



Zika mRNA vaccine induces long-term protective immunity

Siyuan Ding^{1,2,3}, Harry B. Greenberg^{1,2,3}

¹Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305, USA; ²Department of Medicine, Division of Gastroenterology and Hepatology, Stanford University, Stanford, CA 94305, USA; ³Palo Alto Veterans Institute of Research, VA Palo Alto Health Care System, Palo Alto, CA 94304, USA

Correspondence to: Siyuan Ding, 3801 Miranda Ave., Bldg 101, Rm C4-181, Palo Alto, CA 94304, USA. Email: syding@stanford.edu.

Comment on: Pardi N, Hogan MJ, Pelc RS, *et al.* Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* 2017;543:248-51.

Received: 12 May 2017; Accepted: 24 May 2017; Published: 13 July 2017.

doi: 10.21037/amj.2017.05.22

View this article at: <http://dx.doi.org/10.21037/amj.2017.05.22>

If you are wondering what the hype on Zika virus (ZIKV) all over the news is about, here is a fact: In 2015 in Brazil alone, there is an estimate of 1.3 million cases of ZIKV infection (1). The virus is generally spread through *Aedes* mosquito bites but can also be transmitted sexually (2). It has unofficially replaced Ebola virus as the most significant global public health threat. Clinically, the virus has been identified as the etiological agent for microcephaly in newborns and rarely, Guillain-Barre syndrome in adults (1,3). The rapid increase in ZIKV infection cases has prompted a concerted effort on a global scale to develop safe and effective vaccines from scratch. Multiple immunization platforms are currently under study for a ZIKV vaccine, including plasmid DNA, inactivated virus particles, protein subunits and adenovirus-based vector (4-7).

In a recent paper published in *Nature*, Pardi *et al.* (8) generated the first mRNA vaccine against ZIKV and tested its immunogenicity and efficacy to induce antibody response in mice and rhesus macaques. The authors circumvented the common pitfalls associated with mRNA vaccine, such as the undesired activation of the host innate immune system and RNA instability, by chemical synthesis of a 1-methylpseudouridine cap modification at the 5' end and inclusion of additional 5' and 3' UTR sequences and a polyA tail. The central coding region expresses the pre-membrane and envelope (prM-E) glycoproteins derived from a ZIKV strain from a French Polynesia outbreak in 2013 (9). The resultant modified mRNA was subsequently packaged into lipid nanoparticles (LNP) for *in vivo* experiments.

The authors first tested the T cell and B cell responses induced by ZIKV mRNA vaccine as compared to the

poly(I:C)-LNP control in wild-type C57BL/6 and BALB/c mice. A single immunization of 30 µg of prM-E mRNA-LNP via the intradermal route resulted in high levels (titer ranging from 10³ to 10⁵ EC₅₀ depending on the exact quantification assays) of neutralizing antibodies in the serum at weeks 8–12 post vaccination, indicative of potent activation of the humoral immunity in these mice. Importantly, when challenged with ZIKV infection at either 2 or 20 weeks post vaccination, all mice immunized with prM-E mRNA-LNP were protected and exhibited no sign of viremia, as opposed to high copies of viral RNA observed in poly(I:C)-LNP immunized mice. Mortality was not used as the readout since immunocompetent mice do not succumb to ZIKV infection due to their antiviral interferon (IFN) response. ZIKV inoculation induces lethality in *Stat1* or *Ifnar1* knockout mice that lack responsiveness to IFNs (10) but this model was not examined in the current study.

The authors subsequently examined the ZIKV mRNA-LNP vaccine efficacy in rhesus macaques, non-human primates that resemble human more closely than mice in many clinical manifestations of ZIKV infection. Consistent with the previous observation in mice, one single immunization of prM-E mRNA-LNP was sufficient to elicit a potent and durable neutralizing antibody response (10³–10⁴ EC₅₀) in rhesus macaques. Moreover, the vaccinated animals were highly protected from subsequent ZIKV challenge. Only one of five animals showed a small and transient increase in viral RNA copies, barely above the detection threshold, at 3 days post infection. In contrast, high levels of viral RNA copies were found in the circulation of all six unvaccinated control monkeys. Taken together, these data

confirmed the safety and efficacy of this new mRNA vaccine in two different animal models.

In comparison to other commonly used vaccine platforms, the advantages of an mRNA vaccine are substantial: first and foremost, unlike all DNA-based vaccines, mRNA does not integrate into the genome and therefore does not arouse major safety concerns with regard to insertional mutagenesis and potential tumorigenesis; second, mRNA is prone to design and encodes exogenous proteins with high efficiency; and third, mRNA can be propagated at sizable scales for manufacturing. As a proof-of-principle, the same group recently published another article using the same mRNA platform to express HIV broadly neutralizing antibody for therapeutic purposes (11). It is also worth mentioning that around the same time as the current *Nature* paper, an independent mRNA vaccine study on ZIKV was published in *Cell* with similar findings (12). Using several wild-type and knockout mouse models, Richner *et al.* found that their version of mRNA-LNP vaccine, given as two doses (prime and boost) in several *in vivo* experiments, significantly reduced viral loads in the serum, prevented weight loss, and completely protected against ZIKV infection upon challenge.

