Critically ill COVID-19 status associated trait genetics reveals CDK6 inhibitors as potential treatment

Joern Klinger
biotx.ai GmbH

Charles Ravarani
biotx.ai GmbH

Colin Bannard
University of Liverpool  https://orcid.org/0000-0001-5579-5830

Margaretha Lamparter
biotx.ai GmbH

Alexander Schwinges
biotx.ai GmbH

Justin Cope
biotx.ai GmbH

Hannes Baukmann
biotx.ai GmbH

Marco Schmidt (ms@biotx.ai)
biotx.ai GmbH

Article

Keywords: SARS-CoV-2, COVID-9, CDK6 inhibitors, monoclonal antibodies, vaccines

DOI: https://doi.org/10.21203/rs.3.rs-568366/v1

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Critically ill COVID-19 status associated trait genetics reveals CDK6 inhibitors as potential treatment

Joern E. Klinger¹, Charles N. J. Ravarani¹, Colin Bannard², Margaretha R. J. Lamparter¹, Alexander R. E. C. Schwinges¹, Justin L. Cope¹, Hannes A. Baukmann¹, Marco F. Schmidt¹*

¹biotx.ai GmbH, Am Mühlenberg 11, 14476 Potsdam, Germany.
²Institute of Population Health, University of Liverpool, Eleanor Rathbone Building, Bedford Street South, Liverpool L69 7ZA, United Kingdom.

*e-Mail: ms@biotx.ai
Abstract

Despite the recent development of vaccines and monoclonal antibodies preventing SARS-CoV-2 infection, treating critically ill COVID-19 patients still remains a top goal. In principle, drug repurposing – the use of an already existing drug for a new indication – could provide a shortcut to a treatment. However, drug repurposing is often very speculative due to lack of clinical evidence. We report here on a methodology to find and test drug target candidates for drug repurposing. Using UK Biobank data, we matched critically ill COVID-19 cases with healthy controls and screened for significant differences in 33 blood cell types, 30 blood biochemistries, and body mass index. Significant differences in traits that have been associated with critically ill COVID-19 status in prior literature, such as alanine aminotransferase, body mass index, C-reactive protein, and neutrophil cell count, were further investigated. In-depth statistical analysis of COVID-19 associated traits and their genetics using regression modeling and propensity score stratification identified cyclin-dependent kinase 6 (CDK6) as a more promising drug target for the selective treatment of critically ill COVID-19 patients than the previously reported interleukin 6. Four existing CDK6 inhibitors -- abemaciclib, ribociclib, trilaciclib, and palbociclib -- have been approved for the treatment of breast cancer. Clinical evidence for CDK6 inhibitors in treating critically ill COVID-19 patients has been reported. Further clinical investigations are ongoing.
Introduction

The phenotype of critically ill coronavirus disease 2019 (COVID-19) status completely differs from mild or moderate disease, even among hospitalized cases, by an uncontrolled overreaction of the host’s immune system\textsuperscript{1–3} – a so-called virus-induced immunopathology\textsuperscript{4} – resulting in acute respiratory distress syndrome (ARDS). Although the molecular mechanism leading to critical illness due to COVID-19 is still unclear, there is evidence that susceptibility and overreaction of the immune system to respiratory infections are both strongly heritable.\textsuperscript{5,6} A series of genome-wide association (GWA) studies have been conducted to investigate disease pathogenesis in order to find mechanistic targets for therapeutic development or drug repurposing, as treating the disease remains a top goal despite the recent development of vaccines.\textsuperscript{7–10}

