ZINC15 was accessed is missing (month of accession). Which version of ZINC library was used is missing. Most importantly, the reason why ligands conformation was generated is missing.

Results:
- Table 3, the details regarding generation of data appears missing. I feel the detailed legends can be given.

- The current gold standard for molecular activity prediction is MD simulation, this can be tried by authors.

Discussion:
- In the methodology the number of drugs selected were 1283, but here it changed to 1300. This looks inconsistent.

- In the methodology, authors have mentioned that they have generated some conformers. Which conformation of drugs were active?

- Overall, I feel the discussion part appears very weak and lacks substantial evidence for how these drugs could be used in COVID-19.

Conclusions:
- The conclusion can be made simple by reducing the number of sentences. Most of the journals will not accept citations (references) in conclusions. Hence, the authors can give a thought to modify the conclusion by keeping their views only on objectives/ aims.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacy and Pharmacology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 29 Oct 2021

Guillermo A Silva-Martinez, Tecnologico Nacional de Mexico en Celaya, Celaya, Mexico

We thank the reviewer for the detailed revision of our paper and for suggesting improvements.

Abstract:

- *As the pandemic is dynamically changing the numbers, giving a country specific might not hold good. I suggest to add the data related to the world-wide data can be put.*

- *An important observation is the number of drugs from US-FDA can be written the same number as it was mentioned later in the manuscript (1,283 may not be correct).*

Response: We thank the reviewer for this suggestion to improve the impact of our abstract. US-FDA approved drug number was corrected.

Introduction:

- *I feel many places few more additional support from the literature is required. Which can be strengthened by citing references.*

Response: We agree with the reviewer's observation, however, we believe that the introduction provides the necessary information to be in context to the overall objective of our work.

Methods:

- *Molecule selection for docking appears incorrect. It is protein selection for ligand docking. Moreover, authors have optimized the protein structure, which should have been more appropriate. The resolution of 6VW1 is missing. The rationale for taking 6VW1 appears missing in methodology.*
  Database preparation appears incorrect. It can be dataset/ or ligand preparation. When DrugBank/ZINC15 was accessed is missing (month of accession). Which version of ZINC library was used is missing. Most importantly, the reason why ligands conformation was generated is missing.

  Response: We thank the reviewer for the comments and for suggesting improvements. Changes on the subsections mentioned above were made. Resolution of 6VW1, the rationale of using that structure and accession date to databases were added to the manuscript. In the "Protein selection for ligand docking", it is now clearly specified that rigid molecular docking is carrying out. In this type of docking, protein orientation is fixed and only ligands are allowed to vary their orientation, therefore each conformer allow to simulate this variation. We assumed that no further explanation was needed.

Results:

- *Table 3, the details regarding generation of data appears missing. I feel the detailed legends can be given.*

  Response: We shortlisted nine drugs based on their risk of teratogenicity, route of administration, interaction with other drugs, side effects and by their background as pharmacological therapy for the treatment of respiratory diseases. It is described in the Results section, subsection Virtual Screening and

Molecular docking, paragraph 3.

- *The current gold standard for molecular activity prediction is MD simulation, this can be tried by authors.*

Response: We agree with the reviewer comment. Unfortunately, we do not have the computational infrastructure or access to supercomputer cluster to run MD. We are trying to run MD on our workstations, but those simulations will take time. However, we feel that our work is a valid approximation for COVID19 drug repurposing.

Discussion:

- *In the methodology the number of drugs selected where 1283, but here it changed to 1300. this looks in consistency.*

Response: We thank reviewer comment. See response above.

- *In the methodology, authors have mentioned that they have generated some conformers. Which conformation of drugs were active?*

Response: In order to generate active conformers for docking simulation, we impose a 3 kcal/mol limit on strain energy, which means that conformation elucidates under these criteria can occur under physiological conditions and maintain their activity. Methodology section was restructured to be more clear.

- *Overall, I feel the discussion part appears very weak and lacks substantial evidence for how these drugs could be used in COVID-19.*

Response: We thank the reviewer for this comment. We feel that most of the relevant evidence -to date of manuscript submission- regarding COVID19 and the potential inhibitors, were discussed.

