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ARTICLE

Phase I study in healthy participants to evaluate safety, tolerability, and pharmacokinetics of inhaled nezulcitinib, a potential treatment for COVID-19

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Abstract

Nezulcitinib (TD-0903), a lung-selective pan–Janus-associated kinase (JAK) inhibitor designed for inhaled delivery, is under development for treatment of acute lung injury associated with coronavirus disease 2019 (COVID-19). This two-part, double-blind, randomized, placebo-controlled, single ascending dose (part A) and multiple ascending dose (part B) phase I study evaluated the safety, tolerability, and pharmacokinetics (PK) of nezulcitinib in healthy participants. Part A included three cohorts randomized 6:2 to receive a single inhaled dose of nezulcitinib (1, 3, or 10 mg) or matching placebo. Part B included three cohorts randomized 8:2 to receive inhaled nezulcitinib (1, 3, or 10 mg) or matching placebo for 7 days. The primary outcome was nezulcitinib safety and tolerability assessed from treatment-emergent adverse events (TEAEs). The secondary outcome was nezulcitinib PK. All participants completed the study. All TEAEs were mild or moderate in severity, and none led to treatment discontinuation. Overall (area under the plasma concentration-time curve) and peak (maximal plasma concentration) plasma exposures of nezulcitinib were low and increased in a dose-proportional manner from 1 to 10 mg in both parts, with no suggestion of clinically meaningful drug accumulation. Maximal plasma exposures were below levels expected to result in systemic target engagement, consistent with a lung-selective profile. No reductions in natural killer cell counts were observed, consistent with the lack of a systemic pharmacological effect and the observed PK. In summary, single and multiple doses of inhaled nezulcitinib at 1, 3, and 10 mg were well-tolerated in healthy participants, with dose-proportional PK supporting once-daily administration.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There are a number of investigations of orally delivered Janus-associated kinase (JAK) inhibitors for the treatment of coronavirus disease 2019

(COVID-19)–associated cytokine storm. The systemic JAK inhibitor baricitinib appears to be effective for treatment of COVID-19. An inhaled pan-JAK inhibitor for local delivery to the lung, nezulcitinib, was in preclinical development at the start of the COVID-19 pandemic but had not yet been tested in humans.

WHAT QUESTION DID THIS STUDY ADDRESS?

This first-in-human study addresses the safety and pharmacokinetics (PK) of nezulcitinib in healthy participants, and the appropriateness to move nezulcitinib forward in clinical trials in patients with COVID-19 infection.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Nezulcitinib was generally well-tolerated in healthy participants; adverse events were mild or moderate in severity, and none resulted in study discontinuation. The PK of nezulcitinib were consistent with preclinical results suggesting a lung-selective profile and support once-daily dosing.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These results informed an ongoing phase II clinical trial of nezulcitinib for treatment of acute lung injury associated with COVID-19 in symptomatic patients (NCT04402866).

INTRODUCTION

Respiratory complications are a major driver for disease severity and mortality associated with coronavirus disease 2019 (COVID-19).[1] Patients with severe COVID-19 suffer acute lung injury involving diffuse alveolar damage, tissue inflammation, coagulopathy, and microvascular thrombosis; these pathophysiological features cause severe hypoxemia.[2–4] A characteristic feature of severe COVID-19 is uncontrolled production of inflammatory cytokines, causing a "cytokine storm" (cytokine release syndrome [CRS]).[5] Cytokines detected at higher levels in patients with severe COVID-19 relative to those with less severe disease include interleukin (IL)-6, granulocyte colony-stimulating factor, interferon gamma-induced protein 10, monocyte chemotactic protein 1, macrophage inflammatory protein 1 alpha, tumor necrosis factor alpha, IL-10, IL-7, and IL-2.[6,7] CRS causes high levels of systemic inflammation associated with multi-organ dysfunction syndrome.[8]

Many cytokines involved in CRS bind to cell surface cytokine receptors that signal through Janus kinases (JAKs), a family of receptor-associated tyrosine kinases (Tyks).[9] Activation of JAK-signal transducer and activator of transcription (STAT) signaling pathways promotes complex biological functions, such as cell proliferation and differentiation, oxidative stress, and immune regulation.[9] Therefore, pharmacological inhibition of JAK-STAT signaling may address the heterogeneous inflammation associated with COVID-19–related lung injury by reducing production of a broad range of cytokines. In support of this hypothesis, treatment with the JAK inhibitor baricitinib in combination with the antiviral remdesivir significantly shortened recovery time and improved clinical status at day 15 in a randomized controlled trial in patients with COVID-19.[10]

