Are losartan and Imatinib Effective Against SARS-CoV2 Pathogenesis? 
A Pathophysiologic-Based in Silico Study

Reza Nejat1*, Ahmad Shahir Sadr2,3,4,5*

1Former Assistant Professor, Department of Anesthesiology and Critical Care Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2Bioinformatics Research Center, Cheragh Medical Institute and Hospital, Kabul, Afghanistan
3Department of Computer Science, Faculty of Mathematical Sciences, Shahid Beheshti University, Tehran, Iran
4Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran
5School of Biological Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran

*. indicate equal senior authors.

WARNING: As to the nature of in silico modeling studies which are a sort of theoretical swift investigation to determine the best way to start in vivo investigations negating the need for cumbersome time- and budget-consuming other sorts of in vitro studies, we declare that this study does not mean that the proposed drugs are certainly effective in the treatment of COVID 19. Unless robust subclinical studies are conducted all the conclusions might be assumptive.

Abstract

Introduction: A novel virus called SARS-CoV2, of Coronaviridae family, has distributed all around the globe since December in 2019. COVID 19 the disease caused by this virus results in an atypical pneumonia which in some patients eventuates in acute respiratory distress syndrome (ARDS) with high morbidity and mortality. ARDS in COVID 19 has been linked to a sort of cytokine storm inexplicable only through activation of an immunopathological response to the virus but might probably be rather due to a hyperacute destructive surge of angiotensin II. In this context, losartan, an angiotensin receptor blockers (ARBs), has been proposed as a drug with the ability to restore the balance in ACE2/angiotensin (1-7)/Mas and ACE/Ang II/AT1R pathways in tissues to ameliorate lung inflammation. Besides, imatinib, a tyrosine kinase inhibitor, studied priorly in SARS and MERS may be suggested to block the fusion of the virus with the host cell or modulate the immunologic dysregulation in ARDS. In this article we propose a novel insight to the probable pathophysiology of cytokine storm in COVID19 and then we report the results of our in Silico theoretical study on the effects of losartan and imatinib against the virus pathogenesis.

Method: The required protein structures were obtained from Protein Data Bank, the structures were evaluated and purified selectively to achieve the most desirable structure.
The required drugs and small molecules were fully optimized for geometry, electrical and structural properties. Docking study was performed by AutoDock 4 to find the suitable orientation of the molecules in the active site of the protein structures. MD simulations of the protein–small molecule complexes following docking processes were performed with the GROMACS 2018 package. Through redocking, the binding energies and the binding status of the small molecules with the protein structures were evaluated. After performing MD simulation, we analyzed exported RMSD, RMSF, hydrogen bonding and Radius of gyration diagrams.

**Results:** According to our bioinformatic study losartan and imatinib could occupy and distort the binding of RBD with ACE2 through changing the conformational shape of its N-terminal α helix where RBD binds. This results in reduction of the affinity of the virus for ACE2. In addition, losartan and imatinib could pose in the structure of main and papin-like proteases as well as in p38MAPK with high affinity and may affect the behavior of these proteins. Losartan due to disturbing of papin-like protease structure may change the function of this protease with high probability, yet it loses its affinity to this protein to some degree. Binding of furin to imatinib revealed to be of high affinity. Imatinib may prevent S’2 cleavage through inhibiting furin. Losartan due to disturbing of papin-like protease structure may change the function of this protease with high probability. Losartan, vs other ARBs had higher affinity to bind with the protein structures.

**Conclusion:** Implied from reviewing the literature, the proposed novel theory about the pathophysiology of ARDS in COVID19 in this article seems to be legitimate. Considering this theory with our knowledge of losartan and imatinib pharmacokinetic and pharmacodynamic characterizations, and according to the bioinformatic data in our modeling study, it might be concluded that both losartan and imatinib are effective, yet to be approved, in alleviating ARDS in COVID 19 through:

1- their immunomodulatory effects,
2- (in case of losartan) restoring of the balance in physiologic responses in ACE2/angiotensin (1-7)/Mas and ACE/Ang II/AT1R pathways by reducing AT1R-activated downstream cascade in the context of Ang II excess,
3- probable changing the conformational structure of ACE2 and consequently lowering the ability and affinity of the virus to bind to ACE2,
4- (in case of imatinib) probable inhibiting of priming of S protein with furin,
5- probable inhibiting main and papin-like proteases,
6- probable inhibiting p38MAPK and the downstream inflammatory responses,

In this study the effects of other small molecules, such as cholecalciferol and some herbal extracts in few aspects of the virus life cycle were also evaluated. All the results are theoretical and must be validated in subclinical and clinical studies.

**KeyWords:** SARS-CoV2, losartan, imatinib, Ang II, ACE2, FURIN, TMPRSS2, papain-like protease, cytokine storm, ARDS, clinical bioinformatics, in silico study, IFN, cholecalciferol, herbal extracts

**Abbreviation:** ACE: angiotensin converting enzyme; ACE2: type 2 angiotensin converting enzyme; AT1R: angiotensin II type 1 receptor; AT2R: angiotensin II type 2 receptor; Ang II: angiotensin II; Im,nACE2: new ACE2 new ACE2 under the influence of imatinib after 100ns MD simulation; Lo,nACE2: new ACE2 under the influence of losartan after 100ns MD simulation; Mpro: Main protease; Pre-inh: predefined inhibitor in data bank; PLpro: papain-like protease; PRR: pattern recognition receptor
Introduction

Since December 2019, a viral disease called COVID-19 has hunted thousands of people savagely all around the world and the death toll increases in a skyrocketing manner every day. It was elucidated through a breathtaking study that the disease was caused by a coronavirus named 2019-nCoV. Thereafter this virus was named SARS-CoV2 and the disease, COVID 19. Coronavirus are classified in Coronavirinae subfamily, the family Coronaviridae and the order Nidovirales. The subfamily Coronaviridae is composed of four genera: Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Deltacoronavirus (δCoV), and Gammacoronavirus (γCoV). SARS-CoV2 belongs to β-genus. Other members of β-genus of this family, SARS-CoV and MERS-CoV, are the causes of severe acute respiratory syndrome (SARS) and the Middle East Respiratory Syndrome (MERS), respectively.

These pathogens are large, enveloped and positive-sense single stranded RNA viruses. SARS and COVID 19 are very similar in terms of clinical, pathological and radiological features, yet SARS-CoV2 and SARS-CoV are sufficiently divergent in their genomic characteristics to be considered as the causes of two distinct diseases. However, structurally they are 82% homologous despite a few mutations in their amino acid sequences. The genome of SARS-CoV2 consists of 29891 nucleotides encoding 9860 amino acids. Its genomic sequence is:

\[5' -\text{replicase (orf1/ab)-structural proteins [Spike (S)-Envelope (E)- Membrane (M)-Nucleocapsid (N)]-3'}\]

The nucleotide sequence of the genome of the virus contains two main open-reading frames (ORFs), ORF1a and ORF1b, the translation of which results in the expression of two co-terminal replicase polyproteins ppl1a (ORF1a) and ppl1ab (ORF1a & ORF1b together). These polyproteins are cleaved into non-structural proteins (NSPs) by two proteases: a papain-like protease (PLpro) and the main serine type protease (Mpro). The amino acid sequence of these replicating proteins in SARS-CoV2 and SARS-CoV are 94.4% identical. In addition, PLpro as well as some nsps (nsp3) suppress innate immunity through interfering with the production of type I interferon and inhibiting of Toll-like receptor3 (TLR3) and Toll-like receptor 7 (TLR7). In vivo, it has been demonstrated that type I and type III interferon response against SARS-CoV2 is lower than that of seen in respiratory syncytial virus (RSV) and influenza A virus. It seems that SARS-CoV2 like SARS-CoV evade innate immunity successfully.

Viral RNA synthesis leads to genomic and subgenomic RNAs production, the latter serves as mRNA to encode for structural proteins. Nucleocapsid (N), membrane (M) and envelop (E) proteins are structural proteins which determine the different compartments and shape of the virus. Furthermore, a glycoprotein structure called spike (S) protein protruding from the surface of the virus facilitates its entry into the host cells and
determines the host range, tissue tropism and the host immune responses.\textsuperscript{17} As a class 1 fusion protein the characteristic clove-shape viral S protein of coronaviruses is composed of two trimeric subunits, S1 and S2. S1, containing the receptor binding domain (RBD), mediates the attachment of the virus to its receptor on the host cell and S2, the stalk of this glycoprotein, is responsible for virus-cell fusion.\textsuperscript{13, 18, 19} Protein S of SARS-CoV2 has 76\% homology to that of SARS-CoV; their RBDs’ similarity relevant to their amino acid sequence is 74\%.\textsuperscript{18}

The critical point of the virus replication and pathogenesis is entry of the virus into the host cell. In this context, S1 and S2 subunits should be cleaved in a priming process through which, as S1 attaches to the receptor, S2 is exposed to fuse with the host cell membrane. Host cell proteases like furin, transmembrane protease serine protease-2 (TMPRSS2) and cathepsin L are responsible for this cleavage. Furin is highly found in the lungs.\textsuperscript{20} It has been demonstrated that SARS-CoV2, to the contrary of SARS and other bat coronaviruses, has achieved a furin cleavage site in S’2 of protein S, which has resembled its infectivity rather to those of HIV, Ebola virus (EBoV) and some avian influenza viruses.\textsuperscript{21}

The receptor of SARS-CoV2 on the host cells, like that of SARS-CoV, is angiotensin converting enzyme 2 (ACE2).\textsuperscript{22, 23, 24} Apart from some few novel mutations in the SARS-CoV2, the complex of (SARS-CoV2)-ACE2 is highly similar to (SARS-CoV)-ACE2. However, the binding affinity of SARS-CoV2 to its receptor, ACE2, is 10 to 20-fold higher than that of SARS-CoV.\textsuperscript{25}

ACE2, a metallopeptidase and homologue of angiotensin converting enzyme (ACE), is a member of renin-angiotensin system (RAS). RAS is a very complicated network of ligands and receptors which not only exert their effects in a systemic counter/cross regulated signaling pathways but also engage in a set called local RAS that acts in an autocrine and intracrine manner in some organs.\textsuperscript{26} These two collaborative systems contribute to the regulation of cardiovascular system, metabolism, cell growth, salt and electrolyte homeostasis and vascular resistance.\textsuperscript{27, 28}

ACE2 is a mono-carboxypeptidase which removes single amino acids from peptides of RAS. ACE2 is not inhibited by ACE inhibitors like captopril or lisinopril.\textsuperscript{29,30,31} It converts angiotensin I [1-10] and angiotensin II [1-8] to angiotensin [1-9] and angiotensin [1-7], respectively.\textsuperscript{31,32} (31, 32). ACE2 is a functional competitor of ACE as the former converts angiotensin I [1-10] to a less active metabolite, angiotensin [1-9], so that less angiotensin I [1-10] remains available for ACE to be converted to Ang II. Besides, ACE2 converts Ang II [1-8] to angiotensin [1-7]. Opposing to ACE2, ACE degrades angiotensin [1-7] to inactive products like angiotensin [1-5].\textsuperscript{33,34} Angiotensin [1-7] is considered as an active peptide in RAS with antioxidative, anti-inflammatory, antiproliferative/antifibrotic, potent vasodilatory, and anti-thrombotic properties which exerts most of its effect via Mas receptor.\textsuperscript{34,35,36,37} On the other side of this intricate-regulatory RAS, ACE increases Ang II with its oxidative, proinflammatory, proliferative/fibrotic, vasoconstrictive and thrombotic effects which is mostly exerted through angiotensin II type 1 receptor (AT1R). Another receptor for Ang II called angiotensin II type 2 receptor (AT2R) with cell protective and some opposing post-receptor effects to AT1R is distribute in a limited number of organs.\textsuperscript{37,38,39} Stimulating AT1R, Ang II induces mitochondrial dysfunction, ROS
generation through activating NADPH oxidase, production of cytokines such as TNF-α, IL-6 and IL-8 and activation of p38-MAPK and NF-kB pathways. AT1R resides on adipose and many other tissues such as cardiomyocytes, pulmonary vascular endothelial and bronchial epithelial as well as alveolar cells and specially on monocytes and macrophages. It is implicated that ACE2/Ang [1–7]/Mas axis plays a counter-regulatory role against ACE/Ang II [1-8]/AT1R signaling pathway.

ACE2, besides to its expression on cardiovascular system, kidney, small intestine and adrenal, is abundantly distributed in the apical portion of the ciliated nasal and tracheobronchial cells as well as pneumocytes type 1 and type 2. Epithelial cells of the respiratory system including pneumocytes type II are immune competent cells. They contribute to playing an integral part in innate immunity of the lungs through keeping the integrity of the alveoli as a barrier, repairing any damaging insult to type I pneumocytes, enhancing the functions of dendritic cells and macrophages, secreting cytokines and chemokines and even presenting antigens and consequently facilitating the shifting of innate to adaptive immunity.

