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UJEMI PEARLS

# Use of combinational broad-spectrum antiviral cocktail for treating current and emerging coronavirus infections

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**SUMMARY** Coronaviruses pose a serious threat to the human population due to the high mortality rate of some strains such as SARS-CoV and MERS-CoV, and also the circulation of other zoonotic strains that may one day infect the human population. Despite the inevitable threat posed by coronaviruses, currently there are no vaccines, or treatment options available and thus, developing a broad-spectrum therapeutic option is crucial. In this article I will propose a novel solution to this problem by exploring the use of broad-spectrum antivirals such as Remdesivir and AM580 in the form of combinational therapy in order to treat individuals with coronavirus infections. By understanding the mechanism of action of Remdesivir and AM580, we can enhance future drug designs and also apply their principles to other diseases. The feasibility of the use of Remdesivir and AM580 in combinational therapy with respect to coronavirus infections is currently not clear, however, both Remdesivir and AM580 have shown to have a high selectivity index (SI), suggesting that they are safe to use. Additionally, other viral diseases may also benefit from the use of this combinational drug regimen due to the broad-spectrum nature of the drugs. By exploring the use of this drug regimen in dealing with coronavirus infections, not only do I propose an economical solution to a deadly problem, but it also paves the way for the use of similar implementations for other viral diseases.

## INTRODUCTION

The field of virology contains a plethora of viruses which have caused diseases in the human population, resulting in the death of millions of individuals worldwide. One important viral species which has received little attention are coronaviruses. Coronaviruses are positive sense, single-stranded RNA viruses which are enveloped. Embedded in the envelope, coronaviruses contain spike proteins which are utilized for viral entry into the host cells and give the distinct "crown" like appearance with which coronaviruses are commonly associated [1]. They often infect the respiratory tract and hence result in respiratory distress. Coronaviruses can infect animals such as pigs, birds (domestic and wild), bats, rodents, dogs, cats, rabbits, hedgehogs, and cattle, and they can also infect humans [2, 3]. Specifically, there are 6 coronaviruses that can infect humans. Out of the 6 coronaviruses known to infect humans, 4 of them are associated with mild cold-like symptoms [4]. These include a runny nose, fever, fatigue, sore throat and a cough. The other 2 coronaviruses are severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV); these cause more severe symptoms and may be fatal. These symptoms include high fever, shortness of breath, body aches and the development of pneumonia and kidney failure [1, 4].

SARS-CoV was first detected in China in November 2002 and grew into a worldwide outbreak in 2003. The outbreak took researchers and health professionals by surprise and resulted in the infection of 8,098 individuals with 774 of these individuals dying [5]. Approximately 10 years after the SARS-CoV epidemic, coronaviruses again re-surfaced in another deadly outbreak, this time through MERS-CoV. Although first originating from Saudi Arabia in 2012 and mostly being localized to the Middle East, the virus led to the infection of approximately 1,797 individuals with 687 individuals dying from the disease [6]. As can be seen from the numbers, both SARS-CoV and MERS-CoV have staggeringly

**Published Online:** September 2020

**Citation:** Naser Milad Biparva. 2019. Use of combinational broad-spectrum antiviral cocktail for treating current and emerging coronavirus infections. UJEMI PEARLS 4:1-8

**Editor:** François Jean (Ph.D.), University of British Columbia

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high mortality rates of 10% and 38% respectively. To make matters worse, the virus can be easily transmitted, similar to common cold viruses. Modes of transmission include sneezing, coughing, and touching infected areas [1].

The high mortality rate and easy transmission of coronaviruses are very concerning; however, there are neither vaccines nor treatment options currently available for any of the coronaviruses. The only treatment option for an individual is supportive care under quarantine to prevent the spread of the virus to others [7]. To make matters worse, current strains can evolve rapidly due to the nature of the virus and other zoonotic coronavirus strains may evolve to infect humans [1, 7]. If a treatment option is not devised, humans will be left vulnerable to a highly virulent virus with the capability of causing massive outbreaks leading to the death of countless individuals.

