RESEARCH ARTICLE

Network-based Multi-omics Disease–Drug Associations Reveal Drug Repurposing Candidates for Covid-19 Disease Phases

Francis E. Agamah[1] ⊙, **Thomas H. A. Ederveen**[2]⊙, **Michelle Skelton**[1]⊙, **Darren P. Martin**[1]⊙, **Emile R. Chimusa**[3,a,*]⊙, **Peter A. C. 't Hoen**[2,a,*]⊙

[1]Computational Biology Division, Department of Integrative Biomedical Sciences, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
[2]Department of Medical BioSciences, Radboud University Medical Center, Nijmegen, The Netherlands
[3]Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle, Tyne and Wear, NE1 8ST, UK

[a]Joint corresponding authors.
*Correspondence to: Peter A. C. 't Hoen, Medical BioSciences Department, Radboud University Medical Center, Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel: +31-(0)-243619390. E-mail: Peter-Bram.tHoen@radboudumc.nl

Emile R. Chimusa, Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle, Tyne and Wear, NE1 8ST, UK. Tel: +44-(0)191-227-3831. E-mail: emile.chimusa@northumbria.ac.uk

ABSTRACT

Background: The development and rollout of vaccines and the use of various drugs have contributed to controlling the coronavirus disease 2019 (Covid-19) pandemic. Nevertheless, challenges such as the inequitable distribution of vaccines, the influence of emerging viral lineages and immunoevasive variants on vaccine efficacy, and the inadequate immune defense in subgroups of the population continue to motivate the development of new drugs to combat the disease.

Aim: In this study, we sought to identify, prioritize, and characterize drug repurposing candidates appropriate for treating mild, moderate, or severe Covid-19 using a network-based integrative approach that systematically integrates drug-related data and multi-omics datasets.

Methods: We leveraged drug data and multi-omics data and used a random walk with restart algorithm to explore an integrated knowledge graph comprising three subgraphs: (i) a Covid-19 knowledge graph, (ii) a drug repurposing knowledge graph, and (iii) a Covid-19 disease state-specific omics graph.

Results: We prioritized 20 US Food and Drug Administration-approved agents as potential candidate drugs for mild, moderate, and severe Covid-19 disease phases. Specifically, drugs that could stimulate immune cell recruitment and activation including histamine, curcumin, and paclitaxel have potential utility in mild disease states to mitigate disease progression. Drugs like omacetaxine, crizotinib, and vorinostat that exhibit antiviral properties and have the potential to inhibit viral replication can be considered for mild to moderate Covid-19 disease states. Also, given the association between antioxidant deficiency and high inflammatory factors that trigger cytokine storms, antioxidants like glutathione can be considered for moderate disease states. Drugs that exhibit potent anti-inflammatory effects like (i) anti-inflammatory drugs (sarilumab and tocilizumab), (ii) corticosteroids (dexamethasone and hydrocortisone), and (iii) immunosuppressives (sirolimus and

cyclosporine) are potential candidates for moderate to severe disease states that trigger a hyperinflammatory cascade of Covid-19.

Conclusion: Our study demonstrates that the multi-omics data-driven integrative analysis within the drug data enables prioritizing drug candidates for Covid-19 disease phases, offering a comprehensive basis for therapeutic strategies that can be brought to market quickly given their established safety profiles. Importantly, the multi-omics data-driven integrative analysis within the drug data approach implemented here can be used to prioritize drug repurposing candidates appropriate for other diseases.

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the highly contagious and virulent coronavirus responsible for the global outbreak of coronavirus disease 2019 (Covid-19). Between 2020 and 2023, Covid-19 imposed an unprecedented burden on global public health systems, and by December 2023, it had been responsible for at least 770 million reported cases and close to 7 million reported deaths.[1]

The pandemic's containment and the restoration of societal normalcy have been achieved through two key routes: (i) the development and widespread use of SARS-CoV-2 vaccines and (ii) the gradual increase in natural infection-acquired immunity. While progress has been made, critical hurdles remain, notably the need for equitable vaccine distribution and effective treatment options for those unvaccinated or immunocompromised.[2] Furthermore, there are also some concerns surrounding vaccine efficacy against a backdrop of waning immunity[2,3] and the emergence of immunoevasive viral strains.[4,5] It is now a widely held view that Covid-19 is likely to transform into another endemic human coronavirus, possibly with seasonal epidemic waves.[6] Irrespective of the number of infections that occur or the intensiveness with which vaccines are used, it remains uncertain whether true herd immunity of sorts achieved with measles and rubella will ever be achieved for Covid-19.

While data are currently being collected and analyzed to understand how newly evolved SARS-CoV-2 variants might impact the effectiveness of vaccines and the severity of future Covid-19 infection waves, there remains a demand for both host-directed and pathogen-directed drugs that could be utilized to treat the mild, moderate, and severe manifestations of the disease.

In light of this, multiple existing drugs have been sought to treat or control SARS-CoV-2 infection.[7] An example is *ritonavir-boosted nirmatrelvir* (*Paxlovid*) (DrugBank: DB16691), a protease inhibitor used for the treatment of mild to moderate Covid-19 in adults who are at high risk of developing severe symptoms.[8] Additionally, *remdesivir* (Veklury) (DrugBank: DB14761), an adenosine triphosphate (ATP) analog targeting the conserved viral RNA-dependent RNA polymerase, shortens the recovery time for adults hospitalized with Covid-19 infection and pneumonia, while also mitigating disease severity and associated mortality.[9–11] Other virus-directed antiviral drugs like *favipiravir* (DrugBank: DB12466), *molnupiravir* (DrugBank: DB15661), and *ritonavir* (DrugBank: DB00503)

may also potentially improve the health outcomes of Covid-19 patients.[12] Furthermore, a host-directed drug such as *dexamethasone* (DrugBank: DB01234), an anti-inflammatory corticosteroid, has demonstrated its effectiveness in reducing mortality among severely infected patients. It achieves this by modulating inflammation-mediated lung injury, preventing in some cases progression to respiratory failure and death.[13] Another host-directed drug, *aspirin* (DrugBank: DB00945),[14] decreases the risk of complications and mortality in hospitalized Covid-19-infected patients.[14–16]

While these drugs are mainly utilized for severe Covid-19 cases, some virus-directed monoclonal antibodies (e.g., *bebtelovimab, casirivimab*) received emergency use authorization at various stages of the pandemic for managing mild to moderate Covid-19.[17] Additionally, other monoclonal antibodies including the combination of *bebtelovimab*, *casirivimab*, and *imdevimab* and the combination of *bamlanivimab* and *etesevimab* have been useful for managing mild to moderate Covid-19 in adults.[17] These monoclonal antibodies inhibit viral entry into host cells by preventing viral attachment to human *ACE2* receptors.

Host-targeted monoclonal antibodies such as *tocilizumab* (DrugBank: DB06273) and *sarilumab* (DrugBank: DB11767),[18–21] which modulate aberrant immune responses to infection by binding to the host interleukin 6 (IL6) receptor (IL6R), have also been granted emergency use authorization. Specifically, these IL6R-binding monoclonals are used for treating hospitalized adults and pediatric patients (2 years of age and older) who are receiving systemic corticosteroids and require supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation.[21]

Although existing drugs have been recommended for managing Covid-19, concerns have arisen about post-hospitalization effects and the appropriateness of using these drugs in different Covid-19 disease phases.[22–24] Covid-19 exhibits a wide spectrum of symptoms and severities, necessitating a nuanced approach to treatment. Personalized medicine, where the right drug is administered to the right patient group at the right disease phase, could revolutionize Covid-19 treatment strategies. Hence, it would be useful to identify effective drugs that are specific to different phases of the disease, and which could also be potentially applicable to combating any future emergence of other coronaviruses.

Given that the development of new medications is a time-consuming process, the repurposing of existing medications for

other indications may prove to be a viable alternative. However, most studies that have implemented computational methods to identify drug repurposing candidates for Covid-19 have so far leveraged disease–gene associations, protein–protein interaction, and drug–target data, but less consideration is given to the interactions between other biomedical and molecular features specific to different Covid-19 disease phases, such as those recorded in large-scale multi-omics profiling efforts.[25–28] Applying computational multi-omics data-driven analysis to the repurposing of existing medications is, in fact, a potentially and highly efficient means of drug discovery since the pharmacological properties, formulations, and toxicities of such agents are already known.[25–31] In this study, we explore the utility of incorporating disease state-specific omics graphs (DSOGs) along with drug-related data to identify drug repurposing candidates for mild, moderate, or severe Covid-19 disease states. We employ MultiXrank,[32] a random walk with restart (RWR) algorithm that can combine multiple heterogeneous networks and allows for universal multi-layer network exploration. We demonstrate that this integrative multi-omics network-based approach with drug data has the potential to repurpose drugs in different disease states and can be applied to other diseases.

MATERIALS AND METHODS

Study Design and Procedures

The methodology employed in this study (**Figure 1**) encompasses five main steps: (i) curation and pre-processing of data related to the action of drugs and to the molecular omics profiles associated with the different phases of the disease; (ii) multi-layer network-based random walk analysis; (iii) predicting drug repurposing candidates; (iv) drug prediction robustness analysis; and (v) validation of predicted candidate drugs.

Drug Repurposing Knowledge Graph, Covid-19 Knowledge Graph, and DSOGs

We utilized an existing drug repurposing knowledge graph (DRKG) constructed by Ioannidis et al.[31] The DRKG is a biological knowledge graph relating genes, compounds, diseases, biological processes, side effects, and symptoms as of 2020 when it was constructed. The DRKG includes information from six existing databases: (i) Global Network of Biomedical Relationships,[33] (ii) STRING,[34] (iii) IntAct,[35] (iv) Hetionet,[36] (v) DrugBank,[37] and (vi) Drug–Gene Interaction Database.[38] The DRKG includes 97,238 entities classified into 13 different node types (**Table 1**) and consists of 5,874,261 triplets belonging to 107 edge types (**Supplementary Table 1**). We leveraged the gene pathway and gene biological process edge types (**Supplementary Table 1**) in the DRKG to construct pairwise interaction between biological processes and pathways based

Table 1: Description of the Node Types in the Drug Repurposing Knowledge Graph.

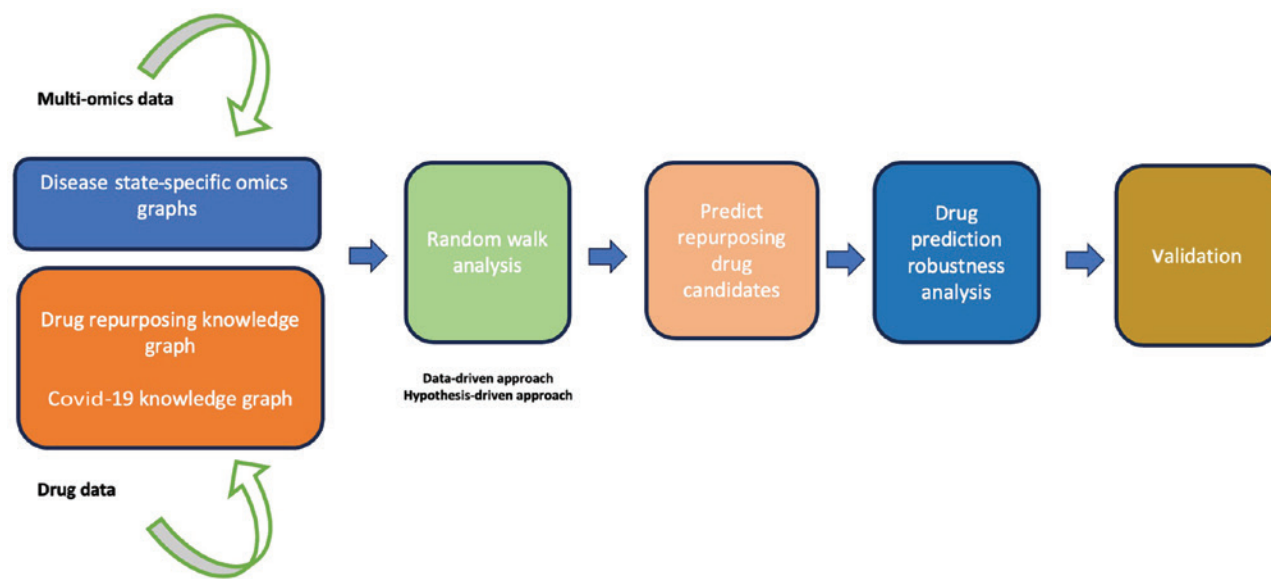| Node Type	Number of Features
Anatomy	400
Anatomical Therapeutic Chemical	4048
Biological process	11,381
Cellular component	1391
Compound	24,313
Disease	5103
Gene	39,220
Molecular function	2884
Pathway	1822
Pharmacologic class	345
Side effect	5701
Symptom	415
Taxonomy	215

Figure 1. Diagram illustrating the workflow implemented in this study. The workflow begins with curating multi-omics data and drug data, followed by a random walk with restart network analysis using both data-driven and hypothesis-driven approaches. Next, we prioritized and characterized candidate drugs, followed by drug prediction robustness analysis. Finally, we concluded the analysis by validating the predicted drug candidates.

on the semantic relation that biological processes and pathways that share similar disease-related genes are indirectly associated. This was achieved by exploring the associations between genes, biological processes, and pathways to investigate the biological processes and pathways enriched among the disease-associated genes. We then paired pathways and biological processes sharing common genes.

We additionally considered the Covid-19 knowledge graph (Covid-19 KG) built by Hsieh et al. in 2021.[29] This Covid-19 KG is notable for its integration of drug data from the Comparative Toxicogenomics Database, specifically data available as of 2021,[39] along with protein–protein interactions involving SARS-CoV-2 and host proteins from a study by Gordon et al.[40] In addition to the virus–host interaction data, we also included SARS-CoV-2 and host protein interactions from IntAct database.[35] The SARS-CoV-2 and host protein interaction data extracted from IntAct were derived from several studies that examined protein–protein interactions between SARS-CoV-2 and humans. Following the merge of the Covid-19 KG and virus–human protein interaction data, the resulting graph represents the interactions between entities belonging to five different node types (**Table 2**) and consists of 33,621 triplets belonging to five edge types (**Supplementary Table 2**).

Furthermore, we considered DSOGs constructed from our previous study[41] for downstream analysis. The DSOGs were constructed by integrating harmonized proteomics, transcriptomics, metabolomics, and lipidomics datasets retrieved from the studies by Overmyer et al.[42] and Su et al.,[43] together with a unified knowledge graph, assembled by merging protein–protein interactome, metabolite–metabolite interactome, transcript–transcript interactome, and lipid–lipid interactome curated from literature and databases.[41] The DSOG consisted of four node types (i.e., protein, transcript, metabolite, and lipid) and nine edge types (i.e., protein–protein, transcript–transcript, metabolite–metabolite, lipid–lipid, protein–transcript, protein–metabolite, transcript–metabolite, protein–lipid, and transcript–lipid).

The process used to construct the graphs is described in Agamah et al.[41] In summary, the World Health Organization Ordinal Scale was used as a disease severity reference to harmonize Covid-19 patient metadata across the studies by Overmyer et al.[42] and Su et al.[43] These harmonized metadata were then used to categorize the multi-omics data into mild, moderate, and severe Covid-19 disease phases. Subsequently, a correlation network approach was implemented to construct co-expression networks for proteomics, transcriptomics, metabolomics, and lipidomics data for each disease state. The co-expression networks generated were integrated/merged based on the disease state and omics data type to construct DSOGs.

Overall, these above data sources were used to construct a unified and integrated knowledge graph comprising three subgraphs including the Covid-19 KG, the DRKG, and the DSOG from which we conducted an in-depth quality check (see the section **Data Pre-processing, Quality Control, and Filtering**), prioritize, characterize, and repurpose specific drugs for the mild, moderate, and severe state of Covid-19.

