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A comparative study of COVID-19 transcriptional signatures between clinical samples and preclinical cell models in the search for disease master regulators and drug repositioning candidates

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an acute viral disease with millions of cases worldwide. Although the number of daily new cases and deaths has been dropping, there is still a need for therapeutic alternatives to deal with severe cases. A promising strategy to prospect new therapeutic candidates is to investigate the regulatory mechanisms involved in COVID-19 progression using integrated transcriptomics approaches. In this work, we aimed to identify COVID-19 Master Regulators (MRs) using a series of publicly available gene expression datasets of lung tissue from patients which developed the severe form of the disease. We were able to identify a set of six potential COVID-19 MRs related to its severe form, namely *TAL1, TEAD4, EPAS1, ATOH8, ERG*, and *ARNTL2*. In addition, using the Connectivity Map drug repositioning approach, we identified 52 different drugs which could be used to revert the disease signature, thus being candidates for the design of novel clinical treatments. Furthermore, we compared the identified signature and drugs with the ones obtained from the analysis of nasopharyngeal swab samples from infected patients and preclinical cell models. This comparison showed significant similarities between them, although also revealing some limitations on the overlap between clinical and preclinical data in COVID-19, highlighting the need for careful selection of the best model for each disease stage.

1. Introduction

The COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 has affected more than 600 million people and has claimed over 6 million lives worldwide between 2020 and 2022. After an initial period of unprecedented infection rates, in the second half of 2022 the number of new cases dropped from more than 2 million per day, at the peak of the pandemic, to approximately 200 thousand per day, and the proportion of asymptomatic or mild cases has increased among those with confirmed COVID-19 diagnosis (Murray, 2022; World Health Organisation, 2022). These alterations on the course of the pandemic and the global improvement of the disease outcomes are directly associated with the development of vaccines and the achievement of a worldwide immunization rate of approximately 60% (Watson et al., 2022). However, despite the apparently imminent end of the pandemic, the emergence of novel variants of concern with higher transmissibility and immune evasion capabilities, such as the Omicron variant (Dhama et al., 2023; Fan et al., 2022), seems to indicate that COVID-19 will likely continue to be present on our daily lives in the near future. Thus, as we do not yet know if the immunization effectiveness of the current vaccination strategies will wane over time, the establishment of outpatient treatments for severe acute COVID-19 is still needed.

COVID-19 is a disease with remarkable symptomatic heterogeneity where patients may present one or more symptoms such as fever, dry

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cough, rhinorrhea, shortness of breath, myalgia, headache, sore throat, fatigue, abdominal pain, anosmia, and symptoms epidemiology may vary between population (Eythorsson et al., 2020; Stokes et al., 2020). Although on the onset of the pandemics 81% cases were mild infections, 19% of the patients experienced a severe case, developing pneumonia, acute respiratory distress syndrome, respiratory failure, and an anomalous cytokine response, which could promote vasculitis, thrombocytosis, and, ultimately, multiple organ failure (Sheikh et al., 2021; Wu and McGoogan, 2020).

The emergence and rapid worldwide spread of SARS-CoV-2 has motivated a surge of *in vitro* model development for application on COVID-19 research, with cell lines derived from both humans and several other species, such as nonhuman primates. These models have been widely used for the isolation of SARS-CoV-2, the study of virus infection etiopathogenesis and the identification of potential drugs for efficient therapeutic interventions (Runft et al., 2022). Up to this date, several drugs have been evaluated as potential COVID-19 therapeutic alternatives, mainly proposed as candidates based on two different strategies: empirical drug repositioning, and *in vitro* experiment with hypothetical or known antiviral compounds (Izda et al., 2021; WHO Solidarity Trial Consortium et al., 2021). However, only a limited number of drugs has shown promising results, such as the intravenous antiviral Remdesivir® and the oral antiviral Paxlovid®. A possible reason for the high failure rate on the prediction of drugs for COVID-19 therapy is that the strategies being adopted might lack the ability to encompass the full complexity of the disease, leading to uncertainties on dosage definition (Agrawal, 2015; Parvathaneni and Gupta, 2020) or incoherence between theoretical and practical models (Hoffmann et al., 2020), which tends to decrease the clinical trials success rate (Khadka et al., 2020).

Remdesivir® is a delayed chain terminator of the viral RNA-dependent RNA polymerase and has been approved in 2020 by the US FDA for the treatment of SARS-CoV-2 infection, despite limitations and controversial efficacy (De Clercq, 2021). Paxlovid® is an inhibitor of SARS-CoV-2 NPS5 main protease and has recently been approved for emergency use by the FDA, being seen as a promising treatment for SARS-CoV-2 infection (Wang and Yang, 2022; Wen et al., 2022).

Nevertheless, despite the promising initial results provided by these antiviral drugs, their efficiency is susceptible to the emergence of variants carrying mutations that can promote resistance to their action mechanisms, once they directly target viral components. Although no mutations conferring resistance to currently employed antivirals have been clinically detected to date, several mutations in the NSP5 protease, the target of the drug Paxlovid®, have already been identified in emerging SARS-CoV-2 lineages (Ullrich et al., 2022), and viral strains with a Remdesivir®-resistant phenotype due to mutations in the viral protein RNA-dependent RNA polymerase, have also been observed *in vitro* (Stevens et al., 2022). This raises a concern that the barriers to the emergence of resistance to antivirals may be substantial, but not insurmountable, and that the search for new therapeutic alternatives is still necessary. An alternative to overcome the resistance emergence issue would be the development of therapeutic strategies that target virus induced host gene expression alterations. Besides decreasing the chance of antiviral resistance development, these strategies could also be adapted for the treatment of infections promoted by several other but currently problematic coronaviruses, as well as by future novel variants that will certainly emerge.

Thus, although essential for preclinical pathogenetic research, once they allow the study of specific cellular targets, the use of cellular models for clinical translation purposes must be carefully evaluated. The characterization of these models for the identification of potential factors that could limit its applicability, as the partial physiological representation of its origin tissue, and evaluation of model's suitability according to the question to be answered, must always be considered to ensure that obtained results are reliable and can be adequately translated to the clinic (Rosa et al., 2021). In this sense, a holistic approach which take into account the whole molecular context of the disease could be a valuable tool in order to better guide the drug repositioning strategies and increase the chance for clinical trials successes.

