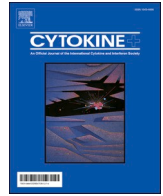




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Randomized trial drug controlled compendious transcriptome analysis supporting broad and phase specific therapeutic potential of multiple candidates in COVID-19

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ABSTRACT

Effective therapies for coronavirus disease 2019 (COVID-19) are urgently needed. Maladaptive hyperinflammation and excessive cytokine release underlie the disease severity, with antiinflammatory and cytokine inhibiting agents expected to exert therapeutic effects. A major present challenge is identification of appropriate phase of the illness for a given intervention to yield optimum outcomes. Considering its established disease biomarker and drug discovery potential, a compendious analysis of existing transcriptomic data is presented here toward addressing this gap. The analysis is based on COVID-19 data related to intensive care unit (ICU) and non-ICU admissions, discharged and deceased patients, ventilation and non-ventilation phases, and high oxygen supplementation. It integrates transcriptomic data related to the effects of, in various cellular treatment models, the COVID-19 randomized clinical trial (RCT) successful drug dexamethasone, and the failed drug, with a potential to harm, hydroxychloroquine/chloroquine. Similarly, effects of various COVID-19 candidate drugs/anticytokines as well as proinflammatory cytokines implicated in the illness are also examined. The underlying assumption was that compared to COVID-19, an effective drug/anticytokine and a disease aggravating agent would affect gene regulation in opposite and same direction, in that order. Remarkably, the assumption was supported with respect to both the RCT drugs. With this control validation, etanercept, followed by tofacitinib and adalimumab, showed transcriptomic effects predictive of benefits in both ventilation and non-ventilation ICU stages as well as in non-ICU phase. On the other hand, canakinumab showed potential for effectiveness in high oxygen supplementation phase. These findings may inform experimental and clinical studies toward drug repurposing in COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19) is an ongoing global public health emergency. Besides vaccines, which may take a long time to cover large populations, effective therapies are urgently required to treat COVID-19 morbidity and reduce mortality [29,10]. COVID-19 pathogenesis involves an initial phase of host immune response to viral infection and accompanying mild clinical symptoms, and, in a small proportion of patients with non-self-resolving illness, a later phase of inappropriate hyperinflammation and excessive cytokine release that may lead to acute respiratory conditions carrying considerable risk of mortality [7]. Cytokine inhibition to reduce hyperinflammation is thus recognized as a rational approach to severe COVID-19 therapy [7]. Evidence from randomized controlled trials (RCTs), considered gold standard, suggests that hydroxychloroquine/chloroquine, on which

much attention was initially focused, do not exert beneficial effects in the illness, and may possibly cause adverse effects [2,5]. In contrast, dexamethasone, an antiinflammatory and immunosuppressive drug, has been found effective in decreasing all-cause mortality and composite disease progression [22]. Regarding others, effectiveness of the IL6 receptor directed antibody tocilizumab has been controversial, with the drug possibly reducing the likelihood of progression to mechanical ventilation [1,19,24]. Also, a trial in hospitalized patients not receiving noninvasive or invasive ventilation has recently reported death risk or respiratory failure lowering effect of the of the JAK inhibitor tofacitinib [14]. On the other hand, the IL1 receptor antagonist anakinra has failed to show benefits in mild-to-moderate COVID-19 pneumonia [9]. Similarly, the anti-IL1B antibody canakinumab has not been found effective in increasing likelihood of survival in severe patients not receiving invasive mechanical ventilation [6]. In a trial with small sample size, the

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tumor necrosis factor alpha signaling inhibitor adalimumab, in combination with the antiviral drug remdesivir and dexamethasone, did not show therapeutic benefit in severe COVID-19 [12]. However, RCTs aside, an observational study has found canakinumab as effective in improving oxygen supplementation and lowering mortality in COVID-19 [20]. Other small molecule cytokine inhibitors and anticytokine biologics that are considered potentially beneficial in COVID-19 include tumor necrosis factor alpha inhibitors adalimumab, infliximab and etanercept, besides others [7,29]. Though supporting evidence is currently lacking, theoretical considerations suggest that non-steroidal anti-inflammatory drugs, which include ibuprofen and diclofenac, besides others, may also possibly benefit COVID-19 patients [11]. A main challenge in COVID-19 therapy at present is to identify which patients are likely to benefit the most from a given treatment, with timing of administration important in achieving optimum outcomes [7,13].

Integrative analysis of existing transcriptomic datasets is an accepted approach to gain mechanistic insights into disease pathophysiology and drug action, and to identify candidate biomarkers, drugs and drug targets [33]. As drug induced transcriptomic alterations show high conservation across cell types, a compendious analysis of drug treatment related gene expression data could prove valuable in therapeutic and adverse effect assessment [17,21]. For example, a drug can be predicted as effective in a given disease if gene expression perturbations associated with the drug and the disease show inverse correlation [18]. Toward addressing the existing gaps in developing effective COVID-19 therapies, presented here is a compendious analysis of human transcriptomic data related to different COVID-19 stages, various RCT tested and other repurposing candidate drugs/anticytokines, and multiple cytokines. The compendious approach is validated first, followed by an unbiased, algorithmic analysis of individual COVID-19 phases.

2. Methods

NCBI's PubMed and Gene Expression Omnibus (GEO) were extensively searched for identifying relevant studies. Search terms included COVID-19, rheumatoid arthritis, severe acute respiratory syndrome coronavirus 2, hydroxychloroquine, chloroquine, dexamethasone, tocilizumab, canakinumab, anakinra, adalimumab, infliximab, etanercept, tofacitinib, diclofenac, acetaminophen, interleukin 1, interleukin 1 alpha, interleukin 1 beta, interleukin 6, tumor necrosis factor alpha, interferon alpha, interferon beta, and interferon gamma, besides alternative names and symbols, and other key words as appropriate. The identified datasets were manually curated and annotated. Wherever provided in full, original author-identified differentially expressed genes (DEGs) were used. If not, adjusted p-value significant DEGs irrespective of \log_2 fold change values were retrieved from GEO datasets by using GEO2R [4] for microarray studies, and GREIN [23] for RNA-seq studies. The dataset associated official gene symbols were directly used as provided or retrieved using DAVID gene ID conversion tool [16], with duplicates removed in excel. Heatmapper [3] was used for generating heatmaps, with DEGs manually arranged for separating exclusively up- and down-regulated genes in samples in the leading heatmap. For examining whether up- and down-regulated genes are equal in number as expected in a random situation, chi-square goodness of fit nominal p-values (<https://www.socscistatistics.com/>) were used. Enrichment of gene ontology biological process terms was found using ToppFun of ToppGene Suite [8], with q-value FDR B&Y considered for statistical significance.