Data Pre-processing, Quality Control, and Filtering

We observed differences in the gene and drug identifiers across the DRKG and Covid-19 KG. To achieve consistency in the identifiers across the datasets, we mapped gene identifiers onto gene symbols using the UniProt database resource[44] and drug identifiers onto drug names using the DrugBank database.[37] To identify clinically approved drugs with known safety profiles and pharmacokinetic properties, we filtered/cleaned the drug-related data by maintaining (i) drug–drug interactions between US Food and Drug Administration (FDA)-approved drug candidates, (ii) drug–protein/gene interactions between FDA-approved drug candidates and proteins/genes, and (iii) biological process/pathway–chemical interactions between biological processes/pathways and FDA-approved chemicals. As our investigation is centered around identifying potential repurposable drugs for Covid-19, we took additional measures to refine our analysis. To prioritize approved drugs with known safety profiles for Covid-19, we specifically removed interactions involving drugs that have been studied (based on literature evidence) and found to lack therapeutic effectiveness in treating Covid-19, as is the case with quinolones like chloroquine and hydroxychloroquine.[45,46] Moreover, we omitted certain endogenous substances including hormones such as progesterone, testosterone, and melatonin, as well as alcohol (ethanol), various compounds like cholesterol, and cocaine, and gases such as oxygen and hydrogen from our analysis. Overall, we utilized about 2782 FDA-approved drugs for downstream analysis.

RWR Network Analysis

The random walk method is a technique for detecting the spread of biological information through networks. The concept behind the random walk method is such that a hypothetical particle exploring the network structure takes discrete steps (walks) in some direction from a seed node.[47] The walk explores different layers based on the premise that related nodes lie close to each other in the network.[47] To perform RWR network analyses, we utilized MultiXrank,[32] an RWR on a multi-layer network algorithm, to explore DSOGs, Covid-19 KG, and DRKG. Whereas the MultiXrank algorithm enables random walk analysis on multiple large multidimensional datasets in a multi-layer network framework, other methods are limited in the combination and heterogeneity of networks that they can handle.[48] In brief, the first step of the algorithm is to create adjacency matrices for the input graphs,

Table 2: Description of the Node Types in the Covid-19 Knowledge Graph.

Node Type	Number of Features
SARS-CoV-2 baits	23
Host genes and drug targets	10,959
Pathways	274
Drugs (chemical/compound)	4266
Biological process (phenotypes)	1893

Covid-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

followed by computing different transition probabilities of the RWR on the graphs. The probabilities are estimated based on the concept that an imaginary particle starts a random walk from the seed node to other nodes in the network. These different transition probabilities describe the walks within a graph and the jumps between graphs. A higher probability score (close to 1) suggests a higher likelihood of walking or jumping between graphs. We made specific adjustments to the algorithm's configuration script based on the input datasets. While the global restart probability was set at 0.7, the intra-layer jump probability and the probability to restart in a specific layer were set to 0.5 and 1, respectively. Other parameters such as inter-layer jump probability were fine-tuned to align with the number of input graph layers. We set parameters in the algorithm to control the behavior of the algorithm to achieve desired outcomes.

As a result of this analysis, we obtained multi-layered graphs that detailed the exploration of seed nodes across various omics layers and drug data, along with a ranked list of features in each graph layer.

RWR analysis on the DRKG, Covid-19 KG, and DSOG

We performed an initial analysis focused on identifying drugs that can be repurposed for Covid-19 without accounting for omics profiles. Thus, we employed the DRKG and Covid-19 KG as the input data sources in the RWR algorithm to predict candidate drugs for Covid-19. The algorithm accepts as layers, monoplex graphs, and/or a combination of monoplex graphs (multiplex). Specifically, in our analysis, edge types with the same node entities including the drug–drug and gene–gene interactions each served as a monoplex and were interconnected by edge types with different node entities including the gene–drug, pathway–gene, phenotype (biological process)–gene, phenotype (biological process)–drug, and SARS-CoV-2–host gene interactions (**Supplementary Tables 1 and 2**).

In subsequent analysis to predict potential drugs for different Covid-19 disease states, we utilized the DRKG, Covid-19 KG, and DSOG as input data. Herein, the input data included the proteomics, transcriptomics, metabolomics, and lipidomics disease state graphs, which also served as individual monoplex layers. Similar to the analysis on the DRKG and Covid-19 KG, graphs with edge types of the same node entities were interconnected by graphs with edge types of different node entities including the protein–transcript, protein–metabolite, transcript–metabolite, protein–lipid, and transcript–lipid interactions from the DSOG which were not utilized in the previous analysis.

Prediction of drugs using the RWR algorithm is based on a network exploration process where simulated particles walk iteratively from one node to one of its neighbors with some probability. In this process, the walk is restricted to restart from seed nodes to prevent the random walker from being trapped in dead ends.[47]

Selection of seeds for RWR based on a hypothesis- and data-driven approach

To select seed nodes for the analysis, we implemented two approaches: (i) a hypothesis-driven approach where we selected seeds based on their impact on disease severity to test the hypothesis of their differential associations with mild, moderate, and severe Covid-19 disease states and (ii) a data-driven approach where we selected, after merging the different co-expression networks, the features with the highest node integrated centrality score in each omics layer as seeds. The hypothesis-driven approach has the advantage of bringing the question being investigated into focus by designing the model with a specific biological hypothesis in mind and exploring variations across disease phases, whereas the data-driven approach enables a more unbiased and informed model.[49,50] Although hypothesis- and data-driven modeling approaches are not mutually exclusive, it is worth noting that this diversity is beneficial: Most model-building tools and models have a specific and clear role; however, at the same time, combining hypothesis- and data-driven approaches in an interoperable way provides an immense impact on our understanding of the disease phases as modeling and integrating data at different biological scales.[49,50]

IL6 and *IL6R* features were used as hypothesis-driven seeds for the random walk analysis because of the evidence for their significant role in the pathology of SARS-CoV-2 and Covid-19.[51–53] *IL6* is a cytokine, a type of signaling molecule involved in various inflammatory and immune responses. The inflammatory response plays a critical role in Covid-19, with an excessive inflammatory response leading to a "cytokine storm" increasing the severity of Covid-19. Since *IL6* interacts with cells via *IL6R*, it has been hypothesized that inhibition of *IL6R* might reduce the likelihood of cytokine storms developing, ameliorate the symptoms of severe Covid-19, and reduce mortality.[48] In this context, we, therefore, used *IL6* and *IL6R* as hypothesis-driven seeds in an RWR analysis that we refer to as our "hypothesis-driven approach."

For an RWR using data-driven seeds, we selected seeds following the approach described in our previous study.[41] Specifically, we selected, after merging the different co-expression networks, the features with the highest integrated centrality score in each omics layer.[41] The features were ranked by leveraging the node degree, closeness, betweenness, and eigenvector centrality metrics to compute an integrated score (see **Supplementary data, Equation 1**). These centrality metrics provide insight into the importance of a node. For instance, the closeness metric measures how close a node is to all other nodes in the network. A lower closeness centrality score indicates the node is on average closer to other nodes, potentially making it a faster "information hub." The degree metric measures the number of connections (edges) a node has with other nodes. A higher degree signifies the node has more direct connections, suggesting it might be more influential or receive more information flow. The betweenness metric captures how often a node lies on the shortest path between other pairs of nodes in the network. A higher betweenness score suggests the node acts as a crucial bridge for information flow within the network. The eigenvector metric considered goes beyond the number of connections and considers the "importance" of a node's neighbors. Nodes with high eigenvector centrality are considered influential due to their connections to other influential nodes. By integrating these diverse perspectives, the calculated

score provides a comprehensive understanding of a node's relative importance within the network structure and its potential role in information flow and communication. Herein, the RWR analysis using the data-driven seeds is referred to as our "data-driven approach."

Ranking candidate drugs for Covid-19 disease states

The RWR approach has the benefit of capturing the global topology of a graph and representing a measure of proximity from all the nodes to the seed(s) based on the graph topology.[54] The measure of proximity between nodes is a relevant measure quantifying how closely connected a node is with the seeds and can be used to rank nodes.[47,54,55] Overall, the network's structure and connectivity patterns impact how random walks propagate through the nodes and influence the ranking of nodes based on their importance and relevance. Highly connected nodes or hubs may attract more random walkers and have a greater influence on the ranking of nodes. In this study, nodes within each layer were ranked based on their measure of proximity to the seed nodes, representing the relatedness of nodes to the seeds. The measure of proximity was the geometric mean of the node's proximity to the seeds.[47]

RESULTS

Predicting Candidate Drugs Using Existing Knowledge Graphs and Hypothesis-driven Seeds

To predict potential Covid-19 drugs, we systematically analyzed the DRKG and Covid-19 KG excluding DSOGs by implementing an RWR analysis (see **Materials and Methods**). The RWR is an approach that allows for the exploration of the DSOGs, Covid-19 KG, and DRKG to identify patterns and prioritize features within the network. The algorithm, MultiXrank, conducts multiple random walks over the graphs, each originating from the seed nodes. These walks iteratively traverse from one node to a neighboring node at random, thus simulating a pattern that results in a multi-layered graph. In our hypothesis-driven approach, we selected *IL6* and *IL6R* as seeds given their established roles as aggravators of the disease. The random walk process restricts the restarts from seed nodes (*IL6* and *IL6R*) during network exploration. The RWR analysis revealed a multi-layered graph describing the random walks from the seed nodes (**Figure 2**) and a set of potential therapeutics (**Table 3**) for the treatment of Covid-19. These included immunosuppressants, vital minerals, anticancer agents, antivirals, antibiotics, angiotensin receptor blockers, and corticosteroids (as detailed in **Table 3**). Notable among these are presently recommended
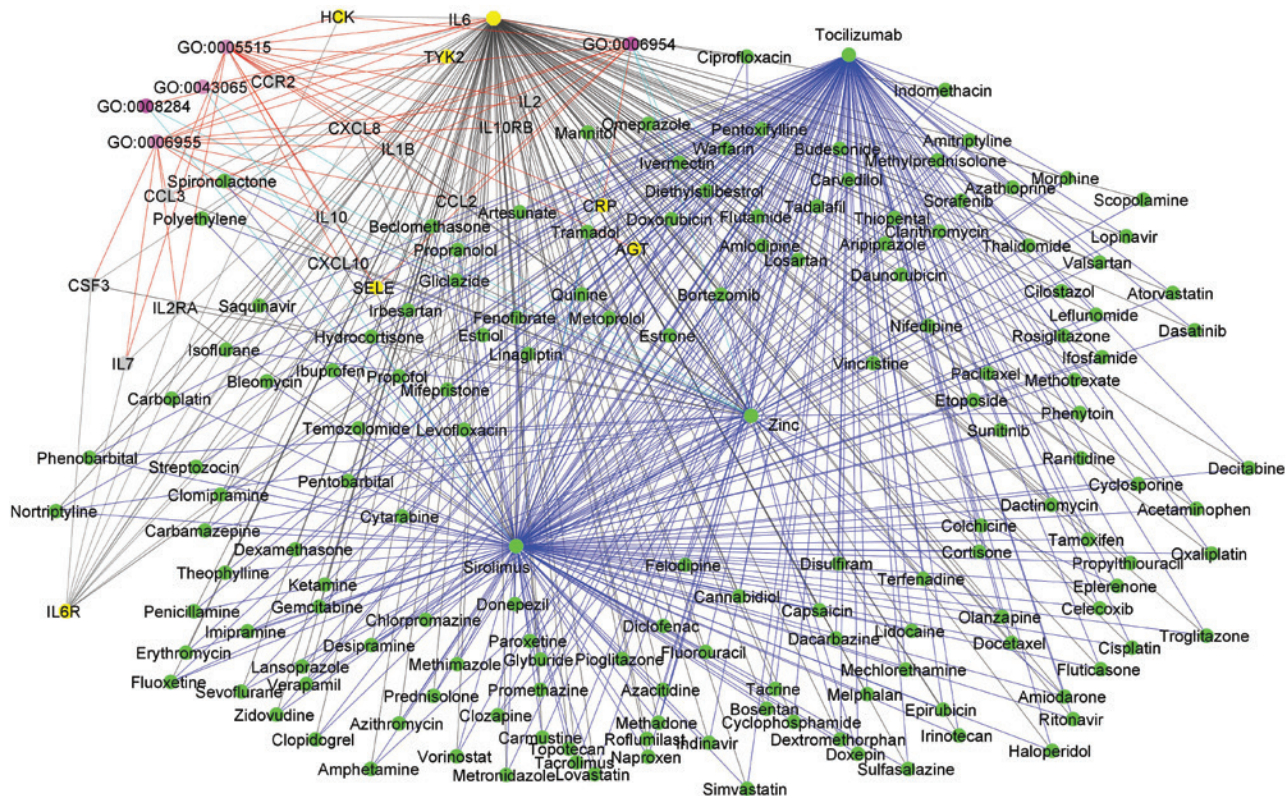

Figure 2. Graph representation of interactions between drugs and other features as observed from predicting candidate drugs using existing knowledge graphs and hypothesis-driven seeds. Blue edges represent interactions between drugs (green nodes). Cyan edges represent interactions between biological processes (pink nodes) and drugs. Red edges represent biological process–proteins (gray nodes) interactions and biological process–transcript (yellow nodes) interactions. Black edges represent drug–protein, drug–transcript, protein–transcript, and transcript–transcript interactions. The graphs were generated by defining filtering criteria based on the node degree between 4 and 1633 in Cytoscape.

Table 3: Top 20 Potential Covid-19 Drugs, Ranked According to Their Measure of Proximity to IL6 and IL6R Seed Nodes as Determined Through the RWR Analysis of the DRKG and Covid-19 KG.

Drug Name	Drug Category	Mechanism of Action Potentially Linked with Covid-19	Measure of Proximity	Reference[a]
Tocilizumab[b]	IL6R antagonist	Suppresses immune response by blocking IL6 signaling	0.0353861	[21]
Zinc	Essential mineral/nutrient	Interferes with viral RNA synthesis to inhibit replication	0.0007941	[123]
Sirolimus	Immunosuppressive drug	Expresses immunomodulatory and anti-inflammatory properties and inhibits the expression of pro-inflammatory cytokines	0.0003784	[124,125]
Choline	Essential nutrient	Supports cell membrane integrity and neurotransmitter function	0.0003440	[126]
Ivermectin	Antiparasitic drug	Inhibits viral replication and modulates the host immune response	0.0003056	[127]
Dactinomycin	Anticancer	Expresses immunomodulatory properties and inhibits viral cellular transcription	0.0002734	[79,80]
Losartan	Angiotensin receptor blocker	Reduces the activity of the renin–angiotensin system	0.0002327	[128]
Ribavirin	Antiviral	Interferes with viral RNA synthesis and replication	0.0002168	[129,130]
Azithromycin	Antibiotic	Expresses antiviral and anti-inflammatory properties	0.0002000	[131]
Tenofovir	Antiviral	Interferes with viral RNA synthesis to inhibit replication	0.0001930	[132]
Acetaminophen	Analgesic	Expresses antipyretic and analgesic effects and inhibits the cyclooxygenase pathways	0.0001665	[133,134]
Dexamethasone	Corticosteroid	Suppresses immune response	0.0001633	[98]
Methotrexate	Immunosuppressive drug	Suppresses immune response	0.0001624	
Cyclosporine	Immunosuppressive drug	Express anti-inflammatory and antiviral properties	0.0001614	[135,136]
Cisplatin	Anticancer		0.0001555	
Tacrolimus	Immunosuppressive drug	Mitigates the hyperinflammatory response	0.0001532	[137]
Indomethacin	Non-steroidal anti-inflammatory drug	Expresses anti-inflammatory properties and reduces pain and fever	0.0001505	[63,138]
Cannabidiol	Cannabinoid	Inhibits viral replication by upregulating the host inositol-requiring enzyme 1α ribonuclease endoplasmic reticulum stress response and interferon signaling pathways	0.0001488	[60]
Doxorubicin	Anticancer	Expresses antiviral and immunomodulatory properties	0.0001486	[61]
Diclofenac	Non-steroidal anti-inflammatory drug	Expresses anti-inflammatory properties and reduces pain and fever	0.0001481	[138]

[a]The references point to publications that have reported the drugs' mechanism of action potentially linked with Covid-19.
[b]Drugs with direct contact to seed nodes as observed in first neighbors.
Covid-19, coronavirus disease 2019; Covid-19 KG, Covid-19 knowledge graph; DRKG, drug repurposing knowledge graph; IL6, interleukin 6; IL6R, interleukin 6 receptor; RWR, random walk with restart.

drugs for treating the disease such as tocilizumab,[21,56,57] dexamethasone,[13] and losartan.[58,59] Furthermore, our analysis pinpointed additional potential pharmaceutical options, such as *cannabidiol* and *doxorubicin*.[60–62] While these have yet to be definitively endorsed for Covid-19 treatment, prior research indicates their potential candidacy based on their efficacy and merits for further experimental validations.[60,63] **Figure 2** shows a multi-layered graph generated from the RWR analysis highlighting interactions involving highly ranked drug candidates and other features such as *IL2*, *IL1B*, *HCK*, and *TYK2*.