In this article, we assessed the COVID-19 molecular signature in clinical lung autopsy samples using differential gene expression analysis, gene ontology (GO), and master regulator (MR) analysis. Further, we explored the results using the connectivity map (CMap) repositioning drug approach in order to search for potentially beneficial drug candidates, which could revert the disease signature. Additionally, to assess the correlation between pre-clinical models and clinical samples, we applied high-throughput analyses of mutual information and summarization of the biological context on publicly available expression data of cells transfected with SARS-CoV-2 and nasopharyngeal swab samples from infected patients, measuring the similarity indexes between them and achieving a comparison-limitation awareness. The experimental workflow is described and summarized in Fig. 1.

2. Methods

The differential expression, Master Regulators, and Connectivity map analysis, further described below, were conducted using the R environment, version 4.1.0. Gene Ontology (GO) analyses were performed on Cytoscape, version 3.8.2 (Shannon et al., 2003). For the regulatory network visualization, we used the RCy3 (Gustavsen et al., 2019) package, on both R and Cytoscape platforms.

2.1. Transcriptional data and differential expression analysis

In this study, we analyzed gene expression datasets from different patient-derived clinical samples and preclinical cell models infected with SARS-CoV-2 (Table 1). These datasets were downloaded from Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) and will be described in brief. The GSE155241 (Han et al., 2021) contains transcriptional data derived from clinical autopsy of lung tissue from three COVID-19 deceased patients and three deceased healthy subjects. The GSE152075 dataset includes data from nasopharyngeal swabs collected from 430 positive and 54 negative SARS-CoV-2 patients (Lieberman et al., 2020). The GSE147507 dataset contains transcriptional data from A549 cells with SARS-CoV-2 multiplicity of infection (MOI) 0.2 and 2, and NHBE cells with MOI 2 after 24 h (Blanco-Melo et al., 2020). The GSE159316 dataset comprises data from Vero cells (kidney epithelial cells extracted from an African green monkey) infected with SARS-CoV-2 MOI 0.01 for 24 h and 48 h (Youk et al., 2020).

For GSE159316, GSE147507 and GSE155241, data quality was verified using the fastqcr package and the transcription quantification was conducted using the salmon package for each dataset independently (Patro et al., 2017). For GSE152075, we used the readily available transcription counts data. We then carried the differential expression analysis using the DESeq2 package (Love et al., 2014), selecting as a differentially expressed gene (DEG) only those with a false discovery rate adjusted p-value (q-value) below or equal to 0.05. To evaluate the systemic effects of COVID-19 in different models, DEGs were used as input for the aforementioned analysis.

2.2. Gene ontology analysis

To evaluate which biological processes were associated in each set of DEG, we performed GO analysis with ClueGO tool (Bindea et al., 2009), searching only for overrepresented biological processes measured by the hypergeometric test and q-value less than or equal to 0.05.

2.3. Master regulators analysis

To infer the COVID-19 MRs we used the transcriptional network centered on transcription factors (TFs) from healthy lung tissue

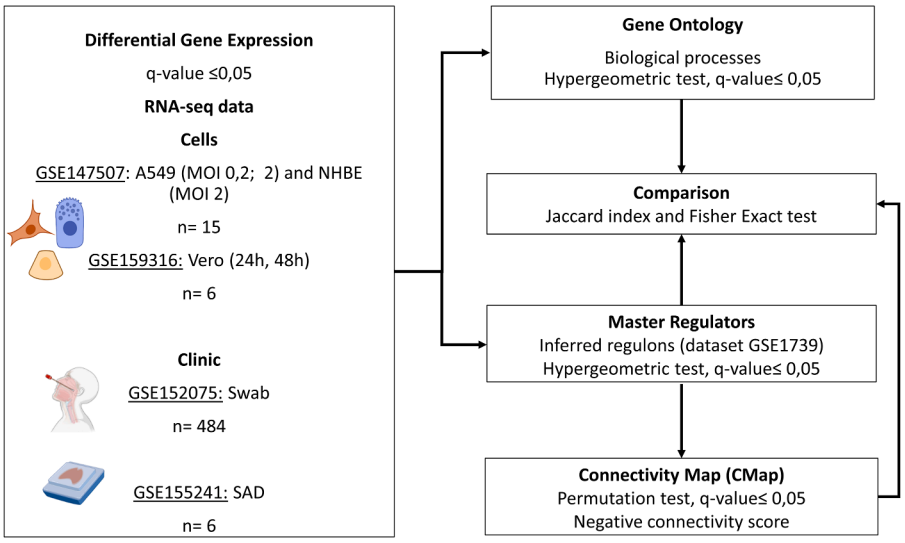

Fig. 1. Scheme representing the workflow adopted in this study.

Table 1
Datasets and their descriptions.

GEO ID	Description	Refs.
GSE147507	dataset contains transcriptional data from A549 cells with SARS-CoV-2 multiplicity of infection (MOI) 0.2 and 2, and NHBE cells with MOI 2 after 24 h	Blanco-Melo et al. (2020)
GSE159316	dataset comprises data from Vero cells (kidney epithelial cells extracted from an African green monkey) infected with SARS-CoV-2 MOI 0.01 for 1 h	Youk et al. (2020)
GSE152075	dataset includes data from nasopharyngeal swabs collected from SARS-CoV-2 positive and negative patients	Lieberman et al. (2020)
GSE155241 small autopsy (SAD)	comprising of transcriptional data derived from clinical autopsy of lung tissue from COVID-19 deceased patients and healthy subjects.	Han et al. (2021)

(GSE23546) inferred by De Bastiani and Klamt (2019), using RTN package (Fletcher et al., 2013). This approach maps significant associations between known TFs and all potential target genes. The groups of inferred target genes associated with each TF are hereinafter referred as its regulatory unit. Next, we searched for regulatory units enriched with DEGs in each dataset analyzed. TFs with regulatory units significantly enriched with differentially expressed target genes were then defined as MRs. To assure a non-causal relationship with each MR, only regulatory units with 100 or more gene hits were considered biologically relevant. Hypergeometric test q-value threshold of 0.05 or less was chosen as significant.