The results of 46 GWA studies comprising 46,562 COVID-19 patients from 19 countries have been combined in three meta-analyses by the COVID-19 Host Genetics Initiative.\textsuperscript{10} Overall, 15 independent genome-wide significant loci associations were reported for COVID-19 infection in general, of which six were found to be associated with critical illness due to COVID-19: 3p21.31 close to CXCR6, which plays a role in chemokine signaling, and LZTFL1, which has been implicated in lung cancer; 12q24.13 in a gene cluster that encodes antiviral restriction enzyme activators; 17q21.31, containing the KANSL1 gene, which has been previously reported for reduced lung function; 19p13.3 within the gene that encodes dipeptidyl peptidase 9 (DPP9); 19p13.2 encoding tyrosine kinase 2 (TYK2); and 21q22.11 encoding the interferon receptor gene IFNAR2. The functions of the genes associated with these six loci are either related to host antiviral defense mechanisms or are mediators of inflammatory organ damage. These results are a
good starting point for a better understanding of host genetics in viral infections. Nevertheless, none of these genes encodes for an established drug target. Consequently, these studies provide no evidence that supports drug repurposing.

We present here an approach for drug repurposing based not on disease genetics but on the genetics of disease associated traits. First, critically ill COVID-19 cases are matched with healthy controls, and the two cohorts are investigated for significant differences in previously reported traits. Traits that differ in cases and controls and that have been associated with critically ill COVID-19 status are further investigated to find and test established drug target genes for drug repurposing.

Results

Screening for critically ill COVID-19 status associated traits

We adopt from prior literature a definition of the phenotype of critically ill COVID-19 cases as patients who were hospitalized due to confirmed SARS-CoV-2 infection and who required respiratory support and/or died due to infection. Using UK Biobank data, we identified 8,153 cases and selected age- and sex-matched healthy controls. In order to explore how the critically ill cohort differed in general from healthy controls, we screened 64 candidate predictive traits (33 blood cell types, 30 blood biochemistries, and body mass index) that had been measured years before the individuals were affected by COVID-19 (Fig. 1). We observed Bonferroni-corrected statistically significant differences ($p < \alpha/n = 0.05/64$) in 36 traits confirmed by independent two-sample t-test and Mann-Whitney U-test. In these measures, cases showed significant differences in various traits that have been described as phenotypes for critically ill COVID-19 status. For instance, relative to healthy controls, cases had higher body mass index (BMI), higher reticulocyte cell
count, higher inflammatory markers such as alanine aminotransferase, C-reactive protein, cystatin C, neutrophil cell count, and higher glycated hemoglobin (HbA1c), but lower HDL and LDL cholesterol as well as lower vitamin D levels.

As an additional comparison group, we selected infectious disease cases from the UK Biobank in order to assess the extent to which these traits are general indicators of predisposition to severe infections. In total 23,348 participants were selected who had been diagnosed with a respiratory infection, acute respiratory distress syndrome, influenza or pneumonia and were hospitalized or had died as a result. Again, cases were matched with healthy controls in order to screen for differences in measures for 33 blood cell types, 30 blood biochemistries, and body mass index. As in the COVID-19 investigation, we here found that cases had higher body mass index (BMI), higher reticulocyte cell count, higher inflammatory markers such as alanine aminotransferase, C-reactive protein, cystatin C, neutrophil cell count, and higher glycated hemoglobin (HbA1c), but lower HDL and LDL cholesterol as well as lower vitamin D levels than healthy controls (Supplementary Information Fig. 1 and Fig. 2).

Traits that have been previously associated with critically ill COVID-19 status and infectious diseases such as alanine aminotransferase, BMI, C-reactive protein, and neutrophil cell count were further investigated.15,16