Predicting Candidate Drugs Using Existing Knowledge Graphs, DSOGs, and Hypothesis-driven Seeds

To discover potential candidate drugs that could be used to specifically treat the disease in either its mild, moderate, or severe phases, we employed the RWR method in a network-based approach utilizing the knowledge graphs (DRKG and Covid-19 KG), alongside the DSOGs encompassing transcriptomics, proteomics, metabolomics, and lipidomics data (see **Materials and Methods**). The outcome of the RWR analysis is a ranked list of potential drugs and a multi-layered graph describing the random walks from the seed nodes (*IL6* and *IL6R*). Across different analyses of the mild, moderate, and severe disease states, we consistently identified the same sets of drugs (**Table 4**) that could potentially be impactful during Covid-19 treatment. Notably, our analysis revealed that drugs known to suppress the immune response and reduce inflammation, including those promoting IL6 production, consistently ranked high across all investigated disease states (**Table 4**). This observation aligns with expectations, considering our choice of *IL6* and *IL6R* as seeds: Both are pivotal biomarkers identified in multiple studies as displaying expression levels that are positively associated with severe disease states.[64,65] These biomarkers are prominently expressed during cytokine storms that are characteristic of severe Covid-19 cases.[65]

We observed differences between the multi-layered graphs generated by the RWR analysis on the mild, moderate, and severe disease states. Specifically, these differences were observed in the levels of connectivity between molecular features and the drug repurposing candidates. We therefore performed network topology analysis on the multi-layered graphs generated from the RWR analysis to explore the differences between these graphs

Table 4: Top 20 Potential Drugs for Mild, Moderate, and Severe Covid-19, Ranked According to Their Measure of Proximity to IL6 and IL6R Seed Nodes as Determined Through the RWR Analysis of the Covid-19 KG, DRKG, and DSOG.

Drug	Drug Category	Mechanism of Action Potentially Linked with Covid-19	Measure of Proximity in the Mild State	Measure of Proximity in the Moderate State	Measure of Proximity in the Severe State	Reference[a]
Dexamethasone	Corticosteroid	Suppresses immune response	0.0033655	0.0033691	0.0033697	98
Sarilumab[b]	Anti-IL6R monoclonal antibody	Suppresses immune response by blocking IL6 signaling	0.0031924	0.0031908	0.0031911	19
Tocilizumab[b]	Anti-IL6R monoclonal antibody	Suppresses immune response by blocking IL6 signaling	0.0031908	0.0031894	0.0031897	21
Zinc	Essential mineral/nutrient	Expresses antiviral properties and interferes with viral RNA synthesis to inhibit replication	0.0002730	0.0002681	0.0002692	123,139
Sirolimus	Immunosuppressive drug	Expresses immunomodulatory and anti-inflammatory properties and inhibits the expression of pro-inflammatory cytokines	0.0001524	0.0001526	0.0001530	124,125
Histamine	Depressor amine	Expresses immunomodulatory and anti-inflammatory properties	0.0001420	0.0001413	0.0001416	140,141
Curcumin	Natural compound	Expresses immunomodulatory and anti-inflammatory properties that inhibit severe inflammation and cytokine storm	0.0001366	0.0001358	0.0001363	142,143
Cyclosporine	Immunosuppressive drug	Expresses anti-inflammatory and antiviral properties	0.0001352	0.0001370	0.0001369	135,136
Doxorubicin	Anticancer	Inhibits the protease-mediated viral entry to the host cell	0.0001314	0.0001316	0.0001318	61
Morphine	Opioid pain medication	Contributes to improving respiratory failure	0.0001301	0.0001281	0.0001284	144
Dactinomycin	Anticancer	Expresses immunomodulatory properties and inhibits viral cellular transcription	0.0001292	0.0001290	0.0001288	79,80
Simvastatin	Lipid-lowering drug	Expresses anti-inflammatory, immunomodulatory properties and reduces viral replication	0.0001290	0.0001289	0.0001287	145,146
Hydrocortisone	Corticosteroid	Expresses anti-inflammatory and immunomodulatory properties	0.0001274	0.0001279	0.0001276	98
Vitamin C	Essential mineral/nutrient	Express antioxidant properties and improves immune function	0.0001268	0.0001269	0.0001276	105
Mycophenolic acid	Immunosuppressive drug	Expresses immunomodulatory properties	0.0001236	0.0001236	0.0001239	99
Methotrexate	Immunosuppressive drug	Expresses immunomodulatory properties	0.0001182	0.0001195	0.0001194	
Dopamine	Catecholamine neurotransmitter	Influences the expression of ACE2	0.0001178	0.0001187	0.0001197	147
Indomethacin	Non-steroidal anti-inflammatory drug	Expresses anti-inflammatory properties and reduces pain and fever	0.0001170	0.0001165	0.0001162	63
Paclitaxel	Anticancer	Express anti-inflammatory and antiviral properties	0.0001159	0.0001159	0.0001161	
Metformin	Biguanide antihyperglycemic	Inhibits viral replication	0.0001158	0.0001158	0.0001164	100

[a]The references point to publications that have reported the drugs' mechanism of action potentially linked with Covid-19.
[b]Drugs with direct contact to seed nodes as observed in first neighbors.
Covid-19, coronavirus disease 2019; Covid-19 KG, Covid-19 knowledge graph; DRKG, drug repurposing knowledge graph; DSOG, disease state-specific omics graph; IL6, interleukin 6; IL6R, interleukin 6 receptor; RWR, random walk with restart.

and how that can provide more insights into the use of predicted drugs across mild, moderate, and severe disease states. First, we evaluated the degree, betweenness, and closeness measures of the drug repurposing candidates and observed relatively similar scores (**Table 5**). The node degree indicates the number of connections a drug has, revealing its involvement in broader disease processes. Higher degrees might suggest broader applicability across multiple diseases. The betweenness centrality measures a drug's "traffic control" role, indicating how often it lies on the shortest paths between other nodes. High betweenness suggests a potential "bridge" molecule connecting different disease pathways. The closeness centrality reflects how quickly information can reach other disease elements or nodes from a candidate drug. High closeness suggests a central position within the disease network, potentially making it a good starting point for treatment. For instance, cyclosporine had a higher degree of 6 in the multi-layered graph generated by the RWR analysis on the moderate disease state as compared to a degree of 5 in the multi-layered graph generated by the RWR analysis on the mild disease state and a degree of 4 in the multi-layered graph generated by the RWR analysis on the severe disease state with a corresponding high betweenness score of 0.0014 and a closeness score of 0.4832, suggesting it might have potential utility for the moderate disease state.

Similarly, mycophenolic acid had a higher degree of 5 in the multi-layered graph generated by RWR for the moderate disease state as compared to a degree of 4 in both the multi-layered graph generated by the RWR analysis on the mild disease state and the multi-layered graph generated by the RWR analysis on the severe disease state with a corresponding high betweenness score of 0.009 and a closeness score of 0.4739, also suggesting it might have potential utility for the moderate disease state.

In **Table 5**, the drugs were ranked according to the node degree (starting from the mild disease state) with the corresponding betweenness and closeness scores. There are variations in the betweenness and closeness scores. For instance, drugs with higher degree scores for specific disease states have high betweenness and closeness scores for that disease state. As shown in **Table 5**, three drugs (dexamethasone, tocilizumab, and sarilumab) had betweenness scores between 0.024 and 0.64 in at least one disease state. The remaining had a betweenness score between 0.0 and 0.007 across all disease states. Additionally, all drugs in **Table 5** had a closeness score between 0.3 and 0.63.

Next, we evaluated the contribution of other molecular features in the networks. To begin with, beyond the central influence of top-ranked drugs (*dexamethasone, tocilizumab,* and *sarilumab*) in the multi-layered graph generated from the RWR analysis on the mild disease state (**Figure 3A**), three key inflammatory-related features, C-C motif chemokine ligand 2 (*CCL2*), C-C motif chemokine ligand 4 (*CCL4*), and negative elongation factor complex member C/D (*NELFCD*), formed distinct subnetworks, acting as crucial hubs that connected seed nodes and promising candidates for drug repurposing. In mild Covid-19 cases, *CCL2* helps recruit monocytes and macrophages, which are essential for fighting the virus as compared to excessive immune cell recruitment in severe disease states.[66,67] Also, *CCL4* levels in mild disease states help recruit necessary immune cells to fight the virus.[68] Thus, using drug repurposing candidates that could influence the recruitment of immune cells in mild disease states could be more appropriate, acknowledging the fact that the development of clinical Covid-19 involves cell activation such as dysfunctional mast cell activation.[69] From the predicted drugs, histamine is a biogenic amine known to attract and activate immune cells, particularly mast

Table 5: Node Degree, Betweenness, and Closeness Centrality Measures for the Drug Repurposing Candidates Predicted Using the Hypothesis-driven Approach.

| Drug Name	Degree, Mild State	Degree, Moderate State	Degree, Severe State	Betweenness, Mild State	Betweenness, Moderate State	Betweenness, Severe State	Closeness, Mild State	Closeness, Moderate State	Closeness, Severe State
Dexamethasone	1633	1632	1633	0.6282	0.5881	0.6293	0.6355	0.6233	0.6228
Tocilizumab	830	829	830	0.11436	0.1008	0.1103	0.4643	0.4482	0.4571
Sarilumab	469	469	469	0.0286	0.0246	0.0283	0.4432	0.4289	0.4373
Sirolimus	7	8	6	0.0063	0.0068	0.0054	0.4831	0.4889	0.4512
Vitamin C	6	6	4	0.0047	0.0034	0.0004	0.4860	0.4525	0.4460
Cyclosporine	5	6	4	0.0007	0.0014	0.0004	0.4769	0.4832	0.4460
Dactinomycin	5	4	4	0.0007	0.0003	0.0004	0.4769	0.4367	0.4460
Paclitaxel	5	5	4	0.0007	0.0009	0.0004	0.4769	0.4739	0.4460
Simvastatin	5	5	4	0.0007	0.0009	0.0004	0.4769	0.4739	0.4460
Hydrocortisone	4	5	4	0.0004	0.0007	0.0004	0.4528	0.4454	0.4460
Zinc	4	5	4	0.0005	0.0011	0.0005	0.3652	0.3903	0.3637
Indomethacin	4	5	4	0.0004	0.0009	0.0004	0.4528	0.4739	0.4460
Mycophenolic acid	4	5	4	0.0004	0.0009	0.0004	0.4528	0.4739	0.4460
Doxorubicin	4	5	4	0.0004	0.0009	0.0004	0.4528	0.4739	0.4460
Methotrexate	4	5	4	0.0004	0.0007	0.0004	0.4528	0.4454	0.4460
Morphine	4	4	3	0.0005	0.0007	0.0003	0.4768	0.4738	0.4459
Curcumin	3	4	3	0.0	0.0001	0.0004	0.4644	0.4716	0.4483
Metformin	3	3	2	0.0	0.0	0.0	0.4644	0.4355	0.4354
Histamine	3	3	1	0.0002	0.0	0.0	0.3866	0.3705	0.3601
Dopamine	1	1	1	0.0	0.0	0.0	0.3615	0.3584	0.3601
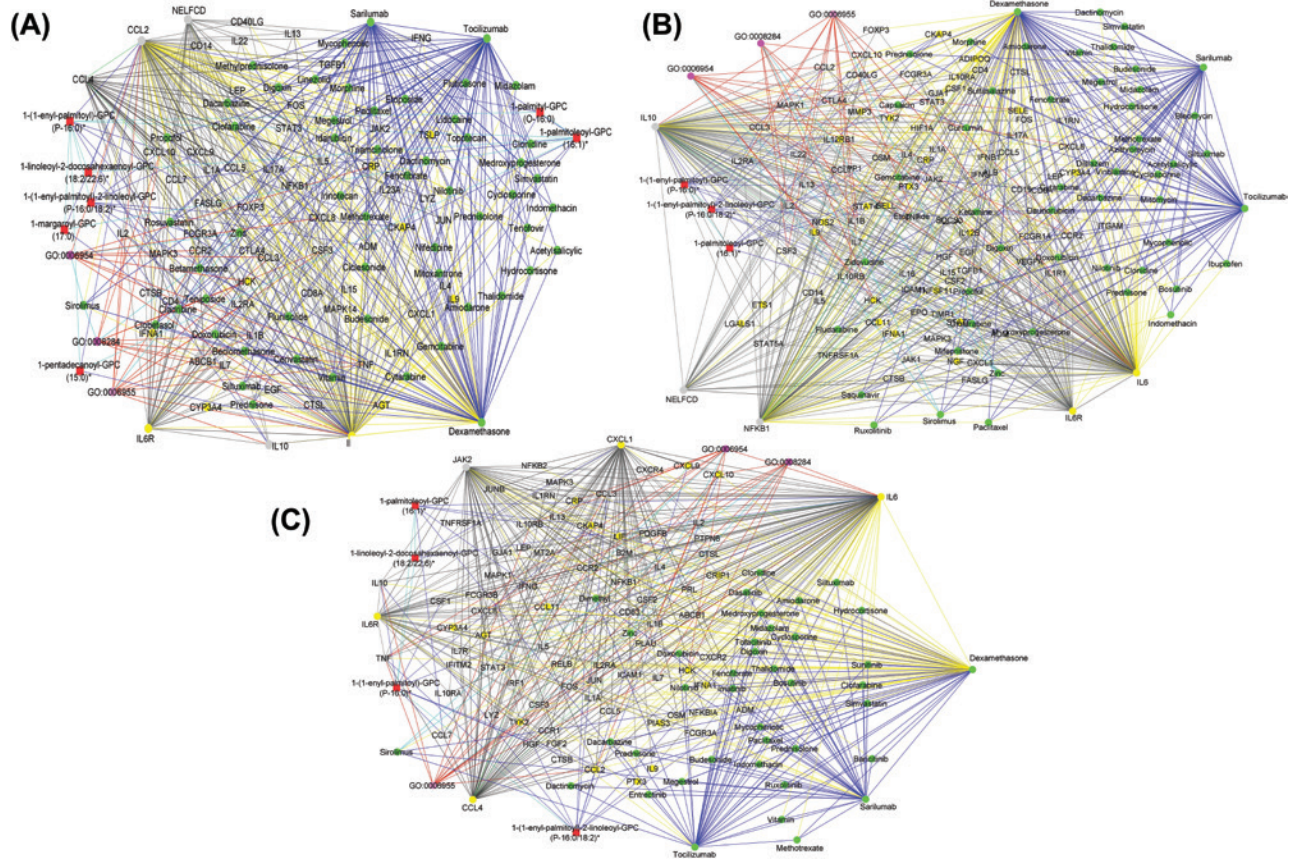
Figure 3. (A) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), and biological process (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, mild disease state-specific omics graphs (DSOGs), and hypothesis-driven seeds. The graph reveals distinct subnetworks formed by hubs CCL2, CCL4, and NELFCD demonstrating extensive interactions with drug candidates, seed nodes (IL6 and IL6R), and other molecular features. (B) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), and biological process (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, moderate DSOGs, and hypothesis-driven seeds. The graph reveals distinct subnetworks formed by hubs NFKB1, IL10, and NELFCD demonstrating extensive interactions with drug candidates, seed nodes (IL6 and IL6R), and other molecular features. (C) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), and biological process (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, severe DSOGs, and hypothesis-driven seeds. The graph reveals distinct subnetworks formed by hubs CXCL1, CCL4, and JAK2 demonstrating extensive interactions with drug candidates, seed nodes (IL6 and IL6R), and other molecular features. Yellow edges represent drug–protein and drug–transcript pairwise interactions. Red edges represent biological process–protein interactions and biological process–transcript interactions. Green edges represent protein–protein interactions. Black edges represent transcript–transcript interactions and protein–transcript interactions. Blue edges represent drug–drug interactions. Light blue edges represent biological process–biological process interactions and biological process–pathway interactions. The graphs were generated by defining filtering criteria based on the node degree between 4 and 1633 in Cytoscape. CCL2, C-C motif chemokine ligand 2; CXCL1, C-X-C motif chemokine ligand 1; IL6, interleukin 6; IL6R, interleukin 6 receptor; JAK2, Janus kinase 2; NFKB1, nuclear factor kappa B subunit 1; NELFCD, negative elongation factor complex member C/D.

