

Online supplementary material to:

**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**

Mickie H. Cheng, MD, PhD^{1*}, Alex J. Mann, MSc², Brian M. Maas, PharmD¹, Tian Zhao, PhD¹,
Melissa Bevan, MBBS, BSc, MRCP², Andrea K. Schaeffer, MS¹, Laura E. Liao, PhD¹, Andrew
P. Catchpole, DPhil², David W. Hilbert, PhD¹, S. Aubrey Stoch, MD¹, Carisa S. De Anda,
PharmD¹

¹Merck & Co., Inc., Rahway, NJ, USA; ²hVIVO Services Ltd., London, United Kingdom

* Corresponding author (Email: mickie.cheng@merck.com)

Table of Contents

Methods S1: Nasal wash sample processing and analysis	2
Methods S2: Conversion rules for viral load assay results into analysis values for VL-AUC calculation	4
Figure S1: Individual (light lines) and mean (heavy lines) mucus weight by study arm (A) in the FAS population and (B) in the FAS-I population	5
Table S1: Full listing of adverse events (regardless of assumed relationship to study intervention or RSV inoculation) from inoculation to the day 28 follow-up visit, by treatment arm in the safety population.....	6

Methods S1: Nasal wash sample processing and analysis

Nasal wash processing for virology analyses. Nasal wash samples were collected for the purposes of diagnosing RSV infection (by qualitative real time [RT]-PCR) in the treatment arm and for viral load analysis (by quantitative RT-PCR and quantitative cell-culture plaque assay) in all arms. Nasal wash samples were transferred to the laboratory immediately after collection on wet ice and were processed as follows:

1. The sample was pulse-vortexed to break down mucus. If mucus clumps persisted, the sample was homogenized by pipette.
2. The aliquots for plaque assay and quantitative RT-PCR were immediately diluted 1:1 with hVIVO's proprietary 2X stabilization transport media (STM) and then transferred to -80°C storage. Aliquots used for qualitative PCR were not diluted.

Qualitative RT-PCR (for diagnosis). The onset of viral shedding (to trigger molnupiravir administration in treatment arm participants) was determined using a real-time reverse transcription-polymerase chain reaction method for the rapid detection of RSV that has previously been described in detail [see: [Kelly G, et al. Use of qualitative integrative cyclor PCR to identify optimal therapeutic dosing time-points in a Respiratory Syncytial Virus Human Viral Challenge Model. J Virol Methods, 2015. 224: 83–90.](#)] To start, 50 µL of nasal wash from each individual sample was mixed with 50 µL of reagent from the Simplexa™ Flu A/B and RSV reaction kit (Focus Diagnostics, Cypress, CA, USA) in a single disc wedge of a Simplexa 8-well Direct Amplification Disc (Focus Diagnostics, Cypress, CA, USA). Direct Amplification Discs then were processed and amplified within a 3M Integrated Cyclor (3M, Saint Paul, MN, USA), which detected the presence of target virus RNA in individual samples using real-time fluorometry. Amplification plots were reviewed for each sample and results reported as 'detected' or 'not detected' using a pre-defined cycle threshold (Ct) cut-off value of Ct >0 and ≤38 as positive for RSV.

Quantitative RT-PCR (for viral load analysis). Respiratory syncytial virus RNA was extracted from nasal wash samples using a Qiagen DSP Virus/Pathogen Midi kit (Qiagen, Hilden, Germany) on the QIASymphony SP module (Qiagen, Hilden, Germany). Each extracted RNA sample was then loaded in triplicate via the QIASymphony AS module (Qiagen, Hilden, Germany) into the QuantiFAST mastermix (Qiagen, Hilden, Germany) containing an RSV Taqman hydrolysis probe (Qiagen, Hilden, Germany) and PCR primers targeting the RSV genome (Qiagen, Hilden, Germany). The 2-step quantitative RT-PCR reaction was performed

using a ViiA7 thermocycler (Applied Biosystems, Waltham, MA, USA) for up to 40 cycles. The quantity of viral RNA was determined by the comparison of Ct values to a calibration standard curve for target-specific copy number; this standard curve was prepared from in vitro-generated nucleic acid copies of the target RSV gene sequences and run with each batch of samples. The RNA quantity mean of the triplicates (as calculated by the ViiA7 software) was then used to calculate the final viral load reported for each sample. During the assay validation experiments, the lower limit of quantification (LLOQ) in the assay was determined as a Ct of 34.4, which corresponded to a viral load of 3.24 log₁₀ copies/mL. The validation experiments involved the repeat testing of known concentrations of virus, performed in accordance with both the US Food and Drug Administration and the International Conference on Harmonisation guidelines. LLOQ was defined as the Ct associated with the lowest concentration of virus at which all replicates were detected within the expected range. The viral load of test samples with a Ct of ≤34.4 was calculated from the standard curve. All positive quantitative RT-PCR results with a Ct of >34.4 were reported as “detected”; however, viral load was not calculated from these samples because the viral load was less than the validated LLOQ of the assay.