In summary, Pardi *et al.* reported a concise and definite study on developing and testing a modified mRNA-based ZIKV vaccine in mice and rhesus macaques. Although the data showed great promise in moving towards clinical trials in humans, much remains to be determined. For instance, there is substantial evidence, at least in mice, that ZIKV immunization induces cross-reactive antibodies, which might enhance dengue virus (DENV) infection and pathology through antibody-dependent enhancement (13,14), a condition that has been noted in several DENV studies where cross-reactive antibodies from a prior DENV infection would actually worsen the subsequent secondary infection with a heterologous DENV serotype (15). In addition, it is important to keep other anti-ZIKV options open, especially given the hope that thousands of *Aedes aegypti* mosquitoes infected with the *Wolbachia* bacteria have just been released in Florida (16), in a new effort to limit the spread of several viruses including ZIKV and DENV. In the meantime, researchers should not slow down the pace for basic research on the molecular biology and pathogenesis of ZIKV infection. Genetic and proteomic screens have proven to be powerful tools to investigate the role of host factors in virus infection (17,18). The recently conducted CRISPR-Cas9 screen on ZIKV is an excellent example of how useful information, including the identification of viral

receptors and other necessary host factors, can be identified from such fundamental studies (19). The development of a reverse-genetics system for ZIKV has also greatly promoted the vaccine studies *in vivo* (20). With all these progresses, it seems likely that a safe and effective vaccine against ZIKV is not too far away.

Acknowledgements

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Dr. Ziwei Li (Department of Molecular, Cell & Developmental Biology, University of California, Los Angeles, USA).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/amj.2017.05.22>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. *N Engl J Med* 2016;374:951-8.
2. CDC. Transmission and Risks. Available online: <https://www.cdc.gov/zika/transmission/> 2017.
3. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016;387:1531-9.

4. Abbink P, Larocca RA, De La Barrera RA, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science* 2016;353:1129-32.
5. Dowd KA, Ko SY, Morabito KM, et al. Rapid development of a DNA vaccine for Zika virus. *Science* 2016;354:237-40.
6. Kim E, Erdos G, Huang S, et al. Preventative Vaccines for Zika Virus Outbreak: Preliminary Evaluation. *EBioMedicine* 2016;13:315-20.
7. Larocca RA, Abbink P, Peron JP, et al. Vaccine protection against Zika virus from Brazil. *Nature* 2016;536:474-8.
8. Pardi N, Hogan MJ, Pelc RS, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* 2017;543:248-51.
9. Cao-Lormeau VM, Musso D. Emerging arboviruses in the Pacific. *Lancet* 2014;384:1571-2.
10. Lazear HM, Govero J, Smith AM, et al. A Mouse Model of Zika Virus Pathogenesis. *Cell Host Microbe* 2016;19:720-30.
11. Pardi N, Secreto AJ, Shan X, et al. Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. *Nat Commun* 2017;8:14630.
12. Richner JM, Himansu S, Dowd KA, et al. Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell* 2017;168:1114-25 e10.
13. Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. *Nat Immunol* 2016;17:1102-8.
14. Stettler K, Beltramello M, Espinosa DA, et al. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* 2016;353:823-6.
15. Beltramello M, Williams KL, Simmons CP, et al. The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity. *Cell Host Microbe* 2010;8:271-83.
16. FKMCD. MosquitoMate Wolbachia Trial. Available online: <http://keymosquitoorg/mosquito-mate-wolbachia-trial/> 2017.
17. Ding S, Mooney N, Li B, et al. Comparative Proteomics Reveals Strain-Specific beta-TrCP Degradation via Rotavirus NSP1 Hijacking a Host Cullin-3-Rbx1 Complex. *PLoS Pathog* 2016;12:e1005929.
18. Li B, Ding S, Feng N, et al. Drebrin restricts rotavirus entry by inhibiting dynamin-mediated endocytosis. *Proc Natl Acad Sci U S A* 2017;114:E3642-E3651.
19. Savidis G, McDougall WM, Meraner P, et al. Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics. *Cell Rep* 2016;16:232-46.
20. Shan C, Xie X, Muruato AE, et al. An Infectious cDNA Clone of Zika Virus to Study Viral Virulence, Mosquito Transmission, and Antiviral Inhibitors. *Cell Host Microbe* 2016;19:891-900.

doi: 10.21037/amj.2017.05.22

Cite this article as: Ding S, Greenberg HB. Zika mRNA vaccine induces long-term protective immunity. *AME Med J* 2017;2:86.