2.4. Connectivity Map analysis

We conducted the CMap drug repositioning method using the PharmacoGx package (Smirnov et al., 2016) and were used as input, for each dataset, the MR candidate regulatory units previously identified. In this analysis, MR target gene expression profile is compared to expression profiles of several cell lines treated with FDA-approved drugs, highlighting a mimetic or opposing transcriptional perturbation. Only the pharmacogenomics signatures with negative Geneset Enrichment Analysis connectivity score were selected as potential candidate drugs, once they can induce gene expression modifications that counteract the ones caused by SARS-CoV-2 infection. The q-value was assessed by a permutation test ($n = 1000$) and its significance threshold was set to 0.05.

2.5. Datasets similarities

We calculated Jaccard indexes and Fisher's exact test tables between preclinical cell models and patients for GO, MR, and proposed drugs by CMap with GeneOverlap package (Shen, 2014). The tables (Supplemental Files) containing all intersections were made at http://bioinformatics.psb.ugent.be/webtools/Venn/, with exception of Supplemental File 3. The UpSet plots were made using Intervene Shiny App (Khan and Mathelier, 2017).

3. Results

3.1. Dataset analysis of lung autopsies

Signature of severe COVID-19

Differential gene expression analysis showed that the clinical lung autopsy dataset derived from deceased severe COVID-19 subjects has 299 DEG when compared to healthy lung samples (Supplemental File 1). The three topmost up and down-regulated DEGs are, respectively, HLA class II histocompatibility antigen DQ beta 1 chain (HLA-DQB1), TNF superfamily member 12/13 (TNFSF12/ TNFSF13), fibroblast activation protein alpha (FAP), cytochrome P450 family 1 subfamily A member 1 (CYP1A1), with $\log_2$FC greater than 4, and DENN domain containing 11 (DENND11), and eukaryotic translation initiation factor 3 subunit C like (EIF3CL), with $\log_2$FC lesser than −4. These genes are markedly associated with juxtacrine regulation (or contact-dependent signaling), extracellular matrix remodeling, cytoskeleton rearrangement, aggrephagy, leukocyte cellular innate-mediated immune response, cytokine production, response to steroid, DNA damage response, and pathways related to integrin, G-protein coupled receptor, nuclear factor kappa B (NFκB), rat sarcoma (Ras), mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK) (Supplemental File 2).

According to our MR analysis, severe COVID-19 transcriptional signature in response to SARS-CoV-2 infection shows a significant modulation in several genes associated to six MRs, namely t-cell acute lymphocytic leukemia 1 (TAL1), TEA domain transcription factor 4 (TEAD4), endothelial PAS domain protein 1 (EPAS1), atonal BHLH transcription factor 8 (ATOH8), ETS-related gene (ERG), and aryl hydrocarbon receptor nuclear translocator like 2 (ARNTL2) (Fig. 2). These MRs are directly related to inflammatory response regulation and to cell

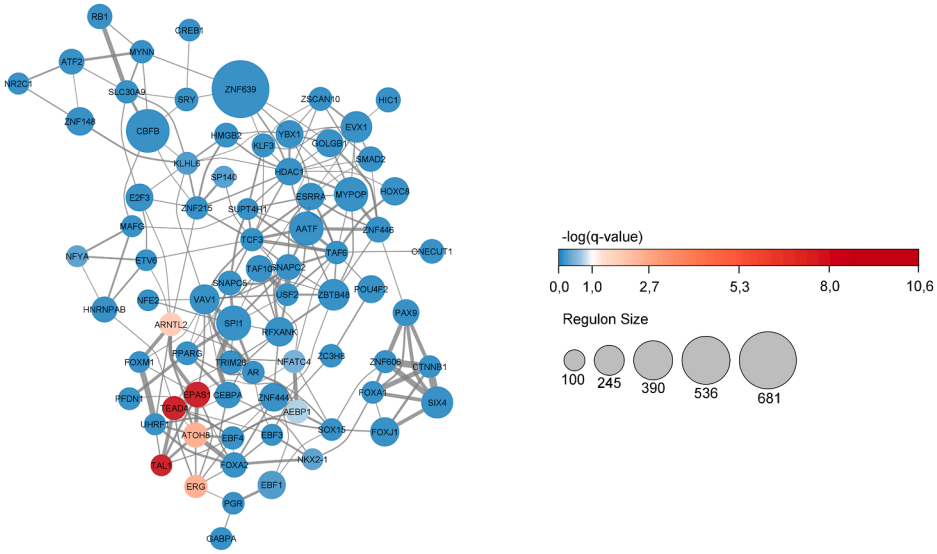

Fig. 2. Healthy lung transcriptional regulatory network centered on transcription factors, with COVID-19 MRs highlighted. Node sizes correspond to the number of predicted gene targets for each transcription factor and the edge width is proportional to the number of mutually regulated genes between each transcription factors pair.

morphogenesis, being potentially involved on the development of two significant features observed in severe COVID-19: the hyper-inflammatory response-induced tissue damage, and the cytoskeleton and cell organelles hijacking.

Drug repositioning candidates to severe COVID-19

Using the CMap approach, we found 52 drugs negatively related to the disease signature (Supplemental File 6), summarized in Table 2. The main classes to which these drugs belong, according to the WHO Anatomical Therapeutic Chemical classification (ATC) databank, are: corticosteroids, antibiotics, including cephalosporins, lincosamide, macrolide and fluoroquinolone, and psychoanaleptics, such as antidepressants and psychostimulants. These results may show which drugs could be helpful to counterbalance the patient's transcriptional profile perturbations caused by severe cases of COVID-19. Further, not only the drug list obtained by CMap are practical for treatment prospection, but also to evaluate how other experimental models behave in comparison with lung tissue.

