Single-nucleus lung transcriptomics and inflammatory responses in lethal COVID-19 reveal potential drugs in advanced-stage clinical trials

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Abstract

There is pressing urgency to identify drugs that allow treating COVID-19 patients effectively. Respiratory failure is the leading cause of death in patients with severe COVID-19, and the host inflammatory response at the lungs remains poorly understood. Therefore, we retrieved data from postmortem lungs from COVID-19 patients and performed in-depth *in silico* analyses of single-nucleus RNA sequencing data, inflammatory protein interactome network, functional enrichment, and shortest pathways to cancer hallmark phenotypes to reveal potential therapeutic targets and drugs in advanced-stage COVID-19 clinical trials. Herein, we analyzed transcriptomics data of 719 inflammatory response genes across 19 cell types (116,313 nuclei) from lung autopsies. The functional enrichment analysis of the 233 significantly expressed genes showed that the most relevant biological annotations were: inflammatory response, innate immune response, cytokine production, interferon production, macrophage activation, thymic stromal lymphopoietin, blood coagulation, IL-1 and megakaryocytes in obesity, NLRP3 inflammasome complex, and the TLR, JAK-STAT, NF- κ B, TNF, oncostatin M, AGE-RAGE signaling pathways. Subsequently, we identified 34 essential inflammatory proteins with both high-confidence protein interactions and shortest pathways to inflammation, cell death, glycolysis, and angiogenesis. Lastly, we propose five small molecules involved in advanced-stage COVID-19 clinical trials: baricitinib, pacritinib, and ruxolitinib are tyrosine-protein kinase JAK2 inhibitors, losmapimod is a MAP kinase p38 alpha inhibitor, and eritoran is a TLR4/MD-2 antagonist. After being thoroughly analyzed in COVID-19 clinical trials, these drugs can be considered for treating severe COVID-19 patients.

Introduction

The first zoonotic transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurred in China in late December 2019¹, and it is the etiological agent of the coronavirus disease 2019 (COVID-19)². Since the World Health Organization (WHO) declared the outbreak of COVID-19 as a pandemic on March 11, 2020, the SARS-CoV-2 infection has led to more than 200 million cases and more than 4 million deaths globally³.

SARS-CoV-2 is the seventh CoV known to infect humans, along with HKU1, OC43, NL63, 229E, SARS-CoV, and Middle East respiratory syndrome (MERS)⁴. The novel coronavirus is a single-stranded positive-sense RNA virus of about 30 kb in length^{5,6}, which encompasses a 5' terminal cap, 14 open reading frames (ORFs) encoding for 29 proteins, and a 3' poly A tail⁷. ORF1a and ORF1ab encode 16 non-structural proteins (nsps) involved in antiviral response (nsp1), viral replication (the nsp3-nsp4-nsp6 complex), the protease 3C^{Pro} (nsp5), the RNA polymerase (the nsp7-nsp8 complex), the single strand RNA binding protein (nsp9), the methyltransferase activity (nsp10 and nsp16), the RNA-dependent RNA polymerase (nsp12), the helicase/triphosphatase (nsp13), the 3'-5' exonuclease (nsp14), the uridine-specific endoribonuclease (nsp15), and the RNA-cap (nsp16)⁸⁻¹⁰. The remaining genes encode structural proteins: the spike (S) glycoprotein, the nucleocapsid (N) protein, the membrane (M) glycoprotein, the envelope (E) protein, and several accessory proteins^{7,11}.

At the molecular level, amino-acid changes that result in reduced fitness are generally removed by negative selection, whereas changes that increase virus fitness are maintained by positive selection ¹². The most significant mutation observed in SARS-CoV-2 is probably the D614G substitution in the S1 subunit of the S protein. This mutation confers a 20% increase in infectivity and is associated with a higher ACE2-binding affinity ¹³. Additionally, SARS-CoV-2 fitness is also enhanced in presence of the E484K mutation, which increases resistance to antibodies ¹⁴.

This transmissibility advantage was further increased to approximately 50% by the emergence of the B.1.1.7 variant ¹⁵. Subsequently more variants have emerged, some of them capable of escaping monoclonal antibodies, partially eluding the polyclonal immune responses induced by previous infection or even allowing re-infections. It should be noted that recent improvements in immune escape are linked to mutations that alter the N-terminal domain (NTD) rather than the receptor-binding domain (RBD) of the S protein, where early and functionally important alterations prevailed ¹⁶. However, improved transmissibility, rather than immunoevasion or increased lethality, are considered as the main route for the virus to become fitter and more viable ¹⁷.

The variants that are being carefully monitored include: A) Variants of concern (VOCs): characterized by increased transmissibility, cause a more severe manifestation of the disease or significant reduction in neutralizing antibodies generated during a previous infection or after vaccination, reduced effectiveness of treatments, or diagnostic detection failures. This group includes the B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and P.1 (Gamma) variants. B) Variants of interest (VOIs): present reduced neutralization by antibodies induced by previous infection or vaccines, reduced efficacy of treatments, potential diagnostic escape and expected increase in transmissibility or severity of COVID-19. Among them, we find the B.1.427 / 429 (Epsilon), B.1.525 (Eta), B.1.526 (Iota), B.1.617.1 (Kappa), C.37 (Lambda), B.1.617.3, P.2 (Zeta), and B.1.621 variants. C) Variants under monitoring: could have properties similar to those of VOCs, however precise information is still lacking. D) High consequence variants: those that have significantly reduced the efficacy of vaccines in relation to previously circulating variants, and additionally cause failures in their diagnosis; however, there are not SARS-CoV-2 variants that rise to the level of high consequence yet ^{18,19}. It is expected that more variants will emerge over time that will need to be closely monitored, since they are a potential threat to public health. Nevertheless, this will not happen indefinitely because -over time- the virus will reach its maximum transmission point, therefore, new variants will not acquire more advantages in terms of infectivity. Thereafter, virus infectivity will stabilize and experience occasional and minimal variations ^{20,21}.

SARS-CoV-2, enriched by the previously mentioned genomic variants, has the ability to infect human body cells –especially in the lung microenvironment– through the angiotensin-converting enzyme 2 (ACE2) protein receptor ⁴. Lung homeostasis necessitates a fine balance between tolerance mechanisms against non-pathogenic agents, pro-inflammatory immune system activation to fight off respiratory tract infections and anti-inflammatory and pro-fibrotic processes that will minimize the immune-mediated tissular lesion and promote tissue remodeling and repair. These complex mechanisms are mediated by a

