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***In vivo* antiviral efficacy and immune dampening effect of zapnometinib emphasize a dual therapeutic potential against COVID-19**

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ABSTRACT

Coronaviruses, in particular the current pandemic strains of SARS-CoV-2, represent an ever-evolving threat to human life. Currently available antiviral drugs are not suitable for severely infected patients in later hyperinflammatory stages of the disease. New therapeutics that inhibit virus propagation and target inflammation would be ideal. We demonstrate that the host targeted MEK inhibitor zapnometinib displays such a dual effect. The drug efficiently reduced virus titers of different SARS-CoV-2 variants and other highly pathogenic human coronaviruses such as SARS-CoV-1 and MERS-CoV *in vitro*. In a Syrian hamster infection model, zapnometinib reduced SARS-CoV-2 viral load and alleviated lung pathology. Drug safety biomarkers, body weight change and clinical observations, indicated that zapnometinib was well tolerated and showed no toxicity. Moreover, the immune dampening effect could be confirmed in an acute lung injury mouse model and *in vitro* experiments in primary human blood cells that demonstrated the reduction of disease related cytokines and chemokines. Thus, in contrast to direct-acting antivirals, zapnometinib displays a dual therapeutic effect highlighting its potential in the treatment of severe COVID-19 and other acute viral infections leading to hyperinflammation.

INTRODUCTION

In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), emerged in humans. The WHO declared COVID-19 as a pandemic due to the rapid person-to-person transmission of SARS-CoV-2 and substantial morbidity and mortality worldwide ¹⁻³. Severity of COVID-19 varies widely from asymptomatic infection to severe disease, with the latter being associated with lower respiratory tract symptoms and pathological hyperinflammation ⁴⁻⁶ that may lead to multiorgan dysfunction, including acute respiratory distress syndrome (ARDS), neurological symptoms, cardiovascular events, and coagulopathies ⁷⁻⁹. While the viral load is high at onset and in the early stages of COVID-19, the hyperinflammatory response plays a more important role in determining disease severity in later stages ¹⁰. Hyperinflammation is evidenced by highly elevated levels of certain proinflammatory cytokines and chemokines that are observed in most COVID-19 patients, especially in patients with severe disease who require intensive care ¹¹. Currently available treatments can be grouped in therapeutic antibodies, repurposed direct-acting antivirals, such as nirmatrelvir, remdesivir, or molnupiravir, and immunomodulating drugs, such as dexamethasone or anti-IL-6 antibodies ¹²⁻¹⁶. They all have their limitations. Therapeutic antibodies became inefficient because of the emergence of new SARS-CoV-2 variants, highlighting the problem of resistance. Direct-acting antivirals must be applied very early in the onset of infection and were not very efficient in treating severely ill patients. Immunomodulators such as dexamethasone showed quite good efficacy in late-stage infections, however, should not be given too early to allow the initial onset of an antiviral immune response. Thus, a drug that is effective throughout the full course of the disease is still missing. Targeting cellular factors represents a new strategy to overcome this gap. The cellular Raf/MEK/ERK signaling pathway is mainly involved in cell proliferation and the regulation of cytokine and chemokine expression ¹⁷. Moreover, inhibition of this pathway leads to modulation of the adaptive immune response toward an effector immune response ¹⁸⁻²⁰. Thus,

inhibition of this pathway may interfere with overshooting immune responses. In addition, we previously demonstrated that targeting the cellular Raf/MEK/ERK signaling pathway leads to viral load reduction and protects mice from a lethal viral challenge during infection with another live threatening respiratory virus, the influenza virus²¹⁻²³. This prompted us to analyze whether inhibition of the pathway would also lead to inhibition of SARS-CoV-2 replication. Indeed we could show that zapnometinib (ATR-002, PD 0184264), an oral MEK inhibitor, not only demonstrated antiviral activity against influenza virus^{22,23} but also against SARS-CoV-2 *in vitro*²⁴. Interestingly, the inhibitor also led to the reduction of expression of some virus-induced cytokines in infected cells²⁴, although the relevance of this observation has still to be examined *in vivo*. Additionally, zapnometinib showed no toxicity and was well tolerated in humans in a phase 1 study in healthy volunteers (NCT04385420). The drug is currently being investigated in a phase 2 clinical trial of hospitalized COVID-19 patients (NCT04776044).

Here, we aimed to assess the therapeutic potential of zapnometinib against various coronaviruses, demonstrating its dual effect *in vivo* with different approaches. The pharmacokinetics, antiviral efficacy, immunomodulatory effect, and biomarker-indicated safety of zapnometinib could be demonstrated in a Syrian hamster SARS-CoV-2 infection model. When administered orally as a single dose starting 4 or 24 h after infection with SARS-CoV-2, zapnometinib significantly decreased viral load and alleviated lung pathology without exerting toxic effects. This was reflected by an apparent lack of treatment-related side effects, body weight changes or changes in the levels of drug safety biomarkers. Furthermore, we revealed that zapnometinib could significantly alleviate hyperinflammation induced by cytokines and chemokines associated with severe COVID-19 in an acute lung injury mouse model and in primary human blood cells. These findings support the potential of zapnometinib as a promising broad-spectrum antiviral therapeutic with a dual effect, particularly in severe viral infections leading to hyperinflammation like COVID-19.

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MATERIALS AND METHODS

Ethics

The hamster experiments were carried out in the Central Animal Facilities of Viroclinics Xplore in Schaijk, the Netherlands, in accordance with the standards of the Dutch law for animal experimentation (2010/63/EU). Ethical approval for the present study was obtained from the institutional Animal Welfare Body under the number AVD277002015283-WP24.

All mouse experiments were conducted following review and approval by the Regional Council Tuebingen (35/9185.81-2/ IM 1/17) and in compliance with the institutional Principles of Animal Welfare and Animal Research.

Animals

Seven- to nine-week-old male Syrian hamsters (Janvier, France) with a body weight of 102-134 g at the time of drug administration were used for the pharmacokinetic study. The same animals were used for the efficacy study at an age of 11-13 weeks and a body weight of 113-148 g. The animals were housed in elongated type 2 IVC group cages with two animals per cage under BSL-II conditions during acclimatization and in elongated type 2 group cages under BSL-III conditions (isolators) during challenge using sawdust as bedding. They were checked daily for overt signs of disease, were fed standard food, and drinking water was provided ad libitum. All biotechnical procedures were performed under 3% isoflurane anesthesia.