3.2. Dataset analysis of COVID-19 preclinical cell models and patients' nasopharyngeal swabs

Signature of COVID-19 preclinical cell models and patients' nasopharyngeal swabs

The number of DEG from SARS-CoV-2 infected *versus* paired controls varies greatly between the different biological samples analyzed (Fig. 3). In brief, the A549 cell line with MOI 2 had 7494 DEGs, being the mostly altered DEGs early growth response 1 (EGR1), basic helix-loop-helix family member E41 (BHLHE41), protein phosphatase 4 regulatory subunit 4 (PPP4R4), keratin 4 (KRT4), epoxide hydrolase 1 (EPHX1), and uroplakin 1B (UPK1B), all of them with $\log_2$FC modulus greater than 3. A549 MOI 0.2 presented 3874 DEG and the most prominently over and under expressed genes were EGR1, also listed among the genes with notoriously altered expression in A549 MOI 2, zinc finger 354 B (ZNF354B), FBJ murine osteosarcoma viral oncogene homolog B (FOSB), lymphocyte antigen 6 family member E (LY6E), carbonic anhydrase (CA9) and cadherin 2 (CDH2), all of them with $\log_2$FC modulus greater than 4. NHBE had 884 DEG, with the greatest alterations being observed on C-X-C motif chemokine ligand (CXCL5), C—C motif chemokine ligand 20 (CCL20), C-X-C motif chemokine ligand 8 (CXCL8), mitochondrially encoded 16S RRNA like 3 (MTRNR2L3), spermatogenesis associated 13 (SPATA13) and trafficking protein particle complex subunit 3 (TRAPPC3), all of them with $\log_2$FC modulus greater than 1. Vero cells at 24 and 48 h after infection had 2609 and 1859 DEG, respectively. The top DEGs from Vero cells at 24 h were 2′−5′-oligoadenylate synthetase like (OASL), C-X-C motif chemokine ligand 10 (CXCL10), G0/G1 switch 2 (G0S2), proenkephalin (PENK), matrix metallopeptidase 10 (MMP10), secreted phosphoprotein 1 (SPP1), all of them with $\log_2$FC modulus greater than 2. While for Vero cells at 48 h were mannose receptor C-type 1 (MRC1), OASL, also listed among the genes with notoriously altered expression in Vero cells at 24 h, ryanodine receptor 1 (RYR1), PENK, also listed among the genes with notoriously altered expression in Vero cells at 24 h, S100 calcium binding protein A2 (S100A2), inhibitor of DNA binding 3 (ID3), all of them with $\log_2$FC modulus greater than 2. Nasopharyngeal swabs collected from positive SARS-CoV-2-infected patients presented 5396 DEG, when compared to negative samples, and caspase 17, pseudogene (CASP17P), proprotein convertase subtilisin/kexin type 1 inhibitor (PCSK1N), AL022578.1, immunoglobulin heavy constant gamma 1 (IGHG1), immunoglobulin heavy constant gamma 3 (IGHG3), immunoglobulin heavy constant mu (IGHM) were the genes with most differentiated expressions, all of them with $\log_2$FC modulus greater than 4.

Interestingly, only four genes are present as DEG in all models, including lung autopsies, namely MAF BZIP transcription factor F (MAFF), cysteine and serine rich nuclear protein 1 (CSRNP1), nuclear factor kappa B inhibitor alpha (NFKBIA), dual specificity phosphatase 1 (DUSP1) from a total of 11,326 unique genes. None of them were consistently up or down regulated across all datasets.

Afterward, GO analysis was performed to establish biological processes related to the gene sets for each experiment. The extensive full results are shown in Supplemental File 2. In brief, all models showed a significant association with biological processes related to immune system regulation, cell death, and stress response. Specific to each dataset, A549 MOI 2 cells DEG are associated with, but not only, Acetyl-CoA metabolism, oxidative stress response, and small GTPase signal transduction, and A549 MOI 0.2 are associated with cell redox homeostasis, virus response, and NFκB pathway regulation. NHBE DEGs had overrepresented processes including nitric oxide biosynthesis, peptidase activity, and acute-phase response. Vero 24 h DEGs show relation with stress granule assembly, nuclear matrix organization, and p53-mediated signal transduction, whereas Vero 48 h DEGs present association with

Table 2
CMap results from SAD with ATC level 1 and 3 (summarized) annotations.

Drug	Connectivity Score	p-value	ATC level 1*	ATC level 3
ajmaline	−0.224	0.021	C	Antiarrhythmics
amoxapine	−0.232	0.021	N	Antidepressants
antazoline	−0.252	0.025	R	Antihistamines
beclometasone	−0.248	0.047	A, D, R	Corticosteroids
benperidol	−0.255	0.014	N	Antipsychotics
betamethasone	−0.247	0.035	A, C, D, R	Corticosteroids
bisoprolol	−0.236	0.049	C	Beta blocking agents
cefalotin	−0.217	0.031	J	Other beta-lactam antibacterials
cefixime	−0.235	0.041	J	Other beta-lactam antibacterials
cetirizine	−0.297	0.000	R	Antihistamines
cimetidine	−0.218	0.020	A	Drugs for peptic ulcer and gastro-esophageal reflux disease
citiolone	−0.210	0.043	A	Liver therapy, lipotropics
clindamycin	−0.235	0.036	D, G, J	Macrolides, lincosamides and streptogramins
clioquinol	−0.260	0.037	D, G, P	Agents against amoebiasis and other protozoal diseases
clofazimine	−0.264	0.014	J	Drugs for treatment of leprosy
diethylcarbamazine	−0.247	0.010	P	Antinematodal agents
emetine	−0.283	0.016	P	Agents against amoebiasis and other protozoal diseases
erythromycin	−0.274	0.003	D, J, S	Macrolides, lincosamides and streptogramins
flumetasone	−0.244	0.045	D	Corticosteroids
flunisolide	−0.251	0.023	R	Corticosteroids
fluocinonide	−0.245	0.018	C, D	Corticosteroids
fluorometholone	−0.263	0.038	C, D, S	Corticosteroids
gabapentin	−0.224	0.045	N	Antiepileptics
griseofulvin	−0.252	0.013	D	Antifungals
lomefloxacin	−0.227	0.018	J, S	Quinolone antibacterials
monobenzone	−0.298	0.004	D	Other dermatological preparations
nialamide	−0.266	0.005	N	Antidepressants
oxybutynin	−0.221	0.030	G	Urologicals
piperidolate	−0.253	0.005	A	Drugs for functional gastrointestinal disorders
piracetam	−0.244	0.009	N	Psychostimulants
propylthiouracil	−0.280	0.006	H	Antithyroid preparations
sertaconazole	−0.255	0.031	D, G	Antifungals
sulfinpyrazone	−0.236	0.013	M	Antigout preparations
verapamil	−0.257	0.018	C	Selective calcium channel blockers with direct cardiac effects
5,109,870	−0.313	0.047	–	–
5,155,877	−0.290	0.025	–	–
blebbistatin	−0.259	0.015	–	–
dioxybenzone	−0.254	0.020	–	–
DL-thiorphan	−0.264	0.012	–	–
epirizole	−0.219	0.032	–	–
etofylline	−0.244	0.042	–	–
eucatropine	−0.237	0.013	–	–
glycocholic acid	−0.236	0.034	–	–
imipenem	−0.260	0.009	–	–
lobelanidine	−0.256	0.016	–	–
lycorine	−0.258	0.043	–	–
N6-methyladenosine	−0.248	0.017	–	–
napelline	−0.273	0.007	–	–
nipecotic acid	−0.252	0.012	–	–
pentamidine	−0.248	0.033	–	–
pseudopelletierine	−0.266	0.039	–	–
puromycin	−0.288	0.013	–	–

