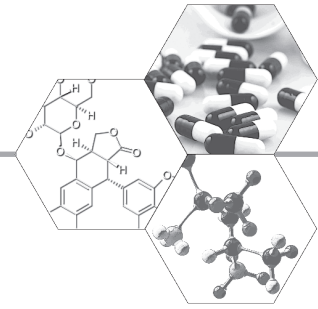

Future treatment strategies for novel Middle East respiratory syndrome coronavirus infection

"Given the similarity of Middle East respiratory syndrome coronavirus with severe acute respiratory syndrome coronavirus, what lessons can we learn from past work on severe acute respiratory syndrome coronavirus, or other positive strand RNA viruses?"

Keywords: cathepsin L inhibitors ■ coronavirus ■ drug resistance ■ inhibitors ■ interferons ■ Middle East respiratory syndrome ■ pseudotyped virus ■ ribavirin ■ severe acute respiratory syndrome ■ small molecule

More than a year ago, a novel coronavirus (CoV), the Middle East respiratory syndrome CoV (MERS-CoV), was first identified as the causative agent of a severe pneumonia in several patients in the Middle East [1]. As of 5 August, 2013, a total of 94 cases have been identified in the Middle East (Saudi Arabia, Jordan, Qatar and the United Arab Emirates), Europe (the UK, France, Italy and Germany) and North Africa (Tunisia), with a case fatality rate of 56%. Since this CoV is closely related to severe acute respiratory syndrome CoV (SARS-CoV), an epidemic that was short-lived but alarming in 2002–2003 that resulted in approximately 8000 cases and 800 deaths, several health agencies that include governmental, public health, clinical and laboratory authorities have all moved rapidly to respond to this new virus outbreak. Following identification of MERS-CoV, details about its genomic sequence and organization, species tropism and host-cell receptor (dipeptidyl peptidase 4) have been reported [2,3].

Although there have been several reports of inhibitors of SARS-CoV, to date, there are no approved drugs or vaccines for the treatment of SARS-CoV. Studies have shown that ribavirin has a very minimal effect in controlling adverse outcomes from SARS-CoV infection. Its use has attracted a lot of criticism due to its unproven and low efficacy and unwarranted side effects [4]. At nontoxic concentrations, ribavirin has no direct *in vitro* activity against SARS-CoV [5]. Among some of the promising agents for the control of SARS-CoV infection are interferons (IFNs). A large number of recombinant IFNs that belong to the three classes (α, β and γ) have been tested for their antiviral activities against SARS-CoV *in vitro*. IFN-α and -β were shown to be active against SARS-CoV, with IFN-β being the most active of the three [6].

More recently, Falzarano *et al.* proposed a potential therapeutic regimen for the treatment of MERS-CoV based on cell-based experiments [7]. In this report, the authors compared the use of both IFN-α2b and ribavirin individually or in combination for the inhibition of MERS-CoV in two different cell lines, Vero and LLC-MK2. When used individually, to completely eliminate the cytopathic effect of SARS-CoV infection, each drug required concentrations as high as 1000 U/ml IFN-α2b (IFN-α2b) and 200 μg/ml ribavirin. However, when the two drugs were combined they acted synergistically, and only 125 U/ml IFN-α2b and 25 μg/ml ribavirin were required to completely eliminate the cytopathic effect. This represented an eight- and 16-fold decrease in the amount of IFN-α2b and ribavirin respectively, which is required to attain the same reduction when used individually.

While the combination therapy portends to be a promising strategy, these concentrations are still very much higher than the current doses at which these two drugs are being used for hepatitis C virus (HCV) infections in humans [8]. High doses of ribavirin have been associated with hemolytic anemia, elevated transaminase levels and bradycardia [9]. Despite the lower doses of these drugs for the treatment of HCV, many side effects accompany this combination therapy, which include depression, suicide, relapse of drug abuse/overdose, bacterial infections and many others [101]. Nonetheless, given the severity of this disease and the lack of any available treatments, this is a promising finding that should be further explored.