Six- to eight-week-old female C57BL/6J mice weighing 20-27 g from the Mouse Facility of the University of Tuebingen (Interfaculty Institute for Cell Biology) were used to establish an inflammatory acute lung injury (ALI) model. The animals were maintained in elongated type 2 IVC cages housing four mice each under BSL-II conditions, fed standard food, and drinking water was provided ad libitum.

Virus and cells

SARS-CoV-2 (BetaCoV/Munich/BavPat1/2020, containing the D614G substitution in the S1 fragment, kindly provided by Dr. C. Drosten, Berlin, Germany) was passaged once in Vero-TMPRSS2 cells and three times in Vero E6 cells and titered in a TCID₅₀ assay as described in the section “Determination of replication-competent virus (TCID₅₀ assay)”. Vero E6 (Cat# CRL-1586) and Vero cells (Cat# CCL-81) used for *in vitro* assays were obtained from ATCC (Manassas, Virginia, USA). The virus strains used for *in vitro* studies at a MOI of 0.001 were the following: SARS-CoV-1: SARS-CoV-HKU-39849; MERS-CoV: MERS-CoV-EMC/2012; SARS-CoV-2 BavPat1: Human 2019-nCoV Isolate, EVAg Ref-SKU:026V-03883; SARS-CoV-2 (Alpha / B.1.1.7): Isolate USA/CA_CDC_5574/2020, BEI Resources NR-54011; SARS-CoV-2 (Beta / B.1.351): Isolate hCoV-19/South Africa/KRISP-K005325/2020, BEI Resources NR-54009.

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers registered with the Biobank of the Department of Immunology at the University of Tuebingen. Ethical approval was obtained upon review by the Ethics Board of the Medical Faculty of the Eberhard Karls University Tuebingen and the University Hospital Tuebingen (887/2020BO2). Briefly, 50 ml whole blood (roughly 5×10^7 PBMCs) of two donors were taken in 50 ml syringes (BD Biosciences, Franklin Lakes, New Jersey, USA) supplied with heparin-natrium (100.000 I.E./ml; Braun, Melsungen, Germany). The whole blood was mixed with PBS (Gibco, Carlsbad, California, USA) in a 1:1 ratio in 50 ml tubes (Greiner Bio-One, Frickenhausen, Germany), and PBMCs were subsequently isolated from the whole blood by density gradient centrifugation using Histopaque®-1077 (Sigma-Aldrich, St. Louis, Missouri, USA) as density gradient medium. 5 ml plasma per donor were preserved for cell culture medium preparation. PBMCs were then purified by washing twice with PBS.

Virospot reduction assay

The *in vitro* antiviral activity assay was performed at Viroclinics Biosciences BV (Rotterdam, the Netherlands) according to the company's standard operating procedures (SOPs). Briefly, Vero E6 cells (for SARS-CoV-2) or Vero cells (for Middle East respiratory syndrome (MERS)-CoV and SARS-CoV-1) were seeded at a density of 1.5×10^4 cells in 96-well plates the day before infection and inoculated with 0.001 MOI of appropriate virus in the absence (virus control wells) and presence of 100, 75, 50, 25 or 0 μ M zapnometinib. The 96-well plates were incubated for 24 and 48 h at 37 °C. The supernatants were harvested after the incubation, and the viral titer was determined in 4 replicates by TrueBlue Immunostaining and spot counting using a CTL Immunospot Image Analyzer. The viral titer measured in the presence of each concentration of zapnometinib was used to determine the 50% effective concentration (EC₅₀) using a previously described method ²⁵.

Drug administration

Zapnometinib was synthesized by Chemcon GmbH (Freiburg, Germany). Before administration to Syrian hamsters, it was freshly dissolved in dimethyl sulfoxide (DMSO) (Carl Roth GmbH, Karlsruhe, Germany) and then further dissolved in 15% Kolliphor EL (Sigma-Aldrich, Germany) and 80% phosphate-buffered saline (PBS) (Gibco, Life Technology Europe B.V., Bleiswijk, the Netherlands). Zapnometinib was administered orally at the indicated doses in a volume of 500 μ L/150 g body weight using a gavage needle.

Pharmacokinetics

Drug administration and blood sampling in Syrian hamsters were performed by Viroclinics Xplore (Schaijk, the Netherlands) and are described in the sections "Drug administration" and "Sampling after inoculation". Specifically, at different time points (0, 0.5, 1, 2, 4, 8, 12, and 24 h) after the administration of a single dose of zapnometinib (15, 30, or 60 mg/kg per oral (p.o.)), ~200 μ L blood was collected retro-orbitally from three animals per time point per dose and

alternating animals between time points (except at 24 h, n = 6 per group) under isoflurane anesthesia using a heparinized capillary tube containing a clot activator (Microvette 500 Z-Gel, Sarstedt). Serum was collected by spinning the tubes at 4000 xg for 10 minutes, aliquoted and stored at <-70 °C until pharmacokinetic analysis of serum. Clinical observations were conducted daily and included ruffled fur, hunched back posture, accelerated breathing and lethargy, and the body weight was monitored before and 24 h post administration. Bioanalysis and pharmacokinetic evaluation were then performed at Prolytic GmbH (Frankfurt, Germany). High-affinity liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used to determine the zapnometinib concentration in hamster serum according to the company's SOPs. During the analytical phase, two batches were evaluated. The acceptance criteria for the calibration standards and quality control (QC) samples were fulfilled for both batches according to the company's SOPs. The calibration curve was in the range of 10-10000 ng/mL for zapnometinib and showed acceptable linearity over the calibration range. In total, 81 study test samples were analyzed. The validity of the method during the analysis of the test samples was ensured by assaying QC samples with known concentrations of zapnometinib; zapnometinib 13C6 was used as the internal standard. The lower limit of quantification (LLOQ) of the method was determined to be 10 ng/mL for zapnometinib in undiluted serum. ANALYST software was used to calculate the concentrations of the test and QC samples based on the corresponding calibration curves. The obtained mean serum concentrations were used for noncompartmental pharmacokinetic evaluation with Phoenix® WinNonLin software (Pharsight Corporation, Mountain View, CA, USA; version 8.1).

Antiviral efficacy study in the Syrian hamster infection model

On day 0, all animals were infected intranasally with SARS-CoV-2 (1×10^3 median tissue culture infectious dose (TCID₅₀) BetaCoV/Munich/BavPat1/2020 (grown and titered at Viroclinics) in a total volume of 0.1 mL diluted in PBS (Gibco, Paisley, UK) and divided between the nostrils.