Nezulcitinib (TD-0903) is a lung-selective pan-JAK inhibitor specifically designed for inhaled delivery. Nezulcitinib demonstrated potent inhibitory activity at purified, isolated kinase domains across all human JAK family members (negative log-transformed inhibition constant [pK_i] values of 10.3, 10.6, 10.2, and 9.2 for JAK1, JAK2, JAK3, and Tyk2, respectively; data on file, Theravance Biopharma). Consistent with potent JAK inhibition observed in biochemical assays, nezulcitinib inhibits cytokine-induced STAT6 phosphorylation in bronchial epithelial cell lines (data on file, Theravance Biopharma). Nezulcitinib exhibits high lung-to-plasma exposure (area under the plasma concentration-time curve from time point 0 to the end of the dosing interval [$AUC_{0\text{-}t}$]) ratios of 58:1, 172:1, and 850:1 following single dose administration by oral aspiration in mouse, inhalation in rat, and inhalation in dog, respectively (data on file, Theravance Biopharma; see Supplementary Materials for additional information), thus demonstrating higher total concentrations in lung tissue compared with plasma. The preclinical profile of nezulcitinib is consistent with a lung-selective agent, with potential to allow for a high level of target engagement in the lungs while minimizing systemically mediated adverse effects. Thus, inhaled nezulcitinib may offer a unique therapeutic profile in the effort to dampen the cytokine storm in COVID-19 by targeting multiple cytokine pathways in the lungs and limiting progression of acute lung injury.

The aim of this study was to evaluate the safety, tolerability, and pharmacokinetics (PK) of nezulcitinib in a first-in-human phase I single ascending dose (SAD) and multiple ascending dose (MAD) study in healthy participants.

METHODS

Study design

This was a phase I, two-part, double-blind, randomized, placebo-controlled SAD (part A) and MAD (part B) study (Figure 1). Each participant was allowed in only one cohort in only one study part. The study participants, site investigators, and site study staff were blinded to the study throughout the study duration. The decision to proceed to administration of the next higher dose was made by the principal investigator and study sponsor.

Dose selection and the starting dose of 1 mg were based on preclinical toxicity data with consideration of the anticipated pharmacologically active dose. The maximum recommended starting dose based on repeat-dose inhalation toxicity studies in the most sensitive species (rat) was 10.8 mg, and an ~ 10-fold lower dose (1 mg) was selected as the starting dose for this trial. The dose range of 1 to 10 mg was anticipated to result in target engagement exceeding 50% in the lungs of humans based on a translational PK/pharmacodynamic modeling approach. The predicted human lung PK profile following inhalation dosing was projected using an allometrically scaled animal compartmental model fitted to lung and plasma PK data collected following the i.v. and inhalation administration of nezulcitinib in the rat and dog.[11] The projected dose range was determined based on the predicted exposure range required to maintain an average free lung concentration of nezulcitinib equal to the half-maximal inhibitory concentration (IC50), as determined in a mouse model of JAK inhibition, and assuming that the pharmacology of JAK inhibition in the mouse lung was also present in humans (see Supplementary Materials for additional information).

The SAD study (part A) enrolled three ascending dose cohorts of eight healthy participants each (6 active and 2 placebo), with each participant receiving a single inhaled dose of nezulcitinib 1, 3, or 10 mg, or matching placebo. Each cohort included a sentinel group of two participants (1 active and 1 placebo) who were dosed at least 24 h before the remaining six participants (5 active and 1 placebo); the remaining participants were only to be dosed if there were no clinically significant safety or tolerability concerns in the sentinel group.

The MAD study (part B) enrolled three ascending dose cohorts of 10 healthy participants each (8 active and 2 placebo). In each cohort, participants received inhaled doses of nezulcitinib 1, 3, or 10 mg, or matching placebo once daily for 7 days.

Throughout the study, all doses were delivered via the PARI eFLow nebulizer system. In vitro aerosol characterization using a cascade impactor for nezulcitinib in the PARI eFlow nebulizer system resulted in an emitted fine particle dose of 40% of the nominal dose across a dose

