

Patterns

Integrative resource for network-based investigation of COVID-19 combinatorial drug repositioning and mechanism of action

Highlights

- COVID-CDR is an integrative platform for *in silico* repositioning of drug combinations
- It prioritizes drugs with potential to interfere with viral or host-virus functions
- It prioritizes potentially synergistic combinations with complementary modes of action
- It also provides much useful drug information, all in one intuitive online platform

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In brief

We present COVID-CDR, a web-based computational platform for *in silico* repositioning of drug combinations against SARS-CoV-2 infection. COVID-CDR constructs a multi-level interactome encompassing drug-target, target-human, and viral-human interactions overlaid on a human PPI network. By leveraging this interactome, COVID-CDR prioritizes potentially synergistic drug combinations as those whose primary targets are in close vicinity to SARS-CoV-2 proteins but holds distinct PPI footprints. The platform also provides diverse information on drugs/drug pairs, offering a multi-evidence solution for investigating drug combination strategies against COVID-19.



Descriptor

Integrative resource for network-based investigation of COVID-19 combinatorial drug repositioning and mechanism of action

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THE BIGGER PICTURE Repurposing of existing medications has been the mainstream focus of anti-COVID-19 drug discovery as it offers rapid and cost-effective solutions for therapeutic development. Repurposing a combination of therapeutic options with complementary but varying mechanisms of action remains a challenge. Our ability to identify effective combinations is limited due to the vast number of possible drug pairs and a lack of convenient tools that can systematically guide the prioritization of a large range of individual drugs or drug combinations with potential value for the treatment of COVID-19. To address this resource gap, we developed COVID-CDR, an integrative network pharmacology-based platform for *in silico* repositioning of drug combinations. COVID-CDR provides a visual representation of the cellular interactome involved in modes of action of the chosen drugs and can be used to quantitatively prioritize drug combinations with the potential to act synergistically against COVID-19.



Development/Pre-production: Data science output has been rolled out/validated across multiple domains/problems

SUMMARY

An effective monotherapy to target the complex and multifactorial pathology of SARS-CoV-2 infection poses a challenge to drug repositioning, which can be improved by combination therapy. We developed an online network pharmacology-based drug repositioning platform, COVID-CDR (<http://vafaee.com/COVID19repositioning.html>), that enables a visual and quantitative investigation of the interplay between the primary drug targets and the SARS-CoV-2-host interactome in the human protein-protein interaction network. COVID-CDR prioritizes drug combinations with potential to act synergistically through different, yet potentially complementary, pathways. It provides the options for understanding multi-evidence drug-pair similarity scores along with several other relevant information on individual drugs or drug pairs. Overall, COVID-CDR is a first-of-its-kind online platform that provides a systematic approach for pre-clinical *in silico* investigation of combination therapies for treating COVID-19 at the fingertips of the clinicians and researchers.

INTRODUCTION

The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has caused a grave threat to public health and an unprecedented loss to the global economy. Worldwide scien-

tific attention has been focused on drug repositioning to rapidly identify interventions for COVID-19 prevention and cure.¹ In addition to time-effective solutions for disease treatment, drug repositioning provides better value health care by reducing cost and avoiding risk, as multiple phases of *de novo* drug



discovery can be bypassed.² An effective monotherapy to target the complex and multi-factorial pathology of SARS-CoV-2 infection poses a challenge to drug development, which can be improved by combination therapy.^{3,4} The increased therapeutic efficacy due to combination therapy could result in lower-dose prescribing, reducing the risk of side effects and toxicity hazards. However, due to the large number of possible drug pairs, our ability to find and verify effective combinations is limited by this combinatorial explosion.⁵

Over the last decade, a variety of computational drug-repurposing methods have been developed. Some of these have been applied to search for new therapeutics against COVID-19 (as recently reviewed⁶), most of which focused on developing monotherapy strategies. Among these methods, network pharmacology approaches that quantify the interplay between the SARS-CoV-2-host interactome and the drug targets in the human protein-protein interaction (PPI) network have offered grounds for prioritizing effective repositioning candidates as both mono- and combination therapies.⁷⁻⁹ However, most of the former network pharmacology studies focused on prioritizing and reporting a few individual drugs or drug pairs (Table S8). There has been a lack of an integrative and accessible platform enabling the investigation of a large set of repositioning drug candidates for their putative efficacy and mechanism of action.

To address this resource gap, we developed COVID-CDR (COVID-19 Combinatorial Drug Repositioning), an integrative web-based computational platform that prioritizes complementary and additive drug combinations for SARS-CoV-2 treatment. COVID-CDR compiles a large set of FDA-approved drugs, investigational compounds previously used to treat COVID-19 symptoms, and drugs in clinical trials for COVID-19 treatment. For a given drug combination, COVID-CDR constructs a multi-level interactome encompassing drug-target, target-human, and viral-human interactions overlaid on a comprehensive human PPI network. By leveraging this network, COVID-CDR prioritizes drugs with primary host protein targets in close vicinity to SARS-CoV-2 proteins, highlighting those that may have potential to interfere with viral or host-virus functions. Moreover, COVID-CDR prioritizes drug combinations with potential to act synergistically through different, yet potentially complementary, pathways. This network-based information is complemented by a diverse drug-drug similarity measurement as well as drug pair synergy in cell lines to offer a rational multi-level, multi-evidence solution for investigating drug combination strategies against COVID-19.