Zapnometinib (100 mg/kg) was administered p.o. at 4 or 24 h post-infection (p.i.). Thereafter, the animals in both groups were treated with 75 mg/kg zapnometinib once daily. The animals in the control group received vehicle 4 h p.i. and once daily thereafter. All animals were euthanized by exsanguination under 3% isoflurane anesthesia on day 4 post-infection (dpi).

Sampling after inoculation

Respiratory tract samples were collected daily during the study from Syrian hamsters infected and treated as described in the in the section “Antiviral efficacy study in the Syrian hamster infection model”. Briefly, throat swabs were collected in virus transport medium (Eagles minimal essential medium containing HEPES buffer, Na bicarbonate solution, L-Glutamine, Penicillin, Streptomycin, BSA fraction V and Amphotericin B), and the samples were aliquoted and stored at $\leq -70^{\circ}\text{C}$. Serum was also collected as described for pharmacokinetics at 0 dpi and 4 dpi and frozen at $\leq -70^{\circ}\text{C}$ until further analysis.

Additionally, upon necropsy, lung, nasal turbinates and trachea samples were collected and stored in 10% formalin for histopathology or frozen at $\leq -70^{\circ}\text{C}$ for virological analysis. For virological analysis, the tissue samples were weighed, homogenized in infection medium and centrifuged at 3000 xg for 10 minutes at room temperature before titration.

Determination of viral load

Detection of replication-competent virus (TCID₅₀ assay): Quadruplicate 10-fold serial dilutions of throat swap and tissue homogenate samples were used to determine the viral titers in confluent layers of Vero E6 cells. Briefly, serial dilutions of samples were prepared and incubated on Vero E6 monolayers for 1 h at 37 °C. Then, the Vero E6 monolayers were washed once with infection medium (DMEM containing L-Glutamine, Penicillin, Streptomycin, Amphotericin B and Fetal Bovine serum) and incubated for 4-6 days at 37 °C, after which the

plates were scored using WST8 (colorimetric assessment). The optical density (OD) of the wells was read at 450 nm (OD₄₅₀) using a microplate reader and compared to the OD of the positive control wells (non-treated, infected cells), which showed a cytopathic effect (CPE). The viral titer (TCID₅₀) per mL or g was calculated using the Spearman-Kärber method.

Detection of viral RNA: The viral titer in throat swab and homogenized tissue samples was determined using an RT-qPCR method. Briefly, RNA was isolated with a Roche MagNA Pure 96 instrument using a MagNA Pure 96 DNA and Viral NA Small Volume Kit, and a TaqMan-based RT-qPCR assay was performed with specific primers (Supplementary Table 1), as described by Corman et al.²⁶. The number of viral copies (CP per mL or g) per sample was calculated by using the resulting Ct value of the sample and the slope, intercept, and upper and lower limits of detection of a log₁₀ dilution series of a viral stock for which the number of copies per dilution was known.

Gross pathology

At the time of necropsy, gross pathology was performed for each animal. All lung lobes were inspected, and the percentage of affected lung tissue was estimated from the dorsal view. In addition, any other abnormalities observed in other organs during full-body gross pathology were recorded. Then, the left lung lobes, trachea and nasal turbinates were preserved in 10% neutral buffered formalin for histopathology, and the right lung lobes and nasal turbinates were subsequently homogenized for TaqMan-based RT-qPCR and virus titration.

Histopathology

Histopathological analysis of the left lung, trachea and left nasal turbinate was performed for all animals. After fixation with 10% formalin for at least 2 weeks, tissue sections were embedded in paraffin and stained with hematoxylin and eosin (H&E) for histological examination. SARS-CoV-2-associated lesions in the lungs were semi-quantitatively assessed

as follows: presence of alveolar edema, alveolar hemorrhage, and type II pneumocyte hyperplasia: 0 = no, and 1 = yes.

Candidate biomarker analysis

Serum samples from hamsters representing pre- (0 dpi) and post-infection (4 dpi) and treatment timepoints were analyzed to assess the levels of different candidate safety biomarkers. Serum proteins were enzymatically fragmented by proteolysis, and protein-specific peptides were targeted for quantification of the corresponding protein biomarker levels by immunoaffinity liquid chromatography tandem mass spectrometry (IA-LC-MS/MS) performed as described previously^{27,28}. For details see supplementary.

Inflammatory ALI mouse model

Mice were anesthetized by intraperitoneal (i.p.) injection of ketamine (10 mg/kg) solution. The mice in both the treatment and control groups (n = 4 each) were treated with 5 mg/kg LPS (O55:B5, Sigma-Aldrich) in PBS (100 µL per mouse) via i.p. injection. At 1 h post-stimulation, the mice in the treatment group were treated with 25 mg/kg zapnometinib via i.p injection, while the mice in the control group were treated with vehicle (1% DMSO in PBS). The mice were euthanized at 6 h post-zapnometinib treatment, and the lungs were preserved in RNAlater (Qiagen, Hilden, Germany).

Lungs were homogenized by a bead beating system (FastPrepTM, MP Biomedicals) and the RNA was isolated using an RNeasy Plus Universal Midi Kit (Qiagen) according to the manufacturer's instructions. RNA quality (A260/A280 ratio ≥ 1.8 and A260/A230 in the range of 2.0-2.2) was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and the isolated RNA was reverse transcribed using an RT² First Strand Kit (Qiagen). Afterward, the generated cDNA was subjected to a real-time RT² Profiler PCR Array using RT² SYBR® Green qPCR Mastermix. For optimal performance, the - RT² ProfilerTM Mouse

Cytokines & Chemokines arrays (PAMM-150ZR, Qiagen) were customized to include 84 genes and 5 housekeeping genes for data normalization (see Supplementary Table 3). For QC, a mouse genomic DNA contamination test, 3 reverse transcription efficiency tests, and 3 PCR array reproducibility tests were included. The data were analyzed using the GeneGlobe portal (Qiagen) according to the $\Delta\Delta C_t$ method. The fold change/regulation was calculated, and the threshold value was 2.

