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Public comment Re: Docket **FDA-2021-N-0758;** consideration of Emergency Use Authorization (EUA) application 000108, submitted by Merck & Co. Inc., for emergency use of molnupiravir oral capsules for treatment of mild to moderate COVID-19 in adults who are at risk for progressing to severe COVID-19 and/or hospitalization.

Summary:

In this Public Comment I express the concern that molnupiravir could accelerate SARS-CoV-2 evolution and lead to the emergence of new transmissible variants able to escape immunity provided by vaccines or previous SARS-CoV-2 infections. I provide mechanistic rationale for how such SARS-CoV-2 evolution and transmission could take place. I summarize current publicly available evidence, including that (i) molnupiravir induces SARS-CoV-2 mutations in treated humans and (ii) infectious SARS-CoV-2 virus is shed by humans during treatment with molnupiravir. I emphasize that this scenario—transient drug-induced mutagenesis followed by a period of high-fidelity viral replication and selection—is different than a more commonly studied scenario of viral evolution with constant mutation rate, and therefore should be carefully analyzed.

A single viral evolutionary event in one of a million molnupiravir-treated patients could change the course of this pandemic for the worse. Therefore, the scientific concern is not whether evolution occurs in every treated person, or whether an immune-escape mutant was already generated in one of a few thousand treated patients. The concern is to understand less common drug-induced viral evolution and transmission events that may be more likely under special circumstances—including those early in the course of treatment, and in individuals with sub-therapeutic doses of the drug and/or weakened immune system. A molnupiravir-induced evolutionary event that produces an immune-escape mutant, if allowed to spread, would negatively impact the entire world's population. Therefore, this Advisory Committee and the FDA, supported by the scientific community, must ensure that such molnupiravir-induced SARS-CoV-2 evolution and transmission of immune-escape variants does not occur.

Detailed description:

Molnupiravir is an orally available small molecule promising to treat Covid-19 when treatment is initiated sufficiently early in the course of the SARS-CoV-2 infections. Molnupiravir is a pro-drug that acts by inducing mutations in the virus (*Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis PMID: 34381216*). In cells, molnupiravir is converted to N^4-hydroxycytidine (NHC) triphosphate, which is mis-incorporated by RNA-dependent RNA polymerase (RdRp). The mis-incorporated NHC subsequently base-pairs with either G or A during viral replication, generating mutants of the virus. At therapeutic (high) concentrations of the drug administered over a sufficiently long time period, this mutagenesis generates highly mutated virus progeny with low viability. In general, this drug-induced lethal mutagenesis and "error catastrophe" resulting in significant reduction of viral load would benefit the infected host and reduce transmission of the virus to others, excellent outcomes for treating a highly pathogenic and transmissible virus like SARS-CoV-2.

This mechanism of molnupiravir's action implies that at lower (sub-therapeutic) concentrations of the drug, NHC would compete less effectively with the cellular pool of ribonucleoside triphosphates and would reduce the degree of mis-incorporation of NHC by RdRp. Therefore, low concentrations of molnupiravir will generate virions that are viable yet more mutated than the virions produced without exposure to the drug. This process would increase the genetic diversity of the viable virus. Publicly available data for other coronaviruses support this expectation, with the extent of NHC-induced viral mutagenesis proportional to concentration of NHC (Fig 4 of *Small-Molecule Antiviral β-d-N^4-Hydroxycytidine Inhibits a Proofreading-Intact Coronavirus with a High Genetic Barrier to Resistance, PMID: 31578288*). Furthermore, SARS-CoV-2 (RdRp gene) sequenced from molnupiravir-treated humans displayed a statistically significant increase in viral mutation rate relative to the virus in untreated controls (*Molnupiravir, an Oral Antiviral Treatment for COVID-19, PMID: 34159342*).

It is likely that there is only a small gap between the high concentrations of the drug that effectively reduce viral viability and the low concentrations of the drug that primarily induce mutagenesis. For example, data for two other coronaviruses (Fig. 3 of *Small-Molecule Antiviral β-d-N^4-Hydroxycytidine Inhibits a Proofreading-Intact Coronavirus with a High Genetic Barrier to Resistance, PMID: 31578288*) show that while 8 μM concentration of NHC induces high mutagenesis and reduces viral titers by about 1000-10,000 fold, a slightly smaller 2 μM concentration of NHC still induces viral mutagenesis, but only with moderate (about 10 fold) reduction of viral titers.