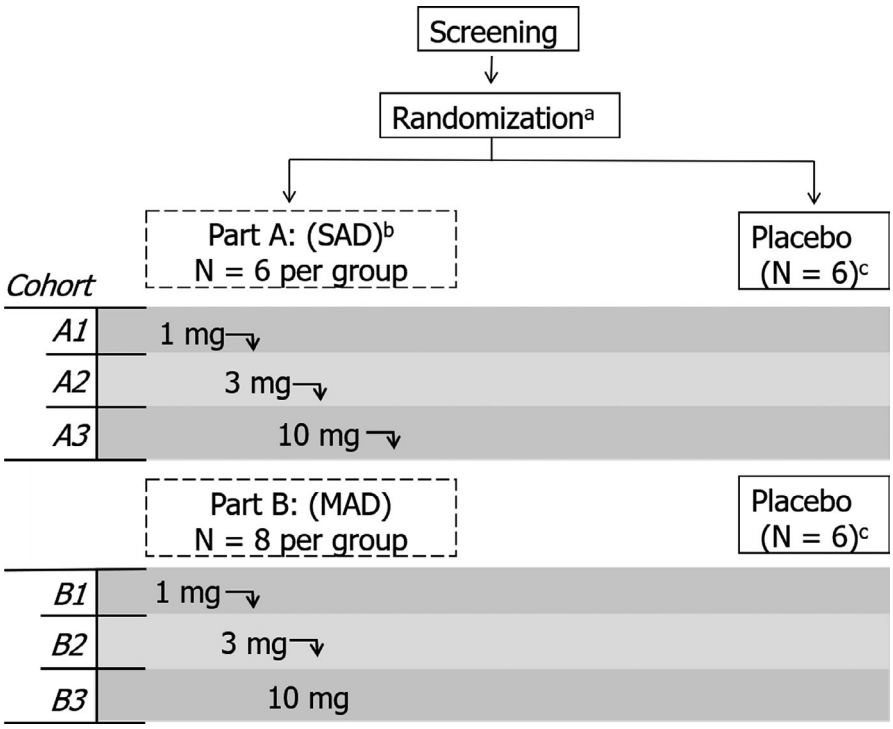

FIGURE 1 Study design overview. (a) Randomization to active or placebo was performed for each cohort. (b) Each cohort in the SAD portion of the study included a sentinel group of two subject, one active and one placebo, (c) $N = 2$ placebo subjects per cohort. MAD, multiple ascending dose; SAD, single ascending dose

range of 0.1–10 mg of nezulcitinib. This is the portion of the dose leaving the nebulizer device that is theoretically available for inhalation and deposition in the airways.

The investigators and/or study sponsor could stop the study at any time in the interest of participant welfare or based on prespecified stopping criteria. The following criteria were prespecified to guide decisions to stop dosing in the study: (1) a clinically or medically significant nezulcitinib-related serious or severe adverse event (AE) experienced by greater than or equal to 1 participant; (2) a moderate nezulcitinib-related AE in the same organ system class in greater than or equal to 2 participants in the same cohort; or (3) nezulcitinib systemic exposure in greater than or equal to 1 participant was predicted to exceed the mean AUC associated with the no observed adverse effect level in rats.

The study was conducted at a single site—the Medicines Evaluation Unit, Manchester, UK. The study protocol and its amendments were reviewed and approved by an independent research ethics committee prior to study initiation, the North West – Greater Manchester Central Research Ethics Committee. The study was conducted in accordance with the principles in the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practices guidelines, and is registered on ClinicalTrials.gov (identifier: NCT04350736) and the European Union Clinical Trials Register (identifier: EudraCT No.: 2020-000577-24). All participants provided written informed consent before starting the trial.

Study population

Male or nonpregnant female healthy participants aged 18 to 50 years with a body mass index between 18 and 32 kg/m^2 (and weighing ≥50 kg) and a forced expiratory volume in 1 s (FEV1) of greater than or equal to 80% predicted were eligible for the study. Key exclusion criteria were history or presence of any clinically significant medical or psychiatric condition; abnormal electrocardiogram (ECG) measurements; personal or family history of congenital long QT syndrome or sudden death; resting supine bradycardia (<40 beats per minute [bpm]) or tachycardia (>100 bpm); history or symptoms of clinically relevant neurologic disease; history of cancer; respiratory tract infection in the previous 6 weeks; excessive alcohol or caffeine consumption; history of drug hypersensitivity; positive results for human immunodeficiency virus, hepatitis, or COVID-19; or recent blood or bone marrow donation.

Individuals underwent a telephone screen for COVID-19 exposure before the scheduled visit for study screening. All participants were required to have a 14-day quarantine period prior to clinical research unit residency.

Participants who completed the initial screening visits were admitted to the clinical research unit 2 to 3 days before study initiation for COVID-19 testing and remained in residence through day 4 (part A) or day 9 (part B) following completion of study assessments. All participants who received greater than or equal to 1 dose of study drug returned to the clinical research unit 1 week (±2 days) after the last study drug administration for follow-up procedures, including AE assessment.

Assessments

The primary outcome was safety and tolerability of nezulcitinib. Safety was assessed throughout the study (i.e., physical examinations, vital signs, 12-lead safety ECGs, spirometry, blood and urine samples for clinical laboratory tests, and treatment-emergent [TE] AEs). AEs were coded using preferred terms from the most current version of the Medical Dictionary for Regulatory Activities. The primary safety end point was the number and severity of TEAEs following single (part A) or multiple (part B) inhaled doses of nezulcitinib and placebo.

The secondary outcome measure was nezulcitinib PK. Blood and urine samples were collected for nezulcitinib PK assessment predose and for 72 h postdose in part A. In part B, serial blood samples were collected through 24 h following the first dose on day 1 and predose through 48 h following dosing on day 7; blood samples for assessment of trough plasma concentrations were also collected predose on the morning of days 3 through 6 from participants in all part B cohorts (see Supplementary Materials). Urine collection and calculation of PK parameters for urine nezulcitinib were performed following a single inhaled dose in part A only. PK parameters were estimated using Phoenix WinNonlin (Certara, Inc; version 8.10; see Supplementary Materials for list of specific PK parameters). Natural killer (NK) cell counts were quantitated by flow cytometry using the Beckman Coulter AQUIOS CL Flow Cytometer System in combination with the AQUIOS Tetra reagents for lymphocyte subset analysis.[12]

Statistical analysis

The study sample size was based on clinical considerations for phase I studies; due to the exploratory nature of this study, no formal power or hypothesis testing was considered to determine sample size. The PK analysis set included all participants who received greater than or equal to 1 dose of study drug and had one evaluable PK

profile, and the safety analysis set included all participants who received greater than or equal to 1 dose of study drug. Demographics, safety, and PK data were summarized descriptively for placebo and nezulcitinib using SAS software (SAS Institute).