* A: Alimentary tract and metabolism; C: Cardiovascular system; D: Dermatologicals; G: Genitourinary system and sex hormones; H: Systemic hormonal preparations, excluding sex hormones and insulins; J: Antiinfectives for systemic use; M: Musculoskeletal system; n: nervous system; P: Antiparasitic products, insecticides, and repellents; R: Respiratory system; S: Sensory organs; –: No annotation.

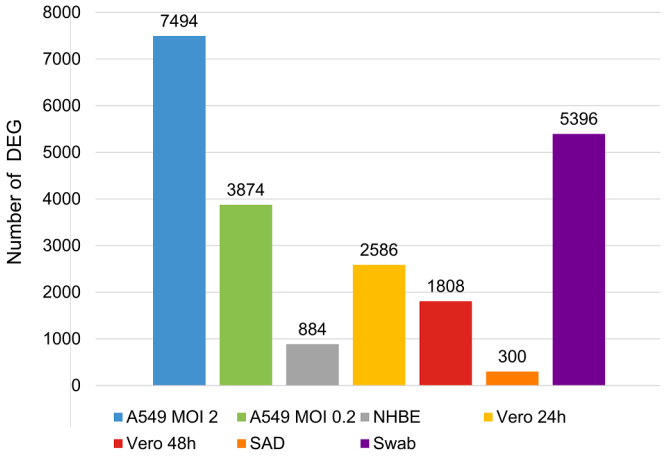

Fig. 3. Differentially Expressed Genes (DEG) total count present in each dataset.

chromatin organization, lipid metabolism, and MAPK pathway regulation. DEGs from nasopharyngeal swabs collected from SARS-CoV-2-positive patients mostly correspond to endoplasmic reticulum stress, juxtacrine signaling, and cell innate immune response.

Reflecting the differential expression analysis, the number of MRs was also remarkably different between experiments (Supplemental File 4). Swab samples from SARS-CoV-2-infected subjects showed 29 altered regulon activities when compared to healthy ones, while for the human-derived cell lines we found that A549 had 33 MRs responding to SARS-CoV-2 MOI 2, A549 MOI 0.2 had 28, and NHBE cells had only 4 MRs associated with SARS-CoV-2 infection. In Vero cells, the number of altered regulons was 20 and 16 for the 24 h and 48 h post incubation times, respectively.

Drug repositioning candidates to preclinical cell models and patients' nasopharyngeal swabs

Regarding the CMap analyses, samples from NHBE cells provided the greatest number of drug candidates, with 118 in total, while there were 101 for A549 MOI 2 and 75 for A549 MOI 0.2. Analysis of Vero cells at 24 h and 48 h data provided 30 and 98 suggested drugs, respectively. Finally, for the nasopharyngeal swabs collected from SARS-CoV-2-positive patients, we were able to identify 105 drug candidates (Fig. 5, Supplemental File 6).

3.3. Similarity indexes between clinical severe COVID-19, nasopharyngeal swabs and preclinical cell models

To compare the transcriptional signature in response to SARS-CoV-2 infection from different preclinical cell models with nasopharyngeal swabs and with the clinical severe COVID-19 samples we calculated de

Jaccard Indexes for gene ontologies, MRs, and drug results between them (Figs. 4 and 5).

Across all cellular models paired with severe clinical samples, the NHBE model has shown the highest Jaccard indexes, with 0.13 for GO comparisons, 0.11 for MRs and 0.02 for CMap results. Whereas for the comparisons with nasopharyngeal swabs collected from SARS-CoV-2 positive patients, A549 MOI 2 cell model was the one with the highest indexes, being 0.33, 0.35, and 0.17 for GO, MRs and CMap results, respectively.

Both severe COVID-19 clinical samples and NHBE share more than two hundred GOs enriched with DEGs (Supplemental File 3), but only the TEAD4 transcription factor was identified as a mutual MR. For the drug repositioning candidates, although no statistical significance was found for the Jaccard index, NHBE and severe COVID-19 clinical samples shared four drugs in common: nialamide, amoxapine, bisoprolol, and puromycin.

Regarding the similarities between nasopharyngeal swab samples and the A549 MOI 2 cell model, more than eight hundred GOs were enriched with DEGs in both analyses, while they shared 18 MRs and 5 drug repositioning candidates, namely stachydrine, mycophenolic acid, tolmetin, GW-8510, and staurosporine. Interestingly, these 5 drugs were also identified as repositioning candidates in the CMap analyses for the other four cell models analyzed. In addition, the transcription factors Y-box-binding protein 1 (YBX1), activating transcription factor 2 (ATF2), GA binding protein transcription factor subunit alpha (GABPA), tripartite motif containing 28 (TRIM28), golgin B1 (GOLGB1), and Maf leucine-zipper transcription factor G (MAFG) were consensus MRs for nasopharyngeal swab samples and all cell models, except the NHBE, which had only MAFG in common with the first (Fig. 6 and Supplemental File 5).