**GWAS results**

We next focused on trait genetics of reported critically ill COVID-19 status associated phenotypes such as BMI, neutrophil cell count, C-reactive protein, and alanine aminotransferase. We ran GWA analyses for these four traits and compared our results with previously reported statistics available from the NHGRI-EBI GWAS
Catalog\textsuperscript{17}. The identified genes were further investigated for already approved drug molecules. We found \textit{IL-6}, encoding for interleukin 6, reported for C-reactive protein\textsuperscript{18} and \textit{CDK6}, encoding for cyclin-dependent kinase 6 (CDK6), reported for BMI\textsuperscript{19} and neutrophil cell count\textsuperscript{20}. Unfortunately, we could not confirm the \textit{IL-6} signal (rs2097677) in our GWA analysis for C-reactive protein (Supplementary Information Fig. 3). We continued our research with the reported drug target for interleukin 6 and interleukin 6 receptor (IL-6R). We furthermore confirmed single nucleotide polymorphisms (SNPs) rs42044 and rs445 in the \textit{CDK6} gene as significant in our GWA analyses for BMI and neutrophil cell count (Supplementary Information Fig. 4). The allele distributions of rs2097677, rs42044, and rs445 in cases and controls can be found in the Supplementary Information Tab. 1 and 2.

\textbf{Regression modeling}

Logistic regression models were built to examine the relationship between a series of candidate predictive traits (age, alanine aminotransferase, BMI, C-reactive protein, and neutrophil cell count) and critically ill COVID-19 status. All of these traits apart from age were significant predictors of critically ill COVID-19 status (as illustrated for neutrophil cell count in Fig. 2). As expected, no relationship between age and disease status could be found, as the members of the control group were matched with the reported cases by age and sex. A drop one analysis revealed that all traits explain unique variance in critically ill COVID-19 status.

\textbf{Propensity score analysis}

Propensity score analysis is a technique for estimating the effect of a treatment on an outcome independent of any observed factors that covary with that treatment and
would otherwise make it impossible to determine causation. We specifically employed propensity score stratification using the propensity function of Imai and van Dyk\textsuperscript{21} in order to determine whether a causal relationship exists between both C-reactive protein and neutrophil cell count and the prevalence of critically ill COVID-19 status independently from other covariates. A weighted average across propensity deciles indicated a significant effect of C-reactive protein (Log Odds Ratio = 0.04; SE = 0.004; \( p = 1.3288\times 10^{-26} \)) and neutrophil cell count (Log Odds Ratio = 0.3; SE = 0.018; \( p = 2.7354\times 10^{-61} \)) on disease status. Tab. 2 shows the neutrophil cell count in the critically ill COVID-19 patients and the control group across the propensity deciles.

**Mendelian randomization**

Mendelian randomization (MR) is a robust and accessible tool to examine the causal relationship between an exposure variable and an outcome from GWAS summary statistics.\textsuperscript{22} We employed two-sample summary data Mendelian randomization to further validate causal effects of neutrophil cell count genes on the outcome of critically ill COVID-19 status. We used independent GWAS summary data for neutrophil cell count (exposure) published by Vuckovic \textit{et al.}\textsuperscript{23} and summary data for critically ill COVID-19 status (outcome) published by the COVID-19 Host Genetics Initiative\textsuperscript{10}. As shown in the Supplementary Information Tab. 3, instrumental variable weight (IVW) was significant with a \( p \) value of 0.01199 when we used a lenient clumping parameter of \( r = 0.2 \) and 1,581 SNPs whereas we observed no significant IVW when we used strict clumping parameters of \( r = 0.01 \) and 567 SNPs (Supplementary Information Tab. 3).
Discussion

We have described a method for identifying drug targets for the treatment of disease based not directly on the genetics of the disease itself, but rather on the genetics of disease-associated traits. Using data from the UK Biobank, we found evidence of a causal relationship between a series of traits and critical illness due to COVID-19. Using genome-wide associations, we identified genetic markers associated with these traits. Based on these two steps, we were able to identify CDK6 as a potential drug target for critically ill COVID-19 status. The four CDK6 inhibitors abemaciclib, palbociclib, ribociclib, and trilaciclib have been already approved for breast cancer and can potentially be repurposed to treat critically ill COVID-19 patients.