RESULTS

Participant demographics and baseline characteristics

Overall, 24 participants were randomized in part A (6 placebo and 18 nezulcitinib; 6 each at single doses of 1, 3, and 10 mg) and 30 participants were randomized in part B (6 placebo and 24 nezulcitinib; 8 each at doses of 1, 3, and 10 mg once daily for 7 days). The majority of participants in part A and part B were men (88% and 80%) and White (100% and 93%), with mean ages of 35.6 and 32.7 years, respectively (Tables S1–S2). Other baseline characteristics, including weight and body mass index (BMI), were generally balanced across cohorts. All participants completed the study and were included in the evaluation of safety and PKs.

Safety

Overall, nezulcitinib was generally well-tolerated as a single inhaled dose (Table 1) and as single daily doses up to 10 mg for 7 days in healthy participants (Table 2). No severe or serious AEs were reported. All TEAEs were assessed as mild or moderate in severity, and none led to discontinuation of study treatment. No clinically significant changes from baseline were observed in vital sign and ECG assessments; similarly, there were no clinically significant changes in chemistry or hematology laboratory parameters, with the exception of a mild AE of asymptomatic elevation in liver function tests in one participant receiving 10 mg nezulcitinib in part B. The participant's alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were slightly above the upper limit of normal (ULN) prior to dosing on day 1 and values increased to 3.8 × ULN (ALT) and approximately 2.8 × ULN (AST) by day 9. The elevations in ALT and AST trended downward from day 9 through follow-up (day 14) and had returned to baseline (screening) values at a repeat laboratory visit (day 19); therefore, the investigator considered the AE resolved. No abnormality in bilirubin, alkaline phosphatase, or albumin was observed in this participant during the study. However, the participant did show signs of emerging hyperlipidemia during the treatment period in conjunction with consumption of a pro-inflammatory diet (processed sugars/carbohydrates) and weight gain while in-residence, and the event of elevated transaminases was deemed not to be related to the study drug.

After a single dose of nezulcitinib or placebo (part A), 15 TEAEs were reported in 10 (42%) participants, with similar rates of TEAEs in participants receiving nezulcitinib and placebo (Table 1). The majority of AEs were mild in severity (1 participant had 1 moderate AE of headache); all resolved during the study, and none were determined by the investigator to be drug-related. The most frequently reported TEAEs were dizziness, headache, and cough reported by three (13%), two (8%), and two (8%) participants each, respectively; all other TEAEs were reported by one participant each.

After 7 days of once-daily nezulcitinib or placebo (part B), 41 TEAEs were reported in 17 (57%) participants, with

TABLE 1 Treatment-emergent adverse events, part A

	Nezulcitinib					
	1 mg ($n = 6$)	3 mg ($n = 6$)	10 mg ($n = 6$)	Total ($n = 18$)	Placebo ($n = 6$)	Overall ($N = 24$)
Total participants with TEAEs[a]	4 (67)	0	2 (33)	6 (33)	4 (67)	10 (42)
Moderate or severe TEAEs[a]	0	0	0	0	0	0
TEAE related to nezulcitinib[a]	0	0	0	0	0	0
All TEAEs in >1 participant						
Dizziness	1 (17)	0	1 (17)	2 (11)	1 (17)	3 (13)
Headache	1 (17)	0	0	1 (6)	1 (17)	2 (8)
Cough	1 (17)	0	1 (17)	2 (11)	0	2 (8)

Note: Data are presented as *n* (%).

Abbreviation: TEAEs, treatment-emergent adverse events.

[a]Percent is based on total number of participants dosed.

TABLE 2 Treatment-emergent adverse events, part B

	Nezulcitinib					
	1 mg ($n = 8$)	3 mg ($n = 8$)	10 mg ($n = 8$)	Total ($n = 24$)	Placebo ($n = 6$)	Overall ($N = 30$)
Total participants with TEAEs[a]	4 (50)	3 (38)	6 (75)	13 (54)	4 (67)	17 (57)
Moderate or severe TEAEs[a]	0	0	2 (25)	2 (8)	1 (17)	3 (10)
TEAE related to nezulcitinib[a]	0	0	1 (13)	1 (4)	0	1 (3)
All TEAEs in >1 participant						
Cough	0	2 (25)	3 (38)	5 (21)	0	5 (17)
Dizziness	1 (13)	1 (13)	1 (13)	3 (13)	1 (17)	4 (13)
Headache	1 (13)	3 (38)	0	4 (17)	0	4 (13)
Medical device site reaction	0	0	1 (13)	1 (4)	1 (17)	2 (7)
Lethargy	1 (13)	0	0	1 (4)	1 (17)	2 (7)

Note: Data are presented as n (%).