Quantitative plaque assay (for viral load analysis). Human epithelial type 2 (HEp-2) cells were seeded into 24-well assay plates at 5×10^5 cells/mL, incubated at 37°C (±2°C) under 5% CO₂ overnight, and used at 80%–90% confluence on the day of the assay. Nasal wash samples were added to the assay plates wells at 4 different dilutions (each dilution in triplicate), followed by incubation for 1 hour at 37°C (±2°C) under 5% CO₂, before addition of methyl cellulose media (Sigma-Aldrich, St. Louis, MO, USA). The plates were incubated for 6 days at 37°C (±2°C) under 5% CO₂. The assay plates were fixed with 10% neutral buffered formalin and stained with hematoxylin solution (0.06% v/v) and eosin Y solution (1% v/v) and then dried overnight. The presence and number of plaques per assay plate were determined by light microscopy. For each dilution, the virus titer in log₁₀ plaque-forming units (PFU)/mL was calculated as the average number of plaques counted for the 3 replicate wells. The overall virus titer in log₁₀ plaque-forming units (PFU)/mL for each nasal wash sample was calculated as the mean titer of all valid dilutions for this individual sample.

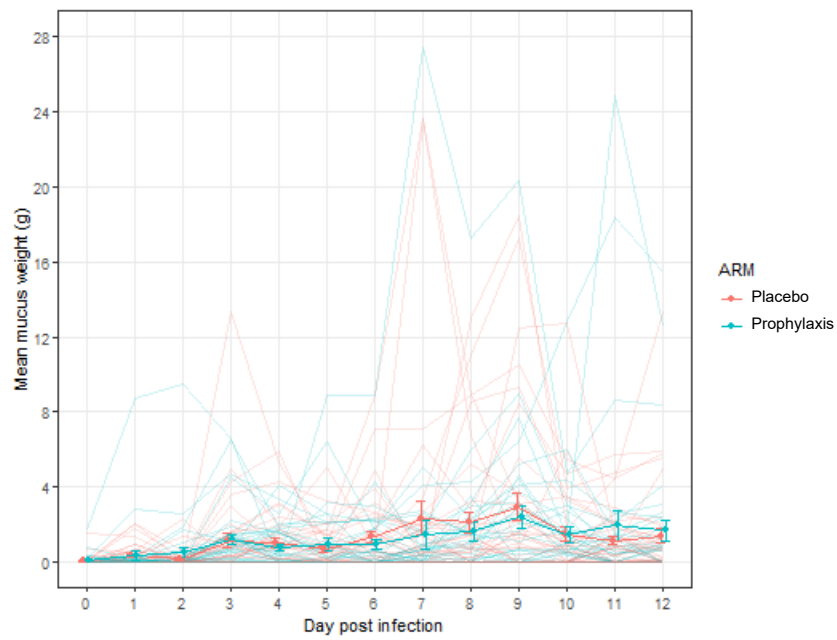
Methods S2: Conversion rules for viral load assay results into analysis values for VL-AUC calculation

Result	Reported result	S-cubed assigned value
Plaque assay (units: log ₁₀ plaque-forming units/mL)		
Quantifiable titer	Actual value	Use reported result
Detected	Detected	1.0
Not detected	Not detected	0.5
Invalid	Invalid	Missing data point
Not tested	[n/a]	Missing data point
Quantitative RT-PCR (units: log ₁₀ copies/mL)		
Quantifiable titer	Actual value	Use reported result
Detected	Detected	1.62
Not detected	Not detected	0
Invalid	Invalid	Missing data point
Not tested	[n/a]	Missing data point

n/a, not applicable. VL-AUC, area under the viral-load time curve.

Figure S1: Individual (light lines) and mean (heavy lines) mucus weight by study arm (A) in the FAS population and (B) in the FAS-I population

(A)



(B)