COVID-CDR also includes a multitude of useful drug data, all in one intuitive platform, including drug structure, drug physicochemical properties, therapeutic class, indications, side effects, induced pathways, and drug-drug interactions, which together form a unique starting point for *in silico* COVID-19 combinatorial drug repositioning. We demonstrate the utility of COVID-CDR for the combination of LY2275796 and cyclosporine and explain the mechanism of action of such combination. To the best of our knowledge, COVID-CDR is the first computational online tool to integrate COVID-19 drug information in the context of virus and human interaction networks, which may facilitate a better understanding of the molecular mechanisms of drug actions for the identification of potentially effective drug combinations and can help in prioritizing therapies for COVID-19 worldwide.

RESULTS

COVID-CDR overview and statistics

Figure 1 shows the COVID-CDR platform content and construction. Eight hundred sixty-seven drugs with reported evidence in treating COVID-19 symptoms or under investigation in trials were pre-compiled (Table S1). Of these drugs, 57% were approved for an indication, 41% are investigational, and >2% were veterinary approved, nutraceutical, or withdrawn. These drugs cover a wide range of therapeutic classes (>200 categories), including antivirals, antibiotics, anticancer, anti-inflammatory, immunomodulatory, immunosuppressive, and anti-coagulant agents. Multiple drug-related information sources, including chemical structure, physicochemical and pharmacological properties, side effects, protein targets, associated pathways, and drug-drug interactions, were compiled from diverse resources for each drug (Table 1) and are accessible to explore from the web interface.

COVID-CDR constructs a multi-dimensional network (Figure 1A) comprising drug-target interactions (867 drugs, 2,228 protein targets, and 4,866 interactions) and high-confidence binding associations between SARS-CoV-2 and human proteins (28 viral proteins, 340 human proteins, and 414 interactions) overlaid on a comprehensive experimentally validated human protein-protein interactome (469,515 PPIs). The SARS-CoV-2-host PPI network was curated from literature^{19,20} and relevant interaction databases.²¹ In addition, we incorporated the SARS-CoV-1 virus-host PPI network, which can serve as a valuable reference due to the close similarity between SARS-CoV-1 and SARS-CoV-2 proteins.²²⁻²⁴ This multi-dimensional interactome (Table S2) has been used to estimate the topological proximity of drug targets to COVID-19-related proteins and quantify the separation of drug targets on the human protein-protein interactome for network-based exploration of efficacious drug combinations (Figure 1C, cf. experimental procedures). In addition to network-based topological metrics, the functional relevance of drug targets to COVID-related cellular biological processes was estimated (Figure 1D).

Furthermore, for each drug pair, structural and functional similarity measures were estimated (Figure 1B, Table S3). Multiple studies suggest that synergy is associated with the functional similarity or dissimilarity of drug pairs.^{25,26} Distinct drug-drug similarity matrices were generated based on chemical structures, target protein sequences, induced pathways, and target protein functions, i.e., cellular components, biological processes, and molecular functions (see experimental procedures). The size of each matrix is 867 by 867, i.e., 751,689, and values range from 0 to 1. The individual similarity matrices were then mean-aggregated to form a combined-score similarity matrix and Z transformed for significance assessment (Table S3). Overall, the network proximity of drug-drug pairs holds negative but insignificant correlation with structural and functional similarities (Figure S2).

To provide in-action examples of studies likely to influence clinical practice, 36 different drug combinations were incorporated into the platform, involving more than 20 different drugs in various clinical trials designed for treating COVID-19 from the ClinicalTrials.gov database (Figure 1E, Table S5). In addition, 150 pairs of COVID-19-related drugs approved by the FDA for

