

Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients

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Abstract

Baricitinib, is an oral Janus kinase (JAK)1/JAK2 inhibitor approved for the treatment of rheumatoid arthritis (RA) that was independently hypothesized, using artificial intelligence (AI)-algorithms, to be useful for the treatment of COVID-19 infection via a proposed anti-cytokine effects and as an inhibitor of host cell viral propagation^{1,2}. We validated the AI-predicted biochemical inhibitory effects of baricitinib on human numb-associated kinase (hNAK) members measuring nanomolar affinities for AAK1, BIKE, and GAK. Inhibition of NAKs led to reduced viral infectivity with baricitinib using human primary liver spheroids, which express hAAK1 and hGAK. We evaluated the in vitro pharmacology of baricitinib across relevant leukocyte subpopulations coupled to its in vivo pharmacokinetics and showed it inhibited signaling of cytokines implicated in COVID-19 infection. In a case series of patients with bilateral COVID-19 pneumonia, baricitinib treatment was associated with clinical and radiologic recovery, a rapid decline in SARS-CoV-2 viral load, inflammatory markers, and IL-6 levels. This represents an important example of an AI-predicted treatment showing scientific and clinical promise during a global health crisis. Collectively, these data support further evaluation of the AI-derived hypothesis on anti-cytokine and anti-viral activity and supports its assessment in randomized trials in hospitalized COVID-19 patients.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently the biggest public health challenge to the biomedical community. Despite multiple public health measures, there remains an urgent need for pharmacologic therapies to treat infected patients, minimize mortality, and optimally, decrease viral shedding and subsequent transmission. Artificial intelligence (AI) allows for rapid drug development¹ including repurposing existing drugs. Algorithms were used to search for approved drugs capable of inhibiting both the inflammatory damage and infectivity associated with SARS-CoV-2^{2,3}.

Baricitinib, an oral inhibitor of Janus kinase (JAK)1 and JAK2⁴ approved for the treatment of moderately- to-severely active rheumatoid arthritis (RA) in adults, was independently hypothesized to be a therapeutic option for COVID-19. It was considered amongst all molecules studied to have a

unique role by virtue of its potential to both inhibit relevant cytokine signaling and have activity against the NAKs, AAK1, and GAK^{2,3}, which stimulate AP-2-mediated host viral propagation⁵⁻⁷. Infection by pathogenic coronaviruses (e.g. SARS and SARS-CoV-2) often results in excessive cytokine and chemokine action with the development of acute respiratory distress syndrome (ARDS)⁸⁻¹³. In patients with moderate-to-severe forms of these diseases, anti-viral cytokine signaling is maintained at inappropriate levels (perhaps due to incomplete viral clearance) causing acute lung injury¹⁴, persistent interferon (IFN) activity, and impaired T cell and antibody responses^{9,15,16}. In the COVID-19 setting, ARDS is the leading cause of death and is associated with high levels of interleukin-6 (IL-6), which appears to be a predictor of mortality¹¹. Therefore, treating hospitalized patients requires both improved viral clearance and restriction of the inflammatory response, with the potential to improve outcomes such as mortality and reduce admissions to intensive care units. The anti-inflammatory benefit of baricitinib has been previously demonstrated through a reduction in a range of JAK-STAT-dependent cytokines. Phase 3 clinical trials providing safety and efficacy data have been conducted or are ongoing for baricitinib treatment in patients with autoimmune diseases including RA, atopic dermatitis, systemic lupus erythematosus, alopecia areata, juvenile idiopathic arthritis, and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE). Now, we provide biochemical and cellular evidence confirming predictions focused on anti-cytokine signaling and potential anti-viral effects for baricitinib, along with a case series, supporting its potential utility in hospitalized COVID-19 patients.

Results & Discussion

Anti-cytokine activity for baricitinib

The anti-cytokine and anti-inflammatory activity of baricitinib was evaluated with a focus on cytokines relevant to COVID-19 infection. We evaluated the *in vitro* pharmacology of baricitinib across relevant leukocyte subpopulations coupled to its *in vivo* pharmacokinetics to determine its effect on distinct cytokine pathways. Concentration response curves combined with exposure data from baricitinib-treated healthy volunteers demonstrate that baricitinib affects cytokine-dependent phosphorylated

STAT (pSTAT) inhibition to varying degrees (Fig. 1A). Baricitinib inhibited signaling of cytokines implicated in COVID-19 infection, including IL-2, IL-6, IL-10, IFN- γ , and G-CSF, with lower IC₅₀ values translating to a greater overall inhibition of cytokine-induced JAK/STAT signaling during the dosing interval (Fig. 1A). Furthermore, baricitinib treatment resulted in a significant reduction ($p < 0.05$) from baseline in plasma IL-6 at week 12 in patients with active RA who had an inadequate response to methotrexate from a phase 2b¹⁷, randomized, placebo-controlled, dose-ranging study (Fig. 1B).

Anti-viral activity for baricitinib

Next, we validated the proposed biochemical inhibitory activity of baricitinib on the numb-associated kinase (NAK) family members AAK1, BIKE, GAK, and STK16, some of which are hypothesized to facilitate viral propagation of coronavirus in epithelial cells^{1,6,7}. Baricitinib activity demonstrated affinity against AAK1 (8.2nM), BIKE (20nM), and GAK (120nM) (Fig. 2); these values are within the exposure range of the approved 2-mg (US and OUS) and 4-mg (OUS) once-daily doses of baricitinib for the treatment of RA¹⁸.

The pharmacokinetics of baricitinib show the unbound fraction of free bioavailable drug in RA patient sera as being 326 nM area under the curve (AUC) and 652 nM (AUC) for baricitinib 2-mg and 4-mg, respectively (data on file). We compared the relative binding affinities of different JAK inhibitors (JAKis) for these NAKs. Notably, amongst JAKis approved for the treatment of RA, baricitinib uniquely demonstrated high affinity for AAK1, BIKE, and GAK, whereas tofacitinib and upadacitinib did not demonstrate high affinity for these kinases (Fig. 2B). The binding affinity of baricitinib for AAK1 and GAK is similar to the binding affinity of baricitinib for JAK1 (5.9nM) and JAK2 (5.7nM)⁴.