cells, basophils, neutrophils, and certain T cells, through specific histamine receptors.[70] Histamine can stimulate mast cell degranulation, leading to the release of *CCL4* and *CCL2* among other inflammatory mediators.[71,72] This suggests a potential indirect link between histamine and these chemokines in inflammatory processes. Paclitaxel is known to modulate the immune system in various ways, including (i) promoting the migration of T cells and other immune cells into tumors, (ii) enhancing the activity of antigen-presenting cells, vital for activating T cells, and (iii) modulating the expression of immune-related genes that influence inflammation and immune responses. Paclitaxel induces the release of cytokines like tumor necrosis factor (*TNF*) and *IL6*

and chemokines like *CCL2* and might, therefore, help control viral infection by stimulating immune cell recruitment and boosting immune responses.[73] Metformin might enhance the activity of certain immune cells like macrophages and natural killer cells, potentially aiding in viral clearance.[74,75] Metformin activates 5' adenosine monophosphate-activated protein kinase (AMPK), a cellular energy sensor that regulates various metabolic and inflammatory processes.[76] AMPK activation can downregulate pro-inflammatory signaling pathways and reduce the production of inflammatory mediators like *TNF* and *IL6*.[77]

The *NELFCD*, as part of NELF, regulates RNA polymerase II pausing, potentially influencing viral RNA synthesis during viral genome

replication.[78] This suggests that drugs with the potential to inhibit SARS-CoV-2 replication could be appropriate for repurposing. Such drugs would include, for example, dactinomycin which, besides having immune-modulatory properties, also inhibits viral genome replication.[79,80]

Analysis of the multi-layered graph generated by the RWR analysis during predictions for the moderate disease state identified *NELFCD*, nuclear factor kappa B subunit 1 (*NFKB1*), and *IL10* as hubs influencing the network based on their high connectivity, forming both direct and indirect pathways between the seed nodes and the top candidates for drug repurposing (**Figure 3B**). *NFKB1* activates genes encoding pro-inflammatory cytokines, chemokines, and adhesion molecules, orchestrating the body's initial response to viral infection and enhancing the severity of Covid-19 symptoms.[81,82] Thus, moderating *NFKB1* activity could mitigate cytokine storms and improve outcomes. Corticosteroids like *dexamethasone* can be used in severe Covid-19 to suppress *NFKB1* activity and reduce inflammation. It is, however, important to note that while dampening *NFKB1* can be beneficial, completely suppressing it could impair the body's ability to fight the virus. Thus, finding the right balance remains crucial. *IL10* is a natural anti-inflammatory cytokine, acting as a brake on the immune response. It helps control excessive inflammation, as disease severity progresses, particularly in moderate disease states, to prevent tissue damage. However, overactive *IL10* production in moderate Covid-19 cases can dampen the immune system's ability to fight the virus, potentially prolonging the infection and allowing persistent viral replication. From our results (**Figure 3B**), drugs with the potential to modulate *IL10* activity could be beneficial during moderate disease states to balance the suppression of excessive inflammation with optimal immune functioning. For instance, *sirolimus* inhibits the mammalian target of the rapamycin (mTOR) pathway, which can indirectly suppress *IL10* production by limiting signal transducer and activator of transcription 3 (*STAT3*) signaling.[83,84] Also, it can dampen the activation of certain immune cells like T cells, which may indirectly decrease *IL10* production. Additionally, *sirolimus* promotes the differentiation and expansion of regulatory T cells, a subset of T cells that naturally suppress inflammation and can promote *IL10* production as part of their suppressive function.[85]

In the multi-layered graph generated by the RWR analysis during predictions for the severe disease state (**Figure 3C**), we observed key inflammation-related features like C-X-C motif chemokine ligand 1 (*CXCL1*), *CCL4*, and Janus kinase 2 (*JAK2*) to establish subnetworks which included both direct and indirect interactions with the seed nodes and top-ranked drug candidates (**Figure 3C**). *JAK2*, a signaling molecule inside immune cells, expresses both inflammatory and anti-inflammatory effects during Covid-19. For its inflammatory role, *JAK2* activates certain signaling pathways, including the *JAK2/STAT3* pathway, that trigger inflammatory responses in the lungs, which may help fight viral infections.[86] For its anti-inflammatory role, *JAK2* also activates pathways promoting tissue repair and regeneration. Thus, drug repurposing candidates with the potential to influence *JAK2* signaling may represent an effective therapeutic strategy for controlling the disease.[87] For instance, *IL6* binds to soluble and transmembrane *IL6R* and the resultant

complex induces homodimerization of gp130, leading to activation of JAK2.[88] This suggests that *sarilumab* and *tocilizumab* targeting the *IL6R* indirectly activate *JAK2* downstream by limiting the IL6-mediated signaling pathway.[88,89] *Sirolimus* targeting an mTOR pathway connected to *JAK2* can be appropriate. Increased levels of *CXCL1* and *CCL4* have been associated with severe disease and hyperinflammatory states, suggesting a potential role in Covid-19 disease progression.[67,90] In general, the drug repurposing candidates (**Table 4**) with anti-inflammatory activities, immunomodulatory activities, and viral replication inhibitory activities have the best potential to manage excessive inflammation and limit viral persistence during the severe disease state. We further observed from the shortest path analysis how these molecular features act as mediators connecting the drug repurposing candidates to the seed nodes across the mild, moderate, and severe disease states (**Supplementary File 4**).

Predicting Candidate Drugs Using Existing Knowledge Graphs, DSOGs, and Data-driven Seeds

To investigate the prediction of drugs that might be differentially applicable to treating different Covid-19 disease states, we used a data-driven approach to identify seeds by computing an integrated node centrality metric score leveraging the node degree, closeness, betweenness, and eigenvector centrality metrics (see **Materials and Methods** and **Supplementary Table 3**).

In the transcriptomics layer, we identified *STAT1* as a seed node. *STAT1* is known to be involved in immune responses and antiviral activity[67] and is reported to be upregulated in mild and severe Covid-19 cases, with the phosphorylation of the gene associated with both the upregulation of *ACE2* expression and the development of severe disease states.[91,92] In the proteomic layer, *superoxide dismutase 2* (*SOD2*) was identified as a seed node. *SOD2* is an essential antioxidant enzyme that protects cells from superoxide radical anions which are known to be significantly under-expressed in the plasma[93] and lung cells of severe Covid-19 patients.[94] In the metabolomics layer, *3-hydroxyoctanoate* was identified as a seed node. This metabolite is generated during medium-chain fatty acid oxidation and serves as a marker for primary defects in beta-hydroxy fatty acid metabolism. It is also affiliated with essential pathways such as those responsible for macrophage activation and platelet aggregation, with increases in 3-hydroxyoctanoate concentrations being associated with asymptomatic Covid-19 infections.[95] In the lipidomics layer, we identified "unknown_mz_815.61548_+_RT_27.063," an uncharacterized lipid associated with disease severity, as a seed node.

Using these seed nodes for the RWR analysis across the various disease states, we identified several potential drug repurposing candidates (**Table 6**) including "natural compounds" (such as *glutathione* and *curcumin*) and inhibitors of signal transduction pathways of protein kinases and cell proliferation (tyrosine, histone deacetylase, and methyltransferase). The results revealed the same drug repurposing candidates across the various disease states (**Table 6**). However, network topology analysis revealed differences between the multi-layered graphs generated by the

Table 6: Top 20 Potential Drugs for Mild, Moderate, and Severe Covid-19, Ranked According to Their Measure of Proximity to STAT1, SOD2, 3-Hydroxyoctanoate, and Unknown_mz_815.61548_+_RT_27.063 Seed Nodes as Determined Through the RWR Analysis of the Covid-19 KG, DRKG, and DSOG.

Drug	Drug Category	Mechanism of Action Potentially Linked with Covid-19	Measure of Proximity in the Mild State	Measure of Proximity in the Moderate State	Measure of Proximity in the Severe State	Reference[a]
Glutathione	Antioxidant	Protects cells from damage caused by oxidative stress	0.0007061	0.0007044	0.0007044	104,105
Crizotinib	Tyrosine kinase inhibitor	Expresses immunomodulatory properties and could inhibit receptor tyrosine kinases and affect cellular processes relevant to viral replication	0.0007022	0.0007005	0.0007002	111
Curcumin	Natural compound	Expresses antiviral, immunomodulatory, and antioxidant properties that may contribute to inhibit inflammation and oxidative stress and reduce lung injury	0.0006966	0.0006948	0.0006951	142
Vorinostat	Histone deacetylase inhibitor	Expresses anti-inflammatory and antiviral properties	0.0006943	0.0006952	0.0006954	112
Vinblastine	Anticancer	Disrupts microtubule dynamics, leading to mitotic spindle dysfunction and cell cycle arrest	0.0006920	0.0007029	0.0007022	148
Iron	Essential mineral/nutrient	Expresses anti-inflammatory and immunomodulatory properties	0.0006917	0.0006899	0.0006944	149
Mebendazole	Antihelminthic	Expresses antiviral and immunomodulatory properties and may interfere with viral replication	0.0006913	0.0006913	0.0006910	101
Podofilox	Topical agent	Stimulates the production of interferon-γ, a cytokine that plays a role in the immune response	0.0006900	0.0007003	0.0007002	150
Valine	Essential amino acid	Protein synthesis	0.0006893	0.0006891	0.0006891	151,152
Acetylcysteine	Antioxidant and glutathione inducer	Expresses antioxidant, immunomodulatory, and mucolytic properties	0.0006878	0.0006877	0.0006878	153
Thimerosal	Methyltransferase inhibitor	Induces Th2-type cytokines via influencing cytokine secretion by human dendritic cells	0.0006826	0.0006824	0.0006824	
Fludarabine	Chemotherapy drug	Inhibits type I interferon-induced expression of ACE2	0.0006813	0.0006811	0.0006812	92
Calcipotriol	Anti-psoriatic	Enhances cell differentiation	0.0006800	0.0006800	0.0006800	
Teniposide	Cytotoxic drug	Inhibits SARS-CoV-2 3-chymotrypsin-like cysteine protease	0.0001087	0.0001091	0.0001096	154
Omacetaxine	Cephalotaxine ester and protein synthesis inhibitor	Inhibits protein translation and interferes with viral replication	0.0001015	0.0000955	0.0001009	155
Etoposide	Topoisomerase II inhibitor	Express anti-inflammatory properties and suppresses cytokine production	0.0001010	0.0000973	0.0001022	156,157
L-glutamine	Amino acid	Expresses immunomodulatory, anti-inflammatory, and antioxidant properties	0.0000945	0.0000943	0.0000942	158,159
Carglumic acid	Analog of N-acetylglutamate	Expresses enzyme properties for processing excess nitrogen produced when the body metabolizes proteins	0.0000942	0.0000939	0.0000939	
Pregabalin	Anticonvulsant drug	Reduces Covid-19-related pain and cough	0.0000591	0.0000591	0.0000591	160
Threonine	Essential amino acid	Expresses immunomodulatory, anti-inflammatory, and antioxidant properties	0.0000579	0.0000578	0.0000579	152

aThe references point to publications that have reported the drugs' mechanism of action potentially linked with Covid-19.
Covid-19, coronavirus disease 2019; Covid-19 KG, Covid-19 knowledge graph; DRKG, drug repurposing knowledge graph; DSOG, disease state-specific omics graph; RWR, random walk with restart; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SOD2, superoxide dismutase 2; STAT1, signal transducer and activator of transcription 1.

RWR analysis (**Figure 4A–C**). Specifically, these differences were observed in the connectivity of the drug repurposing candidates with the corresponding node degree, betweenness centrality, and closeness centrality scores (**Table 7**). This provides more insights into the most appropriate drug repurposing candidates for the different disease states. For instance, *curcumin* had a higher degree of 1076 in the multi-layered graph generated by the RWR analysis on the mild disease state as compared to a degree of 4 in the multi-layered graph generated by the RWR analysis on the moderate disease state and a degree of 2 in the multi-layered graphs generated by the RWR analysis on the severe disease

state. *Podofilox* had a higher degree of 478 in the multi-layered graph generated by the RWR analysis on the severe disease state as compared to a degree of 2 for both multi-layered graphs generated by the RWR analysis on the mild and moderate disease states. Therefore, whereas *curcumin* is an appropriate drug repurposing candidate with potential utility during mild Covid-19, *podofilox* is an appropriate drug repurposing candidate with potential utility during severe Covid-19. Furthermore, *vinblastine* showed a high degree of 1905 in the multi-layered graph generated by the RWR analysis on the moderate disease state and a degree of 1378 in the multi-layered graph generated by the RWR analysis on the
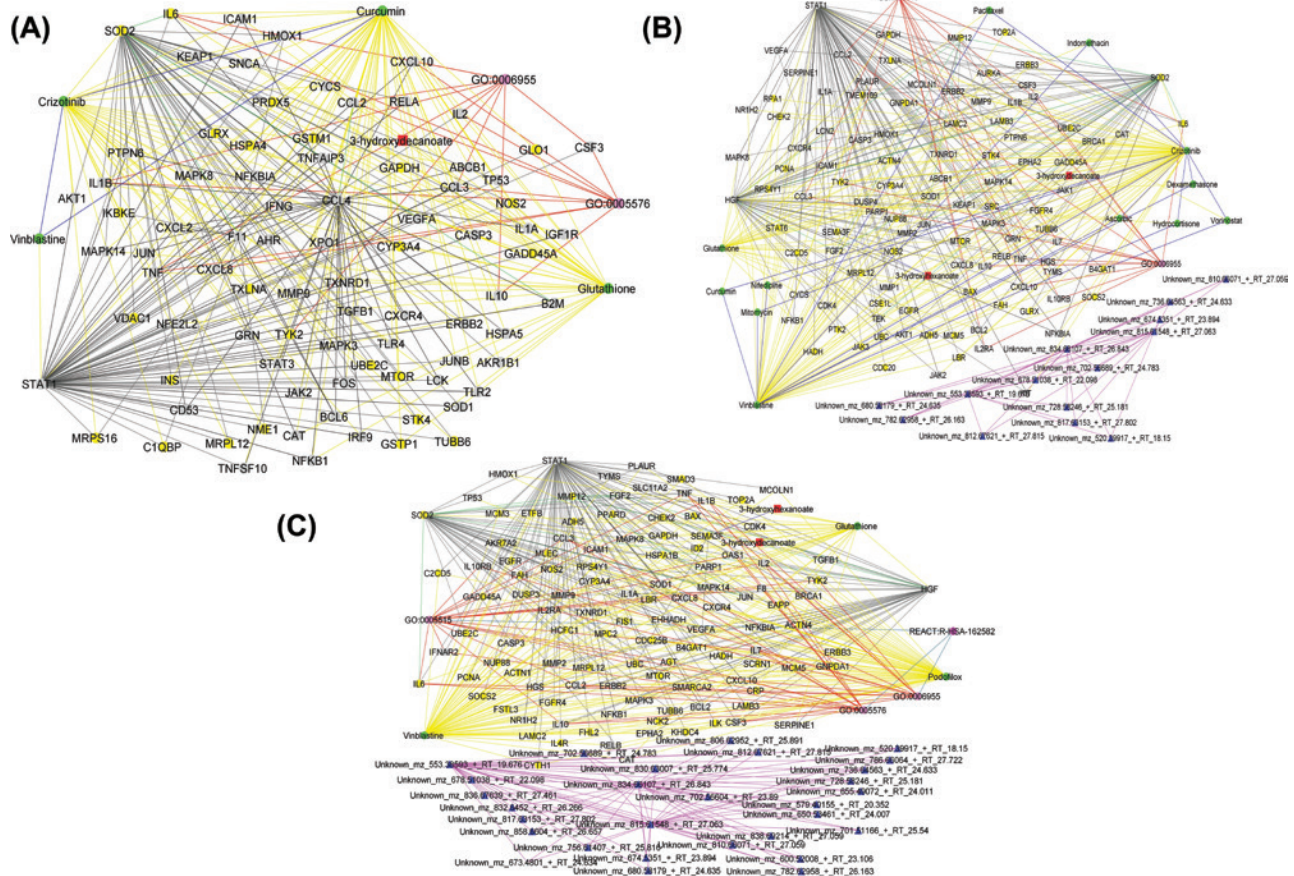