Our procedure worked as follows. We matched reported critically ill COVID-19 cases from the UK Biobank with healthy controls and checked for significant differences between cases and controls in 64 candidate predictive traits. Cases showed significant differences in alanine aminotransferase, BMI, C-reactive protein, and neutrophil cell count. These measures were taken from the individuals concerned years before infection. We hypothesize that the genetic drivers of a disease associated trait indicate a good drug target to treat the disease. In the literature we found the genes $IL-6$, encoding interleukin 6, as a driver of C-reactive protein, and $CDK6$, encoding cyclin-dependent kinase 6, as a driver of BMI and neutrophil cell count. Three drugs have been already approved that inhibit interleukin 6 either directly (siltuximab for Castleman’s Disease) or indirectly via the interleukin 6 receptor (tocilizumab and sarilumab for rheumatoid arthritis). The four drugs abemaciclib, palbociclib, ribociclib, and trilaciclib are already approved for treatment of breast cancer target CDK4/6.
Regression models that tested all traits together showed that C-reactive protein and neutrophil cell count explained independent variance in critically ill COVID-19 status in the presence of other traits such as BMI. Propensity score stratification found evidence of a causal relationship between both C-reactive protein and neutrophil cell count and the prevalence of critically ill COVID-19 status.

It is important to note that Mendelian randomization results did not confirm a causal role for either C-reactive protein or neutrophil cell count. However, MR is typically used where there is a direct relationship between gene and outcome. In our case we are looking for a relationship that is mediated by a viral infection, adding a great deal more noise. This, compounded by the fact that MR needs a larger sample size than we have available, might account for our not finding evidence of a relationship in this analysis.

The role of C-reactive protein in COVID-19 can be explained by the previously-reported disease mechanism. The phenotype of critically ill COVID-19 status completely differs from mild or moderate disease, even among hospitalized cases, by an uncontrolled overreaction of the host’s immune system. The most prominent difference between critically ill and moderate COVID-19 cases is the response to immunosuppressive therapy. In patients without respiratory failure, there is a trend indicating that treatment with corticosteroid dexamethasone is harmful, whereas among patients with critical respiratory failure, it has substantial benefit. Therefore, immunomodulatory therapies, such as interleukin 6 receptor (IL-6R) antagonists tocilizumab and sarilumab have been successfully tested to block the immune system’s overreaction in the form of a cytokine storm in critically ill COVID-19 patients.
The role of neutrophil count in the disease mechanism can also be explained. Neutrophils are white blood cells and an important component of our host defense against invading pathogens. Critically ill COVID-19 status is characterized by infiltration of the lungs with macrophages and neutrophils that cause diffuse lung alveolar damage, the histological equivalent to ARDS.\textsuperscript{27–29} Neutrophils develop a sophisticated network of DNA called neutrophil extracellular traps (NETs) through NETosis, a liberation of web-like structures of nucleic acids wrapped with histones that detain viral particles.\textsuperscript{30} However, ineffective clearance and regulation of NETs result in pathological effects such as thromboinflammation as described above.\textsuperscript{31} On the one hand, NETs are essential and efficient for trapping the virus. And on the other, they cause damage to the organism by triggering highly intense immunological and inflammatory processes. CDK4/6 have been previously described as regulators of NETosis. CDK4/6 inhibitors abemaciclib and palbociclib block NET formation in a dose-responsive manner but do not inhibit the oxidative burst, phagocytosis, or degranulation, indicating that CDK4/6 inhibition specifically affects NET production, rather than generally modulating inflammatory pathways as IL-6 inhibitors do.\textsuperscript{32} There is good reason to think that IL-6 and CDK4/6 might both be good drug targets for the treatment of COVID-19. However, given that neutrophils have a direct role in the thromboinflammatory process in critically ill COVID-19 patients, we believe that blocking neutrophils represents a more selective strategy than suppressing the immune system as a whole. There are two phases of COVID-19 infection (Fig. 3). In the beginning of infection, the virus enters the cell and starts viral replication. Here, vaccines and monoclonal antibodies block the viral entry. Nonetheless, after entry into the cell via the ACE2 receptor, currently, no therapeutic option for intervention exists. After viral infection, first immune reactions are observed such as a decrease
of lymphocyte cell count (lymphopenia). This phase is called the viral response phase. In the beginning of the next phase, the so-called host response phase, an overreaction of the host immune system occurs. The detailed mechanism leading to the overreaction of some patients’ host immune systems is still unknown. From our results here we assume that already manifested inflammations indicated by high C-reactive protein levels and high neutrophil cell count trigger the immune system’s overreaction resulting in thrombotic inflammation in the lungs and, from there, respiratory failure. In summary, the host response decides whether an infectious disease like COVID-19 has a mild course or leads to respiratory failure.