There has been some research into treatment approaches against coronaviruses such as using entry inhibitors. For example, MERS-CoV has been associated with the dipeptidyl peptidase IV (DPP4) entry factor in order for the virus to enter respiratory cells [8]. DPP4 inhibitors are in fact currently available and are used to treat type 2 diabetes; however, there are multiple limitations to that approach making it highly unfeasible [8-11]. Firstly, DPP4 inhibitors such as saxagliptin and alogliptin have been associated with an increased risk of heart failure [10]. In addition to this, they are also associated with a 58% increase in the risk of developing acute pancreatitis and leave patients with an elevated risk of developing inflammatory bowel disease [11]. Aside from the health concerns associated with using DPP4 inhibitors, logistically, such a specific approach makes this treatment option useless against other coronaviruses using different receptors. In addition, viral resistance to the inhibitors is another concern. In order to tackle these issues, a more broad-spectrum approach is required that targets all coronaviruses. In addition to this, combinational approaches utilizing multiple drugs can increase effectivity and reduce resistance.

A putative and novel drug combination that can help treat coronaviruses is the use of a nucleoside analogue called Remdesivir with a retinoic acid derivative called AM580 [12, 13]. Remdesivir was originally developed in 2013 against filovirus infections such as Ebola virus, however it has recently been discovered that its effects are broad spectrum due to targeting viral replication through the RNA-dependent RNA polymerase (RdRp) [12]. AM580 is a retinoic acid derivative that has been shown to reduce virus-induced lipid droplet formations [13]. By using Remdesivir and AM580 in a novel combinational therapy approach, their independent mechanisms of action are expected to offer low viral resistance with high antiviral effectivity (FIG. 1). Additionally, a broad-based approach consisting of using a direct-acting antiviral such as Remdesivir in combination with an indirect-acting antiviral such as AM580 can help pave the way for devising a long-term and effective treatment option against not only coronaviruses, but other viruses as well [12, 13].

## RESEARCH QUESTIONS

Throughout the past decade, there has been little to no progress in finding a feasible treatment option for dealing with coronaviruses and their associated deadly diseases. During both the SARS-CoV and MERS-CoV outbreaks, individuals were left vulnerable to these deadly diseases with doctors being able to do very little to treat them. Thus, it is imperative to find a treatment option in order to minimize fatalities. One approach is to develop a broad-spectrum antiviral cocktail in order to treat all coronaviruses. Specifically, this paper will explore the use of a direct-acting antiviral called Remdesivir in conjunction with an indirect acting antiviral called AM580. In order to fully understand the scope of using this drug regimen, I will first discuss the molecular mechanisms behind both drugs. Next, I will look at the therapeutic feasibility of using this drug regimen. Finally, as this is a broad-spectrum antiviral cocktail, I will explore other viruses in which this treatment option may be applicable.

## PROJECT NARRATIVE

**What is the molecular mechanism of action for Remdesivir and AM580?** In order to further the knowledge of the field regarding drug cocktails, it is important to understand the mechanism behind how these drugs function in order to improve drug regimens.

