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Shutting the gate before the horse has bolted: is it time for a conversation about SARS-CoV-2 and antiviral drug resistance?

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This article provides a brief overview of drug resistance to antiviral therapy as well as known and emergent variability in key SARS-CoV-2 viral sequences. The purpose is to stimulate deliberation about the need to consider drug resistance prior to widespread roll-out of antivirals for SARS-CoV-2. Many existing candidate agents have mechanisms of action involving drug targets likely to be critical for future drug development. Resistance emerged quickly with monotherapies deployed for other pulmonary viruses such as influenza virus, and in HIV mutations in key drug targets compromised efficacy of multiple drugs within a class. The potential for drug resistance in SARS-CoV-2 has not yet been rigorously debated or assessed, and we call for more academic and industry research on this potentially important future threat prior to widespread roll-out of monotherapies for COVID-19 treatment and prevention.

The unprecedented urgency presented by SARS-CoV-2 has resulted in a concerted global effort to rapidly bring forward therapeutic interventions to reduce the morbidity and mortality in the associated disease COVID-19. Severe disease is associated with specific immunopathology in the lung and reticuloendothelial system.[1] Strategies have focused broadly on anti-inflammatory/immunomodulatory interventions to dampen or mitigate the immunopathology, including the widespread use of dexamethasone.[2] Focus is also being placed on antiviral therapeutics for disrupting the life cycle of SARS-CoV-2. The overwhelming majority of therapeutics currently under investigation in clinical trials have emerged from fortuitous repurposing of medicines from the list of those already approved for other indications (e.g. chloroquine, hydroxychloroquine, lopinavir, favipiravir, nitazoxanide, ivermectin). However, several newer agents repurposed from other antiviral drug development programmes (e.g. remdesivir and molnupiravir) have or are also being evaluated. The months and years ahead are sure to bring a new generation of agents specifically developed for SARS-CoV-2. Many putative drugs being repurposed as antiviral agents are being investigated based on antiviral activity in a cell line or animal model in the absence of empirical demonstration of the mechanism of action. This may make many candidates a forlorn hope, but for those drugs that do have a putative mechanism of action, the overwhelming majority target the SARS-CoV-2 replication/transcriptome complex, spike, protease or a host-directed mechanism associated with these aspects of the viral life cycle.[3]

Resistance to antiviral drugs has been identified for other viruses but surprisingly little has been published on the potential for resistance to the putative antivirals currently being explored for SARS-CoV-2. With few exceptions,[4,5] most publications focusing on resistance raise concerns about the consequences of the pandemic and prescribing behaviours for resistance to other pathogens.[6,7] Drug resistance has been reported for other coronaviruses and this was reviewed recently within the literature.[8] As a containment/biological safety level 3 (CL3/BSL3) pathogen, robust study of SARS-CoV-2 resistance requires specialized facilities. However, many investigators also believe that for an acute infection like SARS-CoV-2, the chances of resistant variants emerging are low because of the short courses that should be necessary once potent antivirals emerge. This is despite evidence from other acute respiratory pathogens in which antivirals are used, and that some patients are being managed in primary care settings for over a month. If antiviral resistance does emerge for the first generation of antivirals, the consequences could be profound for existing and future interventions, so the subject of drug resistance warrants greater discussion to reach an evidence-based consensus on the most appropriate means of managing the risk. Several sources of information about SARS-CoV-2 and other viruses present us with concern and these are outlined below.

Two central principles for emergence of resistance are that resistance emerges when an organism continues to replicate in the presence of drug, and that mutations may develop stochastically, but outgrow the common variant in a deterministic fashion.

Treatment-emergent resistance occurs when the high viral loads and mutability of an RNA virus increases the stochastic occurrence, but subsequent transmission of resistance requires opportunity. Perhaps one of the closest paradigms can be drawn from knowledge of therapeutic interventions for influenza virus. Substantial differences exist between influenza virus and SARS-CoV-2, but both are pulmonary viruses with RNA genomes, and both only require short course antiviral interventions. Worryingly, resistance to monotherapies emerges quickly for influenza virus.[9] Adamantanes are no longer recommended for use because of widespread resistance,[10,11] and there is detailed literature on resistance of influenza virus to oseltamivir and favipiravir.[9,12,13] Moreover, the deployment of drug combinations has been empirically demonstrated to reduce emergence of resistance in preclinical models and in clinical trials of influenza virus infection.[14,15] It is important to recognize that for influenza virus some patient groups (e.g. children, infants, immunosuppressed patients) have a very long tail of viral replication even during oseltamivir use. This long window creates the opportunity for transmission of resistance and, so far, specific subgroups in whom prolonged replication occurs have not been identified for SARS-CoV-2. However, widespread deployment of moderately active drugs as prophylactic interventions may present risks that require some additional thought. Unlike influenza virus, SARS-CoV-2 does not yet appear to be seasonal so if drug resistance does emerge, transmission of variants may be ongoing throughout the year. Given similarities to other human coronaviruses, a successful vaccine programme and complementary containment strategies may yet unmask seasonality for SARS-CoV-2.