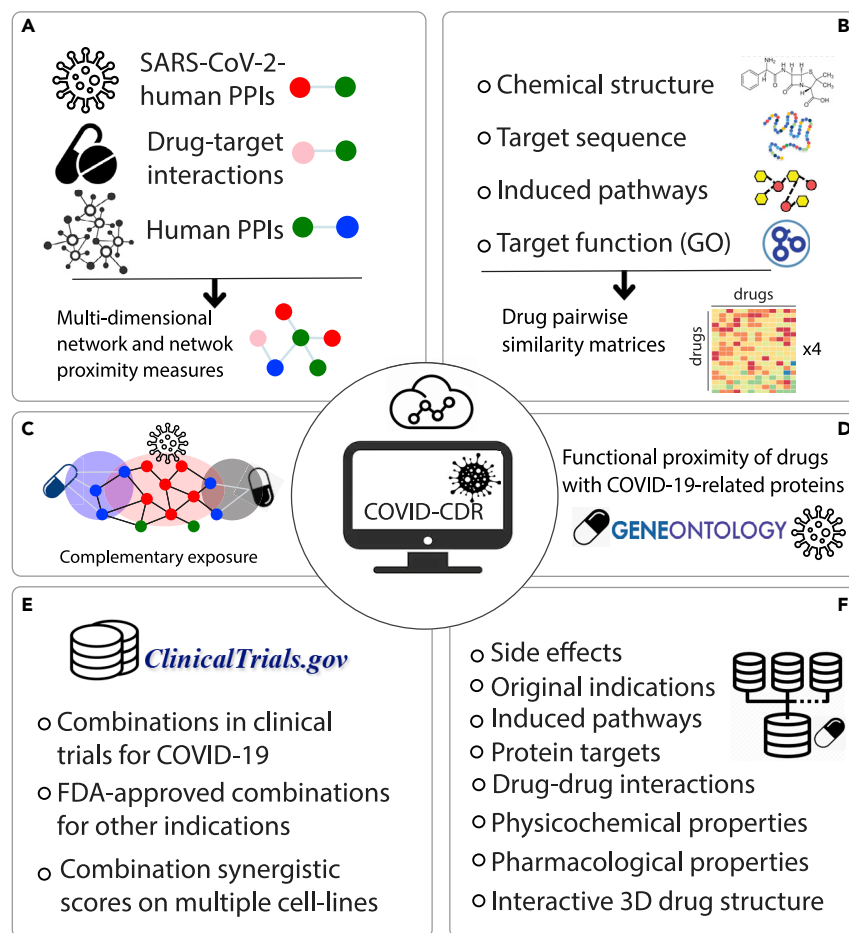


Figure 1. Schematic workflow for the content and construction of COVID-CDR

(A) Multi-dimensional network construction. COVID-CDR encompasses a comprehensive multi-layer interactome that is curated based on the known SARS-CoV-2 protein-human host interactions and interactions of all drugs and their direct targets, along with all experimentally validated human protein-protein interactions.

(B) Drug-drug similarity estimation. A number of drug-drug similarity measures were calculated to determine the similarity index of each possible drug combination (drug chemical structures to estimate drug pairwise chemical similarity, drug-protein targets and protein sequences to estimate sequence-based target similarity, drug-induced pathways and their constituent genes to estimate pathway-based similarities, and GO annotations of protein targets and protein-protein interactions to identify functional similarities).

(C) Network-based *complementary exposure* pattern, where the targets of the drugs hit the virus subnetwork but target separate neighborhoods in the human interactome.

(D) COVID-19 functional proximity estimation. Functional proximity is an added measure that calculates the functional similarity of the COVID-19-related proteins and drug targets.

(E) Curated drug combinations. Users can explore curated drug combinations, i.e., drug combinations under investigation in COVID-19 clinical trials or FDA-approved potential COVID-19 drug combinations. Synergistic scores of specific combinations can be assessed on various cell lines derived from high-throughput screening assays.

(F) Comprehensive information on drugs. Multiple drug-related information sources were compiled and are accessible to explore from the web interface. Abbreviation: GO, gene ontology.

other indications were compiled (Table S6). Table 2 provides statistics and details of external drug combinations included in this platform.

COVID-CDR also incorporates the high-throughput viability screening results related to drug combinations assessed on more than 124 immortalized human cancer cell lines (Figure 1E, Table S7) assembled by Liu and colleagues.²⁷ While a reduction in cancer cell proliferation and/or viability may not be associated with antiviral effects, it indicates that, at least in a different context/endpoint, the evaluated drugs have shown synergistic interaction.

Prioritization of individual drugs based on the topological and functional proximity between known primary targets and SARS-CoV-2 proteins in the PPI network

The network-based drug-repositioning prioritization is based on the notion that for a drug to be efficacious, its target proteins should be within or in the immediate neighborhood of the corresponding subnetwork of the disease-related proteins in the human interactome.^{5,7,28–31} Accordingly, the topological distance from a drug to SARS-CoV-2 proteins was measured as the network-based shortest distance from the drug's primary targets to SARS-CoV-2-related proteins (i.e., disease module) on a hu-

man PPI network (see [experimental procedures](#)). SARS-CoV-2-related proteins considered in this study include viral proteins, human proteins interacting with SARS-CoV-2, and virus entry factors (Table S2). To quantify the significance of the shortest distance between drug and disease module, drug-disease proximity measures were then converted to Z scores (Z) based on permutation tests as previously explained,^{5,7} and the corresponding p values were estimated. For $Z < 0$ (and the corresponding $p < 0.05$), the drug-target subnetwork (i.e., drug module) and the disease module are significantly proximal and often overlap; while for $Z \geq 0$, the drug module and the disease module are distal and thus separated.^{5,32} Overall, 543 drugs topologically overlap with the SARS-CoV-2 module ($Z < 0$), and 118 of them show significant exposure with the disease module ($Z < 0$ and $p < 0.05$, permutation test, Table S1).

The network-based topological proximity of the drug module to the disease module measures the immediate vicinity of drug targets to SARS-CoV-2 proteins on cellular interactome. However, it falls short in capturing the effect of the drug's downstream changes in biological processes perturbed under the impact of the SARS-CoV-2 infection. Hence, the topological proximity was complemented with a measure of drug-disease functional proximity that quantifies the similarity between biological processes significantly enriched (false discovery rate [FDR] < 0.05)

Table 1. Data types, statistics, and details of data sources used to generate COVID-CDR