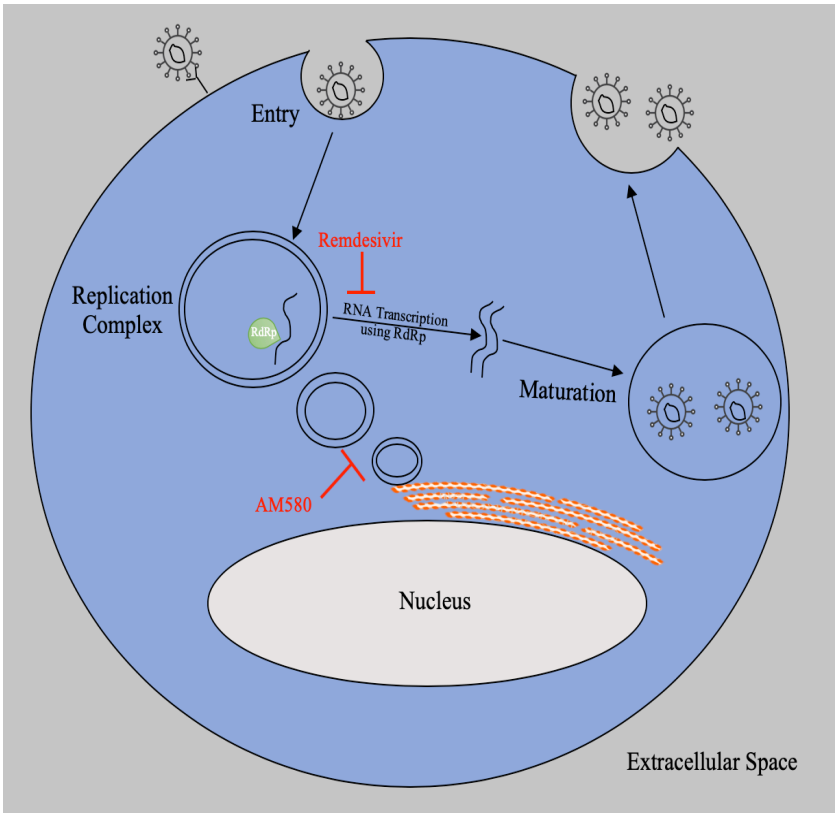


**FIG. 1 Points of action of Remdesivir and AM580.** The small molecule drugs Remdesivir and AM580 both inhibit viral replication; however, they do so in a non-redundant manner. Remdesivir inhibits the functioning of the viral RdRp within the replication complex, and AM580 reduces lipid droplet formation by preventing SREBP interaction with SRE, which is crucial for the formation of the replication complex.

Specifically, Remdesivir and AM580 have different and distinct mechanisms which need to be explored (FIG. 1).

Although the complete mechanism of action of Remdesivir is not clear, it is known that Remdesivir is an adenosine nucleoside analogue prodrug targeting the viral RdRp. The drug in its inactive form is taken up by passive diffusion and is intracellularly anabolised to its active form, a triphosphate metabolite [12, 14]. The adenosine nucleoside analogue then acts as an alternate substrate and RNA-chain terminator for primer extension during viral replication [14]. In terms of the coronavirus lifecycle, this means that once incorporated into the genome, the RdRp is not able to lengthen the nucleotide chain due to the presence of Remdesivir. As a result of this, the coronavirus is not able to create negative or positive strand copies of its RNA. The negative strand acts as a template for the transcription of smaller sub-genomic positive sense RNAs that are used for the synthesis of viral proteins [7, 15]. In addition to this, the negative strand also acts as a template for the synthesis of positive sense RNA genomes [7]. As a result of the interference of Remdesivir with the transcriptional ability of the RdRp, the virus cannot replicate its genome or synthesize proteins. It is hypothesized that the 1′-cyano group of Remdesivir results in increased potency and selectivity towards viral RNA-dependent RNA polymerases. In the case of coronaviruses, Remdesivir specifically targets the nsp12 [14].

AM580 offers a different approach to reducing coronavirus loads. Unlike Remdesivir, AM580 is not a direct-acting antiviral but is rather an indirect-acting antiviral. It is a retinoic acid derivative which specifically targets the sterol pathway. It does so by blocking the interaction of sterol regulatory element-binding protein (SREBP) 1/2 transcription factors with the non-palindromic sterol regulatory elements (SREs) in the promoter/enhancer regions of lipogenic genes (FIG. 2) [13]. The interaction of SREBP 1/2 transcription factors with the SREs is crucial for the synthesis of lipogenic enzymes and the formation of lipid droplets [16]. It has been shown that MERS-CoV induces a drastic upregulation of lipid metabolism genes. The reason behind this is unclear; however, similar to other viruses, it may be used for double-membrane vesicle formation and/or lipophagy [13, 17]. Double-membrane vesicles have been shown to provide a platform for MERS-CoV replication/transcription complexes (FIG. 1). Hence, the reduction in lipid droplets induced by AM580 can hinder coronavirus pathogenesis by interfering with lipid droplet

