against radiological and histopathological pneumonia after MERS coronavirus challenge in rhesus macaques,[10] it remains difficult to interpret the relevance of the immunogenicity parameters that were assessed in humans in the phase 1 trial given our poor understanding of protective natural immune responses. Modjarrad and colleagues sought to bridge this gap in a post-hoc analysis by obtaining samples from ten individuals who had recovered from natural MERS coronavirus infection during the 2015 outbreak in South Korea (convalescent serum and peripheral blood mononuclear cell samples were obtained at a mean of 19·8 months [SD 0·7] after the original MERS diagnosis). Anti-S1 and neutralising antibody titres following natural infection were significantly higher than vaccine-induced responses in the acute phase, but were not different to vaccine in late convalescent samples and at similar post-vaccination timepoints.

Given the differences between S1-ELISA and neutralising antibody responses to GLS-5300, the comparison of post-natural infection versus post-vaccination responses will have an important role in the development of vaccines against sporadically occurring pathogens such as MERS coronavirus. Despite many challenges for development, the platform technologies on which vaccines such as GLS-5300 are based hold promise against novel pathogen threats because of the rapidity with which they can be formulated and manufactured; four MERS coronavirus vaccine candidates that have started phase 1 trials are based on different platform technologies.[6] Completion of the phase 1 trial of GLS-5300 represents an incremental but important step in the development of vaccines against emerging viral global threats.

*In-Kyu Yoon, *Jerome H Kim*
International Vaccine Institute, Gwanak-gu, Seoul, 08826, South Korea (I-KY, JHK)
jerome.kim@ivi.int

I-KY and JHK's institution is collaborating with GeneOne Life Science on a Middle East respiratory syndrome coronavirus vaccine trial in South Korea.

1 FAO-OIE-WHO MERS Technical Working Group. MERS: progress on the global response, remaining challenges and the way forward. *Antiviral Res* 2018; **159:** 35–44.
2 Dighe A, Jombart T, Van Kerkhove MD, Ferguson N. A systematic review of MERS-CoV seroprevalence and RNA prevalence in dromedary camels: implications for animal vaccination. *Epidemics* 2019; published online June 5. DOI:10.1016/j.epidem.2019.100350.
3 Coalition for Epidemic Preparedness Initiatives. Targeting diseases with epidemic potential. 2019. https://cepi.net/research_dev/priority-diseases (accessed June 17, 2019).
4 WHO. List of Blueprint priority diseases. 2019. https://www.who.int/blueprint/priority-diseases/en (accessed June 17, 2019).
5 WHO. Target product profiles for MERS-CoV vaccines. 2017. https://www.who.int/blueprint/what/research-development/MERS_CoV_TPP_15052017.pdf?ua=1 (accessed June 17, 2019).
6 Modjarrad K, Roberts CC, Mills KT, et al. Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial. *Lancet Infect Dis* 2019; published online July 24. http://dx.doi.org/10.1016/S1473-3099(19)30266-X.
7 Skariyachan S, Challapilli SB, Packirisamy S, Kumargowda ST, Sridhar VS. Recent aspects on the pathogenesis mechanism, animal models and novel therapeutic interventions for Middle East respiratory syndrome coronavirus infections. *Front Microbiol* 2019; **10:** 569.
8 Zhao J, Li K, Wohlford-Lenane C, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc Natl Acad Sci USA* 2014; **111:** 4970–75.
9 Agrawal AS, Tao X, Algaissi A, et al. Immunization with inactivated Middle East respiratory syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus. *Hum Vaccin Immunother* 2016; **12:** 2351–56.
10 Muthumani K, Falzarano D, Reuschel EL, et al. A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. *Sci Transl Med* 2015; **7:** 301ra132.

