Review 3: "Small-molecule ligands can inhibit –1 programmed ribosomal frameshifting in a broad spectrum of coronaviruses"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

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Review:

Programmed ribosomal frameshifting is used by many RNA viruses, including coronaviruses, for the expression of their polyproteins. It is an essential regulatory mechanism of viral gene expression regulation which is exceptionally conserved in SARS-CoV-2 (Mei et al. 2021 doi: 10.1093/molbev/msab265). This makes it an attractive potential target for antiviral interventions. Here, Munshi et al. present the results of a large-scale screen of about two thousand FDA-approved drugs for their ability to alter the efficiency of viral programmed ribosomal frameshifting. They tested these drugs using reporter constructs under the control of frameshifting cassettes from representative coronaviruses and identified several drugs potentially affecting frameshifting efficiencies.

This is a promising and important (albeit somewhat preliminary) study that begs for a follow-up. The authors did a good job at eliminating several sources of false positives, e.g. drugs that directly inhibit the activity of the reporters (rather than their expression) as well as general inhibitors of translation. The parallel use of two assays, luminescence reporters in a cell-free system, and fluorescent reporters in cell-based systems further increase the confidence in the presented data.

Nonetheless, it is hard to know how well these reporter assays reflect changes in endogenous levels of ribosomal frameshifting that may be induced by these drugs. For screening purposes, it may be impractical to reproduce all potential factors that could influence frameshifting. Nonetheless, there are emerging evidence suggesting that the efficiency of frameshifting may depend on translational dynamics upstream, e.g. the levels of ribosome loading (Smith et al. 2019, doi: 10.1073/pnas.1910613116; Bhatt et

al 2021, doi: 10.1126/science.abf3546; Carmody et al. 2021, doi: 10.1093/nar/gkab1172) as well as changes in ribosomal frameshifting during the timelines of viral infections (Cook 2021, doi: 10.17863/CAM.77982). Long-ranged RNA interactions exist in real viruses (Ziv et al. 2020, doi: 10.1016/j.molcel.2020.11.004) and several epitranscriptomics modifications have been found in SARS-CoV-2 (Kim et al. 2020, doi: 10.1016/j.cell.2020.04.011). Their potential effect on frameshifting has not yet been studied. The existence of relevant factors outside of narrow frameshifting cassettes (slippery sequences plus stimulatory RNA pseudoknot) is also supported by the authors' own experiments, i.e. differences in observed inhibition between two systems used. The study provides no information on the mechanism of action of these drugs and was not designed for this purpose. These, as well as other unknown factors, have the potential to undermine the initial findings. Thus, I think it will be crucially important to follow up this study with a time-resolved ribosome profiling of infected cells under the treatments with these drugs. In addition to validating initial findings it also would be instrumental for identifying host mRNAs whose translation may be affected by these drugs, it also may help decipher the mechanism of their action.