The cytokine IL-6 plays a central role in host response. On one hand, IL-6 binds to liver cells inducing the release of C-reactive protein that binds to phosphocholine of dead cells and recruits phagocytes. On the other hand, IL-6 stimulates the production of neutrophils and, thus, indirectly induces NETosis. Therefore, it is reasonable to inhibit IL-6 in a therapeutic intervention. However, immunomodulators can only be administered to tackle the overreaction of the immune system. Immunomodulators given in the early infection phase are harmful for patients. In contrast to IL-6 inhibitors, CDK4/6 inhibitors selectively block the NET formation and have no impact on other important host immune reactions such as phagocytosis. Consequently, they can be given earlier in the infection than IL-6 inhibitors, thus filling the therapeutic gap between vaccines and monoclonal antibodies in early infection and immunomodulators in the late stage. Our statistical analyses identified an independent effect of neutrophil cell count on critically ill COVID-19 status, providing evidence that therapeutic intervention at this later stage is still effective. We therefore hypothesize that CDK4/6 inhibitors are superior to IL-6 inhibitors in the treatment of critically ill COVID-19 patients. This is supported by the reported cases
of breast cancer patients and their disease course during CDK4/6 therapy, such as the report of Grinshpun et al. that a breast cancer patient on CDK4/6 inhibitor therapy had a unique disease course, halting the full presentation of the disease. Once the drug was withdrawn, the full classic spectrum of illness appeared, including a bothering desaturation necessitating a prolonged hospital stay for close monitoring of the need for invasive ventilations.

To conclude – we propose CDK6 as a new and plausible drug target and the repurposing of already-approved breast cancer drugs abemaciclib, ribociclib, trilaciclib, and palbociclib as a possible treatment against critically ill COVID-19 status. CDK4/6 inhibitors have the advantage of targeting the thromboinflammation earlier and more selectively than reported IL-6 inhibitors. Additionally, CDK4/6 inhibitors are chemical compounds and are therefore easier to store and administer than monoclonal antibodies. Clinical evidence for CDK6 inhibitors in treating critically ill COVID-19 patients has been already reported. Further clinical investigations are ongoing. We have also presented a novel methodology to find and test drug target candidates for drug repurposing in population data. Our approach is not limited to drug repurposing, but can also be used to validate new drug targets. This highlights the importance of biobanks for global health in a pandemic.

Methods

Recruitment of cases and controls

The COVID-19 phenotype was defined based on the rich information made available by the UK Biobank project, which has been collecting COVID-19 outcomes for their large cohort of patients. The COVID-19 outcomes up until 28th October 2020 were collected, and cases were defined as reported previously. Briefly, severe cases were
defined as patients who died or were hospitalized (cause of death or diagnosis contains an ICD10 code for COVID-19 U071) or were on a ventilator (operation code contains an E85*). Cases were not filtered based on COVID-19 test outcomes. The remaining individuals from the UK Biobank were defined as potential controls. Subsequently, patients of European ancestry were selected, and cases and controls were matched for similar age and sex distributions, resulting in a matched number of cases and controls (total number of subjects 16,307 with 8,153 cases and 8,154 controls). The dataset was used to perform a number of analyses described below.