To extend these activities on NAKs, we evaluated the effect of baricitinib in reducing viral infectivity in 3D primary human liver spheroids infected¹⁹ with purified SARS-CoV-2 and treated with baricitinib. Specifically, we used a 3D spheroid model of primary human liver cells in which hepatocytes retain their transcriptomic, proteomic, and metabolomic phenotype and functionality for multiple weeks¹⁹⁻²¹. SARS-CoV-2 was able to infect human primary liver spheroids in this experimental paradigm, as

demonstrated by a day's post-infection (dpi) dependent increase in viral RNA (Fig. 2C). Importantly, exposure to physiologically relevant concentrations of baricitinib (400 nM and 800 nM) significantly ($p < 0.05$) reduced viral load, corroborating the proposed inhibitory effects of baricitinib on AAK1 and GAK-mediated viral propagation (Fig. 2D). These results suggest that host cells that express these NAKs may serve as a target for baricitinib-mediated reduction in viral propagation.

Clinical case series using baricitinib

Following the recent publications by Richardson *et al.* and Stebbing *et al.*^{1,2}, COVID-19 patients were treated with baricitinib in a pilot study in Milan, Italy. Four patients with bilateral COVID-19 pneumonia, who presented with varying degrees of disease severity (Table 1), were included in this pilot study; 3 individuals (Patients B, C, and D) were clinically unstable with moderate-to-severe disease. All four patients were admitted to the ward from the emergency department in March 2020. As shown in Table 1, Patient A was a female nurse, aged 29. Patient B, a 76-year-old male, had significant co-morbidities (a former smoker, arterial hypertension, chronic obstructive pulmonary disease [COPD], coronary artery disease, and had undergone an aortic aneurysm Endurant II graft repair on February 25, 2020). Patient C, a 56-year-old male, had co-morbidities including a high body mass index (BMI) of 35 and COPD (non-smoker); he suddenly deteriorated a few hours following hospitalization, and was placed on continuous positive airway pressure at the time baricitinib was initiated. Patient D, a 51-year-old male had a BMI of 35. All four patients had detectable plasma IL-6 levels (Fig. 3A) with markedly raised inflammatory markers (C-reactive protein [CRP] and ferritin; Fig. 4A), as would be expected in patients with COVID-19 pneumonia.

As shown in Figure 3A, all four patients showed improvement with baricitinib treatment in signs and symptoms such as cough, fever, and reduction in plasma IL-6 levels, along with a reduction in the SARS-CoV-2 RNA viral load, as detected by the real-time reverse transcription-polymerase chain reaction (RT-PCR) signal from the nasopharyngeal carriage. Stringent criteria were used for RNA detection of SARS-CoV-2 in nasopharyngeal carriage and peripheral blood. Real-time RT-PCR was performed on three distinct viral gene targets²² (Table S2), using the most sensitive target (N gene)

and a stringent cut-off using Ct values ≤ 40 for the analyses illustrated in Figure 3A, in contrast to others, for example in the hydroxychloroquine study (Ct values ≥ 35)². Only two of the patients (Patients A and C) had detectable viral RNA in their peripheral blood (Fig. 3A and Table S2). In addition, all four patients demonstrated improvement in their CRP, ferritin, and D-dimer levels (Fig. 4A). Notably, Patient C, who had the lowest PaO₂ at baseline, showed radiographic improvement in lymphocytic infiltrates when comparing the computed tomography scan from day One to day 19 (Fig. 4C). Overall, a total of 10 days of dosing for patients A, B, and D and 12 days for Patient C, with 4-mg baricitinib once-daily orally in Patients A, C and D, and 2-mg in Patient B (in accordance with the label guidelines) was sufficient to document in all patients improved lung function, resolution of their illness, and reductions in viral load, plasma IL-6, ferritin, and C-reactive protein (CRP) levels.

Baricitinib treatment with doses were associated with a rapid and consistent improvement in clinical, radiologic, virologic, inflammatory, and cytokine measures in this diverse group of patients, including 3 (Patients B, C, and D) who were deemed to be moderately- to-severely unwell and at high risk of deteriorating rapidly (i.e. those apart from the 29-year-old nurse).

There were nominal changes in lymphocyte counts throughout the course of treatment with baricitinib in all four patients (Fig. 3B). Neutrophil and total white blood cell counts tracked with improvement in disease severity (Fig. 4A). Transient increases in liver aminotransferases were observed in all four patients, with no changes in other liver enzymes or bilirubin (Fig. 4B); however, these liver enzyme elevations improved within 72 hours without interrupting baricitinib treatment, suggesting that these elevations may not be causally related to baricitinib treatment, but may be reflective of disease severity (Fig. 4B). In general, these data demonstrate that treatment with baricitinib in these four patients with bilateral COVID-19 pneumonia was well tolerated. Collectively, this limited case series provides preliminary evidence that baricitinib treatment may lower inflammatory burden and may result in a rapid reduction in disease severity in COVID-19 patients.