4. Discussion

COVID-19 is a severe illness which has high transmission and mortality rates, and is associated with many long-term sequelae in convalescent patients. This disease can affect the lower respiratory tract in moderate and severe cases, manifest as pneumonia and diffuse alveolar damage, and can culminate in the development of an acute respiratory syndrome (Carsana et al., 2020). Thus, molecular data from clinical samples such as lung biopsies of patients with COVID-19 is of great value for the elucidation of viral infection-induced host response in the most severe cases. Unfortunately, up to the moment of our analyses, data of this nature remain very limited. Here we analyzed the GEO database GSE155241 containing high throughput expression profiling data from lung autopsies of three COVID-19 deceased patients along with equivalent data from three healthy controls. However small, the available datasets allowed us to identify a significant number of DEGs and subsequently infer potential MRs of the severe COVID-19 pathology. The identification of disease MRs have previously been successfully used in cancer research and several other fields for elucidation of disease mechanisms and prospection of novel therapeutic strategies (Jarada et al., 2020).

A known clinical feature of severe COVID-19 is the extensive damage caused by a hyper-inflammatory response (Anka et al., 2021). The TAL1,

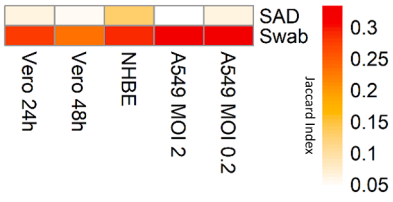

Fig. 4. Jaccard index heatmap for GO results comparison between preclinical cell models with nasopharyngeal swabs and severe COVID-19 signatures. All comparison had significant Fisher's Exact test p-value.

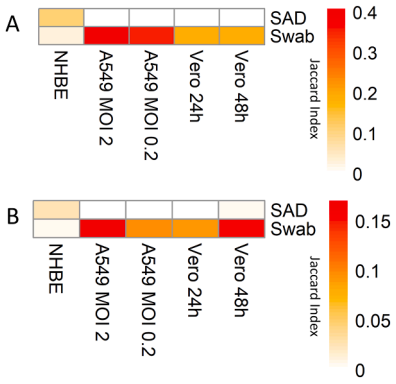

Fig. 5. Jaccard index heatmap for comparison between preclinical cell models with nasopharyngeal swabs and severe COVID-19 signatures for MR analysis (A) and CMap (B). The "*" sign indicates a p-value below 0.05 and "**" a p-value under 0.001.

ERG, TEAD4, and ARNTL2 transcription factors, identified as severe COVID-19 MRs, have already been shown to be involved in inflammation regulation.

Previous reports described the presence of a NFκB/ cAMP response element-binding (CREB) regulatory element in *TAL1* promoter region, also showing that the increased expression of this transcription factor leads to cytokine production in macrophages (Terme et al., 2008) and is associated with histone acetyl-transferase p300, promoting interleukin 6 (IL-6) expression (Huang et al., 1999; Ntranos and Casaccia, 2016) which has been recently described as a major player COVID-19 related cytokine storm. Indeed, serum levels of IL-6 were also shown to effectively assess disease severity and predict outcome in patients with COVID-19 (Elshazli et al., 2020; Liu et al., 2020) (Fig. 7).

On the other hand, the transcription factor ERG seems to control anti-inflammatory pathways by controlling the transcription of interleukin 8 (IL-8), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule (VCAM), and by inhibiting proinflammatory signaling pathways and activation of NFκB, and tumor necrosis factor alpha (TNF-α) induced inflammation, in endothelial cells (Sperone et al., 2011; Yuan et al., 2009). Further, its expression is significantly reduced by proinflammatory molecules, such as TNF-α and lipopolysaccharides (Yuan et al., 2009), suggesting that its downregulation may be required for proinflammatory signaling.

Regarding the TEAD4, cancer cells overexpressing this transcription factor showed immune system process and immune response pathways such as antigen processing and presentation, natural killer cell mediated cytotoxicity, and T cell receptor signaling pathway differentially activated when compared to controls. The differential expression of the TEAD genes family also showed significant association with different types of immune cells infiltration in the tumor environment (Ren et al., 2021; Wang et al., 2021a). In the same way ARTL2 co-express with genes mainly associated with positive regulation of cytokine production, regulation of innate immune response and cellular responses to molecules of bacterial origin and shown positive correlation with infiltration of CD8+ T, macrophages, neutrophils and dendritic cells in tumors (Pan et al., 2021; Wang et al., 2021b).

Besides inflammation, SARS-CoV-2 hijacks cell organelles and cytoskeleton, making profound morphologic changes, such as syncytium formation (Wen et al., 2020). EPAS1 and ATOH8 are both related to cell morphogenesis, migration and proliferation, and such processes are deeply related to cytoskeleton dynamics (Fang et al., 2014; Islam et al., 2020; Provenzano and Keely, 2011). Indeed, EPAS1 and dexamethasone-activated glucocorticoid receptor NR3C1 promotes PTK6 expression, a tyrosine kinase that regulates RHO/Ras pathway, a critical hub for cytoskeleton organization. More importantly, EPAS1 (also known as hypoxia-induced factor 2 alpha, HIF2A), TAL1, ATOH8,