Abbreviation: TEAEs, treatment-emergent adverse events.

[a]Percent is based on total number of participants dosed.

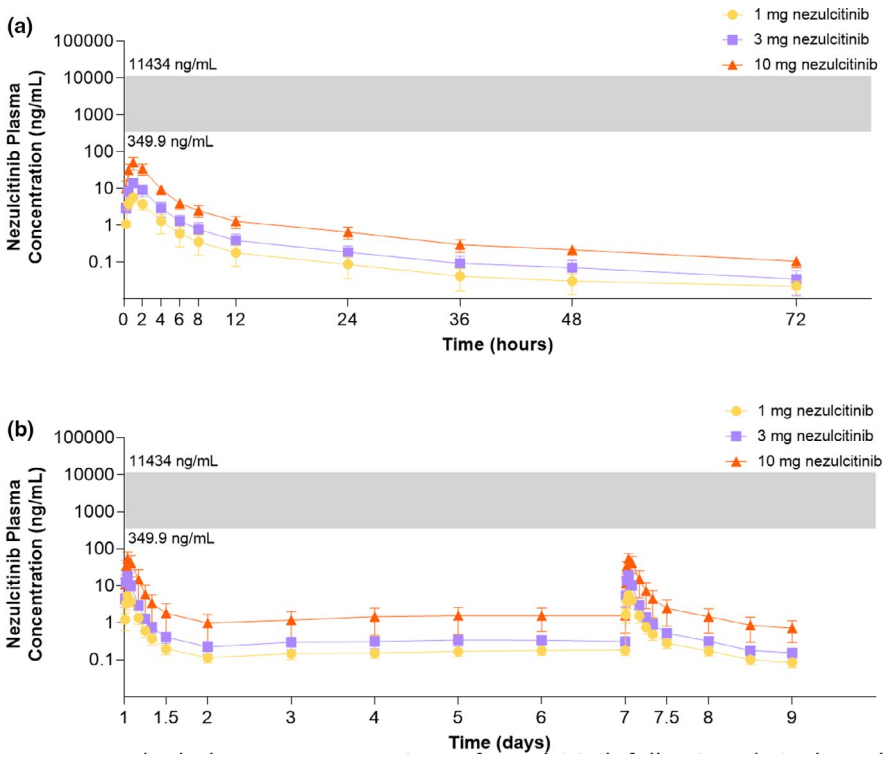

FIGURE 2 Mean (SD) plasma concentrations of nezulcitinib following (a) single and (b) multiple inhaled doses of 1 mg (yellow), 3 mg (purple), and 10 mg (orange). Data are shown as mean ± SD. Shaded grey box reflects the range of inhibitory potency demonstrated by nezulcitinib against cytokine-induced STAT phosphorylation in human immune and bronchial epithelial cells. STAT, signal transducer and activator of transcription

similar rates of TEAEs in participants receiving nezulcitinib and placebo (Table 2). The majority of AEs were mild in severity (4 participants had 5 moderate AEs of stiff neck, headache, nausea, toothache, and vomiting), all resolved during the study, and only one AE was determined by the investigators to be drug-related (tickly cough in the 10 mg group). The most frequently reported TEAEs were cough (5 [17%] participants), dizziness (4 [13%] participants), headache (4 [13%] participants), lethargy (2 [7%] participants), and ECG electrode rash (2 [7%] participants); all other TEAEs were reported by one participant each.

Pharmacokinetics

In general, following single and multiple doses of nezulcitinib, plasma concentrations of nezulcitinib demonstrated rapid absorption, with a time to maximum concentration (T_{max}) of ~ 1 h, then declined in a biphasic manner with a terminal elimination half-life of ~ 24 h. For part A, mean (SD) plasma exposures (C_{max} and $AUC_{0\text{-}t}$) of nezulcitinib following a single dose (Figure 2a) increased proportionally with increasing doses of nezulcitinib (1 mg, 3 mg, and 10 mg).

Geometric mean C_{max} values at 1 h following inhaled single doses of nezulcitinib 1, 3, and 10 mg were 5.5, 13.8, and 47.3 ng/ml (Table 3), respectively. Geometric mean terminal elimination half-life values ranged from 18.4 to 24.6 h. Urinary recovery of nezulcitinib was low; cumulative excretion in the urine was less than 1% of the administered dose (Table 3).

TABLE 3 Plasma and urine PK parameters in part A

	Nezulcitinib		
	1 mg (*n* = 6)	**3 mg (*n* = 6)**	**10 mg (*n* = 6)**
AUC_{0-t}, ng•h/ml	18.7 (44.5)	45.2 (33.3)	156.7 (40.2)
C_{max}, ng/ml	5.5 (30.2)	13.8 (24.3)	47.3 (46.6)
T_{max}, h, median (range)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
CL/F, L/h	52.2 (44.8)	64.7 (33.8)	62.4 (39.8)
V_z/F, L	1386 (24.0)	2297 (33.9)	2195 (46.0)
$t_{1/2}$, h	18.4 (34.3)	24.6 (11.8)	24.4 (7.5)
Cum%Dose	0.97 (38.3)	0.72 (17.5)	0.84 (41.6)

Note: Values are geometric mean (CV%) unless otherwise indicated.