Analysis of cytokine and chemokine levels in human PBMCs

2×10^6 human PBMCs from two donors each were suspended in 1 mL RPMI 1640 medium (Gibco) containing 5% autologous plasma, 1% penicillin/streptomycin (Sigma-Aldrich), and 1% L-glutamine (Sigma-Aldrich) and were treated with 1 $\mu\text{g/mL}$ LPS from *E. coli* O55:B5 (prepared in PBS) and 10 $\mu\text{g/mL}$ zapnometinib (prepared in DMSO and diluted in cell culture medium to a final DMSO concentration of 0.1%) for 6 h at 37 °C and 5% CO₂. The supernatants (cell culture medium) were collected and stored at -20 °C until analysis by ELISA. Ready-to-use ELISA kits (Invitrogen, Waltham, Massachusetts, USA) were used according to the manufacturer's instructions to determine the levels of the following cytokines: IL-1 β , IL-6, IL-8, IP-10, MCP-1, MIP-1 α , and TNF- α . Briefly, the samples and standards were added to ELISA plates precoated with specific antibodies against the cytokines of interest. Biotinylated detection antibodies binding to the cytokines of interest were added to the wells followed by a horseradish peroxidase (HRP)-streptavidin conjugate binding to the biotinylated detection antibodies. Unbound cytokines, detection antibodies, and HRP-streptavidin conjugate were removed by washing. Tetramethylbenzidine (TMB) substrate was added and the color change in the wells was used as readout for the ELISA. The optical density (OD) at 450 nm was read using a SpectraMAX Plus 384 microplate reader. For IL-1 β , IL-8, MCP-1, and TNF- α the OD was also read at a reference wavelength of 620 nm, and for IL-6 at a reference wavelength of 550 nm. For IP-10 and MIP-1 α no reference wavelength was used. Cytokine and chemokine

concentrations were calculated following the manufacturer's instructions for interpolating unknown concentrations from a standard curve.

Statistical analysis

Unless otherwise noted, data were collected in MS Excel, and statistical analyses were performed using GraphPad Prism software v8 (La Jolla, CA, USA). Statistical details for each experiment are described in the corresponding figure legends. Differences in viral titer, lung injury severity or body weight were analyzed by one-way ANOVA. Differences in the mRNA expression of cytokines were assessed by analyzing replicate data using ordinary one-way ANOVA with the Kruskal-Wallis test or using Student's t-test. Differences in cytokine expression at the protein level were analyzed using unpaired t-test with Welch's correction for normally distributed data, otherwise Mann-Whitney test. Differences in biomarkers were evaluated using Brown-Forsythe ANOVA with the Dunnett T3 correction. P values < 0.05 were considered statistically significant.

RESULTS

Zapnometinib inhibits various coronaviruses *in vitro*.

In our recent publication we demonstrated the antiviral efficacy of zapnometinib against various SARS-CoV-2 strains in cell culture²⁴. Here, this *in vitro* analysis of the antiviral efficacy of zapnometinib against SARS-CoV-2 (BavPat1 and the alpha and beta variants) was extended to other highly pathogenic human coronaviruses (MERS-CoV and SARS-CoV-1) and evaluated using a Virospot reduction assay. A reduction in the viral titer of at least 1 log₁₀ in magnitude, correlating to a 90% reduction, was achieved with 75-100 µM zapnometinib compared to the solvent control for all tested viruses (Fig. 1). For SARS-CoV-2 BavPat1 and the beta variant, 100 µM zapnometinib achieved a 2-log₁₀ reduction (99%) (Fig. 1 c, d). Furthermore, at 75 µM, zapnometinib achieved a reduction of more than 80% for all the viruses tested, while 50 µM zapnometinib achieved a reduction of approximately 50% for SARS-CoV-1 and SARS-CoV-2 BavPat1 (Fig. 1a, c). The EC₅₀ values ranged from 39.0 µM (SARS-CoV-1) to 62.6 µM (SARS-CoV-2 beta) for all the viruses tested (Table 1). In summary, *in vitro* antiviral efficacy of zapnometinib against various coronaviruses could be shown, confirming its broad antiviral therapeutic potential that is, for a host-cell targeting drug, most probably not dependent on upcoming variations due to virus mutations, which is an important drug feature explicitly in the current SARS-CoV-2 pandemic.

Pharmacokinetics of zapnometinib in Syrian hamsters

Next to the *in vitro* efficacy of a drug, the therapeutic potential and efficacy must be confirmed with *in vivo* studies. Prior to the *in vivo* efficacy study, we studied the pharmacokinetics of zapnometinib in Syrian hamsters to better understand the metabolism of zapnometinib in this animal. In addition, the tolerability of zapnometinib was verified by conducting clinical observations. To investigate the dose proportionality, clearance, and safety of zapnometinib in the hamsters used as model animals, a single dose of 15, 30 or 60 mg/kg zapnometinib was

administered p.o. to groups of 6 animals. Blood samples were collected for analysis after 30 minutes and 1, 2, 4, 8, 12, and 24 h from 3 alternating animals per group. The serum concentration time curves for all doses showed a monophasic progression following oral administration. A proportional increase was observed until the maximum observed plasma concentration ($C_{\max}$) was reached after 2 h for the 15 and 30 mg/kg doses and after 4 h for the 60 mg/kg dose (Fig. 2). Moreover, the area under the concentration-time curve (AUC) and the $C_{\max}$ values increased proportionally with the dose. The apparent terminal half-life was comparable for all dose groups and was in the range of 2-3 h (Table 2). Notably, at the highest dose (60 mg/kg), the concentration of zapnometinib after 24 h (prior to the next treatment) was 5.32 $\mu\text{g/mL}$ (Table 2; Fig. 2). Based on previous work in our laboratory, 10 $\mu\text{g/mL}$ is required for 50% MEK inhibition. Thus, higher doses are needed to achieve a serum concentration exceeding 10 $\mu\text{g/mL}$ at 24 h, ensuring effective and constant MEK inhibition. Additionally, all doses were well tolerated, and no adverse events upon zapnometinib treatment were reported. Therefore, based on the present data, a loading dose of 100 mg/kg followed by a daily dose of 75 mg/kg was used for the efficacy study in the Syrian hamster infection model.

