0739

Ultrasensitive Assay Assessment of Host DNA Mutations in Molnupiravir-Treated SARS-CoV-2

Shuntai Zhou[1], Nathan Long[1], Collin S. Hill[1], Micheal Vernon[1], Michael Clark[1], Jessica R Keys[1], Dinelka Nanayakkara[1], Sai Mugi[1], Becky Straub[1], Lakshmanane Premkumar[1], Katie R Mollan[1], Timothy P Sheahan[1], Ronald Swanstrom[1], William A Fischer[1], David A Wohl[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, USA

BACKGROUND

RNA viruses exhibit high genetic diversity due to rapid replication and error-prone polymerases. Molnupiravir, a prodrug of N-hydroxycytidine (NHC), exploits this vulnerability by inducing transition mutations during viral RNA synthesis and has demonstrated broad antiviral activity. However, because NHC can be incorporated during host DNA replication, concerns remain regarding potential host mutagenesis.

Here, we used ultra-accurate error-corrected sequencing to simultaneously assess mutations in host DNA and viral RNA following NHC exposure in people with acute COVID-19. Using peripheral blood, we quantified low-frequency host DNA mutations and characterized antiviral mutational signatures across doses and time points.

METHODS

- Participants were those in the VISION study, a prospective longitudinal observational study of adults with self-reported diagnosis of SARS-CoV2 infection within 7 days of enrollment.
- Participants who initiated or planned to initiate oral nirmatrelvir/ritonavir (NMV/r) or molnupiravir (MOV) within 48 h of study entry were eligible. Treated participants were and age- and sex-matched to untreated controls.
- Whole blood specimens were collected at study Weeks 0, 2, and 4 for mutagenesis assays. Nasopharyngeal (NP) swab specimens were obtained at Week 0 and Day 5.
- Two ultra-sensitive assays were applied to detect host DNA mutations. MutaFlow PIG-a assay and TwinStrand DuplexSeq V2 mutagenesis panel.
- Multiplexed Primer ID sequencing was performed to measure viral RNA sequence diversity in samples with detectable SARS-CoV-2.
- A linear mixed-effects model (LMM) was used to compare changes in DNA sequence diversity across treatment groups; exact nonparametric tests were applied for sensitivity analyses.

Table 1. Baseline characteristics of study participants

	MOV (N=5)	NMV/r (N=13)	Control (N=11)	Total (N=29)	p value
Age, y/o					0.1
Median (IRQ)	70 (67-73)	53 (50-74)	50 (42-55)	53 (47-70)	
Sex					0.23
Male (%)	2 (40%)	9 (69%)	4 (36%)	15 (52%)	
Race					0.53
Caucasian	5 (100%)	12 (92%)	11 (100%)	28 (97%)	

We did not observe evidence of increased host DNA mutation frequency or accumulated viral mutations in outpatients with COVID-19 treated with molnupiravir in a real-world study.

RESULTS

- 29 participants enrolled and analyzed in this study. MOV (N=5), NMV/r (N=13), Control (N=11). Participant demographics are reported in **Table 1**.
- DuplexSeq assays achieved a median sequencing depth of 600 million duplex bases per specimen and a median DNA mutation rate of 2.6×10^{-7}. We did not detect effects by treatment group, visit time, or their interaction on DNA mutation rates. One MOV participant had the highest DNA mutation rate but lacked signature MOV mutation patterns (**Figure 1**).
- In the PIG-a assay, mutant RBCs and RETs were quantified per 10^6 RBCs or RETs. LMMs again showed no detectable treatment effects on mutant RBC or RET frequencies (**Figure 2**).
- Among two MOV participants with detectable SARS-CoV-2 at Day 5, viral RNA mutation density decreased, and no fixed mutations detected compared with baseline specimens (**Figure 3**).
- In vitro experiment using mouse embryo fibroblasts cultured with different concentrations of NHC demonstrated a time- and dose-dependent increase in host DNA mutation frequencies (**Figure 4**).