The infectious disease phenotype was created based on UK Biobank data for respiratory infections, acute respiratory distress syndrome, influenza, and pneumonia with hospitalization or death as a result. We aggregated hospital in-patient and death register data from over 337,000 individuals in UK Biobank for ICD codes corresponding to J00-J06, J09-J18, J80, and J20-J22 (UK Biobank Fields 41202, 41204, 40001, 40002, 41201, and 41270 for diseases and 40001 and 40002 for deaths). The remaining individuals from the UK Biobank were defined as potential controls. European ancestry was carried over and selected based on UK Biobank phenotype as well as covariates information (age and sex) for the resulting dataset. Covariate distributions were matched to result in the same number of cases and controls. Variants reported by Pairo-Castineira et al.⁸ and Ellinghaus et al.⁷ as well as variants reported by the ClinVar database³⁴ for the genes reported by the papers were included in the dataset.

**GWAS**

The UK Biobank genotypes for the cases and controls were extracted to create a dataset that was then submitted to a series of quality control steps with an aim to
remove biases in the downstream analysis as described in Marees et al.\textsuperscript{35} First we filtered SNPs and individuals based on their missingness in the dataset. This excludes SNPs that have a high proportion of subjects where genotyping information is unavailable or of poor quality. Similarly, individuals where a large proportion of SNPs could not be measured were excluded. This was achieved in two steps, where first a lenient threshold of 0.2 (i.e. > 20\%) was applied to remove the clear outliers, followed by a more stringent threshold of 0.02 (i.e. > 2\%). SNP filtering was performed before individual filtering. Next, all variants not on autosomal chromosomes were removed. Next, variants that deviate from Hardy-Weinberg equilibrium were removed in a two-step process whereby we first applied a lenient threshold of 1e-6, followed by a more stringent threshold of 1e-10. This is a common indicator of genotyping errors. Thereafter, individuals were filtered out based on their heterozygosity rates which can indicate sample contamination. Individuals deviating by more than 3 standard deviations from the mean of the rate from all samples were filtered out. To assess the heterozygosity rate per sample, those variants that were in linkage disequilibrium with each other were extracted by scanning the genome at a window size of 50 variants, a step size of 5, and a pairwise correlation threshold of 0.2. Next, related individuals were removed. To achieve this, their identity by descent coefficients (IBD) were calculated and only one individual per related cluster was kept. Then, the small proportion of missing genotypes were imputed and additional variants reported by Pairo-Castineira \textit{et al.}\textsuperscript{8} and Ellinghaus \textit{et al.}\textsuperscript{7} as well as variants reported by ClinVar database\textsuperscript{34} were included in the dataset from the UK Biobank imputed variants. This yielded a dataset with a total number of 335,332 quality controlled variants. Finally, the population structure of the samples was analyzed in two stages to identify internal stratifications, which was used to filter out any
individuals not predicted close enough to a European reference cohort. A genome-wide association analysis was performed with the R-package SAIGE\textsuperscript{36} to score individual variants for their association with critically ill COVID-19 status.

**Regression modeling**

Logistic regression models were fitted using the \texttt{glm} function in R (www.R-project.org). A drop one model comparison procedure was performed in order to determine whether each of a set of traits accounts for unique variance in critically ill COVID-19 disease status.

**Propensity score analysis**

Using the method of Imai and Van Dyk\textsuperscript{21}, we regressed neutrophil count on BMI, age, and gender and then used the resulting model predictively in order to generate propensity values for our population. We then stratified the population into propensity deciles (the members of which are approximately matched in terms of their propensity), estimated the effects of z-transformed neutrophil count on disease status within each band using logistic regression, and calculated a weighted average.