Adsorption of recombinant protein S of SARS-CoV to ACE2 was shown to result in the downregulation of ACE2. Internalization of this receptor with the virus into the host cell or shedding of ACE2 in the airways are potential causes of this phenomenon. Releasing of ACE2 from the epithelial cells of the airways is a dynamic phenomenon that occurs constitutively and may upregulate in response to various stimuli. Intriguingly, protein S in binding with ACE2 induces ADAM-17/TACE as a sheddase to separate the ectodomain subunit of ACE2. Shedding of ACE2 is associated with the production of TNF-α which was argued to be the initiating cause in inflammation of the lung in SARS. Cytoplasmic domain of ACE2 plays an important role in this process as its mutation reduces SARS-CoV entry into target cells and release of TNF-α is abolished as well. Some experts believe that ADAM17 is not involved in entry of SARS-CoV2 into the host cells and TMPRSS2 and cathepsin-L share in this process. As a debating subject to be studied more, it is also suggested that TMPRSS2 and ADAM17/TACE compete with each other in facilitating the viral-cell entry. Anyhow, binding of S protein to ACE2 results in hyperacute downregulation of ACE2 which deregulates the balance in local RAS pathways in favor of ACE/Ang II [1-8]/AT1R in the lungs. In this context, hyperacute upregulation of local intracrine Ang II [1-8]/AT1R in the setting of invasion of huge number of SARS-CoV2 is not encountered with appropriate negative physiological feedback with ACE2. Furthermore, Ang II has been demonstrated to decline ACE2 expression and function via lysosomal degradation mediated by AT1R. Henceforth, Ang II/AT1R sets on fire locally to provoke lung inflammation through pro-inflammatory, cytokine inducing, proliferative, thrombotic and tissue destructive effect as well as activating platelet derived growth factor receptor (PDGFR) which might spread to other organs like kidneys.

So far, we have a deadly virus with distinct replication and pathogenesis which:

1- evades innate immunity via avoiding IRF3, TLR3 and TLR7 pathways by its nsp3 and papin-like proteases and elicit a moderate immune response,
2- contains protein S requiring to be primed by furin and host cell proteases to facilitate its fusion with the host cell  
3- downregulates its receptor (ACE2) after attachment  
4- dysregulates the balance between two opposing axes of local RAS in the lungs in favor of Ang II with all the destructive and fibrotic properties in the lungs

As there is not any available anti-viral drug or an imminent potential vaccine against this deadly virus, it seems wise to find a way to reduce the death toll by preventing or mitigating the respiratory failure or through breaking the life cycle of the virus itself till a comprehensive preventive or curing measure is introduced.

We hypothesized that re-balancing Ang II/ACE2 with angiotensin receptor blockers and subsiding immunopathological changes by immunomodulators might alleviate the severity of the disease and may reduce the morbidity and mortality rates in COVID 19. To prove our hypothesis losartan (an ARB) and imatinib (a tyrosine kinase inhibitor) were chosen to be studied in an in silico study. It is noteworthy that imatinib had been introduced in the treatment of SARS and MERS. Besides, low doses of imatinib was demonstrated to have the ability to enhance innate immune responses. Intriguingly, imatinib also was effective in attenuating LPS-induced acute lung injury in an animal study.

**Method:**

**Preparation of the Protein Structures**

The required protein structures were obtained from Protein Data Bank (PDB) according to Table 1:

| Macromolecule | Sequence Length | Organism | ID |
|---------------|-----------------|----------|----|
| SARS-CoV-2 spike receptor-RBD bound to ACE2 | 603/229 | Homosapiens/SARS-Cov2 | 6m0j |
| Angiotensin Converting Enzyme 2 (ACE2) | 615 | Homo sapiens | 1r4l |
| COVID-19 main protease | 306 | SARS-Cov2 | 6lu7 |
| MAP Kinase p38 | 379 | Homo sapiens | 1a9u |
| Furin | 482 | Homo sapiens | 6hz |
| Papain-like protease | 316 | SARS-CoV | 3mj5 |
| Angiotensin II type 1 receptor & Angiotensin II | 425/8 | Homo sapiens | 6os0 |

Proteins were studied for the date of publishing, crystallography techniques, the resolution, accompaniment of predefined inhibitor ("Pre-inh") and any required reconstruction due to probable missing of amino acids in their sequence vs the sequence of reference protein. The structures were observed by visualizing softwares (UCSF
chimera⁶⁷, Pymol⁶⁸, Swiss-PdbViewer⁶⁹ to determine their unique protein chains and whether the structure is accompanied by other undesired molecules like (water, ions....) and to purify selectively to achieve the most desirable structure.

**Preparation of Small Molecules**

The required drug and small molecules were obtained from Structure Data Bank such as Pubchem database⁷⁰ and Drug bank database⁷¹ according to the (Table 2 and Table 3). The structures of all the drugs and small molecules were imported through gauss view, and then fully optimized geometries and properties of the electronic and structural properties of all molecules were derived by means of the density functional theory (DFT) method⁷² with B3LYP functional⁷³. For all systems, a geometrical optimization and calculation were performed using the STO-3G⁷⁴ based set. The calculations were carried out using the Gaussian 03 package⁷⁵. The program Open Babel⁷⁶ was used to generate SMILES strings from the optimized structure representation, using them for a similarity study by drug bank Chemical Structure Search with 0.5 to 0.7 Similarity threshold.

**Table 2. the list of required drugs and small molecules**

| Small Molecules       | Pubchem CID | Approved drug | Plant extract |
|-----------------------|-------------|---------------|---------------|
| Azadirachtin          | 5281303     |               | ✓             |
| Caftaric acid         | 6440397     |               | ✓             |
| Chicoric acid         | 5281764     |               | ✓             |
| Chlorogenic acid      | 1794427     |               | ✓             |
| Cholecalciferol       | 5280795     |               | ✓             |
| Curcumin              | 969516      |               | ✓             |
| Curcumin Solfate      | 66645351    |               | ✓             |
| Dasatinib             | 3062316     |               | ✓             |
| Hesperidin            | 10621       |               | ✓             |
| Hydroxychloroquine    | 3652        |               | ✓             |
| Imatinib              | 5291        |               | ✓             |
| Milk Thistle          | 1548994     |               | ✓             |
| Ramipril              | 5362129     |               | ✓             |
| Riluzole              | 5070        |               | ✓             |
| Silibinin             | 31553       |               | ✓             |
| Trandolapril          | 5484727     |               | ✓             |

**Docking Simulation**

Docking study was performed by AutoDock4⁷⁷ to find the suitable orientation of the molecules in the active site of the protein structures. AutoDockTools 1.5.4 (ADT) was used to prepare input PDBQT files and to calculate a grid box. A special grid map appropriate for each structural size (table 4) around the active site of proteins were used. The center of the grid box was aligned to the coordinates of the “Pre-inh”™. A Lamarckian genetic algorithm (LGA) was used for the searching of the status of binding sites. Every Lamarckian job was comprised of 250 runs. The final structures were grouped and
classified according to the most favorable binding energy. This procedure was applied to all small molecules in a similar manner. A more negative score determines which of these small molecules are more likely to dock with a protein structure (target protein) and enter into more favorable interactions. The docking model of each protein (table) complex with its “Pre-inh” was generated by AutoDock 4. The reliability of the applied docking protocol was assessed by redocking each “Pre-inh” into the active site of its protein structure.

Table 3. List of angiotensin II receptor blocker (ARBs)

| Drug molecules | Pubchem CID |
|----------------|-------------|
| Pratosartan    | 9802561     |
| Tasosartan     | 60919       |
| Losartan       | 3961        |
| Candesartan    | 2541        |
| Irbesartan     | 3749        |
| Fimasartan     | 9870652     |
| Telmisartan    | 65999       |
| Forasartan     | 132706      |
| Azilsartan     | 135415867   |
| Saprisartan    | 60921       |
| Olmesartan     | 158781      |
| Valsartan      | 60846       |
| Eprosartan     | 5281037     |
| Saralasin      | 6324663     |

Table 4 grid box dimensions for each structure

| Macromolecules | Steps     | Grid points | Spacing Å | Grid Center |
|----------------|-----------|-------------|-----------|-------------|
| ACE2           | 1st Docking | 80 × 100 × 80 | 0.375     | 40 × 6.0 × 29 |
| ACE2           | 2nd Docking | 75 × 70 × 80  | 0.375     | 13 × 15 × 20  |
| ACE2           | 3rd Docking | 120 × 85 × 100 | 0.600    | 39 × 3.0 × 22 |
| Mpro           |           | 80 × 100 × 100 | 0.375     | −15 × 13 × 70 |
| p38MAPK        |           | 70 × 70 × 70  | 0.375     | 4 × 16 × 29   |
| PLpro          | Before MD  | 80 × 80 × 80  | 0.375     | −13 × 45 × −36 |
| PLpro          | After MD   | 80 × 80 × 80  | 0.375     | 40 × 45 × 47  |
| Furin          |           | 80 × 100 × 80 | 0.375     | 40 × 45 × 47  |

After exposing of the small molecules with the structures, we obtained docking energy for each [structure-small molecule] complex. The clusters of docking energies were determined for 250 posing status and the relevant numerical tables for each complex was studied.

MD Simulation
Molecular dynamic (MD) simulation is a method to study the dynamicity of the protein structure during a defined time period to characterize the behavior and stability of the structure. Gromacs software executed MD simulation in this study for 100ns.

MD simulations of the protein-small molecules complexes following docking process were performed with the GROMACS 2018 package using the GROMOS96 43a1 force field. The conformation status for ACE2 and PLpro complexes with theirs ligands with the highest affinity were selected as the initial conformation for MD simulations. First the topology parameters of protein were created and the complex was immersed in a cubic box of simple point charge (SPC) water molecules. The “solvated system” (protein, ions, small molecule and water) was neutralized by adding required counterions Na or Cl. To equilibrate the system, the solutes (Proteins, counterions, and small molecules) were subjected to the position-restrained dynamics simulation (NVT and NPT) at 299.177 K for 1000 ps. Finally, the full system was subjected to an MD production run for 100 ns at 300 K temperature and 1 bar pressure.

MD simulation was performed for ACE2 crystal, ACE2-SARS-CoV mutated RBD (refer to results), imatinib-ACE2, losartan-ACE2 and imatinib-PLpro, losartan-PLpro complexes.

Redocking study after MD simulation

After MD simulation of complexes for 100ns, the ligands were separated. Through redocking, the binding energies and the binding status of the small molecules with the protein structures was evaluated.

Analysis

RMSD, RMSF, hydrogen bonding and radius of gyration diagrams exported after performing MD simulation were analyzed by qtgrace. Ligplot and poseview were used for determining the hydrogen bonding, hydrophobic and pi-pi interactions after docking and MD simulation. Visual analyzing was done with ucsf chimera and pymol.

Configurations of computational systems:

In this study we used multiple computational systems by different configurations as Table 5:

| System   | Operating Systems | CPU          | GPU |
|----------|-------------------|--------------|-----|
| Server 1 | Linux 3.10        | 32 logical cores E5-2697 | CUDA |
| Server 2 | Linux 4.15        | 32 logical cores E5-2650 | ×    |
Results:

Losartan and imatinib bind to ACE2 with low energy (high affinity).

We exposed losartan and imatinib molecules to ACE2 (before MD simulation). Docking of imatinib and losartan in association with other selected small molecules with ACE2 were performed in three grid boxes with three distinct dimensions (Table 4).

In addition, docking energies for all the small molecules selected based on LBDD and SBDD for binding with ACE2 were assessed based on coordinates (grid box 1) of predefined inhibitor in crystal structure of ACE2. Among the molecules with the highest affinities to ACE2, small molecules with more tendency to contact with N-terminal α helix were selected to perform docking in grid box (grid box 2) relevant to the binding site of ACE2 with RBD. Grid box 3 for the whole structure of ACE2 was set only for imatinib and losartan to check the compatibility and reliability of hotspots and docking energies with the previous results in other grid boxes. The binding energies for all grid boxes and small molecules under study are mentioned in Table 6.

The positions of losartan and imatinib with the most affinity for ACE2 in grid box 1 vs the position of predefined inhibitor were determined (Figure 1).

Table 6. Docking Energies for Small molecules with ACE2 in 3 distinct grid boxes
Losartan and imatinib could change the conformational structure of ACE2 persistently.

In order to study the persistency of losartan-ACE2 and imatinib-ACE2 complexes, we performed 100ns MD simulation for each complex. Two new ACE2 (nACE2) under the influence of each of these ligands were exported: losartan (Lo,nACE2) and imatinib (Im,nACE2). RMSD, RMSF, radius of gyration and h-bonding diagrams of each complex was obtained (Figure 2).
The structure of the two complexes were superimposed on the structure of ACE2 crystal to evaluate the degree of changes in conformational shape of each complex. The data showed that the conformational structures of ACE2 in binding with both losartan and imatinib changed significantly at the binding site of ACE2 to RBD (Figure 3). It is expected that losartan and imatinib lengthen the binding distance between ACE2 to SARS-CoV2 RBD due to relocation of contributing residues in ACE2 (Table 7).
Figure 3. A: SARS-CoV2-ACE2 complex. B: 1-Im,nACE2, 2-super-imposition of ACE2 and Im,nACE2, 3-super-imposition of α-helix of ACE2 and Im,nACE2. C: 1-Lo,nACE2, 2-super-imposition of ACE2 and Lo,nACE2, 3-super-imposition of α-helix of ACE2 and Lo,nACE2. D: hydrophobic and hydrogen bonds of SARS-CoV2RBD-ACE2.
Table 7. Relocation of Carbon alpha of N-terminal helix of ACE2

| RBD     | H-Bond length Å | Hydrophobic | Atom C(α) | Imatinib (Å) | Losartan (Å) |
|---------|-----------------|-------------|-----------|--------------|--------------|
| Asn487(R) | 2.69            |            | GLN 24.A  | 6.32         | 6.06         |
| Lys417(R) | 2.90            |            | ASP 30.A  | 3.46         | 3.24         |
| Tyr449(R) | 2.70            | ✓           | HIS 34.A  | 2.59         | 2.37         |
| Thr500(R) | 2.71            |            | ASP 38.A  | 3.69         | 2.18         |
| Tyr49(R)  | 2.79            |            | TYR 41.A  | 1.98         | 1.80         |
| Asn487(R) | 2.69            |            | GLN 42.A  | 3.33         | 2.05         |
| Gly502  (R)| 2.78            |            | Tyr 83.A  | 7.58         | 6.50         |
| N-Terminal |                 |            | SER 19.A  | 9.24         | 7.71         |

The binding energy of losartan and imatinib with Lo,nACE2 and Im,nACE2 after performing 100ns MD simulation changed.