variety of tissue-resident and recruited cell types. The pulmonary alveolar epithelium is mainly composed of alveolar type I cells (AT1), which are essential for the gas-exchange function of the lungs, and alveolar type II cells (AT2), which are best known for their functions in synthesizing and secreting pulmonary surfactant factors²². Airway epithelial cells are central players in mucociliary clearance of the lungs. They produce a variety of antimicrobial substances, cytokines, and growth factors that mediate leukocyte recruitment, modulation of innate and adaptive immunity, and tissue repair^{23,24}. They also constitute the first cells that contact invading pathogens and are responsible for early pathogen recognition and induction of the antiviral state through pro-inflammatory cytokines and type I interferon secretion²⁵. Pulmonary endothelial cells are localized in the interface between the pulmonary tissue and the bloodstream. Their strategic location is also reflected in their pleiotropic functions that range from gas interchange to regulating vascular tone and facilitating immune cell recruitment and diapedesis upon receiving pro-inflammatory stimuli²⁶. Mast cells are innate immune cells, involved in immune defense and surveillance. They are filled with secretory granules, which upon activation, release bioactive mediators to fight pathogens or induce allergic reactions²⁷. Macrophages are key sentinel cells residing in peripheral tissues that detect pathogen invasion or tissue damage and initiate acute inflammatory processes triggering recruitment and activation of innate and –in second step- adaptive immune responses. Macrophages are also key inducers of the respiratory burst, professional antigen-presenting cells and tissue repair and remodeling mediators²⁸. Dendritic cells form a heterogeneous population of the immune system that have a wide array of immune functions. Conventional dendritic cells bridge the innate and adaptive immune responses as they are constantly sampling antigens from the airways and/or the infected lung tissue, migrate to T-cell areas of secondary lymphoid organs and present it to T lymphocytes thereby activating them²⁹. Monocytes, subsets of leukocytes mostly originated from myeloid progenitors in the bone marrow, are able to differentiate into macrophages or dendritic cells in peripheral tissues. They seed tissues with enough macrophages to replace their loss through infection and tissue damage and can adopt specific macrophage or dendritic cell phenotypes depending on the cytokine milieu they encounter upon arrival to the inflamed tissue³⁰. Natural killer (NK) cells are lymphocytes of the innate immune system, that play a main role in anti-viral and anti-tumor responses^{31–33}. They can identify and kill infected or stressed cells by releasing perforin and granzymes or by death receptor signaling (FasL/Fas interactions and the subsequent induction of apoptosis). NK cells can also release IFN γ upon activation, thereby contributing to naive T helper cell activation and differentiation and classical activation of macrophages³⁴. The CD4 + T helper cell population orchestrates the innate and adaptive immune responses in acute and chronic viral infections by secreting a panel of immunomodulatory cytokines. These cells also play a key role for the establishment of long-term cellular and humoral antigen-specific immunity, which is the basis of long-term protection induced by a plethora of viral infections and vaccines³⁵. The cytotoxic CD8 + T cells play a pivotal role in controlling infections caused by intracellular pathogens. These cells can be considered the adaptive immunity counterparts of NK cells, but unlike their innate immunity counterparts CD8 + T cells are activated by specific pathogen or tumor-derived antigen presented on class I major histocompatibility complex molecules (MHC I). The three major mechanisms of action of these cells are quite similar to NK cell functions: a) direct killing of

infected or tumor cells by release of perforin and granzymes, b) indirect destruction of cells via death receptor signaling (Fas/FasL interactions), and c) secretion of cytokines that can direct and potentiate immune responses of nearby cells ³⁶. Treg cells are potent immunosuppressive cells that play a vital role in maintaining immune homeostasis and in the prevention of autoimmune responses by suppressing the activation of conventional T-cells ^{37,38}. B cells have a key role in the humoral adaptive immune response and –once activated- are responsible for the production of antigen-specific immunoglobulins ³⁹. Plasma blasts are terminally differentiated populations of effector B cells cells, which produce antibodies, providing immunity during initial exposure to a pathogen and mediating the protective effects of vaccination ⁴⁰. Fibroblasts are key cells in the wound repair process and tissue scarring. They participate in the immune response by producing cytokines and chemokines that initiate the recruitment and retention of bone marrow-derived immune effector cells ⁴¹. Smooth muscle cells provide the main support for the vessel wall structure and regulate vascular tone to maintain intravascular pressure and tissue perfusion ⁴². Lastly, neuronal cells release neurotransmitters and neuropeptides that allow fast communication with immune cells, maintaining homeostasis and fighting infections. Neuroimmune interactions are also implicated in several chronic inflammatory conditions ⁴³.

Previous studies have reported profound SARS-CoV-2-induced transcriptional and immunological changes in animal models ⁴⁴, as well as in bronchoalveolar lavage fluid (BALV) ⁴⁵, nasopharyngeal ⁴⁶, and human blood samples ⁴⁷. However, the respiratory failure is the leading cause of death in patients with severe COVID-19 disease and the host inflammatory response at the lung tissue level remains poorly understood ^{48,49}. To shed light on this physiological response, we retrieved data from COVID-19 autopsies and performed in-depth *in silico* analyses of single-nucleus RNA sequencing (snRNA-seq) data, inflammatory protein-protein interactome (iPPI) network, miRNome enrichment, gene ontology (GO), and the shortest paths to cancer hallmark phenotypes to reveal potential therapeutic targets and drugs in advanced stage COVID-19 clinical trials.

Methods

Protein sets. We have retrieved a total of 719 inflammatory response proteins from the David Bioinformatics Resource (<https://david.ncifcrf.gov/>) ⁵⁰ using the gene ontology (GO) term: 0006954 inflammatory response. We have also retrieved the 332 human proteins physically interacting with 26 of the 29 SARS-CoV-2 proteins proposed by Gordon *et al* ¹⁰.

Single-nucleus RNA sequencing data. Melms *et al* have previously published the molecular single-cell lung atlas of lethal COVID-19 through the snRNA-seq technology needed to profile hard-to-dissociate tissues ^{51,52}. Motivated by this study, we performed an in-depth *in silico* analysis comparing the transcriptional data of 719 genes involved in the inflammatory response between 9608 alveolar type I cells, 11341 alveolar type II cells, 7332 airway epithelial cells, 1845 B cells, 7586 CD4 + T cells, 3561 CD8 + T cells, 2814 cycling NK / T cells, 1083 dendritic cells, 5386 endothelial cells, 21472 fibroblast cells, 25960 macrophages, 1438 mast cells, 3464 monocytes, 2141 NK cells, 2017 neuronal cells, 5391 plasma

cells, 1437 smooth muscle cells, 649 Treg cells, and 1788 other epithelial cells. The snRNA-seq database was taken from the 'COVID-19 Studies' section of the Single Cell Portal (https://singlecell.broadinstitute.org/single_cell/covid19), and the transcriptomics data of 116,313 nuclei was taken from 'Columbia University / NYP COVID-19 Lung Atlas' study (https://singlecell.broadinstitute.org/single_cell/study/SCP1219/columbia-university-nyp-covid-19-lung-atlas?cluster=UMAP&spatialGroups=-&annotation=cell_type_intermediate-group-study&subsample=all#study-summary)⁵¹.

The criteria of the analysis of the lung transcriptomics data was the following: 'uniform manifold approximation and projection (UMAP)' as load cluster, 'cell type intermediate' as selected annotation, and 'all cells' as subsampling threshold. Additionally, we adjusted the mRNA expression taking into account the Z-scores, that is, overexpressed mRNAs with Z-scores ≥ 2 and underexpressed mRNAs with Z-scores ≤ -2 . Regarding visualization of transcriptomics data, we designed dot plots to visualize the percentage of cells expressing a certain gene, box plots to compare the mean Z-score across cell types, and scatter plots of 2D UMAPs to visualize significantly expressed multiple genes per subpopulation cell, and biological annotations across cell types.