It appears that clinically used 800 mg dosage of molnupiravir is not far above this gap between the low (mutagenic to SARS-CoV-2 but not lethally so) and the high (lethally mutagenic) concentrations of the drug. This supposition is suggested by two observations from human clinical studies (*Molnupiravir, an Oral Antiviral Treatment for COVID-19, PMID: 34159342, https://www.medrxiv.org/content/10.1101/2021.06.17.21258639v1*). (i) First, rate of reduction in SARS-CoV-2 viral RNA load in nasopharynx (Fig. 2C) is similar among the 800 mg, 400 mg, 200 mg, and placebo groups. The differences are not significant (Table 2) for any group at day 3 and day 14 while significant for the 800 mg group at days 5 and 7. (ii) Second, even on day 3 of treatment (Table 2), all four groups had at least one participant positive for infectious SARS-CoV-2 virus, with only the 800 mg group having a statistically significantly lower percentage of positivity relative to placebo. Note that a negative viral culture test from a nasopharyngeal swab does not prove complete elimination of viable virus from the patient; it shows that the viral titer was decreased below the limit of detection—unspecified, but known to be dependent on the experimental details and the cell lines used—of the viral culture assay. Furthermore, a negative culture result from a single sampling location (e.g. nasopharyngeal) does not exclude the possibility that infectious virus is still present in other body compartments, e.g. the lung, a source of aerosols involved in SARS-CoV-2 transmission. These considerations suggest that in at least some individuals molnupiravir—especially early in the course of treatment and/or when used at sub-therapeutic dosage—may generate infectious yet more mutated SARS-CoV-2 virus that could be transmitted to others.

Sub-therapeutic drug concentrations or sub-therapeutic drug exposure durations of molnupiravir could be encountered via at least three mechanisms. First is biodistribution in the human body. If the drug is distributed non-uniformly, it could achieve lower concentrations and shorter exposures in some body compartments than others. Because SARS-CoV-2 infects a wide range of cells and tissues, one would need to be concerned, in particular, with any compartment that might experience the combination of low drug concentration and high SARS-CoV-2 viral load. SARS-CoV-2 evolution in one compartment could lead to spread of mutant virus to other compartments, and other individuals, post-treatment. Second is physiological. For example, patients with vomiting or diarrhea may not achieve full therapeutic concentration. Food intake is known to affect peak molnupiravir concentrations. Myriad other physiological factors could lead to differences in the drug's pharmacokinetics among patients across large, genetically diverse cohorts. Third is behavioral and socioeconomic. For example, as is known from studies of antibiotics, the real-world usage may deviate from expected. Patients may not complete the full course of treatment during post-exposure prophylaxis or if their infection is—or becomes—asymptomatic. Patients may also split treatment or split pills with others, especially in socioeconomically disadvantaged areas.

The possibility that molnupiravir-induced generation of mutated infectious SARS-CoV-2 virus would accelerate evolution needs to be considered from first principles and tested experimentally, without assuming that traditional steady-state analysis of viral evolution applies. One would typically reason that viruses are naturally teetering on the edge of the "error catastrophe," having already optimized their mutation rate, and therefore one would assume that any additional increase in mutation rate would not accelerate evolution. This assumption could apply at steady state (e.g., when considering the mutation rate due to normal error rate of viral polymerases)—under such conditions, increases in mutation rate would increase viral diversity, but this effect would be offset by the decrease of viability of progeny due to decreased fidelity of viral replication. However, the scenario being considered here is different: molnupiravir-induced transient increase in mutation rate of the virus, followed by the removal of the drug exposure (or transmission to an untreated individual) and therefore return to high-fidelity viral replication under selection pressure from the immune system. SARS-CoV-2 viral immune escape likely requires simultaneous appearance of multiple mutations. Molnupiravir-induced mutagenesis, followed by high-fidelity viral replication and selection, could be a more efficient way to achieve such immune escape than evolution under constant mutation rate—and should be carefully analyzed. Of particular concern are treated individuals with low-level, but non-zero, immunity against the current SARS-CoV-2 sequences, providing sufficient pressure on the virus to select for new variants, but not enough pressure to clear the virus.

Viral evolution would be especially concerning if the mutated and/or evolved virus is transmitted to others to enable further evolution and spread. As stated above, infectious virus has been isolated from humans early in the course of molnupiravir treatment and/or those receiving reduced dosages of the drug, indicating that this risk of viral transmission after molnupiravir treatment cannot be ignored. For example, sub-therapeutic concentrations of the drug in a vaccinated, immunocompromised individual could promote rapid evolution of vaccine-resistant mutants that could be transmitted to others during or after molnupiravir treatment. Such transmission among multiple individuals with reduced immunity (e.g. in a long-term care facility) would be especially concerning as it could provide an extended period of time for the mutants to undergo selection and additional evolution.