Redocking for losartan with Lo,nACE2, imatinib with Im,nACE2, losartan with Im,nACE2 and imatinib with Lo,nACE2 were performed. The binding energies are as the followings (Table 8):

Table 8. Docking Energies for Lo,nACE2 and Im,nACE2 bound to imatinib and losartan after 100ns MD simulation

| nACE2   | Binding energy (Kcal/mole) |
|---------|-----------------------------|
| Losartan |                             |
| Imatinib |                             |
| Lo,nACE2 |-11.99                       |
| Im,nACE2 |-8.47                        |
| Im,nACE2 |-17.78                      |

Available ARBs vs losartan could only bind to ACE2 with reasonable but lower affinity

ARBs were searched for on Kyoto Encyclopedia of Genes and Genomes (84). Docking was done for each member of ARBs with ACE2 crystal structure. The energy binding for each item was obtained. Losartan in association with two unavailable ARBs, pratosartan and tasosartan, was in the upper three ranking of binding energy.71 (Table 9)
The mutated new amino acids in the sequence of SARS-CoV2 RBD were replaced on SARS-CoV RBD; MD-simulation was performed for new structure

In the beginning of our study (mid of February 2020) due to the lack of crystal structure of SARS-CoV2 RBD we replaced 22 defined mutated amino acids in SARS-CoV2 RBD on the corresponding place on SARS-CoV RBD to achieve a RBD structure with the most similarity to the real crystal structure of SARS-CoV2 RBD, assuming that S proteins in these viruses are 76% homologous. MD simulation of 100ns was performed after accomplishing this replacement to achieve a persistently stable structure with the most homology to RBD-ACE2 complex of SARS-CoV2. RMSD, RMSF, H-bonding and radius of gyration diagrams are available. Due to the publishing of crystal structure of RBD-ACE2 complex of SARS-CoV2 by X-ray defraction (resolution of 2.45 Å) we quitted using the achieved RBD-ACE2 complex and continued our bioinformatic study on the new published one (Figure 4).
*Imatinib and losartan could occupy the space where the predefined inhibitor (“Pre-inh”) in crystal structure of the main protease (Mpro) of SARS-CoV2 poses; they might act as an inhibitor of Mpro.*

We exposed imatinib, dasatinib, losartan, cholecalciferol, silibinin, hydroxychloroquin and the other small molecules to crystal structure of Mpro. It was elucidated that imatinib in association with dasatinib, cholecalciferol and losartan had higher affinity to Mpro. It shows that these ligands based on their affinity probably behave as inhibitors of Mpro, considering CADD theories. (Figure 5) (Table 10)
Imatinib and losartan could occupy the space where the “Pre-inh” in crystal structure of the furin of SARS-CoV2 poses. Imatinib shows higher affinity but losartan has lower affinity vs the inhibitor; imatinib might act as an inhibitor of furin.

We exposed imatinib, dasatinib, losartan, cholecalciferol, silibinin, hydroxychloroquin and the other small molecules to crystal structure of furin. It was demonstrated that imatinib had higher affinity to furin. But dasatinib, cholecalciferol and losartan and the other molecules showed up with lower but reasonable affinity to furin. Considering CADD theories, it shows that imatinib based on its affinity will probably inhibit furin function. (Figure 6) (table 11)

It is worth mentioning that redocking of predefined inhibitor of furin in its crystal structure obtained from PDB was disturbed and showed error in the process. We optimized the structure and performed docking for the second round to earn a reliable reference for binding energy.
Imatinib and losartan could occupy with higher affinity the space where the “Pre-inh” in crystal structure of p38MAPK poses; they might act as an inhibitor of p38MAPK.

We exposed imatinib, losartan, olmesartan, cholecalciferol, silibinin and the other small molecules to crystal structure of p38 MAPK. It was implicated that imatinib, losartan, olmesartan, cholecalciferol and silibinin had higher affinity to p38 MAPK. Considering CADD theories, it shows that imatinib, losartan, olmesartan, cholecalciferol and silibinin based on their affinity will probably inhibit p38MAPK function. (Table 12)
Table 12. Docking Energies for the Small Molecules with p38MAPK (Kcal/Mol)

| Small Molecules       | Binding Energy |
|-----------------------|----------------|
| Imatinib              | -11.71         |
| Losartan              | -9.31          |
| Cholecalciferol       | -8.82          |
| Olmesartan            | -8.69          |
| Silibinin             | -8.48          |
| Pre-Inh               | -8.42          |
| Hesperidin            | -8.36          |
| Curcumin              | -7.43          |
| Hydroxychloroquine    | -6.92          |

![Docking of p38MAPK, Imatinib & Losartan](image)

**Figure 7.** Position of Imatinib and Losartan in complex with p38MAPK with PDBID: 1a9u after Docking simulation. A: Ribbon view of complex (left) and Poseview of interaction with imatinib (Right) B: Ribbon view of complex (left) and Poseview of interaction with Losartan (right)

**Imatinib could occupy with higher affinity the space where “Pre-inh” in crystal structure of papin-like protease (PLpro) poses; imatinib might act an inhibitor of PLpro.**
We exposed imatinib, cholecalciferol, losartan, silibinin, curcumin, hydroxychloroquine and even olmesartan to crystal structure of PLpro. All the compounds showed high affinity to PLpro but lower than imatinib and “Pre-inh”; Considering CADD theories (R) imatinib might act as an inhibitor of PLpro function. (Table 13)(Figure 8)

Table 13. Docking Energies of Small Molecules for binding with PLpro (Kcal/Mol), before and after 100ns

| Small Molecules | Before 100ns | After 100ns |
|-----------------|--------------|-------------|
| Imatinib        | -12.13       | -16.24      |
| Pre-Inh         | -11.15       | -14.15      |
| Cholecalciferol | -10.05       | -10.05      |
| Losartan        | -9.21        | -9.21       |
| Silibinin       | -8.93        | -8.93       |
| Curcumin        | -8.53        | -8.53       |
| Hydroxychloroquine | -8.34     | -8.34       |
| Hesperidin      | -8           | -8          |
| Olmesartan      | -7.32        | -7.32       |

Figure 8. Position of Imatinib and Losartan in complex with PLpro with PDBID: 3mj5, before and after 100ns MD simulation and redocking. A: Ribbon and poseview of complex of PLpro with imatinib before 100ns MD simulation, B: Ribbon and poseview of complex PLpro with imatinib after 100ns MD simulation C: Ribbon and poseview of complex of PLpro with losartan before 100ns MD simulation, D: Ribbon and poseview of complex PLpro with losartan after 100ns MD simulation
Angiotensin II (Ang II) – Imatinib Interaction

We exposed imatinib and Ang II. The result shows that imatinib binds with Ang II with binding energy of $-8.64 \text{ Kcal/mol}$. 

Figure 9. Diagrams of Losartan-PLpro and Imatinib-PLpro complexes after 100ns MD simulation; A: RMSD; B: Rg; C: RMSF; D: H-Bonding

Figure 10. Angiotensin II – Imatinib Interaction in Surface view (Left) and poseview of interaction with two hydrogen bonds with Phe8 and pi-pi interaction with Tyr4 (right)
Discussion

A new insight to pathophysiology of ARDS in COVID19

The fatality and the change of the life style imposed by COVID19 have resulted in uprising death and economic burden in all countries, respectively. Despite the breathtaking efforts done, the development of acute respiratory distress syndrome (ARDS) as the culprit of high morbidity and mortality in infection with SARS-CoV2 has remained elusive. The replication of the virus starts soon after it incubates in the upper respiratory tract. Thereafter the replicas are released and pour into the lower airways and alveoli in huge number (about 10-1000 virion/μl in its peak on day 5-6 of infection).85 59(85). Type II pneumocytes and bronchial epithelial cells are immune competent cells and possess high amount of ACE2.86,87,88 With the knowledge that the affinity of SARS-CoV2 to ACE2 is 10-20 times more than that of SARS-CoV it is legitimate that these sentinel cells get infected easily and in great number.89

Penetrating into the host cells, the viruses introduce their genome to the cytoplasm to replicate more numbers of complete viruses or their fragments. Using a conserved pathway led by papain-like protease and nsp3, SARS-CoV2 evade immunologic recognition by pattern recognition receptor (PRR) and avoid triggering interferon (IFN) response (significant lack of IFN I and III expression in covid19) as well as activating dendritic cells. Consequently, the innate immunity becomes inefficient in ensuing an adaptive response.10,11,14,90 Apart from ARDS, COVID19 compared to influenza and respiratory syncytial disease, is a moderate illness, since SARS-CoV2 elicits a limited antiviral response.15 In COVID 19, dendritic cells fail to process the antigens properly and to migrate to prime naïve T cells in the local lymph nodes.91,92 Lung CT-scans of patients approve this phenomenon as lymphadenopathy in the lungs is scarce or absent on the images.93,94,95 Recently, ARDS in COVID19 has been attributed to a kind of cytokine storm phenomenon. If this virus evades innate immunity and spread insidiously how come the destructive cytokine storm involving the lungs or other organs ensues?! What is the triggering factor?

Relying too much on the clinical studies has resulted in ignorance of some physiological local tissue responses that may convert to systemic harmful reactions if not dealt with immediately. Cytokine storm is triggered from some deregulations in immunological responses which may eventuate into sudden outpouring of pro-inflammatory and tissue destructive cytokines. The origin of this devastating process might be infectious or non-infectious.96 Whenever innate immunity fails to switch to adaptive responses with proper priming of T cells, the pathogen is not cleared out in a well-orchestrated manner. Instead, unrestrained sequential release of cytokines ensues. Amplifying this response, if not halted soon, eventuates to this storm.97,98 The fragile texture of lung tissue with its high exposure to different antigens requires that suppressive responses (immunomodulators) be meticulously coordinated with promoting responses (immunosensors). Otherwise, dysregulation of modulators and sensors will be hazardous.99

SARS-CoV2 and SARS-CoV replicate rapidly in host cells with early high viral load.57,100 It seems that replicating of CoVs is abortive and delayed in dendritic, monocyte/macrophages and lymphocytes.11,101,102 SARS-CoV2 infects type II alveolar and bronchial epithelial cells which contribute to innate immunity. SARS-CoV and SARS-CoV2, in entry to the host cells, downregulate ACE2 and induce imbalance in ACE2/angiotensin[1-7]/Mas and ACE/Ang II/AT1R pathways in favor of the latter.51,103
the virus evades the immune system, hyperacute imbalance (low ACE2/ACE ratio) of the two opposing RAS pathways in favor of pro-inflammatory, proliferative, prothrombotic, tissue destructive and pro-apoptotic Ang II might be the igniting or at least a robust major co-stimulatory factor in inducing cytokine storm. Preliminary clues to this theory are:

1- Ang II level has been reported to be higher in infected patients with SARS-CoV2 than in non-infected healthy people.\textsuperscript{104}

2- ACE2 is tissue protective for the lungs in acid- or sepsis-induced ARDS in mice.\textsuperscript{105}

3- Mechanical-stress induced ARDS is correlated to activation of [Nox1(NADPH-oxidase1)-MK(midkine)-Notch2-ACE] pathway.\textsuperscript{106}

Although probable contribution of RAS to evolving ARDS was suggested in SARS previously\textsuperscript{103} it seems that many latent aspects of the involving pathways and the role they may play to solve this puzzle have recently been elucidated. There are three orders of RAS in our body: systemic-hormonal, tissue-local (with paracrine and autocrine effects) and cellular-subcellular (with intracrine effects).\textsuperscript{26,107,108} It is noteworthy that while ACE is detectable in 20% of capillary endothelium of non-respiratory organs this peptidase is expressed on the entire endothelial cells of the alveolar capillaries.\textsuperscript{109} Thence, lungs can be considered as the major organ producing Ang II.

Invasion of SARS-CoV2 in huge number downregulates ACE2 in the host cells outrageously so that the balance in tissue-local and cellular-subcellular RAS disrupts hyper-acutely. Accordingly, the host cells lose their ability to adapt to or defeat against the consequences of sudden gush of Ang II with adequate negative feedback responses by ACE2. It is worth mentioning that in healthy people, intra-cellular Ang II content in some tissues may reach up to 1000 times higher than that of plasma.\textsuperscript{110} In ACE2 deficiency, Ang II is not hydrolized to angiotensin[1-7] (with its cytoprotective effect) or to other less active metabolites. Consequently, hyperacute excessive content of Ang II exerts rather untoward chaotic pathological effects in an intracrine (intracellular) and autocrine (cell to the same cell) manner and even through spilling over extracellularly, in a paracrine (cell to different neighboring endothelial, macrophages, monocytes, vascular smooth muscle cells or fibroblasts) and endocrine (cell to circulation) fashion. On the other hand, some studies demonstrated that Ang II itself downregulates ACE2 expression through internalization, lysosomal degradation and AT1R-mediated ROS activated ERK/p38 MAPK pathway which promotes TACE/ADAM17 activity as well.\textsuperscript{53,111,112}

Ang II increases reactive oxygen species (ROS) through AT1R-dependent induction of NADPH oxidase (Nox), mostly Nox2 and Nox4.\textsuperscript{109,113} In this pathway which is dependent on intra- and extra-cellular calcium, phospholipase C (PLC) and protein kinase C (PKC) are involved.\textsuperscript{109} Even though ROS as a signaling molecule contributes to cell homeostasis, its overproduction may lead to cell damage.\textsuperscript{114} Intriguingly, ROS upregulates the production of Ang II in a positive feedback response. In the absence of ACE2, this amplifies the production of ROS dramatically.\textsuperscript{115,116} ROS causes DNA damage and mitochondrial dysfunction.\textsuperscript{117,118} It has been reported in animal studies that Ang II through AT1R reduces mitochondrial number and prosurvival genes (Nampt and sirtuin3).\textsuperscript{119} ROS, in turn, through opening mitochondrial K-ATP channels and disturbing mitochondrial membrane potential, upregulates mitochondrial ROS (mtROS) production in a positive feedback response.\textsuperscript{120} Ang II, directly, through activating of type 2 Ang II receptor (AT2R) residing on mitochondrial membrane inhibits mitochondrial respiration (NO-dependent) and increases mtROS.\textsuperscript{106} mtROS functions as a triggering signaling molecule for production of pro-inflammatory cytokines.\textsuperscript{121} It was reported in influenza that
regulated amount of mtROS induces interferon γ (IFNγ) to restrain infection. But when mtROS rises up excessively, striking upregulation of pro-inflammatory cytokines must be expected. mtROS was shown to activate NLRP3 which induces IL-1 and IL-18 production. Furthermore, ROS activates inflammatory responses by inducing redox-sensitive transcriptional factors like NF-kB and activator protein 1 (AP1).

