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ORIGINAL RESEARCH

A Phase 2a, Randomized, Placebo-Controlled Human Challenge Trial to Evaluate the Efficacy and Safety of Molnupiravir in Healthy Participants Inoculated with Respiratory Syncytial Virus

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ABSTRACT

***Introduction*:** Human respiratory syncytial virus (RSV) infections can result in hospitalization and/or death among vulnerable populations. Molnupiravir is a prodrug of ß-D-N4-hydroxycytidine, which has broad-spectrum preclinical activity against RNA viruses. We conducted a pilot trial evaluating molnupiravir for RSV infection.

***Methods*:** Double-blind, placebo-controlled, phase 2a human challenge study in healthy adults (≥ 18 to ≤ 55 years old). Eligible participants were randomized 1:1:1 to molnupiravir prophylaxis (5 days, 800 mg twice daily; followed by placebo), molnupiravir treatment (5 days, 800 mg twice daily; started on day 5, unless triggered earlier by positive PCR test; preceded and followed by placebo), or placebo. Study intervention was administered for 11 days, from day −1 (evening), prior to inoculation with RSV on day 0 (morning). For 10 days, quarantined participants reported symptoms thrice daily and underwent nasal wash sample collection twice daily. Primary efficacy endpoints (assessed by quantitative viral culture on plaque assay) were peak viral load (PVL) in all participants (for prophylaxis) and area under the viral-load time curve (VL-AUC) in participants with confirmed infection (for treatment). Adverse events were assessed from day 0 to day 28.

***Results*:** Forty participants each were randomized to prophylaxis and placebo and 36 to treatment. Molnupiravir was not statistically significant from placebo in either primary endpoint: with prophylaxis, the difference in mean $\log_{10}$ PVL was −0.29 plaque-forming units (PFU)/ml (90% CI −1.16, 0.58; $p=0.578$), and with treatment, the difference in mean $\log_{10}$ VL-AUC was −2.69 day*PFU/ml (90% CI −6.17, 0.79; $p=0.201$). Molnupiravir treatment resulted in significantly faster symptom resolution: 6.0 days versus 8.5 days with placebo (hazard ratio: 2.24 [95% CI: 0.99, 5.07]; $p=0.0459$). Adverse event rates were comparable between arms.

***Conclusions*:** Although the primary endpoints were not met, modest, non-significant benefits with molnupiravir treatment were seen across virologic endpoints, along with significantly faster symptom resolution.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s41030-025-00289-z.

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Key Summary Points

Why carry out the study?

Antiviral treatment options for vulnerable patients with RSV infections are urgently needed, and the broad-spectrum antiviral agent molnupiravir has potential utility in this setting, based on molnupiravir's mechanism of action and limited preclinical data.

We conducted a phase 2a, randomized, double-blind, 3-arm, human challenge study comparing efficacy and safety of molnupiravir prophylactic and triggered treatment against placebo, to assess whether molnupiravir would achieve meaningful reductions in RSV viral load under controlled conditions.

What was learned from the study?

A benefit of molnupiravir over placebo was seen across all efficacy endpoints (including the primary virologic endpoints), but the magnitude of viral load reductions observed in the small treatment subgroup (less than 40 participants) did not reach statistical significance.

In this study population of healthy volunteers, molnupiravir was found to be well tolerated, with an adverse event profile indistinguishable from placebo and no new safety issues identified.

While molnupiravir treatment led to significantly faster time to symptom resolution than placebo, the virologic benefits of molnupiravir observed in this pilot trial were only modest and not significantly different from placebo.

INTRODUCTION

Human respiratory syncytial virus (RSV), an enveloped, single-stranded RNA virus, is a highly communicable pathogen and a common cause of acute lower respiratory infections in adults [1–5]. RSV generally occurs in clear seasonal patterns with a geographical gradient [2, 6, 7]. Its two major strains RSV-A and RSV-B (both with multiple genotypes) circulate concurrently [1, 2, 7]. The virus is readily transmitted through close contact with aerosolized droplets or fomites and inoculation via nasal or ocular membranes [1, 2]. Infections have a 2- to 8-day incubation period and are initially limited to nasopharyngeal tissues but can progress to bronchiolitis and pneumonia [1]. Acquired immunity to RSV does not provide long-term protection, and reinfections throughout a person's lifespan are frequent [1, 2, 6, 8]. Healthy adults generally only develop mild disease [1, 2, 9], but serious complications can occur in high-risk populations (e.g., ≥ 75 years old, immunocompromised, and/or with significant pulmonary/cardiovascular comorbidities) [2, 3, 5, 10–15]. Annually, around 1.5 million RSV infections were estimated to occur among elderly adults in industrialized countries [16], associated with 60,000–160,000 hospitalizations and 6000–10,000 deaths in the United States alone [17]; the true burden was potentially several-fold higher [18–20]. Of note, these estimates were obtained prior to the recent introduction of RSV vaccines, which are now approved for prevention of severe RSV disease in high-risk adults [21, 22]. Similarly, RSV disease in infants and vulnerable young children can be effectively prevented through maternal vaccination and passive immunization with the monoclonal antibodies palivizumab (short-acting) or nirsevimab (long-acting) [23, 24]. For the treatment of acute RSV infections, however, the only currently available option is ribavirin, which is associated with safety concerns and unclear efficacy and is only approved (in its aerosolized form) for pediatric patients [2, 25–27]. Antiviral agents to treat RSV infection in high-risk adults are therefore a significant unmet need.

One potential option is molnupiravir, an orally administered prodrug of the small-molecule nucleoside-analogue

ß-D-N4-hydroxycytidine (NHC) with broad-spectrum activity against RNA viruses and a high barrier to the development of resistance [28–33]. Incorporation of NHC during viral replication causes an accumulation of nucleotide errors randomly distributed throughout the viral genome, ultimately leading to production of only nonviable, noninfectious virions [33–35]. Having shown preclinical activity against SARS-CoV-2 in vitro and in animal studies [31, 32, 36], molnupiravir's efficacy in treating COVID-19 was subsequently confirmed in clinical trials and real-world studies [37–40]. Limited published preclinical data, i.e., a plaque assay and a murine infection model, suggest potential activity of NHC against RSV [29].

While such preclinical results are an early indication that investigational agents might be able to treat RSV infections, evaluating clinical efficacy is challenging given the vulnerable target populations [41, 42]. To address these risks and challenges, it is a fairly standard approach in RSV clinical research to first conduct phase 2a human challenge trials as gateway studies prior to considering clinical trials in high-risk patients [41, 43–45]. Human challenge studies represent very early therapeutic intervention in participants (generally a homogenous study population of healthy volunteers) who receive a fixed inoculum of RSV intranasally. Participants are then intensively followed for virologic, symptomatic, and safety outcomes in a strict quarantine setting, with RSV viral load as the primary biomarker for exploring antiviral activity against RSV infections in humans. The magnitude of viral load reduction achieved under such highly controlled conditions is assumed to correlate with the likelihood that the investigational agent would achieve significant efficacy in actual target populations [41, 43–45]. Human challenge studies can thus yield valuable data to inform potential follow-on trials that assess clinical outcomes in appropriate patient populations—however, they are not a substitute for those trials.