Data type	Statistics	Details	Data source
Drug identifiers, drug names, and clinical status	867 drugs, including 487 approved drugs	–	DrugBank ¹⁰ , ClinicalTrials.gov ¹¹ , literature
Drug physicochemical properties	16 distinct properties per drug	molecular weight, hydrogen bond acceptors/donors, ring count, molecular refractivity and polarizability, CAS number, SMILES, InChI, IUPAC name, etc.	DrugBank ¹⁰
Drug pharmacological properties	16 distinct properties per drug	description, indication, mechanism of action, target names, toxicity, pharmacodynamics, metabolism, half-life, route of elimination, etc.	DrugBank ¹⁰
Drug chemical structures	726 structures	structure-data file (SDF) format	DrugBank ¹⁰
Drug target-protein sequences	2,393 unique protein sequences	FASTA format	DrugBank ¹⁰
Drug-target network	2,228 and 4,866 drug-target pairs	composed of drugs and their targets from human and other organisms (e.g., SARS-CoV-2, SARS-CoV, etc.)	DrugBank ¹⁰
Drug-induced pathways	298, 459, 226, 1,530, and 112, pathways from KEGG, WikiPathways, BioCarta, Reactome, and Panther databases, respectively	based on the overrepresentation analyses of drug targets with pathway constituents (hypergeometric test, $p \leq 0.05$)	KEGG ¹² , WikiPathway ¹³ , BioCarta ¹⁴ , Reactome ¹⁵ , and Panther ¹⁴
Gene ontology terms and annotations	446 CC, 1,151 MF, and 5,103 BP terms, and a total of 250,734 protein-GO term associations	gene ontology terms across categories of cellular components (CC), molecular functions (MF), and biological processes (BP)	EnrichR ¹⁴
Protein-protein interactions (PPIs)	469,515 PPIs	validated and computationally predicted human PPIs	I2D ¹⁶
Drug indications and therapeutic classes			TTD ¹⁷ , DrugBank ¹⁰
Drug side effects	139,756 drug-side effect associations	information on marketed medicines and their recorded adverse drug reactions	SIDER ¹⁸
Drug-drug interactions	413,898 drug-drug interactions	information on potential changes in the action or side effects of a drug caused by administration with another drug	DrugBank ¹⁰

by a drug module (drug primary targets and their direct interactors in PPI) and the disease module (SARS-CoV-2-related proteins). The similarity between drug- and disease-associated biological processes was estimated using a gene ontology-based semantic similarity measure, which leverages on the ontology graph structure and information content to estimate similarities among gene ontology terms.³³ Table S4 shows biological processes enriched by SARS-CoV-2-related proteins (FDR < 0.05). Drug-disease functional proximities range between 0 and 1 with a mean value of $\mu = 0.29$ (Figure S1A). Overall, the higher the similarity is, the greater the effect of the drug would be in perturbing disease-related mechanisms. Similarity measures were standardized to Z scores, and the corresponding one-tailed p values (i.e., $P[X > x]$) were estimated; 306 drugs hold a Z score $> \mu$, and among them 82 have $p < 0.05$ (Table S3). SARS-CoV-2 functional proximities of drugs are inversely correlated to the corresponding topological proximities (Pearson's correlation coefficient -0.413) and hold relatively weak linear relationship ($R^2 = 0.17$), indicating that these two measurements are complementary rather than being redundant, justifying the integration (Figure S1B).

Prioritization of drug combinations based on the difference in PPI footprint of drugs

For drugs whose known primary targets are topologically and functionally proximal to SARS-CoV-2-related proteins, combinations can be prioritized based on the separation of drug-target modules in PPI. It has been previously hypothesized that different drug-target modules have different network-based footprints; two drugs are pharmacologically distinct if the footprints of the drug-target modules are topologically separated.⁵ A drug combination is therefore putatively effective if it follows a complementary exposure pattern (Figure 1C), indicating that targets of individual drugs (in a combination) overlap with the disease module but target separate neighborhoods on the interactome. Accordingly, for each drug pair A and B, a network separation measure, s_{AB} , was estimated as the mean shortest distance within the interactome between the targets of two drugs (Equation 3, experimental procedures). For $s_{AB} < 0$, drug target subnetworks overlap, while for $s_{AB} \geq 0$, they are separated on the interactome. Hence, complementary exposure implies that $s_{AB} \geq 0$, $z_A < 0$, and $z_B < 0$.

Table 2. Details about external drug combinations that are used in the COVID-CDR interface

Data type	Statistics	Combination type	Details	Data source
Experimental drug combinations	6,181 drug-combinations	dual combinations only	combinations experimented with in various cell lines in different settings	drugCombDB ²⁷
Combinations in clinical trials	36 drug-combinations	dual, triple, and quadruple combinations	combinations that are related to 867 COVID-19 drugs found in clinical trials in various phases	ClinicalTrials.gov ¹¹
FDA-approved combinations	150 drug combinations	dual, triple, and quadruple combinations	FDA-approved combinations that are related to 867 COVID-19 drugs	drugCombDB ²⁷

For FDA-approved drug pairs, $\text{median}(s_{AB}) = 1.0$ and $\text{median}(Z \text{ scores}) = -0.75$, and 31% of drug pairs follow the complementary exposure criteria. Note that these drugs are not meant to overlap with the SARS-CoV-2 module, as they are approved for other indications. However, 84% of FDA-approved drug pairs show distinct PPI footprints, i.e., $s_{AB} \geq 0$ ($p = 0.0016$, Fisher's exact test with hypergeometric null distribution). For drug pairs in COVID-19 clinical trials, $\text{median}(s_{AB}) = 0.833$ and $\text{median}(Z \text{ scores}) = -0.47$. Of drug pairs with human and/or SARS-CoV-2/SARS-CoV-2 primary targets, 25% follow the complementary exposure criteria and 75% have distinct PPI footprints ($s_{AB} \geq 0$) with at least one drug in close proximity to the disease module ($z_B < 0$ or $s_{AB} < 0$); $p = 0.138$ using Fisher's exact test with hypergeometric null distribution.