As an inconclusive subject, over-expression of TNF-α during hyper-acute shedding of ACE2 by ADAM17 through synergism with Ang II may aggravate the situation by inducing oxidative stress via NF-kB and p38 MAPK dependent pathways.

In addition, Ang II through AT1R was reported to amplify oxidative stress by distorting iron homeostasis, increasing labile ferrous iron and expression of ferritin in endothelial cells. Even though ferritin may show an antioxidative effect, it has been described in mice that ferritin may act as a local cytokine and activate NF-kB and MAPK-mediating pathway. This response results in rise of inducible NO synthase (iNOS) of about 100-fold and IL-1β and RANTES 50-fold with a small increase in intercellular adhesion molecule (ICAM). Ferritin may suppress adaptive immune response, as well.

Ang II by activating AT1R induces expression of TNF-α (presented already in the scene), IL-1β, IL-6, IL-8, MCP-1 and even IL-10 through a NF-kB and activating protein 1 (AP-1) transcriptional factors. Aggravating to these pro-inflammatory effects, Ang II by activating AT1R increases vascular permeability in the lung by release of prostaglandins and vascular endothelial growth factor (VEGF). Disruption of endothelial-epithelial barrier in alveoli and increase in permeability of endothelium rises the fluid in the alveolar sacs that should be cleared out by epithelial Na channels (ENaCs). In rats, endogenous activation of AT1R by Ang II downregulates ENaC expression and disturbs pouring out the extra fluid.

Of these cytokines induced by Ang II, IL-6 plays a more special role in immunopathological effect of Ang II. It induces signaling processes associated with JAK2/STAT1/3 activating pathway which promotes many genes contributing to the production of signaling molecules like cytokines, adaptors, receptors and protein kinases. In this context regulation of differentiation of monocytes into macrophages mediated by the expression of macrophage colony-stimulating factor, upregulation of B-cell IgG production, downregulation of dendritic cell maturation by activation of the STAT3 signaling pathway and the promotion of the Th2 response by inhibiting Th1 polarization are attributed to IL-6. Tocilizumab (atemra) achieved its fame in COVID19 through its inhibitory effect against the receptor of IL-6.

Ang II promotes production and release of IL-6 and IL-8 from human cultured adipocytes by NF-kB-mediated pathway to which AT1R rather than AT2R contributes. It is demonstrated that in the obese IL-6 plasma level is closely correlated to body mass index (BMI). Ang II-induced over expression of IL-6 in adipose tissue might be the reason why obesity is a risk factor in severity of COVID19. This cytokine has been found to increase platelet and immune cell aggregation through a T-cell dependent mechanism by Ang II. Compatible with the theory of Ang II-mediated immunopathology in COVID 19, this might be the reason why patients with COVID19 are prone to vascular thrombosis. IL-6 level has been correlated to the severity of COVID19. This cytokine, in association with IL-1 and TNF-α, is the major inducer of CRP production in the liver. In addition, Ang II, induces CRP expression in hepatocytes in a time- and dose-dependent manner through
activation of AT1R and resulting from ROS-MAPK-(NF-kb) pathway independent of IL-1β and IL-6.\textsuperscript{141}

During an immunologic reaction to a pathogen, switching from innate to adaptive immunity requires that naïve CD4+ T cells (Th0) be differentiated to effector T-helper cells: Th1 (producing IL-2, IFNγ, lymphotoxins) and Th2 (producing IL-4, -5, -13) which help to activate cytotoxic CD8+ T cells (CTLs) and B cells, respectively.\textsuperscript{142} As a potent pyrogenic cytokine, IL-6 in synergism with IL-7 and IL-15 promotes the differentiation and cytolytic activity of CD8+ T cells.\textsuperscript{132} Different cytokines may also promote Th0 cell to differentiate to two opposing classes of T cells: anti-inflammatory T-regulatory (Treg) cells and pro-inflammatory Th17.\textsuperscript{142} Ang II through AT1R-PKA-proteosome pathway and activation of STAT1 and NF-κB promotes differentiation of Th0 to Th1. It has been demonstrated that in shifting from Th0 to Th1 or Th2, Ang II upregulates the production of IFNγ (10-fold), IL-2 (18-fold), IL-4 (3.5-fold) and IL-10 (1.5-fold). In addition, Ang II increases T-box transcription factor mRNA (Tbet, marker for Th1) and GATA3 mRNA (marker for Th2) by 38 and 1.6-fold, respectively. Amazingly, losartan, AT1R blocker, has been shown to inhibit markers for Th1 differentiation without having any effect on that for Th2.\textsuperscript{143} It is noteworthy that Th1 is differentiated to Th17 in the presence of IL-6 and TGF-β.\textsuperscript{132,143} Th17 induces synthesis of IL-17, IL-21, IL-22 and TNF-α. TNF-α, itself in the presence of IL-6 and IL-1β may promote differentiation of T cells to Th17.\textsuperscript{143,144} High level of IL-17 in patients with ARDS suggests its contribution to this syndrome. This cytokine in a model of influenza and LPS induced acute lung injury has been associated with neutrophil recruitment and increase in alveolar layer permeability.\textsuperscript{145}

There seems to be a local RAS in DCs, T cells and NK cells with a complete enzymatic repertoire enabling them to synthesize and metabolize Ang II and even AT1R and AT2R. It has also been described that these cells not only respond to Ang II but they have tendency to migrate to this peptide. Thenceforth, Ang II may orchestrate recruitment of leukocytes to the site of inflammation. Ang II induces synthesis of CCL5/RANTES chemokine in T cells and NK cells. It shows that Ang II may direct chemotaxis of cells possessing CCR1, CCR3 and CCR5 and even regulate proliferation of T cells via CCR5.\textsuperscript{146}

Activation of AT1R by Ang II has been associated with apoptosis in pneumocytes.\textsuperscript{147} It seems that alveolar and bronchial cell death may restrain the distribution of the virus. But even low concentration of IL-6 (upregulated by Ang II to high levels in cytokine release syndrome) in synergism with IL-17 (secreted by Th17) is able to induce expression of prosurvival proteins Bcl-2 and Bcl-xl which inhibit cell destruction by CD8+ cytotoxic T cells and prevent apoptosis of pneumocytes through STAT3 and NF-kB signaling.\textsuperscript{148} IL-22, a member of IL-10 anti-inflammatory cytokine family, secreted by Th17 also prevents apoptosis of pulmonary endothelial cells and may ameliorate ARDS through inducing JAK2/STAT3 pathway.\textsuperscript{149} But it should be taken into account that over-expression of Ang II upregulates IFNγ (10-fold) and IL-2 (18 fold) (pro-inflammatory cytokines) much more than IL-4 (3.5-fold) and IL-10 (1.5-fold) (anti-inflammatory cytokines).\textsuperscript{143} It seems that in this milieu of highly complicated set of pro-inflammatory cytokines some anti-inflammatory molecules prevent apoptosis and cells death of the respiratory epithelial and as a theory even help giant cells produced in COVID 19 to survive.

Destruction of the pulmonary tissue encompasses the interstitial and basal collagen-elastin structures as Ang II upregulates matrix metalloproteinases (MMPs) in vascular smooth muscle cells: MMP2 [through AT1R-mediated extra-cellular signal-regulated
kinase (ERK)1/2 activation], MMP1, MMP3 and MMP9 (via AT1R-ROS mediated NF-kB and AP-1 pathways). These proteinases regulate remodeling and turn-over of extracellular matrix (ECM) and promote smooth muscle and endothelial cell proliferation and migration resulting in vascular wall fibrosis which eventually may end up in pulmonary hypertension if lasts.\textsuperscript{150,151,152,153} It has been reported in an animal study that ACE2 deficiency results in activation MMP and STAT3 pathway which may promote lung injuries.\textsuperscript{154}

Macrophages are important in the healing process. These cells should switch from M1 (with more pro-inflammatory cytokines) to M2 (with more IL-4 and IL-13 dependent IL-10) phenotype to resolve the inflammation. In oxidative stress macrophages exhibit more AT1R. Activating AT1R impairs efferocytosis (clearance of apoptotic cells) and interferes with resolution of the inflammatory cascade.\textsuperscript{155,156} MerTK (a tyrosine kinase) which shifts DCs from pro-inflammatory to anti-inflammatory status increases survival of macrophages in acidic environment.\textsuperscript{157} Ang II induces shedding of MerTK off the cell membrane through AT1R/ROS/p38MAPK/ADAM17 pathway.\textsuperscript{158} Consequently, Ang II impairs switching M1 to M2. Continuation of pro-inflammatory status result in activation of MMPs and inducing of ECM remodeling processes. Failure of Tregs to show up due to the predominance of IL-6 promoting Th17 may prevent effective efferocytosis. In this milieu, pro-fibrotic IL-13 and TGF-\(\beta\) may lead to lung fibrosis. Ang II through AT1R induces fibrotic changes in the lungs with direct and indirect effects. It has been shown in transgenic mice that Ang II stimulates lung fibroblasts/myofibroblast proliferation and synthesis of ECM. It induces production of TGF-\(\beta\) and connective tissue growth factor (CTGF).\textsuperscript{159,160} Ang II in synergism with TGF-\(\beta\) may promote fibrosis in many organs including the lungs.\textsuperscript{161} On the other hand, angiotensin [1-7], the product of ACE2, has been considered an antifibrotic molecule.\textsuperscript{36} Oxidative stress may promote pulmonary fibrosis through deregulation of sirtuin3.\textsuperscript{162}

**Losartan, an AT1R blocker, in COVID 19**

Ang II was previously considered as a factor that might play a role in ARDS.\textsuperscript{163} There is a reliable amount of evidence that losartan, an ARB, is effective in ameliorating ARDS.\textsuperscript{164,165,166} Considering the above proposed pathophysiology of ARDS in COVID19 with the new insight that hyper-acute dysregulation of AngII/ACE2 due to the dramatic downregulation of ACE2 might be the cause of cytokine storm and ARDS, led us choose losartan, an AT1R blocker, to inhibit the post receptor effects of Ang II just to moderate the pathological effects of ACE2 deficiency in this disease. In this context, striking upregulation of AT1R by Ang II in a positive feedback manner which might occur in the lungs similar to other organs in oxidative stress could not be ignored, bearing in mind that dramatic downregulation of ACE2 in invasion of huge number of virions and the consequent drastic impairment of hydrolyzing of the intracellular content of Ang II occur in a very short time.\textsuperscript{163} Reviewing the literature, it was clear that losartan, candesartan and olmesartan were used to modulate local RAS in previous studies.\textsuperscript{131,143,150,159,167} But losartan probably due to its more hydrophobicity and availability might have been chosen more. Losartan was effective in many studies in suppressing pro-inflammatory effects of cytokines due to its immunomodulatory properties or even in preventing lung fibrosis; it can be considered as an ameliorating drug in ARDS in COVID 19.\textsuperscript{39,153,157,168,169}
As a debating subject some of the experts according to some animal studies, not proved in other animal or human studies, suggested that ARBs might upregulate ACE2 expression on the cells and thus increase the viral load in the body.\textsuperscript{170,171,172} In those studies, overexpression of ACE2 after administration of ARBs needed 25-28 days to appear. Furthermore, the impact of hyper-acute increase in the level of local Ang II in the lungs, heart and kidneys in COVID 19 within a short time is much more dangerous. In addition, it seems that the infectivity of the virus, like that of HIV might decrease with the increase of ACE2 content on the cell membrane. Entry of the virus to the cell is a complicated multifactorial subject. It is also dependent on the presence of cell membrane proteases. Upregulation of ACE2 content of the cell membrane is not necessarily associated with an increase in other engaging factors in cell entry such as TMPRSS2. Overexpression of ACE2 without concomitant increase in TMPRSS2 results in the presence of extra ACE2 unable to lead the entry of the virions but capable of entrapping and decreasing the infectivity of the virus. As ADAM17 induces shedding of ACE2, free extra ACE2 is released to the airways with the ability to trap the inhaled viruses. Consequently, upregulation of ACE2 increases entrapment of the virus.\textsuperscript{173,174,175} There is not any reported adverse effect of ARBs in COVID 19 and due to favorable effects of these drugs scientific societies in Europe and the USA recommended that ARBs be continued in patients with hypertension or heart diseases. Even two official studies on losartan in the treatment of out and in-patients with COVID 19 have recently been conducted.\textsuperscript{176}