3. Results

First, in order to ascertain the applicability of a compendious transcriptomic approach in the present context, most frequently occurring DEGs among various tissue types in severe COVID-19 as a whole were matched with DEGs associated with drug/anticytokine and cytokine treatments in diverse conditions (Fig. 1A). Notably, the disease related

genes in general showed opposite and same direction of regulation in drugs/anticytokines and cytokines, in that order (Fig. 1B). This clear separation of drugs/anticytokines and cytokines was on expected lines, given the accepted potential of these therapeutic agents in and pathophysiology of severe COVID-19. Conspicuously, hydroxychloroquine/chloroquine mainly showed same regulation, which is consistent with inefficacy and adverse effects of the drugs observed in the RCT meta-analysis mentioned above. It was notable that dexamethasone and tocilizumab, with established and recent evidence for benefits in severe COVID-19 RCTs, both showed a statistically significant or a trend for higher representation of opposite regulation. Also, it was notable that the remaining RCT drug anakinra, with no evidence for effectiveness in mild-to-moderate COVID-19 and a present lack of any reported RCT in severe illness, showed opposite regulation. Given these findings, the analysis supported the therapeutic potential of the non-RCT anticytokines canakinumab, adalimumab, etanercept, and tofacitinib, not infliximab though, in severe COVID-19. Further, disease associated DEGs showed enrichment of biological process terms related to defense response, myeloid cell activation, and innate immune response (Fig. 1C). As enrichment of these terms was on expected lines, the results supported the significance of DEGs used in the analysis. For further proof-of-concept, the analysis was repeated by replacing severe COVID-19 with rheumatoid arthritis, to which the present set of drugs/anticytokines and cytokines in general are therapeutically and pathophysiologically relevant (Fig. 1D). Again, as expected, the two groups were overall separated on the basis of directionality of rheumatoid arthritis associated differential gene expression (Fig. 1E). Besides, the gene ontology terms showing enrichment in rheumatoid arthritis DEGs (Fig. 1F) were non-overlapping with that in COVID-19 DEGs (Fig. 1C), confirming general applicability of the compendious approach.

With the proof-of-concept obtained using heterogeneous samples encompassing multiple studies, analysis henceforth was focused on repeating the steps with various COVID-19 stages, for isolating stage-specific effects. To avoid confounding factors in comparative analysis, samples of patients representing different disease stages in a single study or samples of a single patient representing different stages were used. First, DEGs in various blood immune cells of a floor and discharged patients were compared with that in an intensive care unit (ICU) and deceased patient (Fig. 2A). As expected from known pathophysiology, the DEGs associated with the former patient (Fig. 2B), both the patients (Fig. 2C), and the latter patient (Fig. 2D) showed an increasing trend for separation between drugs/anticytokines and cytokines. Hydroxychloroquine/chloroquine showed a trend for disease mimicking effect across categories, with the effect being highly significant in the last one. Among other drugs/anticytokines, the floor and discharged patient associated DEGs showed significant inverse match for tocilizumab, anakinra, etanercept, infliximab, and tofacitinib, with the second and the third biologics exhibiting higher significance (Fig. 2B). The ICU and deceased patient associated DEGs, on the other hand, exhibited highly significant inverse match for tocilizumab, canakinumab, anakinra, adalimumab, etanercept, and diclofenac, and significant inverse match for dexamethasone and acetaminophen (Fig. 2D). Further, gene ontology enrichment analysis of DEGs associated with the floor and discharged patient (Fig. 2E), both the patients (Fig. 2F), and ICU and deceased patient (Fig. 2G) showed changing pattern of biological process terms. Conspicuously, whereas IFNG signaling was uniquely enriched in the first two categories, the last category showed unique enrichment for defense response to virus and related terms. Overall, the results supported stage specific effects of the drugs/anticytokines.

Next, DEGs in various blood immune cells of an ICU patient in non-ventilation stage and in invasive mechanical ventilation stage, following which the patient was discharged, were compared (Fig. 3A). The DEGs associated with pre-ventilation stage (Fig. 3B), both stages (Fig. 3C), and ventilation stage (Fig. 3D) showed a much pronounced separation between drugs/anticytokines and cytokines in the second category compared to the first, and in the first category compared to the third.