Figure 4. (A) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), lipids (blue nodes), and biological processes and pathways (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, mild disease state-specific omics graphs (DSOGs), and data-driven seeds. The graph reveals distinct subnetworks formed by hub CCL4, demonstrating extensive interactions with drug candidates and other molecular features including seed nodes (STAT1 and SOD2). (B) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), lipids (blue nodes), and biological processes and pathways (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, moderate DSOGs, and data-driven seeds. The graph reveals distinct subnetworks formed by the hub HGF, demonstrating extensive interactions with drug candidates and other molecular features including seed nodes (STAT1 and SOD2), as well as a subnetwork formed among lipids. (C) Graph representation of the interaction between drugs (green nodes), proteins (yellow nodes), transcripts (gray nodes), metabolites (red nodes), lipids (blue nodes), and biological processes and pathways (pink nodes) as observed from predicting candidate drugs using existing knowledge graphs, severe DSOGs, and data-driven seeds. The graph reveals distinct subnetworks formed by the hub HGF, demonstrating extensive interactions with drug candidates and other molecular features including seed nodes (STAT1 and SOD2), as well as a subnetwork formed among lipids. Yellow edges represent drug–protein and drug–transcript pairwise interactions. Red edges represent biological process–protein interactions and biological process–transcript interactions. Pink edges represent pairwise interactions between lipids. Green edges represent protein–protein interactions. Black edges represent transcript–transcript interactions and protein–transcript interactions. Dark blue edges represent drug–drug interactions. Light blue edges represent biological process–biological process interactions and biological process–pathway interactions. The graphs were generated by defining filtering criteria based on the node degree between 4 and 1633 in Cytoscape. CCL4, C-C motif chemokine ligand 4; HGF, hepatocyte growth factor; SOD2, superoxide dismutase 2; STAT1, signal transducer and activator of transcription 1.

Table 7: Node Degree, Betweenness, and Closeness Centrality Measures for the Drug Repurposing Candidates Predicted Using the Data-driven Approach.

Drug Name	Degree, Mild State	Degree, Moderate State	Degree, Severe State	Betweenness, Mild State	Betweenness, Moderate State	Betweenness, Severe State	Closeness, Mild State	Closeness, Moderate State	Closeness, Severe State
Crizotinib	1919	1947	2	0.5147	0.4171	0.0025	0.5478	0.5297	0.3987
Curcumin	1076	4	2	0.3281	0.0005	0.0	0.5389	0.4317	0.3983
Glutathione	212	234	118	0.0397	0.0350	0.0439	0.3532	0.3611	0.3657
Vinblastine	4	1905	1378	0.0009	0.4787	0.6440	0.4364	0.5639	0.5556
Acetylcysteine	2	2	1	0.0	0.0	0.0	0.3106	0.3028	0.2743
Calcipotriol	2	2	1	0.0	0.0	0.0	0.3178	0.3186	0.3370
Etoposide	2	2	2	0.0	0.0	0.0001	0.3987	0.3993	0.3715
Fludarabine	2	3	2	0.0	0.0	0.0	0.3178	0.3902	0.3983
Iron	2	2	1	0.0	0.0	0.0	0.3106	0.3028	0.2743
Mebendazole	2	2	1	0.0	0.0	0.0	0.3178	0.3186	0.3370
Omacetaxine	2	2	1	0.0	0.0	0.0	0.2438	0.2466	0.2460
Podofilox	2	2	478	0.0	0.0	0.1438	0.3178	0.31863	0.4092
Teniposide	2	2	2	0.0	0.0	0.0001	0.3987	0.3993	0.3715
Thimerosal	2	2	1	0.0	0.0	0.0	0.3106	0.3028	0.2743
Valine	2	2	1	0.0	0.0	0.0	0.3106	0.3028	0.2743
Vorinostat	2	4	2	0.0009	0.0005	0.0	0.4199	0.4317	0.3983
Carglumic acid	1	1	1	0.0	0.0	0.0	0.2610	0.2653	0.2678
L-glutamine	1	1	1	0.0	0.0	0.0	0.2610	0.2653	0.2678
Threonine	1	2	1	0.0	0.0	0.0	0.3106	0.3028	0.2743
Pregabalin	1	1	1	0.0	0.0	0.0	0.3539	0.3463	0.3463

severe disease state as compared to a degree of 4 in the multi-layered graph from the RWR analysis on the mild disease state. This suggests that *vinblastine* might be most effective in tackling advanced disease stages. Also, *crizotinib* showed a high degree of 1919 in the multi-layered graph generated by the RWR analysis on the mild disease state and a degree of 1947 in the multi-layered graph generated by the RWR analysis on the moderate state as compared to a degree of 2 in the multi-layered graph from the RWR analysis on the severe disease state; this indicates that it might be most appropriate for treatment of the mild and moderate stages of Covid-19 (**Table 7**). Similarly, *glutathione* showed a high degree of 212 in the multi-layered graph generated by the RWR analysis on the mild disease state and a degree of 234 in the multi-layered graph generated by the RWR analysis on the moderate disease state as compared to a degree of 118 in the multi-layered graph from the RWR analysis on the severe disease state; this suggests that it can be a promising drug repurposing candidate for the mild and moderate disease states. Noticeably, nodes with higher degree scores (**Table 7**) have higher betweenness and closeness scores revealing how often these nodes lie on the shortest paths between other nodes in the network and mediate how quickly information can reach other disease-related features from a candidate drug (**Supplementary File 5**). Such nodes have high relevance within biological systems and, besides their specific biological activities, might also facilitate communication and synergy between biological pathways, making them key targets for the management of the disease.

Analysis of the multi-layered graphs generated by the RWR analysis revealed several features that establish subnetworks (**Figure 4**). Specifically, we observed the *CCL4* to establish subnetwork in the mild disease state and hepatocyte growth factor

(*HGF*) to establish subnetworks in both the moderate and severe disease states. *HGF* expresses anti-inflammatory properties and plays a complex and multifaceted role in the battle against Covid-19.[96] While it initially acts as a crucial player in lung tissue repair following viral damage, its activity can also contribute to excessive inflammation if not properly regulated.[96] *HGF* can activate certain signaling pathways that promote inflammation in moderate to severe cases. On the other hand, upregulation of *HGF* represents a robust counter-regulatory mechanism employed by the host immune response to counteract pro-inflammatory cytokines.

Drug Prediction Robustness Analysis

The three highest-ranked candidate drugs yielded by both the hypothesis-driven approach (*dexamethasone*, *sarilumab*, and *tocilizumab*) and the data-driven approach (*glutathione*, *crizotinib*, and *curcumin*) are all known to be efficacious in controlling moderate to severe Covid-19. However, the high rankings of these drugs (based on the measures of proximity) are expected simply because their efficacy during moderate to severe Covid-19 treatment has been comprehensively reported in the literature up to 2021. These reports are reflected in the Covid-19 KG layers of our networks.

In this section, we removed direct interactions between the top three predicted potential drug candidates identified using the hypothesis-driven and data-driven approaches and other features (such as drugs, proteins, and transcripts) to assess the influence of these interactions and features on the drug predictions. We assessed the robustness of the drug predictions in both the hypothesis-driven and data-driven approaches by repeating the RWR analysis (as described in the **Materials and Methods** section)

after individually and collectively removing direct interactions associated with the three highest-ranked candidate drugs and examining changes in the drug rankings.

These analyses yielded a relatively consistent trend (**Supplementary Files 1 and 2**): Drugs such as *sirolimus*, *histamine*, *cyclosporine,* and *vorinostat* that initially ranked below the drugs that were removed tended to achieve higher rankings following the removal of the initial top-ranked drug candidates. The measure of proximity of the drugs that attained elevated rankings varied from one drug removal experiment to the next, but generally increased relative to the measure of proximity that the drugs obtained in the absence of any exclusions (**Supplementary Files 1 and 2**).

Furthermore, the drug removal analyses revealed additional candidate drugs that were not apparent in the absence of drug removal. For instance, with the hypothesis-driven approach, when we removed *dexamethasone* and *tocilizumab* simultaneously for each disease state, drugs like *dinoprostone* and *perhexiline* emerged among the top drug candidates across the mild, moderate, and severe disease states (**Supplementary File 1**). Similarly, when we removed sarilumab, we observed *ketamine, acetylsalicylic acid* (aspirin), and *menadione* in the top 20. Concurring with the individual drug removal, when *dexamethasone, tocilizumab*, and *sarilumab* were collectively removed, we observed that *dinoprostone*, *perhexiline*, *menadione*, iron, and *ketamine* all entered the top 20 for the disease states.

When we excluded the top-ranked drug candidates that were revealed by the data-driven approach (*glutathione, crizotinib,* and *curcumin*), we observed similar ranking score changes to those seen with the drug candidates identified by the hypothesis-driven approach. For example, when *glutathione* was removed, the measure of proximity of both *L-glutamine* and *carglumic acid* dropped substantially (from 0.0000945 and 0.0000942 to 0.0000022 and 0.0000594, respectively) resulting in lower rankings, whereas thimerosal disappeared completely (**Supplementary File 2**). This observation could be partly because (i) *thimerosal* interacted with nodes that established connections with *glutathione* and *crizotinib* and (2) *thimerosal* established direct interactions with *glutathione* and *crizotinib*.

When *glutathione*, *crizotinib*, and *curcumin* were collectively removed, several notable drug candidates surfaced among the top hits, including *penicillamine, pregabalin, dexamethasone*, *midostaurin,* and *treprostinil* (**Supplementary File 2**). Similar to when *crizotinib* and *curcumin* were individually removed, the measure of proximity of *L-glutamine* and *carglumic acid* increased from 0.0000945 and 0.0000942 to 0.0001621 and 0.0001629 for *crizotinib* removal and from 0.0000945 and 0.0000942 to 0.0001306 and 0.0001308 for *curcumin* removal, respectively (**Supplementary File 2**).

In Silico Validation of Top Hit Candidate Drugs

To validate the Covid-19 drug predictions, we aimed to investigate how enriched the potential candidate drugs are as anti-Covid drugs in other databases. Specifically, we conducted RWR analyses using hypothesis-driven seeds on drug data extracted from DrugCombDB (version 2.0), a drug resource database.[97] We

implemented these analyses to investigate whether we were able to predict known efficacious Covid-19 drugs (**Table 4**). Among the top-ranked drugs (**Supplementary File 3**) revealed by these analyses were *dexamethasone* (rank 1), *simvastatin* (rank 7), *cyclosporine* (rank 8), *hydrocortisone* (rank 9), *paclitaxel* (rank 11), *indomethacin* (rank 15), and *methotrexate* (rank 16).

DISCUSSION

In this work, we employed computational analyses for the prediction of drug repurposing candidates tailored for disease state-specific Covid-19 treatment. Leveraging a combination of knowledge graphs (DRKG and Covid-19 KG), along with Covid-19 DSOGs generated from experimental proteomics, transcriptomics, metabolomics, and lipidomics data, enabled the identification of various drug repurposing candidates that could potentially be usable as treatments for specific Covid-19 disease states. In our study, we recognize the importance of edge information in characterizing the interactions between nodes as described in **Supplementary Tables 1 and 2**. Within the framework of the RWR algorithm, each layer is made up of interactions of specific edge types, such as drug–drug, drug–protein, and drug–transcript interactions, which can offer unique insights into the connectivity and relationships within the network, including the potential indirect associations between nodes and their impact on network dynamics. We implemented MultiXrank, a random walk algorithm capable of handling multiple multi-layered graphs and integrated drug data to predict candidate drugs for the mild, moderate, and severe Covid-19 disease states. The analysis resulted in the generation of multi-layered graphs that described the exploration of seed nodes across different DSOGs. One of the key advantages of the RWR analysis compared to the naïve first neighbor method is its ability to explore distant nodes in multi-layered graphs beyond just the immediate neighbors of a given seed node. The RWR analysis enables us to "restart" random walks from the seed nodes, traverse the network iteratively, and assign restart probabilities to nodes based on their likelihood of being visited. By incorporating restart probabilities and iteratively exploring the network based on random walks, RWR can uncover connections to nodes that may not be directly adjacent to the seed but are still given network connectivity and influence.

Although the hypothesis-driven and data-driven methodologies differed, the findings from both approaches have contributed to prioritizing potential drug repurposing candidates for mild, moderate, and severe Covid-19. We recommend considering both data- and hypothesis-driven approaches in studies utilizing multiple-source omics datasets. The hypothesis-driven approach revealed mostly drugs known to suppress the immune response and reduce inflammation, including those promoting IL6 production. In contrast, the data-driven approach revealed more diverse drugs including "natural compounds" (such as *glutathione* and *curcumin*) and inhibitors of signal transduction pathways of protein kinases and cell proliferation (tyrosine, histone deacetylase, and methyltransferase). The random walk analysis using both the hypothesis-driven and data-driven approaches yielded distinct multi-layered graphs (**Figures 3 and 4**) characterized by different

hubs and interactions with the candidate drugs, highlighting the unique perspectives offered by each method. With these differences, a consistent finding emerged from both approaches: Cross-layer interactions between omics features and drug repurposing candidates play a role in the dynamics of the drugs at the different disease states.