Conclusions

The pharmaceutical interventions for the treatment of COVID-19 patients proposed to date include testing anti-viral mechanisms, either in combination or alone, along with anti-malarials and

immunomodulators¹². One such potential pharmacological approach is the use of immunomodulators in the subset of patients who develop a cytokine storm associated with pulmonary involvement including ARDS that leads to a rapid deterioration of the co-morbid conditions in such patients (Supplementary Figure 1)^{10,12,13}. In this report, we demonstrate that baricitinib is an inhibitor of cytokines implicated in ICU-bound COVID-19 patients, which have been collated from several reports, including IL-2, IFN- γ , IL-6, IL-10, and G-CSF^{10,11,13}. Elevated IL-6 and hyperferritinemia were predictors of death in COVID-19 patients in China^{11,13}. The relevance of IL-6 in COVID-19 associated ARDS was recently shown in an open-label study where blockade with tocilizumab, an IL-6R antibody, resulted in a rapid recovery of peripheral oxygen saturation and recovery from febrile signs and symptoms²⁴. Baricitinib has been previously shown to inhibit IL-6-induced MCP-1 production from human peripheral blood mononuclear cells⁴. In addition to a marked and rapid reduction in levels of human serum CRP in adult RA patients previously described¹⁷, baricitinib treatment also reduced the mean change from baseline of plasma IL-6 in adult RA patients. The anti-inflammatory effects of baricitinib have also been demonstrated by the reduction of serum levels of IFN- γ , IP-10, GM-CSF, and MCP-1 in pediatric patients with steroid-dependent chronic inflammation, resulting in control of disease activity and the ability to wean or taper steroids²⁵.

Another pharmacologic strategy could include targeting members of the NAK family (AAK1, GAK, BIKE, and STK16) that activate the AP-2 scaffolding protein vital to viral entry and propagation^{6,7}. Previous reports demonstrated inhibiting such kinases was effective in reducing cellular infectivity for viruses that rely on the activity of AP-2 such as HCV, Dengue, HIV, SARS, and Ebola^{6,7,26,27}. While baricitinib demonstrated potent inhibition of several NAKs that phosphorylate the AP-2 adaptor and reduced viral infectivity in primary human liver spheroids, it is still unknown whether this inhibition inhibits SARS-CoV-2 propagation in COVID-19 infected patients.

The aforementioned four COVID-19 patients offer support to the mechanism of action of baricitinib as an anti-cytokine and a potential anti-viral agent. Reductions in inflammatory markers of COVID-19

disease, such as IL-6, CRP, and ferritin are consistent with expected anti-inflammatory activity. While the consistent reduction in viral RNA observed in all four patients (measured from their nasopharyngeal carriage) is suggestive of reduction in viral load, this could be a consequence of the natural ability in these patients to clear the virus. At the time of manuscript submission, only a few days have elapsed since cessation of baricitinib. Active clinical, biochemical, and virologic surveillance of each patient is ongoing to monitor any sequelae, including the possible appearance of an immune reconstitution inflammatory syndrome or molecular evidence of *de novo* SARS-CoV-2 replication in the respiratory tract or peripheral blood.

Given many unknowns concerning this novel SARS-CoV-2 virus, it is necessary to consider safety concerns alongside potential efficacy. As an effective immunomodulatory agent used for the treatment of RA, baricitinib was associated with an increased risk of infections such as upper respiratory tract infections and herpes zoster²⁸. Baricitinib treatment in RA patients has been associated with increased incidence of deep vein thrombosis (DVT), and it is recommended that hospitalized patients with higher risk of DVT should consider appropriate prophylactic measures with use of baricitinib²⁸. As baricitinib is an inhibitor of IFN-responsive genes²⁵, its potential impact on the subsequent development of protective humoral and cell-mediated anti-viral immunity needs to be assessed in the context of COVID-19 infection. Of relevance, RA patients on long-term baricitinib treatment achieved satisfactory humoral responses to pneumococcal conjugate (PCV-13) vaccination suggesting that humoral responses remain partially intact²⁹. Changes seen in lymphocyte subsets in this limited clinical case series suggest that baricitinib treatment does not reduce T cell subsets (CD3⁺ and CD4⁺) or NK cells. It is too early to ascribe observed reductions of SARS-CoV-2 RNA loads in the nasopharyngeal carriage to a putative antiviral activity of baricitinib, since the underlying T cell subset levels were maintained in these treated patients. It is, however, reassuring to underscore that after treatment with baricitinib Patient A seroconverted, and we observed persistence of serum anti-SARS-CoV-2 IgG in all patients.

This is consistent with previous reports wherein T cell and various B cell subsets remained within

normal reference ranges after initiation of baricitinib treatment in RA patients³⁰. Baricitinib has a well-established safety profile in patients with RA over long-term treatment; in 10,127 patients-year of exposure and up to 6.9 years duration, the incidence rate of serious infection was 2.8/100-patient years and stable over time³¹. Vigilance for detecting serious adverse events and appropriate management of potential complications would be essential given the inhibition on IFN-responsive genes by baricitinib²⁵. Therefore, the impact of baricitinib on the subsequent development of protective humoral and cell-mediated anti-viral immunity in COVID-19 patients must be evaluated. Importantly, baricitinib is administered orally once a day, and has a low cytochrome P₄₅₀ inhibitory activity with a low drug-drug interaction risk; its short half-life (approximately 12 hours in RA patients) and renal elimination as the main clearance mechanism make it fast to wash-out if necessary²⁸. Collectively, these data provide evidence that baricitinib could be tested as an effective intervention strategy to stem the cytokine storm and viral propagation seen in hospitalized COVID-19 patients. The finding that baricitinib is a potent AAK1/BIKE/GAK inhibitor that may reduce host cell infectivity, along with reaffirmation of its anti-cytokine profile, provide reasons to study this intervention in randomized clinical trials. Results from such trials will be central to manage effective clinical care as this outbreak continues to expand across the globe.

Methods

Leukocyte preparation and experimental design

Leukocyte preparation and experimental design were largely performed as previously described³².

Details pertinent to this study are described in the Supplementary Appendix.

IL-6 changes in baricitinib-treated RA patients

Patient samples were obtained from the double-blind, randomized, placebo-controlled, phase 2b study NCT01469013. Patients had moderate-to-severe active adult onset RA, despite stable methotrexate treatment. Patients (N = 145) were randomized (2:1:1:1:1) to placebo or once-daily oral 1-mg, 2-mg, 4-mg, or 8-mg baricitinib for 12 weeks¹⁷. Plasma IL-6 levels were analyzed using an enzyme immunoassay. A two-sided t-test was used to compare baricitinib 2-mg and 4-mg IL-6 levels with

placebo.