Database access and usage notes

Figure 2 shows the COVID-CDR web interface. The user can query drug combinations simply by using the search option and can start with two drugs of choice (Figure 2A). If required, additional drugs can be added on top of the built network to explore a combination of three or more drugs. When the drug-targets network is displayed, each node type is highlighted with a specific color: pink nodes indicate drugs, blue nodes are human proteins directly targeted by the drug, while green nodes are other human host proteins, red nodes indicate SARS-CoV-2 proteins, and purple nodes indicate other viral proteins. Users can simply hover on the individual drug to check the information related to the drug, such as its therapeutic class, primary indication, and disease topological and functional proximities. While being selected, details of a drug and its target information can be observed by clicking a small brain tab in the top right (Figure 2B), which displays physiochemical properties of the queried drug, its chemical structure in an interactive 3D view, and its pharmacological properties, providing an all-in-one view for further investigation of the drug of interest. The platform also provides the flexibility of querying any drug beyond the 867 pre-compiled drugs, by using the customize tab in the top right (Figure 2B) to upload drug-target interactions into the platform. The drug will be integrated into the in-screen network and drug-disease functional and topological proximity measures as well as drug-pair separation measures will be estimated in real time.

Upon completion of network rendering, the user can observe pairwise multi-modal drug similarity information and their network separation score by interacting with the tab at the bottom of the interface (Figure 2B). The induced subnetwork of the queried drug(s) in the network view is also interactive and

query-able, and upon selection of an edge, a PubMed query is made with its incident nodes (e.g., protein-pair or drug-protein), and the search results of the literature list are displayed as a table in a modal window. Under the curated combinations tab the user can also check the network for clinical drug combinations by clicking the clinical trial tab at top left; these 40 selected bi- or tri-drug combinations are currently in ongoing clinical trials for COVID-19 treatment (Figure 2C). In addition, the network-based action mechanisms of FDA-approved drug combinations can be explored. The synergism or antagonism of drug combinations across various cancer cell lines can be viewed as well under the curated combinations tab (Figure 2C). All these files can be downloaded from the download tabs at the top front page of COVID-CDR interface.

Case study: LY2275796 and cyclosporine combination therapy

We sought to use our platform to identify drug combinations that may provide effective synergistic therapy in potentially treating SARS-CoV-2 infection along with displaying well-defined mechanism-of-action by the implemented functional and network-based analyses. The utility of COVID-CDR and its integrated network-based system medicine approaches is showcased by the combination of LY2275796 and cyclosporine. Our network analysis indicates that LY2275796 and cyclosporine synergistically target a SARS-CoV-2-associated host protein subnetwork by a "complementary exposure" pattern, offering potential combination regimens for the treatment of SARS-CoV-2 (Figure 3). The targets of both drugs hit the SARS-CoV-2 host subnetwork (overlap with the disease module), but the targets separate neighborhoods in the human interactome network. Briefly, the negative value of the topological network proximity for both drugs suggested proximity to the disease module (LY2275796, $Z \text{ score} = -1.68$, $p = 0.01$; cyclosporine, $Z \text{ score} = -2.24$, $p = 0.01$). Simultaneously, the higher positive value for functional proximity for both drugs (LY2275796, $Z \text{ score} = 4.42$, $p = 4.86 \times 10^{-6}$; cyclosporine, $Z \text{ score} = 2.40$, $p = 0.008$) indicated significant similarity between the biological processes targeted by these drugs and the perturbed cellular processes in SARS-CoV-2 infection, implying potentially high effectiveness of each drug. Moreover, the two drugs denote a positive separation score ($s_{AB} = 0.46$) between the submodules, suggesting no overlap between the targets of LY2275796 and cyclosporine and thus the efficacy of the combination therapy.

All viruses require host protein synthesis machinery for replication before release and infection of neighboring cells. Numerous promising antiviral therapies against SARS-CoV-2