Cum%Dose and CLr are presented for urine data only. All other PK parameters are for plasma data only.

Abbreviations: AUC_{0-t}, area under the plasma concentration-time curve from 0 to the last measurable concentration; CL/F, apparent total body clearance from plasma; C_{max}, maximum concentration; Cum%Dose, cumulative percentage of administered dose excreted; CV%, coefficient of variation; PK, pharmacokinetic; $t_{1/2}$, time taken for half of initial dose to be eliminated from the body; T_{max}, time to C_{max}; V_z/F, apparent volume of distribution during terminal phase after non-i.v. administration.

For part B, mean (SD) plasma exposures (C_{max} and AUC_{0-t}) of nezulcitinib following 7 days of once-daily dosing (Figure 2b) increased proportionally with increasing doses of nezulcitinib (1 mg, 3 mg, and 10 mg). Steady-state plasma concentrations of nezulcitinib were reached by the end of day 4 or sooner, based on analysis of daily plasma trough concentration values following once-daily dosing of nezulcitinib. Day 7 geometric mean plasma trough concentration steady-state values following once-daily inhaled doses of nezulcitinib 1, 3, and 10 mg were 0.2, 0.3, and 1.2 ng/ml, respectively. Dose-normalized systemic exposure (AUC for the dosing interval [AUC_{tau}] and steady-state C_{max}) did not appear to appreciably change following repeated administration of nezulcitinib, suggesting no clinically meaningful drug accumulation in plasma. Mean accumulation ratios for AUC from 0 to 24 h (AUC_{0-24}) values of nezulcitinib were ~ 1.1-fold to 1.2-fold following the last dose on day 7 and were similar to those based on C_{max} (Table 4). Geometric mean plasma C_{max} steady-state values (day 7) at 1 h following once-daily inhaled doses of nezulcitinib 1, 3, and 10 mg were 5.6, 17.6, and 49.6 ng/ml, respectively (Table 4).

Maximal plasma exposures following single and multiple doses of nezulcitinib were low relative to concentrations expected to result in systemic target engagement. The steady-state maximal plasma exposures were at least 25-fold (at 3 mg) and 7-fold (at 10 mg) lower than the concentration producing the most potent half-maximal JAK inhibition (IC50) in a cell-based assay of 12.6 nM, when the IC50 value is adjusted for human plasma protein binding of 98.1%.

TABLE 4 Plasma nezulcitinib pharmacokinetic parameters on day 7 in part B

	Nezulcitinib		
	1 mg (*n* = 8)	**3 mg (*n* = 8)**	**10 mg (*n* = 8)**
AUC_{tau}, ng•h/ml	21.0 (25.7)	51.6 (32.5)	177.8 (65.0)
$C_{max,ss}$, ng/ml	5.6 (24.1)	17.6 (42.6)	49.6 (46.4)
$T_{max,ss}$, h, median (range)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (0.5–1.1)
DN $C_{max,ss}$, ng/ml/mg	5.6 (24.1)	5.9 (42.6)	5.0 (46.4)
$C_{trough,ss}$, ng/ml	0.2 (29.5)	0.3 (39.0)	1.1 (83.6)
DN AUC_{tau}, ng•h/ml/mg	21.0 (25.7)	17.2 (32.5)	17.8 (65.0)
Ra_{AUC}	1.2 (20.5)	1.1 (19.9)	1.1 (52.1)
Ra_{Cmax}	1.1 (20.4)	1.0 (24.4)	1.0 (42.8)

Note: Data presented as geometric mean (geometric CV%) unless otherwise noted.

The Ra AUC was calculated as follows: AUC_{tau}/AUC_{0-24} (from day 1).

The Ra C_{max} was calculated as follows: $C_{max,ss}/C_{max}$ (from day 1).

Abbreviations: AUC_{tau}, area under the plasma concentration-time curve from 0 to the end of the dosing interval; $C_{max,ss}$, maximum concentration at steady state; $C_{trough,ss}$, trough concentration at steady state; CV%, coefficient of variation; DN, dose normalized; Ra_{AUC}, accumulation ratio based on AUC; Ra_{Cmax}, accumulation ratio based on C_{max}; $T_{max,ss}$, time to $C_{max,ss}$.

Natural killer cell counts

As there were no changes in standard hematology parameters, absolute NK cell counts in whole blood samples were evaluated after multiple-dosing in part B to assess the potential for systemic pharmacologic effects associated with JAK inhibition by nezulcitinib. No reductions in NK cells were observed relative to baseline in participants receiving placebo or nezulcitinib at any dose level (1, 3, or 10 mg) explored in the study (Figure 3).