**Imatinib, an Abl tyrosine-protein kinase inhibitor**

It is wise mentioning that patients with cytokine storm may experience hypotension in their extremes of severity of the disease. With the knowledge that losartan may aggravate hypotension and emphasizing that the initial release of TNF-\(\alpha\) simultaneous with binding of RBD and ACE2 and consequent shedding of ACE2 may have co-stimulatory effect in starting of cytokine storm it seems to be rational to recommend another immunomodulator in association with losartan to stop the storm. This combination provides the possibility to reduce the required dosage of each drug. There is conflicting evidence that systemic corticosteroids may be hazardous to patients with COVID 19.\textsuperscript{177} Recently, it has been described that glucocorticoids may increase Th17 in T cell culture of healthy human.\textsuperscript{178,179} Reviewing the literature, tyrosine kinase inhibitors were previously introduced in the treatment of SARS, MERS and ARDS. Abelson tyrosine-protein kinase 2 (Abl 2) is needed in replication of SARS-CoV and MERS-CoV. In this family of viruses, imatinib hinders the initial phases of the virion replication by inhibiting fusion of the virion at the endosomal membrane.\textsuperscript{58,180,181} Imatinib, with immunomodulatory effects, has also been suggested to have subsiding effects specially against vascular leak in ARDS.\textsuperscript{182,183,184} There is also a report that low dose imatinib was effective in reducing pulmonary blood pressure in dogs.\textsuperscript{185} In another report inhaled imatinib was also used as a drug to subside pulmonary hypertension.\textsuperscript{186}

Amazingly, Imatinib was found to have an inhibitory effect against Ang II impact on vascular smooth muscle cells in dissection of the aorta in mice.\textsuperscript{187} Furthermore, expression of MHC class I and II, production of co-stimulatory molecules and secretion of cytokines and chemokines in monocyte-derived dendritic cells decrease in the presence of imatinib. This tyrosine kinase inhibitor subsides phosphatidylinositol 3-kinase/Akt pathways and downregulates exhibition of NF-kB in the nucleus.\textsuperscript{188} Cultured
human monocytes are morphologically and functionally suppressed in the presence on imatinib which reduces the ability of these cells to synthesize IL-6 and TNF-α and to respond efficiently to M-CSF and GM-CSF stimulation. In an in vitro study, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines. In vitro, imatinib could inhibit expression of TNF-α, IL-6, IFNγ and IL-17 in cultured splenocyte of mice with arthritis in a dose dependent manner. In monocytes and macrophages, TNF-α production was reduced by imatinib while IL-10 expression did not change. Imatinib in mice with hyper-reactive airway disease could subside peri-bronchial eosinophil cell accumulation and decrease secretion of IL-4 and IL-13 by Th2 as well as CCL2, CCL5 and CCL6 chemokines.

**In Silico Study**

We conducted an In silico modeling study to investigate the probable inhibitory or modulatory effect of losartan and imatinib in some critical points of the life cycle of SARS-CoV2. In this study we used bioinformatic tools (refer to the method) to assess the effect of the suggested drugs on important points found in the proposed theoretical pathophysiology, as well. It was elucidated that both losartan and imatinib could bind to ACE2 with lower docking energy (higher affinity) relative to the “Pre-inh” (reference ligand, table 2). It does not mean that these drugs could inhibit the catalytic property of this carboxypeptidase: neither of these two drugs has been reported to exhibit any inhibitory property against ACE2, yet. Intriguingly, cholecalciferol showed similar behavior.

As a novel finding, we have demonstrated that losartan and imatinib could distort the binding site of SARS-CoV2 RBD to ACE2. According to our study there are seven points on the α-helix arm of ACE2 molecule located between glycine 24 and lysine 353 (fig 2A and 2D, table 3) where SARS-CoV2 RBD and ACE2 (acceptor-donor) may establish hydrogen (H) bonds (refer to the method and results). The distance between the acceptor-donor residues at these points are between 2.69-2.90Å (table 3). It means that hydrogen bond energies between SARS-CoV2 RBD and ACE2 based on the distance of acceptor and donor residues, without considering the hydrophobic contacts, are of moderate magnitude and mostly electrostatic. Considering the low docking energy (high affinity) of losartan and imatinib to ACE2 which even dropped more (resulted in more affinity) after 100ns of exposure of these two drugs to ACE2 in MD simulation, it is obvious that the bond between losartan and imatinib with ACE2 is stable enough. In our modeling study, ACE2, after 100ns, exhibited significant translocation of the α-helix in its structure where RBD binds. According to table 3 this change in conformational shape of ACE2 made relocation of all 7 points of binding of ACE2 to SARS-CoV2 RBD for at least 1.80 Å. But
in some binding residues the change in location was more significant (more than 3.00 Å) which means that H-bonds at these points after the relocation might not be strong and stable enough:

1- for losartan in four out of seven binding points,
2- for imatinib in six out of seven binding points.

According to this modeling it is expected that the affinity of the virus to its receptor might decrease in the presence of these two drugs. In addition, our study showed that losartan among other available ARBs in the market has the highest affinity to ACE2 (table 5). It is implicated that other ARBs might not efficacious in docking to ACE2 to make an effective and stable similar conformational structural change in the receptor of the virus. In addition, in COVID 19 we aimed at inhibiting local intra-cellular rather than systemic RAS. Except telmisartan, other ARBs compared to losartan are more soluble in water. Therefore, losartan may penetrate into the cell more efficiently.

Furthermore, we could find that both losartan and imatinib as well as cholecalciferol could pose in Mpro, PLpro and MAPK molecules in the position of their “Pre-inh” with higher affinity. These docking energies in our model are important because they determine how these ligands may probably affect the behavior of the proteases.

Of all the proposed ligands, imatinib could pose favorably in furin structure with higher affinity relative to its “Pre-inh”. Considering high expression of furin in the lungs in the entry of SARS-CoV2 to the target cell, if the inhibitory effect of imatinib against furin is approved in in vivo studies it can be regarded as an inhibitor that hinders entry of SARS-CoV2 to the target cell. Imatinib, cholecalciferol and losartan due to their effective docking to PLpro and Mpro with higher affinity relative to “Pre-inh” might be successful in preventing those proteases from letting the virus evade innate immunity or start replication. Although in the case of PLpro the docking energy for losartan and cholecalciferol were higher (lower affinity) than that of “Pre-inh”, the magnitude of energies (<-9.21) were low enough to consider them as probable inhibitor of PLpro. Studying RMSD diagram for 100ns MD simulation of losartan-PLpro complex, we could find that a sudden change of about 0.25 nm (2.5 Å) in the mean position of its molecules occurred in about 60ns that continued till the end of MD simulation for 100ns with a rise (fall in affinity) in the docking energy [from -9.21 (Kcal/mole) before MD simulation to -7.83 (Kcal/mole) post-hoc]. According to RMSF diagram almost the first 30 residues of PLpro showed the most fluctuations during 100ns simulation. These changes may indicate that losartan might affect the conformational shape and probably the function of PLpro at the expense of losing its affinity to the protein to some degree. Perhaps longer dynamic study is needed to explain the real behavior of PLpro in the presence of losartan.

Losartan increases bradykinin concentration up to 2-fold but the risk of angioedema due to losartan is very low (0.1-0.4%). Bradykinin induces allergic inflammatory responses to which p38 MAPK may contribute. Besides, bradykinin activates airway fibroblast/myofibroblasts through MAPK pathway. In many of the pathophysiological destructive pathways in COVID 19 the signature of MAPK is evident. Surprisingly, in this modeling in silico study losartan and imatinib showed to have significant tendency to bind with p38 MAPK. This might indicate that these drug ligands may have inhibitory effect against p38MAPK and its downstream pathways and may inhibit untoward bradykinin-
dependent or responses, as well. In our *In silico* study, imatinib also showed its tendency to bind to Ang II. It should be investigated if imatinib could change the function of Ang II.

We, at Bazarganan Hospital, Tehran, Iran, in some patients (who accepted to be given losartan after being informed and written consent was taken) administered low doses of losartan (6.25-12.5 mg twice a day in non-hypertensive patients for 14-20 days) along with the anti-viral drugs (lopinavir/ritonavir) according to the approved national therapeutic protocols in patients with COVID19. Below (Figure 11) two slices (the same level and condition) of spiral lung CT-scan belonging to one patient taken on the day of admission and 4 days later, show significant clearing of ground glass opacities after administration of losartan. The patient’s dyspnea subsided significantly after three doses of 6.25 mg of losartan.

![Spiral lung CT-scan of a 50 years old patient with COVID19; A: Apr 6, 2020; B: Apr 10, 2020](image)

There comes an argument regarding increased mortality in hypertensive patients with COVID19 who have already been using ARBs for a long time. How if losartan can be protective against COVID19 these patients are more at risk of being infected by SARS-CoV2. Surprisingly, in an animal study it was demonstrated that chronic administration of losartan upregulates AT1R and PKCσ, both the latters increase the vulnerability of myocardium to ischemia and ischemia/reperfusion injuries. Considering the above proposed hypothetical pathophysiology for the cytokine storm, hyperacute increase in Ang II in hypertensive patients with COVID19 who have been using ARBs may pose them to increased downstream effects of AT1R activation more than that seen in normotensive patients who has not used losartan.

We did not prescribe imatinib because its aerosolized form should be manufactured then be allowed to administer after successful subclinical studies. In addition, our study should be validated in clinical studies which at least for losartan it is under way in the USA.

**Conclusion**
COVID 19 is a viral disease which involves the lung with an ARDS-like pathology, the major cause of death in this disorder. As the receptor of this virus is ACE2, a member of RAS family, which in entry of the virus to target cell downregulates in favor of pro-inflammatory ACE/Ang II/AT1R pathway it seems that imbalance of two opposing limbs of RAS contributes to the immunopathological features of this disorder. Shedding of ACE2 after contact with virus S protein is one the causes of downregulation of ACE2. As a debating theory, TNF-α is released in this process which as a pro-inflammatory cytokine aggravates the ignited inflammation. As there is not any efficient anti-viral drug or imminent vaccine against SARS-CoV2 it seems logic that treatment of ARDS in COVID 19 will be an effective measure in reducing the death toll of this infection.

In order to regulate RAS, which according to the above discussed pathophysiology, results definitely in modulation of the imbalance in pro- and anti-inflammatory responses and to boost anti-inflammatory potentials to subside the cytokine storm we selected losartan and imatinib to be investigated as probably efficient therapy in COVID 19 in an in Silico modeling study.

According to the findings in this study and the preliminary clinical evidences, we suggest low dose systemic losartan and inhaled aerosolized low dose imatinib be studied in a subclinical setting in treating ARDS in patients with COVID 19. In this manner while losartan by blocking AT1R antagonizes Ang II effects, aerosolized imatinib may modulate the local immunological responses. Based on our study both losartan and imatinib may change the behavior of PLpro. If this property of these drugs is approved in further subclinical studies it means that PLpro may not be able to let the virus evade innate immunity. This may provide the opportunity that PRRs recognize and present the virus meticulously to adaptive immunity apparatus. Furthermore, this combination may reduce the probability of binding of the virus to ACE2.

This modeling in silico study does not mean with certainty that these drugs would treat ARDS in COVID19. According to the proposed theory and these findings we were to shed a light on a novel route that might decline the fatality rate of this disease. Besides, these therapeutic measures do not obviate any need for future investigations in finding immediate novel anti-viral drugs or vaccine.

**Acknowledgement:**

The authors would like to dedicate this article to:

Physicians and all medical personnel around the world especially to the authorities and medical staff at Bazarganan Hospital, Tehran, Iran, who have been sacrificing their lives to give special medical care to patients with COVID19. Among them we would like to name our colleagues at this hospital:

a. Dr. Nasser Iravanimanesh, CEO, Head of the Hospital,
b. Dr. Reza Ebadi, Infectious Disease Specialist,
c. Dr. Maryam Afkar, Radiologist,
d. Emergency Medicine Specialists,
e. All the personnel of ICU (specially the respectable nurses), Imaging Department and Emergency Room,
References:

1) Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine. 2020 Jan 24.

2) Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Version 2. Nat Microbiol. 2020;5(4):536–44. doi: 10.1038/s41564-020-0695-z. Epub 2020 Mar 2. PMCID: PMC7095448.

3) Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging microbes & infections. 2020 Jan 1;9(1):221-36.

4) Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronavirus. Nature reviews Microbiology. 2019 Mar;17(3):181-92.

5) Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020 Feb 22;395(10224):565-74.

6) Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DS, Du B. Clinical characteristics of coronavirus disease 2019 in China. New England Journal of Medicine. 2020 Feb 28.

7) Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet respiratory medicine. 2020 Apr 1;8(4):420-2.

8) Chung M, Bernheim A, Mei X, Zhang N, Huang M, Zeng X, Cui J, Xu W, Yang Y, Fayad ZA, Jacobi A. CT imaging features of 2019 novel coronavirus (2019-nCoV). Radiology. 2020 Apr;295(1):202-7.

9) Snijder EJ, Decroly E, Ziebuhr J. The nonstructural proteins directing coronavirus RNA synthesis and processing. In Advances in virus research 2016 Jan 1 (Vol. 96, pp. 59-126). Academic Press.

10) Mielech AM, Chen Y, Mesecar AD, Baker SC. Nidovirus papain-like proteases: multifunctional enzymes with protease, deubiquitinating and deISGylating activities. Virus research. 2014 Dec 19;194:184-90.

11) Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. InCoronaviruses 2015 (pp. 1-23). Humana Press, New York, NY.

12) Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral research. 2018 Jan 1;149:58-74.

13) Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Mar;579(7798):270-3.