**Mendelian randomization**

We used independent GWAS summary data for neutrophil cell count (exposure) published by Vuckovic \textit{et al.}\textsuperscript{23} (GCST90002398 downloaded January 15th 2021) and summary data for critically ill COVID-19 status (outcome) published by the COVID-19 Host Genetics Initiative (https://www.covid19hg.org/results - COVID19hg
GWAS meta-analyses round 5 release date January 18th 2021). Two-sample MR analyses were done as previously described.\textsuperscript{10}

**Acknowledgment**

The research has been conducted using the UK Biobank Resource under Application no. 36226. We thank Radi Hilaneh for making Fig. 3. The research work was supported by the *Investitionsbank des Landes Brandenburg* (ILB), the European Regional Development Fund (ERDF), and the European Social Fund+ (ESF+). Access to the UK Biobank was funded by the EIT Health Digital Sandbox program to access European biobank data (grant number 2019-DS1001-3754). We also thank the program ‘digital solutions made in Brandenburg’ (digisolBB) for its continued support.

**Competing interests**

J.E.K., C.N.J.R., M.R.J.L., H.A.B., J.L.C., and M.F.S are employees of biotx.ai GmbH. A.R.E.S was an employee of biotx.ai GmbH.

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Figures & Tables

Figure 1

Fig. 1. Bonferroni corrected statistically significant differences in 36 traits confirmed by Mann-Whitney U-test. In these measures, taken years prior to infection, cases showed significant differences in the characteristics in various traits that have been later described as phenotypes associated with critical illness due to COVID-19. For instance, cases had higher body mass index (BMI), higher reticulocyte cell count, higher inflammatory markers such as alanine aminotransferase, C-reactive protein, cystatin C, neutrophil cell count, and higher glycated hemoglobin (HbA1c), but lower HDL and LDL cholesterol as well as lower vitamin D levels than healthy controls.
Fig. 2. Regression model uncovered that neutrophil cell count measured years prior to infection correlates with the prevalence of critical illness due to COVID-19.
**Fig. 3.** COVID-19 infection stages. In the beginning of the infection the virus enters the cell and starts viral replication. Here, vaccines and monoclonal antibodies block the viral entry. After entry into the cell, no therapeutic option for intervention currently exists. First, in the viral response phase, immune reactions such as a decrease of lymphocyte cell count (lymphopenia) are observed. Then, in the host response phase, an overreaction of the host immune system occurs. While the details of the mechanism behind the overreaction of some patients’ immune systems is still unknown, a central role is attributable to the cytokine IL-6. On one hand, IL-6 binds to liver cells, inducing the release of C-reactive protein, which binds to phosphocholine of dead cells and recruits phagocytes. On the other hand, IL-6 stimulates the production of neutrophils and, thus, indirectly induces NETosis. Therefore, it is reasonable to inhibit IL-6 in a therapeutic intervention. However, while
immunomodulators could be administered to tackle the overreaction of the immune system, they are harmful for patients in the early infection phase. In contrast to IL-6 inhibitors, CDK4/6 inhibitors selectively block the NET formation and have no impact on other important immune reactions such as phagocytosis. Consequently, they can be given earlier in the infection than IL-6 inhibitors, filling the therapeutic gap between vaccines and monoclonal antibodies in early infection and immunomodulators in the later stage.
Table 1

Critically ill COVID-19 status was regressed on age, alanine aminotransferase, BMI, C-reactive protein, and neutrophil cell count. All traits other than age were found to explain unique variance in disease status. This table reports log likelihood ratios, standard errors and likelihood ratio tests from the drop one procedure for each.

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<th>$p$ value</th>
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Table 2

Tab. 2. Propensity score matching was employed in order to determine whether a causal relationship exists between neutrophil cell count and the prevalence of critically ill COVID-19 status. A weighted average across propensity deciles indicates a significant effect of neutrophil cell count (Log Odds Ratio = 0.3; SE = 0.018; \( p = 2.7354e-61 \)) on disease status. The table shows the neutrophil cell count in the critically ill COVID-19 and control groups across the propensity deciles.