formation which ultimately prevents viral replication [13]. The role of lipid droplets in the lifecycles of other coronaviruses is still unclear and must be further explored. Thus, both Remdesivir and AM580 appear to primarily act by inhibiting viral replication. Whereas Remdesivir does so by preventing the functioning of the RdRp, AM580 does so in a different manner by reducing the lipid droplets required by the virus in order to form the replication complexes. As a result, both small molecules offer non-overlapping mechanisms that, when used together, can significantly reduce viral replication [13, 14].

**What is the therapeutic feasibility of Remdesivir and AM580?** In order to estimate the therapeutic feasibility of an antiviral drug regimen, two important factors must be considered. The first factor is the selectivity associated with the drugs. It is vital that the proposed treatment option shows tolerable toxicity and tolerable off-target/side effects at effective concentrations. The second important aspect of feasibility which must be considered is viral resistance. If a treatment option is devised, then it is imperative that it is a long-term solution and that viral resistance does not develop. If resistance does develop, then it can be fatal if other treatment options are not present as well as economically costly. Both factors must be considered when trying to determine the feasibility of Remdesivir and AM580.

In terms of the selectivity of Remdesivir and AM580, they differ due to the nature of the drugs. Remdesivir is a direct-acting antiviral and hence, by nature, has low cytotoxicity as it selectively targets viral components; the $CC_{50}$ value of Remdesivir for SARS-CoV and MERS-CoV has been determined using human airway epithelial cells to be greater than 10 µM. As for the effectivity of the drug, it shows a high potency with a mean $EC_{50}$ value of 0.074 µM for both SARS-CoV and MERS-CoV. Thus, Remdesivir shows a selectivity index (SI) of at least 130:1 [12]. AM580 also has fairly good selectivity. AM580 has shown an $EC_{50}$ value of 0.25 µM for MERS-CoV and a value of 1.11 µM for SARS-CoV. Additionally, it has a selectivity index (SI) of 507:1 with respect to MERS-CoV and 114:1 for SARS-CoV [13]. By comparison, morphine has an SI of 70:1 and it has been reported that a ratio of 10:1 is the minimal acceptable index [18, 19]. Thus, both Remdesivir and AM580 are well within the acceptable range. However, what is imperative to be tested is the selectivity index of the drugs when used in a combinational drug regimen. This will be dependent on the dosages of each drug used however, based on their individual values, the regimen will likely have a fairly high selectivity index. One of the main benefits of a high selectivity index is that it allows the dosage to be increased allowing for later administration of the drug [12]. Often, diseases aren't diagnosed from the onset of symptoms; hence, having some flexibility in administration time and dosage helps increase the therapeutic window of the drug. Additionally, a high selectivity index allows for the dosage to be varied based on epidemiological needs, such as increasing the dosage in the unlikely event resistance develops or as a pre-emptive measure to prevent resistance development.

