

Molnupiravir-induced mutagenesis and the risk of emergence of new SARS-CoV-2 variants of concern

Public comments re: FDA-2021-N-0758 by Rustem Ismagilov

- I have no conflicts of interest with authorization of molnupiravir or with Merck & Co.
- I am a professor at Caltech and have discussed this topic with other scientists, however the opinions presented here are my own.
- My comments outlining these concerns (<https://www.regulations.gov/comment/FDA-2021-N-0758-0015>) provide some additional mechanistic details. They were written before the Meeting Materials became available.

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SARS-CoV-2 evolution continues to be a concern as of Nov 2021, with the potential for many additional combinations of mutations beyond the Delta variant

The B.1.1.529 variant was first reported to WHO from South Africa on 24 November 2021. The epidemiological situation in South Africa has been characterized by three distinct peaks in reported cases, the latest of which was predominantly the Delta variant. In recent weeks, infections have increased steeply, coinciding with the detection of B.1.1.529 variant. The first known confirmed B.1.1.529 infection was from a specimen collected on 9 November 2021.

This variant has a large number of mutations, some of which are concerning. Preliminary evidence suggests an increased risk of reinfection with this variant, as compared to other VOCs. The number of cases of this variant appears to be increasing in almost all provinces in South Africa. Current SARS-CoV-2 PCR diagnostics continue to [https://www.who.int/news/item/26-11-2021-classification-of-omicron-\(b.1.1.529\)-sars-cov-2-variant-of-concern](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern)

Molnupiravir mechanism of action is viral mutagenesis and, under non-ideal conditions, could lead to production of highly mutated but viable SARS-CoV-2 virus

- Molnupiravir induces viral mutations (PMID: 34381216)
- High and constant concentrations of such drug applied over long periods of time increase mutation rate so much that it leads to “lethal mutagenesis” (PMID: 17202214)
- In humans, such “lethal mutagenesis” may not always be achieved
 - Heterogeneous pharmacokinetics in diverse populations -> lower drug concentrations in some individuals
 - “Refugia” body compartments with lower drug concentrations
 - Sub-therapeutic usage of the drug
 - Transient nature of the treatment from each dose
 - Short-term (5 day) treatment
 - The emergence of beneficial (to the virus) mutations that raise the threshold of lethal mutagenesis (PMID: 17202214)
- Coronaviruses have low basal mutation rate (~0.03 mutations/genome/ replication for SARS-CoV-1) because of proofreading (PMID: 20463816)
- Therefore, molnupiravir-induced multiple simultaneous mutations followed by off-drug replication under immune selection pressure could produce unusual, highly mutated viruses that escape the immune response

In treated humans, on average, molnupiravir increases mutations in SARS-CoV-2 genome, including the spike gene

participants (pooled MOV and placebo) were identified and tabulated. Note that NGS analyses were generally restricted to samples collected up to Day 5 (EOT), so these analyses would not identify changes that emerged or persisted at later timepoints.

Results of these analyses are summarized in [Table 8](#). Consistent with the MOV mechanism of action, a greater proportion of participants in the MOV arms relative to the placebo arm had at least one treatment-emergent amino acid substitution or other structural nucleotide change (deletion, insertion) detected in the spike gene, and amino acid changes were scattered throughout the coding sequence. A total of 81 emergent

p. 32 of AMDAC-20211130-Backgrounder-FDA.pdf from <https://www.fda.gov/advisory-committees/advisory-committee-calendar/november-30-2021-antimicrobial-drugs-advisory-committee-meeting-announcement-11302021-11302021#event-materials>

Just a few cases of SARS-CoV-2 evolution and transmission of immune-escape variants among millions of molnupiravir-treated patients could change the course of this pandemic

- To understand the risk of molnupiravir-induced SARS-CoV-2 evolution, one must analyze and monitor the “outlier” evolutionary events (and not only the effect of molnupiravir on mutations on average).
- Even a 1:10,000 probability of molnupiravir-induced SARS-CoV-2 evolution and transmission of an immune-escape variant is highly significant when millions of patients are treated.