14) Li SW, Wang CY, Jou YJ, Huang SH, Hsiao LH, Wan L, Lin YJ, Kung SH, Lin CW. SARS coronavirus papain-like protease inhibits the TLR7 signaling pathway through removing Lys63-linked polyubiquitination of TRAF3 and TRAF6. International journal of molecular sciences. 2016 May;17(5):678.
15) Blanco-Melo D, Nilsson-Payant B, Liu WC, Moeller R, Panis M, Sachs D, Albrecht R. SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems. bioRxiv. 2020 Jan 1.

16) Yoshikawa T, Hill TE, Yoshikawa N, Popov VL, Galindo CL, Garner HR, Peters CJ, Tseng CT. Dynamic innate immune responses of human bronchial epithelial cells to severe acute respiratory syndrome-associated coronavirus infection. PLoS one. 2010;5(1).

17) Li F. Structure, function, and evolution of coronavirus spike proteins. Annual review of virology. 2016 Sep 29;3:237-61.

18) Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications. 2020 Mar 27;11(1):1-2.

19) White JM, Delos SE, Brecher M, Schornberg K. Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. Critical reviews in biochemistry and molecular biology. 2008 Jan 1;43(3):189-219.

20) Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral research. 2020 Apr 1;176:104742.

21) Li X. A furin cleavage site was discovered in the S protein of the 2019 novel 248 coronavirus. Chinese Journal of Bioinformatics (In Chinese).;18:1-4.

22) Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov;426(6965):450-4.

23) Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of virology. 2020 Mar 17;94(7).

24) Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Wong SK, Huang IC, Xu K, Vasilieva N, Murakami A. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. The EMBO journal. 2005 Apr 20;24(8):1634-43.

25) Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 Mar 13;367(6483):1260-3.

26) Marshall RP. The pulmonary renin-angiotensin system. Current pharmaceutical design. 2003 Apr 1;9(9):715-22.

27) Parodi-Rullan R, Barreto-Torres G, Ruiz L, Casasnovas J, Javadov S. Direct renin inhibition exerts an anti-hypertrophic effect associated with improved mitochondrial function in post-infarction heart failure in diabetic rats. Cellular Physiology and Biochemistry. 2012;29(5-6):841-50.

28) Goossens GH, Blaak EE, Arner P, Saris WH, Van Baak MA. Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. International journal of obesity. 2007 Feb;31(2):382-4.

29) Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circulation research. 2000 Sep 1;87(5):e1-9.
30) Reddy Gaddam R, Chambers S, Bhatia M. ACE and ACE2 in inflammation: a tale of two enzymes. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy). 2014 Aug 1;13(4):224-34.
31) Riviere G, Michaud A, Breton C, VanCamp G, Laborie C, Enache M, Lesage J, Deloof S, Corvol P, Vieau D. Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension in the adult rat. Hypertension. 2005 Nov 1;46(5):1169-74.
32) Arendse LB, Danser AJ, Poglitsch M, Touyz RM, Burnett JC, Llorens-Cortes C, Ehlers MR, Sturrock ED. Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. Pharmacological reviews. 2019 Oct 1;71(4):539-70.
33) Dilauro M, Burns KD. Angiotensin-(1-7) and its effects in the kidney. The Scientific World Journal. 2009;9:522-35.
34) Bader M. ACE2, angiotensin-(1–7), and Mas: the other side of the coin. Pflügers Archiv-European Journal of Physiology. 2013 Jan 1;465(1):79-85.
35) Watanabe T, Barker TA, Berk BC. Angiotensin II and the endothelium: diverse signals and effects. Hypertension. 2005 Feb 1;45(2):163-9.
36) Uhal BD, Li X, Piasecki CC, Molina-Molina M. Angiotensin signalling in pulmonary fibrosis. The international journal of biochemistry & cell biology. 2012 Mar 1;44(3):465-8.
37) Reddy R, Asante I, Liu S, Parikh P, Liebler J, Borok Z, Rodgers K, Baydur A, Louie SG. Circulating angiotensin peptides levels in Acute Respiratory Distress Syndrome correlate with clinical outcomes: A pilot study. PloS one. 2019;14(3).
38) Manabe S, Okura T, Watanabe S, Fukuoka T, Higaki J. Effects of angiotensin II receptor blockade with valsartan on pro-inflammatory cytokines in patients with essential hypertension. Journal of cardiovascular pharmacology. 2005 Dec 1;46(6):735-9.
39) Nahmod KA, Vermeulen ME, RAIDEN S, Salamone G, Gamberale R, Fernández-Calotti P, Alvarez A, Nahmod V, Giordano M, Geffner JR. Control of dendritic cell differentiation by angiotensin II. The FASEB Journal. 2003 Mar;17(3):491-3.
40) Skurk T, van Harmelen V, Hauner H. Angiotensin II stimulates the release of interleukin-6 and interleukin-8 from cultured human adipocytes by activation of NF-κB. Arteriosclerosis, thrombosis, and vascular biology. 2004 Jul 1;24(7):1199-203.
41) SA Capettini L, Montecucco F, Mach F, Stergiopulos N, AS Santos R, F da Silva R. Role of renin-angiotensin system in inflammation, immunity and aging. Current pharmaceutical design. 2012 Mar 1;18(7):963-70.
42) Parodi-Rullan R, Barreto-Torres G, Ruiz L, Casasnovas J, Javadov S. Direct renin inhibition exerts an anti-hypertrophic effect associated with improved mitochondrial function in post-infarction heart failure in diabetic rats. Cellular Physiology and Biochemistry. 2012;29(5-6):841-50.
43) Rosenfeld CR, Zagariya AM, Liu XT, Willis BC, Fluhart S, Vidyasagar D. Meconium increases type 1 angiotensin II receptor expression and alveolar cell death. Pediatric research. 2008 Mar;63(3):251-6.
44) Santos RA, Ferreira AJ, Verano-Braga T, Bader M. Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. J endocrinol. 2013 Jan 18;216(2):R1-7.
45) Imai Y, Kuba K, Penninger JM. The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Experimental physiology. 2008 May 1;93(5):543-8.

46) Arendse LB, Danser AJ, Poglitsch M, Touyz RM, Burnett JC, Llorens-Cortes C, Ehlers MR, Sturrock ED. Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. Pharmacological reviews. 2019 Oct 1;71(4):539-70.

47) Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2004 Jun;203(2):631-7.

48) Jia H. Pulmonary angiotensin-converting enzyme 2 (ACE2) and inflammatory lung disease. Shock. 2016 Sep 1;46(3):239-48.

49) Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. Journal of virology. 2005 Dec 15;79(24):15511-24.

50) Kannan S, Huang H, Seeger D, Audet A, Chen Y, Huang C, Gao H, Li S, Wu M. Alveolar epithelial type II cells activate alveolar macrophages and mitigate P. aeruginosa infection. PLoS one. 2009;4(3).

51) Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, Simmons G, Hofmann H, Kuri T, Weber F, Eichler J. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. Journal of Virology. 2010 Jan 15;84(2):1198-205.

52) Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Yamamoto N, Sasazuki T, Ishizaka Y. Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. Proceedings of the National Academy of Sciences. 2008 Jun 3;105(22):7809-14.

53) Palau V, Riera M, Soler MJ. ADAM17 inhibition may exert a protective effect on COVID-19. Nephrology Dialysis Transplantation. 2020 Apr 15.

54) Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020 Mar 5.

55) Deshotels MR, Xia H, Sriramula S, Lazartigues E, Filipeanu CM. Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an angiotensin II type 1 receptor–dependent mechanism. Hypertension. 2014 Dec;64(6):1368-75.

56) Coleman CM, Sisk JM, Mingo RM, Nelson EA, White JM, Frieman MB. Abelson kinase inhibitors are potent inhibitors of severe acute respiratory syndrome coronavirus and middle east respiratory syndrome coronavirus fusion. Journal of virology. 2016 Oct 1;90(19):8924-33.

57) Napier RJ, Norris BA, Swimm A, Giver CR, Harris WA, Laval J, Napier BA, Patel G, Crump R, Peng Z, Bornmann W. Low doses of imatinib induce myelopoiesis and enhance host anti-microbial immunity. PLoS pathogens. 2015 Mar;11(3).

58) Kim IK, Rhee CK, Yeo CD, Kang HH, Lee DG, Lee SH, Kim JW. Effect of tyrosine kinase inhibitors, imatinib and nilotinib, in murine lipopolysaccharide-induced
acute lung injury during neutropenia recovery. Critical Care. 2013 Jun 1;17(3):R114.

59) The Protein Data Bank H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne (2000) Nucleic Acids Research, 28: 235-242. doi:10.1093/nar/28.1.235

60) Lan J2, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020 Mar 30:1-9.

61) Towler P3, Staker B, Prasad SG, Menon S, Tang J, Parsons T, Ryan D, Fisher M, Williams D, Dales NA, Patane MA. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. Journal of Biological Chemistry. 2004 Apr 23;279(17):17996-8007.

62) Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y. Structure of Mpro from COVID-19 virus and discovery of its inhibitors. bioRxiv. 2020 Jan 1.

63) Wang Z, Canagarajah BJ, Boehm JC, Kassisà S, Cobb MH, Young PR, Abdel-Meguid S, Adams JL, Goldsmith EJ. Structural basis of inhibitor selectivity in MAP kinases. Structure. 1998 Sep 15;6(9):1117-28.

64) Van Lam6 van T, Ivanova T, Hardes K, Heindl MR, Morty RE, Böttcher-Friebertshäuser E, Lindberg I, Than ME, Dahms SO, Steinmetzer T. Design, synthesis, and characterization of macrocyclic inhibitors of the proprotein convertase furin. ChemMedChem. 2019 Mar 22;14(6):673-85.

65) Ghosh 7AK, Takayama J, Rao KV, Ratia K, Chaudhuri R, Mulhearn DC, Lee H, Nichols DB, Baijii S, Baker SC, Johnson ME. Severe acute respiratory syndrome coronavirus papain-like novel protease inhibitors: design, synthesis, protein–ligand X-ray structure and biological evaluation. Journal of medicinal chemistry. 2010 Jul 8;53(13):4968-79.

66) Wingler8 LM, Skiba MA, McMahon C, Staus DP, Kleinhenz AL, Suomivuori CM, Latorraca NR, Dror RO, Lefkowitz RJ, Kruse AC. Angiotensin and biased analogs induce structurally distinct active conformations within a GPCR. Science. 2020 Feb 21;367(6480):888-92.

67) UCSF 9Chimera--a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.

68) Delano, 10W. L. The PyMOL Molecular Graphics System; DeLano Scientific: San Carlos, CA, 2002.

69) Guex, 11N. and Peitsch, M.C. (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 18, 2714-2723.

70) Kim S, Chen J, 12Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L. PubChem 2019 update: improved access to chemical data. Nucleic acids research. 2019 Jan 8;47(D1):D1102-9.

71) Wishart DS, 13Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, Assempour N. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic acids research. 2018 Jan 4;46(D1):D1074-82.

72) Nagy, Á. 14Density functional. Theory and Application to Atoms and Molecules. Phys. Rep. 1998, 298, 1–79.
73) Becke 15AD. Density-functional exchange-energy approximation with correct asymptotic behavior. Physical review A. 1988 Sep 1;38(6):3098.
74) McKean 16DC, Edwards HG, Lewis IR, Mastryukov VS, Boggs JE, Leong MK. Infrared and Raman spectra of 1, 1-dibromodisilanes and STO-3G+ calculations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 1996 Feb 1;52(2):199-205.
75) Frisch17 MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery Jr JA, Vreven T, Kudin KN, Burant JC, Millam JM. Gaussian 03, Revision C. 02. Wallingford, CT: Gaussian. Inc.[Google Scholar]. 2004.
76) O'Boyle18 2NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. Journal of cheminformatics. 2011 Dec;3(1):33.
77) Morris 19GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry. 2009 Dec;30(16):2785-91.
78) Berendsen20 HJ, van der Spoel D, van Drunen R. GROMACS: a message-passing parallel molecular dynamics implementation. Computer physics communications. 1995 Sep 2;91(1-3):43-56.
79) van 21Gunsteren WF, Daura X, Mark AE. GROMOS force field. Encyclopedia of computational chemistry. 2002 Apr 15;2.
80) Dolatkhah22 Z, Javanshir S, Sadr AS, Hosseini J, Sardari S. Synthesis, molecular docking, molecular dynamics studies, and biological evaluation of 4 h-chromone-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate derivatives as potential antileukemic agents. Journal of chemical information and modeling. 2017 Jun 26;57(6):1246-57.
81) Turner 23PJ. XMGRACE, Version 5.1. 19. Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton, OR. 2005.
82) Wallace 24AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein engineering, design and selection. 1995 Feb 1;8(2):127-34.
83) Stierand 25K, Rarey M. PoseView--molecular interaction patterns at a glance. Journal of cheminformatics. 2010 May;2(1):1-.
84) Kanehisa26 M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research. 2000 Jan 1;28(1):27-30.
85) Pan Y, Zhang D, Yang P, Poon LL, Wang Q. Viral load of SARS-CoV-2 in clinical samples. The Lancet Infectious Diseases. 2020 Apr 1;20(4):411-2.
86) Mossel EC, Wang J, Jeffers S, Edeen KE, Wang S, Cosgrove GP, Funk CJ, Manzer R, Miura TA, Pearson LD, Holmes KV. SARS-CoV replicates in primary human alveolar epithelial II cell cultures but not in type I-like cells. Virology. 2008 Mar 1;372(1):127-35.
87) Chuquimia OD, Petursdottir DH, Rahman MJ, Hartl K, Singh M, Fernández C. The role of alveolar epithelial cells in initiating and shaping pulmonary immune responses: communication between innate and adaptive immune systems. PLoS one. 2012;7(2).
88) Zhao MQ, Amir MK, Rice WR, Enelow RI. Type II Pneumocyte–CD8+ T-Cell Interactions: Relationship between Target Cell Cytotoxicity and Activation. American journal of respiratory cell and molecular biology. 2001 Sep 1;25(3):362-9.
89) Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, Qi F, Bao L, Du L, Liu S, Qin C. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Research. 2020 Mar 30:1-3.