We conducted a phase 2a, randomized, double-blind, three-arm, single-center human challenge study comparing the efficacy and safety of molnupiravir prophylactic and triggered treatment against placebo. Our goal was to assess if molnupiravir would yield meaningful reductions in RSV viral load under highly controlled conditions in healthy participants at low risk of complications from RSV disease.

METHODS

Study Design

Protocol MK-4482-017 was a phase 2a, double-blind, randomized, placebo-controlled human challenge study evaluating the efficacy and safety of molnupiravir in healthy adults experimentally inoculated with RSV and conducted at a single clinical research unit (hVIVO, London, United Kingdom) from November 2022 to June 2023 (clinicaltrials.gov NCT05559905). The trial comprised three arms (i.e., prophylactic administration, triggered dosed treatment, and matched placebo) and proceeded in three phases: screening (from day –90 through day –4, relative to inoculation on day 0), quarantine (day –3 through day 12), and follow-up (day 13 through day 28).

The study was conducted in accordance with principles of Good Clinical Practice (which reflect the ethical principles that have their origin in the Declaration of Helsinki) and was approved by the appropriate institutional review board (South Central - Berkshire B Research Ethics Committee) and regulatory agencies. Written informed consent was obtained from all participants prior to their participation in the trial.

Participants, Randomization, and Intervention

The trial was conducted in female and male participants ≥ 18 to ≤ 55 years old. To be eligible for the trial, potential participants had to be in good health based on medical history, physical examination, vital sign measurements, spirometry, electrocardiogram, and laboratory safety tests (including hemoglobin and platelet levels at the lower limit of normal or above, total white cell count ≥ 3000/µl,

and absolute neutrophil count ≥ 1500/μl). Participants were also required to have a total body weight of ≥ 50 kg and a body mass index between ≥ 18 kg/m^2 and ≤ 35 kg/m^2.

Pregnant or breastfeeding women were ineligible for trial participation. Other key exclusion criteria were: any active respiratory infection at day 0; any signs/symptoms of upper or lower respiratory tract infection within 4 weeks prior to study entry; history of any clinically significant abnormalities or diseases; ongoing (or resolved within less than 12 months prior to study entry) depression and/or anxiety; history of rhinitis (unless mild or currently inactive for at least 30 days); significant abnormalities of nasal and/or nasopharyngeal anatomy; forced expiratory volume in 1 s < 80%; creatinine clearance ≤ 60 ml/min; cigarette use of ≥ 10 pack years; use (or anticipated use) of concomitant medications (including vitamins and supplements) beginning 2 weeks prior to the first dose until the final follow-up visit; and any vaccine received within 4 weeks prior to inoculation or anticipated before the final follow-up visit. Importantly, potential participants also had to exhibit no or low (i.e., in the lower quartile for immunogenicity) serum levels of RSV A Memphis 37b strain-specific antibodies, as measured by a cell-based neutralization assay, at screening and within 90 days prior to receiving study intervention.

Eligible participants were randomized 1:1:1 to the prophylaxis, treatment, and placebo arms according to a computer-generated allocation schedule without stratification. To ensure at least 31 evaluable participants per treatment arm (to maintain the planned statistical power), participants who withdrew or discontinued from the trial were to be replaced with a new randomized participant according to a protocol-defined replacement allocation schedule. Participants randomized to the placebo arm received 11 days of placebo twice daily starting in the evening of day −1 with the last dose on the morning of day 10. Participants randomly assigned to the prophylaxis arm received 5 days of molnupiravir (800 mg every 12 h) starting in the evening of day −1 and were subsequently switched to matching placebo starting in the evening of day 4 through the morning of day 10. Those randomized to the treatment arm received placebo starting in the evening of day −1 and were switched to 5 days of molnupiravir (800 mg every 12 h) approximately 12 h after the conditions to trigger treatment had been met; treatment was triggered (a) based on a qualitative integrative cycler PCR test [46] positive for RSV infection between the afternoon of day 2 and the morning of day 5 or (b) in the evening of day 5 if no PCR test had been positive prior to that time. Participants in this arm who had completed a full 5-day course of molnupiravir prior to day 10 were switched back to placebo, with the last dose also on the morning of day 10.

Switching between molnupiravir and placebo was managed by unblinded study staff who otherwise did not have any additional role in the trial. Participants, investigators, and all other study personnel remained fully blinded throughout the trial.

Trial Schedule and Assessments

Potential participants who had provided Health Research Authority (HRA) generic screening consent proceeded to the generic screening phase, during which general health assessments were conducted. The first step of the screening process was the determination of anti-RSV A Memphis 37b-specific antibodies using a foci reduction serum microneutralization assay (FRNA). Screened participants who were identified as being eligible for the trial were invited to be admitted to quarantine between day −3 to day −1. Upon admission to the quarantine unit, participants had to be consented onto the trial before final eligibility assessments were performed. Participants remained in the quarantine unit until day 12, when they were discharged after completion of all morning assessments; participants who discontinued the trial were discharged prior to day 12.

Quarantined participants received their first dose of study intervention (i.e., either molnupiravir or placebo, depending on which study arm they had been randomized to) in the evening of day −1 and were intranasally inoculated with approximately 10^4 plaque-forming units (PFU) of RSV A Memphis 37b on the morning of day 0, as long as did they did not present

with any active respiratory infection at that time. The assessment for respiratory infection included clinical evaluation on days –3 to 0 and a respiratory pathogen screen upon admission on day –3 or day –2 using nasopharyngeal swabs evaluated via the BioFire® Respiratory 2.1 Panel (bioMérieux, Salt Lake City, UT, USA).

Beginning on day –1, while quarantined, participants completed a standardized 13-item paper questionnaire three times per day to assess for symptoms of RSV infection. Eleven symptoms (i.e., runny nose, stuffy nose, sneezing, sore throat, earache, malaise/tiredness, muscle and/or joint ache, chilliness/feverishness, cough, and chest tightness) were self-rated on a scale from 0 to 3 while two symptoms (i.e., shortness of breath and wheezing) were rated on a scale from 0 to 4. Ratings were defined as follows: "no symptoms" as grade 0; "just noticeable" as grade 1; "clearly bothersome from time to time but does not interfere with me doing my normal daily activities" as grade 2; "quite bothersome most/all of the time and stops me from participating in activities" as grade 3; and "symptoms at rest" as grade 4.

Nasal wash samples were collected twice daily (12 ± 1 h apart) from the morning of day 2 through the morning of day 12. All nasal wash samples underwent testing for RSV viral load by both quantitative RT-PCR (see Supplementary Material, Methods S1) and quantitative viral culture assessed by plaque assay (see Supplementary Material, Methods S1), and those samples collected from day 2 up to and including the morning of day 5 also underwent RSV infectivity testing via a previously described qualitative integrative cycler PCR method [46]. Nasal discharge weight per 24 h was determined for each participant for days 0 through 12. A detailed description of nasal wash sample processing and the subsequent analyses can be found in the Supplementary Material (Methods S1).