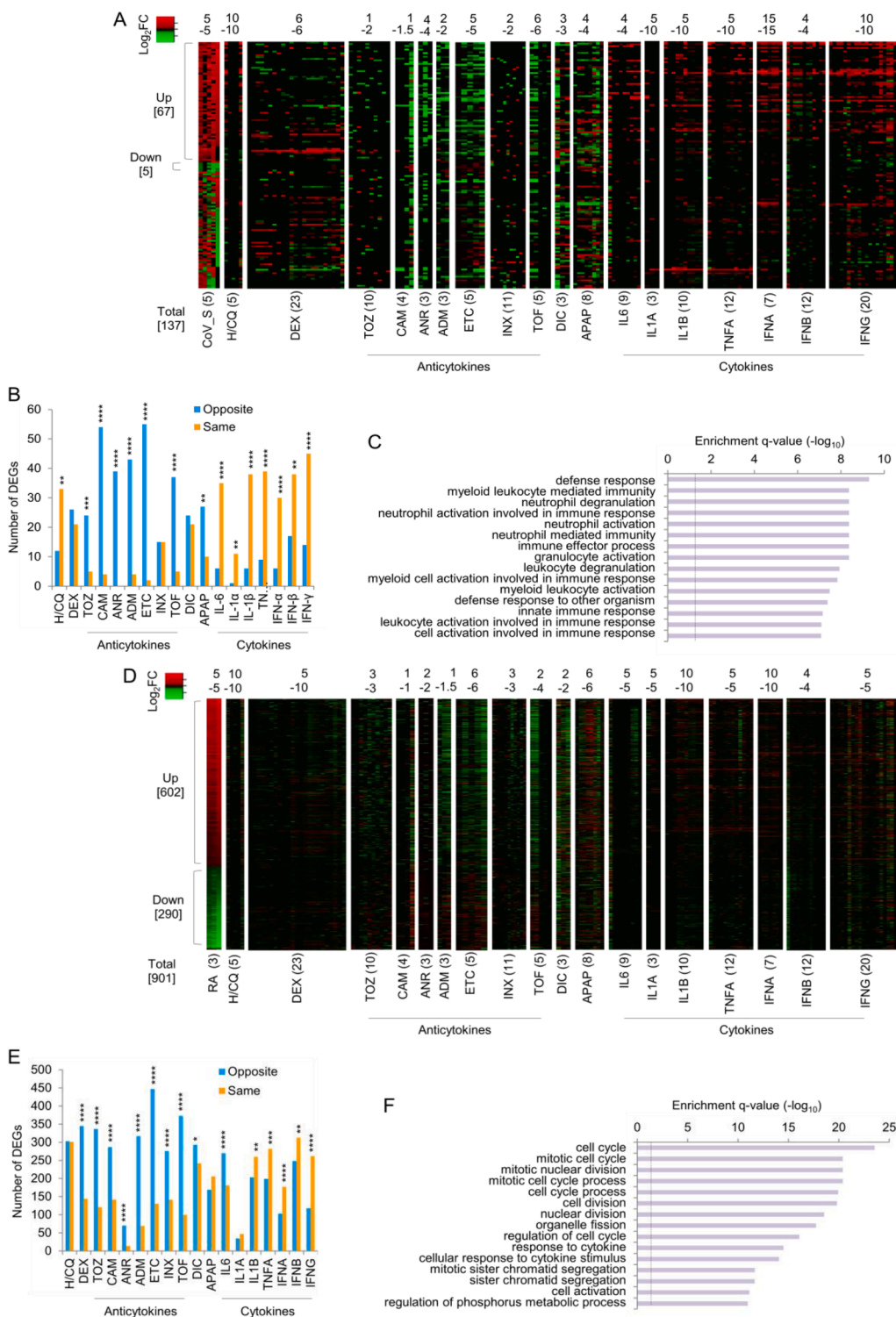


Fig. 1. Proof-of-concept analysis. (A) Heatmap of most frequent DEGs in severe COVID-19 (CoV-S) samples, representing 39, 3, 2, 8, and 2 patients from left to right, is juxtaposed with heatmaps of corresponding DEGs in indicated drug and cytokine treated samples. COVID-19 heatmap shows, from top to bottom, genes that are exclusively up-, exclusively down-, and either up- or down-regulated in disease samples. Numbers in square brackets and parentheses represent gene and sample numbers, in that order. ADM, adalimumab; ANR, anakinra; APAP, acetaminophen; CAM, canakinumab; DEX, dexamethasone; DIC, diclofenac; ETC, etanercept; FC, fold change; IFNA, interferon alpha; IFNB, interferon beta; IFNG, interferon gamma; IL1A, interleukin 1 alpha; IL1B, interleukin 1 beta; IL6, interleukin 6; INX, infliximab; TOF, tofacitinib; TOZ, tocilizumab; TNFA, tumor necrosis factor alpha. Sample details along with references and log₂FC values are given in **Supplementary Table 1**. (B) Bar chart showing numbers of DEGs that are exclusively up- or down-regulated in a given drug or cytokine set and the direction of regulation is opposite or same as that of the corresponding exclusively up- or down-regulated CoV-S genes. Nominal p-values of chi-square goodness of fit tests are indicated; * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 . Rest as mentioned in A. (C) Bar chart showing biological process enrichment in exclusively up- and down-regulated CoV-S genes combined. Dashed line indicates false discovery rate q-value cut-off for significance. Top 15 gene ontology terms are shown. Full set is presented in **Supplementary Table 2**. (D) Heatmap of most frequent DEGs in rheumatoid arthritis (RA) samples is juxtaposed with heatmaps of corresponding DEGs in indicated drug and cytokine treated samples. RA heatmap shows, from top to bottom, genes that are exclusively up-, exclusively down-, and either up- or down-regulated in all disease samples. Sample details along with references and log₂FC values are given in **Supplementary Table 3**. Rest as mentioned in A and B. (E) Bar chart showing numbers of DEGs that are exclusively up- or down-regulated in a given drug or cytokine set and the direction of regulation is opposite or same as that of the corresponding exclusively up- or down-regulated RA genes. Rest as mentioned in A and B. (F) Bar chart showing biological process enrichment in exclusively up- and down-regulated RA genes combined. Top 15 gene ontology terms are shown. Full set is presented in **Supplementary Table 4**.