Some of the drugs identified through both hypothesis-driven and data-driven approaches are being, or have already been, tested in various clinical trials to assess their efficacy and effectiveness in the treatment of Covid-19. For instance, from the hypothesis-driven approach, corticosteroids such as *dexamethasone* and *hydrocortisone* have demonstrated an association with lower 28-day all-cause mortality in critically ill patients with Covid-19,[98] including *mycophenolic acid*, which has been investigated and validated to reduce mortality and hospital stays in patients with moderate to severe Covid-19,[99] *indomethacin*, which has been found in clinical trials to be safe and effective for treating mild and moderate Covid-19 cases,[63] and the diabetes medication *metformin*, which exhibits potential in reducing prolonged illness by inhibiting virus replication when administered during the acute phase of Covid-19.[100] From the data-driven approach, the antihelminthic drug *mebendazole* enhanced innate immune responses and restored inflammation to normal levels in symptomatic non-hospitalized Covid-19 patients during a recent clinical trial.[101] Also, *etoposide* has been investigated for its potential to treat severe disease, albeit with observed adverse events that warrant further investigation.[102]

In general, immunosuppressive drugs might have a beneficial effect in the moderate to severe phase of Covid-19 because it is in this phase dysregulated pro-inflammatory immune responses can precipitate tissue damage and result in acute respiratory distress syndrome, organ failure, and mortality.[103] On the other hand, drugs predicted using the data-driven approach either had antioxidant properties (such as glutathione, and curcumin) or were inhibitors of tyrosine kinase, histone deacetylase, methyltransferase, and protein synthesis (**Table 7**). The antioxidants can protect immune system cells and those directly targeted by SARS-CoV-2 from oxidative stress. For example, *glutathione* is an antioxidant assumed to have a vital role in maintaining the balance of reactive oxygen species (ROS) and aids in diverse cellular processes including immune responses.[104,105] Notably, oxidative stress reflects an imbalance between ROS generation and antioxidation mechanisms[106] and plays an important role in Covid-19 onset, progression, and severity,[107–109] possibly by exacerbating inflammation and tissue damage.[110] This, therefore, suggests that *glutathione's* capability to counteract ROS and diminish oxidative stress holds promise for mitigating some of the adverse effects inflicted by the virus.[104] *Glutathione* and *SOD2* bring unique strengths such that their combined efforts provide a multi-layered defense against oxidative stress and its harmful consequences. By neutralizing superoxide radicals, *SOD2* sets the stage for glutathione to efficiently handle other free radicals and detoxify the cell. Also, given the aggressive inflammatory response and the production of cytokines occurring during severe Covid-19 disease states, some known inhibitors of receptor tyrosine kinases and cell proliferation,

such as *crizotinib* and *vorinostat*, have been investigated as Covid-19 treatments.[111,112] For instance, a recent study has shown that histone deacetylase inhibitors modulate immune responses in stimulated monocytes,[112] whereas tyrosine kinase inhibitors have the potential to reverse pulmonary insufficiency because of their anti-inflammatory activities, cytokine suppression activities, or antifibrotic activities.[111]

Overall, the prioritized drug repurposing candidates (**Tables 3, 4, and 7**) exhibit the potential to target a multitude of specific biological pathways and gene ontology processes that are associated with Covid-19 outcomes. Among these candidates are those that have shown promise in treating other diseases or conditions such as cancer, malaria, viral infections, and obstructive pulmonary disease. For instance, *glutathione* shows activity against HIV, influenza A, and hepatitis C by inhibiting viral replication and modulating immune response.[113] *Curcumin* shows activity against HIV, influenza A, hepatitis C, and dengue virus by inhibiting viral entry and replication.[114] *Vorinostat* shows activity against HIV by inhibiting viral replication and disrupting HIV-1 latency in patients on antiretroviral therapy.[115] Consequently, there is the possibility of repurposing these drugs to combat Covid-19 and other virus-induced conditions.

The approach implemented in this study is relevant to identifying drugs that warrant further exploration. Our methodology leading to the findings has been controlled through leveraging a data-driven approach complemented by a hypothesis-driven approach to minimize bias and ensure the robustness of findings effectively. However, validation procedures on interactome datasets such as (i) training a model to predict with which targets a previously unseen drug will interact and (ii) finding new putative interactions between drugs and other omics data types already in the datasets, can be implemented to reduce bias.[116] It is important to mention that some of the drugs that were highly ranked in our hypothesis-driven and data-driven analyses as potential Covid-19 treatments have not, to our knowledge, been tested before in the context of Covid-19 treatment. These included *podofilox*, *calcipotriol*, *vinblastine*, *etoposide*, and *carglumic acid* identified from the data-driven approach and *paclitaxel* identified from the hypothesis-driven approach. The shortest path analysis revealed molecular features that were close to the drugs (**Supplementary Files 4 and 5**). Considering the drugs generated from the data-driven approach, *podofilox*, for example, inhibits topoisomerase I by stabilizing the covalent complex formed between the enzyme and a broken DNA strand.[117] This prevents religation, causing DNA damage and eventually cell death.[117] *Podofilox* is known to down-regulate SOD2 expression in cancer cells and indirectly modulate *SOD2* activity, impacting ROS levels and influencing cell survival and death. The ROS impact on Covid-19 progression[107–109] suggests that *podofilox* may have a potential role in Covid-19 treatment. Also, *etoposide* possesses an immunosuppressive effect. While suppressing certain immune cells, *etoposide* may also selectively eliminate abnormal or activated T cells involved in the inflammatory process.[118] This can be beneficial in some inflammatory conditions, potentially mitigating immune-mediated damage. Additionally, *etoposide* has the potential to influence the

production of certain cytokines and signaling molecules involved in immune communication.[119] In the context of Covid-19, this could have both pro-inflammatory and anti-inflammatory effects. *Vinblastine* can modulate the production of certain cytokines, signaling molecules that orchestrate immune responses.[120]

Furthermore, the analysis conducted indicated that ritonavir, an HIV protease inhibitor utilized in combination with nirmatrelvir in the potent COVID-19 treatment paxlovid, was assigned a lower ranking in our data-driven analysis. This may be attributed to the characteristics of the exploited knowledge graphs that contained limited information about the impacts of *ritonavir* on the transcriptomics, proteomics, lipidomics, and metabolomics of human cells. As a result, the topology of the networks that we used was biased in favor of ranking better-researched compounds like *dexamethasone* and *tocilizumab*. In our analysis, we did not identify *nirmatrelvir* among the ranked drug candidates either. This observation is partly attributed to the choice of seeds for the RWR analyses and also to the fact we focus here on the omics networks from the host because *nirmatrelvir* targets the viral genome (polyprotein 1ab) and could, therefore, not be captured by the network exploration.

This analysis, drawing on diverse datasets, has provided valuable insights that contribute to ongoing efforts to combat endemic Covid-19 and the long-term health consequences of repeated SARS-CoV-2 infections. While some of the identified drugs have been implemented in disease management, several promising candidates are yet to be investigated for Covid-19 disease treatment and require experimental validation which is beyond the scope of this study. However, the predictions provide a starting point for further experimental validation and clinical investigations. Ensuring the safety and efficacy of new Covid-19 drugs requires rigorous experimental and clinical testing and validation. *In vitro* analyses and clinical trials must be conducted to determine the cytotoxicity, optimal dosages, administration protocols, and potential interactions with other medications. These experiments would ultimately be needed to provide actual proof that many of the less well-studied drug repurposing candidates that we have identified could indeed be used to effectively treat Covid-19. Importantly, the algorithmic framework implemented in this study can be translated to other diseases to investigate relevant drug repurposing candidates and to explore the dynamics among drugs and multi-omics features in a multi-layered network. We recommend considering both data- and hypothesis-driven approaches in studies utilizing multiple-source omics datasets.

LIMITATIONS

Considering the limitations of the DRKG and Covid-19 KG data, which predate large-scale drug evaluations, incorporating more recent drug information is crucial for future studies. While this study identified potential drugs for acute Covid-19 treatment, it did not address long Covid or the impact of comorbidities and disease severity. Thus, future investigations should explicitly explore treatment options for long Covid Furthermore, our

drug prediction analysis did not account for Covid-19 comorbidities and recommends further studies to refine drug prediction analysis specific for mild, moderate, or severe Covid-19-infected patients experiencing other infections. Additionally, we acknowledge that incorporating information about pharmacokinetics, pharmacodynamics, and drug effects such as activator, or inhibitor, status or details on drug synergy could provide a more comprehensive understanding of the therapeutic implications. To maximize the potential of our approach, future work should incorporate pharmacokinetics, pharmacodynamics, drug effect information, and drug synergy analysis. By systematically evaluating how the therapeutic activities of different drugs might combine, we can identify and prioritize the most promising combination therapies for further testing and development in the fight against Covid-19. While we have not used dynamic networks in this study, we compared static networks in the mild, moderate, and severe phases of Covid-19 and drew conclusions on the drugs that may be effective in each of these phases. We did not account for temporal changes such as the dynamic nature of biological interactions in the network. We acknowledge this limitation and recognize the implications it may have on the relevance of the results, especially in scenarios where temporal dynamics may play a critical role. We recommend future studies to incorporate temporal changes and time-dependent processes, as well as methods to explore time series data and time-dependent processes.[121,122]

CONCLUSION

This study explored an integrative multi-layered network approach to identify drugs for repurposing against Covid-19 disease phases. We analyzed multi-omics data (proteomics, transcriptomics, metabolomics, and lipidomics) and drug-related data (DRKG and Covid-19 KG) using the RWR technique. Notably, we conducted RWR analyses in both hypothesis-driven and data-driven manners, incorporating information specific to disease severity levels (mild, moderate, severe) via dedicated DSOGs. Our multi-layered network approach successfully identified potential drug candidates for repurposing against mild, moderate, and severe Covid-19. The incorporation of disease state-specific omics data significantly influenced the predicted drug candidates. Both immunosuppressive and pathway-targeting mechanisms emerged as potential approaches for Covid-19 treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

The authors have consented for the work to be published.

DATA AND CODE AVAILABILITY

To adhere to FAIR principles and facilitate replication of our approach, we provide a containerized workflow with an expanded

AUTHOR CONTRIBUTIONS

FEA, ERC, and PACH conceived the study. FEA conducted the data analysis and prepared the first draft. PACH, TAHE, MS, DPM, and ERC supervised the work. THAE, MS, ERC, DPM, and PACH contributed to the revision of the article. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

REFERENCES

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020;20(5): 533–534. DOI: 10.1016/S1473-3099(20)30120-1

2. Menni C, May A, Polidori L, et al. COVID-19 vaccine waning and effectiveness and side-effects of boosters: A prospective community study from the ZOE COVID study. Lancet Infect Dis. 2022;22(7): 1002–1010. DOI: 10.1016/S1473-3099(22)00146-3

3. Ferdinands JM, Rao S, Dixon BE, et al. Waning of vaccine effectiveness against moderate and severe COVID-19 among adults in the US from the VISION network: Test negative, case-control study. Br Med J. 2022;379: e072141. DOI: 10.1136/bmj-2022-072141

4. Lau JJ, Cheng SMS, Leung K, et al. Real-world COVID-19 vaccine effectiveness against the Omicron BA. 2 variant in a SARS-CoV-2 infection-naive population. Nat Med. 2023;29(2): 348–357. DOI: 10.1038/s41591-023-02219-5

5. Zhang, Y, Banga Ndzouboukou JL, Gan M, Lin X, Fan X. Immune evasive effects of SARS-CoV-2 variants to COVID-19 emergency used vaccines. Front Immunol. 2021;12: 771242. DOI: 10.3389/fimmu.2021.771242

6. Telenti A, Arvin A, Corey L, et al. After the pandemic: Perspectives on the future trajectory of COVID-19. Nature. 2021;596(7873): 495–504. DOI: 10.1038/s41586-021-03792-w

7. Kiplin Guy R, DiPaola RS, Romanelli F, Dutch RE. Rapid repurposing of drugs for COVID-19. Science. 2020;368(6493): 829–830. DOI: 10.1126/science.abb9332

8. Hammond J, Leister-Tebbe H, Gardner A, et al. Oral nirmatrelvir for high-risk, nonhospitalized adults with Covid-19. N Engl J Med. 2022;386(15): 1397–1408. DOI: 10.1056/NEJMoa2118542

9. Beigel JH, Ansems K, Dahms K, et al. Remdesivir for the treatment of Covid-19. N Engl J Med. 2020;383(19): 1813–1826. DOI: 10.1002/14651858.CD014962.pub2

10. Diaz GA, Christensen AB, Pusch T, et al. Remdesivir and mortality in patients with coronavirus disease 2019. Clin Infect Dis. 2022;74(10): 1812–1820. DOI: 10.1093/cid/ciab698

11. WHO Solidarity Trial Consortium; Pan H, Peto R, et al. Repurposed antiviral drugs for Covid-19 – interim WHO solidarity trial results. N Engl J Med. 2021;384(6): 497–511. DOI: 10.1056/NEJMoa2023184

12. Dryden-Peterson S, Kim A, Kim AY, et al. Nirmatrelvir plus ritonavir for early COVID-19 in a large US health system: A population-based cohort study. Ann Intern Med. 2023;176(1): 77–84. DOI: 10.7326/M22-2141

13. RECOVERY Collaborative Group; Horby P, Lim WS, et al. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med. 2021;384(8): 693–704. DOI: 10.1056/NEJMoa2021436

14. Ma S, Su W, Sun C, et al. Does aspirin have an effect on risk of death in patients with COVID-19? A meta-analysis. Eur J Clin Pharmacol. 2022;78(9): 1403–1420. DOI: 10.1007/s00228-022-03356-5

15. RECOVERY Collaborative Group. Aspirin in patients admitted to hospital with COVID-19 (RECOVERY): A randomised, controlled, open-label, platform trial. Lancet. 2022;399(10320): 143. DOI: 10.1016/S0140-6736(21)01825-0

16. Chow JH, Rahnavard A, Gomberg-Maitland M, et al. Association of early aspirin use with in-hospital mortality in patients with moderate COVID-19. JAMA Network Open. 2022;5(3): e223890. DOI: 10.1001/jamanetworkopen.2022.3890

17. Liew MNY, Kua KP, Huey Lee SW, Wong KK. SARS-CoV-2 neutralizing antibody bebtelovimab-a systematic scoping review and meta-analysis. Front Immunol. 2023;14: 1100263. DOI: 10.3389/fimmu.2023.1100263

18. Sivapalasingam S, Lederer DJ, Bhore R, et al. Efficacy and safety of sarilumab in hospitalized patients with coronavirus disease 2019: A randomized clinical trial. Clin Infect Dis. 2022;75(1): e380–e388. DOI: 10.1093/cid/ciac153

19. Benucci M, Giannasi G, Cecchini P, et al. COVID-19 pneumonia treated with sarilumab: A clinical series of eight patients. J Med Virol. 2020;92(11): 2368–2370. DOI: 10.1002/jmv.26062

20. Mushtaq MZ, Mahmood SBZ, Almas A, Ather Wasti S, Ahsan Ali S. Tocilizumab in critically ill COVID-19 patients: An observational study. Int Immunopharmacol. 2022;102: 108384. DOI: 10.1016/j.intimp.2021.108384

21. Gupta S, Wang W, Hayek SS, et al. Association between early treatment with tocilizumab and mortality among critically ill patients with COVID-19. JAMA Intern Med. 2021;181(1): 41–51. DOI: 10.1001/jamainternmed.2020.6252

22. Heskin J, Pallett SJC, Mughal N, et al. Caution required with use of ritonavir-boosted PF-07321332 in COVID-19 management. Lancet. 2022;399(10319): 21–22. DOI: 10.1016/S0140-6736(21)02657-X

23. Nevalainen OP, Pallett SJC, Mughal N, et al. Effect of remdesivir post hospitalization for COVID-19 infection from the randomized SOLIDARITY Finland trial. Nat Commun. 2022;13(1): 6152. DOI: 10.1016/S0140-6736(21)02657-X

24. Kocks J, Kerkhof M, Scherpenisse J, et al. A potential harmful effect of dexamethasone in non-severe COVID-19: Results from the COPPER-pilot study. ERJ Open Res. 2022;8(2): 00129-2022. DOI: 10.1183/23120541.00129-2022

25. Panda S, Kumari L, Badwaik HR, Shanmugarajan D. Computational approaches for drug repositioning and repurposing to combat SARS-CoV-2 infection. In: Parihar A, Khan R, Kumar A, Kaushik AK, Gohel H, editors. Computational approaches for novel therapeutic and diagnostic designing to mitigate SARS-CoV2 infection. Amsterdam: Elsevier; 2022. pp. 247–265.