Use of reverse genetics to inform Ebola outbreak responses

Published **Online**
July 9, 2019
http://dx.doi.org/10.1016/S1473-3099(19)30346-9

See **Articles** page 1023

For **Ebola virus sequence information obtained from the Democratic Republic of the Congo outbreaks** see http://virological.org/t/drc-2018-viral-genome-characterization/230

Since 1976, Ebola viruses have caused sporadic outbreaks and epidemics throughout central and west Africa. In recent years, the size and duration of these outbreaks has grown exponentially, as exemplified by over 28 000 cases with more than 11 000 deaths in the 2013–16 west African epidemic. An ongoing outbreak in the Ituri and North Kivu provinces of the Democratic Republic of the Congo, began in July 2018 and has spilled over to Uganda, resulting in 2181 cases and 1459 deaths (as of June 17, 2019).[1,2] By contrast, previous outbreaks never reached more than a few hundred cases and were generally short-lived, likely because of their emergence in relatively isolated locations. Factors leading to the scale and duration of the 2013–16 west African epidemic included, but were not limited to, a highly mobile society, inadequate public health infrastructure, and absence of an approved vaccine. Many important advances in the understanding of Ebola virus infection and recovery were made during this outbreak, which contributed to the refinement of medical countermeasures including vaccines, therapeutics, and diagnostics.

The development of reverse genetics systems (ie, techniques for the generation of infectious

virus entirely from cloned genetic constructs) has substantially contributed to our understanding of the molecular pathogenesis of a number of high-priority viruses, including Ebola.[3] The technique has also proven invaluable for the generation of recombinant experimental vaccines against these agents, including the primary vaccine in use in the current outbreak in DR Congo, rVSV-ZEBOV.[4] In 2001–02, the first filovirus reverse genetics systems were described and valuable information about the role of viral proteins was examined;[5,6] since then, the technique has been used in vaccine efforts for a number of filoviruses,[4] and to understand the host adaptation[7–9] and attenuation mechanisms.[10]

In *The Lancet Infectious Diseases*, Laura K McMullan and colleagues[11] present the first occasion in which reverse genetics technology has been used to generate an Ebola virus isolate using sequence information from an ongoing filovirus outbreak where isolates were not readily available. The engineered isolate contains a fluorescent reporter protein (Zoanthus green fluorescent protein) that allows virus growth to be quantified by measuring fluorescence in infected cells. McMullan and colleagues assessed the efficacy of investigational therapies against Ebola virus disease and the sensitivity of diagnostic assays approved for emergency use in the 2018 outbreak. Importantly, the authors showed that investigational therapies being tested in clinical trials in DR Congo (including remdesivir and ZMapp monoclonal antibodies)[12] are effective against viruses known to be circulating in the outbreak. The Ebola diagnostic assays approved for emergency use by the US Centers for Disease Control and Prevention and WHO detected Ituri Ebola virus with high sensitivity despite primer site binding mismatches.

Ensuring that medical countermeasures against Ebola viruses are available and effective is crucial to timely and targeted outbreak management, and indeed a number of promising vaccine candidates, therapeutics, and rapid diagnostic assays have been approved for emergency use.[1] However, countermeasures are all of limited value if they cannot reach the target population because of safety concerns caused by civil conflict in affected areas. Furthermore, their efficacy is dependent on the availability of information about the exact virus isolate responsible for the outbreak. Although heroic efforts have been made by Congolese scientists and those with the Institut National de Recherche Biomédicale (Kinshasa, DR Congo) and United States Army Medical Research Institute of Infectious Diseases (Fort Detrick, MD, USA) to ensure sequence information is available to the scientific community, only predictive inferences can be made about the efficacy of any medical countermeasure used in the current outbreak. During the 2013–16 west African epidemic, viral isolates were made available to the scientific community shortly after the onset of the outbreak, which clearly assisted in the generation and validation of experimental vaccines, therapeutics, and diagnostics against the new Makona outbreak strain.[13–15] Additionally, a lot was learned about the pathogenesis of the 2013–16 Makona strain through the use of animal models, including ferrets and non-human primates.[16–18]

The findings by McMullan and colleagues[19] support the continued testing of investigational therapies against Ebola virus disease in the ongoing outbreak, and have renewed confidence in the Ebola assays approved for emergency use. Further work is needed to characterise host responses to infection with this engineered Ebola isolate compared with other Ebola isolates. In vivo verification of antiviral and vaccine candidate efficacy is of equal importance to strengthen global confidence in new medical countermeasures and make the necessary refinements.