Blood samples to measure concentrations of NHC in plasma and concentrations of NHC-TP in peripheral blood mononuclear cells were collected throughout the study (i.e., for the evening dose of study intervention on day –1 and the morning dose on days 2 [NHC only], 4, 5 [NHC only], 6, and 7) at the following time points: predose (within 3 h prior to the first dose on day –1 or within 30 min prior to each subsequent dose), 0 to < 1 h, 1 to < 12 h, and 12 h. A detailed description of the bioanalysis methods used was published previously [47]. In brief, both NHC (analytical range: 0.00386–3.86 µM) and NHC-TP (analytical range: 0.01–4.0 µM) concentrations were determined using a validated high-performance liquid chromatography tandem mass spectrometry method [48]. Key pharmacokinetic parameters assessed were maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the concentration-time curve from time 0 to 12 h ($AUC_{0–12}$), AUC to the last quantifiable concentration ($AUC_{0–last}$), AUC extrapolated to infinity ($AUC_{0–inf}$), accumulation ratio (AR), and apparent half-life ($t_{1/2}$). AUC $_{0–inf}$ and $t_{1/2}$ were determined following the last dose only. The accumulation ratio was calculated using C_{max} and $AUC_{0–12}$ on day –1 versus day 11. All pharmacokinetic parameter values were estimated through noncompartmental modeling methods in Phoenix WinNonlin version 8.1 (Certara, Princeton, NJ, USA).

Prior to discharge from quarantine, a rapid viral antigen test (or alternatively a PCR test) for RSV infection was performed at the discretion of the investigator if indicated for any reason. To further minimize the risk of passing the challenge virus on to others, participants were counseled to avoid contact with vulnerable people (including children, elderly, nursing home residents, immunocompromised people, and those with severe lung disease) for 2 weeks after departing quarantine. All inoculated participants were followed for safety until day 28 ± 3 days.

Endpoints

Efficacy was assessed in two analysis populations, i.e., the full analysis set (FAS; defined as all randomized participants who received both the RSV inoculation and ≥ 1 dose of study intervention) and the full analysis set—infected (FAS-I; defined as all participants in the FAS population who had laboratory-confirmed RSV infection). Laboratory-confirmed RSV infection from nasal wash samples was defined as ≥ 2 independent, quantifiable RT-PCR measurements over 2 days and/or ≥ 1 positive, quantifiable

plaque assay measurement. The primary efficacy endpoint for prophylaxis was peak viral load (PVL), defined as the maximum viral load from day 2 to day 12 as determined by quantitative plaque assay, in the FAS analysis population. The primary efficacy endpoint for treatment was the area under the viral-load time curve (VL-AUC) based on quantitative plaque assay results, from initiation of study intervention (i.e., molnupiravir or placebo) through the morning of day 12 in the FAS-I analysis population.

Important secondary endpoints for prophylaxis included the following (all analyzed in the FAS population): PVL determined by quantitative RT-PCR; VL-AUC determined by plaque assay; VL-AUC determined by quantitative RT-PCR; infectivity rate (defined as ≥ 1 positive plaque assay test); plaque assay-confirmed symptomatic RSV infection; and total clinical symptoms, expressed as area under the curve over time of total symptom scores (TSS-AUC) from day 2 to day 12 in the morning (i.e., planned discharge from quarantine). Important secondary endpoints for triggered treatment included the following (all analyzed in the FAS-I population): VL-AUC determined by quantitative RT-PCR; PVL determined by plaque assay; time to confirmed negative RSV test (determined by plaque assay) after the initiation of study intervention (i.e., molnupiravir or placebo); total clinical symptoms, expressed as area under the curve over time of total clinical symptoms (TSS-AUC) after initiation of study intervention; and time to symptom resolution after initiation of study intervention.

Safety (i.e., the incidence of adverse events [AEs] from initiation of study intervention to day 28) was assessed in the safety population, defined as all participants who received at least one dose of study intervention as treated. AEs were classified as all-cause AEs (i.e., irrespective of their potential relationship to study intervention) and treatment-related AEs (i.e., those determined by the investigator to be related to study intervention). AEs related to the RSV challenge (from viral challenge to day 28) were assessed separately.

Statistical Analysis

VL-AUC was determined using the trapezoid method and the actual timepoints of sample collection. Rules applied to convert viral load assay results to analysis values for VL-AUC calculation are detailed in Supplementary Material Methods S2. To assess the efficacy of molnupiravir in the prophylaxis setting, mean PVL (on the $\log_{10}$ scale) for the prophylaxis and placebo arms was calculated using an analysis of variance (ANOVA) linear model with study arm as a fixed categorical effect. The difference in mean PVL between molnupiravir and placebo (with the corresponding two-sided 90% confidence interval [CI]) was determined from this model, with a two-sided p value provided to test whether this difference was statistically significant. To assess the efficacy of molnupiravir in the treatment setting, mean VL-AUC (on the $\log_{10}$ scale) for the treatment and placebo arms was also determined using an ANOVA linear model with study arm as a fixed categorical effect, and the difference in mean VL-AUC between molnupiravir and placebo (with the corresponding two-sided 90% CI and a two-sided p value for significance testing) was calculated. If the assumptions of a linear model were not met, Wilcoxon rank-sum tests were to be applied instead. For all analyses of symptom-based secondary endpoints, only ten items (i.e., runny nose, stuffy nose, sneezing, sore throat, earache, malaise/tiredness, muscle and/or joint ache, cough, and shortness of breath) of the 13-item RSV symptom questionnaire were included, to match prior RSV human challenge studies which had generally only assessed these ten symptoms. No multiplicity adjustments were made in any of the analyses. AEs were tabulated and summarized descriptively. All analyses were conducted with the use of SAS version 9.4 (SAS Institute Inc, Carey, NC, USA).

The planned enrollment total for this trial was 105 participants, with 35 participants per study arm. The evaluable sample size was assumed to be 35 participants per arm, with an expected dropout rate of around 5% (i.e., two out of 33 participants per arm). The prophylaxis arm had ~ 80.9% power to detect a 65% decrease (molnupiravir versus placebo) in PVL, assuming a coefficient of variation (CV) in PVL of 0.917 with a two-sided alpha of 0.05. The treatment arm had ~ 80.5% power to detect a 70% decrease (molnupiravir versus placebo) in

VL-AUC, assuming a CV in VL-AUC of 0.767 with a two-sided alpha of 0.05. Assuming an infection rate of 61% (based on a single positive quantitative plaque assay from day 2 to day 12), a sample size of 31 per arm would maintain 80% power. The assumed CV in VL-AUC or PVL and the assumed infection rate were based on the results of previous RSV human challenge studies conducted by hVIVO (London, United Kingdom).