Functional enrichment analysis. The functional enrichment analysis gives curated signatures of gene sets generated from omics-scale experiments^{11,53,54}. We performed the enrichment analysis to validate the correlation between significantly expressed genes and biological annotations related to lethal COVID-19. The enrichment was calculated using g:Profiler version e101_eg48_p14_baf17f0 (<https://biit.cs.ut.ee/gprofiler/gost>) to obtain significant annotations (Benjamini-Hochberg FDR q-value < 0.001) related to gene ontology: biological processes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways, Reactome signaling pathways, and Wikipathways^{53–57}. Lastly, the expression of genes involved in significant annotations was visualized in scatter plots, and the significant terms related to lethal COVID-19 pathology were manually curated.

miRNome enrichment analysis. The Gene Set Enrichment Analysis (GSEA) (<https://www.gsea-msigdb.org/gsea/index.jsp>) is a powerful analytical method for interpreting gene expression data that share common biological functions or regulations⁵⁸. Therefore, we performed a miRNome enrichment analysis using the 'microRNA targets' option to compute overlaps between miRNAs and significantly expressed mRNAs in > 50% of lung cells from lethal COVID-19 patients. Lastly, we proposed the most significant miRNAs with a false discovery rate (FDR) q-value < 0.01.

Inflammatory protein-protein interactome network. The iPPI network with zero node addition and a highest confidence cutoff of 0.9 was created between the human proteins physically associated with SARS-CoV-2 and human proteins involved in the pulmonary inflammatory response. This network was generated using the human proteome from the Cytoscape StringAPP^{59,60}, which imports protein interactions from the STRING database⁶⁰. The degree centrality represents the number of edges the node has in a network^{11,61,62}, and it was calculated using the CytoNCA app⁶³. The network elements were organized through the organic layout producing a clear representation of complex networks, and the iPPI

network was visualized through the Cytoscape software v.3.7.1⁶⁴. Finally, we ranked the inflammatory response proteins with the highest protein-protein interactions to the human-SARS-CoV-2 proteins.

Shortest paths from inflammatory response proteins to cancer hallmark phenotypes. CancerGenNet (<https://signor.uniroma2.it/CancerGeneNet/>) is a resource that links frequently altered proteins to cancer hallmark phenotypes⁶⁵. This bioinformatic tool, curated by SIGNOR⁶⁶, is based on experimental information that allows to infer likely paths of causal interactions linking proteins to oncogenic phenotypes. The shortest distance scores or paths from proteins to cancer phenotypes were programmatically implemented using the shortest path function of *igraph* R package⁶⁵. Hence, we calculated the shortest distance scores of positive regulation from the inflammatory response proteins with the highest confidence interactions to the human-SARS-CoV-2 proteins to the inflammation, cell death, angiogenesis, and glycolysis hallmark phenotypes.

Drugs involved in current COVID-19 clinical trials. The Open Targets Platform version 21.06 (<https://www.targetvalidation.org>) is comprehensive and robust data integration for access to and visualization of potential drug targets associated with several diseases including COVID-19⁶⁷. This platform has developed the COVID-19 Target Prioritization Tool (<https://covid19.opentargets.org/>) that integrates molecular data from the ChEMBL database⁶⁸ to provide an evidence-based framework to support decision-making on potential drug targets for COVID-19. Lastly, this platform shows all drugs in clinical trials associated with target proteins, detailing its modality, mechanism of action, phase, status, type of drug, target class, and clinical trial number⁶⁷.

Results

Single-nucleus RNA sequencing data. Single-nucleus biology is a powerful approach of omics medicine, needed to profile hard-to-dissociate tissues, that provides unprecedented resolution to the cellular underpinnings of biological processes in order to find druggable targets for complex diseases^{52,69,70}. Here, we identified 233 inflammatory response genes with significant expression in 116,313 nuclei belonging to 19 different lung cell types. Genes with the highest mean Z-score (3.26) and the most significant p-value (0.001) were identified in neural cells, followed by B cells (3.24; 0.001), mast cells (3.14; 0.002), fibroblast cells (3.0; 0.003), alveolar type II cells (2.96; 0.003), cycling NK / T cells (2.94; 0.003), endothelial cells (2.89; 0.004), macrophages (2.88; 0.004), airway epithelial cells (2.76; 0.006), alveolar type I cells (2.74; 0.006), NK cells (2.73; 0.006), dendritic cells (2.70; 0.007), smooth cells (2.68; 0.007), Treg cells (2.67; 0.008), plasma cells (2.62; 0.009), monocytes (2.47; 0.014), other epithelial cells (2.41; 0.016), CD4 + T cells (2.39; 0.017), and CD8 + T cells (2.24; 0.025) (Fig. 1).

Figure 2 shows scatter plots of significant mean log normalized gene expression and dot plots of genes with the highest percentage of cells expressing per lung cell type. *MECOM* has the highest percentage of cells expressing in alveolar type I cells, *LRRK2* in alveolar type II cells, *ELF3* in airway epithelial cells, *PXK* in B cells, *CAMK4* in CD4 + T cells, *AOAH* in CD8 + T cells, *HMGB1* in cycling NK / T cells, *CIITA* in dendritic cells, *RBPJ* in macrophages, *KIT* in mast cells, *SLC11A1* in monocytes cells, *APP* in neuronal cells, *AOAH*

in NK cells, *CALCRL* in endothelial cells, *RORA* in fibroblasts, *ASH1L* in plasma cells, *FN1* in smooth muscle cells, and *SGMS1* in Treg cells. Lastly, the 26 inflammatory response genes significantly expressed in more than 50% of lung cells were *ABR*, *ACER3*, *AOAH*, *APP*, *ASH1L*, *ATM*, *CALCRL*, *CAMK1D*, *CAMK4*, *CD163*, *CIITA*, *EGFR*, *FN1*, *HDAC9*, *IL18R1*, *IL1R1*, *KIT*, *LRRK2*, *LYN*, *MECOM*, *PRKCA*, *PRKCZ*, *RBPJ*, *RORA*, *SLC11A1*, and *SLIT2* (Supplementary Table 1).