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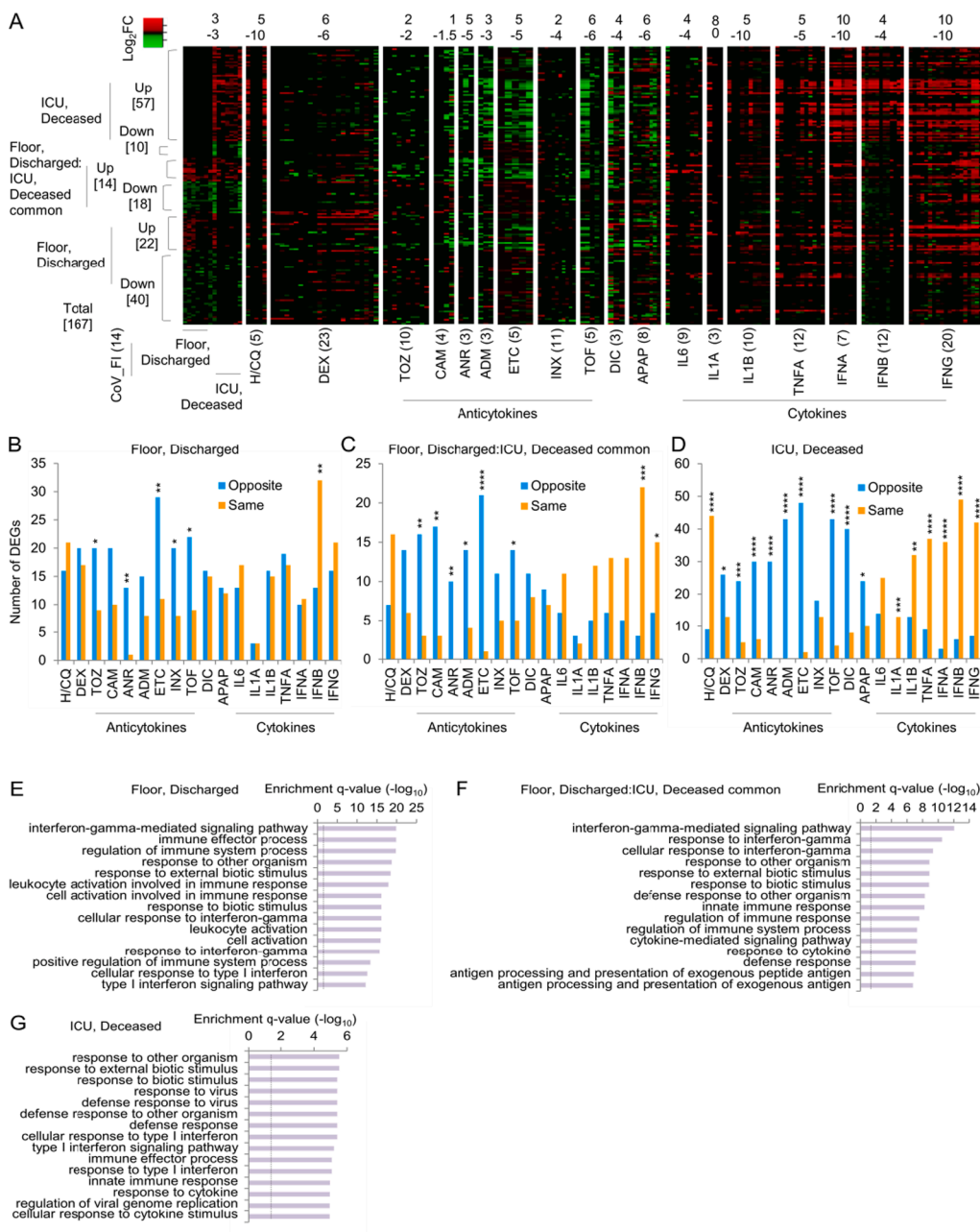


Fig. 2. Comparison of floor and discharged with ICU and deceased patients. (A) Composite heatmap representing 100 most frequent DEGs in various blood immune cell types in a floor and discharged COVID-19 patient and in an ICU and deceased COVID-19 patient (CoV-FI) is juxtaposed with heatmaps of corresponding DEGs in indicated drug and cytokine treated samples. Genes that are exclusively up- or down-regulated in CoV-FI are arranged in the heatmap in order to display, from bottom to top, discharged specific set, discharged and deceased common set, and deceased specific set. Sample details along with references and \log_2FC values are provided in **Supplementary Table 5**. Rest as mentioned in **Fig. 1**. (B-D) Bar chart showing numbers of DEGs that are exclusively up- or down-regulated in a given drug or cytokine set and the direction of regulation is opposite or same as that of the corresponding exclusively up- or down-regulated disease genes specific to discharged (B), common to discharged and deceased (C), and specific to deceased (D) sets. Nominal p-values of chi-square goodness of fit tests are indicated; * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 . Rest as mentioned in **Fig. 1**. (E-G) Bar chart showing biological process enrichment in exclusively up- and down-regulated disease genes specific to discharged (E), common to discharged and deceased (F), and specific to deceased (G) sets. Dashed lines indicate false discovery rate q-value cutoff for significance. Top 15 gene ontology terms are shown for each gene set. Full results are presented in **Supplementary Table 6**.

Like previous comparison between floor and discharged patient, and ICU and deceased patient (Fig. 2B-D), hydroxychloroquine/chloroquine showed statistically significant or a trend for disease mimicking effect in all the categories (Fig. 3B-D). Among other drugs/anticytokines, etanercept, anakinra, and adalimumab and tofacitinib showed decreasingly significant inverse match with pre-ventilation DEGs (Fig. 3B). On the other hand, dexamethasone, etanercept and diclofenac, and canakinumab, anakinra and acetaminophen showed decreasingly significant inverse match with ventilation DEGs (Fig. 3D). The matches were in general more significant in pre-ventilation than ventilation stage. Also, compared to ventilation, pre-ventilation appeared more similar in drug/anticytokine and cytokine profiles to the previous ICU and deceased patient (Fig. 2D). This similarity was also observed at the level of gene ontology analysis (Fig. 3E-G), wherein defense response to virus and related terms that were uniquely enriched in ICU and deceased patient (Fig. 2G) showed unique enrichment in pre-ventilation stage (Fig. 3E). These findings together suggested that ventilation improved the illness,

which is consistent with the fact that the patient was discharged thereafter.