Conclusions:

- *The conclusion can be made simple by reducing the number of sentences. Most of the journals will not accept citations (references) in conclusions. Hence, the authors can give a thought to modify the conclusion by keeping their views only on objectives/ aims.*

Response: We thank the reviewer for this observation. This section was restructured.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 June 2021

https://doi.org/10.5256/f1000research.55408.r85900

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Fernando Yepes-Calderon
GYM Group SA, Cali, Colombia

Authors use molecular docking tools to test existing medicine in their efficacy to impede the S protein-ACE2 binding and thus, disabling the entry point of Cov2 to human cells.
The authors use a solid justification to support this project. Since medicine creation is an expensive and long-lasting task, verifying if some active molecules in already FDA-approved medicine might block the binding between the S-protein and the ACE2 is an excellent alternate path.

The document is generally well organized and pleasant to read. I suggest adding some corrections to the abstract as follows.

Background
A new coronavirus outbreak, firstly reported in Wuhan, China, in December 2019, causes COVID-19, which symptoms are similar to SARS-atypical pneumonia. Worldwide, around 116 million cases and 2.57 million deaths are reported, with new cases increasing mortality every day. To date, there is no specific commercial treatment to control the infection.

Repurpose drugs targeting the angiotensin-converting enzyme 2 (ACE2) receptor represent an alternative strategy to block the binding of CoV2's protein S and forestall virus adhesion, internalization, and replication in the host cell.

Methods
We performed rigid molecular docking using the receptor-binding domain at the S1 subunit of the S protein (RBDS1)-ACE2 (PDB ID:6VW1) interaction site and 1,283 FDA-approved drugs. The docking score, frequency of the drug in the receptor site, and interactions at the binding site residues were used as analyzing criteria.

Results
This research yielded 40 drugs labeled as potential inhibitors of RBD S1-ACE2 interaction. Among the inhibitors, compounds such as ipratropium, formoterol, and fexofenadine can be found. Specialists employ these drugs to treat chronic obstructive pulmonary diseases, asthma, and almost any respiratory infection.
Conclusions: Our results will serve as the basis for *in vitro* and *in vivo* studies to evaluate the potential use of those drugs to generate
affordable and convenient therapies to treat COVID-19.

The proposed modifications focus on keeping the communications global. The CoV2 numbers in Mexico are absent in this suggested abstract since the authors provide worldwide numbers. I also shortened some sentences and fixed some wrongly used singulars.

1. Correct the hyphenation in the whole document. If the authors are using a latex template, use the command \hyphenation{co-rrect, se-pa-ra-tion, sche-me}.

Events: Janu-ary and associ-ated (intro, paragraph 2), lig-and (methods, paragraph 2).

2. Introduction, page 3, paragraph 2. Current events suggest that Covid19 is not only lethal in the elder or patients with chronic diseases. I might suggest deleting this statement.

3. Introduction, page 3, paragraph 2. something is missing in this paragraph. Also, the statement may be misleading; the actual spread and high contamination levels are proof of non-efficiency. If isolation and the other measurements were effective, what would be the sense for the proposal?

4. In subsection "Database preparation," you have information already provided in the first subsection of the methods.

5. Discussion, page 7, paragraph 1. In "ACE2 expression it is" the authors might have two nouns in the same sentence.

Regarding the methodology, the authors used a standard: importing the macromolecule, edit or delete water molecules, add hydrogens, add charges, and the ligand. Did the authors have any particular issue during the process? In case affirmative, add it to assert reproducibility.

The list of potential inhibitors is highly appreciated. The authors presented the interactions of the selected drugs nicely.

I suggest the authors reinforcing their discussion with arguments derived from the mutation capacity of the retrovirus that might render current vaccination not entirely compelling. Therefore, the presented alternatives could set as plausible options for treatment.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical devices development, Medical Imaging, Methods development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 29 Oct 2021

Guillermo A Silva-Martinez, Tecnologico Nacional de Mexico en Celaya, Celaya, Mexico

We thank you for the detailed revision of our paper and for suggesting improvements. Also, we want to apologize for the long delay on our response, we were waiting for a second reviewer report to edit our manuscript.

Authors use molecular docking tools to test existing medicine in their efficacy to impede the S protein-ACE2 binding and thus, disabling the entry point of Cov2 to human cells.
The authors use a solid justification to support this project. Since medicine creation is an expensive and long-lasting task, verifying if some active molecules in already FDA-approved medicine might block the binding between the S-protein and the ACE2 is an excellent alternate path.

