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Screening of FDA-Approved Drugs for Treatment of Emerging Pathogens

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ABSTRACT: The current outbreaks of Middle East Respiratory Syndrome (MERS) and Ebolavirus (EboV) have revealed a gap in the development and availability of drugs to treat these infections. To date, there are no approved treatments for patients infected with MERS coronavirus (MERS-CoV), a virus that continues to infect new patients and that has now spread from the Middle East to Asia. Despite a downward trend in the number of new EboV cases in West Africa, new infections are still occurring, and many patients continue to suffer from this illness. People infected with MERS and Ebola viruses receive only supportive care in hopes of recovery. Investigation into repurposing drugs approved by the FDA is gaining interest. To identify better treatment strategies, several groups have used drug screens to repurpose FDA-approved drugs as inhibitors of MERS-CoV and EboV.

Between 2012 and July 7, 2015, MERS-CoV infected 1368 people, causing 487 deaths in 26 countries with a case fatality rate of 38% according to the World Health Organization (WHO). In the spring of 2015, MERS-CoV spread to Asia, infecting 185 people and resulting in 36 deaths. During the 2014−2015 Ebola outbreak, 27,678 people have been infected, with 11,276 deaths, and Ebolavirus (EboV) infections continue to spread. Currently, there are no approved therapeutics to treat MERS or Ebola patients. The development of novel antiviral compounds that inhibit these viruses can take over 10 years and hundreds of millions of dollars to go from the bench to U.S. Food and Drug Administration (FDA) approval. With the rapid response needed during the outbreak of an emerging virus, the time frame must be shortened for therapeutic development. To combat the lack of available therapies for treatment of MERS and Ebola, high-throughput screens (HTS) have been developed to identify potential FDA-approved drug candidates that could be repurposed to treat these diseases. Recent studies using HTS to examine FDA-approved drugs have identified compounds that inhibit MERS-CoV as well as EboV.

The virus life cycle for MERS-CoV and EboV can be broken down into discrete steps. These steps include internalization of the virion, trafficking of the virion to endosomal compartments, fusion of the virion with the endosomal membrane, replication of viral RNA and proteins, and packaging of the virion and virus egress. Each of these steps of the virus life cycle can be targeted for therapeutic inhibition. For viruses that enter through receptor-mediated endocytosis, flow cytometry and co-immunoprecipiation assays are used to identify compounds that reduce receptor levels or inhibit virion binding, respectively. Immunostaining and confocal microscopy is used to identify inhibitors of virion trafficking to appropriate endosomal compartments. Endosomal fusion assays, utilizing pseudotyped viruses, are used to identify compounds that block virus release from endosomes. Multiple methods to identify inhibitors of viral RNA and protein production are used including RT-PCR for viral mRNA and Western blotting or ELISAs for viral proteins. Additionally, electron microscopy and viral titer assays can identify drugs that inhibit virus egress from infected cells. This type of screening not only identifies FDA-approved drugs with off-target antiviral activity but also narrows the drug mechanism of action against virus infection.

Recent advances in HTS using pseudotyped viruses or virus-like particles (VLPs) instead of wild-type live viruses have enabled the broader and safer screening of potential antiviral compounds against highly pathogenic BSL3 and BSL4 viruses such as MERS-CoV and EboV. Virus fusion assays using pseudotyped virus can identify drugs that prevent virus−endosome fusion and subsequent release of the viral genome into the host cell cytoplasm. In the studies below, pseudotyped viruses and VLPs are used in place of live virus to evaluate FDA-approved drugs for off-target effects that inhibit virus entry due to their relative ease of use at BSL2 containment rather than BSL3 or BSL4. To study MERS-CoV, the pseudovirions express the virus Spike protein on their surface. For Ebola, the pseudovirions express the viral glycoprotein (GP).[1] The VLPs also express β-lactamase (β-lac) fused to a viral protein.[1] Cells are loaded with a β-lac substrate, and upon infection and virus fusion, β-lac is released and the substrate is cleaved. This cleavage can be measured by flow cytometry or other methods. Cells can be treated with compounds of interest prior to infection or at different time points post-infection. Cleavage of the β-lac substrate is then quantitated. Use of pseudotyped virus or VLPs in BLS-2 laboratories makes this method of investigation available to a majority of researchers.