Subsequently, the analysis was repeated with DEGs in serial whole blood samples drawn on day 4–9, 11, 12 and 18 of symptom onset in a severe COVID-19 patient, with day 4–8 being the period of high and day 9–18 that of low or no supplemental oxygen, following which the patient was discharged (Fig. 4A). Remarkably, high supplemental oxygen associated DEGs (Fig. 4B), unlike high supplemental oxygen neutral DEGs (Fig. 4C), clearly separated drugs/anticytokines from cytokines based on direction of gene regulation. Hydroxychloroquine/chloroquine showed highly significant disease mimicking regulation for high oxygen associated DEGs, and significant inverse regulation for high oxygen neutral DEGs. Among other drugs/anticytokines, canakinumab, anakinra, adalimumab, etanercept, and tofacitinib showed highly significant, and infliximab showed significant inverse match in the former category (Fig. 4B). However, in the latter category, it was notable that dexamethasone, adalimumab, and tofacitinib showed significant disease

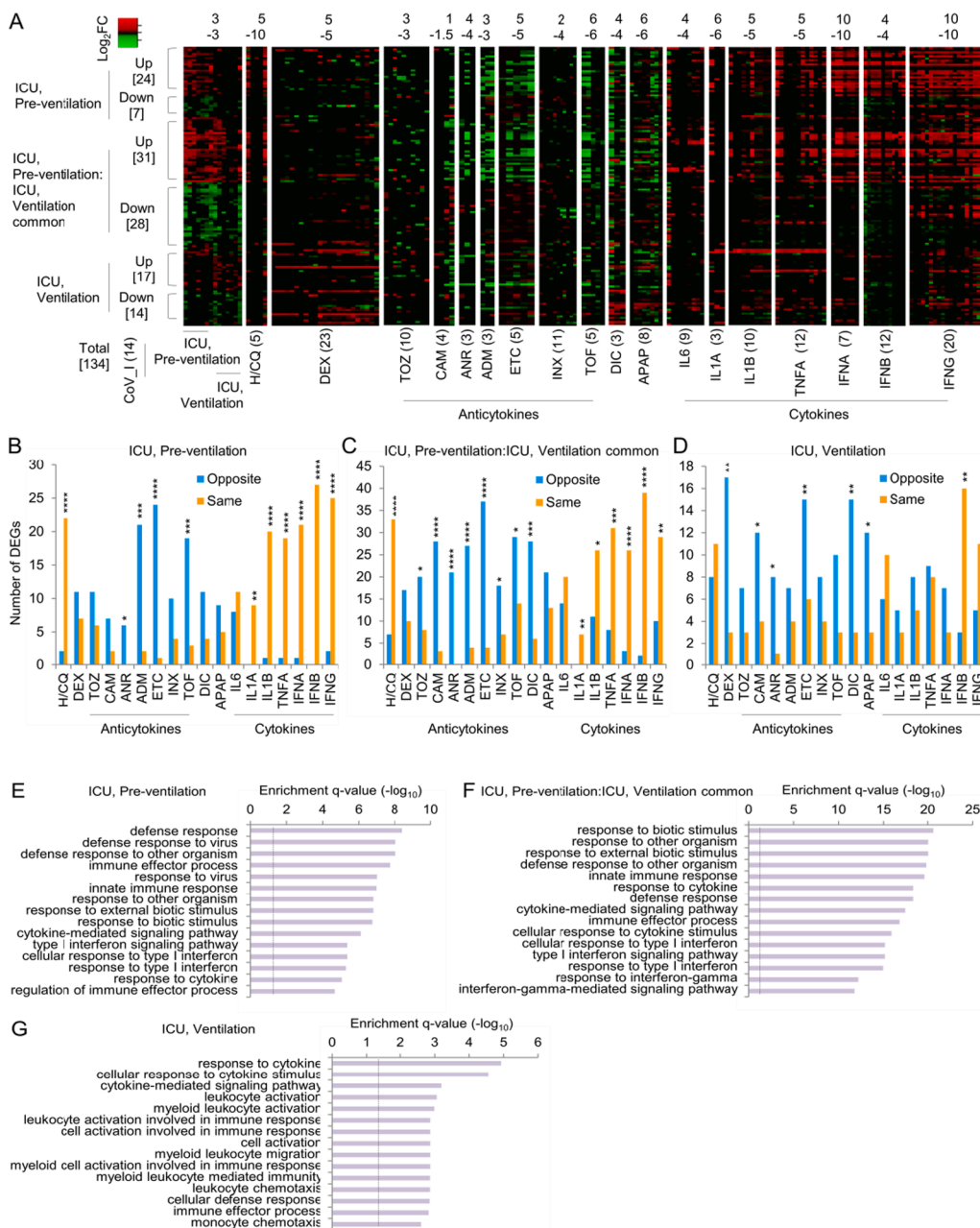


Fig. 3. Comparison between pre-ventilation and ventilation ICU stages. (A) Composite heatmap representing 100 most frequent DEGs in pre-ventilation and ventilation stages in various blood immune cell types in a single COVID-19 ICU (CoV_I) patient is juxtaposed with heatmaps of corresponding DEGs in indicated drug and cytokine treated samples. Genes that are exclusively up- or down-regulated in CoV-I are arranged in the heatmap in order to display, from top to bottom, pre-ventilation specific set, pre-ventilation and ventilation common set, and ventilation specific set. Sample details along with references and log₂C values are provided in **Supplementary Table 7**. Rest as mentioned in **Fig. 1**. (B-D) Bar chart showing numbers of DEGs that are exclusively up- or down-regulated in a given drug or cytokine set and the direction of regulation is opposite or same as that of the corresponding exclusively up- or down-regulated disease genes specific to pre-ventilation (B), common to pre-ventilation and ventilation (C), and specific to ventilation (D) sets. Nominal p-values of chi-square goodness of fit tests are indicated; * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. Rest as mentioned in **Fig. 1**. (E-G) Bar chart showing biological process enrichment in exclusively up- and down-regulated disease genes specific to pre-ventilation (E), common to pre-ventilation and ventilation (F), and specific to ventilation (G) sets. Dashed lines indicate false discovery rate q-value cutoff for significance. Top 15 gene ontology terms are shown for each gene set. Full results are presented in **Supplementary Table 8**.

mimicking activity, whereas canakinumab exhibited highly significant inverse match (**Fig. 4C**). Gene ontology enrichment also showed clear differences between the two categories, with the former uniquely enriched in terms related to defense response, cytokine production, innate immune response, and inflammatory response (**Fig. 4D**), and the latter to adaptive immune response, lymphocyte activation, T cell activation, and leukocyte proliferation, besides others (**Fig. 4E**). Overall, the results supported the stage specific therapeutic and adverse effects of drugs/anticytokes.

