Merck & Co. and may own stock and/or stock options in Merck & Co. G. P. is an employee of Emory University and has a financial interest in molnupiravir. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Reply to Troth et al

To the Editor—We thank Troth et al for the opportunity to extend the discussion of our data on the mutagenicity of N^4-hydroxycytidine (rNHC) [1]. We view our work as providing the proof of concept showing that as rNHC is phosphorylated to its active ribonucleoside 5′-triphosphate, the ribonucleoside 5′-diphosphate intermediate that is the immediate precursor to the ribonucleoside 5′-triphosphate also plays the equivalent role of an intermediate precursor for the synthesis of 2′-deoxyribonucleoside 5′-diphosphate (by the activity of ribonucleotide reductase). This is the normal pathway for the synthesis of DNA precursors used from bacteria to humans; thus, it should not be a question of whether the mutagenic form of dNHC (2'-deoxyribose form of rNHC) as a precursor to DNA is formed, but rather what the impact is. On this point we have unpublished cell-based data supporting conversion of rNHC to dNHC, albeit at low intracellular levels. Also, the near identity of rNHC to cytidine (the addition of a single oxygen atom) makes it likely that rNHC and cytidine undergo similar metabolism in the cell.

Although we easily demonstrated the mutagenic potential of rNHC in a cell culture model, Troth et al note their negative data using 2 in vivo model systems. Negative results must be viewed in the context of assay sensitivity. NHC mutagenesis will occur in dividing cells. Do the in vivo assays focus on dividing cells, and what is the limit of detection of new mutations when dividing cells are assessed? How do we scale these negative results to a human who may live for years? Mutagenesis is not an acute toxicity but, rather, would be revealed over a long period in cancer rates and germline mutations.

Troth et al raise several questions concerning our experimental approach. First, they question our use of a 32-day drug exposure rather than a 3- to 6-hour exposure. Since rNHC has to be taken into the cell then metabolized to become a DNA precursor, a 3- to 6-hour exposure would likely result in a negative result (it would likely fail as an antiviral agent also). Short exposures are relevant to chemicals that derivatize DNA, not for metabolic precursors. Thus, it is important to think about the mechanism of mutagenesis when choosing a test for mutagenic potential, both in vitro and in vivo.

We used a short-term (5-day) cell toxicity/cytostatic assay. Troth et al suggest this should have been a 32-day assessment. While we did not do this, we also did not notice a difference in growth rate in the presence of 3 μM rNHC during the multiple rounds of cell passage.

Our results using a gene knockout model demonstrate the mutagenic potential for the host, but in our adaptation of the hypoxanthine phosphoribosyltransferase knockout model it is difficult to establish a mutation rate given that multiple rounds of cell replication and drug incorporation

occurred. Highly accurate sequencing of DNA is likely better suited to ask this question. Ultimately the rate of mutagenesis in cell culture is less relevant than the long-term consequences of exposure to a mutagen during treatment.

Troth et al pose several questions about solubility and purity. No solubility or pH issues have been noted by visual inspection, and the concentrations chosen span those obtained in the blood with anti–human immunodeficiency virus nucleoside analogs and also recently reported by Painter and colleagues studying blood levels of rNHC in people dosed with molnupiravir [2]. In addition, using these concentrations we found a dose-response pattern for incorporation of mutations in viral RNA, inconsistent with drug precipitation during culture conditions. As to purity, it would be more relevant to repeat this experiment with clinical-grade material, which we would be happy to do.

It is hard to argue that a ribonucleoside precursor to both RNA and DNA goes into one but not the other. Also, the known mutagenicity of hydroxylamine (which generates dNHC in DNA) suggests that if DNA repair could target such a small change in a base, it must do so in an incomplete way. This leads to the conclusion that treatment with molnupiravir will lead to mutations in host DNA in dividing cells. Using negative results to justify this risk as being unimportant is to create a blind spot for potential long-term harm. Until a better understanding of treatment with molnupiravir is achieved, we would argue that its use should be limited to people with co-factor risks for coronavirus disease 2019 who are likely to receive the greatest benefit while being exposed to the unknown long-term risks of exposure to this mutagen.

Notes

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Potential conflicts of interest. The University of North Carolina is pursuing intellectual property protection for Primer ID and R. S. has received nominal royalties. All other authors report no potential conflicts of interest.

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Potential Effect of Previous Human Coronavirus NL63 Infection on the Rate of Infection and the Clinical Course of Coronavirus Disease 2019

To the Editor—In a study of neutralizing antibodies for human coronavirus (HCoV) NL63 by Henss et al [1], the severity of coronavirus disease 2019 (COVID-19) appeared to be correlated with low HCoV-NL63 neutralizing activity, and patients with severe COVID-19 had no high-level NL63-neutralizing antibodies. However, some severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–naive individuals analyzed in that study had high NL63-neutralizing antibodies, so the authors considered it worthwhile to explore the hypothesis that preexisting immunity to NL63 or other common cold coronaviruses might reduce the risk of severe disease.

To examine this hypothesis, we tested for the presence of HCoV-NL63 antibodies, using the human anti-HCoV-NL63 immunoglobulin G enzyme-linked immunosorbent assay kit produced by Creative Diagnostics. We tested 4 groups: individuals who were negative for SARS-CoV-2 (control group), those who tested negative even though they took care of family members with COVID-19 (high-risk contacts), patients with mild COVID-19, and patients with severe COVID-19.

In the control group (negative for SARS-CoV-2), 3 of 42 individuals tested were positive for HCoV-NL63 antibodies. This is consistent with the presumed positivity in the general population. In high-risk contacts (individuals