Adeyemi O Adedeji

Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
Author for correspondence:
Tel.: +1 573 882 4338
Fax: +1 573 884 9676
E-mail: aoadedeji@ucdavis.edu

Stefan G Sarafianos

Christopher Bond Life Sciences Center, Department of Molecular Microbiology & Immunology, Department of Biochemistry, University of Missouri School of Medicine, Columbia, MO 65211, USA

FUTURE SCIENCE part of fsg

Given the similarity of MERS-CoV with SARS-CoV, what lessons can we learn from past work on SARS-CoV, or other positive strand RNA viruses? MERS-CoV was recently shown to use dipeptidyl peptidase 4 as its functional receptor to gain entry into cells [3]. While this virus is utilizing a receptor different than the one used by SARS and other respiratory coronaviruses, there appears to be some similarities in their mechanisms of entry. MERS-CoV has been shown to require cathepsins B and L for entry, similar to SARS-CoV [10]. Cathepsin L inhibitors have been shown to prevent SARS-CoV entry in SARS-CoV-pseudotyped assays (SARS-CoVenv/HIV core) [11]. Published cathepsin L inhibitors include oxocarbazate, MDL28170 and SSAA09E1. MDL28170 and oxocarbazate inhibit SARS-CoV-pseudotyped virus with EC_{50} values of approximately 100 and 300 nM, respectively [12,13]. These two compounds appear to be peptidomimetic in nature, thereby limiting their use only to parenteral administration if they were to be considered for therapeutic purposes. SSAA09E1, a non-peptidomimetic small molecule that inhibited SARS-CoV-pseudotyped virus with an EC_{50} of approximately 7 μM can also inhibit live SARS-CoV in Vero cells [Adedeji AO, Sarafianos SG, Unpublished Data]. Therefore, any of these cathepsin L inhibitors would be worth testing for inhibition of MERS-CoV as well as of other coronaviruses that require cathepsin L for entry.

In thinking about treatments of viral infections, one has to also consider the challenge of drug resistance, which is the result of the ability of viruses to rapidly evolve and escape treatments through multiple and diverse mechanisms of resistance. There is an extensive body of clinical data on development of resistance against antivirals that are used in the clinic for the treatment of HIV, hepatitis B virus, and HCV infections. For HIV, remarkable progress has been made with highly active antiretroviral therapies, which are based on combinations of drugs that almost exclusively target directly the virus. Highly active antiretroviral therapies regimens significantly suppress the viral load in the blood plasma of HIV patients, resulting in significant decreases in HIV-related deaths. However, prolonged treatment inevitably results in resistance to all of these available drugs [14–16]. Similar benefits have been reported in the treatment of hepatitis B virus-infected patients with antiretrovirals.

For many years, HCV has been treated with a combination therapy of PEGylated IFN and ribavirin. Clinical trials have shown sustained virological response rates of approximately 50% (efficacy), which does not even provide equivalent results in clinical practice [17]. HCV has also evolved various mechanisms to develop resistance to these therapies. For example, HCV circumvents the IFN response by blocking downstream signaling actions via STAT1, STAT2, IRF9 and JAK-STAT pathways [18,19]. Hence, MERS-CoV can also develop resistance to IFN treatment.

> *"The recent report that interferon-α2b and ribavirin have some synergistic activity in cell-based assays is indeed encouraging, as these drugs are currently used to treat hepatitis C and are therefore widely available..."*

Ribavirin is a nucleoside analog that after activation by host kinases to a nucleotide, it can be used by viral polymerases and incorporated into the nascent viral genomes, causing mutations by allowing base mismatches. During the amplification of HCV replicons, error frequency is known to increase upon ribavirin treatment. Hence, ribavirin's antiviral activity is likely to result from error catastrophe caused by increased mutagenesis of viral genomes thereby reducing the fitness of the virus. Another mechanism through which ribavirin may exhibit its antiviral activity is by depletion of intracellular GTP pools (by inhibition of the cellular IMPDH by the 5′-monophosphate metabolite of ribavirin) [20]. This mechanism may be useful in explaining the drug's general cytotoxic and anti-DNA replication effect as well as some effect on DNA viral replication.

While reports of viral resistance to ribavirin are rare, its reliance on host factors to be able to convert to its mono- and tri-phosphate active forms stands as a potential problem. Falzarano *et al.* showed the difference in the efficacies of ribavirin depending on the cell types, as ribavirin was more effective in LLC-MK2 cells than the Vero cells [7]. Since MERS-CoV naturally infects respiratory epithelial cells, it will be more expedient to monitor the effect of ribavirin in respiratory epithelial cells to see if comparable viral inhibition will be observed.

With limited efficacy of this therapy, coupled with multiple side effects and resistance

development, many scientists have been working on IFN-free therapy that involves development and the use of small-molecule compounds that directly inhibit the viral replication proteins. The efficacies of these compounds are being tested alone or in combination with ribavirin or PEGylated IFNs. These compounds are referred to as 'direct acting antivirals'. Examples include the recently approved boceprevir and telaprevir NS3/4A protease inhibitors, as well as NS5A inhibitors and NS5B polymerase inhibitors that are in clinical trials [21]. For coronaviruses, several reports of SARS-CoV small-molecule compounds that inhibit the viral replication proteins, or prevent entry of the virus into cells, are well documented [11,12,22,23]. All these compounds, with known mechanisms of action, introduce potential leads for the development of MERS and other CoV therapeutics.