90) Hackbart M, Deng X, Baker SC. Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors. Proceedings of the National Academy of Sciences. 2020 Apr 7;117(14):8094-103.

91) Manfredi AA, Capobianco A, Esposito A, De Cobelli F, Canu T, Monno A, Raucci A, Sanvito F, Doglioni C, Nawroth PP, Bierhaus A. Maturing dendritic cells depend on RAGE for in vivo homing to lymph nodes. The Journal of Immunology. 2008 Feb 15;180(4):2270-5.

92) Martin-Fontecha A, Lanzavecchia A, Sallusto F. Dendritic cell migration to peripheral lymph nodes. In Dendritic Cells 2009 (pp. 31-49). Springer, Berlin, Heidelberg.

93) Chung M, Bernheim A, Mei X, Zhang N, Huang M, Zeng X, Cui J, Xu W, Yang Y, Fayad ZA, Jacobi A. CT imaging features of 2019 novel coronavirus (2019-nCoV). Radiology. 2020 Apr;295(1):202-7.

94) Zhou S, Wang Y, Zhu T, Xia L. CT features of coronavirus disease 2019 (COVID-19) pneumonia in 62 patients in Wuhan, China. American Journal of Roentgenology. 2020 Mar 5:1-8.

95) Ng MY, Lee EY, Yang J, Yang F, Li X, Wang H, Lui MM, Lo CS, Leung B, Khong PL, Hui CK. Imaging profile of the COVID-19 infection: radiologic findings and literature review. Radiology: Cardiothoracic Imaging. 2020 Feb 13;2(1):e200034.

96) Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiol. Mol. Biol. Rev. 2012 Mar 1;76(1):16-32.

97) Tufet M. T cells calm the storm. Nature Reviews Immunology. 2007 Nov;7(11):834-5.

98) Kim KD, Zhao J, Auh S, Yang X, Du P, Tang H, Fu YX. Adaptive immune cells temper initial innate responses. Nature medicine. 2007 Oct;13(10):1248-52.

99) Weitnauer M, Mijošek V, Dalpke AH. Control of local immunity by airway epithelial cells. Mucosal immunology. 2016 Mar;9(2):287-98.

100) Ng ML, Tan SH, See EE, Ooi EE, Ling AE. Proliferative growth of SARS coronavirus in Vero E6 cells. Journal of General Virology. 2003 Dec 1;84(12):3291-303.

101) Wang X, Xu W, Hu G, Xia S, Sun Z, Liu Z, Xie Y, Zhang R, Jiang S, Lu L. SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cellular & Molecular Immunology. 2020 Apr 7;1:1-3.

102) Zhou J, Chu H, Chan JF, Yuen KY. Middle East respiratory syndrome coronavirus infection: virus-host cell interactions and implications on pathogenesis. Virology journal. 2015 Dec 1;12(1):218.

103) Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nature medicine. 2005 Aug;11(8):875-9.

104) Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China Life Sciences. 2020 Mar;63(3):364-74.
105) Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005 Jul;436(7047):112-6.

106) Zhang R, Pan Y, Fanelli V, Wu S, Luo AA, Islam D, Han B, Mao P, Ghazarian M, Zeng W, Spieth PM. Mechanical stress and the induction of lung fibrosis via the midkine signaling pathway. American journal of respiratory and critical care medicine. 2015 Aug 1;192(3):315-23.

107) Re RN. Role of intracellular angiotensin II. American Journal of Physiology-Heart and Circulatory Physiology. 2018 Apr 1;314(4):H766-71.

108) Abadir PM, Foster DB, Crow M, Cooke CA, Rucker JJ, Jain A, Smith BJ, Burks TN, Cohn RD, Fedarko NS, Carey RM. Identification and characterization of a functional mitochondrial angiotensin system. Proceedings of the National Academy of Sciences. 2011 Sep 6;108(36):14849-54.

109) Garrido AM, Griendling KK. NADPH oxidases and angiotensin II receptor signaling. Molecular and cellular endocrinology. 2009 Apr 29;302(2):148-58.

110) Fazeli G, Stopper H, Schinzel R, Ni CW, Jo H, Schupp N. Angiotensin II induces DNA damage via AT1 receptor and NADPH oxidase isoform Nox4. Mutagenesis. 2012 Nov 1;27(6):673-81.

111) Koka V, Huang XR, Chung AC, Wang W, Truong LD, Lan HY. Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. The American journal of pathology. 2008 May 1;172(5):1174-83.

112) Patel VB, Clarke N, Wang Z, Fan D, Parajuli N, Basu R, Putko B, Kassiri Z, Turner AJ, Oudit GY. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. Journal of molecular and cellular cardiology. 2014 Jan 1;66:167-76.

113) Bernard K, Hecker L, Luckhardt TR, Cheng G, Thannickal VJ. NADPH oxidases in lung health and disease. Antioxidants & redox signaling. 2014 Jun 10;20(17):2838-53.

114) Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cellular signalling. 2012 May 1;24(5):981-90.

115) Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: key regulators in vascular health and diseases. British journal of pharmacology. 2018 Apr;175(8):1279-92.

116) Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. Critical Care. 2017; 21:305.

117) Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. The FASEB Journal. 2003 Jul;17(10):1195-214.

118) Cadet J, Davies KJ. Oxidative DNA damage & repair: an introduction. Free Radical Biology and Medicine. 2017 Jun 1;107:2-12.

119) Benigni A, Corna D, Zoa C, Sonzogni A, Latini R, Salio M, Conti S, Rottoli D, Longaretti L, Cassis P, Morigi M. Disruption of the Ang II type 1 receptor promotes longevity in mice. The Journal of clinical investigation. 2009 Mar 2;119(3):524-30.

120) Vajapey R, Rini D, Walston J, Abadir P. The impact of age-related dysregulation of the angiotensin system on mitochondrial redox balance. Frontiers in physiology. 2014 Nov 24;5:439.
121) Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. Journal of Experimental Medicine. 2011 Mar 14;208(3):417-20.

122) Kim HJ, Kim CH, Ryu JH, Kim MJ, Park CY, Lee JM, Holtzman MJ, Yoon JH. Reactive oxygen species induce antiviral innate immune response through IFN-λ regulation in human nasal epithelial cells. American journal of respiratory cell and molecular biology. 2013 Nov;49(5):855-65.

123) Shi Q, Lei Z, Cheng G, Li D, Wang Q, Luo S, Yang H, Jia H. Mitochondrial ROS activate interleukin-1β expression in allergic rhinitis. Oncology letters. 2018 Sep 1;16(3):3193-200.

124) Bernstein KE, Khan Z, Giani JF, Cao DY, Bernstein EA, Shen XZ. Angiotensin-converting enzyme in innate and adaptive immunity. Nature Reviews Nephrology. 2018 May;14(5):325.

125) Takahashi M, Suzuki E, Takeda R, Oba S, Nishimatsu H, Kimura K, Nagano T, Nagai R, Hirata Y. Angiotensin II and tumor necrosis factor-α synergistically promote monocyte chemoattractant protein-1 expression: roles of NF-κB, p38, and reactive oxygen species. American Journal of Physiology-Heart and Circulatory Physiology. 2008 Jun;294(6):H2879-88.

126) Mayr M, Duerrschnid C, Medrano G, Taffet GE, Wang Y, Entman ML, Haudek SB. TNF/Ang-II synergy is obligate for fibroinflammatory pathology, but not for changes in cardiorenal function. Physiological reports. 2016 Apr;4(8):e12765.

127) Ishizaka N, Saito K, Mori I, Matsuzaki G, Ohno M, Nagai R. Iron chelation suppresses ferritin upregulation and attenuates vascular dysfunction in the aorta of angiotensin II–infused rats. Arteriosclerosis, thrombosis, and vascular biology. 2005 Nov 1;25(11):2282-8.

128) Tajima S, Tsuchiya K, Horinouchi Y, Ishizawa K, Ikeda Y, Kihira Y, Shono M, Kawazoe K, Tomita S, Tamaki T. Effect of angiotensin II on iron-transporting protein expression and subsequent intracellular labile iron concentration in human glomerular endothelial cells. Hypertension Research. 2010 Jul;33(7):713-21.

129) Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. International immunology. 2017 Sep;29(9):401-9.

130) Sauter NS, Thienel C, Plutino Y, Kampe K, Dror E, Traub S, Timper K, Bédat B, Pattou F, Kerr-Conte J, Jehle AW. Angiotensin II induces interleukin-1β–mediated islet inflammation and β-cell dysfunction independently of vasoconstrictive effects. Diabetes. 2015 Apr 1;64(4):1273-83.

131) Guo F, Chen X L, Wang F, et al. Role of Angiotensin II Type 1 Receptor in Angiotensin II-Induced Cytokine Production in Macrophages. J Interferon Cytokine Res 2011; 31(4): 351-361

132) Velazquez-Salinas L, Verdugo-Rodríguez A, Rodriguez L L, Borca M V. The Role of Interleukin 6 During Viral Infections. Front Microbiol. 2019; 10: article 1057

133) Deng J, Wang DX, Deng W, Li CY, Tong J. The effect of endogenous angiotensin II on alveolar fluid clearance in rats with acute lung injury. Canadian respiratory journal. 2012;19(5):311-8.

134) Weidanz JO, Jacobson LM, Muehrer RJ, Djamali A, Hullett DA, Sprague J, Chiriva-Internati M, Wittman V, Thekkumkara TJ, Becker BN. AT1R blockade reduces IFN-γ production in lymphocytes in vivo and in vitro. Kidney international. 2005 Jun 1;67(6):2134-42.
135) Lighter J, Phillips M, Hochman S, Sterling S, Johnson D, Francois F, Stachel A. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission. Clinical Infectious Diseases. 2020 Apr 9.

136) Senchenkova EY, Russell J, Yildirim A, Granger DN, Gavins FN. Novel Role of T Cells and IL-6 (Interleukin-6) in Angiotensin II–Induced Microvascular Dysfunction. Hypertension. 2019 Apr;73(4):829-38.

137) Klok FA, Kruip MJ, van der Meer NJ, Arbous MS, Gommers DA, Kant KM, Kaptein FH, van Paassen J, Stals MA, Huisman MV, Endeman H. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thrombosis Research. 2020 Apr 10.

138) Herold T, Jurinovic V, Arnreich C, Hellmuth JC, von Bergwelt-Baildon M, Klein M, Weinberger T. Level of IL-6 predicts respiratory failure in hospitalized symptomatic COVID-19 patients. medRxiv. 2020 Jan 1.

139) Eklund CM. Proinflammatory cytokines in CRP baseline regulation. Advances in clinical chemistry. 2009 Jan 1;48:111-36.

140) Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arteriosclerosis, thrombosis, and vascular biology. 2002 Oct 1;22(10):1668-73.

141) Zhao J, Liu J, Pang X, Wang S, Wu D, Zhang X, Feng L. Angiotensin II induces C-reactive protein expression via AT1-ROS-MAPK-NF-κB signal pathway in hepatocytes. Cellular Physiology and Biochemistry. 2013;32(3):569-80.

142) Martinez NE, Sato F, Kawai E, Omura S, Chervenak RP, Tsunoda I. Regulatory T cells and Th17 cells in viral infections: implications for multiple sclerosis and myocarditis. Future virology. 2012 Jun;7(6):593-608.

143) Qin XY, Zhang YL, Chi YF, Yan B, Zeng XJ, Li HH, Liu Y. Angiotensin II regulates Th1 T cell differentiation through angiotensin II type 1 receptor-PKA-mediated activation of proteasome. Cellular Physiology and Biochemistry. 2018;45(4):1366-76.

144) Zheng Y, Sun L, Jiang T, Zhang D, He D, Nie H. TNFα promotes Th17 cell differentiation through IL-6 and IL-1β produced by monocytes in rheumatoid arthritis. Journal of immunology research. 2014;2014.

145) Li JT, Melton AC, Su G, Hamm DE, LaFemina M, Howard J, Fang X, Bhat S, Huynh KM, O’Kane CM, Ingram RJ. Unexpected role for adaptive αβTh17 cells in acute respiratory distress syndrome. The Journal of Immunology. 2015 Jul 1;195(1):87-95.

146) Jurewicz M, McDermott DH, Sechler JM, Tinckam K, Takakura A, Carpenter CB, Milford E, Abdi R. Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II–induced inflammation. Journal of the American Society of Nephrology. 2007 Apr 1;18(4):1093-102.

147) Lukkarinen HP, Laine J, Aho H, Zagariya A, Vidyasagar D, Kääpä PO. Angiotensin II receptor inhibition prevents pneumocyte apoptosis in surfactant-depleted rat lungs. Pediatric pulmonology. 2005 Apr;39(4):349-58.

148) Hou W, Jin YH, Kang HS, Kim BS. Interleukin-6 (IL-6) and IL-17 synergistically promote viral persistence by inhibiting cellular apoptosis and cytotoxic T cell function. Journal of virology. 2014 Aug 1;88(15):8479-89.

149) Ren W, Wang Z, Wu Z, Hu Z, Dai F, Chang J, Li B, Liu H, Ruan Y. JAK2/STAT3 pathway was associated with the protective effects of IL-22 on aortic dissection with acute lung injury. Disease markers. 2017;2017.
150) Browatzki M, Larsen D, Pfeiffer CA, Gehrke SG, Schmidt J, Kranzhöfer A, Katus HA, Kranzhöfer R. Angiotensin II stimulates matrix metalloproteinase secretion in human vascular smooth muscle cells via nuclear factor-κB and activator protein 1 in a redox-sensitive manner. Journal of vascular research. 2005;42(5):415-23.