Functional enrichment analysis. The functional enrichment analysis was performed using g:Profiler to obtain significant GO: biological processes, KEGG signaling pathways, Reactome signaling pathways, and Wikipathways related to lethal COVID-19 (Benjamini-Hochberg FDR $q < 0.001$)^{11,53,54}. Figure 3 shows scatter plots of significantly expressed genes ($n = 233$) in lung cells of lethal COVID-19 autopsies. After a manual curation of biological annotations, the most significant GO terms were inflammatory response (5.9×10^{-241}), cytokine production (9.5×10^{-62}), innate immune response (1.0×10^{-30}), macrophage activation (1.1×10^{-29}), toll-like receptor signaling pathway (3.8×10^{-15}), type I and II interferon production (1.8×10^{-13}), the Janus Kinase (JAK) / Signal Transducers and Activators of Transcription (STAT) signaling pathway (9.0×10^{-8}), NF- κ B signaling pathway (2.0×10^{-6}), thymic stromal lymphopoietin (TSLP) (4.5×10^{-6}), TNF signaling pathway (4.9×10^{-6}), blood coagulation (5.6×10^{-6}), oncostatin M signaling pathway (5.9×10^{-6}), AGE-RAGE signaling pathway (5.9×10^{-6}), IL-1 and megakaryocytes in obesity (6.8×10^{-6}), and NLRP3 inflammasome complex (2.5×10^{-4}) (Supplementary Table 2).

miRNome enrichment analysis. After identifying the significantly expressed genes in lung cells of lethal COVID-19 autopsies, we performed the GSEA analysis to compute overlaps between miRNAs and mRNAs⁵⁸. Figure 4 shows a circos plot of the 18 most significant miRNAs (FDR q -value < 0.01) overlapped with the 19 significantly expressed genes in $> 50\%$ of lung cells. The most significant miRNAs were MIR6867_5P ($q = 0.002$), MIR2662 ($q = 0.002$), MIR32_3P ($q = 0.002$), MIR548A0_5P_MIR548AX ($q = 0.003$), MIR570_3P ($q = 0.004$), MIR338_5P ($q = 0.005$), MIR144_3P ($q = 0.005$), MIR4711_3P ($q = 0.005$), MIR628_5P ($q = 0.005$), MIR548AJ_3P_MIR548X_3P ($q = 0.005$), MIR1290 ($q = 0.006$), MIR4496 ($q = 0.007$), MIR12136 ($q = 0.007$), MIR9718 ($q = 0.007$), MIR875_3P ($q = 0.008$), MIR4698 ($q = 0.008$), MIR3941 ($q = 0.009$), and MIR4789_3P ($q = 0.009$).

Inflammatory protein-protein interactome network. We generated the iPPI network encompassing 265 nodes and 2052 edges (Fig. 5). Of them, 159 pulmonary inflammatory response proteins had a mean of degree centrality of 8 and 108 human-SARS-CoV-2 proteins had a mean of degree centrality of 7.2. The top ten inflammatory response proteins with the highest degree centrality were APP (38), NFKB1 (36), STAT3 (34), C3 (31), ITGAM (29), FN1 (26), PTAFR (24), JAK2 (22), EGFR (20), and LYN (20). The top ten human-SARS-CoV-2 proteins with the highest degree centrality were GNB1 (29), GNG5 (25), RHOA (23), ITGB1 (22), STOM (20), RAB14 (20), PRKAR2B (17), RAB8A (17), PRKACA (17), and ANO6 (16). Additionally, 111 pulmonary inflammatory response proteins had the highest confidence interactions (cutoff = 0.9) with human-SARS-CoV-2 proteins, being the top ten: C3 (11 interactions), FN1 (11), NFKB1

(10), RPS19 (10), CTSC (9), HSPD1 (9), APP (8), ITGAM (8), SNAP23 (8), and MAPK14 (7) (Supplementary Table 3).

Shortest pathways from inflammatory response proteins to cancer hallmark phenotypes. We analyzed the 111 pulmonary inflammatory response proteins with the highest confidence interactions (cutoff = 0.9) to human-SARS-CoV-2 proteins in order to find the shortest pathways toward inflammation, cell death, angiogenesis, and glycolysis according to Iannuccelli *et al*⁶⁵. Figure 6A shows box plots encompassing proteins with the shortest distance scores to cancer hallmark phenotypes related to COVID-19 pathology. Cell death was the phenotype with the shortest mean of distance score (2.82), followed by inflammation (3.06), glycolysis (3.12), and angiogenesis (3.79). Figure 6B shows a Venn diagram integrating inflammatory proteins with shortest pathways to biological phenotypes related to COVID-19. We found 34 essential inflammatory response proteins with shortest pathways simultaneously to inflammation, glycolysis, cell death, and angiogenesis (Supplementary Table 4). Figure 6C details the ranking of inflammatory response proteins with positive response and shortest distance to cell death, inflammation, glycolysis, and angiogenesis. The top ten essential proteins with shortest pathways of positive regulation to cell death were ATM (1.20), NFKBIA (1.42), TNFRSF1B (1.64), APP (1.73), MAPK14 (1.73), PRKCZ (1.93), TLR4 (1.97), JAK2 (2.26), TGFB1 (2.35), and MECOM (2.36). The top ten essential proteins with shortest pathways of positive regulation to inflammation were PTGS2 (0.53), PRKCZ (1.39), NFKB1A (1.71), MAPK14 (1.92), TNFRSF1B (2.40), TLR4 (2.44), ATM (2.45), MECOM (2.57), PIK3CG (2.63), and EGFR (2.64). The top ten essential proteins with shortest pathways of positive regulation to glycolysis were ATM (1.67), CD28 (1.84), EGFR (1.98), TNFAIP3 (2.00), HGF (2.03), CYLD (2.09), PRKCZ (2.21), EPHA2 (2.25), JAK2 (2.27), and PIK3CG (2.34). The top ten essential proteins with shortest pathways of positive regulation to angiogenesis were TGFB1 (0.86), STAT3 (1.97), MAPK14 (1.98), EGFR (2.48), JAK2 (2.58), ATM (2.91), NFKBIA (2.96), PRKCZ (3.13), TLR4 (3.35), and PTAFR (3.46) (Supplementary Table 5). Lastly, Fig. 7 details all shortest pathways and distance scores of positive regulation from the 34 essential proteins to the inflammation phenotype.

Drugs involved in current COVID-19 clinical trials. Figure 8 details the current status of COVID-19 clinical trials regarding to essential inflammatory proteins, according to the Open Targets Platform⁶⁷. There are 5 drugs (small molecules) that are being analyzed in 8 clinical trials in advanced stages (phases III and IV) for 3 essential inflammatory proteins. Baricitinib is a tyrosine-protein kinase JAK1/2 inhibitor that acts on the JAK proteins and it is being studied in 4 clinical trials in phase III (NCT04640168, NCT04693026, NCT04421027, and NCT04401579). Similarly, pacritinib and ruxolitinib are tyrosine-protein kinase JAK1/2 inhibitors. They are currently being evaluated in a phase III clinical trial NCT04404361 and NCT04362137, respectively. Losmapimod is a MAP kinase p38 alpha inhibitor that acts on the MAPK14 protein and it is being studied in a phase III clinical trial (NCT04511819). Lastly, eritoran is a toll-like receptor 4/MD-2 antagonist that acts on the TLR4 protein and it is being studied in one clinical trial in phase IV (NCT02735707).

Discussion

Since the identification of patient zero in China, a wide spectrum of clinical features have been discovered in severe COVID-19. For instance, dyspnea, acute respiratory distress syndrome (ARDS)⁷¹, respiratory failure, lung edema, severe hypoxemia, cardiac arrhythmias, lymphopenia⁷², hyperferritinemia, rhabdomyolysis, intravascular coagulopathy^{73,74}, and pulmonary thromboembolism⁷⁵. Nowadays, it is known that SARS-CoV-2 not only causes respiratory tract infection, but also skin, kidneys, blood, and central neural system pathologies⁷⁶. Therefore, it is imperative to continuously review the clinical manifestations and physiopathological mechanisms of the SARS-CoV-2 infection, especially with the appearance of new genomic variants.