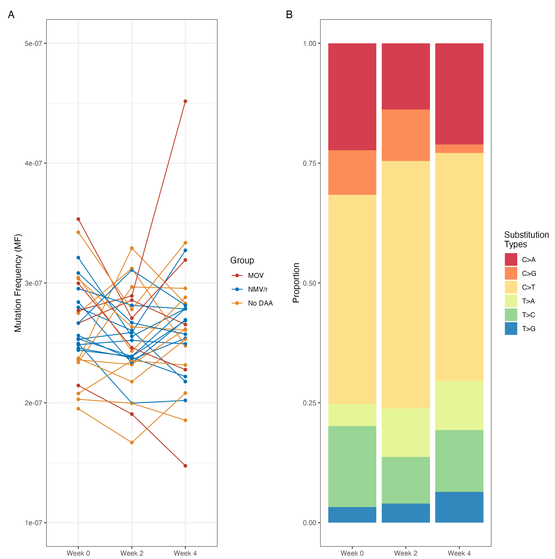


Figure 1. Host DNA mutation frequencies and mutation spectra measured by Duplex Sequencing in VISION host genetic substudy.

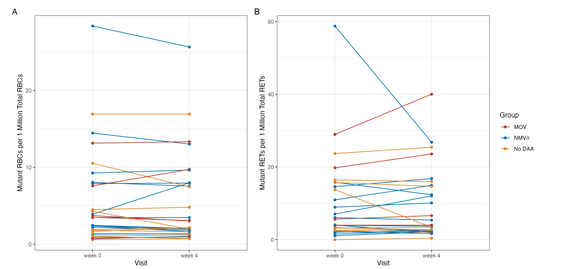


Figure 2. PIG-a mutant frequencies. Each line represents an individual participant, colored by treatment group. No systematic differences were observed across treatment groups.

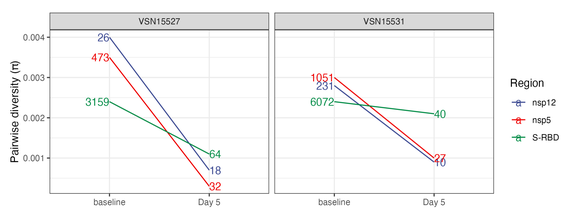


Figure 3. Change of pairwise diversity in viral genomes at baseline and Day 5 for two participants in the MOV group. The numbers in the figure indicate the number of TCS (sampling depth) for each sequenced region.

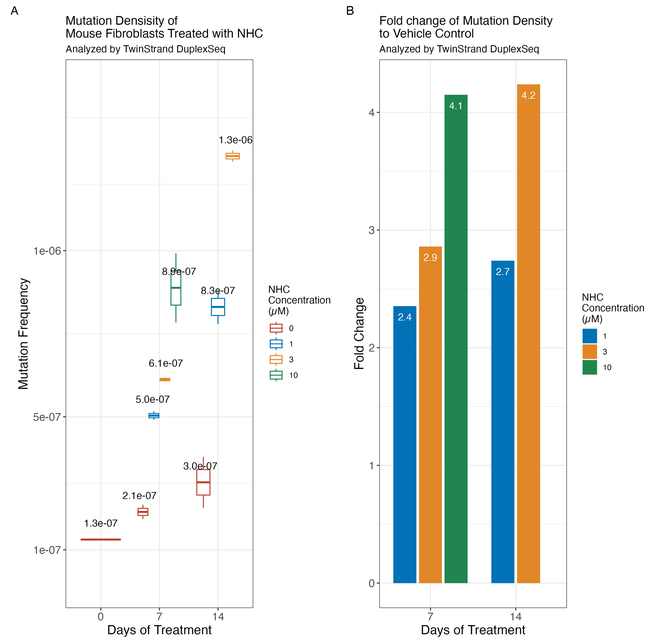


Figure 4. NHC induces host DNA mutagenesis in mouse embryonic fibroblasts.

CONCLUSIONS

- This real-world study applied ultra-sensitive assays to evaluate host and viral mutational outcomes following antiviral treatment for COVID-19.
- Within the limits of sample size and tissue accessibility, we did not observe evidence of increased host DNA mutation or accumulation of viral mutations from individuals treated with a short course of oral molnupiravir.
- Controlled in vitro experiments confirmed the sensitivity of the sequencing approach used.
- These findings highlight important differences between experimental models and real-world clinical settings and underscore the value of evaluating mutagenic antivirals directly in treated human populations.

Funding: North Carolina Policy Collaboratory: UNC-CH-184

PLAIN LANGUAGE SUMMARY

We examined whether the COVID-19 antiviral molnupiravir causes genetic changes in people treated with the drug. Using highly sensitive testing methods, we did not find evidence of increased mutations in 5 MOV-treated outpatients or their virus after short-term treatment, although mutations were observed in laboratory cells exposed to the drug under controlled conditions.