The document is generally well organized and pleasant to read. I suggest adding some corrections to the abstract as follows.

Response: We thank the reviewer for the detailed revision of our paper and for suggesting improvements. We are pleased that you found the structure of the writing to be pleasant.

Background
A new coronavirus outbreak, firstly reported in Wuhan, China, in December 2019, causes COVID-19, which symptoms are similar to SARS-atypical pneumonia. Worldwide, around 116 million cases and 2.57 million deaths are reported, with new cases increasing mortality every day. To date, there is no specific commercial treatment to control the infection.

Repurpose drugs targeting the angiotensin-converting enzyme 2 (ACE2) receptor represent an alternative strategy to block the binding of CoV2's protein S and forestall virus adhesion, internalization, and replication in the host cell.

Methods
We performed rigid molecular docking using the receptor-binding domain at the S1 subunit of the S protein (RBDS1)-ACE2 (PDB ID:6VW1) interaction site and 1,283 FDA-approved drugs. The docking score, frequency of the drug in the receptor site, and interactions at the binding site residues were used as analyzing criteria.

Results
This research yielded 40 drugs labeled as potential inhibitors of RBD S1-ACE2 interaction. Among the inhibitors, compounds such as ipratropium, formoterol, and fexofenadine can be found. Specialists employ these drugs to treat chronic obstructive pulmonary diseases, asthma, and almost any respiratory infection.

Conclusions: Our results will serve as the basis for in vitro and in vivo studies to evaluate the potential use of those drugs to generate affordable and convenient therapies to treat COVID-19.

- **Response: Taking into account your contribution, in the manuscript the abstract has been improved and better organized.**

The proposed modifications focus on keeping the communications global. The CoV2 numbers in Mexico are absent in this suggested abstract since the authors provide worldwide numbers. I also shortened some sentences and fixed some wrongly used singulars.

1. *Correct the hyphenation in the whole document. If the authors are using a latex template, use the command \hyphenation{co-rrect, se-pa-ra-tion, sche-me}.*
   Events: Janu-ary and associ-ated (intro, paragraph 2), lig-and (methods, paragraph 2).

   Response: We thank the reviewer for this observation. The hyphenation has been corrected in the whole manuscript.

2. *Introduction, page 3, paragraph 2. Current events suggest that Covid19 is not only lethal in the elder or patients with chronic diseases. I might suggest deleting this statement*.

   Response: We agree with the reviewer's observation, however it's important to remark that adult individuals (over 40 years) and patients with comorbidities are prone to death by COVID 19 disease. This paragraph was restructured.

3. *Introduction, page 3, paragraph 2. something is missing in this paragraph. Also, the statement may be misleading; the actual spread and high contamination levels are proof of non-efficiency. If isolation and the other measurements were effective, what would be the sense for the proposal?*

   Response: We thank the reviewer for this comment. This paragraph was rewritten.

4. *In subsection "Database preparation," you have information already provided in the first subsection of the methods.*

   Response: We thank to reviewer for this comment. Changes were made in this section.

5. *Discussion, page 7, paragraph 1. In "ACE2 expression it is" the authors might have two nouns in the same sentence.*

   Response: We thank the reviewer for this comment. Changes were made in this section.

Regarding the methodology, the authors used a standard: importing the macromolecule, edit or delete water molecules, add hydrogens, add charges, and the ligand. Did the authors have any particular issue during the process? In case affirmative, add it to assert reproducibility.

Response: We do not have any issue during the structure preparation; therefore, reproducibility can be achieved without any problem.

The list of potential inhibitors is highly appreciated. The authors presented the interactions of the selected drugs nicely.

Response: We appreciate that the reviewer finds our data well-presented.

I suggest the authors reinforcing their discussion with arguments derived from the mutation capacity of the retrovirus that might render current vaccination not entirely compelling. Therefore, the presented alternatives could set as plausible options for treatment.

Response: We thank the reviewer for suggesting improvements to our discussion section. Reinforced of the discussion section were made in the manuscript taking reviewers suggestion.

Competing Interests: No competing interests were disclosed.