Alternative Screening Approaches for Discovery of Middle East Respiratory Syndrome Coronavirus Inhibitors

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Two coronaviruses causing severe respiratory disease and high mortality rates emerging within the past dozen years reinforces the need for clinically efficacious antivirals targeting coronaviruses. Alternative screening approaches for antivirals against the recently emergent Middle East respiratory syndrome coronavirus (MERS-CoV) may provide lead compounds to address this need. Two *Antimicrobial Agents and Chemotherapy* (AAC) papers screened libraries of approved compounds that may potentially be repurposed as MERS-CoV antivirals. A third AAC paper showed that a previously described severe acute respiratory syndrome coronavirus (SARS-CoV) helicase inhibitor also has activity against MERS-CoV.

Three papers available in this issue of *Antimicrobial Agents and Chemotherapy* (AAC) address antiviral drug discovery for the recently emergent Middle East respiratory syndrome coronavirus (MERS-CoV) (1, 2, 3).

Over the past few decades, antiviral drug discovery and development have generally followed a path from viral target definition through assay development and subsequent compound screening to find a lead compound. Once a lead compound is found, there are routes for lead compound optimization to increase efficacy which may involve crystallography or molecular modeling studies and also optimization for an improved pharmacokinetic profile. This route usually includes animal safety studies. Concurrent studies to address cell culture antiviral activity usually include the selection of resistant virus. For viral targets, the question generally is not whether one can select for resistance but rather what does it take to select for resistance. This may include both the number of mutations necessary to select for resistant virus and also the replicative fitness of the resistant virus.

Following an approach consistent with the typical pathway described above, Adedeji and colleagues had previously described a severe acute respiratory syndrome coronavirus (SARS-CoV) nsp13 helicase inhibitor, SSYA10-001 using a commercially available library and a SARS-CoV biochemical helicase assay (4). The compound had low micromolar activity both in the biochemical assay and also in a SARS-CoV replicon assay. In the current study, this compound was tested as a potential inhibitor of MERS-CoV and also the related coronavirus murine hepatitis virus (MHV) and found to be active against both viruses, although its efficacy was slightly diminished compared with what had been reported for SARS-CoV (3). They also use a molecular modeling approach to address compound interaction with the SARS-CoV helicase and define potential residues that may be involved in compound binding. Amino acid substitutions at these residues were introduced into the SARS-CoV enzyme, and these mutated enzymes showed reduced inhibition by SSYA10-001. Virological (or replicon) resistance selection was not addressed in the paper, and it would be interesting to see whether the virus conforms to one's predictions. This is always an interesting question especially given the efficient replication capacity and the lack of polymerase proof-reading capability of these viruses, which helps ensure a diverse pool of genomes available for antiviral resistance selection. The authors suggest that this compound may serve as a lead com-

pound for development of a broad-spectrum anticoronavirus inhibitor. A potential added benefit may be that the definition of a viral helicase inhibitor may stimulate added attention to this function as a validated antiviral target.

Two other papers take an alternative viral target neutral approach by screening small libraries of FDA-approved compounds in MERS-CoV antiviral assays (1, 2). Since licensed inhibitors may have previously defined pharmacokinetic and safety profiles, the suggestion is that this repurposing approach may shorten the time to provide clinically useful inhibitors of MERS-CoV. This may be a reasonable suggestion, although there are several points mentioned in the Discussions of both papers that should be noted.

(i) There have been previous attempts to define licensed compounds as repurposed antivirals. One example relevant to these current papers regards chloroquine, which was found as a positive hit in their MERS-CoV assays (1, 2). The authors note that this compound was tested previously against SARS-CoV infectivity in mouse models and was not efficacious in this system (5).

(ii) It is important when analyzing screening data to note any related compounds tested in the screen that did not score as hits in an attempt to define some level of structure activity relationship. One example is the description of lopinivir as a low micromolar inhibitor of MERS-CoV. Lopinivir is an HIV protease inhibitor (PI), but the proteases of HIV and coronaviruses fall into different mechanistic classes of proteases. de Wilde and colleagues provide a list of their compound library as supplemental information and discuss whether inhibition is peculiar to lopinivir or to HIV PIs in general (1).

(iii) If a licensed compound has been optimized to be an inhibitor of some cellular function, does that mean that inhibition in the virus assay is mediated via inhibition of that defined cellular target? This becomes especially relevant if there is a substantial differential between the cellular target and virological 50% effec-

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tive concentrations (EC_{50} s). Similarly, if the inhibitor found in the screen is a known inhibitor of a cellular function, then it may be difficult to select for resistant virus. Given the large replicative pool and the lack of coronavirus polymerase proofreading capability, it is still essential to attempt to select for resistant virus, and the authors recognize this concern and note that resistance selection studies are being addressed. While it may be possible to optimize lead compounds based on cell culture antiviral activity, if the repurposed compounds are meant to serve as potential leads for coronavirus inhibitors, then target definition is also an important concern to provide a basis for lead optimization.

Following the SARS coronavirus outbreak in 2002 to 2003, resolution of the outbreak was largely through the implementation of public health measures. Treatment with antiviral agents did not play a major role, as there were no specific coronavirus antivirals available for patient treatment. de Wilde and colleagues (1) note that it may take up to 10 years to move from antiviral discovery to clinical development of efficacious antivirals. In the 11 to 12 years since the SARS outbreak, a clinically useful antiviral has not been defined for SARS-CoV. Now 2 years into the emergence of MERS-CoV, hopefully that timeline can be shortened. By defining potential coronavirus inhibitors, these papers may provide repurposed antivirals or lead structures that can be developed as coronavirus inhibitors. Whether repurposed compounds provide clinical benefit for an infected patient necessitates input by

infectious disease clinicians to determine the utility of these coronavirus inhibitors.

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