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<tr>
<td>neutrophil cell count in (10^9) cells/liter</td>
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**Supplementary information**

**SI Fig. 1.** Distribution of neutrophil cell count in critically ill COVID-19 cases (orange) and healthy controls (blue).
SI Fig. 2. As an additional comparison group we selected infectious disease cases from the UK Biobank. In total 23,348 participants had been diagnosed with a respiratory infection, acute respiratory distress syndrome, influenza, or pneumonia and were hospitalized or died as a result. Again, the cases were matched with healthy controls in order to screen 64 candidate predictive traits for statistically significant differences in these two groups. As in the COVID-19 investigation, we found that cases had higher body mass index (BMI), higher reticulocyte cell count, higher inflammatory markers such as alanine aminotransferase, C-reactive protein, cystatin C, neutrophil cell count, and higher glycated hemoglobin (HbA1c), but lower HDL and LDL cholesterol as well as lower vitamin D levels than healthy controls.
SI Fig. 3. Manhattan plot of C-reactive protein indicating that we did not reproduce the reported *IL-6* signal (rs2097677) on chromosome 7.
SI Fig. 4. Manhattan plot of neutrophil cell count showing that we reproduce the reported CDK6 signal (rs445) on chromosome 7.
**SI Tab. 1.** Distribution of allele carriers rs2097677 (*IL-6*), rs445 (*CDK6*), and rs42044 (*CDK6*) in cases and controls.

<table>
<thead>
<tr>
<th>Allele Carrier</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2097677 AA</td>
<td>492</td>
<td>504</td>
</tr>
<tr>
<td>rs2097677 AG</td>
<td>2944</td>
<td>2981</td>
</tr>
<tr>
<td>rs2097677 GG</td>
<td>4418</td>
<td>4445</td>
</tr>
<tr>
<td>rs445 CC</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td>rs445 CT</td>
<td>1365</td>
<td>1419</td>
</tr>
<tr>
<td>rs445 TT</td>
<td>6417</td>
<td>6446</td>
</tr>
<tr>
<td>rs42044 GG</td>
<td>581</td>
<td>632</td>
</tr>
<tr>
<td>rs42044 GT</td>
<td>3140</td>
<td>3102</td>
</tr>
<tr>
<td>rs42044 TT</td>
<td>4133</td>
<td>4191</td>
</tr>
</tbody>
</table>
**SI Tab. 2.** Distribution of allele combination carriers rs445 (CDK6) and rs42044 (CDK6) in cases and controls.

<table>
<thead>
<tr>
<th></th>
<th>rs42044 GG</th>
<th>rs42044 GT</th>
<th>rs42044 TT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs445 CC</strong></td>
<td>352 cases / 372 ctrls</td>
<td>2348 cases / 2295 ctrls</td>
<td>3709 case / 3768 ctrls</td>
</tr>
<tr>
<td><strong>rs445 TC</strong></td>
<td>199 cases / 230 ctrls</td>
<td>754 cases / 778 ctrls</td>
<td>411 cases / 408 ctrls</td>
</tr>
<tr>
<td><strong>rs445 TT</strong></td>
<td>30 cases / 30 ctrls</td>
<td>38 cases / 29 ctrls</td>
<td>13 cases / 15 ctrls</td>
</tr>
</tbody>
</table>
The two sample MR analyses here showed that for neutrophil cell count as exposure and critically ill COVID-19 status as outcome no significant effect was detected while using strict clumping parameters. This approach is limited by the quality and sample size of GWA studies of critically ill COVID-19 status.

<table>
<thead>
<tr>
<th>Clumping parameters</th>
<th>SNPs used</th>
<th>beta</th>
<th>SE</th>
<th>IVW p-value</th>
<th>Pleiotropy test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposure:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neutrophil cell count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outcome:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>critically ill COVID-19 status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenient (r = 0.2)</td>
<td>1,581</td>
<td>-0.11139</td>
<td>0.04433</td>
<td>0.01199</td>
<td>negative</td>
</tr>
<tr>
<td>Strict (r = 0.01)</td>
<td>567</td>
<td>0.01135</td>
<td>0.06987</td>
<td>0.87095</td>
<td>negative</td>
</tr>
</tbody>
</table>