Antiviral efficacy of zapnometinib in the Syrian hamster infection model

An *in vivo* study was designed to investigate the efficacy of zapnometinib administered p.o. in SARS-CoV-2-infected hamsters. In this efficacy study, all animals were challenged with 1×10^3 TCID₅₀ SARS-CoV-2 via intranasal administration, which induces both high levels of viral replication in the respiratory tract and significant histopathological changes in the lungs ²⁹. Control animals were treated with vehicle (5% DMSO, 15% Kolliphor in PBS) beginning at 4 h p.i., while the experimental animals were treated with the following regimen: one loading dose of 100 mg/kg zapnometinib (4 or 24 h p.i.) followed by 75 mg/kg zapnometinib once per day until the day of sacrifice (4 dpi). During the challenge phase, the animals were weighed,

clinical observations were monitored upon treatment and infection, and throat swabs were taken daily (Fig. 3a). At 4 dpi, all animals were euthanized for virological and pathological analyses. For the analysis of viral titers, throat swabs were analyzed by titration for the detection of replication competent virus and additionally subjected to TaqMan-based RT-qPCR for the detection of viral RNA to determine the viral copy number. At 2 dpi, the viral load in the throat swabs was comparable among all three treatment groups (Fig. 3b). At 3 dpi, the TCID₅₀ assay indicated that zapnometinib treatment of both treatment groups resulted in a significant reduction in the amount of replication-competent virus in the throat swabs, with reductions of 1.1 log₁₀ ($p = 0.0134$) and 1.3 log₁₀ ($p = 0.0191$) noted in the groups treated at 4 h p.i. and 24 h p.i., respectively (Fig. 3b, top row). These results were supported by the RT-qPCR data, which demonstrated viral titer reductions (number of viral copies) of 1.1 log₁₀ ($p = 0.0089$) and 1.2-log₁₀ ($p = 0.0090$) in animals treated at 4 h p.i. and 24 h p.i., respectively, compared to control animals (Fig. 3b, bottom row). By contrast, at 4 dpi, viral titers were still lower in the throat swabs of drug-treated animals, but the differences were not statistically significant (Fig. 3 b).

A similar pattern was observed for the infectious viral load in the nasal turbinates at 4 dpi. Treatment starting at 4 h p.i. significantly reduced the infectious viral titer by 1.3 log₁₀ ($p = 0.0335$). These data were further confirmed by RT-qPCR, which showed a reduction of 1.1 log₁₀ ($p = 0.0145$) when treatment was started at 4 h p.i. (Fig. 3c). However, there was no significant reduction in viral load when treatment was started at 24 h p.i. .

To gain further insight into how treatment affects disease progression beside the influence on viral load, the lung lesions of the infected animals were evaluated by gross pathology at 4 dpi, and the percentage of affected lung area was estimated. The percentage of lung injury in left lobe lung tissue was significantly reduced in both treated groups ($p = 0.0282$ for treatment beginning at 4 h p.i., $p = 0.0314$ for treatment beginning at 24 h p.i.) compared to the vehicle-treated group, indicating a beneficial effect of zapnometinib treatment (Fig. 3d). Next to the

gross pathology analysis of the lungs, a more detailed examination was performed. Histopathological evaluation of the lungs revealed that pulmonary changes were mostly mild in all treatment groups, however, inflammation was less severe in the zapnometinib-treated groups than in the vehicle-treated control group (Fig. 3f). Hamsters treated with the vehicle showed alveolitis with many inflammatory cells, including macrophages and neutrophils in the alveolar septa and lumina, hemorrhage and edema in the alveolar lumina and type II pneumocyte hyperplasia. Hamsters treated with zapnometinib exhibited reduced alveolitis with less cellular inflammatory infiltrate containing few macrophages and neutrophils and no hemorrhage, edema, or type II pneumocyte hyperplasia (Fig. 3f). Comparing the lungs of all treatment groups revealed that alveolar edema was less frequent in animals treated beginning at 4 h p.i. than in control animals and absent in animals treated beginning at 24 h p.i. Additionally, intra-alveolar hemorrhage, which is suggestive of epithelial damage, was comparable between animals treated beginning at 4 h p.i. and vehicle-treated animals, whereas it was absent in animals treated beginning at 24 h p.i. (Fig. 3g). The alveolar epithelium showed variable scoring of hyperplasia among the different treatment groups (type II hyperplasia), which is related to regeneration. Animals treated beginning at 4 h p.i. were less affected, and no hyperplasia was observed in animals treated beginning at 24 h p.i. (Fig. 3g).

In general, treatment was well tolerated in both groups, as no significant weight differences were observed among all groups; mean body weight loss was higher than 5% and comparable among all groups (ranging from 8-11%) (Fig. 3e). In addition, no treatment related adverse effects were observed in the zapnometinib treated groups compared to the vehicle-treated group. Taken together, these results demonstrate that zapnometinib treatment could significantly reduce viral load in the respiratory tract, alleviated lung pathology and was well-tolerated in the Syrian hamster SARS-CoV-2 infection model.

Levels of drug safety biomarkers

To further evaluate drug safety, serum samples collected pre- (0 dpi) and post-infection (4 dpi) and zapnometinib treatment were used for the assessment of candidate protein biomarkers indicating organ injury. Various candidate biomarkers were analyzed to assess drug-induced vascular injury (DIVI)³⁰ and drug-induced liver injury (DILI)^{30,31}. The levels of high mobility group box 1 (HMGB1; an indicator of necrosis)^{32,33} and glutamate dehydrogenase (GLDH; an indicator of liver damage)³⁴ were measured to assess liver injury (DILI), and p-selectin (SELP) was used as an early indicator of endothelial damage to blood vessels (DIVI). There were no prominent fold changes in the levels of the examined biomarkers between before and after infection and treatment of the animals, and the levels were comparable among all groups at each timepoint (Fig. 4). Collectively, these results provide further evidence that the selected treatment regimen (100 mg/kg zapnometinib followed by 75 mg/kg beginning at 4 or 24 h p.i.) was not toxic to hamsters.

Effect of zapnometinib on the levels of proinflammatory cytokines and chemokines

Beside demonstrating the antiviral efficacy, we also examined the potential immunomodulatory properties of zapnometinib. In a first approach, we compared the expression of cytokines and chemokines in serum samples from the hamsters of the efficacy study by ELISA, but there were no significant differences among all the groups after infection and treatment at 4 dpi (Supplementary Fig. 1). This was also generally observed in RT-qPCR analysis from lung tissue samples of the same animals at 4 dpi (Supplementary Fig. 2). Thus, the immune dampening effect of zapnometinib could not be shown by comparing serum and lung tissue cytokine and chemokine levels before (0 dpi) and after infection and treatment (4 dpi) in the Syrian hamster SARS-CoV-2 infection model. For this reason, we used an LPS-induced acute lung injury (ALI) mouse model to mimic the disease severity and to evaluate the anti-inflammatory properties of zapnometinib as well as its capacity to reduce cytokine storm. Using this virus-independent