DISCUSSION

In this first-in-human study, inhaled nezulcitinib administered as single and multiple doses of 1, 3, and 10 mg was generally well-tolerated in healthy participants with an acceptable safety profile over the 7-day dosing period. The nezulcitinib plasma exposures increased in a dose-proportional manner after single and multiple doses. Nezulcitinib steady-state plasma concentrations were achieved with a once-daily dosing period in part B (7 days), with accumulation ratios of ~ 1, suggesting no significant plasma drug accumulation. This lack of accumulation and the terminal elimination half-life of ~ 24 h support a once-daily dosing regimen. Systemic exposure to nezulcitinib was not associated with any changes in hematological parameters, including NK cell counts.

No severe or serious TEAEs were reported, all AEs were mild or moderate in severity, and none led to discontinuation of study treatment. There were no clinically significant changes in vital signs, ECG, and chemistry or hematology laboratory parameters except for one asymptomatic increase in liver function tests. Transaminase elevations may occur in up to 20% of placebo-treated phase I trial participants, possibly due to restricted activity with maintained or increased calorie intake; consumption of a high-carbohydrate high-calorie diet in a phase I unit was associated with weight gain and elevated levels of transaminases and triglycerides in healthy volunteers.[13,14] Therefore, taken in the context of observed weight gain and dyslipidemia for the subject in this study, the elevated transaminases were not considered to be related to the study drug. Similar rates of TEAEs were reported in participants receiving nezulcitinib and placebo. The most frequently reported TEAEs were cough, dizziness, and headache following multiple-dose administration.

Overall (AUC) and peak (C_{max}) plasma exposures of nezulcitinib were low and increased in a dose-proportional manner from 1–10 mg in both parts of the study. Steady-state plasma concentrations of nezulcitinib were achieved over the 7-day course of dosing in part B. Accumulation ratios for nezulcitinib plasma AUC and C_{max} values were close to one following 7 days of once-daily dosing, suggesting no clinically meaningful drug accumulation in plasma. The terminal elimination half-life of ~ 24 h is supportive of once-daily dosing, assuming the terminal elimination phase in plasma is driven by egress of drug from the lungs. This assumption is consistent with preclinical data demonstrating lung-to-plasma ratios based on AUC of 58:1, 172:1, and 850:1 in mice, rats, and dogs, respectively. The mechanism(s) facilitating egress of nezulcitinib from lung (e.g., diffusion and active transport) as well as the relative egress via systemic (i.e., drug entering the blood) and nonsystemic routes are unknown.

Approximately 1% of the administered dose was recovered in urine as nezulcitinib in part A. Although the bioavailability following inhaled administration in humans is not known, it is likely that cumulative renal excretion of 1% represents a minor pathway of elimination. This is consistent with preclinical data showing that nezulcitinib

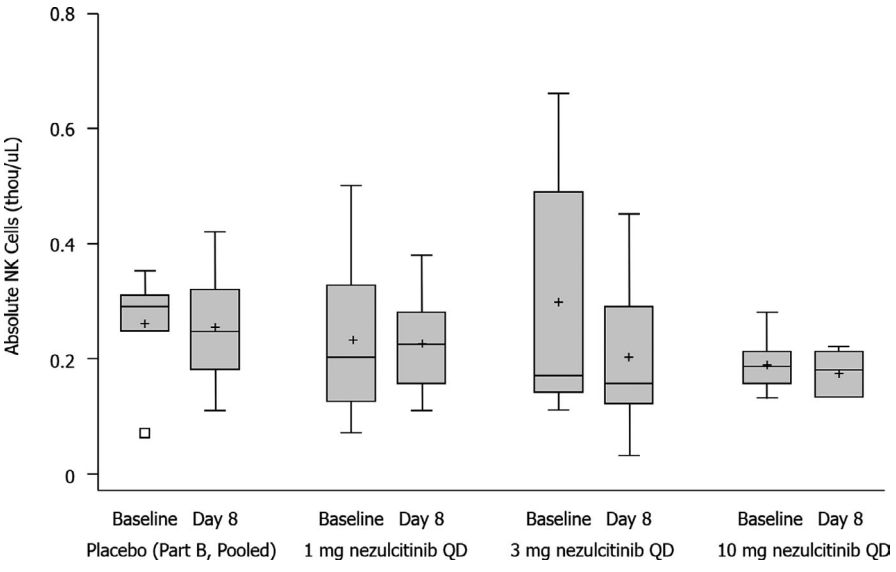

FIGURE 3 Absolute NK cell count following multiple inhaled doses of placebo, 1, 3, and 10 mg nezulcitinib. Upper and lower whiskers represent the largest and smallest observed values within 1.5 times the IQR from the upper and lower quartiles (q3 and q1). The horizontal line represents the median value, and the + represents the mean value. Baseline samples were collected predose. IQR, interquartile range; NK, natural killer

undergoes metabolism in the rat, with a minor amount eliminated in the urine and bile as unchanged compound. The primary metabolite of nezulcitinib identified in rat and dog hepatocytes was a sulfation product, whereas an oxidation, methylation, and glucuronidation product was primary in human hepatocytes. Nezulcitinib demonstrated extensive conversion to the sulfation product in dog lung S9 fractions fortified with 3′-phosphate-5′-phosphosulfate, but was relatively stable in rat and human lung S9 (data on file, Theravance Biopharma). The sulfation product was detected following inhaled administration of nezulcitinib in rats and dogs, and was also detected in human plasma samples in the present study (data not shown). No metabolites unique to humans have been identified to date.