151) Wang C, Qian X, Sun X, Chang Q. Angiotensin II increases matrix metalloproteinase 2 expression in human aortic smooth muscle cells via AT1R and ERK1/2. Experimental Biology and Medicine. 2015 Dec;240(12):1564-71.

152) Wang XM, Shi K, Li JJ, Chen TT, Guo YH, Liu YL, Yang YF, Yang S. Effects of angiotensin II intervention on MMP-2, MMP-9, TIMP-1, and collagen expression in rats with pulmonary hypertension. Genet Mol Res. 2015 Mar 6;14(1):1707-17.

153) Guo YS, Wu ZG, Yang JK, Chen XJ. Impact of losartan and angiotensin II on the expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in rat vascular smooth muscle cells. Molecular medicine reports. 2015 Mar 1;11(3):1587-94.

154) Hung YH, Hsieh WY, Hsieh JS, Liu C, Tsai CH, Lu LC, Huang CY, Wu CL, Lin CS. Alternative roles of STAT3 and MAPK signaling pathways in the MMPs activation and progression of lung injury induced by cigarette smoke exposure in ACE2 knockout mice. International journal of biological sciences. 2016;12(4):454.

155) Yamamoto S, Yancey PG, Zuo Y, Ma LJ, Kaseda R, Fogo AB, Ichikawa I, Linton MF, Fazio S, Kon V. Macrophage polarization by angiotensin II-type 1 receptor aggravates renal injury-acceleration of atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2011 Dec;31(12):2856-64.

156) Keidar S, Heinrich R, Kaplan M, Aviram M. Oxidative stress increases the expression of the angiotensin-II receptor type 1 in mouse peritoneal macrophages. Journal of the Renin-Angiotensin-Aldosterone System. 2002 Mar;3(1):24-30.

157) Anwar A, Keating AK, Joung D, Sather S, Kim GK, Sawczyn KK, Brandao L, Henson PM, Graham DK. Mer tyrosine kinase (MerTK) promotes macrophage survival following exposure to oxidative stress. Journal of leukocyte biology. 2009 Jul;86(1):73-9.

158) Zhang Y, Wang Y, Zhou D, Zhang LS, Deng FX, Shu S, Wang LJ, Wu Y, Guo N, Zhou J, Yuan ZY. Angiotensin II deteriorates advanced atherosclerosis by promoting MerTK cleavage and impairing efferocytosis through the AT1R/ROS/p38 MAPK/ADAM17 pathway. American Journal of Physiology-Cell Physiology. 2019 Oct 1;317(4):C776-87.

159) Wang J, Chen L, Chen B, Meliton A, Liu SQ, Shi Y, Liu T, Deb DK, Solway J, Li YC. Chronic activation of the renin-angiotensin system induces lung fibrosis. Scientific reports. 2015 Oct 23;5:15561.

160) Proto JD, Doran AC, Gusarova G, Yurdagul Jr A, Sozen E, Subramanian M, Islam MN, Rymond CC, Du J, Hook J, Kuriakose G. Regulatory T cells promote macrophage efferocytosis during inflammation resolution. Immunity. 2018 Oct 16;49(4):666-77.

161) Murphy AM, Wong AL, Bezuhlly M. Modulation of angiotensin II signaling in the prevention of fibrosis. Fibrogenesis & tissue repair. 2015 Dec 1;8(1):7.

162) Sosulski ML, Gongora R, Feghali-Bostwick C, Lasky JA, Sanchez CG. Sirtuin 3 deregulation promotes pulmonary fibrosis. The Journals of Gerontology: Series A. 2017 May 1;72(5):595-602.
163) Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, Hall R, Poirier G, Ronco JJ, Tidswell M, Hardes K. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Critical Care. 2017 Dec;21(1):234.

164) Ruthman CA, Festic E. Emerging therapies for the prevention of acute respiratory distress syndrome. Therapeutic advances in respiratory disease. 2015 Aug;9(4):173-87.

165) Ruthman CA, Festic E. Emerging therapies for the prevention of acute respiratory distress syndrome. Therapeutic advances in respiratory disease. 2015 Aug;9(4):173-87.

166) Kim J, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK, Lee SM. Effect of Renin-Angiotensin System Blockage in Patients with Acute Respiratory Distress Syndrome: A Retrospective Case Control Study. Korean journal of critical care medicine. 2017 May;32(2):154.

167) Garcia GE. ANG II receptor antagonists as modulators of macrophages polarization. American Journal of Physiology-Renal Physiology. 2010 Apr;298(4):F868-9.

168) Mitra AK, Gao L, Zucker IH. Angiotensin II-induced upregulation of AT1 receptor expression: sequential activation of NF-κB and Elk-1 in neurons. American Journal of Physiology-Cell Physiology. 2010 Sep;299(3):C561-9.

169) Wang F, Huang L, Peng ZZ, Tang YT, Lu MM, Peng Y, Mei WJ, Wu L, Mo ZH, Meng J, Tao LJ. Losartan inhibits LPS+ ATP-induced IL-1beta secretion from mouse primary macrophages by suppressing NALP3 inflammasome. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2014 Sep 10;69(9):680-4.

170) Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation. 2005 May 24;111(20):2605-10.

171) Soler MJ, Ye M, Wysocki J, William J, Lloveras J, Battle D. Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan. American Journal of Physiology-Renal Physiology. 2009 Feb;296(2):F398-405.

172) Guo, J., Huang, Z., Lin, L. and Lv, J., 2020. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease: A Viewpoint on the Potential Influence of Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers on Onset and Severity of Severe Acute Respiratory Syndrome Coronavirus 2 Infection. Journal of the American Heart Association, 9(7), p.e016219.

173) Sanchis-Gomar F, Lavie CJ, Perez-Quilis C, Henry BM, Lippi G. Angiotensin-Converting Enzyme 2 and Antihypertensives (Angiotensin Receptor Blockers and Angiotensin-Converting Enzyme Inhibitors) in Coronavirus Disease 2019. InMayo Clinic Proceedings 2020 Apr 4. Elsevier.

174) Perico L, Benigni A, Remuzzi G. Should Covid-19 concern nephrologists? Why and to what extent? The emerging impasse of angiotensin blockade. Nephron. 2020 Mar 23:1-9.

175) Vaduganathan M, Vardeny O, Michel T, McMurray JJ, Pfeffer MA, Solomon SD. Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. New England Journal of Medicine. 2020 Mar 30.
176) South AM, Tomlinson L, Edmonston D, Hiremath S, Sparks MA. Controversies of renin–angiotensin system inhibition during the COVID-19 pandemic. Nature Reviews Nephrology. 2020 Apr 3;1-3.

177) Veronese N, Demurtas J, Yang L, Tonelli R, Barbagallo M, Lopalco P, Lagolio E, Celotto S, Pizzol D, Zou L, Tully MA. Use of Corticosteroids in Coronavirus Disease 2019 Pneumonia: A Systematic Review of the Literature. Frontiers in Medicine. 2020 Apr 24;7:170.

178) Liu Q, Zhou YH, Yang ZQ. The cytokine storm of severe influenza and development of immunomodulatory therapy. Cellular & molecular immunology. 2016 Jan;13(1):3-10.

179) de Castro Kroner J, Knoke K, Kofler DM, Steiger J, Fabri M. Glucocorticoids promote intrinsic human TH17 differentiation. Journal of Allergy and Clinical Immunology. 2018 Nov 1;142(5):1669-73.

180) Coleman CM, Sisk JM, Mingo RM, Nelson EA, White JM, Frieman MB. Abelson kinase inhibitors are potent inhibitors of severe acute respiratory syndrome coronavirus and middle east respiratory syndrome coronavirus fusion. Journal of virology. 2016 Oct 1;90(19):8924-33.

181) Ruella M, Kenderian SS, Shestova O, Klichinsky M, Melenhorst JJ, Wasik MA, Lacey SF, June CH, Gill S. Kinase inhibitor ibrutinib to prevent cytokine-release syndrome after anti-CD19 chimeric antigen receptor T cells for B-cell neoplasms. Leukemia. 2017 Jan;31(1):246-8.

182) Rizzo AN, Sammani S, Esquinca AE, Jacobson JR, Garcia JG, Letsiou E, Dudek SM. Imatinib attenuates inflammation and vascular leak in a clinically relevant two-hit model of acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015 Dec 1;309(11):L1294-304.

183) Stephens RS, Johnston L, Servinsky L, Kim BS, Damarla M. The tyrosine kinase inhibitor imatinib prevents lung injury and death after intravenous LPS in mice. Physiological reports. 2015 Nov;3(11):e12589.

184) Rizzo AN, Aman J, van Nieuw Amerongen GP, Dudek SM. Targeting Abl kinases to regulate vascular leak during sepsis and acute respiratory distress syndrome. Arteriosclerosis, thrombosis, and vascular biology. 2015 May;35(5):1071-9.

185) Arita S, Arita N, Hikasa Y. Therapeutic effect of low-dose imatinib on pulmonary arterial hypertension in dogs. The Canadian Veterinary Journal. 2013 Mar;54(3):255.

186) Pitsiou G, Zarogoulidis P, Petridis D, Kioumis I, Lampaki S, Organtzis J, Porpodis K, Papaiwannou A, Tsiouda T, Hohenforst-Schmidt W, Kakolyris S. Inhaled tyrosine kinase inhibitors for pulmonary hypertension: a possible future treatment. Drug design, development and therapy. 2014;8:1753.

187) Sun T, Chen S, Dong N, Zhou X, Li H. Imatinib inhibits angiotensin II-induced aortic dissection through the c-Abl signaling pathway. Int J Clin Exp Pathol. 2017 Jan 1;10(5):5316-24.

188) Appel S, Rupf A, Weck MM, Schoor O, Brümmedorf TH, Weinschenk T, Grünebach F, Brossart P. Effects of imatinib on monocyte-derived dendritic cells are mediated by inhibition of nuclear factor-κB and Akt signaling pathways. Clinical Cancer Research. 2005 Mar 1;11(5):1928-40.

189) Dewar AL, Doherty KV, Hughes TP, Lyons AB. Imatinib inhibits the functional capacity of cultured human monocytes. Immunology and cell biology. 2005 Feb;83(1):48-56.
190) Berlin AA, Lukacs NW. Treatment of cockroach allergen asthma model with imatinib attenuates airway responses. American journal of respiratory and critical care medicine. 2005 Jan 1;171(1):35-9.

191) Wolf AM, Wolf D, Rumpold H, Ludwikczek S, Enrich B, Gastl G, Weiss G, Tilg H. The kinase inhibitor imatinib mesylate inhibits TNF-α production in vitro and prevents TNF-dependent acute hepatic inflammation. Proceedings of the National Academy of Sciences. 2005 Sep 20;102(38):13622-7.

192) Akashi N, Matsumoto I, Tanaka Y, Inoue A, Yamamoto K, Umeda N, Tanaka Y, Hayashi T, Goto D, Ito S, Sekiguchi K. Comparative suppressive effects of tyrosine kinase inhibitors imatinib and nilotinib in models of autoimmune arthritis. Modern rheumatology. 2011 Jun 1;21(3):267-75.

193) Larmonier N, Janikashvili N, LaCasse C J, et al. Imatinib mesylate inhibits CD4+CD25+ regulatory T cell activity and enhances active immunotherapy against BCR-ABL negative tumors. J Immunol. 2008 November 15; 181(10): 6955–6963

194) Napier RJ, Norris BA, Swimm A, Giver CR, Harris WA, Laval J, Napier BA, Patel G, Crump R, Peng Z, Bornmann W. Low doses of imatinib induce myelopoiesis and enhance host anti-microbial immunity. PLoS pathogens. 2015 Mar;11(3).

195) Sisk JM, Frieman MB, Machamer CE. Coronavirus S protein-induced fusion is blocked prior to hemifusion by Abl kinase inhibitors. The Journal of general virology. 2018 May;99(5):619.

196) Dannenberg JJ. An Introduction to Hydrogen Bonding By George A. Jeffrey (University of Pittsburgh). Oxford University Press: New York and Oxford. 1997. Page 12. ISBN 0-19-509549-9.

197) Oferkin IV, Katkova EV, Sulimov AV, Kutov DC, Sobolev SI, Voevodin VV, Sulimov VB. Evaluation of docking target functions by the comprehensive investigation of protein-ligand energy minima. Advances in bioinformatics. 2015;2015.

198) Campbell DJ, Krum H, Esler MD. Losartan increases bradykinin levels in hypertensive humans. Circulation. 2005 Jan 25;111(3):315-20.

199) Pantsar T, Poso A. Binding affinity via docking: fact and fiction. Molecules. 2018 Aug;23(8):1899.

200) Golias C, Charalabopoulos A, Stagikas D, Charalabopoulos K, Batistatou A. The kinin system-bradykinin: biological effects and clinical implications. Multiple role of the kinin system-bradykinin. Hippokratia. 2007 Jul;11(3):124.

201) Sabatini F, Luppi F, Petecchia L, Di Stefano A, Longo AM, Eva A, Vanni C, Hiemstra PS, Sterk PJ, Sorbello V, Fabbri LM. Bradykinin-induced asthmatic fibroblast/myofibroblast activities via bradykinin B2 receptor and different MAPK pathways. European journal of pharmacology. 2013 Jun 15;710(1-3):100-9.

202) Song MA, Dasgupta C, Zhang L. Chronic losartan treatment up-regulates AT1R and increases the heart vulnerability to acute onset of ischemia and reperfusion injury in male rats. PloS one. 2015;10(7).