26. Tomazou M, Bourdakou MM, Minadakis G, et al. Multi-omics data integration and network-based analysis drives a multiplex drug repurposing approach to a shortlist of candidate drugs against COVID-19. Brief Bioinform. 2021;22(6): bbab114. DOI: 10.1093/bib/bbab114

27. Wang J. Fast identification of possible drug treatment of coronavirus disease-19 (COVID-19) through computational drug repurposing study. J Chem Inf Model. 2020;60(6): 3277–3286. DOI: 10.1021/acs.jcim.0c00179

28. Mahdian S, Ebrahim-Habibi A, Zarrabi M. Drug repurposing using computational methods to identify therapeutic options for COVID-19. J Diabetes Metab Disord. 2020;19: 691–699. DOI: 10.1007/s40200-020-00546-9

29. Hsieh K, Wang Y, Chen L, et al. Drug repurposing for COVID-19 using graph neural network and harmonizing multiple evidence. Sci Rep. 2021;11(1): 23179. DOI: 10.1038/s41598-021-02353-5

30. Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. Cell Discov. 2020;6(1): 14. DOI: 10.1038/s41421-020-0153-3

31. Ioannidis VN, Song X, Manchanda S, et al. Drkg-drug repurposing knowledge graph for Covid-19. arXiv preprint arXiv:2010.09600. 2020

32. Baptista A, Brière G, Baudot A. Random walk with restart on multilayer networks: From node prioritisation to supervised link prediction and beyond. BMC Bioinformatics. 2024;25(1): 1–19. DOI: 10.1186/s12859-024-05683-z

33. Sosa DN, Derry A, Guo M, et al. A literature-based knowledge graph embedding method for identifying drug repurposing opportunities in rare diseases. In: Pacific symposium on biocomputing 2020. Singapore: World Scientific; 2019.

34. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: Customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49(D1): D605–D612. DOI: 10.1093/nar/gkaa1074

35. Del Toro N, Shrivastava A, Ragueneau E, et al. The IntAct database: Efficient access to fine-grained molecular interaction data. Nucleic Acids Res. 2022;50(D1): D648–D653. DOI: 10.1093/nar/gkab1006

36. Himmelstein DS, Lizee A, Hessler C, et al. Systematic integration of biomedical knowledge prioritizes drugs for repurposing. Elife. 2017;6: e26726. DOI: 10.7554/eLife.26726

37. Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46(D1): D1074–D1082. DOI: 10.1093/nar/gkx1037

38. Freshour SL, Kiwala S, Cotto KC, et al. Integration of the drug–gene interaction database (DGIdb 4.0) with open crowdsource efforts. Nucleic Acids Res. 2021;49(D1): D1144–D1151. DOI: 10.1093/nar/gkaa1084

39. Davis AP, Grondin CJ, Johnson RJ, et al. Comparative toxicogenomics database (CTD): Update 2021. Nucleic Acids Res. 2021;49(D1): D1138–D1143. DOI: 10.1093/nar/gkaa891

40. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816): 459–468. DOI: 10.1038/s41586-020-2286-9

41. Agamah FE, Ederveen TH, Skelton M, Martin D, Chimusa ER, Hoen PAT. Network-based integrative multi-omics approach reveals biosignatures specific to COVID-19 disease phases. Front Mol Biosci. 2024;11. DOI: 10.3389/fmolb.2024.1393240

42. Overmyer KA, Shishkova E, Miller IJ, et al. Large-scale multi-omic analysis of COVID-19 severity. Cell Syst. 2021;12(1): 23–40.e7. DOI: 10.1016/j.cels.2020.10.003

43. Su Y, Chen D, Yuan D, et al. Multi-omics resolves a sharp disease-state shift between mild and moderate COVID-19. Cell. 2020;183(6): 1479–1495.e20. DOI: 10.1016/j.cell.2020.10.037

44. UniProt Consortium. UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Res. 2021;49(D1): D480–D489. DOI: 10.1093/nar/gkaa1100

45. Hinkson CR. COVID-19 treatment guidelines. National Institutes of Health; 2022. https://digitalcommons.providence.org/publications/6499

46. Bhimraj A, Morgan RL, Shumaker AH, et al. Infectious Diseases Society of America Guidelines on the treatment and management of patients with coronavirus disease 2019 (COVID-19). Clin Infect Dis. 2020: ciaa478. DOI: 10.1093/cid/ciaa478

47. Baptista A, Gonzalez A, Baudot A. Universal multilayer network exploration by random walk with restart. Commun Phys. 2022;5(1): 1–9. DOI: 10.1038/s42005-022-00937-9

48. Agamah FE, Bayjanov JR, Niehues A, et al. Computational approaches for network-based integrative multi-omics analysis. Front Mol Biosci. 2022;9: 967205. DOI: 10.3389/fmolb.2022.967205

49. Arazi A, Ribeiro RM, Perelson AS, Hacohen N. Human systems immunology: Hypothesis-based modeling and unbiased data-driven approaches. Semin Immunol. 2013;25: 193–200. DOI: 10.1016/j.smim.2012.11.003

50. Eriksson O, Bhalla US, Blackwell KT, et al. Combining hypothesis-and data-driven neuroscience modeling in FAIR workflows. Elife. 2022;11: e69013. DOI: 10.7554/eLife.69013

51. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet. 2020;395(10229): 1054–1062. DOI: 10.1016/S0140-6736(20)30566-3

52. Chen LY, Hoiland RL, Stukas S, Wellington CL, Sekhon MS. Confronting the controversy: Interleukin-6 and the COVID-19 cytokine storm syndrome. Eur Respir J. 2020;56(4): 2003006. DOI: 10.1183/13993003.03006-2020

53. Coomes EA, Haghbayan H. Interleukin-6 in COVID-19: A systematic review and meta-analysis. Rev Med Virol. 2020;30(6): 1–9. DOI: 10.1002/rmv.2141

54. Lv B, Yu W, Wang L, McCann JA. Efficient processing node proximity via random walk with restart. In: Web technologies and applications: 16th Asia-Pacific Web Conference, APWeb 2014, Changsha, China, September 5–7, 2014. Proceedings 16. Berlin: Springer; 2014. DOI: 10.1007/978-3-319-11116-2

55. Jung J, Shin K, Sael L, Kang U. Random walk with restart on large graphs using block elimination. ACM Trans Database Syst. 2016;41(2): 1–43. DOI: 10.1145/2901736

56. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): A randomised, controlled, open-label, platform trial. Lancet. 2021;397(10285): 1637–1645. DOI: 10.1016/S0140-6736(21)00676-0

57. Samaee H, Mohsenzadegan M, Ala S, Maroufi SS, Moradimajd P. Tocilizumab for treatment patients with COVID-19: Recommended medication for novel disease. Int Immunopharmacol. 2020;89: 107018. DOI: 10.1016/j.intimp.2020.107018

58. Bengtson CD, Montgomery RN, Nazir U, et al. An open label trial to assess safety of losartan for treating worsening respiratory illness in COVID-19. Front Med. 2021;8: 630209. DOI: 10.3389/fmed.2021.630209

59. Puskarich MA, Ingraham NE, Merck LH, et al. Efficacy of losartan in hospitalized patients with COVID-19–induced lung injury: A randomized clinical trial. JAMA Netw Open. 2022;5(3): e222735. DOI: 10.1001/jamanetworkopen.2022.2735

60. Nguyen LC, Yang D, Nicolaescu V, et al. Cannabidiol inhibits SARS-CoV-2 replication through induction of the host ER stress and innate immune responses. Sci Adv. 2022;8(8): eabi6110. DOI: 10.1126/sciadv.abi6110

61. Sajid Jamal QM, Alharbi AH, Ahmad V. Identification of doxorubicin as a potential therapeutic against SARS-CoV-2 (COVID-19) protease: A molecular docking and dynamics simulation studies. J Biomol Struct Dyn. 2022;40(17): 7960–7974. DOI: 10.1080/07391102.2021.1905551

62. Singh MB, Vishvakarma VK, Lal AA, Chandra R, Jain P, Singh P. A comparative study of 5-fluorouracil, doxorubicin, methotrexate, paclitaxel for their inhibition ability for Mpro of nCoV: Molecular docking and molecular dynamics simulations. J Indian Chem Soc. 2022;99(12): 100790. DOI: 10.1016/j.jics.2022.100790

63. Ravichandran R, Mohan SK, Sukumaran SK, et al. An open label randomized clinical trial of indomethacin for mild and moderate hospitalised Covid-19 patients. Sci Rep. 2022;12(1): 6413. DOI: 10.1038/s41598-022-10370-1

64. Jones SA, Hunter CA. Is IL-6 a key cytokine target for therapy in COVID-19? Nat Rev Immunol. 2021;21(6): 337–339. DOI: 10.1038/s41577-021-00553-8

65. Del Valle DM, Kim-Schulze S, Huang HH, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020;26(10): 1636–1643. DOI: 10.1038/s41591-020-1051-9

66. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe. 2020;27(6): 992–1000.e3. DOI: 10.1016/j.chom.2020.04.009

67. Lucas C, Wong P, Klein J, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature. 2020;584(7821): 463–469. DOI: 10.1038/s41586-020-2588-y

68. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8(4): 420–422. DOI: 10.1016/S2213-2600(20)30076-X

69. Malone RW, Tisdall P, Fremont-Smith P, et al. COVID-19: Famotidine, histamine, mast cells, and mechanisms. Front Pharmacol. 2021;12: 633680. DOI: 10.21203/rs.3.rs-30934/v2

70. Ennis M, Tiligada K. Histamine receptors and COVID-19. Inflamm Res. 2021;70: 67–75. DOI: 10.1007/s00011-020-01422-1

71. Thangam EB, Jemima EA, Singh H, et al. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: The hunt for new therapeutic targets. Front Immunol. 2018;9: 1873. DOI: 10.3389/fimmu.2018.01873

72. Carlos D, Sá-Nunes A, de Paula L, et al. Histamine modulates mast cell degranulation through an indirect mechanism in a model IgE-mediated reaction. Eur J Immunol. 2006;36(6): 1494–1503. DOI: 10.1002/eji.200535464

73. Pashmforosh M, Shariati S, Nezhad HA, Haghighat M. Possible benefits of paclitaxel therapy for COVID-19. Pharm Biomed Res. 2022. 8(2): 91–94. DOI: 10.18502/pbr.v8i2.11022

74. Chen X, Guo H, Qiu L, Zhang C, Deng Q, Leng Q. Immunomodulatory and antiviral activity of metformin and its potential implications in treating coronavirus disease 2019 and lung injury. Front Immunol. 2020;11: 2056. DOI: 10.3389/fimmu.2020.02056

75. Samuel SM, Varghese E, Büsselberg D. Therapeutic potential of metformin in COVID-19: Reasoning for its protective role. Trends Microbiol. 2021;29(10): 894–907. DOI: 10.1016/j.tim.2021.03.004

76. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Investig. 2001;108(8): 1167–1174. DOI: 10.1172/JCI13505

77. Salt IP, Palmer TM. Exploiting the anti-inflammatory effects of AMP-activated protein kinase activation. Expert Opin Invest Drugs. 2012;21(8): 1155–1167. DOI: 10.1517/13543784.2012.696609

78. Kim D, Lee JY, Yang JS, et al. The architecture of SARS-CoV-2 transcriptome. Cell. 2020;181(4): 914–921.e10. DOI: 10.1016/j.cell.2020.04.011

79. El Bairi K, Trapani D, Petrillo A, et al. Repurposing anticancer drugs for the management of COVID-19. Eur J Cancer. 2020;141: 40–61. DOI: 10.1016/j.ejca.2020.09.014

80. Sperry MM, Oskotsky TT, Marić I, et al. Target-agnostic drug prediction integrated with medical record analysis uncovers differential associations of statins with increased survival in COVID-19 patients. PLoS Comput Biol. 2023;19(5): e1011050. DOI: 10.1371/journal.pcbi.1011050

81. Zhou Q, Zhang L, Dong Y, et al. The role of SARS-CoV-2-mediated NF-κB activation in COVID-19 patients. Hypertens Res. 2023;47: 375–384. DOI: 10.1038/s41440-023-01460-2

82. Davies DA, Adlimoghaddam A, Albensi BC. The effect of COVID-19 on NF-κB and neurological manifestations of disease. Mol Neurobiol. 2021;58(8): 4178–4187. DOI: 10.1007/s12035-021-02438-2

83. Jørgensen P, Wang JE, Almlöf M, et al. Sirolimus interferes with the innate response to bacterial products in human whole blood by attenuation of IL-10 production. Scand J Immunol. 2001;53(2): 184–191. DOI: 10.1046/j.1365-3083.2001.00862.x

84. Boor PP, Metselaar HJ, Mancham S, van der Laan LJ, Kwekkeboom J. Rapamycin has suppressive and stimulatory effects on human plasmacytoid dendritic cell functions. Clin Exp Immunol. 2013;174(3): 389–401. DOI: 10.1111/cei.12191

85. Battaglia M, Stabilini A, Draghici E, et al. Rapamycin and interleukin-10 treatment induces T regulatory type 1 cells that mediate antigen-specific transplantation tolerance. Diabetes. 2006;55(1): 40–49. DOI: 10.2337/diabetes.55.01.06.db05-0613

86. Ravid JD, Leiva O, Chitalia VC. Janus kinase signaling pathway and its role in COVID-19 inflammatory, vascular, and thrombotic manifestations. Cells. 2022;11(2): 306. DOI: 10.3390/cells11020306

87. Khaledi M, Sameni F, Yahyazade S, et al. COVID-19 and the potential of Janus family kinase (JAK) pathway inhibition: A novel treatment strategy. Front Med. 2022;9: 961027. DOI: 10.3389/fmed.2022.961027

88. Tanaka T, Narazaki M, Kishimoto T. Interleukin (IL-6) immunotherapy. Cold Spring Harbor Perspect Biol. 2018;10(8): a028456. DOI: 10.1101/cshperspect.a028456

89. Gajjela BK, Zhou M-M. Calming the cytokine storm of COVID-19 through inhibition of JAK2/STAT3 signaling. Drug Discov Today. 2022;27(2): 390–400. DOI: 10.1016/j.drudis.2021.10.016

90. Laing AG, Lorenc A, Del Molino Del Barrio I, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. Nat Med. 2020;26(10): 1623–1635. DOI: 10.1038/s41591-020-1038-6

91. Rincon-Arevalo H, Aue A, Ritter J, et al. Altered increase in STAT1 expression and phosphorylation in severe COVID-19. Euro J Immunol. 2022;52(1): 138–148. DOI: 10.1002/eji.202149575

92. Xiu H, Gong J, Huang T, et al. Fludarabine inhibits type I interferon-induced expression of the SARS-CoV-2 receptor angiotensin-converting enzyme 2. Cell Mol Immunol. 2021;18(7): 1829–1831. DOI: 10.1038/s41423-021-00698-5

93. Žarković N, Jastrząb A, Jarocka-Karpowicz I, et al. The impact of severe COVID-19 on plasma antioxidants. Molecules. 2022;27(16): 5323. DOI: 10.3390/molecules27165323

94. Zarkovic N, Jakovcevic A, Mataic A, et al. Post-mortem findings of inflammatory cells and the association of 4-hydroxynonenal with systemic vascular and oxidative stress in lethal COVID-19. Cells. 2022;11(3): 444. DOI: 10.3390/cells11030444

95. Bustamante S, Yau Y, Boys V, et al. Tryptophan metabolism 'hub'gene expression associates with increased inflammation and severe disease outcomes in COVID-19 infection and inflammatory bowel disease. Int J Mol Sci. 2022;23(23): 14776. DOI: 10.3390/ijms232314776