NCT01469013 was conducted in accordance with ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All investigation sites received approval from the appropriate authorized institutional review board or ethics committee. All patients provided written informed consent before the study-related procedures were undertaken.

Human NAK assays and viral infection

AAK1, BIKE, GAK, and STK16 binding assays were performed with DNA-tagged recombinant human proteins derived from HEK-293 cells or *E. coli*. Further experimental details are described in the Supplementary Appendix.

SARS-CoV-2 infection of human liver spheroids

Protein levels of AAK1 and GAK were pulled out from tandem mass tag (tmt)-based proteomics datasets³³ generated from total protein of 192 spheroids at the Clinical Proteomics Mass Spectrometry facility (Science for Life Laboratory, Stockholm, Sweden). Both proteins had a Protein Spectrum Match (PSM) level of >1 in all analyzed samples. The corresponding RNA expression values were obtained by Bulk RNA sequencing (poly-A) of a minimum of 100 ng total RNA at the National Genomics Infrastructure (NGI) facility at Science for Life Laboratory, Stockholm, Sweden.

SARS-CoV-2 (Genbank accession number MT093571) was isolated from a nasopharyngeal sample of a patient in Sweden on Vero E6 cells. Cryopreserved PHH (BioIVT, USA) were thawed and seeded into 96- well ultra-low attachment plates (Corning) with 1,500 cells per well as previously described¹⁹. Spheroids were pre-exposed to baricitinib for 24h starting five days after cell seeding. At day six of culture, cells were exposed to SARS-CoV-2 at a multiplicity of infection of 0.1 in triplicate for 48h. After 48h, spheroids were washed with PBS, pooled (32 wells/condition) and lyzed using TrizolTM (ThermoFisher). RNA was extracted using Direct'zol mini-kit (Zymo-research) and relative level of viral RNA was determined by qRT-PCR as previously described³⁴.

Clinical case series

Study design and participants

On the basis of the two reports of AI-derived discovery of baricitinib in COVID-19^{1,2}, the Sacco Baricitinib Study Group and Imperial College developed a protocol to assess baricitinib in a small case series of patients, acknowledging potential risks of pulmonary infections^{35,36} based on its mechanism of action while leveraging the hypothesis for a potential therapeutic benefit in COVID-19. Here, we describe the cases from the independent research conducted. Ethics and local Institutional Review Board approval was authorized on March 16, 2020 (number 14581/2020), which provided permission to treat three patients with bilateral COVID-19 pneumonia with baricitinib. Three patients (Patients A, B, and C) were immediately recruited that day, having been admitted to the wards from the emergency department. A supplementary approval was sought on March 25, 2020 and obtained to treat a fourth patient (Patient D) for validation purposes.

Inclusion criteria for compassionate use of an investigational oral medicine (albeit one that was approved in another indication) for patients with signs of severe illness at diagnosis or secondary clinical aggravation (respiratory symptoms or general signs) was based on World Health Organization (WHO) criteria for severe pneumonia caused by SARS-CoV-2³⁷.

Written informed consent was obtained from each patient, and 4-mg oral baricitinib was administered once-daily according to the label for treatment of RA with a 2-mg lower dose as per the label, for 10–12 days.

Criteria for patient discharge with recovery were from the European Centre for Disease Prevention and Control guidelines³⁸. The open-access Clinical Characterization Protocol for Severe Emerging Infections of the International Severe Acute Respiratory and Emerging Infection Consortium (supported by WHO), which has been updated and used in response to COVID-19³⁹.

Procedures

Clinical samples for SARS-CoV-2 diagnostic testing were obtained according to WHO guidelines⁴⁰ and are described in detail in the Supplementary Appendix, including Table S1. These four patients continue to be monitored for COVID-19 disease at the time of manuscript submission.

Statistical analysis

The concentration response curve fitting for NAK binding assay as previously described was normalized by dividing by the mean of three replicates of the 0 nM treatment condition. Four-parameter logistic curves were fit with the normalized assay signal as the response and \log_{10} compound concentration as the independent variable using the R drc package (R version 3.6.0 and drc version 3.0). The curve top parameter was fixed to a value of one while all other parameters were estimated in the curve fitting.

Equilibrium affinity constant (Kd) values were determined using the absolute half maximum efficacy concentration (EC_{50}) values estimated from the logistic model.

Statistical methods for half maximum inhibitory concentration (IC_{50}) values for cytokine signaling, selection of cases for analysis, fitting, and selection of CRC curves for anti-cytokine activity of baricitinib, and estimation of daily percent pSTAT inhibition are described in the Supplementary Appendix.

Data availability

Eli Lilly and Company data are available upon request at vivli.org. Access to data is provided after a proposal has been approved by an independent review committee identified for this purpose and after receipt of a signed data sharing agreement. Access to data and documents will be provided in a secure data sharing environment. For details on submitting a request, see the instructions provided at www.vivli.org. In your request, please include the following accession/reference numbers so that we can identify the related publication and specific dataset that you're trying to access.

Table

Table 1. Clinical characteristics of patients in baricitinib COVID-19 case-study

	Patient A	Patient B	Patient C
Age (years)	29	76	57
Gender	Female	Male	Male
Coexisting conditions	None	AH, COPD, CAD	AH, COPD
BMI	22	28	29
Medications	combined oral contraception	losartan, propranolol, PPI, aspirin, low mol weight heparin	losartan, hydrochlorothiazide, inhaled beclamethasone
Smoking	No	Ex	No
Symptoms at presentation	fever, dry cough, myalgias	fever, dry cough	fever, dry cough, dyspnea
Blood oxygen levels (PaO ₂) at presentation (mmHg)	111	81	62
Alveolar oxygen gradient (KPa)	0	4.7	12.9
SpO ₂ (%)	98	97	94
RR/min	26	32	28
T°C	37	38.5	39
Baricitinib dose/days on treatment	4-mg for 10 days	2-mg for 10 days	4-mg for 10 days
Antibiotic therapy	none	none	ceftriaxone, azithromycin

AH, high blood pressure; BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; RR, respiratory rate. Patient A tested negative for

pregnancy. All patients tested 325 negative for HIV and Tuberculosis (QuantiFERON-TB Gold Plus).