RESULTS

Participants

In total, 116 participants were randomized into this trial (40 participants each to the prophylaxis and placebo arms and 36 participants to the treatment arm) and received the RSV inoculation (Fig. 1). Of these, 115 in total received at least one dose of molnupiravir or placebo, 110 (94.9% in the prophylaxis, 100.0% in the treatment, and 92.5% in the placebo arms) completed their assigned study intervention, and 114 completed the trial (95.0% in the prophylaxis, 100.0% in the treatment, and 100.0% in the placebo arms). The FAS primary analysis population consisted of 39 participants from the prophylaxis arm and 40 from the placebo arm, and the FAS-I primary analysis population comprised 23 participants from the treatment arm and 26 from the placebo arm (Fig. 1).

Baseline characteristics of participants were generally well balanced between the three study arms (Table 1). Overall, most of the participants were male (61.7%), of white race (73.9%), and not of Hispanic/Latino ethnicity (99.1%). The mean age was 27.3 years, the mean weight was 76.4 kg, and the mean body mass index was 25.3 kg/m^2.

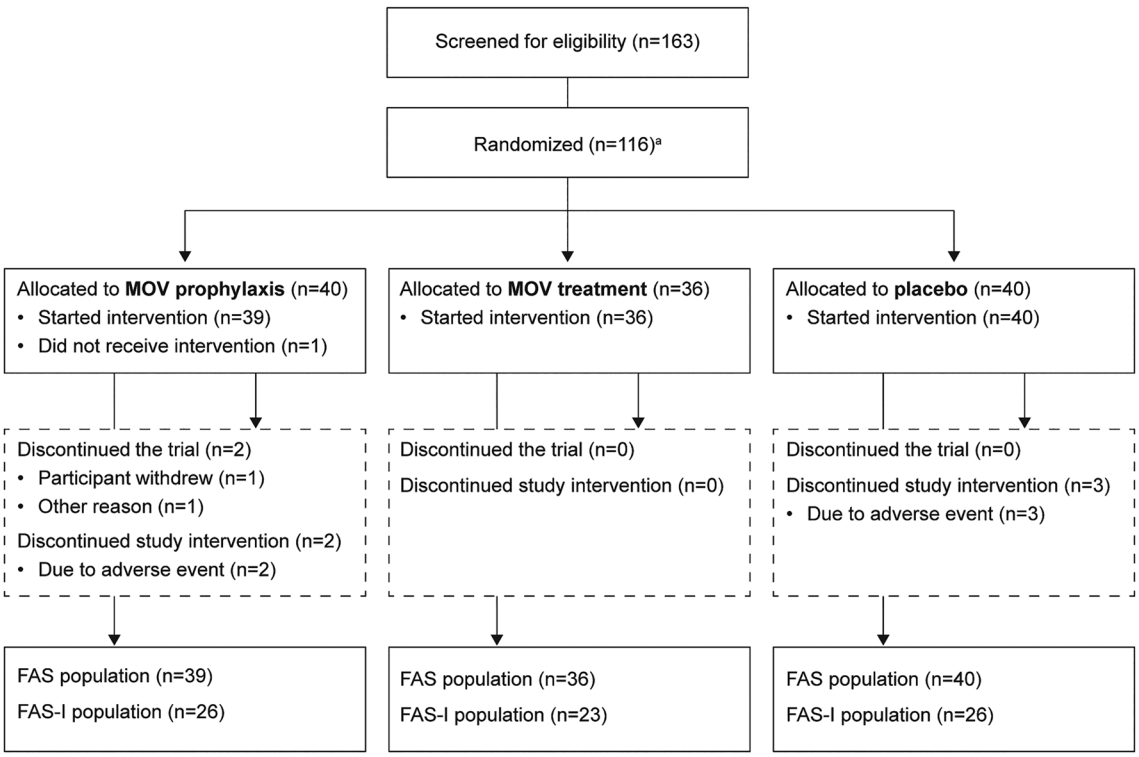


Fig. 1 CONSORT diagram indicating the flow of participants through the trial. [a]All randomized participants were inoculated with RSV. *FAS* full analysis set (defined as all randomized participants who received both the RSV inoculation and ≥ 1 dose of study intervention), *FAS-I* full analysis set-infected (defined as all participants in the FAS population who had confirmed RSV infection), *MOV* molnupiravir

Table 1 Participant baseline characteristics (safety population)

	Prophylaxis	Treatment	Placebo
Participants in population	39	36	40
Sex			
Male, *n* (%)	25 (64.1)	23 (63.9)	23 (57.5)
Female, *n* (%)	14 (35.9)	13 (36.1)	17 (42.5)
Age			
18 to 64 years, *n* (%)	39 (100.0)	36 (100.0)	40 (100.0)
Median (range), years	25.0 (18 to 42)	27.5 (19 to 51)	25.0 (18 to 46)
Race			
Asian, *n* (%)	3 (7.7)	3 (8.3)	2 (5.0)
Black/African American, *n* (%)	4 (10.3)	4 (11.1)	7 (17.5)
Multiple, *n* (%)	1 (2.6)	4 (11.1)	2 (5.0)
White, *n* (%)	31 (79.5)	25 (69.4)	29 (72.5)
Ethnicity			
Hispanic/Latino, *n* (%)	0 (0.0)	1 (2.8)	0 (0.0)
Not Hispanic/Latino, *n* (%)	39 (100.0)	35 (97.2)	40 (100.0)
Weight			
Median (range), kg	73.5 (50.2 to 98.8)	76.5 (50.7 to 106.7)	77.1 (58.0 to 120.8)
BMI			
Median (range), kg/m^2	24.3 (18.6 to 34.2)	25.2 (20.4 to 31.4)	25.3 (20.1 to 34.8)

BMI body mass index, *n* number of participants per intervention arm and row title

Efficacy

All randomized participants were inoculated with RSV. Of the 114 participants who completed the trial, the culture-confirmed infectivity rate (defined as ≥ 1 positive plaque assay test) was 22/38 (57.9%) in the prophylaxis arm, 20/36 (55.6%) in the treatment arm, and 22/40 (55.0%) in the placebo arm. Similarly, the infection rates as confirmed by quantitative RT-PCR were 26/38 (68.4%), 23/36 (63.9%), and 26/40 (65.0%) in the prophylaxis, treatment, and placebo arms, respectively. Most of the infections in the prophylaxis arm were symptomatic (Table 2).

In the prophylaxis setting, there was no statistically significant difference between molnupiravir and placebo in the primary endpoint of PVL determined by plaque assay (Table 2): the difference in mean $\log_{10}$ PVL was −0.29 plaque-forming units (PFU)/ml (90% CI: −1.16, 0.58; $p = 0.578$), approximately equivalent to a 10% reduction with molnupiravir. A supportive analysis was conducted in which this endpoint was analyzed using a nonparametric Wilcoxon Rank-Sum test, and the results were consistent with those of the primary analysis (Table 2).