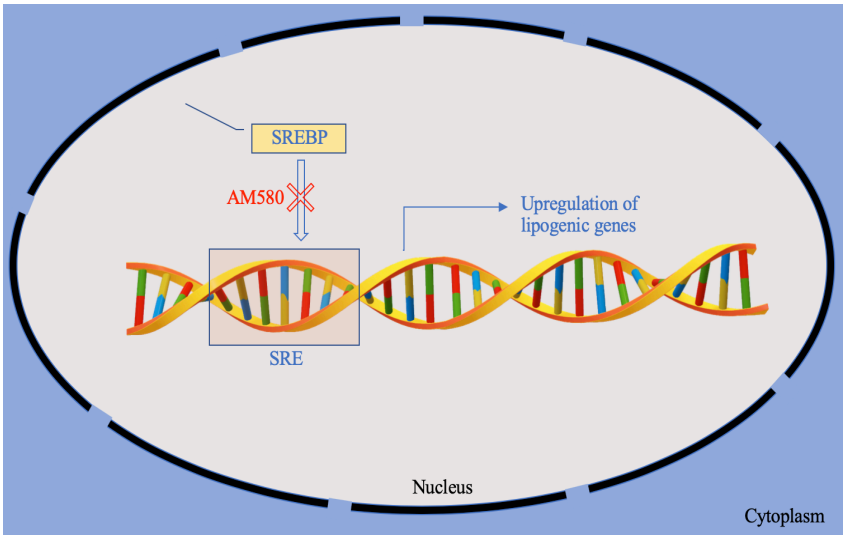


**FIG. 2 Mechanism of action of AM580 in reduction of lipogenic gene transcription.** The drug AM580, has been implicated with blocking the interaction of SREBP with the SRE region located in the nucleus. As a result of this, the SREBP cannot bind to the SRE region and cannot result in the upregulation of lipogenic genes. Elevated lipogenic gene levels have been associated with coronavirus infection.

As stated, resistance is a crucial factor that must be considered when discussing the long-term feasibility of any proposed drug regimen. Out of both Remdesivir and AM580, it is more likely that the viruses will develop resistance to Remdesivir as it is a direct-acting antiviral targeting a viral mechanism [12, 14]. This is an even bigger issue when discussing coronaviruses as they are positive sensed RNA viruses which can mutate quickly [7, 15]. Additionally, nucleoside analogues have been shown to be ineffective against coronaviruses due to the viruses encoding an nsp14 exonuclease (ExoN) which removes those analogues from the virion genome [12]. However, in the case of Remdesivir, the ExoN appears to be rather ineffective at removing Remdesivir. It is not clear exactly why this is; however, it is known that the ExoN does recognize Remdesivir, but it simply cannot remove it effectively. This was demonstrated by nsp14 ExoN knockout studies which showed a drastic increase in Remdesivir effectivity. Moreover, in terms of the development of resistance to Remdesivir over time, researchers found that 2 mutations in the nsp12 RdRp resulted in a 5.6-fold increase in resistance to Remdesivir. However, it is important to note that the mutations in the nsp12 RdRp resulted in a fitness cost and the virions that were resistant to Remdesivir failed to compete with wildtype virions during coinfection over multiple passages [12]. With respect to AM580, no resistance has been reported [13].

Another important consideration is that one of the main novelties of this proposed regimen is that it will include both Remdesivir and AM580 and as a result, viral resistance to both drugs simultaneously is very unlikely to occur. More specifically, resistance to Remdesivir is very likely to form, resistance against AM580 is improbable (being an IAA) and resistance against the combination of the two drugs is most unlikely to form. This is because both drugs operate in a non-redundant manner. Specifically, although both target the replication step of the coronavirus life cycle, they do so by targeting different processes required by the virus in order to propagate infection [12, 13]. Thus, even if a virion does develop resistance to Remdesivir, it is very unlikely that the same virion will also be resistant against AM580. As a result, resistance to both drugs is highly unlikely, improving the therapeutic feasibility of this regimen for long-term use in future cases as well. This is of crucial importance as Remdesivir is currently the most promising drug with respect to treating coronavirus infections and if resistance arises to this drug, it can prove to be fatal. Of course, the decrease in resistance associated with a combinational drug therapy is hypothetical and must be confirmed using in-vivo testing.