Single-cell biology provides unprecedented resolution to the cellular underpinnings of biological processes in order to find therapeutically actionable targets for complex diseases^{69,70}. Melms *et al* have previously published the molecular single-cell lung atlas of lethal COVID-19⁵¹. Motivated by this study, we performed an in-depth *in silico* analysis comparing the transcriptional data of 719 inflammatory response genes across 19 lung cell types belonging to COVID-19 autopsies. The functional enrichment analysis of the 233 significantly expressed inflammatory genes showed that the most significant biological annotations were inflammatory response (5.9×10^{-241}), cytokine production (9.5×10^{-62}), innate immune response (1.0×10^{-30}), macrophage activation (1.1×10^{-29}), TLR signaling pathway (3.8×10^{-15}), type I and II interferon production (1.8×10^{-13}), JAK-STAT signaling pathway (9.0×10^{-8}), NF- κ B signaling pathway (2.0×10^{-6}), thymic stromal lymphopoietin (4.5×10^{-6}), TNF signaling pathway (4.9×10^{-6}), blood coagulation (5.6×10^{-6}), oncostatin M signaling pathway (5.9×10^{-6}), AGE-RAGE signaling pathway (5.9×10^{-6}), IL-1 and megakaryocytes in obesity (6.8×10^{-6}), and the NLRP3 inflammasome complex (2.5×10^{-4}).

The innate immune response is the first line of defense against new invading pathogens⁷⁷. Pattern recognition receptors (PRRs) are capable of recognizing molecules with conserved motifs commonly shared by pathogen groups⁷⁸. Recognition by these receptors triggers innate immune responses and induces multiple IFN and pro-inflammatory cytokine secretion in COVID-19 patients⁷⁹. Upon ligand recognition PRRs initiate a signaling pathway that activate key transcription factors, such as NF- κ B, AP-1, and interferon regulatory factors (IRF3 and IRF7) that induce pro-inflammatory cytokines and type I interferon. Type I IFNs are responsible for inducing the JAK-STAT signaling pathway to activate IFN-stimulated genes and develop the “anti-viral state” in the infected organism^{80,81}.

Interferon is a cytoplasmic glycoprotein with antiviral activity. This cytokine is another contributing factor in the humoral immunological response against respiratory viruses^{82,83}. Through a bronchoalveolar lavage in severely ill patients, evidence of local induction of interferon and the stimulation of interferon genes was found. In contrast, minimal levels of interferon were found in peripheral blood of severely ill patients⁸⁴. This increased cytokine production with limited interferon levels might be due to an antagonist mechanism of the nsp1 protein against interferon signaling⁸⁵. Regarding the genetics underlying severe COVID-19, Zhang *et al* concluded that genetics may determine the clinical course of

SARS-CoV-2 infection identifying mutations in genes involved in the regulation of type I and III IFN immunity⁸⁶, and Bastard et al identified high titers of neutralizing autoantibodies against type I IFN- α 2 and IFN- ω in 10% of patients with severe COVID-19⁸⁷.

Macrophages are cells that perform crucial functions in the immune system, from the phagocytosis of the viruses and bacteria to maintaining homeostasis⁸⁸. Precisely, macrophages produce high amounts of pro-inflammatory cytokines in patients with ARDS, who present an activated state known as cytokine storm or macrophage activation syndrome⁸⁹. The overexpression of cytokines (i.e., TNF- α , IL-2, IL-10, IL-1, and IL-6) leads to a hyperinflammatory response, which has been reported as a remarkable feature of SARS-CoV-2 infection^{90–92}. IL-6 plays a main role in the severity of COVID-19, while TNF- α and IL-1 β trigger the NF- κ B signaling pathway^{93,94}. The excessive production of cytokines leads to development of pathological symptoms, such as lung damage, cell death, severe pneumonia, ARDS, lung fibrosis, and multiple organ failure^{93,95}. Hence, this cytokine storm plays a crucial role in the progression of SARS-CoV-2 infection and is considered as one of the main causes of lethal COVID-19^{92,93}.

TNF is considered as one of the most important pro-inflammatory cytokines, affecting different parts of the immune system and regulating various pathological and physiological processes⁹⁶. Therefore, the TNF α -NF- κ B axis is considered as a potential therapeutic target in COVID-19⁹⁷. Initially, NF- κ B is present within the cytoplasm, after activation of I κ B through phosphorylation of I κ B kinase, NF- κ B is activated and translocated to the nucleus where it regulates the transcription of various target genes^{98,99}. To date, SARS-CoV-2-mediated NF- κ B activation has been observed in several cells such as macrophages of liver, kidney, lung, central nervous system, cardiovascular system, and gastrointestinal system. This causes a chronic production of IL-1, IL-2, IL-6, IL-12, TNF- α , LT- α , LT- β , GM-CSF, and several chemokines, leading to the aforementioned pathological symptoms¹⁰⁰. Catanzaro *et al* have recently published a report analyzing the role of the TNF α -NF- κ B pathway in COVID-19. In their report, it was suggested that inhibiting this axis may prevent pulmonary complications in COVID-19 patients⁹⁷. This was also observed in SARS-CoV infection. NF- κ B expression was elevated in the lungs of recombinant SARS-CoV-1-infected mice, while NF- κ B inhibitors reduced SARS-CoV-related expanding survival of these mice¹⁰¹.

The cytokine signaling depends on the JAK and STAT which are phosphorylated and activated upon cytokines binding to their receptors. The STAT homodimers translocate into the nucleus, where they upregulate the transcription of several genes that participate not only in cytokine production but also in apoptosis, immune regulation, and cell cycle differentiation¹⁰². In the context of SARS-CoV-2 infection, inhibition of the JAK-STAT pathway seems as promising approach to prevent cytokines storm in fatal cases or in patients with comorbidities that express high levels of inflammatory markers such as of IL-6, TNF α , IL-17a, GM-CSF, and G-CSF¹⁰³. In fact, the GenOMICC GWAS study suggests that individuals with a variant on chromosome 19: 10,466,123 that affects expression of tyrosine kinase 2 (TYK2), member of the JAK family, could be associated with a host-driven inflammatory response that leads to severe lung injury¹⁰⁴. Thus, several clinical trials have shown that baricitinib, a JAK inhibitors possesses a good

safety and efficacy profiles in reducing cytokine levels of severe COVID-19 patients without side effects¹⁰⁵.

Nevertheless, the JAK/STAT pathway is also necessary to mediate the immune response to clear viral infections and prolonged inhibition of the pathway could lead to immunosuppression and prolonged infections¹⁰⁶. For instance, SARS-CoV-2 is able to hijack the JAK/STAT pathway in order to increase its proliferation by evading the immune response. Li *et al*/ showed that SARS-CoV-2 infected cell had a decreased expression of JAK1, JAK2, TYK2, and STAT2 proteins. This is explained by action of viral nsp1, ORF6, and ORF8 that prevent the phosphorylation of STAT1 and STAT3 to inhibit IFN production^{107,108}. Therefore, the timeline for administration of JAK/STAT inhibitors should be carefully analyzed since reducing the hyperinflammation could affect viral clearance. Due to the narrow therapeutic window of JAK/STAT inhibitors, dosage should aim to restore the immune response homeostasis.