Table 2 Primary and important secondary efficacy endpoints comparing molnupiravir prophylaxis with placebo in the FAS analysis population

Endpoint	Molnupiravir (95% CI)	Placebo (95% CI)	Difference (90% CI)	*p* value
Primary endpoint—Prophylaxis				
PVL by plaque assay, least-squares mean log_{10} PFU/ml[a]	2.55 (1.81, 3.30)	2.84 (2.12, 3.57)	− 0.29 (− 1.16, 0.58)	0.578
Number of participants, *n*	32	34	–	–
Supportive analysis using Wilcoxon rank-sum test, median log_{10} PFU/ml	2.23	3.37	*n/c*	0.666
Virology secondary endpoints				
PVL by qRT-PCR, least-squares mean log_{10} copies/ml[a]	4.66 (3.66, 5.66)	4.80 (3.81, 5.79)	− 0.14 (− 1.31, 1.04)	0.849
Number of participants, *n*	38	39	–	–
VL-AUC by plaque assay, least-squares mean day*log_{10} PFU/ml[a]	7.56 (4.95, 10.17)	8.17 (5.64, 10.70)	− 0.62 (− 3.65, 2.42)	0.736
Number of participants, *n*	32	34	–	–
VL-AUC by qRT-PCR, least-squares mean day*log_{10} copies/ml[a]	17.62 (11.70, 23.53)	19.54 (13.70, 25.39)	− 1.93 (− 8.88, 5.02)	0.646
Number of participants, *n*	38	39	–	–
Infectivity rate (by plaque assay), %[b,c]	57.9 (40.8, 73.7)[c]	55.0 (38.5, 70.7)[c]	2.9 (-19.4, 25.7)[d]	*n/c*
Infectivity rate (by qRT-PCR), %[e,c]	68.4 (51.4, 82.5)[c]	65.0 (48.3, 79.4)[c]	3.4 (-18.3, 24.5)[d]	*n/c*
Symptomatic RSV infection, %[b,c]	42.1 (26.3, 59.2)[c]	45.0 (29.3, 61.5)[c]	− 2.9 (− 25.7, 19.4)[d]	*n/c*
Symptom-based secondary endpoints				
TSS-AUC, least-squares mean	7.50 (4.18, 10.81)	9.59 (6.36, 12.82)	− 2.09 (− 5.97, 1.78)	0.371
Number of participants, *n*	38	40	–	–

CI confidence interval, *FAS* full analysis population, *n* number of participants in the specific arm and analysis population who contributed to the respective endpoint analysis, *n/c* not calculated, *PFU* plaque-forming units, *PVL* peak viral load from day 2 to day 12, *qRT-PCR* real-time quantitative polymerase chain reaction, *TSS-AUC* area under the curve over time of total clinical symptoms from day 2 to day 12 in the morning, *VL-AUC* area under the viral-load time curve from initiation of study intervention to day 12

[a]Least-squares means, difference between least-squares means, confidence intervals, and *p* value were all calculated using an analysis of variance (ANOVA) model with study arm as fixed effect

[b]Defined as at least 1 positive plaque assay in participants who completed the trial ($n = 38$ molnupiravir, $n = 40$ placebo)

[c]The 95% confidence interval was based on the exact binominal method proposed by Clopper and Pearson

[d]The 95% confidence interval was based on the exact method proposed by Chan and Zhang

[e]Defined as any two quantifiable qRT-PCR measurements reported over 2 consecutive days, starting two days post-viral challenge (day 2) up to day 12 (quarantine discharge)

Table 3 Primary and important secondary efficacy endpoints comparing molnupiravir triggered treatment with placebo in the FAS-I analysis population

Endpoint	Molnupiravir (95% CI)	Placebo (95% CI)	Difference (90% CI)	*p* value
Primary endpoint—treatment				
VL-AUC by plaque assay, least-squares mean day*$\log_{10}$ PFU/ml[a]	7.02 (3.91, 10.14)	9.72 (6.93, 12.50)	− 2.69 (− 6.17, 0.79)	0.201
Number of participants, n	20	25	–	–
Supportive analysis using Wilcoxon rank-sum test, median day*$\log_{10}$ PFU/ml	5.99	9.88	*n/c*	0.273
Virology secondary endpoints				
VL-AUC by qRT-PCR, least-squares mean day*$\log_{10}$ copies/ml[a]	20.58 (13.00, 28.16)	26.51 (19.38, 33.64)	− 5.93 (− 14.61, 2.75)	0.257
Number of participants, *n*	23	26	–	–
PVL by plaque assay, least-squares mean $\log_{10}$ PFU/ml[a]	2.85 (1.96, 3.74)	3.53 (2.74, 4.33)	− 0.69 (− 1.68, 0.31)	0.253
Number of participants, *n*	20	25	–	–
Symptom-based secondary endpoints				
TSS-AUC, least-squares mean	4.74 (1.76, 7.72)	6.57 (3.76, 9.37)	− 1.83 (− 5.25, 1.60)	0.377
Number of participants, *n*	23	26	–	–

CI confidence interval, *EAS* full analysis population, *n* number of participants in the specific arm and analysis population who contributed to the respective endpoint analysis, *n/c* not calculated, *PFU* plaque-forming units, *PVL* peak viral load from day 2 to day 12, *qRT-PCR* real-time quantitative polymerase chain reaction, *TSS-AUC* area under the curve over time of total clinical symptoms from day 2 to day 12 in the morning, *VL-AUC* area under the viral-load time curve from initiation of study intervention to day 12

[a]Least-squares means, difference between least-squares means, confidence intervals, and *p* values were all calculated using an analysis of variance (ANOVA) model with study arm as fixed effect

In the treatment setting, there was also no statistically significant difference between molnupiravir and placebo in the primary endpoint of VL-AUC determined by plaque assay (Table 3): the difference in mean $\log_{10}$ VL-AUC was − 2.69 day*PFU/ml (90% CI: − 6.17, 0.79; $p = 0.201$), approximately equivalent to a 28% reduction with molnupiravir. A supportive analysis was conducted in which this endpoint was analyzed using a nonparametric Wilcoxon Rank-Sum test, and the results again were consistent with those of the primary analysis (Table 3).