Declarations

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Author Contributions

VK wrote the first draft of the manuscript. VK, VML, and REH designed the laboratory experiments, analyzed, and interpreted the data. JS, SO, GC, PJR, and MC wrote the protocol, obtained ethical approval, collated case studies, and interpreted the data. JS, VK, SdB, SO, GC, VM, VML, AM, PJR, JART, BJN, REH, GR, NLB, DES, AC, and MC participated in the analyses and interpretation of data, wrote or critically reviewed the manuscript, and reviewed and approved the final version.

Declarations of Interest

JS is editor-in-chief of Oncogene. JS has sat on a number of scientific advisory boards, including BenevolentAI, and consults with Lansdowne partners and Vitruvian, and since these findings in patients, consults with Eli Lilly and Company; he sits on the Board of Directors for BB Biotech Healthcare Trust.

VML is founder, CEO, and shareholder of HepaPredict AB. In addition, VML discloses consultancy work for EnginZyme AB. AM and VM have no COI to disclose. SO, MC, and GC report no conflicts of interest. PJR is an employee of BenevolentAI. VK, SdB, JART, BJN, REH, GR, NLB, DES, and AC are employees and shareholders of Eli Lilly and Company.

Role of the Funding Source

The non-clinical studies and IL-6 assay from NCT01469013 were designed and analyzed by representatives of Eli Lilly and Company. The clinical case series was not funded or approved by Eli Lilly and Company and discussions between Eli Lilly and Company and the Sacco Baricitinib Study

Group did not occur until patients had been dosed and preliminary results obtained. Similarly, Eli Lilly and Company performed the non-clinical *in vitro* analyses and IL-6 assay from NCT01469013 independently. The Sacco Baricitinib Study Group funded the clinical case series, and representatives (including those from Imperial College, London) had a role in study design, data collection, and data analysis. All authors participated in data analysis and interpretation, draft and final manuscript review, and provided critical comment, including the decision to submit the manuscript for publication with medical writing support from Eli Lilly and Company; all authors reviewed and approved the final submitted version. Venkatesh Krishnan had full access to all data and had final responsibility for the decision to submit for publication.

Additional information

Supplementary Information is available for this manuscript. Correspondence and requests for materials should be addressed to: Venkatesh Krishnan

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References

1. Richardson, P. et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet* 2020; 395(10223): e30-e1.
2. Stebbing, J. et al. COVID-19: combining antiviral and anti-inflammatory treatments. *Lancet Infect Dis* 2020; 20(4):400-402.
3. Schneider, P. et al. Rethinking drug design in the artificial intelligence era. *Nat Rev Drug Discov* 2019.
4. Fridman, J.S. et al. Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: preclinical characterization of INCB028050. *J Immunol* 2010; 184(9): 5298-5307.

5. Inoue, Y. et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. *J Virol* 2007; 81(16): 8722-8729.
6. Bekerman, E. et al. Anticancer kinase inhibitors impair intracellular viral trafficking and exert broad-spectrum antiviral effects. *J Clin Invest* 2017; 127(4): 1338-1352.
7. Owczarek, K. et al. Early events during human coronavirus OC43 entry to the cell. *Sci Rep* 2018; 8(1): 7124.
8. Cameron, M.J. et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol* 2007; 81(16): 8692-8706.
9. Huang, K.J. et al. An interferon-gamma-related cytokine storm in SARS patients. *J Med Virol* 2005; 75(2): 185-194.
10. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395(10223): 497-506.
11. Ruan, Q., Yang, K., Wang, W., Jiang, L., Song, J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020.
12. Siddiqi, H.K., Mehra, M.R. COVID-19 Illness in Native and Immunosuppressed States: A Clinical- Therapeutic Staging Proposal. *J Heart Lung Transplant* 2020. doi: 10.1016/j.healun.2020.03.012.
13. Zhou, F. et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; 396(10229): 1054-1062.
14. Nicholls, J.M. et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003; 361(9371): 1773-1778.

15. Cui, W. et al. Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome. *Clin Infect Dis* 2003; 37(6): 857-859.
16. Shi, Y. et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ* 2020.
17. Tanaka, Y. et al. Efficacy and Safety of Baricitinib in Japanese Patients with Active Rheumatoid Arthritis Receiving Background Methotrexate Therapy: A 12-week, Double-blind, Randomized Placebo- controlled Study. *J Rheumatol* 2016; 43(3): 504-511.
18. Shi, J.G. et al. The pharmacokinetics, pharmacodynamics, and safety of baricitinib, an oral JAK 1/2 inhibitor, in healthy volunteers. *J Clin Pharmacol* 2014; 54(12): 1354-1361.
19. Bell, C.C. et al. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. *Sci Rep* 2016; 6: 25187.
20. Bell, C.C. et al. Transcriptional, Functional, and Mechanistic Comparisons of Stem Cell-Derived Hepatocytes, HepaRG Cells, and Three-Dimensional Human Hepatocyte Spheroids as Predictive In Vitro Systems for Drug-Induced Liver Injury. *Drug Metab Dispos* 2017; 45(4): 419-429.
21. Vorrink, S.U. et al. Endogenous and xenobiotic metabolic stability of primary human hepatocytes in long-term 3D spheroid cultures revealed by a combination of targeted and untargeted metabolomics. *FASEB J* 2017; 31: 2696-2708.
22. Corman, V.M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 2020; 25(3).
23. Gautret, P. et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020:

105949.