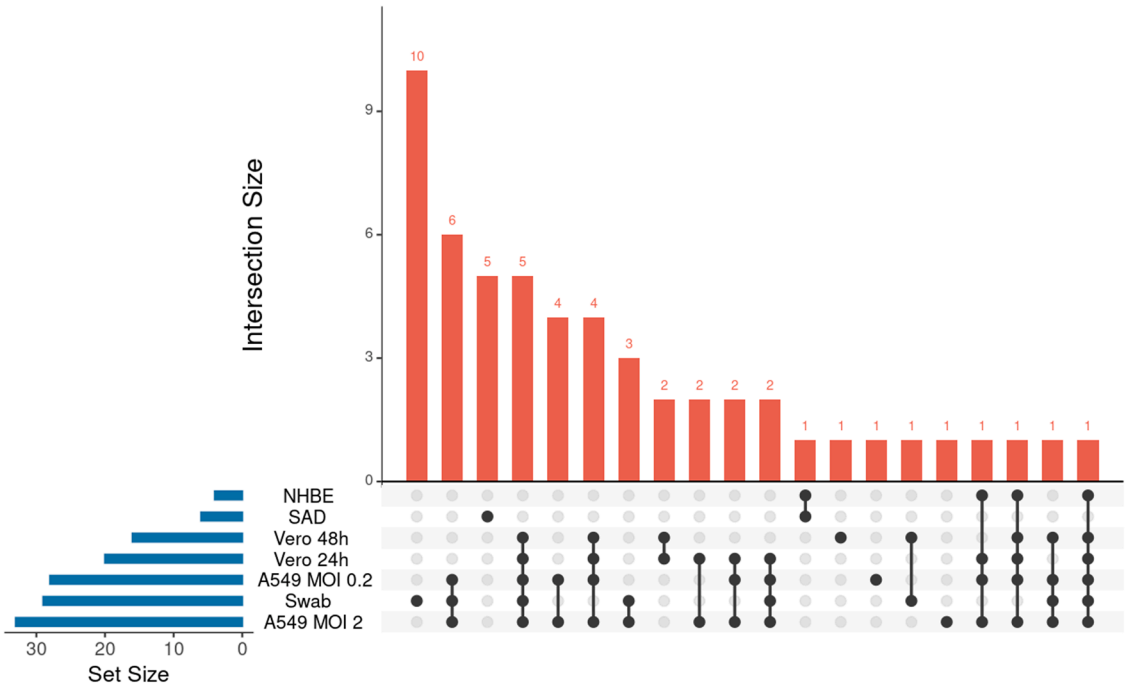

Fig. 6. UpSet plot representing the number of exclusive and shared MRs for all models. The right blue bars show the total number of MRs in each dataset, and the red bars show the intersection size. Solitary and connected dots indicate which datasets are demonstrated above. Sets with no elements are not shown.

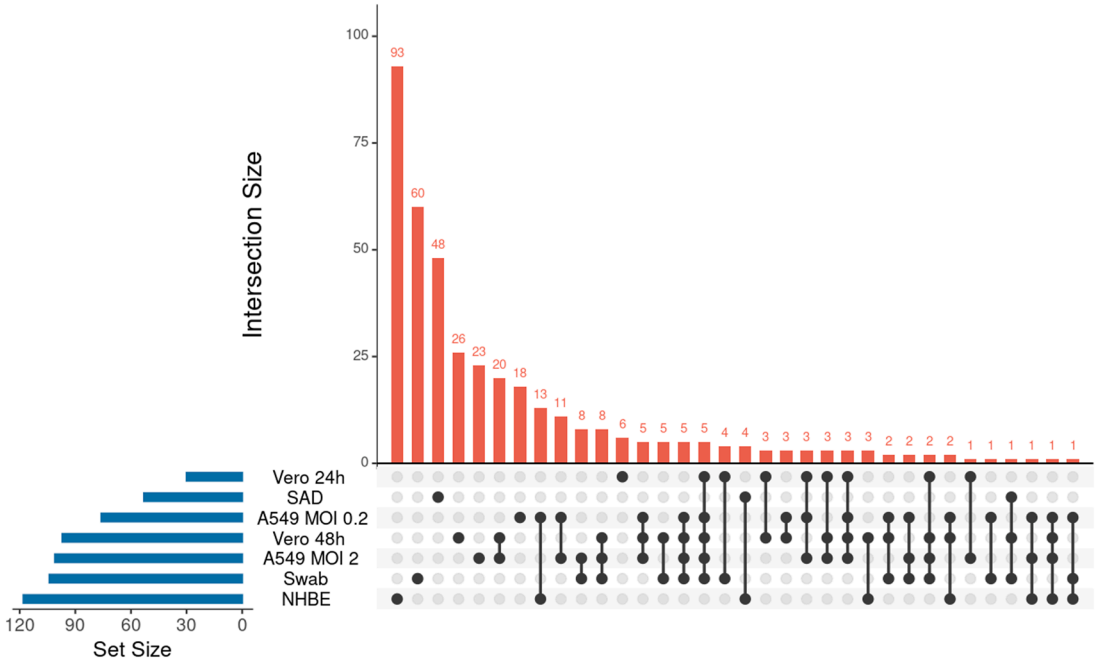

Fig. 7. UpSet plot representing the number of exclusive and shared drugs for all models. The right blue bars show the total number of drugs in each dataset, and the red bars show the intersection size. Solitary and connected dots indicate which datasets are demonstrated above. Sets with no elements are not shown.

EGR and ARNTL2 interact with hypoxia-induced factor 1 alpha (HIF1A) and hypoxia responsive elements standing out a cellular adaptation to severe hypoxia found in SARS-CoV-2-infected lung cells (Goardon et al., 2006; Islam et al., 2020; Kumar et al., 2020; Looney et al., 2017; Morikawa et al., 2019; Poloznikov et al., 2021). Even HIF prolyl hydroxylase inhibitors were proposed as therapeutic candidates for COVID-19 management, intending to improve hypoxic response and alleviate COVID-19 worsening due to downregulation of angiotensin-converting enzyme 2 (ACE2) and ferritin, an iron storage protein involved with chronic inflammation anemia (Poloznikov et al., 2021).

According to the WHO ATC classification system annotations, the drug repositioning candidates identified for severe COVID-19 clinical samples were mainly inflammatory response regulation drugs, such as corticosteroids, different antibiotics, and psychoanaleptics drugs such as antidepressants and psychostimulants (Table 2). Indeed, the first drugs used to treat COVID-19 severe cases, and further proved to improve outcome and significantly reduce disease mortality rates, were corticosteroid and cytokine inhibitors, such as dexamethasone and tocilizumab

(Aziz et al., 2021; RECOVERY Collaborative Group et al., 2021), which suggests that targeting the immune system could be a promising strategy for COVID-19 treatment.

Among the drugs suggested by our analyses, flunisolide belongs to the corticosteroids class and is prescribed to allergic rhinitis. Treatment by inhalation of flunisolide has already been demonstrated to promote a significant inhibitory effect on interleukin 1 (IL-1) and TNF of alveolar macrophages (Bewig and Barth, 1993). In addition, in mild to moderate asthmatic patients, a disease related to infiltration of the major and small airways with chronic inflammatory cell, HFA-flunisolide improve the lung function and reduced eosinophils and eosinophil associated cytokines and chemoattractants, such as interleukin 5 (IL-5), in both the peripheral and the central airways (Hauber et al., 2003). Interestingly, this drug also was suggested as potential therapeutic strategy for COVID-19, based on *in silico* analysis by Nunnari et al. (2020).