The incidence of thrombotic events in COVID-19 patients responsible for strokes and heart attacks raises the concern about the abnormal coagulation patterns and poor prognosis in the actual pandemic. Tang *et al*/ reported that 71.4% of non-surviving COVID-19 patients met the criteria for disseminated intravascular coagulation and presented high levels of coagulation-related biomarkers such as D-dimer and fibrin degradation products¹⁰⁹. The mechanisms of the coagulopathy are not clear; however, some reports indicate that dysregulated immune responses are involved in such processes. Exacerbation of inflammatory cytokines promoting proliferation of megakaryocytes, lymphocyte cell-death, hypoxia, endothelial damage contributing to ischemia and organ dysfunction, and the association between autoantibodies and neutrophil extracellular traps seem to be involved in the abnormal thrombotic events in COVID-19 patients^{110–112}.

Oncostatin M is a cytokine involved in homeostasis and chronic inflammation that has pleiotropic functions such as cell differentiation and proliferation, and it is present in hematopoietic, immunological, and inflammatory networks¹¹³. One of the most important functions of oncostatin M is the stimulation of the chemokines CCL1, CCL7 and CCL8 in primary human dermal fibroblasts at a faster kinetics than IL-1 β or TNF- α ¹¹⁴. In 2020, it was proposed as a new mortality biomarker in patients with acute respiratory failure supported by venous-venous extracorporeal membrane oxygenation¹¹⁵. In the case of COVID-19, an increase of OSM plasma levels and other inflammatory mediators was detected; this finding was correlated with the severity of disease and the increase of bacterial products in plasma¹¹⁶. Finally, OSM is curiously elevated in obese patients and upon recognition by its specific receptor (OSMR β) induces obesity and insulin resistance conditions¹¹⁷.

Obesity is one of the main risk factors associated with lethal COVID-19, and levels of pro-inflammatory cytokines increase under this pathology¹¹⁸. Low NAD⁺ levels in obese individuals decrease the activity of SIRT1, a molecule that modulates cytokine production¹¹⁹. However, the excess of amino acid availability hyperactivates the mTOR signaling pathway increasing viral replication and inflammatory response¹²⁰. Additionally, because adipose tissue has a considerable level of ACE2 expression, viral

shedding increases, as well as the production of pro-inflammatory factors ¹²¹. This inflammatory process contributes to thrombotic problems, a probable cause of multiorgan failure, which has been evidenced by the presence of elevated levels of megakaryocytes in COVID-19 autopsies ^{122,123}.

Thymic stromal lymphopoietin is an epithelial cytokine normally produced by airway epithelial cells. It has been associated with T-helper type 2 (Th2) responses in allergic diseases, highlighting its role in inflammatory disease pathogenesis. It has been discovered that TSLP can be triggered by respiratory viral infections, bacteria, allergens and injuries ¹²⁴. TSLP acts upon cells with TSLP receptor such as hematopoietic progenitor cells, eosinophils, basophils, mast cells, airway smooth muscle cells, group 2 innate lymphoid cells, lymphocytes, dendritic cells and monocytes/macrophages. When several immune mediators were measured in patient's plasma suffering from influenza A (H1N1) and COVID-19, TSLP levels were significantly upregulated in COVID-19 patients. This fact suggests a possible contribution of TSLP in COVID-19 pathogenesis and perhaps aids differential diagnosis ¹²⁵. Besides, since TSLP concentration was reported to be higher in severely affected than in mild and moderated COVID-19 cases, it may be potentially used as a biomarker for disease severity ¹²⁶.

Optimal NLRP3 inflammasome activation is crucial for host immune defense against several pathogenic infections ¹²⁷. SARS-CoV-2 activates inflammasomes, which are large multiprotein assemblies that are broadly responsive to pathogen-associated cellular insults, leading to secretion of cytokines and an inflammatory form of cell death ¹²⁸. However, excessive activation can lead to systemic inflammation and tissue damage which are detrimental to the host ¹²⁹. Patients with severe COVID-19 have been found to have higher serum concentrations of pro-inflammatory cytokines and chemokines such as granulocyte-colony stimulating factor (GCSF), monocyte chemoattractant protein 1 (MCP1), TNF, IL-6, and IL-1 β compared with healthy individuals. A unified mechanism for NLRP3 inflammasome activation has not been proposed yet; however, some researchers have found that SARS-CoV-2 ORF-8b interacts with the LRR domain of NLRP3 inflammasome activating IL-1 β secretion in THP-1 macrophages ¹³⁰. Findings suggest that SARS-Cov-2 infection leads to NLRP3 inflammasome activation, caspase-1 cleavage, and the release of IL-1 β stimulating pyroptosis in peripheral blood mononuclear cells from severe COVID-19 patients ¹³¹.

In a biological system approach, SARS-CoV-2 employs a suite of virulent proteins that interacts with host targets to extensively rewire the flow of information and cause COVID-19 ^{11,132-134}. The human proteins physically associated with SARS-CoV-2 are the first line of host proteins ¹⁰, which also interact with proteins involved in a wide spectrum of signaling pathways and biological processes within lung cells. In this study, we identified 111 pulmonary inflammatory response proteins with the highest confidence interactions to human-SARS-CoV-2 proteins, being the top ten: C3, FN1, NFKB1, RPS19, CTSC, HSPD1, APP, ITGAM, SNAP23, and MAPK14.

Subsequently, we analyzed these 111 inflammatory response proteins to identify those with the shortest pathways to four cancer hallmark phenotypes. Inflammation is a hallmark of cancer observed in patients

with SARS-CoV-2 infection ¹³⁵. The chronic inflammatory process causes cell death ^{136,137}, angiogenesis ¹³⁸, and during the peak of inflammation, immune cells preferentially use glycolysis as a source of energy ¹³⁹. These facts provide a biological rationale to analyze and prioritize the inflammatory response proteins with the shortest distance scores to these biological phenotypes. Consequently, we identified 34 essential inflammatory response proteins highly associated with cell death, glycolysis, and angiogenesis. These proteins were: PTGS2, PRKCZ, NFKBIA, MAPK14, TNFRSF1B, TLR4, ATM, MECOM, PIK3CG, EGFR, JAK2, LYN, CYLD, PRKCQ, STAT3, TGFB1, RBPJ, TNFAIP3, NOTCH1, IGF1, CD28, CCL5, PTAFR, FPR1, EDNRA, EDNRB, CYSLTR1, CNR2, HGF, EPHA2, FN1, CSF1, PTGFR, and APP.

The SARS-CoV-2 infection of lung epithelial cells activates caspase-8 to trigger the three major cell death pathways, including apoptosis, pyroptosis, and necroptosis. Cell death and inflammatory responses are intimately linked during SARS-CoV-2 infection ¹⁴⁰. Lastly, analysis of postmortem lung sections of lethal COVID-19 patients has revealed that inflammatory responses from lung epithelial cells may induce infiltration of inflammatory cells that trigger strong immune pathogenesis ¹³⁷.