Overall, there were no significant differences between molnupiravir and placebo in terms of VL-AUC and PVL for both prophylaxis (Table 2, Fig. 2A) and treatment (Table 3, Fig. 2B), regardless of assay used. In the treatment group, the median (1st quintile, 3rd quintile) time to confirmed negative plaque assay from initial administration of study intervention was 5.0 (4.0, not reached) days with molnupiravir and 5.0 (5.0, 6.0) days with placebo; the corresponding hazard ratio was 1.72 (95% CI: 0.83, 3.57) and the *p* value for the difference was $p = 0.1708$. There were also no significant differences observed

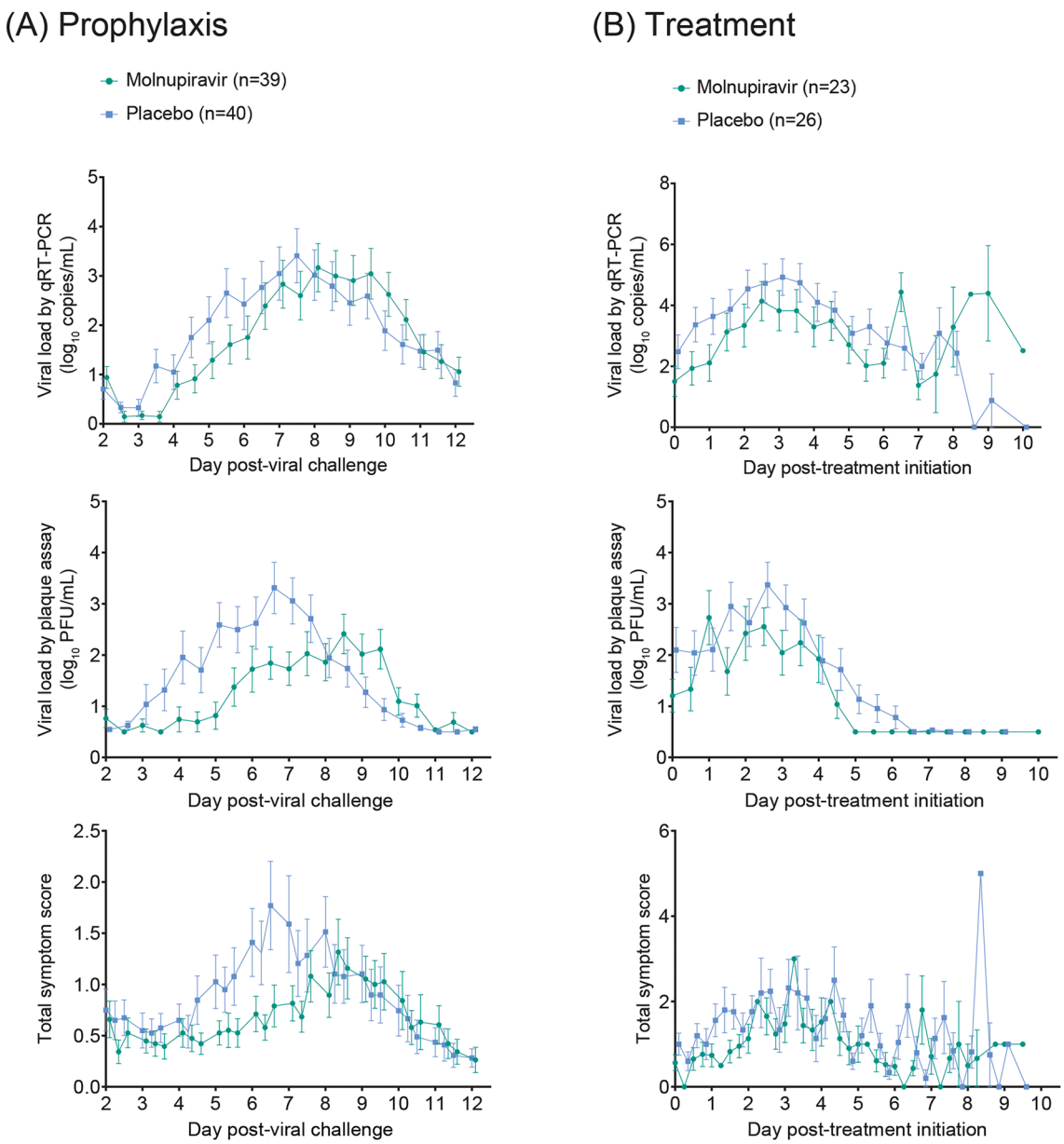
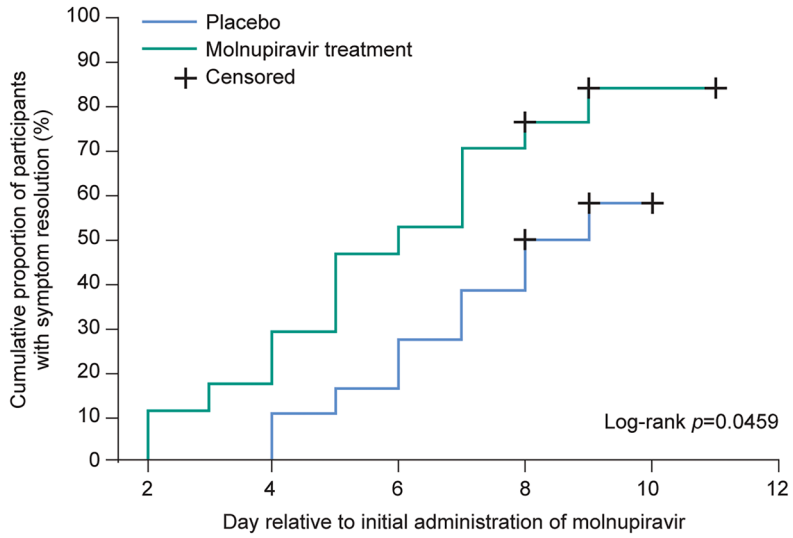


Fig. 2 Mean (standard error) viral load and total clinical symptoms for **A** molnupiravir prophylaxis in the FAS analysis population and **B** molnupiravir triggered treatment in the FAS-I analysis population. For treatment, the number of participants with data available became less as time progressed, resulting in greater variability in the depicted mean results for viral load by qRT-PCR and in total symptom score, after day 7. The lower limits of quantification were 3.24 log_{10} copies/ml in the qRT-PCR assay and 2 log_{10} PFU/ml in the plaque assay. *n* number of participants in the FAS primary analysis population (prophylaxis) or FAS-I primary analysis population (treatment)

in terms of total clinical symptoms (Tables 2, 3, Fig. 2). In the treatment arm, the median (1st quintile, 3rd quintile) time to symptom resolution from initial administration of study intervention was 6.0 (4.0, 8.0) days with molnupiravir and 8.5 (6.0, not reached) days with placebo (Fig. 3); the corresponding hazard ratio was 2.24 (95% CI: 0.99, 5.07) and the difference between arms was statistically significant ($p = 0.0459$). Finally, there were no significant



Number of participants at risk						
Placebo	18	18	15	11	1	0
Molnupiravir treatment	17	14	9	5	1	0
Number of events inside period						
Placebo	0	3	4	3	0	0
Molnupiravir treatment	3	5	4	2	0	0

Fig. 3 Kaplan–Meier curves of time to symptom resolution from initial administration of study intervention in the FAS-I analysis population of the molnupiravir treatment arm ($n=23$) versus the placebo arm ($n=26$). Participants without confirmed symptom resolution by the end of the trial were censored at their last date of symptom scoring

Table 4 Adverse event rates (safety population)

	Prophylaxis		Treatment		Placebo	
	n	(%)	*n*	(%)	*n*	(%)
Participants in safety population	39		36		40	
At least 1 AE	13	(33.3)	15	(41.7)	13	(32.5)
At least 1 drug-related[a] AE	2	(5.1)	1	(2.8)	3	(7.5)
At least 1 serious AE	0	(0.0)	0	(0.0)	0	(0.0)
At least 1 serious drug-related[a] AE	0	(0.0)	0	(0.0)	0	(0.0)
Died	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued treatment due to an AE	2	(5.1)	0	(0.0)	3	(7.5)
Discontinued treatment due to a drug-related[a] AE	1	(2.6)	0	(0.0)	1	(2.5)
Discontinued treatment due to a serious AE	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued treatment due to a serious drug-related[a] AE	0	(0.0)	0	(0.0)	0	(0.0)

[a]Determined by the investigator to be related to study intervention

differences in mean total mucus weight, which was 14.4 (standard deviation [SD]: ± 22.3) g with molnupiravir prophylaxis vs. 15.0 (SD: ± 18.4) g with placebo in the FAS population and 13.1 (SD: ± 21.4) g with molnupiravir triggered treatment vs. 17.2 (SD: ± 18.8) g with placebo in the FAS-I population. Individual and mean mucus weights over time are shown in the Supplementary Material (Figure S2).