24. Xu, X. et al. Effective treatment of severe COVID-19 patients with tocilizumab. <http://www.chinaxiv.org/abs/202003.00026>. (2020).
25. Sanchez, G.A.M. et al. JAK1/2 inhibition with baricitinib in the treatment of autoinflammatory interferonopathies. *J Clin Invest* 2018; 128(7): 3041-3052.
26. Neveu, G. et al. AP-2-associated protein kinase 1 and cyclin G-associated kinase regulate hepatitis C virus entry and are potential drug targets. *J Virol* 2015; 89(8): 4387-4404.
27. Chaudhuri R, Lindwasser OW, Smith WJ, Hurley JH, Bonifacino JS. Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. *J Virol* 2007; 81(8): 3877-3890.
28. Olumiant. Prescribing information. Eli Lilly and Company; 2019. Accessed April 09, 2020. <http://pi.lilly.com/us/olumiant-uspi.pdf>.
29. Winthrop, K.L. et al. Evaluation of pneumococcal and tetanus vaccine responses in patients with rheumatoid arthritis receiving baricitinib: results from a long-term extension trial substudy. *Arthritis Res Ther* 2019; 21(1): 102.
30. Tanaka, Y. et al. Characterization and Changes of Lymphocyte Subsets in Baricitinib-Treated Patients With Rheumatoid Arthritis: An Integrated Analysis. *Arthritis Rheumatol* 2018; 70(12): 1923- 1932.
31. Genovese, M. et al. Safety profile of baricitinib for the treatment of rheumatoid arthritis up to 7 Years: an updated Integrated safety analysis [abstract]. *Arthritis Rheumatol* 2019; 71 (suppl 10).
32. McInnes, I.B. et al. Ex vivo comparison of baricitinib, upadacitinib, filgotinib, and tofacitinib for cytokine signaling in human leukocyte subpopulations [abstract].

Arthritis Rheumatol 2017; 69 (suppl 10).

33. Oliva-Vilarnau et al., *Adv Sci*, personal communication, manuscript in review.
34. Monteil, V. et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 2020. doi: 10.1016/j.cell.2020.04.004.
35. Favalli, E.G., Biggioggero, M., Maioli, G., Caporali, R. Baricitinib for COVID-19: a suitable treatment? *Lancet Infect Dis* 2020.
36. Richardson, P.J., Corbellino, M, Stebbing, J. Comment to Baricitinib for COVID-19: a suitable treatment? *Lancet Infect Dis* 2020.
37. World Health Organization. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: Interim guidance V 1.2. World Health Organization. (2020). [https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)
38. European Centre for Disease Prevention and Control. Infection prevention and control for the care of patients with 2019-nCoV in healthcare settings. European Centre for Disease Prevention and Control. (2020). <https://www.ecdc.europa.eu/en/publications-data/infection-prevention-and-control-care-patients-2019-ncov-healthcare-settings>
39. Dunning, J.W. et al. Open source clinical science for emerging infections. *Lancet Infect Dis* 2014; 14(1): 8-9.
40. World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance. World Health Organization. (2020). <https://apps.who.int/iris/bitstream/handle/10665/331501/WHO-COVID-19-laboratory-2020.5-eng.pdf?sequence=1&isAllowed=y>
41. Pan, F. et al. Time Course of Lung Changes On Chest CT During Recovery From 2019 Novel Coronavirus (COVID-19) Pneumonia. *Radiology* 2020: 200370.

42. Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR. HKU Med. https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf?sfvrsn=af1aac73_4 (accessed April 5, 2020).
43. Diagnostic detection of 2019-nCoV by real-time RT-PCR. Berlin, Germany. 2020. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed April 5, 2020).