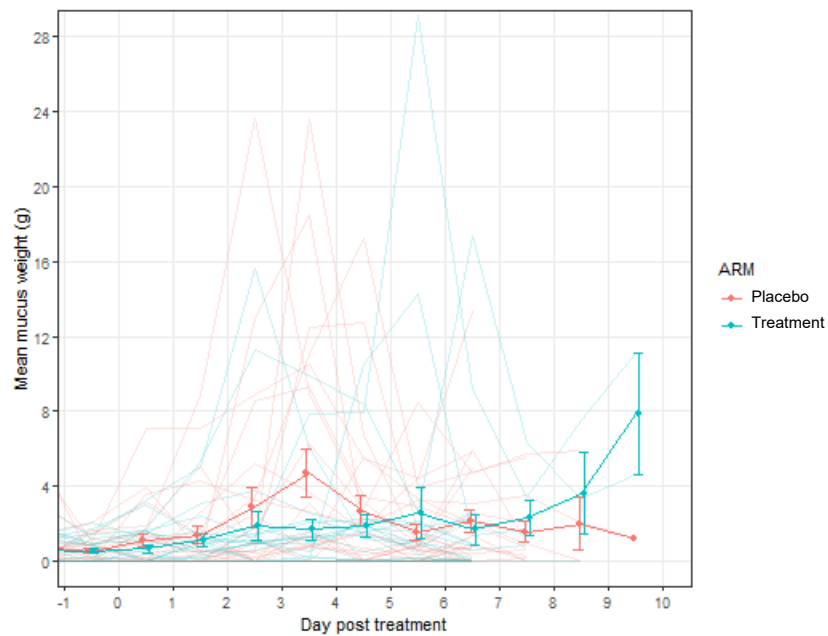


Table S1: Full listing of adverse events (regardless of assumed relationship to study intervention or RSV inoculation) from inoculation to the day 28 follow-up visit, by study arm in the safety population.

	Prophylaxis		Treatment		Placebo		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Participants in population	39		36		40		115	
with one or more adverse events	13	(33.3)	15	(41.7)	13	(32.5)	41	(35.7)
with no adverse events	26	(66.7)	21	(58.3)	27	(67.5)	74	(64.3)
Blood and lymphatic system disorders	0	(0.0)	1	(2.8)	1	(2.5)	2	(1.7)
Lymphopenia	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Neutropenia	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Ear and labyrinth disorders	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Ear pain	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Eye disorders	1	(2.6)	0	(0.0)	1	(2.5)	2	(1.7)
Eyelid skin dryness	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Ocular hyperaemia	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Gastrointestinal disorders	1	(2.6)	2	(5.6)	2	(5.0)	5	(4.3)
Abdominal pain	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Diarrhoea	1	(2.6)	0	(0.0)	1	(2.5)	2	(1.7)
Flatulence	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Mouth ulceration	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Nausea	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Toothache	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)

General disorders and administration site conditions	1	(2.6)	2	(5.6)	0	(0.0)	3	(2.6)
Catheter site pain	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Chest discomfort	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Chest pain	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Immune system disorders	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Hypersensitivity	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Infections and infestations	3	(7.7)	3	(8.3)	2	(5.0)	8	(7.0)
COVID-19	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Cellulitis	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Ear infection	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Influenza	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Tinea versicolour	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Upper respiratory tract infection	1	(2.6)	2	(5.6)	0	(0.0)	3	(2.6)
Investigations	2	(5.1)	0	(0.0)	1	(2.5)	3	(2.6)
Alanine aminotransferase increased	1	(2.6)	0	(0.0)	1	(2.5)	2	(1.7)
Hepatic enzyme increased	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Musculoskeletal and connective tissue disorders	2	(5.1)	5	(13.9)	1	(2.5)	8	(7.0)
Back pain	1	(2.6)	1	(2.8)	0	(0.0)	2	(1.7)
Musculoskeletal chest pain	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Musculoskeletal pain	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Rhabdomyolysis	0	(0.0)	1	(2.8)	1	(2.5)	2	(1.7)
Synovial cyst	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Temporomandibular joint syndrome	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)

Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Osteoma	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Nervous system disorders	1	(2.6)	1	(2.8)	2	(5.0)	4	(3.5)
Cluster headache	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Headache	0	(0.0)	1	(2.8)	1	(2.5)	2	(1.7)
Syncope	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Psychiatric disorders	2	(5.1)	2	(5.6)	2	(5.0)	6	(5.2)
Anxiety	1	(2.6)	0	(0.0)	2	(5.0)	3	(2.6)
Depressed mood	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Panic attack	1	(2.6)	1	(2.8)	0	(0.0)	2	(1.7)
Panic reaction	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)
Respiratory, thoracic and mediastinal disorders	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Nasal congestion	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Skin and subcutaneous tissue disorders	3	(7.7)	2	(5.6)	2	(5.0)	7	(6.1)
Dermatitis, contact	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Dry skin	1	(2.6)	0	(0.0)	0	(0.0)	1	(0.9)
Rash	1	(2.6)	1	(2.8)	0	(0.0)	2	(1.7)
Rash erythematous	1	(2.6)	1	(2.8)	0	(0.0)	2	(1.7)
Skin lesion	0	(0.0)	0	(0.0)	1	(2.5)	1	(0.9)
Skin mass	0	(0.0)	1	(2.8)	0	(0.0)	1	(0.9)

Adverse event terms are from MedDRA Version 26.