Any evidence for uncommon, but potentially highly impactful “extensive molnupiravir-driven SARS-CoV-2 mutagenesis and selection” should not be overlooked or dismissed

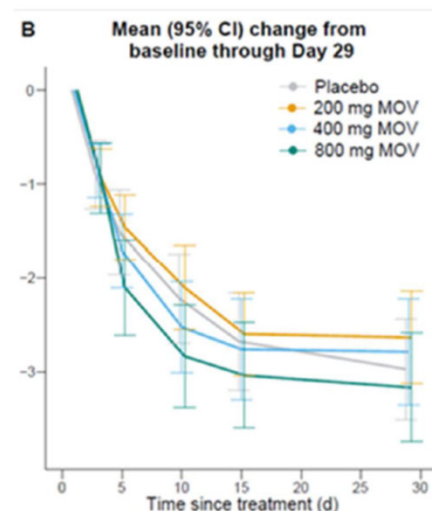
In a few individual participants, numerous treatment-emergent spike changes were detected in association with other changes elsewhere in the genome, as noted above for the MK-4482-001 participant with treatment-emergent Δ Y145, N501Y, and P681H. It is unclear if this reflects extensive MOV-driven mutagenesis and selection, coinfection with multiple SARS-CoV-2 variants, or a technical issue.

p. 35 of AMDAC-20211130-Backgrounder-FDA.pdf from <https://www.fda.gov/advisory-committees/advisory-committee-calendar/november-30-2021-antimicrobial-drugs-advisory-committee-meeting-announcement-11302021-11302021#event-materials>

- All samples from these few individual participants should be re-sequenced and reanalyzed and the mutations and the extent of evolutionary shift should be compared to the ones in the B.1.1.529 omicron variant.
- These data will clarify molnupiravir-driven SARS-CoV-2 evolutionary trajectory, inform the coinfection hypothesis, and eliminate any technical issues.
- Both existing and new sequencing data should be publicly released.
- Seeing examples of such extensive mutagenesis from just a couple hundred treated individuals would be extremely concerning. These data would help estimate the risk of molnupiravir-driven evolution of new, highly mutated SARS-CoV-2 variants of concern (such as B.1.1.529) when millions of people are treated.

Transmission risk of molnupiravir-induced mutants cannot be ignored

- While SARS-CoV-2 is highly transmissible, “culturable virus was rarely detected across the entire study population”, calling for cautious interpretation and consideration of potential issues with sampling sites, preanalytical sample handling, and unspecified limit of detection of the viral culture assay.
- Culturable virus was detected in molnupiravir-treated individuals on day 3 of treatment. Transmission may occur via aerosols formed in the lung; the level of culturable virus in the lung was not assessed.
- While theory predicts that “lethal mutagenesis” should drive down viral load and reduce transmission, the reality of in vivo treatment is more complex (<https://pubmed.ncbi.nlm.nih.gov/17202214/>) due to “refugia” compartments, transient nature of the treatment, and the emergence of beneficial (to the virus) mutations.
- On average, treated outpatient population showed reduction of viral load. However, viral load reduction proceeded at similar rates in the treated 200 mg, 400 mg, and 800 mg treatment groups and the placebo group, with modest differences relative to the typical 10^9 copy/mL range of viral loads. Thus, it cannot be assumed that the same viral load reduction would occur, and would be sustained post-treatment, in immunocompromised patients which do not rapidly clear the virus without treatment. The viral load reduction was highly variable.
- A key concern is not the average reduction of transmission upon molnupiravir treatment. Even uncommon transmission events which could drive the spread of the highly mutated viruses are of significant concern.



Summary: molnupiravir-induced mutagenesis and production of new SARS-CoV-2 variants of concern

- Data suggest that extensive SARS-CoV-2 evolution and selection may have already occurred in a few molnupiravir-treated individuals to produce highly mutated viruses of concern. Let's not assume that they are technical artifacts, because recent emergence of the highly mutated SARS-CoV-2 variant of concern B.1.1.529 shows such extreme evolutionary events do occur and do have global impact.
- Public release of these data, and additional sequencing data from these molnupiravir-treated individuals is urgently needed.
- It would be prudent to obtain and analyze viral sequencing data from the MK-4482-001 inpatient trial, in which numerically higher proportion of participants died in all three molnupiravir-treated groups (5.5%, 11.0%, 4.2%) compared with placebo (2.7%). One should exclude the possibility that drug-induced viral evolution and immune escape played any role in these deaths.
- The potential for transmission of SARS-CoV-2 mutants generated by molnupiravir treatment, especially in immunocompromised patients and during treatment, cannot be eliminated based on the current data.
- If molnupiravir is used in 1,000,000s of people, even rare (1:10,000 or 1:100,000) molnupiravir-induced SARS-CoV-2 evolutionary and transmission events could change the course of the pandemic.
- Generating multiple new SARS-CoV2 variants per year, each able to escape immunity, each with a different genotype, and each with a different protein sequence of the vaccine target, would overwhelm pandemic response capacity in any country, and in low-income countries in particular.
- Additional data and, as needed, rapid engagement of the broad scientific community would help evaluate the probability of rare, but potentially catastrophic, molnupiravir-induced SARS-CoV-2 evolutionary and transmission events.
- The Sponsor, the Advisory Committee, and the FDA must take all possible steps and use the best science possible to ensure that such molnupiravir-induced mutagenesis and production of new SARS-CoV-2 variants of concern does not occur.