Figures

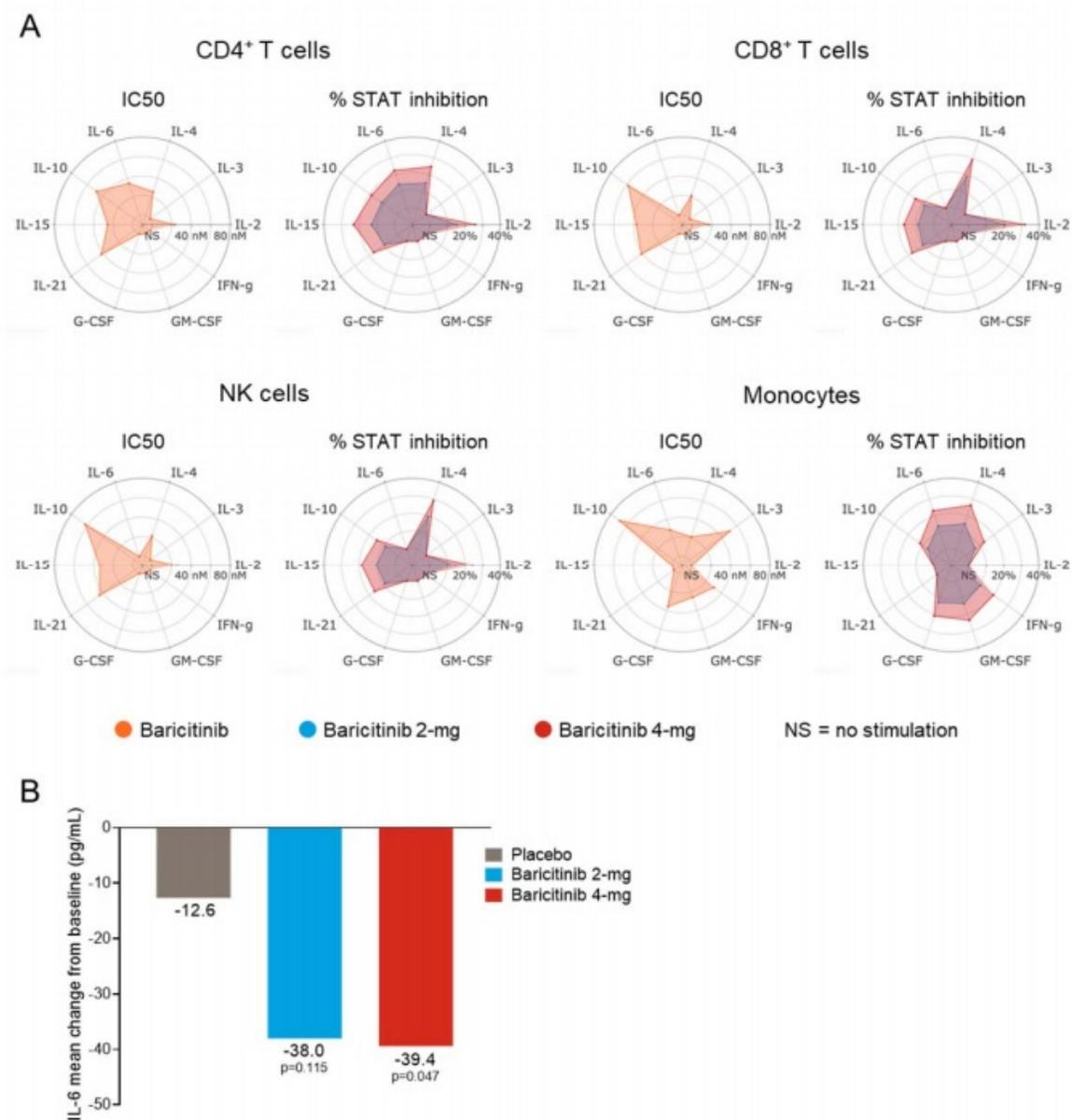


Figure 1

Anti-cytokine activity of baricitinib. (A) IC₅₀s for baricitinib (orange) in cytokine-stimulated human CD4⁺ T cells, CD8⁺ T cells, NK cells, and monocytes are shown for IL-2/pSTAT5, IL-4/pSTAT6, IL-3/pSTAT5, IL-6/pSTAT3, IL-10/pSTAT3, IL-15/pSTAT5, IL-21/pSTAT3, G-CSF/pSTAT3, GM-CSF/pSTAT5, and IFN γ /pSTAT1. Average daily percent STAT inhibition was calculated. (B) Mean change from baseline in IL-6 plasma levels at week 12 from RA patients treated with placebo (n=47), baricitinib-2mg (n=24), and baricitinib 4-mg (n=23) in the phase 2b randomized, placebo-controlled study NCT01469013. p values are for comparisons of baricitinib 2-mg and 4-mg with placebo. IC₅₀, half maximum inhibitory concentration; NK, natural killer; pSTAT, phosphorylated signal transducer and activator of transcription.

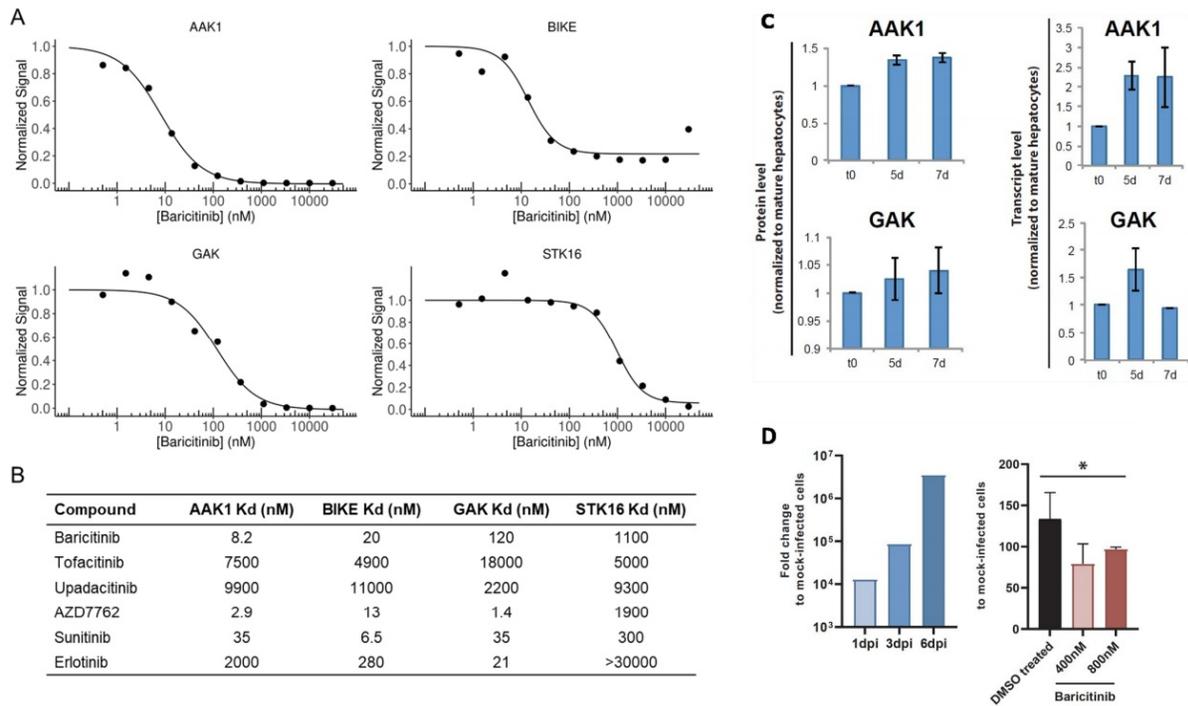


Figure 2

NAK binding affinities of baricitinib and other comparators. (A and B) Binding affinities in Kd are shown for baricitinib (A and B), tofacitinib, upadacitinib, AZD7762 (positive control), and broad-spectrum tyrosine kinase inhibitors sunitinib and erlotinib (B). Kd values were estimated from an 11-point concentration response curve with fixed top parameters and a four-parameter logistic model. Kd, equilibrium affinity constant. (C) Protein and mRNA expression of relevant NAKs in primary human liver organoids. (D). Time course of days (one, three, and six days) post infection (dpi) using liver spheroids and SARS-CoV-2 at 0.1 and 1.0 multiplicity of infection (MOI). Viral load in infected (MOI 0.1) measured after treatment of infected organoids, five days post-infection, with 400 nM and 800 nM of baricitinib for 48 hours (*p<0.05).

values > 40 were defined as negative. A dashed horizontal line indicates the cut-off. IL-6 values are shown in the same graph. Time of baricitinib treatment is highlighted (Patients A, B, and D for 10 days, Patient C for 12 days). (B) Levels of CD3+, CD4+, CD8+, B cells (CD19+) and NK cells (CD3-, CD16+, CD56+) were determined. (C) Total serum IgG, IgA, and IgM levels are shown for all patients. Ct, cycle threshold; E, envelope membrane; ED, emergency department; N, nucleocapsid protein; NK, natural killer; RdRp, RNA-dependent RNA polymerase.