In conclusion, the recent report that IFN-α2b and ribavirin have some synergistic activity in cell-based assays is indeed encouraging, as these drugs are currently used to treat hepatitis C and are therefore widely available even though they are imperfect but with known pharmacological properties. Moreover, the study highlights how past research in related viruses can provide useful insights in the treatment of emerging diseases.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

References

1 Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* 367(19), 1814–1820 (2012).

2 van Boheemen S, de Graaf M, Lauber C *et al.* Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio* 3(6), e00473–12 (2012).

3 Raj VS, Mou H, Smits SL *et al.* Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495(7440), 251–254 (2013).

4 Cyranoski D. Critics slam treatment for SARS as ineffective and perhaps dangerous. *Nature* 423(6935), 4 (2003).

5 Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet* 361(9374), 2045–2046 (2003).

6 Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Treatment of SARS with human interferons. *Lancet* 362(9380), 293–294 (2003).

7 Falzarano D, de Wit E, Martellaro C, Callison J, Munster VJ, Feldmann H. Inhibition of novel beta coronavirus replication by a combination of interferon-α2b and ribavirin. *Sci. Rep.* 3, 1686 (2013).

8 Manns MP, McHutchison JG, Gordon SC *et al.* PEGinterferon α-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358(9286), 958–965 (2001).

9 Booth CM, Matukas LM, Tomlinson GA *et al.* Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 289(21), 2801–2809 (2003).

10 Gierer S, Bertram S, Kaup F *et al.* The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies. *J. Virol.* 87(10), 5502–5511 (2013).

11 Adedeji AO, Severson W, Jonsson C, Singh K, Weiss SR, Sarafianos SG. Novel inhibitors of severe acute respiratory syndrome coronavirus entry that act by three distinct mechanisms. *J. Virol.* 87(14), 8017–8028 (2013).

12 Shah PP, Wang T, Kaletsky RL *et al.* A small-molecule oxocarbazate inhibitor of human cathepsin L blocks severe acute respiratory syndrome and ebola pseudotype virus infection into human embryonic kidney 293T cells. *Mol. Pharmacol.* 78(2), 319–324 (2010).

13 Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proc. Natl Acad. Sci. USA* 102(33), 11876–11881 (2005).

14 Sarafianos SG, Marchand B, Das K *et al.* Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. *J. Mol. Biol.* 385(3), 693–713 (2009).

15 Singh K, Marchand B, Kirby KA, Michailidis E, Sarafianos SG. Structural aspects of drug resistance and inhibition of HIV-1 reverse transcriptase. *Viruses* 2(2), 606–638 (2010).

16 Ren J, Stammers DK. Structural basis for drug resistance mechanisms for non-nucleoside inhibitors of HIV reverse transcriptase. *Virus Res.* 134(1–2), 157–170 (2008).

17 Feuerstadt P, Bunim AL, Garcia H *et al.* Effectiveness of hepatitis C treatment with pegylated interferon and ribavirin in urban minority patients. *Hepatology* 51(4), 1137–1143 (2010).

18 Shao RX, Zhang L, Peng LF *et al.* Suppressor of cytokine signaling 3 suppresses hepatitis C virus replication in an mTOR-dependent manner. *J. Virol.* 84(12), 6060–6069 (2010).

19 Kumthip K, Chusri P, Jilg N *et al.* Hepatitis C virus NS5A disrupts STAT1 phosphorylation and suppresses type I interferon signaling. *J. Virol.* 86(16), 8581–8591 (2012).

20 Leyssen P, De Clercq E, Neyts J. The anti-yellow fever virus activity of ribavirin is independent of error-prone replication. *Mol. Pharmacol.* 69(4), 1461–1467 (2006).

21 Shah N, Pierce T, Kowdley KV. Review of direct-acting antiviral agents for the treatment of chronic hepatitis C. *Expert Opin. Investig. Drugs* 22(9), 1107–1121 (2013).

22 Adedeji AO, Singh K, Calcaterra NE *et al.* Severe acute respiratory syndrome coronavirus replication inhibitor that interferes with the nucleic acid unwinding of the viral helicase.

Antimicrob. Agents Chemother. 56(9), 4718–4728 (2012).

23 Jacobs J, Grum-Tokars V, Zhou Y *et al.* Discovery, synthesis, and structure-based optimization of a series of *N*-(*tert*-butyl)-2-(*N*-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease. *J. Med. Chem.* 56(2), 534–546 (2013).

■ Website

101 Interferon and ribavirin treatment side effects. www.hepatitis.va.gov/provider/reviews/treatment-side-effects.asp#S1X