model, we could demonstrate the direct effect of zapnometinib on cytokine and chemokine expression. Mice were challenged by i.p. injection of 5 mg/kg LPS and treated with 25 mg/kg zapnometinib after 1 h post-stimulation, and alterations in cytokine and chemokine gene expression patterns were evaluated by comparison with untreated control mice in an RT² profiler PCR array. As shown in a volcano plot, the expression of most of 84 genes that encode for key secreted proteins central to inflammation, like IL-1 β , IL-6, MCP-1 (CCL2), IL-8 (CXCL1), IP-10 (CXCL10) and MIP-1 α (CCL3), was downregulated after zapnometinib treatment compared to control treatment (Fig. 5a). Importantly, the levels of the *Ifna2* gene encoding for IFN- α , a key regulator of the innate antiviral immune response, were not significantly downregulated ($p = 0.118$). The expression of various important genes known to be elevated in COVID-19, such as *Il6*, *Cxcl1* and *Tnf*³⁵ was significantly reduced in the lung tissues of zapnometinib-treated mice (Fig. 5b).

In addition to the animal model, we also wanted to show the immunomodulatory properties of zapnometinib in a primary human cell type, namely freshly isolated human PBMCs. PBMCs isolated from two healthy donors were challenged with 1 μ g/mL LPS from *E. coli* O55:B5 (prepared in PBS) and treated with 10 μ g/mL zapnometinib (prepared in 0.1% DMSO) for 6 h. Cytokine and chemokine expression analysis by ELISA confirmed the anti-inflammatory properties of zapnometinib in human PBMCs. Expression levels of IL-1 β , IL-6, IL-8, IP-10, MCP-1, MIP-1 α , and TNF- α were significantly reduced upon treatment with zapnometinib for 6 h compared to the untreated control group (Fig. 5c, Supplementary Fig. 3). Taken together, experiments confirm the anti-inflammatory properties of zapnometinib *in vivo* and in primary human blood cells, showing significant reduction of cytokines and chemokines that are known to be elevated in COVID-19.

DISCUSSION

Recent cell culture studies provided first indications that the MEK inhibitor zapnometinib has anti-SARS-CoV-2 activity *in vitro* and downmodulated expression of candidate cytokines, suggesting that the drug may have a dual benefit in COVID-19. The present work aimed to evaluate whether these *in vitro* activities would also translate to an impact in an infected organism, indicating whether zapnometinib displays a dual therapeutic effect *in vivo*. Therefore, we analyzed antiviral efficacy as well as the immunomodulatory impact of zapnometinib in a well-established Syrian hamster SARS-CoV-2 infection model and in other models of hyperinflammatory infectious diseases. Given that *in vitro* activities of antiviral compounds are not always reflected *in vivo*, it is highly substantial that a dual effect of zapnometinib could be demonstrated *in vivo*, as evidenced by reduced levels of both viral RNA and infectious particles in hamster throat swabs and nasal turbinates, as well as an ameliorative effect on lung pathology. Moreover, by using drug-safety biomarkers it could be shown that zapnometinib was not toxic in the hamster model. All the results emphasize the therapeutic potential of zapnometinib against coronavirus infections correlated with hyperinflammatory symptoms. Zapnometinib, a host targeted antiviral (HTA) inhibits the cellular Raf/MEK/ERK signaling pathway that is pivotal for replication of various viruses³⁶. As by February 2022, nirmatrelvir, molnupiravir and remdesivir are considered for treatment against COVID-19^{16,37-39}. In the Syrian hamster model of SARS-CoV-2 infection, zapnometinib showed a significant effect on viral load reduction 3 dpi (Fig. 3b) comparable to that of the orally administered nucleoside analog molnupiravir³⁷. Furthermore, zapnometinib treatment resulted in a reduction in lung pathology, indicating therapeutic potential. We do not expect zapnometinib, as a host targeted antiviral agent, to exert mutagenic effects on the virus. The mutagenic potential of nucleoside analog antivirals such as remdesivir or molnupiravir in the treatment of viral diseases raises concerns about enhancing viral evolution. This should not occur with host targeted antivirals since they do not interfere with viral RNA replication. Another major advantage of host targeted antivirals is a high barrier towards the emergence of viral resistance since the virus cannot easily

replace the missing cellular function. Finally, since viral dependence on cellular factors should not rapidly change during pathogen evolution, activity should be preserved also against new and upcoming variants. In line with the claim, it is of importance to stress that zapnometinib was also active against other highly pathogenic human SARS-CoV viruses, such as SARS-CoV-1 and MERS-CoV, as well as all tested variants of SARS-CoV-2 (Fig. 1).

A major emphasis of the study was to investigate the impact of the immunomodulatory effect of zapnometinib *in vivo*, as a second beneficial activity beside its antiviral action. We did not observe a significant difference in cytokine and chemokine expression at 4 dpi in the SARS-CoV-2 infected Syrian hamster, suggesting that this model may not be suited to represent hyperinflammatory courses of COVID-19. This assumption is in line with a previous study reporting that in the SARS-CoV-2-challenged Syrian hamster model, the levels of the cytokines and chemokines increase only very early after infection in lung tissue and only to a small extent in the serum ⁴⁰. Therefore, we selected an *in vivo* model that on the one hand represents a fulminant hyperinflammatory disease and on the other hand is independent of the dynamics of pathogen infection. The latter model feature is important to discriminate between direct anti-inflammatory activities or indirect activities primarily due to the reduction of pathogen titers. Acute lung injury (ALI) is an inflammatory condition caused by pathogen infection, sepsis, trauma, ischemia, or reperfusion. The most severe form of ALI is termed acute respiratory distress syndrome (ARDS), which is also a clinical feature in severe COVID-19 ⁴¹. The excessive inflammatory response in the ALI model was induced by bacterial LPS stimulation. Zapnometinib treatment resulted in a massive reduction of hyperinflammatory cytokine responses, indicating the immunomodulatory potency of the drug *in vivo*.

In addition, we analyzed zapnometinib potency in a primary human cell model, namely freshly isolated PBMCs. Cytokine reduction was also observed in PBMCs, demonstrating a similar immunomodulatory effect. These studies emphasize the important anti-inflammatory potential of zapnometinib.