Recent studies showed that SARS-CoV-2 rewires human monocytes in a high glucose culture medium. This induces viral replication and cytokine production, and might be the reason why people suffering from diabetes, obesity and other related metabolic diseases are more susceptible to developing severe COVID-19 ¹³⁹. For instance, people with type 2 diabetes show an increased glucose metabolism due to hyperglycemia, which may boost SARS-CoV-2 pathogenesis ¹³⁹. Codo *et al* proved that glycolytic flux is essential for SARS-CoV-2 impact ¹⁴¹. Through several assays, they inhibited glycolysis by blocking 2-deoxy-D-glucose (2-DG) and glycolytic enzymes 6-phospho-fructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3) and lactate dehydrogenase A (LDH-A), as a consequence, they observed that both viral replication and cytokine response stopped ¹⁴¹. The metabolic transcription factor HIF-1 α activity and related genes are strongly stimulated in SARS-CoV-2 infected blood monocytes isolated from severe COVID-19 patients ¹⁴¹. HIF-1 α is also a major glycolysis regulator, when inhibited, viral replication and cytokine expression were also blocked. Overall, these experiments showed that high glucose concentration and glycolysis are essential for SARS-CoV-2 replication, inflammatory response, and upregulation of ACE2 ¹⁴¹.

Angiogenesis occurs in response to the activation of acute inflammation or chronic systemic hypoxia pathways that increase the expression of proteins and factors (HIF-1 α , VEGF, NO) associated with its development ¹⁴². During the SARS-CoV-2 infection, local endothelial damage, known as endotheliitis, is associated with acute inflammation of the outermost endovascular layers, triggering a cascade of reactions that result in endothelial inflammation, platelet aggregation, and impaired laminar flow ^{138,143}. In the context of COVID-19 disease, the reported vasoconstriction and subsequent hypoxia, stimulate the formation of new blood vessels by promoting branching of pre-existing blood vessels (intussusception) and *de novo* angiogenesis that contributes to the already established systemic hypoxia ¹⁴⁴. This process together with the systemic hypoxia observed in severe COVID-19 patients cause a structural and

functional reorganization of the pulmonary tissue, which ultimate function is to allow an adequate gas exchange between the tissue and the cells ¹⁴².

Regarding drugs against COVID-19 disease, in this study we propose five small molecules (ruxolitinib, baricitinib, pacritinib, losmapimod, and eritoran) that after being thoroughly analyzed in COVID-19 clinical trials, these drugs can be considered for treating severe COVID-19 patients.

A systematic review and meta-analysis published by Walz *et al*/ concluded that Janus kinase-inhibitor treatment is significantly associated with positive clinical outcomes in terms of mortality, intensive care unit admission, and discharge ¹⁴⁵. Ruxolitinib is a tyrosine-protein kinase JAK1/2 inhibitor ¹⁴⁶ that is currently used for myelofibrosis and polycythemia vera, both hematologic malignancies. The use of ruxolitinib in these diseases is based on its ability of being a kinase inhibitor, which mediates the signaling of a number of cytokines and growth factors that are important in hematopoiesis and immune function. Based on this principle, it is reasonable then, from a clinical point of view, to use this drug to specifically manage cytokine storm in COVID-19 ¹⁴⁷. According to Yan *et al*, ruxolitinib normalized interferon signature genes and all complement gene transcripts induced by SARS-CoV-2 in lung epithelial cell lines. They proposed that combination therapy with JAK inhibitors and drugs that normalize NF- κ B-signaling could potentially have clinical application for severe COVID-19 ¹⁴⁶. Baricitinib is a tyrosine-protein kinase JAK1/2 inhibitor ¹⁴⁸ mainly used for rheumatoid arthritis, and among its pharmacological properties it has an antiviral effect on the entry of a virus ¹⁴⁹. At the moment, baricitinib is approved by the WHO, the Food and Drug Administration (FDA) of the United States, and the National Institutes of Health (NIH) for emergent use in severe pneumonia due to COVID-19 ¹⁵⁰. The use of baricitinib is indicated in COVID-19 critically ill patients with high oxygen needs despite the use of dexamethasone (the only approved corticosteroid), however it should not be used when IL-6 inhibitors such as tocilizumab have been started, given that its combined use has not yet been tested as well as its safety. The known efficacy of Baricitinib is from emerging data from an unpublished article where the 27.8% of participants receiving baricitinib vs 30.5% receiving placebo progressed (primary endpoint, odds ratio 0.85, 95% CI 0.67–1.08; $p = 0.18$), and the all-cause mortality was 8.1% for baricitinib and 13.1% for placebo, corresponding to a 38.2% reduction in mortality (hazard ratio [HR] 0.57, 95% CI 0.41–0.78; nominal $p = 0.002$) ¹⁵¹. Pacritinib is also a protein-kinase inhibitor mainly focused on JAK2 and FLT3 protein targets. This small molecule has been developed for the treatment of myelofibrosis ¹⁵². On the other hand, losmapimod is a MAP kinase p38 α inhibitor that has been investigated for the prevention of chronic obstructive pulmonary disease and cardiovascular disease ¹⁵³. The therapeutic hypothesis for the use of losmapimod in COVID-19 is that increased mortality is caused by p38 MAPK-mediated exaggerated acute inflammatory response resulting in SARS-CoV-2 infection. Lastly, eritoran is a Toll-like receptor 4 / MD-2 antagonist that downregulates the intracellular generation of pro-inflammatory cytokines IL-6 and TNF- α in human monocytes, and has been developed for the treatment of severe sepsis. Shirey *et al* examined how antagonizing TLR4 signaling has been effective experimentally in ameliorating acute lung injury and lethal infection in challenge models triggered by acute lung injury-inducing viruses ¹⁵⁴.

Considering the enormous pressure that health systems are facing due to the COVID-19 pandemic and the continuous need to present and implement comprehensive health strategies that can address the global situation; mainly after the emergence of different variants, it is imperative to recognize the urgent need to diminish the gaps between research and the implementation of public health measures. In fact, it may be unprecedented in the history of science to know how many research articles related to COVID-19 have been submitted and published. However, according to Park *et al*, the research community has emphasized on “the new norm of publishing: quantity over quality” and this is also related to the well known problems that clinical trials faced even before the pandemic ¹⁵⁵. This is of particular interest to our research given that we acknowledge that clinical trials are essential in evidence-based medicine, and consequently, in the decision making process of public health policies and strategies. Relevantly, the need to smartly invest not only in randomized clinical trials but also in large-scale clinical trials with master protocols and conducted by coordinated and collaborative structures, as also supported by Park *et al*¹⁵⁵. These clinical trials networks are essential to coordinate actions between clinical researchers and health practitioners, also promoting knowledge sharing, leadership, and cost-time reductions. In addition, it is critical to decentralize, improve and increase clinical trials in low and middle-income countries, as current evidence shows large inequalities and concentrations of funds and information in high-income countries ¹⁵⁶. This holds true especially for Latin America, one of the most affected regions in the world by the pandemic ¹⁵⁷.