Betamethasone, another candidate drug inferred by our analysis, also belongs to the corticosteroids class. Interestingly, this drug has already been suggested as candidate drug for COVID-19 elsewhere by an integrative multi-omic study which used interactome, proteome, transcriptome, and bibliome data (Barh et al., 2020), and it was under clinical trial at sites where dexamethasone is not available, but these results are not available to this date (NCT04509973).

The antibiotics cephalosporin, lincosamide, macrolide and fluoroquinolone were also identified by our CMap analyses. Although controversial, due to the dissemination of antimicrobial resistance (Lucien et al., 2021), the use of antibiotics on COVID19 treatment has been widely discussed, due to its potential antiviral and anti-inflammatory effects suggested by several *in vitro* and *in silico* studies (Durojaiye et al., 2021; Marciniec et al., 2020). However, conflicting results and scarcity of randomized controlled trials for these drugs exposes the need for deeper studies on the effectiveness of these therapeutic strategies (Popp et al., 2021).

The same situation applies in some extent to psychoanaleptics, such as antidepressants, which are thought to also induce the reduction of pro-inflammatory factor levels (Eyre et al., 2016; Hashioka, 2011), having weak and inconsistent clinical evidences for its beneficial effects on late COVID19 treatment, in general derived from low quality and poorly designed studies or anecdotal claims (Hannestad et al., 2011; Hoertel et al., 2021). But what differentiates antimicrobials from psychoanaleptics is that at least one promising antidepressant (fluvoxamine) showed significant effects preventing hospitalization in patients, when treated in early stages of COVID-19 (Reis et al., 2022). The proposed mechanism of action for fluvoxamine are (i) serotonin transport inhibition, thus preventing immune system inflammatory activation; (ii) inhibiting acid sphingomyelinase viral entry domain; (iii) preventing viral replication as sigma-1 receptor chaperone agonist (Hashimoto et al., 2022).

Lastly, verapamil and bisoprolol, drugs classically used for treatment of cardiovascular conditions, have been suggested to hamper virus entry on host cells (Heriansyah et al., 2020; Navarese et al., 2020). Verapamil is a voltage-gated Ca^{2+} channel blocker that potentially inhibits the virus early-entry by a spike protein dependent membrane fusion mechanism (Navarese et al., 2020). Also, it has anti-inflammatory properties, presumably based on leukocyte cytokine secretion hindering (Das et al., 2009; Eteraf-Oskouei et al., 2017). Bisoprolol is a drug used for hypertension treatment, and could be beneficial for COVID-19 treatment on a two-way basis: it decreases ACE2 expression in lung, which is a receptor linked to SARS-CoV-2 host cell infection, by interfering in the sympathetic branch of renin-angiotensin-aldosterone system, and also diminishes circulatory cytokines (Heriansyah et al., 2020).

In an attempt to search for alternative molecular data that potentially reflect the signature of the disease from more accessible biological samples, we also analyzed a dataset of upper airway respiratory tract, specifically from nasopharyngeal swab samples from positive COVID-19 patients (GSE152075 with 484 patient samples). Understandably, the similarity between pulmonary and upper airway respiratory tract COVID-19 signatures was very small, being potentially a reflection of the different cell populations that make up the upper and lower respiratory tracts, since it is known that swabs have mostly ciliated epithelial cells (Matelski et al., 2020).

Surprisingly most of the preclinical cellular models analyzed in this work, with exception of NHBE, are more similar to the upper tract nasopharyngeal swab samples than clinical samples derived from severe COVID-19 lung autopsies. A549 cells are derived from human lung adenocarcinoma and seem that changes in cytogenetics, as well as the insertion of *ACE2*-overexpressing vector, might make the cells behave more like as epithelial cells. Vero cells were isolated from green monkey nephrons, and are widely used to replicate virus cultures. Despite the ontogenetic discrepancy between kidney and lung and the evident phylogenetic interspecific molecular differences, they have been used during the COVID-19 pandemics for drug repositioning candidates prospection (Harcourt et al., 2020; Khoshdel Rad et al., 2020; Someswara Rao and Viswanadha Raju, 2016; Warburton et al., 2010). However, our results show that Vero cells have a lower degree of similarity with the different clinical samples than A549 and NHBE, suggesting that this cell model, although a very useful tool for viral replication studies, is less indicated for the elucidation of COVID19 molecular cell response.

5. Conclusion

Our study shows a potential relevance of immunomodulatory drugs to counteract severe COVID-19 from a holistic perspective, in addition to suggest a plethora of antibiotics, psychoanaleptics, beta-blockers, and anti-hypertensive drugs as candidates for novel COVID19 therapeutic strategies to be investigated.

Further, different COVID-19 cell models were analyzed and their translational potential were evaluated. Our results showed that NHBE cells are more reliable for studying mechanisms and simulating the context of lung tissue gene expression, whereas A549, at both MOI 2 and 0.2, are probably more adequate to model the nature of upper respiratory tract epithelium. However, it is worth noting that for drug repositioning means, none of the cellular models analyzed have emerged as a statistically reliable source for lung clinical data. As final considerations, we emphasize that the *in silico* approach described in this report has already been successfully applied in the context of several other diseases (De Bastiani et al., 2018; De Bastiani and Klamt, 2019; Vargas et al., 2021, 2018), although comparison of *in vitro* and clinical models should be further studied to optimize translational medicine.

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CRediT authorship contribution statement

Henrique Chapola: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Marco Antônio de Bastiani:** Conceptualization, Formal analysis, Methodology. **Marcelo Mendes Duarte:** Writing – review & editing. **Matheus Becker Freitas:** Writing – review & editing. **Jussara Severo Schuster:** Writing – review & editing. **Daiani Machado de Vargas:** Conceptualization, Investigation, Writing – review & editing. **Fábio Klamt:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2023.199053.

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