Finally, for combined comparison across all COVID-19 stages, namely, floor and discharged (**Fig. 2B**), ICU and deceased (**Fig. 2D**), ICU and pre-ventilation (**Fig. 3B**), ICU and ventilation (**Fig. 3D**), and high supplemental oxygen (**Fig. 4B**), the number of drug/anticytokine DEGs showing direct or inverse match in the respective stages was converted in terms of percent (**Fig. 5**). In this secondary analysis, hydroxy-chloroquine/chloroquine showed disease mimicking effect in ICU and deceased, ICU and pre-ventilation, high supplemental oxygen, ICU and

ventilation, and floor and discharged, in decreasing order. The drugs/anticytokes showed various extent of inverse match with disease stages, wherein etanercept ranked highest in floor and discharged, ICU and deceased, and ICU and pre-ventilation, whereas dexamethasone and canakinumab ranked highest in ICU and ventilation, and high supplemental oxygen, in that order (**Fig. 5**). Depending on stage, the second ranking drugs/anticytokes included tofacitinib, adalimumab, diclofenac, and etanercept, and third ranking included dexamethasone, tocilizumab, canakinumab, infliximab, diclofenac, tofacitinib, etanercept, and adalimumab. Regarding disease mimicking effect of cytokines, interferon beta ranked first in 4 of the 5 stages, and interferon gamma in one (**Fig. 5**). Among second ranking cytokines, interferon gamma figured in 4 of the 5 stages, and IL1B in one. Third ranking cytokines included IL6, tumor necrosis factor alpha, and interferon alpha.