Even a very low probability (e.g. 1 in 10,000 treated individuals) of molnupiravir-induced evolution and transmission of an immune-escape SARS-CoV-2 variant is a serious threat in a drug that may be used in millions of individuals. However, no data have been provided to properly estimate this probability. Interim analysis of the Phase 3 MOVe-OUT trial reported results from only 385 molnupiravir-treated individuals, with no information provided on the extent of viral mutation, evolution, or transmission. In addition to analyzing averaged data from a limited number of participants, it is critical to report and analyze the full distribution of results and calculate the likely shapes of each distribution, so the lower-probability "tails" of distributions can be properly analyzed and modeled. For example, if concentrations of molnupiravir corresponding to 200 mg dosage induce viral mutations but do not substantially reduce viral loads, one would need to establish the fraction of treated patients that do not exceed these sub-therapeutic concentration with the 800 mg dosage. Thus, the evaluation of the risk of using molnupiravir will require additional data and careful statistical analyses to estimate the probability of a rare, but potentially catastrophic event.

Data in four areas deserve particular attention, as outlined below. Some of these data could likely be obtained using existing samples. Additional studies would be critical, in particular those including individuals with the reduced ability to clear the virus, and those providing viral sampling from multiple body sites.

1. Deep sequencing of both SARS-CoV-2 viral RNA and infectious virus shed during molnupiravir treatment starting from day 1. These data would help estimate how quickly viral mutations appear in treated individuals, the level of mutations in the shed infectious virus, and the risk of transmitting mutated virus early in the course of treatment. These data will also clarify the risk of viral evolution in individuals who don't complete the full course of treatment.
2. Deep longitudinal sequencing of SARS-CoV-2 viral RNA and any infectious virus shed after the 5-day treatment is completed. These data would clarify whether and how often mutation and selection continue

to occur post-treatment, and whether mutants are likely originating from reservoirs within the body that are not being sampled.

3. Deep sequencing of both SARS-CoV-2 viral RNA and infectious virus shed during and after sub-therapeutic molnupiravir dosages (e.g. 400 mg and 200 mg) or in individuals who do not reach the full therapeutic concentration of the drug. These data will help quantify the increase in probability of viral evolution and transmission with decreased drug concentration, especially in individuals in which the concentration of the drug may be at the lowest edge of the distribution for biological, behavioral, or socioeconomic reasons.
4. Household transmission studies that monitor close contacts of molnupiravir-treated individuals for infection with mutated SARS-CoV-2 virus. As outlined above, not detecting infectious virus in one sampling site (e.g. nasopharynx) does not guarantee that transmission cannot occur (e.g. from the aerosols produced in the lungs). Detecting even rare events of such transmission would be particularly concerning.

Such data and analyses would clarify the risk of molnupiravir-induced viral evolution and transmission. If the drug were to be authorized, these data would also suggest the most critical time periods (e.g. during early treatment) or the key populations (e.g. immunocompromised individuals in congregate settings) for which infection control measures are particularly critical to reduce the probability of spreading mutated SARS-CoV-2 virus.

To summarize, the concern outlined here is not the evolution of a molnupiravir-resistant mutant, but rather molnupiravir-promoted "gain-of-function" evolution of rare mutants that escape naturally acquired or vaccine-induced immunity. Such mutants, if transmissible, would impact billions of people around the world, rather than only the molnupiravir-treated individuals. Therefore, the potential impact of molnupiravir on SARS-CoV-2 viral evolution and transmission should be quantitatively evaluated and carefully considered when weighing the benefits and risks of using molnupiravir.

Every deliberation and decision by this Advisory Committee and the FDA is consequential. However, given the potential impact on the world, this decision—whether to authorize molnupiravir and under what conditions—may be among the most consequential decision of them all. The scientific community and the FDA have a history of using best possible science to make difficult decisions that protected countless lives, going back to not approving thalidomide for pregnant women in the U.S., even though it was approved and used in Europe. Given that we are in a pandemic, it is not surprising that more complete, rigorous science is not available to fully inform this important decision. However, I hope these comments are helpful to the Advisory Committee and the FDA.

Sincerely,

Rustem Ismagilov

Disclosures: I have no financial interests in Merck or its competitors. While I acknowledge informative feedback from a number of scientists over the past four weeks, the views expressed here are my own.