96. Perreau M, Suffiotti M, Marques-Vidal P, et al. The cytokines HGF and CXCL13 predict the severity and the mortality in COVID-19 patients. Nat Commun. 2021;12(1): 4888. DOI: 10.1038/s41467-021-25191-5

97. Liu H, Zhang W, Zou B, Wang J, Deng Y, Deng L. DrugCombDB: A comprehensive database of drug combinations toward the discovery of combinatorial therapy. Nucleic Acids Res. 2020;48(D1): D871–D881. DOI: 10.1093/nar/gkz1007

98. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group; Sterne JAC, Murthy S, et al. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: A meta-analysis. J Am Med Assoc. 2020;324(13): 1330–1341. DOI: 10.1001/jama.2020.17023

99. Sajgure A, Kulkarni A, Joshi A, et al. Safety and efficacy of mycophenolate in COVID-19: A nonrandomised prospective study in western India. Lancet Reg Health Southeast Asia. 2023;11: 100154. DOI: 10.1016/j.lansea.2023.100154

100. Bramante CT, Buse JB, Liebovitz DM, et al. Outpatient treatment of COVID-19 and incidence of post-COVID-19 condition over 10 months (COVID-OUT): A multicentre, randomised, quadruple-blind, parallel-group, phase 3 trial. Lancet Infect Dis. 2023;23(10): 1119–1129. DOI: 10.1016/S1473-3099(23)00299-2

101. El-Tanani M, Ahmed KA, Shakya AK, Ammari WG, Al-Shudifat AE. Phase II, double-blinded, randomized, placebo-controlled clinical trial investigating the efficacy of mebendazole in the management of symptomatic COVID-19 patients. Pharmaceuticals. 2023;16(6): 799. DOI: 10.3390/ph16060799

102. Halpin M, Lerner A, Sagar M, et al. A prospective, single-center, randomized phase 2 trial of etoposide in severe COVID-19. medRxiv:2023.06.05.23290969 [Preprint]. 2023 [cited 2023 Jun 06]. DOI: 10.1101/2023.06.05.23290969

103. Schoot TS, Kerckhoffs APM, Hilbrands LB, van Marum RJ. Immunosuppressive drugs and COVID-19: A review. Front Pharmacol. 2020;11: 1333. DOI: 10.3389/fphar.2020.01333

104. Nair A, Sharma P, Tiwary MK. Glutathione deficiency in COVID19 illness-does supplementation help? Saudi J Anaesth. 2021;15(4): 458. DOI: 10.4103/sja.sja_320_21

105. DE Flora S, Balansky R, La Maestra S. Antioxidants and COVID-19. J Prevent Med Hyg. 2021;62(1 Suppl 3): E34–E45. DOI: 10.15167/2421-4248/jpmh2021.62.1S3.1895

106. Alam MS, Czajkowsky DM. SARS-CoV-2 infection and oxidative stress: Pathophysiological insight into thrombosis and therapeutic opportunities. Cytokine Growth Factor Rev. 2022;63: 44–57. DOI: 10.1016/j.cytogfr.2021.11.001

107. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med Hypotheses. 2020;143: 110102. DOI: 10.1016/j.mehy.2020.110102

108. Ntyonga-Pono M-P. COVID-19 infection and oxidative stress: An under-explored approach for prevention and treatment? Pan Afr Med J. 2020;35(Suppl 2): 12. DOI: 10.11604/pamj.2020.35.2.22877

109. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. Arch Med Res. 2020;51(5): 384–387. DOI: 10.1016/j.arcmed.2020.04.019

110. Laforge M, Elbim C, Frère C, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. Nat Rev Immunol. 2020;20(9): 515–516. DOI: 10.1038/s41577-020-0407-1

111. Weisberg E, Parent A, Yang PL, et al. Repurposing of kinase inhibitors for treatment of COVID-19. Pharm Res. 2020;37: 1–29. DOI: 10.1007/s11095-020-02851-7

112. Ripamonti C, Spadotto V, Pozzi P, et al. HDAC inhibition as potential therapeutic strategy to restore the deregulated immune response in severe COVID-19. Front Immunol. 2022;13: 841716. DOI: 10.3389/fimmu.2022.841716

113. Wróblewska J, Wróblewski M, Hołyńska-Iwan I, et al. The role of glutathione in selected viral diseases. Antioxidants. 2023;12(7): 1325. DOI: 10.3390/antiox12071325

114. Jennings MR, Parks RJ. Curcumin as an antiviral agent. Viruses. 2020;12(11): 1242. DOI: 10.3390/v12111242

115. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature. 2012;487(7408): 482–485. DOI: 10.1038/nature11286

116. van Laarhoven T, Marchiori E. Biases of drug–target interaction network data. In: Comin M, editor. Pattern recognition in bioinformatics: 9th IAPR International Conference, PRIB 2014, Stockholm, Sweden, August 21-23, 2014. Proceedings 9. Berlin: Springer; 2014. pp. 22–23. DOI: 10.1007/978-3-319-09192-1_3

117. Takimoto CH. Anticancer drug development at the US National Cancer Institute. Cancer Chemother Pharmacol. 2003;52: 29–33. DOI: 10.1007/s00280-003-0623-y

118. Johnson TS, Terrell CE, Millen SH, Katz JD, Hildeman DA, Jordan MB. Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. J Immunol. 2014;192(1): 84–91. DOI: 10.4049/jimmunol.1302282

119. Bailly C. Etoposide: A rider on the cytokine storm. Cytokine. 2023;168: 156234. DOI: 10.1016/j.cyto.2023.156234

120. Wang YN, Wang YY, Wang J, Bai WJ, Miao NJ, Wang J. Vinblastine resets tumor-associated macrophages toward M1 phenotype and promotes antitumor immune response. J Immunother Cancer. 2023;11(8): e007253. DOI: 10.1136/jitc-2023-007253

121. Gao Q, Wen T, Deng Y. A novel network-based and divergence-based time series forecasting method. Inform Sci. 2022;612: 553–562. DOI: 10.1016/j.ins.2022.08.120

122. Hesthaven JS, Pagliantini C, Rozza G. Reduced basis methods for time-dependent problems. Acta Numer. 2022;31: 265–345. DOI: 10.1017/S0962492922000058

123. Ben Abdallah S, Mhalla Y, Trabelsi I, et al. Twice-daily oral zinc in the treatment of patients with coronavirus disease 2019: A randomized double-blind controlled trial. Clin Infect Dis. 2023;76(2): 185–191. DOI: 10.1093/cid/ciac807

124. Patocka J, Kuca K, Oleksak P, et al. Rapamycin: Drug repurposing in SARS-CoV-2 infection. Pharmaceuticals. 2021;14(3): 217. DOI: 10.3390/ph14030217

125. Karsulovic C, Lopez M, Tempio F, Guerrero J, Goecke A. mTORC inhibitor sirolimus deprograms monocytes in "cytokine storm" in SARS-CoV2 secondary hemophagocytic lymphohistiocytosis-like syndrome. Clin Immunol. 2020;218: 108539. DOI: 10.1016/j.clim.2020.108539

126. Chowdhury P, Pathak P. Neuroprotective immunity by essential nutrient "Choline" for the prevention of SARS CoV2 infections: An in silico study by molecular dynamics approach. Chem Phys Lett. 2020;761: 138057. DOI: 10.1016/j.cplett.2020.138057

127. Schwartz E. Does ivermectin have a place in the treatment of mild Covid-19? New Microbes New Infect. 2022;46: 100985. DOI: 10.1016/j.nmni.2022.100985

128. Mirjalili M, Soodejani MT, Raadabadi M, Dehghani A, Salemi F. Does losartan reduce the severity of COVID-19 in hypertensive patients? BMC Cardiovasc Disord. 2022;22(1): 116. DOI: 10.1186/s12872-022-02548-2

129. Xu Y, Li M, Zhou L, et al. Ribavirin treatment for critically ill COVID-19 patients: An observational study. Infect Drug Resist. 2021: 5287–5291. DOI: 10.2147/IDR.S330743

130. Khalili JS, Zhu H, Amanda Mak NS, Yan Y, Zhu Y. Novel coronavirus treatment with ribavirin: Groundwork for an evaluation concerning COVID-19. J Med Virol. 2020;92(7): 740–746. DOI: 10.1002/jmv.25798

131. Patel J, Berezowski I, Abdelmonem A, Taylor D, Pourmand A. Azithromycin for mild-to-moderate COVID-19. Lancet Respir Med. 2021;9(10): e99. DOI: 10.1016/S2213-2600(21)00379-9

132. Li G, Park LS, Lodi S, et al. Tenofovir disoproxil fumarate and coronavirus disease 2019 outcomes in men with HIV. AIDS. 2022;36(12): 1689–1696. DOI: 10.1097/QAD.0000000000003314

133. Kim J-W, Yoon S, Lee J, Lee S. Serious clinical outcomes of COVID-19 related to acetaminophen or NSAIDs from a nationwide population-based cohort study. Int J Environ Res Public Health. 2023;20(5): 3832. DOI: 10.3390/ijerph20053832

134. Manjani L, Desai N, Kohli A, Arya R, Woods C, Desale S. Effects of acetaminophen on outcomes in patients hospitalized with COVID-19. Chest. 2021;160(4): A1072. DOI: 10.1016/j.chest.2021.07.992

135. Pathania YS. Cyclosporine: Hope for severe COVID-19? BMJ Support Palliat Care. 2021. DOI: 10.1136/bmjspcare-2020-002681

136. Fenizia C, Galbiati S, Vanetti C, et al. Cyclosporine A inhibits viral infection and release as well as cytokine production in lung cells by three SARS-CoV-2 variants. Microbiol Spectr. 2022;10(1): e0150421. DOI: 10.1128/spectrum.01504-21

137. Belli LS, Fondevila C, Cortesi PA, et al. Protective role of tacrolimus, deleterious role of age and comorbidities in liver transplant recipients with Covid-19: Results from the ELITA/ELTR multi-center European study. Gastroenterology. 2021;160(4): 1151–1163.e3. DOI: 10.1053/j.gastro.2020.11.045

138. World Health Organization. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with COVID-19. 2020. https://www.who.int/news-room/commentaries/detail/the-use-of-

non-steroidal-anti-inflammatory-drugs-(nsaids)-in-patients-with-covid-19

139. Rheingold SZ, Raval C, Gordon AM, Hardigan P. Zinc supplementation associated with a decrease in mortality in COVID-19 patients: A meta-analysis. Cureus. 2023;15(6): e40231. DOI: 10.7759/cureus.40231

140. Mashauri HL. Covid-19 histamine theory: Why antihistamines should be incorporated as the basic component in Covid-19 management? Health Sci Rep. 2023;6(2): e1109. DOI: 10.1002/hsr2.1109

141. Al-Kuraishy HM, Al-Rubiey HF, Al-Buhadily AK, Al-Gareeb AI. Anti-histamines and Covid-19: Hype or hope. J Pak Med Assoc. 2021;71(12): 144–148.

142. Suresh MV, Francis S, Aktay S, Kralovich G, Raghavendran K. Therapeutic potential of curcumin in ARDS and COVID-19. Clin Exp Pharmacol Physiol. 2023;50(4): 267–276. DOI: 10.1111/1440-1681.13744

143. Vahedian-Azimi A, Abbasifard A, Rahimi-Bashar F, et al. Effectiveness of curcumin on outcomes of hospitalized COVID-19 patients: A systematic review of clinical trials. Nutrients. 2022;14(2): 256. DOI: 10.3390/nu14020256

144. Hudzik B, Nowak J, Zubelewicz-Szkodzinska B. Consideration of immunomodulatory actions of morphine in COVID-19-Short report. Eur Rev Med Pharmacol Sci. 2020;24(24): 13062–13064. DOI: 10.26355/eurrev_202012_24213

145. Teixeira L, Temerozo JR, Pereira-Dutra FS, et al. Simvastatin downregulates the SARS-CoV-2-induced inflammatory response and impairs viral infection through disruption of lipid rafts. Front Immunol. 2022;13: 820131. DOI: 10.3389/fimmu.2022.820131

146. Vitiello A, La Porta R, Ferrara F. Correlation between the use of statins and COVID-19: What do we know? BMJ Evid Based Med. 2022;27(2): 126–127. DOI: 10.1136/bmjebm-2020-111589

147. Attademo L, Bernardini F. Are dopamine and serotonin involved in COVID-19 pathophysiology? Eur J Psychiatry. 2021;35(1): 62. DOI: 10.1016/j.ejpsy.2020.10.004

148. Salerni BL, Bates DJ, Albershardt TC, Lowrey CH, Eastman A. Vinblastine induces acute, cell cycle phase-independent apoptosis in some leukemias and lymphomas and can induce acute apoptosis in others when Mcl-1 is suppressed. Mol Cancer Ther. 2010;9(4): 791–802. DOI: 10.1158/1535-7163.MCT-10-0028

149. Suriawinata E, Mehta KJ. Iron and iron-related proteins in COVID-19. Clin Exp Med. 2023;23: 969–991. DOI: 10.1007/s10238-022-00851-y

150. Han J, Hu S, Hu Y, et al. Discovery of podofilox as a potent cGAMP-STING signaling enhancer with antitumor activity. Cancer Immunol Res. 2023;11(5): 583–599. DOI: 10.1158/2326-6066.CIR-22-0483

151. Wu J, Zhao M, Li C, Zhang Y, Wang DW. The SARS-CoV-2 induced targeted amino acid profiling in patients at hospitalized and convalescent stage. Biosci Rep. 2021;41(3): BSR20204201. DOI: 10.1042/BSR20204201

152. Páez-Franco JC, Torres-Ruiz J, Sosa-Hernández VA, et al. Metabolomics analysis reveals a modified amino acid metabolism that correlates with altered oxygen homeostasis in COVID-19 patients. Sci Rep. 2021;11(1): 6350. DOI: 10.1038/s41598-021-85788-0

153. Izquierdo-Alonso JL, Pérez-Rial S, Rivera CG, Peces-Barba G. N-acetylcysteine for prevention and treatment of COVID-19: Current state of evidence and future directions. J Infect Public Health. 2022;15(12): 1477–1483. DOI: 10.1016/j.jiph.2022.11.009

154. Bharadwaj S, Azhar EI, Kamal MA, et al. SARS-CoV-2 Mpro inhibitors: Identification of anti-SARS-CoV-2 Mpro compounds from FDA approved drugs. J Biomol Struct Dynam. 2022;40(6): 2769–2784. DOI: 10.1080/07391102.2020.1842807

155. Ma H, Wen H, Qin Y, et al. Homo-harringtonine, highly effective against coronaviruses, is safe in treating COVID-19 by nebulization. Sci China Life Sci. 2022;65(6): 1263–1266. DOI: 10.1007/s11427-021-2093-2

156. Hamizi K, Aouidane S, Belaaloui G. Etoposide-based therapy for severe forms of COVID-19. Med Hypotheses. 2020;142: 109826. DOI: 10.1016/j.mehy.2020.109826

157. Aoyagi T, Sato Y, Baba H, et al. Case report: Successful treatment of five critically ill coronavirus disease 2019 patients using combination therapy with etoposide and corticosteroids. Front Med. 2021;8: 718641. DOI: 10.3389/fmed.2021.718641

158. Cengiz M, Borku Uysal B, Ikitimur H, et al. Effect of oral L-glutamine supplementation on Covid-19 treatment. Clin Nutr Exp. 2020;33: 24–31. DOI: 10.1016/j.yclnex.2020.07.003

159. Durante W. Glutamine deficiency promotes immune and endothelial cell dysfunction in COVID-19. Int J Mol Sci. 2023;24(8): 7593. DOI: 10.3390/ijms24087593

160. Pektaş S, Gürsoy C, Gümüş Demirbilek S. The use of pregabalin in intensive care unit in the treatment of Covid-19-related pain and cough. J Coll Physicians Surg Pak. 2021;31(7): 143. DOI: 10.29271/jcpsp.2021.Supp2.S143