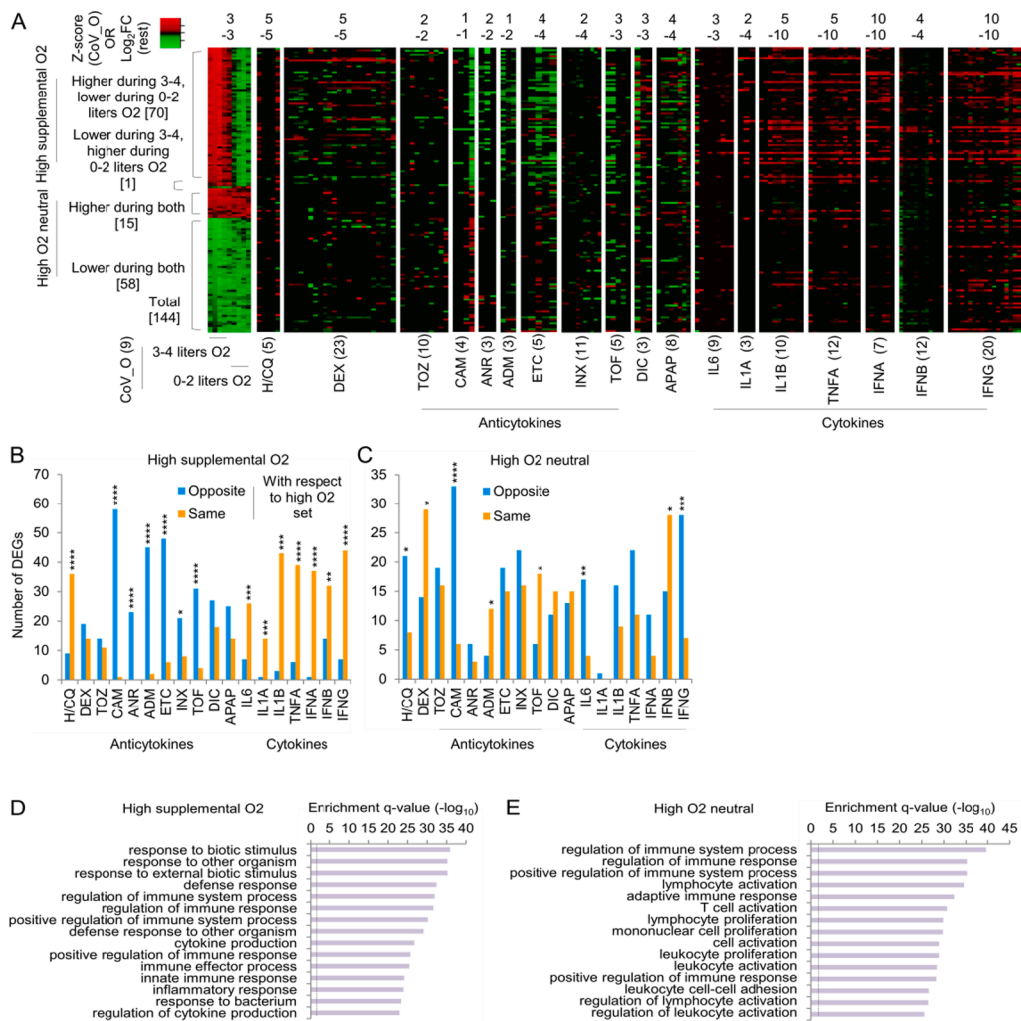


Fig. 4. Analysis of supplemental oxygen requirement. (A) Composite heatmap showing genes with exclusively higher or lower z-score transformed and normalized counts in blood samples of a single patient during days of 3–4 L of supplemental oxygen irrespective of expression levels during days of 0–2 L of supplemental oxygen (CoV_O) is juxtaposed with heatmaps of corresponding DEGs in indicated drug and cytokine treated samples. CoV_O genes are arranged in the heatmap in order to display, from top to bottom, higher counts in samples representing higher supplemental oxygen and lower in samples representing lower supplemental oxygen, vice versa, higher counts in both sample types, and lower counts in both sample types. Sample details along with references and Z-score or Log₂FC values are provided in **Supplementary Table 9**. Rest as mentioned in **Fig. 1**. (B, C) Bar chart showing numbers of DEGs that are exclusively up- or down-regulated in a given drug or cytokine set and the direction of regulation is opposite or same as that of the corresponding genes with higher or lower counts in higher oxygen supplementation samples (B), and that of the corresponding genes with exclusively higher or lower counts in both higher and lower supplementation samples alike (C). Nominal p-values of chi-square goodness of fit tests are indicated; * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 . Rest as mentioned in **Fig. 1**. (D, E) Bar chart showing biological process enrichment in genes with opposite (D) and same (E) directionality of regulation between higher and lower supplemental oxygen. Dashed lines indicate false discovery rate q-value cutoff for significance. Top 15 gene ontology terms are shown for each gene set. Full results are presented in **Supplementary Table 10**.

4. Discussion

The present analysis first demonstrated the capability of a compendious transcriptomic approach in broadly revealing therapeutic and pathophysiological mechanisms in immune diseases including COVID-19, and then focused on identifying windows in the course of the illness that would be appropriate for intervention with different drugs/anticytokines. The phases mainly used to identify these windows were represented by non-ICU, high oxygen supplementation, pre-ventilation, ventilation, and deceased patients. The drugs/anticytokines for which therapeutic effectiveness and adverse effect RCT profiles were known, namely, hydroxychloroquine/chloroquine, dexamethasone, tocilizumab, and anakinra, served as controls. First, consistent with its failure in showing benefits and its association with adverse events in RCTs, hydroxychloroquine/chloroquine showed disease mimicking transcriptomic profile in general. This supports the argument that undertaking further studies toward finding any phase specific use of hydroxychloroquine/chloroquine in COVID-19 may not be justified [28]. With respect to dexamethasone, it showed a disease-counteracting transcriptomic profile in relation to invasive mechanical ventilation and deceased categories, and a trend for the same in relation to the other

disease stages. It was notable that in contrast to the above, the drug showed disease mimicking effect with respect to high oxygen neutral category. These results are remarkably consistent with the finding of the largest dexamethasone RCT, that the glucocorticoid lowered mortality in patients receiving mechanical ventilation and oxygen support, and possibly harmed patients who were not receiving respiratory support [26]. Further, tocilizumab showed high disease-counteracting effect in relation to deceased, followed by non-ICU, and a trend for the same in relation to other phases. These results are also largely consistent with COVID-19 RCT evidence for tocilizumab, showing its effectiveness in reducing the likelihood of mechanical ventilation and death but not in improving survival [30], effectiveness in improving survival and other outcomes in patients with hypoxia and systemic inflammation [27], and ineffectiveness in moderate [32] illness. As regards anakinra, it showed highest disease-counteracting effect in deceased and high oxygen supplementation, followed by non-ICU, and then by pre-ventilation and ventilation phase. Considering that the existing RCT evidence for anakinra is limited to mild-to-moderate COVID-19 [9], it is notable at this time that non-randomized cohort studies support effectiveness of the biologic in severe disease in terms of reducing mortality and mechanical ventilation [25]. With the results reported here overall in line with RCT