Our laboratory, along with others, evaluated 290 compounds for use against SARS-CoV and MERS-CoV using HTS in 2014.[2] Using live SARS-CoV and MERS-CoV, our screen identified 27 drugs with antiviral activity against both viruses. Candidates spanned several drug families, including antibacterial and antiparasitic agents, as well as inhibitors of cathepsin, DNA metabolism, estrogen receptor, neurotransmitters, sterol

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metabolism, protein processing, and kinase activity.[2] Another HTS, by de Wilde et al., tested 348 compounds and found 4 with activity against both live SARS-CoV and MERS-CoV.[3] In both studies, cells in culture were treated prior to infection, potentially biasing results to those drugs that inhibit a step in virus entry.[2,3] As a result, some overlap was observed in these screens from the two separate studies. As a method to identify drugs that inhibit early steps in SARS-CoV and MERS-CoV entry, we are currently using SARS-CoV and MERS-CoV Spike pseudotyped viruses to identify drugs that specifically inhibit virus−endosome fusion. Two drugs validated in this manner have shown promising protection in a lethal mouse model of SARS-CoV (M.F., unpublished data).

Therapeutic candidates for treatment of EboV infection have also been identified by HTS.[4,5] In a 2014 study by Kouznetsova et al., over 600 FDA-approved compounds were screened for their ability to inhibit EboV entry, and 53 inhibited entry of Ebola VLPs.[4] A 2015 study by Johansen et al. screened approximately 2600 approved drugs and found 80 to be effective against EboV entry.[5] In the work by Johansen et al., multiple assays were used to validate drug effectiveness against EboV. After an initial screen, a pseudovirus entry assay was done specifically to identify entry inhibitors that block virus fusion.[5] The assays used in these screens demonstrate that HTS pseudovirus entry assays can be used to rapidly get drugs into animal models and potentially into human trials.

Two criteria must be met before any compound identified through HTS could be used to treat MERS and Ebola patients. First, the drug must be effective at controlling virus replication, which could be accomplished by targeting any of the steps in the virus life cycle. Second, in the context of infection, the compound will have to be tested to ensure that it does not modulate the host immune response and exacerbate disease symptoms. In vivo studies leveraging the protective response of a candidate drug against a potentially induced detrimental immune response are needed to ensure the safety of a chosen drug for each pathogen. Comparison of innate and adaptive immune features using flow cytometry of immune markers would identify a potentially harmful shift in immune profiles that could detract from the drugs' usefulness in patients. Knowing the mechanism of action of these drugs will inform decisions for delivery of drug cocktails, the components of which would inhibit specific steps in the virus life cycle. This type of combination therapy could be used to create a treatment that inhibits different steps in virus entry, or of the virus life cycle, thereby enhancing antiviral effects.

Drug development is costly, and transitioning a drug to market for human use in the United States and European Union typically spans a decade or more.[6,7] As the MERS-CoV and EboV outbreaks have shown, this strategy is not effective when dealing with emerging pathogens. Finding new uses for FDA-approved drugs can be an efficient way to identify new therapeutics. High-throughput screens of FDA-approved drugs is a rapid and cost-effective way of identifying candidate therapies to combat diseases for which there are no current treatments. These drugs have already been used to treat human patients, dosing has been studied, and toxicities and side effects are known. This makes using drugs that have already gone through the FDA-approval process good candidates for testing as therapeutics against new pathogens. The creation of the FDA drug and compound database in 2011[8] underscores the usefulness of this approach to treating rare or emerging diseases.

Drugs that inhibit steps in the life cycle of known coronaviruses including SARS-CoV and MERS-CoV may be effective against outbreaks caused by yet unknown coronaviruses. Similarly, drugs that inhibit EboV entry or other steps in the virus life cycle may prevent a future outbreak of a similar virus. More importantly, this process for drug identification and testing has implications beyond treatment of MERS-CoV and EboV infections. With changing climate and globalization, humans and other species are moving rapidly to new parts of the world. This greatly increases the odds of future outbreaks due to interactions with naïve populations. If we are to combat pathogens that will emerge in the future, we must use every tool available. This includes repurposing drugs originally designed to treat other diseases and using rapid methods for the identification of antiviral compounds to treat outbreaks that will occur in the future.

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Notes

The authors declare no competing financial interest.

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