*Robert W Cross, *Thomas W Geisbert*
Galveston National Laboratory, Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX 77550-0610, USA (RWC, TWG)
twgeisbe@utmb.edu

TWG has patents US 7,635,485 and US 8,017,130 issued to the US Government, patents US 7,838,658 and US 8,716,464 issued to the US Government and Arbutus Biopharma, and patent US 8,796,013 issued to Boston University. RWC declares no competing interests.

1 WHO. Ebola situation reports: Democratic Republic of the Congo. World Health Organisation. https://www.who.int/csr/don/13-june-2019-ebola-drc/en/ (accessed June 18, 2019).
2 CDC. 2014-2016 Ebola outbreak in west Africa. Centers for Disease Control and Prevention. https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/index.html (accessed June 18, 2019).
3 Stobart CC, Moore ML. RNA virus reverse genetics and vaccine design. *Viruses* 2014; **6:** 2531–50.
4 Geisbert TW, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis* 2011; **204** (suppl 3)**:** S1075–81.
5 Neumann G, Feldmann H, Watanabe S, Lukashevich I, Kawaoka Y. Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. *J Virol* 2002; **76:** 406–10.
6 Volchkov VE, Volchkova VA, Muhlberger E, et al. Recovery of infectious Ebola virus from complementary DNA: RNA editing of the GP gene and viral cytotoxicity. *Science* 2001; **291:** 1965–69.

7 Ebihara H, Takada A, Kobasa D, et al. Molecular determinants of Ebola virus virulence in mice. *PLoS Pathog* 2006; **2:** e73.

8 Volchkova VA, Dolnik O, Martinez MJ, Reynard O, Volchkov VE. Genomic RNA editing and its impact on Ebola virus adaptation during serial passages in cell culture and infection of guinea pigs. *J Infect Dis* 2011; **204** (suppl 3)**:** S941–46.

9 Volchkov VE, Chepurnov AA, Volchkova VA, Ternovoj VA, Klenk HD. Molecular characterization of guinea pig-adapted variants of Ebola virus. *Virology* 2000; **277:** 147–55.

10 Hartman AL, Towner JS, Nichol ST. A C-terminal basic amino acid motif of Zaire ebolavirus VP35 is essential for type I interferon antagonism and displays high identity with the RNA-binding domain of another interferon antagonist, the NS1 protein of influenza A virus. *Virology* 2004; **328:** 177–84.

11 McMullan LK, Flint M, Chakrabarti A, et al. Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis. *Lancet Infect Dis* 2019; published online July 9. http://dx.doi.org/10.1016/S1473-3099(19)30291-9.

12 Friedrich MJ. Multidrug Ebola trial underway in Democratic Republic of Congo. *JAMA* 2019; **321:** 637.

13 Cross RW, Mire CE, Feldmann H, Geisbert TW. Post-exposure treatments for Ebola and Marburg virus infections. *Nat Rev Drug Discov* 2018; **17:** 413–34.

14 Tembo J, Simulundu E, Changula K, et al. Recent advances in the development and evaluation of molecular diagnostics for Ebola virus disease. *Expert Rev Mol Diagn* 2019; **19:** 325–40.

15 Venkatraman N, Silman D, Folegatti PM, Hill AVS. Vaccines against Ebola virus. *Vaccine* 2018; **36:** 5454–59.

16 Cross RW, Speranza E, Borisevich V, et al. Comparative transcriptomics in Ebola Makona-infected ferrets, nonhuman primates, and humans. *J Infect Dis* 2018; **218** (suppl 5)**:** S486–95.

17 Versteeg K, Menicucci AR, Woolsey C, et al. Infection with the Makona variant results in a delayed and distinct host immune response compared to previous Ebola virus variants. *Sci Rep* 2017; **7:** 9730.

18 Marzi A, Feldmann F, Hanley PW, Scott DP, Günther S, Feldmann H. Delayed disease progression in cynomolgus macaques infected with Ebola virus makona strain. *Emerg Infect Dis* 2015; **21:** 1777–83.