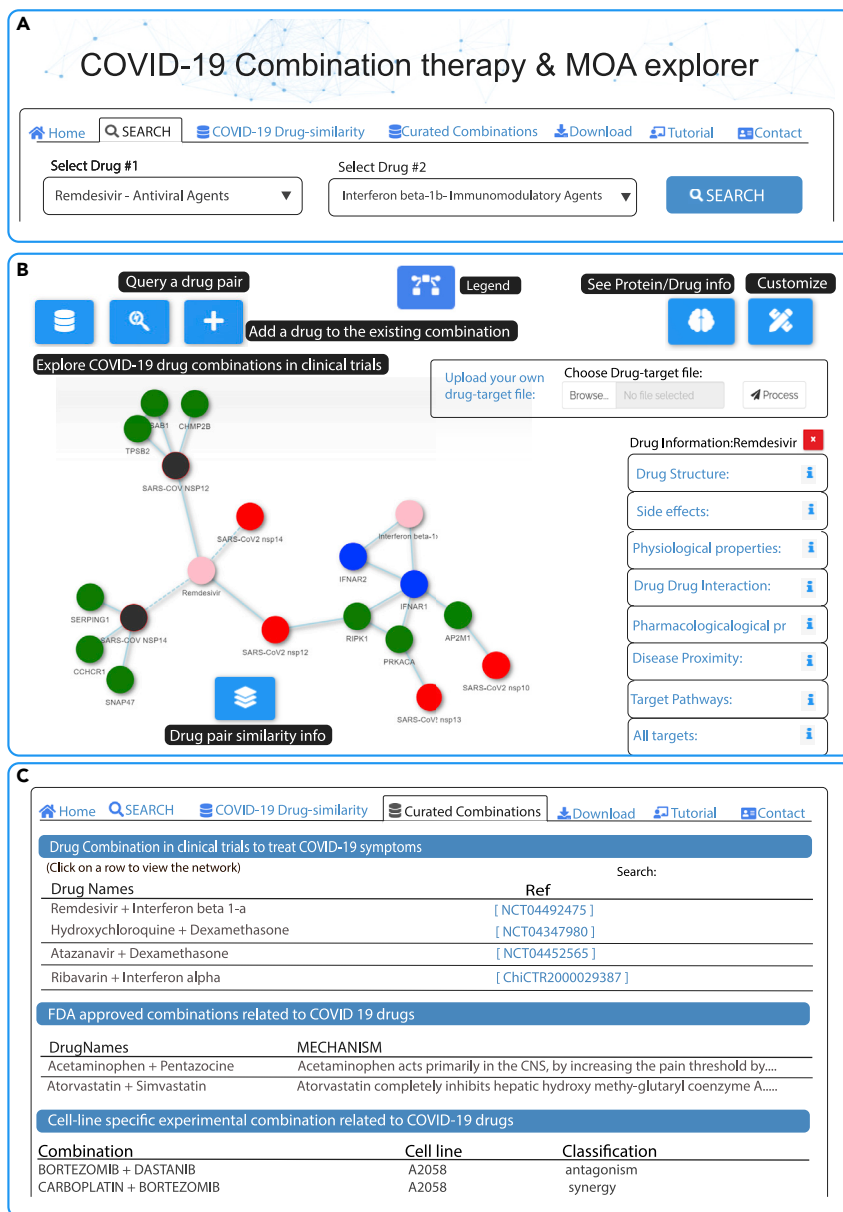


Figure 2. An overview of the COVID-CDR web interface

(A) The user can query drug combinations simply by using the search option and can start with two drugs of choice.

(B) The specific queried drug combination and drug-targets network gets displayed. Users can add another drug to the same combination or query a different drug combination (top left tab). Any drug beyond those pre-compiled can also be added into the network by specifying drug-target interactions via a file upload (customize tab, top right). The solid lines indicate known/confirmed interactions between drug and target proteins, whereas dashed lines indicate predicted interactions based on the similarity of SARS-CoV-2 to other H-CoVs. A color code for the nodes is available via the legend icon on top (pink, drugs; blue, human proteins directly targeted by the drug; green, other human host proteins; red, SARS-CoV-2 proteins; and purple, other viral proteins). Details of drug-target information can be assessed by clicking a small brain tab (top right), which displays detailed information of the queried drug. The user can observe pairwise multi-modal drug similarity information and their network separation score using the tab at the bottom of the graphical user interface.

(C) Under the Curated Combinations tab, the user can also check the network for COVID-19 clinical drug combinations by clicking the clinical trial tab at the top. In addition, the network-based mechanism of action (PPI footprint) of FDA-approved combinations related to COVID-19 drugs can be explored (middle). The sensitivities of various cancer cell lines to the chosen drug combinations can be viewed as well (bottom).

are being investigated with the hope to stop the virus from utilizing the host machinery and thus prevent its replication and spread. The translation of most of the viral (subgenomic) mRNAs is believed to be cap dependent, which displays a requirement for eukaryotic initiation factor 4F (eIF4F), a heterotrimeric complex consisting of eIF4E, the cap-binding protein; eIF4A, an RNA helicase; and eIF4G, a large scaffolding protein needed for the recruitment of 40S ribosomes.³⁴ LY2275796 inhibits the eIF4E complex and its activating kinases, MNK1/2, and is currently in phase 1 development as the second antisense anticancer drug.³⁵ Inhibition of eIF4A³⁶ or eIF4F,³⁷ the catalytic subunits of eIF4F, is shown to lead to apoptosis in selected cancer models. EIF4E, F, and G proteins are involved in tumor progression, angiogenesis, and metastases.^{38,39} Inhibiting eIF4E inhibits the Ras-Mnk and PI3-AKT-mTOR pathways, which are key nodes where the RAS and PI3K pathways come together and con-

trol the production of multiple oncoproteins,^{40,41} which are also important in SARS-CoV-2 infection.^{42–44} Targeting this translational pathway could lead to the development of new, more effective antiviral therapies to fight COVID-19.

In combination with LY2275796, we added cyclosporine, an effective immunosuppressive agent that is often used for prophylaxis of organ rejection. Cyclosporine is a calcineurin inhibitor that is shown to inhibit the replication of SARS-CoV, MERS-CoV, and human immunodeficiency virus at very low doses.⁴⁵ From the perspective of our analyzed network, cyclosporine would inhibit the cyclophilin functions of SARS-CoV-2 by hampering the peptidyl-prolyl isomerase activity (PPIA) (Figure 3). PPIA is a proinflammatory protein that stimulates activation of NF- κ B and ERK, JNK, and p38 MAP kinases.^{46,47} Cyclosporine may also act by indirectly inhibiting multiple SARS-CoV-2 proteins (Figure 1).⁴⁸

Importantly, cyclosporine has demonstrated improved clinical outcomes of patients with severe H1N1 pneumonia and acute respiratory failure in SARS-CoV-2 infection by preventing the production of interleukin-2, an essential cytokine in the cytokine release storm experienced during coronavirus infection.^{45,48,49} Cyclosporine may also significantly limit the severity of sepsis