Safety

AE rates were comparable between the prophylaxis, treatment, and placebo arms (Table 4), with 33.3%, 41.7%, and 32.5%, respectively, reporting ≥ 1 all-causality AE and 5.1%, 2.8%, and 7.5%, respectively, reporting ≥ 1 drug-related AE. No deaths or serious adverse events occurred in this trial, and only two participants (i.e., one each in the prophylaxis and placebo arms) discontinued study intervention due to a treatment-related AE. A full listing of all AEs, regardless of assumed relationship to study intervention, is shown in the Supplementary Material (Table S1). The reported drug-related AEs were diarrhea and rash (one participant each in the prophylaxis arm), nausea (one participant in the treatment arm), and hypersensitivity, increased alanine aminotransferase, and nasal congestion (one participant each in the placebo arm). In addition, three participants exhibited ≥ 1 AE deemed as related to the viral challenge: one participant in the prophylaxis arm with increased hepatic enzyme, one participant in the treatment arm with headache, and one participant in the placebo arm with ear pain and mouth ulcer; none of these viral challenge-related AEs were serious.

Pharmacokinetics

Mean geometric (geometric CV%) pharmacokinetic parameters for NHC in plasma for participants in the prophylaxis arm were as follows: $AUC_{0\text{-}12}$ of 9270 (28.4%) h*ng/ml, C_{max} of 3640 (33.5%) ng/ml, and C_{trough} of 25.1 (55.6%) ng/ml. Mean geometric (geometric CV%) pharmacokinetic parameters for NHC in plasma for participants in the treatment arm were: $AUC_{0\text{-}12}$ of 8100 (87.9%) h*ng/ml, C_{max} of 3010 (111.5%) ng/ml, and C_{trough} of 19.8 (66.8%) ng/ml. The differences in NHC pharmacokinetics between the two arms were driven by a single participant in the treatment arm, who had an abnormal pharmacokinetic profile (on day 7 only) that was consistent with a missed dose. Trough measurements of NHC-TP in peripheral blood mononuclear cells from this participant at other sampling time points were within the range of those from other trial participants, suggesting this particular participant did not systematically miss doses across the study intervention period. Exclusion of this participant resulted in geometric mean (geometric CV%) estimates for $AUC_{0\text{-}12}$ and C_{max} of 9100 (30.2%) h*ng/ml and 3470 ng/ml (33.5%), respectively, thus making the results in the treatment arm consistent with those from the prophylaxis arm.

DISCUSSION

In this rigorously designed phase 2a, randomized, double-blind, single-center human challenge trial, treatment of experimental RSV infection with molnupiravir showed consistent trends towards efficacy compared with placebo. However, the primary endpoint in the treatment setting, i.e., viral load AUC (determined by plaque assay) in participants with confirmed RSV infection, did not reach statistical significance. Molnupiravir treatment did also not perform significantly better than placebo in the secondary virology endpoints, including peak viral load and time to negative PCR. Notably, molnupiravir significantly reduced the time to symptom resolution by approximately 2.5 days over placebo in the treatment setting, but the difference in total symptom scores did not reach statistical significance. This trial included a prophylaxis arm, in which molnupiravir also exhibited a consistent trend towards improvement over placebo in the primary and all secondary virologic (and symptom-based) endpoints. However, none of these differences

were statistically significant. In terms of safety, molnupiravir resulted in all-cause AE and drug-related AE rates similar to those observed with placebo. Molnupiravir was well tolerated: only 4% of participants experienced a drug-related AE, and AE-related discontinuations of molnupiravir were rare (i.e., in only one of the 71 participants who received molnupiravir). The safety profile of molnupiravir was consistent with previous clinical studies, including phase 3 trials for treatment and prophylaxis of COVID-19 [38, 39, 47–51]. No new potential safety issues were identified. Of note, our study had very high completion rates of study therapy (96% of all participants) and of trial assessments (98% of participants), which facilitates results interpretation.

The preclinical data supporting the activity of molnupiravir and its active moiety NHC against RSV were rather limited in comparison to that against other RNA viruses, for which multiple studies support strong in vitro activity (in both plaque and cytopathic effect assays) as well as preclinical in vivo efficacy when administered after infection (as post-exposure prophylaxis and/or treatment). For example, NHC and molnupiravir have been shown as consistently active against SARS-CoV-2 variants and influenza virus in a variety of in vitro assays and animal models [28, 29, 32, 36, 52–55]. In contrast, the published preclinical data against RSV consist of only a single study showing in vitro activity in a plaque assay and in vivo efficacy when administered prior to infection (as pre-exposure prophylaxis) in a murine infection model [29]. Despite these limited preclinical data, a phase 2a trial was justified, given there is no clear preclinical cutoff indicating likely clinical efficacy and considering the high unmet need for RSV antiviral therapies. Additional in vitro evaluation of NHC against RSV found that the compound inhibited plaque formation but was inactive in a cytopathic effect assay [data on file]. Such apparent potency variations between assays are attributable to differences in assay conditions, such as cell lines used, multiplicity of infection, and incubation times. Nevertheless, the modest efficacy results of our RSV challenge study may reflect the limited and mixed preclinical data of NHC against this specific virus.