Dexamethasone, a glucocorticoid that is currently used to treat severe COVID-19, inhibited cytokines production, mainly by interfering with NF- κ B signaling in animal models of LPS-induced ALI and of human PBMCs^{42,43}. Dexamethasone also regulates type I interferons (IFNs), the first line of defense against severe viral respiratory infections^{41,44,45}. Tocilizumab and baricitinib, two immunomodulatory compounds recommended to treat severe COVID-19, also demonstrated efficacy in dampening various cytokines and chemokines in ALI animal models and in human PBMCs. A clear influence on interference with type I IFN could not be demonstrated. Since both drugs lead to inhibition of NF- κ B, an influence on type I IFN can be assumed since NF- κ B is a major regulator of the IFN-beta enhanceosome⁴⁶. In contrast to the NF- κ B cascade, interfering with the Raf/MEK/ERK signaling pathway will not dampen type I IFNs and downstream IFN regulated genes as shown in this report and in our previous work²⁴.

The studies conducted here show that zapnometinib, in comparison to dexamethasone, tocilizumab and baricitinib, showed a comparable immunomodulatory potential in animal models and in human PBMCs. This illustrates the advantage of MEK-inhibitors dampening the proinflammatory cytokine response that is the cause of severe hyperinflammation, but not the innate antiviral IFN response that is essential as a first line of defense against virus infection. Further clinical studies are needed to confirm these findings.

Overall, the study illustrates that zapnometinib might have great therapeutic potential with a novel approach and broad efficacy. This represents a clear benefit over current therapies. The standard therapies target either the virus itself or the hyperinflammatory response. Zapnometinib, by inhibiting the Raf/MEK/ERK signaling pathway, demonstrates a dual effect, namely viral load reduction and dampening of the pro-inflammatory cytokine and chemokine response. This discriminates the drug from most if not all COVID-19 drugs licensed or under development.

A phase 2 clinical trial of zapnometinib in hospitalized COVID-19 patients is ongoing (NCT04776044). Its immunomodulatory effect could make zapnometinib superior to other

antiviral drugs in the treatment of hospitalized patients, in whom the inflammatory response has a strong influence on disease development. Because MEK inhibition leads to modulation rather than full suppression of the immune response^{47,48}, zapnometinib might also be effective against post-COVID syndromes associated with SARS-CoV-2 infection. In line with our previous studies²⁴, our results presented here underline the value of zapnometinib alone or in combination with other drugs that act at different stages of viral infection and disease progression, which could be the best possible strategy for controlling the disease and might be an important step forward in the current COVID-19 pandemic. On top of that, zapnometinib may still have other implications in infectious diseases. The drug recently received an orphan drug designation for the treatment of hantavirus infections by the FDA and a phase 2 clinical trial against influenza is in preparation. This demonstrates the potential of zapnometinib as a broad acting anti-infective compound against acute viral infections.

FIGURE LEGENDS

Figure 1: **Zapnometinib inhibits different coronaviruses *in vitro*.** The dose-dependent inhibitory effect of zapnometinib (100, 75, 50 and 25 μ M) compared to a control (virus-infected cells, not treated) was analyzed using a Virospot reduction assay (four replicates per concentration). The calculated EC₅₀ obtained through this assay are shown in Table 1. Data were obtained after 48 h of incubation in 3% serum for all viruses except for SARS-CoV-1 (24 h). **a** SARS-CoV-1 **b** MERS-CoV **c** SARS-CoV-2 BavPat1 **d** SARS-CoV-2 Beta **e** SARS-CoV-2 Alpha. The data are presented as the mean + SD. The data were analyzed by one-way ANOVA. * $p < 0.05$, **** $p < 0.0001$.

Figure 2: **Pharmacokinetic analysis of zapnometinib in Syrian hamsters.** Three different doses (15, 30 and 60 mg/kg) of zapnometinib were administered orally to Syrian hamsters (n =

6 per group), and serum was collected 0.5, 1, 2, 4, 8, 12 and 24 hours later (alternately from 3 animals per group). The zapnometinib concentration in the serum was determined by HPLC-MS/MS. The data are presented as mean serum concentration vs. time profiles. The dashed line shows the critical plasma level for MEK inhibition (approx. 10 µg/mL).

Figure 3: Study of the efficacy of zapnometinib against SARS-CoV-2 in a Syrian hamster

infection model. a Study schedule. On day 0, all animals were infected intranasally with 1×10^3 TCID₅₀ SARS-CoV-2. zapnometinib (100 mg/kg) was given beginning 4 h p.i. or 24 h p.i. Thereafter, the animals in both groups were treated with 75 mg/kg zapnometinib once daily until sacrifice. The control group received vehicle (5% DMSO, 15% Kolliphor, 80% PBS) starting 4 h p.i. Body weight was measured, and throat swabs were taken daily. At 4 dpi, all animals were sacrificed. Nasal turbinate tissues were subsequently homogenized and subjected to virus titration. Blood was collected for further analysis, the lungs were inspected, and the percentage of affected lung tissue was estimated from the dorsal view. The left lung lobe was collected for histopathological examination. The study schedule was created on biorender.com.

b Infectious viral titers in throat swabs collected at 2, 3 and 4 dpi expressed as log₁₀ TCID₅₀ of control (vehicle-treated) and zapnometinib-treated SARS-CoV-2-infected hamsters (upper row) or expressed as genome copies per g determined by RT-qPCR (bottom row). **c** Infectious viral loads in nasal turbinates collected from control (vehicle-treated) and zapnometinib-treated SARS-CoV-2-infected hamsters at 4 dpi expressed as log₁₀ TCID₅₀ per g nasal turbinate tissue analyzed via the TCID₅₀ assay (left) or as copies per g determined by PCR (right). The dashed lines represent the lower limit of detection (0.8 log₁₀ TCID₅₀/mL for titration and 1.9 log₁₀ CP/mL for RT-qPCR). **d** Percentage of lung area with pathological signs in control (vehicle-treated) and zapnometinib-treated SARS-CoV-2-infected hamsters at 4 dpi. **e** Weight loss at 4 dpi as a percentage of the body weight measured at the time of infection. Individual data

(dots) and median values (horizontal lines) are presented. The bars represent the min. and max. values. **f** Histopathologic changes at 4 dpi in the lungs of hamsters infected with SARS-CoV-2 and treated with zapnometinib or treated with the vehicle only. Hamsters treated with the vehicle showed alveolitis with hemorrhage (arrowheads) and edema (*) in the alveolar lumina and type II pneumocyte hyperplasia (arrows) (A and C). Hamsters treated with zapnometinib exhibited reduced alveolitis with less cellular inflammatory infiltrate and no hemorrhage, edema, or type II pneumocyte hyperplasia (B and D). Representative images for each group. Hematoxylin and eosin staining; 40x magnification (A and C) and 200x magnification (B and D). **g** Lung slides were examined and scored for the presence of alveolar hemorrhage, edema and type II pneumocyte hyperplasia (0 = no, 1 = yes). Mean and individual values are presented. All data are from a single experiment. The number of animals per group was as follows: $n_{\text{(Vehicle)}} = 6$; $n_{\text{(Treatment +4 h p.i.)}} = 6$; $n_{\text{(Treatment +24 h p.i.)}} = 5$. The data were analyzed by one-way ANOVA. * $p < 0.05$.