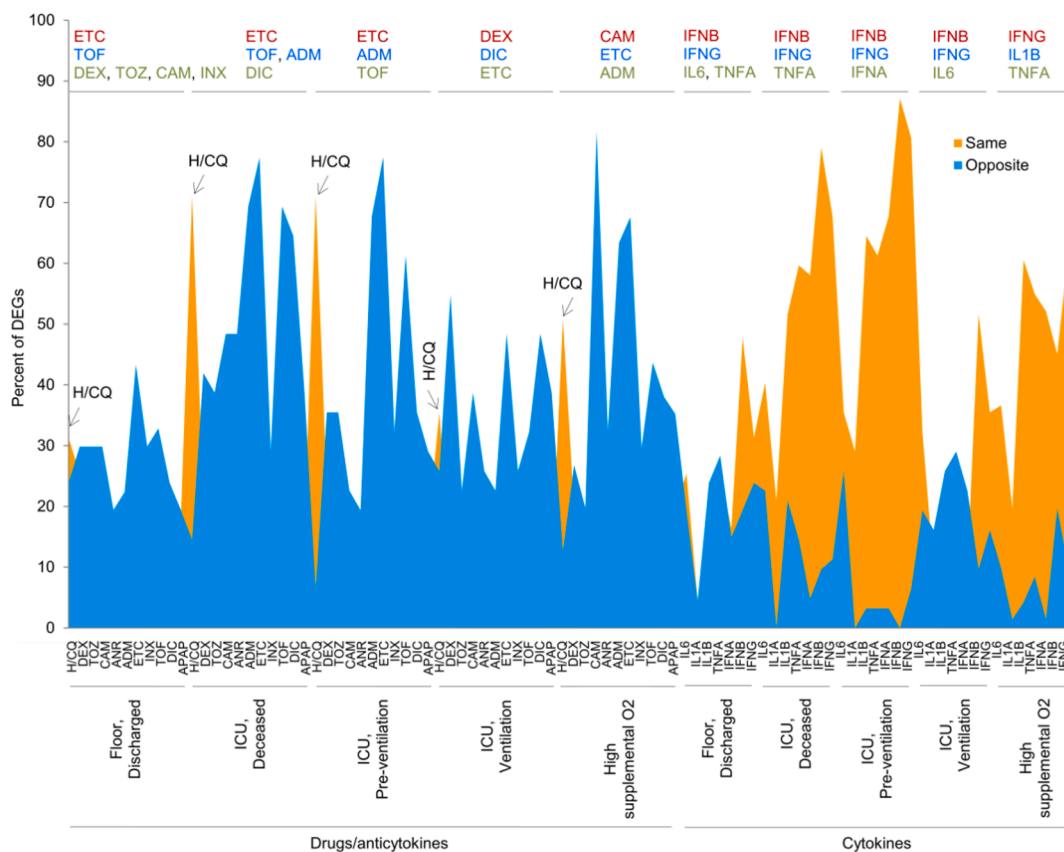


Fig. 5. Integration of therapeutic and pathophysiological profiles across major disease stages. Area chart showing percent of drug/anticytokine and cytokine treatment associated DEGs with regulatory direction opposite to or same as that in COVID-19 samples in CoV-FI (Fig. 2), CoV-I (Fig. 3), and CoV_O (Fig. 4), in that order. Top ranking drugs/anticytokines with highest percent of opposite regulatory direction, and top ranking cytokines with highest percent of same regulatory direction are highlighted in red, blue, and green, indicating rank 1, 2, and 3, respectively. Same directionality of H/CQ DEGs in all five disease stages is also indicated. Rest as mentioned in Fig. 1.

findings, the present analysis suggests that etanercept, followed by tofacitinib and adalimumab, is a promising broad spectrum candidate for severe COVID-19 treatment. It also supports dexamethasone like therapeutic activity of diclofenac in ventilation phase. Effectiveness of canakinumab in high oxygen supplementation stage is also suggested. Regarding variations in therapeutic potential observed here for the tumor necrosis factor alpha inhibitors etanercept, adalimumab, and infliximab, the observations may not be surprising given previously noted differences in clinical efficacy in various inflammatory and immune diseases, and in molecular mechanism of action of these inhibitors [15]. Cumulatively, based on the ranking of drugs/anticytokines found in the final combined analysis presented here, it is strongly recommended that etanercept, for which no COVID-19 RCT has been reported yet, is trialled in ICU patients at pre-ventilation phase as well as at critical phase of the disease with top priority, followed by trial in non-ICU patients. Further, adalimumab, for which negative evidence from a small RCT alone is available at this time [12], should be trialled in pre-ventilation as well as critical phase. Tofacitinib, that has shown benefits in RCT in non-ventilation phase [14], may prove beneficial also in critical phase of the disease. Canakinumab, with no positive evidence in severe patients not receiving invasive mechanical ventilation [6], may exert beneficial effects in less severe, hypoxic patients.

The present observations that individual cytokine inhibitors within a single class differ with respect to their therapeutic potential in COVID-19, and cytokine inhibitors in general show differential therapeutic potential among COVID-19 phases relate to currently ongoing discussion on seemingly contradictory or inconsistent clinical findings. For example, whereas the JAK inhibitor tofacitinib has been found to reduce the risk of death or respiratory failure among COVID-19 hospitalized

patients in a RCT [14], a registry study has shown that people who were on rheumatoid arthritis treatment with JAK inhibitor tofacitinib, baricitinib, or upadacitinib at the time of COVID-19 clinical onset had worse COVID-19 severity [31]. These contradictory findings have been explained in terms of both disease phase and drug class. First, in light of the observation that glucocorticoids, which are known to benefit when given to moderate-to-severe COVID-19 patients, are associated with worse outcomes among people on baseline treatment at the time of infection, the timing of JAK inhibitor use relative to COVID-19 phase may explain the contradictory findings [31]. Like glucocorticoids, baseline use of JAK inhibitors at the time of COVID-19 infection may enhance and dampen viral reproduction and normal immune response, in that order, whereas use of these inhibitors at clinical deterioration may ameliorate pathological hyperinflammatory response underlying disease severity [31]. Alternatively, given that tofacitinib, baricitinib, and upadacitinib act on different Janus kinases, it has been considered possible that the contradictory findings between RCT, which was based on tofacitinib alone, and registry study, which was based on all the three drugs combined, relate to individual drug, not class, specific effects [31]. In another example, a review of COVID-19 RCTs of the IL6 inhibitor tocilizumab suggests that while the treatment is likely to benefit severe patients requiring high-flow nasal cannula therapy, non-invasive ventilation, or mechanical ventilation, it probably does not benefit non-severe patients [1]. These differences in RCT findings are consistent with the growing appreciation for the need to determine the appropriate timing and disease phase for a given treatment candidate in COVID-19, in order to maximize potential benefits and minimize potential harms [7,13]. The possibility of therapeutic differences between tocilizumab and another IL6 inhibitor sarilumab in COVID-19 has also been raised,

suggesting relevance of considering individual agent and drug class effects in interpreting treatment outcomes [1,24].

A limitation of the present transcriptomic approach could have been that COVID-19 treatments affected interpretation of results. However, samples used for proof-of-concept analysis and for comparison between deceased and discharged patients were not associated with COVID-19 treatments, whereas the samples used for comparison between non-ventilation stage and invasive mechanical ventilation stage were commonly associated with azithromycin treatment, and samples used for comparison between high and low or no oxygen supplementation commonly associated with lopinavir-ritonavir treatment. These details suggest that treatment effects might not have significantly impacted the present findings.

5. Conclusion

In conclusion, integrative analysis of available transcriptomic data is overall consistent with therapeutic success and failure of agents tested in RCTs in COVID-19, and, above and over that, supports effectiveness of various other drugs/anticytokines that are yet to be examined in RCTs but are considered promising candidates for drug repurposing in the illness. The analysis suggests that these drugs/anticytokines may exert therapeutic benefits across multiple COVID-19 stages or may act in stage specific manner. These *in silico* findings would however require experimental validation. Nevertheless, the results presented may offer a molecular basis for prioritizing and designing clinical studies on drug repurposing in COVID-19. Most notably, the tumor necrosis factor alpha inhibitor etanercept, for which COVID-19 RCT evidence is presently lacking, has emerged as a highly promising candidate with therapeutic potential in multiple phases of the disease.

CRedit authorship contribution statement

Abhay Sharma: Conceptualization, Methodology, Data curation, Visualization, Writing – original draft, 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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cyto.2021.155719>.

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