Although a number of commentators suggest that coronaviruses 'evolve slowly' or issue similar statements, this is not the case. Resistance to therapeutics will be driven by two processes creating a diverse viral population. The first is the presence of SNPs. Here the nucleotide substitution frequency of SARS-CoV-2, $\sim 1 \times 10^{-3}$ substitutions per year,[16] is within the same magnitude as Ebola virus at 1.42×10^{-3}.[17] However, as a natural by-product of the mechanism used in the generation of viral subgenomic mRNAs, discontinuous transcription during negative strand synthesis, coronaviruses also undergo recombination. This manifests in insertion of both viral and non-viral sequence and deletion of viral sequence (called indels). The continuous emergence of SARS-CoV-2 variants presents an additional pause for thought even if only currently in terms of the implications for variation in spike glycoprotein and replication/transcription complex. Mutations in the viral spike sequence emerge within 3–4 passages through Vero cells,[18,19] and the virus becomes fully murine adapted in its spike sequence in just 6–10 serial infections through mice.[20,21] The pace at which spike mutations occur is exacerbated by the observation that mink-adapted strains of the virus were able to transmit back to humans and subsequently from human-to-human.[22,23] At the time of writing, the full consequences of the new spike variants that have been detected in the UK, South Africa and Brazil are yet to be realized.[24,25] However, the changes in spike associated with B.1.351 (South Africa) and P.1 (Brazil) demonstrated partial resistance to casirivimab and full resistance to bamlanivimab *in vitro*,[26] making them the first chilling examples of mutations that arose without a selective pressure, which are simultaneously transmissible and able to compromise antiviral therapeutic candidates. In preliminary evaluations, selected resistance to bamlanivimab also arose in patients treated for mild/moderate COVID-19 and was much less prevalent when bamlanivimab was given as a combination with etesevimab.[27] They at least provide further evidence of selection for and subsequent transmission of SARS-CoV-2 variants, and further variation likely becomes statistically more probable with an augmented replication and transmission rate.

Currently, variants of the spike glycoprotein have been conclusively demonstrated to emerge rapidly and transmit, but there is good reason to believe mutations in other key drug targets are also occurring, such as the P323L substitution in nsp12, the viral RNA-dependent RNA polymerase, and the key component of the replication/transcription complex. Studies of remdesivir with murine hepatitis virus demonstrated selection of two drug resistance mutations at a sequence in the polymerase that is conserved amongst coronaviruses.[28] Such mutations did not occur in rhesus macaques infected with SARS-CoV-2 and have not been reported in humans treated with the drug,[29,30] but the drug has been predominantly used in severe disease. Can we be sure that because resistance has not emerged it will not emerge, and that it will not rapidly transmit in the community if it does? In some cases with other viruses, resistance to one drug confers resistance to other drugs in the same class.[31–33] Can we afford to be complacent about resistance with such valuable downstream drug targets as the polymerase and the protease? For HIV and HCV, success was only realized after development of potent dual or triple drug combinations. Antiviral synergy or additivity has been reported *in vitro* for several drug combinations.[34–36] Even in the absence of pharmacological synergy, statistical probability of dual or triple drug resistance is greatly diminished with the need for multiple drug resistance mutations in multiple viral genes within the same viral genome, and this underlines the rationale for combination antiviral therapy for other viruses like HIV and HCV. Given even the smallest chance of class-wide resistance compromising drugs in current development, can we afford to risk placing a widespread selective pressure on such valuable drug targets with monotherapies of sub-optimal potency? If a successful antiviral is identified, will we rapidly put ourselves back at square one by too hastily rolling it out rapidly as a monotherapy? There is considerable evidence from animal coronaviruses and SARS-CoV-2 of high variability with insertions/deletions being a hallmark trait of this genus.[19,37,38] Current sequencing efforts have been focused on capturing information on SNPs to aid in molecular epidemiology and pipelines are now being optimized to also identify indels. Therefore, additional caution may be needed to ascertain the rate and extent to which SARS-CoV-2 variants are emerging and were already present but just not identified.

Only time will tell if drug resistance will be a problem for SARS-CoV-2, but the urgency of the pandemic necessitates urgent decision making that may have downstream consequences. *A priori* resistance testing can be conducted quickly with the existing laboratory model systems that have emerged for SARS-CoV-2, and standardized protocols for routine evaluation of the threat for single agents and combinations should be rapidly developed through academic–industry and industry–industry collaborations. Is it time to pause and consider drug

combinations so that more haste does not result in less speed towards victory against COVID-19?

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