There are a number of reported investigations of JAK inhibitors for treatment of COVID-19–associated cytokine storm.[5,15] Baricitinib demonstrated benefit in combination with remdesivir in reducing time to recovery and accelerating improvement in clinical status in patients with severe COVID-19.[10] Smaller studies with baricitinib demonstrated reduced clinical symptoms, improved respiratory function characteristics, reduced intensive care unit admission, and reduced mortality versus controls in hospitalized patients with COVID-19.[16,17] Similarly, ruxolitinib added to standard of care resulted in numerically faster clinical improvement and a reduced mortality rate versus controls in patients with severe COVID-19; however, ruxolitinib did not meet its primary end point in the phase III RUXCOVID study.[18,19] The drugs were generally well-tolerated in these trials; however, concerns have been raised about potential increased risk for infection and thromboembolism with systemic JAK inhibitors, the latter adverse effect being particularly concerning given observations of severe hypercoagulability in patients with COVID-19.[20,21]

Administration of an inhaled JAK inhibitor for the treatment of acute lung injury associated with COVID-19–related cytokine storm may provide benefit locally in the lungs while reducing the potential for systemically mediated adverse effects documented for the systemic JAK inhibitor class. The PK of inhaled nezulcitinib are consistent with this lung-selective approach, with low plasma exposures observed after inhaled administration. One challenge of this approach is that the plasma concentrations do not represent local concentrations at the site of action in the lungs. As such, it is difficult to assess the anticipated biological activity in the lungs based on plasma concentrations obtained in this population of healthy participants. Maximal plasma exposures of nezulcitinib were ~ 20-fold and ~ 7-fold lower than the most potent cell-based JAK IC50 (adjusted for plasma protein binding) at dose levels of 3 and 10 mg, respectively, suggesting that systemic JAK inhibition is unlikely to manifest to a significant degree after inhaled administration. The lack of reduction in NK cell counts at any dose level in the study is also consistent with the lack of systemic JAK inhibition; in contrast, marked reductions in NK cell counts have been observed in as little as 2 weeks following administration of systemic JAK inhibitors, such as tofacitinib.[22] Other systemically mediated hematological changes associated with JAK inhibition, including neutrophil and hemoglobin reductions as well as lipid changes, were not observed with inhaled administration of nezulcitinib.

In COVID-19, viral particles lodge and proliferate in the respiratory tract, where they multiply and spread to other tissues.[23] Systemically administered JAK inhibitors, such as baricitinib and ruxolitinib, have demonstrated benefit in treating patients with severe COVID-19.[10,18] Nezulcitinib is administered via nebulization and thus may allow for a higher level of target engagement in the lungs and lower systemic burden compared to systemic JAK inhibitors. This inhaled profile may allow for additional benefits in controlling lung inflammation and acute lung injury in patients with severe COVID-19 while minimizing the risk of systemic adverse effects relative to systemic JAK inhibitors.

This first-in-human study of inhaled nezulcitinib was conducted during the start of the COVID-19 pandemic in order to accelerate the development of this drug for the treatment of acute lung injury associated with COVID-19. Specific procedures were introduced into the study design to minimize the risk of participants with COVID-19 infection. The small sample sizes at each dose level provided adequate initial information on safety and PK to allow for progression of nezulcitinib into clinical trials involving patients with COVID-19.

In conclusion, the data indicate that inhaled nezulcitinib was generally well-tolerated in healthy participants, and the PK results support the potential for once-daily administration. These results support further testing of nezulcitinib in the prevention of cytokine storm associated with acute lung injury in patients hospitalized with COVID-19. A phase II part 1 clinical trial assessed the safety and efficacy of nezulcitinib 1, 3, and 10 mg in patients with severe COVID-19. Based on all evidence, nezulcitinib 3 mg was advanced for further evaluation to phase II part 2 and is currently ongoing (NCT04402866).[24]

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CONFLICT OF INTEREST

N.D.P., A.L., D.L.B., and K.C. are employees of Theravance Biopharma US, Inc.; and shareholders in Theravance Biopharma, Inc. D.S. reports personal fees from Theravance Biopharma Ireland Limited during the conduct of the study; and personal fees from AstraZeneca; Boehringer Ingelheim; Chiesi; Cipla; Genentech; GlaxoSmithKline; Glenmark; Menarini; Mundipharma; Novartis; Peptinnovate; Pfizer; Pulmatrix; Theravance Biopharma Ireland Limited; and Verona outside this work.

AUTHOR CONTRIBUTIONS

N.D.P. and D.L.B. wrote the manuscript. N.D.P., K.C., and D.L.B. designed the research. D.S. and K.C. performed the research. N.D.P., D.L.B., and A.L. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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