It is important to carefully consider all confounding factors that may have negatively impacted the observed efficacy in our study population, such as treatment window, RSV inoculum, pharmacokinetics, and sample size. Similar to previous RSV challenge studies, treatment initiation was triggered based on a positive PCR test (conducted twice daily) or initiated 5 days after experimental inoculation if PCR results up to this point had been negative [56–61]. An important strength of our trial was the inclusion of a prophylaxis arm, which allowed us to assess whether earlier treatment initiation might have improved outcomes. Since virologic outcomes with the prophylactic intervention were similar to those with triggered treatment, the modest viral load reduction observed with molnupiravir was likely not related to the triggered treatment window. It also does not appear that outcomes in our trial (which carefully enrolled only participants with low or no immunity to the challenge RSV strain) were impacted by the experimental viral inoculation. The ~ 65% infectivity rate observed with placebo was sufficiently high; a rate of ≥ 50%, which allows antiviral activity to be evaluated in a relatively small trial population, is the target for challenge studies. The observed placebo infectivity rate also suggests that the utilized RSV inoculum performed comparably to other challenge trials employing the same viral strain [56–59, 62, 63]. The viral load dynamics observed in placebo participants were also consistent with comparable challenge studies [56, 61], further confirming the integrity of the RSV strain used in our trial. Underexposure to NHC does also not explain the modest virologic effect observed, given that NHC plasma pharmacokinetic parameters were consistent with the current body of evidence, including a phase 1 trial in healthy adults [47, 64, 65]. The molnupiravir dosing regimen selected for our study (i.e., 800 mg twice daily for 5 days) has demonstrated a good safety and tolerability profile in multiple prior clinical trials [38, 39, 49–51], as well as clinical efficacy in treating COVID-19 [38–40]. We selected this same regimen for our trial, since data from in vitro assays and in vivo preclinical models suggested that similar levels of NHC may be effective against

RSV and SARS-CoV-2 [data on file, 29, 32]. Due to the study's small sample size, relative outliers in the efficacy results had a disproportionate impact on reported means. It is unknown if a trial enrolling more participants might have shown greater treatment differences between molnupiravir and placebo.

While phase 2 trials of investigational antimicrobial therapies are often conducted in the target population with the respective infection, this approach is difficult with potential RSV therapeutics. Clinically important populations for RSV antivirals are those most at risk of mortality and hospitalization (e.g., infants, heavily immunocompromised patients, and elderly patients with comorbidities), who by their very nature are extremely vulnerable to adverse outcomes. RSV clinical research therefore often relies on phase 2a human challenge studies as the first step in evaluating novel treatments, because experimental RSV infection of healthy adults under controlled conditions appropriately minimizes the risks of serious morbidity or death. Our trial with molnupiravir was rigorously conducted and robustly designed in line with regulatory guidance [45] and prior RSV human challenge studies, which makes the collected data suitable for informing potential follow-on studies and effective comparison with previously published challenge models. For example, to facilitate comparison of symptom data with similar studies, all of which utilized a ten-point symptom scale, we based our analyses on the same ten symptoms although we collected data on three additional symptoms. Notably, viral load for the primary endpoints was assessed by quantitative viral culture on plaque assay, which detects levels of viable, infectious virus. This is particularly important in the case of molnupiravir: since the drug's unique mechanism of action results in production of non-viable, non-infectious virus [33–35], changes in viral RNA levels as measured by PCR cannot fully reflect the drug's antiviral efficacy. While virologic outcomes are the most important endpoint in experimental challenge trials, clinical outcomes (including mortality rates, hospitalization rates, and symptom reductions) are considerably more relevant in patients with severe RSV disease. However, a lack of meaningful viral load reductions under these highly controlled conditions is generally assumed to indicate a low likelihood of achieving clinical efficacy in the less controlled setting of phase 2b trials [41, 43–45].

The main benefits of the human challenge model, i.e., enrolling healthy volunteers at low risk of complications from RSV disease, its ability to explore antiviral activity using a small sample size, and a study design that represents a highly controlled model of human disease, may also present theoretical limitations. For example, unlike real-world scenarios in which RSV treatment would frequently not be initiated for several days after symptom onset (or perhaps not until hospitalization), human challenge studies initiate treatment upon first evidence of infection. This aspect of trial design powers challenge studies to detect vial load reductions beyond those mediated by the natural immune response—early treatment initiation before peak viral titers are reached allows investigational therapies to show antiviral effects before host responses can shut down viral replication. On the other hand, this same feature may also be the main reason why RSV human challenge trials with promising virology results have thus far all failed to translate into successful follow-on phase 2b studies evaluating those same investigational therapies. These 2b trials conducted in patients with more severe RSV infections may have been designed in such a way that treatment initiation was too late, especially when compared to the corresponding challenge trials, since antiviral treatment for acute respiratory infections needs to be initiated soon after first symptoms in order to achieve meaningful clinical benefits [51, 66, 67]. Conversely, since our virology results suggested only modest activity, successful phase 2b trials may be even less likely with molnupiravir than with antivirals that had previously shown considerable promise in human challenge studies. Another potential limitation of the human challenge model is the choice of trial participants, since viral dynamics in healthy, younger adults are different from those in patients with weaker immune systems. Molnupiravir treatment may have yielded greater viral load reductions relative to placebo had we enrolled high-risk elderly or

immunocompromised participants. However, based on extensive prior experience with similar challenge studies, our trial was adequately powered to detect significant differences in its study population.

Irrespective of these considerations, additional analysis of challenge study data through modeling can provide valuable insights. Modeling was previously used to predict outcomes with other investigational agents, for example by simulating results using different dosing regimens or in other study populations [62, 68]. Simulations using an RSV viral dynamics model that was developed from our trial data suggested that molnupiravir is unlikely to yield significant viral load reductions in immunocompromised participants infected with RSV even with higher doses and/or longer treatment duration [data not shown]. Considering the limitations of human challenge trials, it may be conceivable that molnupiravir would be efficacious if evaluated in an appropriate patient population with RSV disease and using a clinical (instead of a virologic) primary endpoint. Only such a trial can ultimately confirm or refute the potential utility of molnupiravir as RSV treatment. However, the totality of our data and the associated modeling all suggest that follow-on clinical trials in this setting may not demonstrate the desired efficacy.

CONCLUSIONS

- In this study population of healthy volunteers, molnupiravir was found to be well tolerated, and the safety profile was consistent with previous observations.
- A modest benefit of molnupiravir over placebo was seen across all virologic endpoints (including the primary endpoints), but the magnitude of viral load reductions observed in the small treatment subgroup (less than 40 participants) of this phase 2a pilot trial did not reach statistical significance.
- In the treatment subgroup, molnupiravir resulted in significantly faster symptom resolution than placebo.

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Data Availability. The data sharing policy, including restrictions, of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD), is available at https://trialstransparency.msdclinicaltrials.com/policies-perspectives.aspx. Requests for access to the clinical study data can be submitted via email to the Data Access mailbox (dataaccess@msd.com).

Declarations

Conflict of Interest. Mickie Cheng, Brian M. Maas, Tian Zhao, Andrea K. Schaeffer, Laura E. Liao, S. Aubrey Stoch, and Carisa S. De Anda are employees of the trial sponsor Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD), who may own stock and/or hold stock options in Merck & Co., Inc., Rahway, NJ, USA. Alex Mann, Melissa Bevan, and Andrew Catchpole are employees of hVIVO, which contracted with the trial sponsor to conduct this clinical trial.

Ethical approval. The study was conducted in accordance with principles of Good Clinical Practice (in accordance with the ethical principles that have their origin in the Declaration of Helsinki) and was approved by the appropriate institutional review board (South Central - Berkshire B Research Ethics Committee; REC reference 22/SC/0314) and all appropriate regulatory agencies. Written informed consent was obtained from all participants prior to their participation in the trial.

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