The role of health research is fundamental in the response to COVID-19, considering the importance of data sharing and assuring efficiency, equity, and effectiveness in the diverse processes. Contradictorily, a large number of clinical trials might never be completed and others are done with doubtful methodologies ^{155,158}. Thus, analyzing potential drugs targets for COVID-19, especially the ones which can serve for severe cases, need an urgent and efficient development of well designed and managed clinical trials, which can provide potential interventions that help people to live longer, diminish long-term effects, manage pain and/or possible disabilities; not to mention the possible positive effects on the reduction of hospitalization costs, both at the individual level and in terms of possible savings for the national health system. As another study also mentioned, the potential and benefits of repositioning clinical trials are directed to use the already available information of safe and affordable generic drugs and propose “potential, prompt, cost- effective, and safe solutions for the public and global health problems, with a human-centered approach” ¹¹. This is also conveyed by the Pan American Health Organization (PAHO), which adds to the benefits, the idea of having already pharmaceutical formed supply chains ¹⁵⁹.

Finally, as other authors have contributed, the current global research situation must be guided towards a collaborative and synergetic approach instead of being conceived as a competitive and isolated process. The COVID-19 pandemic assures the need to eliminate structural barriers that increase health inequalities, and in this perspective, benefits, knowledge, and of course potential treatments must be available for all, in order to achieve universal health coverage and equity.

Declarations

Data Availability Statement

The datasets generated for this study are included in this published article (and its Supplementary Information files).

Author Contributions

AL-C conceived the subject and the conceptualization of the study. NCK gave conceptual advice and valuable scientific input. AL-C, PG-R, AA-S, AL-S, EA, AAP-M, KN-J, AM, and FA-E did data curation and supplementary data. AL-C did funding acquisition. All authors wrote and edited the manuscript. Lastly, all authors reviewed and approved the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

Figure 1

Significantly expressed genes across lung cell types. Box plots show the number of significantly expressed genes, their Z-scores, and p-values per each lung cell type. Neural cells were the cell type with the highest mean Z-score, followed by B cells, mast cells, fibroblast cells, alveolar type II cells, cycling natural killer / T cells, endothelial cells, macrophages, airway epithelial cells, alveolar type I cells, natural

killer cells, dendritic cells, smooth cells, Treg cells, plasma cells, monocytes, other epithelial cells, CD4+ T cells, and CD8+ T cells.

Figure 2

Transcriptomics data of 116,313 lung nuclei from lethal COVID-19 patients. UMAPs show the mean log normalized expression of significantly expressed genes per lung cell type. Dot plots show the ranking of genes with the highest percentage of cells expressing. UMAP: uniform manifold approximation and

projection for dimension reduction; NK: natural killer; OG: overexpressed genes; and UG: underexpressed genes.

Figure 3

Functional enrichment analysis. UMAPs show the most significant genes per lung cell type involved in gene ontology biological processes and signaling pathways. The most significant biological term was inflammatory response, followed by cytokine production, innate immune response, macrophage

activation, Toll-like receptor signaling pathway, interferon production, JAK-STAT signaling pathway, NF-κB signaling pathway, thymic stromal lymphopoietin, TNF signaling pathway, blood coagulation, oncostatin M signaling pathway, AGE-RAGE signaling pathway, IL-1 and megakaryocytes in obesity, and NLRP3 inflammasome complex. UMAP: uniform manifold approximation and projection for dimension reduction.

Figure 4

miRNome enrichment analysis. Circos plot show a GSEA to compute overlaps between the 18 most significant miRNAs (FDR q-value < 0.01) and the 19 significantly expressed genes in > 50% of lung cells.

The most significant miRNA was MIR6867_5P, followed by MIR2662, MIR32_3P, MIR548A0_5P_MIR548AX, MIR570_3P, MIR338_5P, MIR144_3P, MIR4711_3P, MIR628_5P, MIR548AJ_3P_MIR548X_3P, MIR1290, MIR4496, MIR12136, MIR9718, MIR875_3P, MIR4698, MIR3941, and MIR4789_3P. GSEA: gene set enrichment analysis.

Figure 5

Inflammatory protein-protein interactome network. iPPI network made up of 265 nodes and 2052 edges. Of them, 159 pulmonary inflammatory response proteins had a mean of degree centrality of 8 and 108 human-SARS-CoV-2 proteins had a mean of degree centrality of 7.2. The top ten inflammatory response proteins with the highest degree centrality were APP, NFKB1, STAT3, C3, ITGAM, FN1, PTAFR, JAK2, EGFR, and LYN. The top ten human-SARS-CoV-2 proteins with the highest degree centrality were GNB1, GNG5, RHOA, ITGB1, STOM, RAB14, PRKAR2B, RAB8A, PRKACA, and ANO6. Additionally, 111 pulmonary inflammatory response proteins had the highest confidence interactions (cutoff = 0.9) with human-SARS-CoV-2 proteins, being the top ten: C3, FN1, NFKB1, RPS19, CTSC, HSPD1, APP, ITGAM, SNAP23, and MAPK14.

Figure 6

Shortest paths to cancer hallmark phenotypes. A) Box plots encompassing inflammatory response proteins with the shortest mean of distance score per phenotype. Cell death was the phenotype with the shortest paths, followed by inflammation, glycolysis, and angiogenesis. B) Venn diagram of inflammatory response proteins with shortest paths to hallmarks of cancer related to COVID-19. C)

Ranking of the most essential proteins with shortest paths to cell death, inflammation, glycolysis, and angiogenesis.

Figure 7

Essential proteins with the shortest distance score to the inflammation phenotype. The essential proteins with positive regulation to inflammation were PTGS2, PRKCZ, NFKBIA, MAPK14, TNFRSF1B, TLR4, ATM,

MECOM, PIK3CG, EGFR, JAK2, LYN, CYLD, PRKCQ, STAT3, TGFB1, RBPJ, TNFAIP3, NOTCH1, IGF1, CD28, CCL5, PTAFR, FPR1, EDNRA, EDNRB, CYSLTR1, CNR2, HGF, EPHA2, FN1, CSF1, PTGFR, and APP.

Drug	Class / Type	Target	Structure	Pharmacological indication	Clinical trial
Baricitinib DB11817	Pyrrolopyrimidines / small molecule	JAK2		Tyrosine-protein kinase JAK2 inhibitor	NCT04640168, NCT04693026, NCT04421027, NCT04401579
Pacritinib DB11697	Phenol ethers / small molecule	JAK2		Tyrosine-protein kinase JAK2 inhibitor	NCT04404361
Ruxolitinib DB08877	Pyrrolopyrimidines / small molecule	JAK2		Tyrosine-protein kinase JAK2 inhibitor	NCT04362137
Losmapimod DB12270	Pyridines and derivatives / small molecule	MAPK14		MAP kinase p38 alpha inhibitor	NCT04511819
Eritoran DB04933	Organooxygen compounds / small molecule	TLR4		Toll-like receptor 4 / MD-2 antagonist	NCT02735707

Figure 8

Drugs involved in advanced-stage COVID-19 clinical trials. Drug name, class type, druggable target, structure, pharmacological indication, and clinical trial number related to small molecules involved in advanced-stage COVID-19 clinical trials.

Supplementary Files

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