**What other viruses could be treated by the proposed combinational drug regimen?**

One of the major benefits of this novel antiviral regimen is its ability to target more than one virus. As mentioned previously, Remdesivir was originally developed and used against Ebola virus, however, researchers discovered that it may be effective against coronaviruses as well [12, 14]. A study testing 55 antiviral compounds against 8 RNA and DNA viruses discovered that 7 of these antiviral compounds may have one or more viral targets that were otherwise unknown [20]. Thus, it is possible that the proposed Remdesivir and AM580 drug combination may have more than one target.

The reasoning behind this is that both Remdesivir and AM580 target viral processes which are not specific to only coronaviruses. In the case of Remdesivir, it primarily targets RNA viruses. This is because most RNA viruses require an RdRp in order to replicate and Remdesivir targets the RdRp [21]. So far, Remdesivir has been shown to be effective against SARS-CoV, MERS-CoV, Mouse hepatitis virus (MHV), and Ebola virus [12]. Additionally, using human lung cells, Remdesivir has also been shown to be effective against treating bat coronaviruses, pre-pandemic bat coronaviruses, and circulating contemporary human coronaviruses [22]. As an extension of this, Remdesivir may also be effective against hepatitis C virus (HCV), dengue, and Zika virus (ZIKV) since they are all single-stranded positive-sense RNA viruses that utilize a highly similar RdRp [23-25].

On the other hand, AM580 also offers broad-spectrum activity. Specifically, it has been experimentally shown to be effective against SARS-CoV, MERS-CoV, ZIKV, influenza A virus, and enterovirus-A71 [13]. Due to the wide array of viruses requiring lipid droplets in order to support their life cycles, AM580 may have other viral targets as well, for example dengue virus and HCV. Dengue virus utilizes autophagy in order to provide energy for viral replication [26], while HCV utilizes lipid droplets as storage organelles for the assembly of