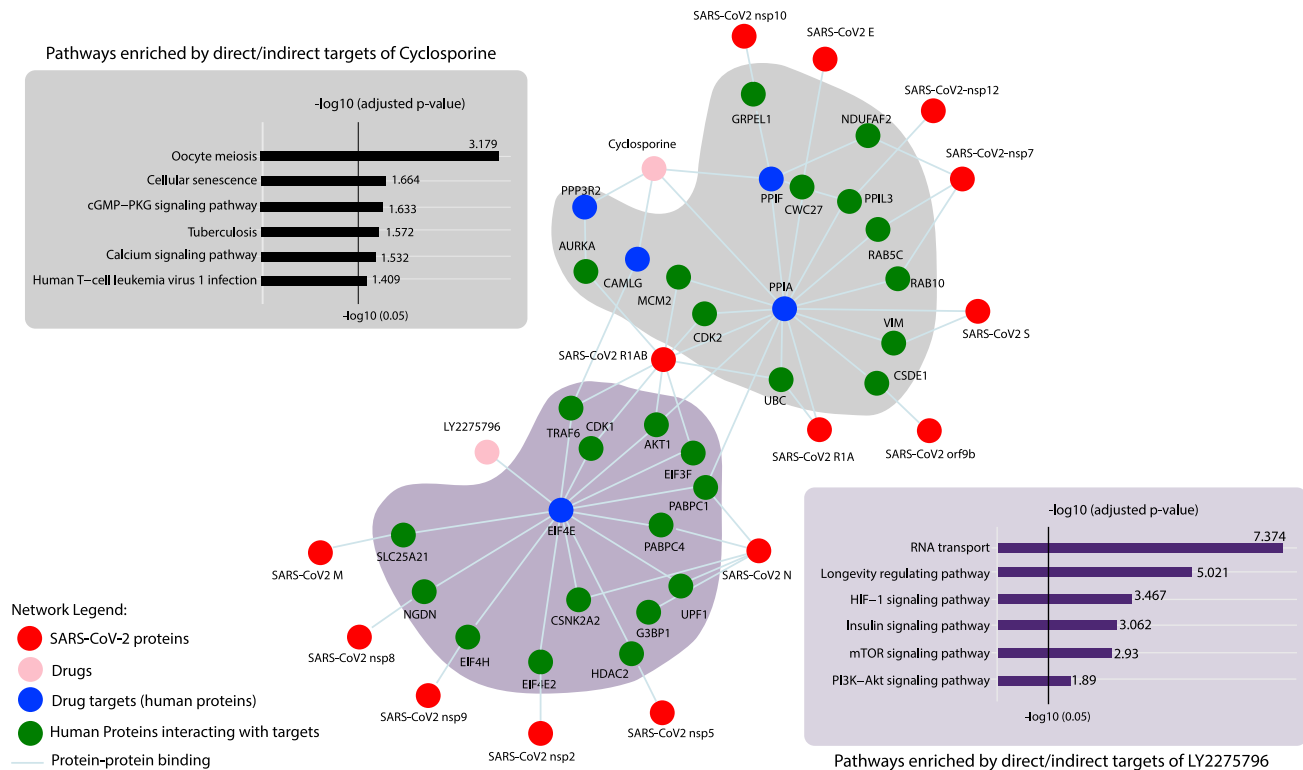


Figure 3. Integrated network visualization generated for a pairwise combination of LY2275796 (cap independent translation inhibitor-glycosides) and cyclosporine (calcineurin inhibitor-immunosuppressant)

The top indicates possible exposure mode of the SARS-CoV-2-associated protein module to the drug cyclosporine. The top left plot shows pathways significantly enriched by direct and indirect targets of cyclosporine (i.e., proteins directly interacting with targets on human PPI). The bottom shows the drug-disease module for LY2275796 and pathways significantly enriched by direct and indirect targets of LY2275796.

and/or inflammation-induced acute lung injury and post-cardiac arrest in SARS-CoV-2 patients.⁵⁰ It has been consistently reported to improve lung function via mitochondrial processes, including PTP inhibition.⁵¹ Altogether, our network analyses and literature evidence suggested that combining LY2275796 and cyclosporine can offer a potential combined therapeutic approach for SARS-CoV-2.

DISCUSSION

COVID-CDR contribution compared with related studies

While a number of clinical trials are proposed to test the efficacy of repurposed drugs against COVID-19, prioritization of many drug candidates has been mostly unstructured.⁵² Following a network pharmacology approach that quantitatively analyzes the vicinity of drug targets to H-CoV proteins on a human PPI network, Zhou et al.⁷ predicted specific mono- and combination therapies as potential treatments for COVID-19. The major limitation of this study is the lack of availability of the SARS-CoV-2-human interactome at the time, so the predictions were made based on host proteins associated with other H-CoV species. Moreover, the study does not offer an accessible and generalizable platform to explore other combinations beyond those few drugs predicted by the study. Gordon et al.¹⁹ constructed the first human-SARS-CoV-2 protein interaction map based on affinity purification-mass spectrometry and identified

potential repurposing candidates whose primary targets directly interact with SARS-CoV-2 proteins. This molecular landscape of the human-SARS-CoV-2 protein interaction has offered grounds for various drug-repurposing strategies^{3,53-55} and a way toward elucidating the mechanisms of viral infection.^{56,57} While multiple *in silico* studies have now proposed analysis of the virus-host-drug network to prioritize “individual” drug candidates as potential COVID-19 monotherapies, only a few studies identified combination therapies with the potential to act synergistically against SARS-CoV-2 infection (Table S8). In addition, while a GitHub code is available in some cases, these studies often overlooked providing an accessible implementation to conduct the network-based proximity analyses of individual or drug pairs as potential COVID-19 mono- or combination therapies. Among these, CoVex⁵⁷ is the only online platform that enables a visual exploration of the SARS-CoV-2 virus-host-drug interactome for drug-repositioning prediction. CoVex implements several network-based algorithms to prioritize repositionable drugs for COVID-19. The platform, however, is not intuitively applicable to drug combination prioritization. It lacks in providing an option for users to start with their own choice of drugs and does not provide comprehensive drug or drug-pair information.