Figure 4: Candidate safety biomarker analysis. The levels of candidate biomarkers in serum samples from hamsters collected before infection with SARS-CoV-2 (0 dpi, prior to infection and zapnometinib treatment) and at 4 dpi and after zapnometinib treatment were analyzed by IA-LC-MS/MS. High mobility group box 1 protein (HMGB1, an indicator of necrosis), glutamate dehydrogenase (GLDH, an indicator of liver damage), and p-selectin (SELP, an indicator of endothelial damage) levels in the zapnometinib-treated and vehicle-treated groups are shown. The treatment conditions described below the graphs refer to the treatment that the animals received after infection. Individual data and median values are presented. The number of animals per group was as follows: $n_{\text{(Vehicle)}} = 6$; $n_{\text{(Treatment +4 h p.i.)}} = 6$; $n_{\text{(Treatment +24 h p.i.)}} = 5$. One data point from the vehicle-treated group is not depicted in the graph showing GLDH levels (4 dpi) because it was outside of the axis limit (252 ng/mL). The data were analyzed by one-way ANOVA. P values < 0.05 were considered statistically significant.

629

630 **Figure 5: The levels of cytokines and chemokines associated with COVID-19 are reduced**
631 **after treatment with zapnometinib** **a, b** mRNA expression of different cytokines and
632 chemokines in acute lung injury (ALI) model mice after LPS (5 mg/kg) stimulation and
633 zapnometinib treatment (25 mg/kg) 1h post stimulation, while the mice in the control group
634 were treated with vehicle (1% DMSO in PBS). The mRNA levels were determined at 6 h post
635 treatment by an RT² profiler PCR array. **a** The volcano plot shows genes that showed significant
636 expression changes. Representative genes mainly associated with COVID-19 were highlighted.
637 The log₂ of the fold change in gene expression is plotted on the x-axis, and statistical
638 significance is plotted on the y-axis. The two dashed vertical lines indicate the fold change
639 threshold, while the horizontal line indicates the p value threshold. Regardless of statistical
640 significance, red and blue indicate upregulation and downregulation, respectively, while black
641 indicates no change in gene expression. Genes represented by data points in the far upper left
642 (downregulated) and far upper right (upregulated) sections met the fold change and p value
643 thresholds, while the genes represented by the data points between the two vertical lines showed
644 no change in gene expression. **b** Representative expression. Mean values + SD are shown. The
645 data were analyzed using the $\Delta\Delta C_t$ method and normalized to the mean expression levels of 5
646 housekeeping genes. The P values for the difference in replicate (-DeltaCt) expression data for
647 each gene between the control and treatment groups were calculated by Student's t-test. ** $p <$
648 0.01 , **** $p < 0.0001$. **c** Reduction in cytokine expression after zapnometinib treatment in
649 LPS-stimulated human PBMCs from one healthy donor. Cells were simultaneously stimulated
650 with 1 $\mu\text{g/mL}$ LPS and treated with 10 $\mu\text{g/mL}$ zapnometinib for 6 h. The expression of cytokines
651 in the supernatants was analyzed by ELISA. The data are presented as the mean $\pm$ SD of
652 technical replicates. $n_{(\text{IL-1}\beta; \text{IL-6; MCP-1; MIP-1}\alpha; \text{TNF-}\alpha)} = 6$; $n_{(\text{IP-10})} = 9$; $n_{(\text{IL-8 control})} = 5$; $n_{(\text{IL-8 zapnometinib-treated})} = 6$. The
653 differences in the levels of all cytokines of interest between the control and zapnometinib-

treated groups were analyzed with unpaired t-test with Welch's correction. $** p < 0.01$; ****
 $p < 0.0001$.

T-values and degrees of freedom: IL-1b: $t=25.08$, $df=3.509$. IL-6: $t=18.61$, $df=2.036$. IL-8:
 $t=8.733$, $df=2.001$. IP-10: $t=15.95$, $df=2.049$. MCP-1: $t=56.76$, $df=2.305$, MIP-1a: $t=10.04$,
 $df=2.012$. TNF-a: $t=7.261$, $df=3.771$ (ALI mouse model).
IL-1b: $t=12.11$, $df=9.089$. IL-6: $t=15.20$, $df=9.996$. IL-8: $t=5.024$, $df=4.783$. IP-10: $t=13.26$,
 $df=11.01$. MCP-1: $t=14.22$, $df=5.028$, MIP-1a: $t=14.75$, $df=9.941$. TNF-a: $t=4.544$, $df=6.154$
(PBMCs).

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AUTHOR CONTRIBUTIONS

Conceptualization: Y.F., SL and O.P.; methodology: G.A., H.H., L.W., L.dW.; formal analysis:
Y.F., O.P., L.dW., H.H., L.W., A.S., S.S.; investigation: H.H., L.W., L.dW.; histology: JvdB.,
M.D. resources: O.P.; writing – original draft: Y.F. and O.P.; writing – review & editing:
H.H., L.W., O.Poe., V.A., A.S., L.dW., JvdB., Y.F., SL and O.P.; visualization: H.H., L.W.,
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COMPETING INTERESTS

O. Planz has an ownership interest in Atriva Therapeutics GmbH and is a consultant for Atriva Therapeutics GmbH. Y. Füll and L. Waidele are employees of Atriva Therapeutics GmbH.

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Figure 1: *in vitro* efficacy

Figure 2: Pharmacokinetics

Figure 3: Efficacy study

a

b

Throat swab viral titer (TCID₅₀)

Throat swab viral titer (RT-qPCR)

c

Nasal turbinates titer (RT-qPCR)

d

Lung injury

e

Body weight loss (%)

f

g

Figure 4: Biomarker

4 dpi

HMG B1

SELP

GLDH

idp 0

HMG B1

SELP

GLDH

Figure 5: ALI Mouse Model

Figure 5: ALI mouse

b

Figure 5: Cytokines PBMCs

C

Supplementary Files

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