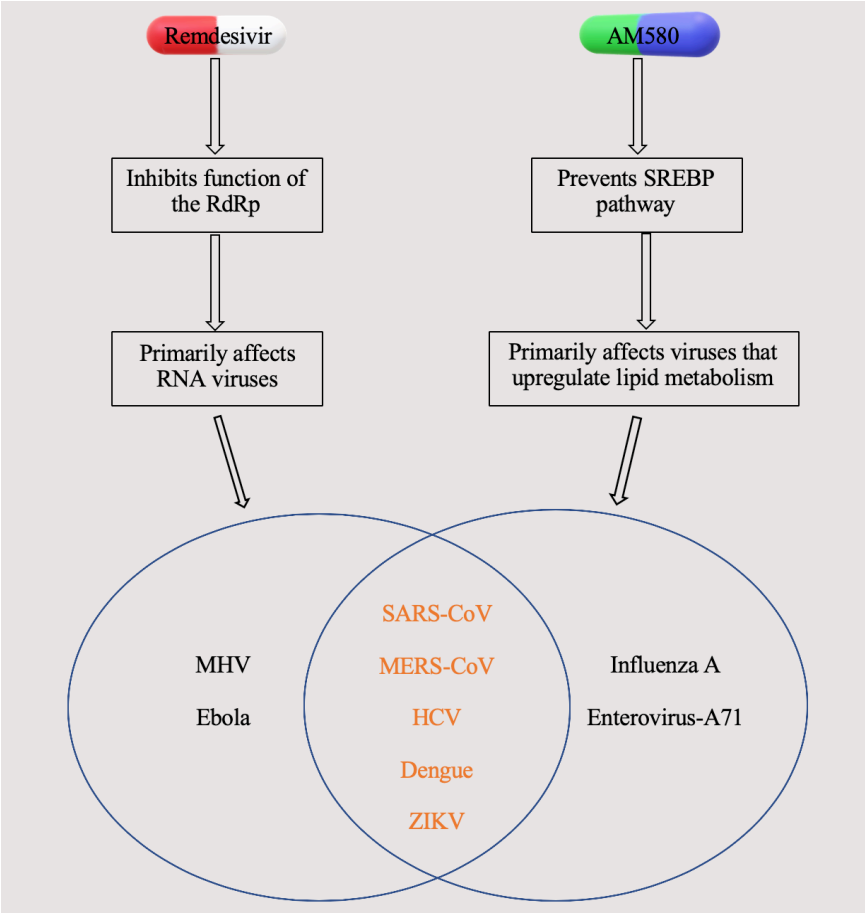


**FIG. 3 Overview of the confirmed and putative targets of Remdesivir and AM580.** Both Remdesivir and AM580 can act as broad-spectrum antivirals. Although they differ in approach as Remdesivir targets the RdRp and AM580 targets the SREBP pathway, there are overlapping diseases which may be able to be treated using the proposed combinational therapy.

infectious HCV virions [27]. Thus, with AM580 interfering with such a critical component of the life cycle of many viruses, the use of the drug has the potential to expand beyond coronaviruses.

Thus, both Remdesivir and AM580 could target viruses such as HCV, dengue virus, and ZIKV which could be further explored (FIG. 3). This will allow researchers to determine the effectiveness of such a combinational therapy with respect to other viruses and utilize this drug regimen to its maximum capacity. By doing so, this drug regimen can be mass-produced for other viral targets and this can help reduce costs. Additionally, it can provide a treatment option for some viruses in which currently we don't have any treatments.

## CONCLUSIONS

The recent outbreaks of SARS-CoV and MERS-CoV have shown that coronaviruses are a major threat to global health; however, they have received little attention with respect to a long-term solution. Taken together, SARS-CoV and MERS-CoV have resulted in over 1,400 deaths and have high mortality rates of 10% and 38% respectively [5, 6]. Alarmingly, pre-pandemic coronaviruses such as SARS-CoV- and MERS-CoV-like bat coronaviruses have been shown to replicate efficiently in primary human airway cells [12]. If such viruses migrate to the human population, it is possible that a deadly outbreak such as that experienced in 2002 with SARS-CoV and 2012 with MERS-CoV may be expected.

Due to the lack of a vaccine currently available, the only means of combatting such an issue is to develop an effective treatment solution [7]. Various treatments such as DPP4 inhibitors have been explored; however, they offer limited usability [8-11]. Thus, as of now, no treatment strategy or vaccine option is available and in the event of a coronavirus outbreak, quarantines are the only option [7]. Quarantines however cannot prevent the death of those already infected and as indicated by the high mortality rate of SARS-CoV and MERS-CoV, infected individuals are likely to die. Consequently, a novel approach such as using Remdesivir with AM580 is essential in order to achieve a long-term solution that can

help prevent the death of thousands of individuals [12, 13]. Such an antiviral cocktail is similar in principle to that used for the treatment of other viruses such as HIV.

The proposed antiviral cocktail provides multiple advantages due to the nature of its component small molecule drugs. Whereas Remdesivir targets viral replication through inhibition of the RdRp, AM580 targets viral replication and possibly other viral life cycle stages through inhibition of lipid droplet upregulation (FIG. 1). Additionally, both drugs exhibit low cytotoxicity, high effectivity and low viral resistance [12-19]. As a result of this, once used in conjunction, the antiviral cocktail has the potential to provide a potent treatment option that can have lasting impacts. Moreover, the broad-spectrum nature of the proposed cocktail allows for greater practicality in terms of diagnosis, administration and costs. Taking these all into account, this novel antiviral cocktail can provide a much-needed, effective and long-lasting solution to not only combatting the imminent coronavirus threat, but also reducing the burden of other diseases such as HCV, dengue and ZIKV (FIG. 3) [22-27]. Furthermore, the lessons learned using this field of research can be applied to drug development for other viruses as well and can help progress the field of therapeutics as a whole with regards to treating viral infections.

**ACKNOWLEDGEMENTS**

I would like to thank Dr. François Jean for his constant support, guidance and thoughtful feedback throughout the development of this project. I would also like to thank my MICB 406 peers for their considerate input and support.

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