Overall, to the best of our knowledge, COVID-CDR is the first computational online platform for *in silico* combinatorial drug repositioning that allows visual and quantitative investigation of any individual drug for its potential to interfere with viral

functions, as well as any drug pairs for their potency to act synergistically against SARS-CoV-2 infection. In addition, COVID-CDR compiles a wealth of other very useful information on individual drugs (i.e., drug structure and physicochemical and pharmacological properties, drug-drug interactions, side effects, induced pathways) and drug pairs (drug-drug similarity measures, drug-drug interactions, and cell line viability synergy scores), all in one place, in a very intuitive way. Together, COVID-CDR provides an easy and holistic approach to explore the crucial SARS-CoV-2-human interactome and may provide added promising targets for therapeutic intervention that can be tested in pre-clinical studies.

Limitation and future directions

The potentially best-performing drug combinations for treatment of SARS-CoV-2 are ranked in the COVID-CDR platform based on their *in silico* scores assessed using network computation methods. The current version of COVID-CDR does not support context specificity and relies on a general PPI network. This does not guarantee that the same interaction occurs *in vivo* in every cell type, as the proteomes of each cell type differ. COVID-CDR will be enhanced by incorporating gene expression profiles of SARS-CoV-2 infection across multiple cell models, organoids, and human samples into the protein interactome. Accordingly, the network proximity measures are estimated based on a subnetwork of the protein interactome that is active in the context of the interest (as per user's choice). Furthermore, the current disease module mainly includes the human proteins directly interacting with SARS-CoV-2. The COVID-CDR disease module, however, will be expanded by incorporating cell-line-specific genome-wide CRISPR screens to include host factors critical for SARS-CoV-2 infection beyond those directly interacting with the virus. Moreover, the current version of COVID-CDR does not *predict* drug combinations and can be mainly used for querying drug pairs of interest or ranking drug pairs based on the measure of interest (i.e., network separation, topological or functional proximity). The platform can be enhanced by incorporating an automated drug pair screening by developing a multi-objective optimizer to identify a Pareto set of drug pairs whose PPI fingerprint is separated in PPI, yet both drugs are functionally and topologically proximal to the SARS-CoV-2 module in any specific context (cell lines, organoid models, or human samples). COVID-CDR is also not exempt from some common drawbacks in integrative data analysis tools regarding data availability and comprehensiveness. For instance, COVID-CDR is limited to specific organisms (i.e., human, SARS-CoV-2, and partially to SARS-CoV/SARS-CoV-1), and antivirals with targets in other organisms (i.e., favipiravir, an antiviral used to manage influenza) are not integrable into the network. In addition, an important step in drug discovery is the assessment of the ADME (absorption, distribution, metabolism, excretion) properties of compounds that can be partially evaluated *in silico*⁵⁸ and *in vitro*.⁵⁹ Information on possible ADME drug-drug interactions is missing in the current version of the COVID-CDR. Furthermore, the drug-target network was built considering only protein targets; hence, nucleic acid targets were not included. These issues could be partially mitigated by a more extensive integration of data from a wider variety of databases. In addition, despite some similarities in epithelial-mesenchymal transitions between cancer cell

lines and the SARS-CoV-2-induced epithelial-mesenchymal transitions in lung cell lines,⁶⁰ cancer cell lines may not be the most appropriate representative for identifying the synergy/antagonism of drug combinations. More physiologic models, like primary cell cultures and organoid models, are required to better understand this process in a way that is decoupled from the transformed nature of the cancer cell lines.

EXPERIMENTAL PROCEDURES

Full experimental procedures are provided in the [supplemental information](#).

Resource availability

Lead contact

Further information and requests for resources should be directed to the lead contact, Fatemeh Vafaee (f.vafaee@unsw.edu.au).

Materials availability

No materials were used in this study.

Data and code availability

To ensure the reproducibility of COVID-CDR, we have made the whole code-base (including any intermediate curation, processing, and the web application) freely available for non-commercial uses in GitHub (first release DOI: <https://doi.org/10.5281/zenodo.5089231>). The code and interface are well documented, and the database update is implemented as an HPC-powered and parallel processing-enabled, semiautomated pipeline to accommodate anytime system upgradation. The platform is accessible via http://vafaeeelab.com/COVID19_repositioning.html.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.patter.2021.100325>.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.K.M.A., S.F., and F.V.; methodology, A.K.M.A. and F.V.; data curation, A.K.M.A., S.F., S.C., and S.W.; software development, A.K.M.A.; analysis, S.F.; formal analysis, A.K.M.A. and F.V.; writing (relevant sections in original draft), A.K.M.A.; writing – original draft, S.F. and F.V.; writing – review & editing, A.K.M.A., S.F., A.C., S.W., and F.V.; validation, S.F.; visualization, A.K.M.A.; preparation of results, F.V.; supervision, F.V.; resources, F.V.; funding acquisition, F.V.; project administration, F.V.

DECLARATION OF INTERESTS

F.V. is a member of *Patterns* journal advisory board. The authors declare no other competing interests.

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