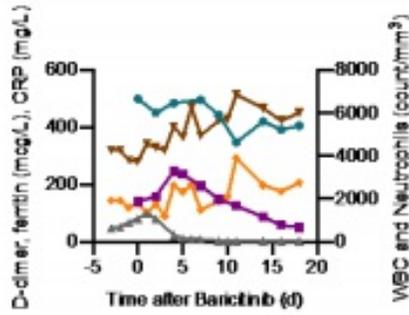
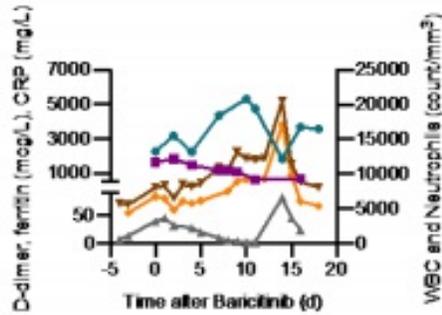
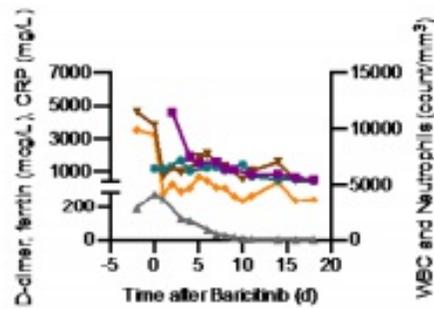
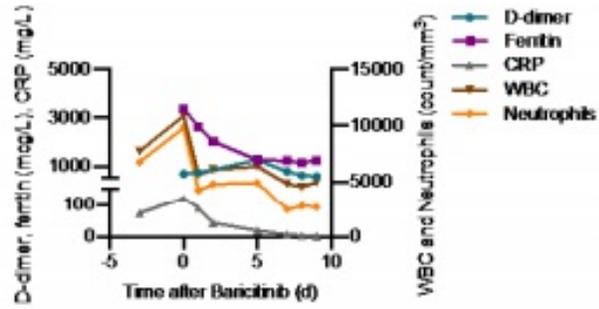
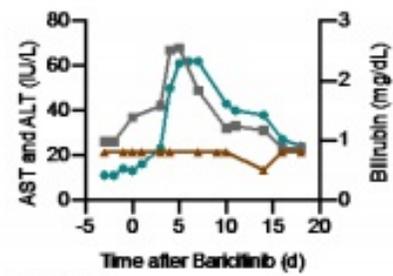
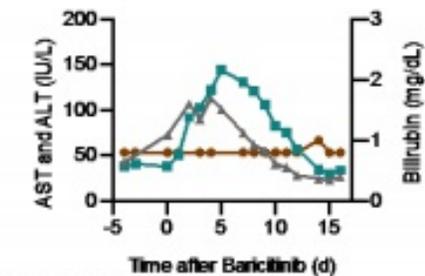
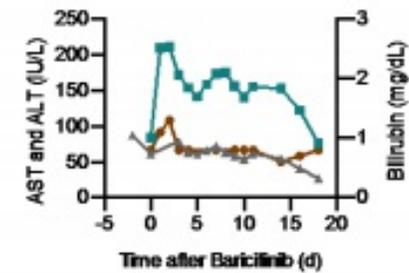
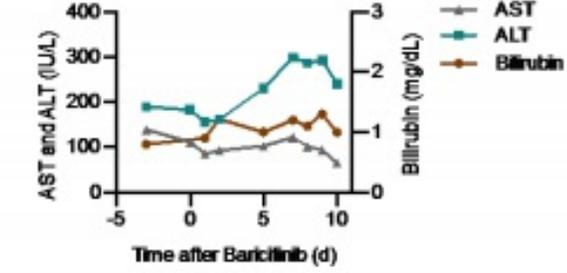
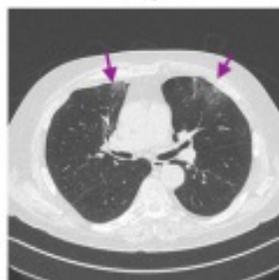
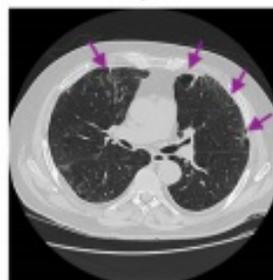
A**Patient A****Patient B****Patient C****Patient D****B****Patient A****Patient B****Patient C****Patient D****C****Day 1****Day 19**

Figure 4

Standard laboratory and radiologic features of the four COVID-19 cases treated with baricitinib. (A) Levels of D-dimer, CRP, ferritin, white blood cells, and neutrophils are shown for all patients. (B) Levels of AST, ALT, and bilirubin are shown for all patients. (C) Chest CT scan for Patient C on day one and 19 from symptom onset showing clinical improvement over time. Day one CT scan shows ground glass opacity (arrows) sub-pleurally in the lower lobes bilaterally (early stage one according to Pan et al⁴¹). Day 19 CT scan shows that consolidation was gradually absorbed with evident residual fibrosis and emphysema bubbles (arrows) in the site of the early lesions (absorption stage four according to Pan et al⁴¹). ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C- reactive protein; CT, computed tomography; WBC, white blood cells.

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