Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Zika virus (ZIKV) is a mosquito-borne flavivirus that can cause devastating microcephaly and other congenital syndromes in infants born to infected pregnant women as well as Guillain-Barré syndrome in infected infants born to infected pregnant women as well as newborns. In this issue of Molecular Therapy, Sariyer et al.\(^2\) present evidence demonstrating that rilpivirine, a US Food and Drug Administration (FDA)-approved HIV drug, inhibits ZIKV RNA-dependent RNA polymerase (RdRp), leading to potent antizika activity in cell cultures and mice. The study highlights the potential of repurposing clinically approved drugs for possible prevention and treatment of ZIKV infection and its associated disease.

Although emerging infectious diseases have been recognized for many decades, the last 20 years have been marked by the unprecedented emergence of several devastating epidemics of zoonotic RNA viral diseases, such as severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), Ebola, Nipah, chikungunya, West Nile, yellow fever, and ZIKV. Given the unpredictable nature of emerging pathogens, approaches for rapid development of countermeasures are actively pursued by both academia and industry. Besides vaccine and transmission control (e.g., vector control for mosquito-borne pathogens), antiviral therapy is an important countermeasure to prevent, treat, and block viral transmission.\(^3\) Because new drugs usually take more than 10 years to develop, drug repurposing has been actively pursued as a rapid approach for emerging and reemerging pathogens. Compared with conventional screening of compound libraries for inhibitors, drug repurposing has the advantages of speed and low cost. In response to the recent emergence of ZIKV, several groups have performed drug-repurposing screening.\(^4,5\) These screenings have identified active compounds against ZIKV. However, those inhibitors have low potency in cell culture, and their in vivo efficacy has not been reported. Taking the repurposing approach, Sariyer et al.\(^2\) have now identified an HIV drug, rilpivirine, as an anti-ZIKV inhibitor in both cell cultures and mouse models by blocking the viral RdRp (Figure 1).

First, Sariyer et al.\(^2\) found that human primary astrocytes are more susceptible to ZIKV infection than several other human cell types, including fetal microglia, fetal neuron, and neural progenitor cells. Using ZIKV infection of astrocytes as a screening assay, they identified rilpivirine as a ZIKV inhibitor from eight FDA-approved HIV nucleoside and non-nucleoside reverse transcriptase drugs. Rilpivirine is a non-nucleoside inhibitor and suppressed ZIKV RNA replication by \(\sim 70\% \) at 5 \(\mu M\) concentration. The higher susceptibility of astrocytes than other cell types to ZIKV infection is intriguing because previous studies suggested that ZIKV preferentially infected human neural progenitor cells.\(^6,\) Since ZIKV was proposed as a potential oncolytic virotherapy for glioblastoma,\(^7,9\) understanding the susceptible cell types in human brains is essential to gauge potential adverse effect caused by non-specific infection and killing of non-tumor cells. It remains to be tested if rilpivirine also inhibits closely related flaviviruses, such as dengue, yellow fever, West Nile, and Japanese encephalitis viruses. Nevertheless, the authors have established the antiviral activity of rilpivirine against ZIKV in cell culture.

Second, Sariyer et al.\(^2\) provided five lines of evidence to support that rilpivirine targets ZIKV RdRp (Figure 1A). (1) Computational docking suggests that rilpivirine may bind to the palm site of RdRp protein. (2) Differential scanning fluorimetry showed that rilpivirine binds to recombinant RdRp with a \(K_D\) of 100 nM. (3) The compound inhibited RdRp activity in a biochemical polymerase assay with a single digit micromolar half maximal inhibitory concentration (IC\(_{50}\)), which agrees with the antiviral potency in cell cultures. (4) A mutant RdRp protein containing 14 amino acid substitutions at the compound-binding site (suggested by computational modeling) was not inhibited or bound by rilpivirine. (5) Overexpression of wild-type RdRp protein in ZIKV-infected cells, but not the 14-amino acid mutant protein, competed for rilpivirine binding and thus alleviated compound-mediated antiviral activity. Although these results have established the mode-of-action of rilpivirine, two future directions may be pursued to further strengthen the antiviral mechanism. (1) Solving the crystal structure of rilpivirine-RdRp complex (through compound soaking or co-crystallization) will uncover the exact compound binding pocket and enable the rational design of analogs with improved potency. (2) The selection of ZIKV variants resistant to rilpivirine will identify mutations that are likely mapped to the viral NS5 RdRp gene.

Third, Sariyer et al.\(^2\) demonstrated the in vivo efficacy of rilpivirine in a ZIKV interferon \(\alpha/\beta\) receptor (IFNAR)\(^+/−\) mouse (type I interferon receptor knockout) model (Figure 1B). Treatment of ZIKV-infected IFNAR\(^+/−\) mice with rilpivirine reduced organ viral burden and weight loss, improved clinical score, and prevented death. In the un-treated mouse brain, viral RNA was detected in astrocytes, microglia/macrophage, T cells, and neurons, but not neural progenitor cells. In addition, ZIKV infection caused significant inflammation and abundant apoptotic/necrotic cell damage. Treatment

\(^1\)Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX 77555, USA.

**Correspondence:** Pei-Yong Shi, Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, Texas TX 77555, USA.

E-mail: peshi@utmb.edu

---

Xuping Xie\(^1\) and Pei-Yong Shi\(^1\)

https://doi.org/10.1016/j.ymthe.2019.10.004
with rilpivirine reduced the levels of viral RNA in the brain hippocampus and frontal cortex and prevented apoptotic/necrotic cell damage, but it did not eliminate inflammation. These results indicate that rilpivirine suppressed viral replication and disease development. However, it was notable that compound treatment did not completely prevent weight loss or brain inflammation, suggesting that further improvement of potency may be required for better in vivo efficacy. Nevertheless, the in vivo potency of rilpivirine seems better than sofosbuvir, a hepatitis C virus (HCV) nucleoside drug that was reported with anti-ZIKV activity. Specifically, rilpivirine completely protected ZIKV-infected mice from death, whereas sofosbuvir and other nucleoside inhibitors only conferred partial protection.

The study by Sariyer et al.7 has provided a good example of repurposing clinical drugs for potential treatment of ZIKV infection. For any repurposed drug to work, the compound exposure level must be greater than the efficacious concentration for the repurposed indication. Since the human exposure levels of approved drugs are usually known, a compound with the human exposure level above the EC90 value (a drug concentration required to inhibit 90% of viral replication) may be readily advanced to clinical trials for the new indication.11 As rilpivirine inhibits HIV-1 and ZIKV with EC90 in the single-digit nanomolar and micromolar range, respectively, its potency against ZIKV needs to be improved to ensure clinical efficacy in patients. To achieve this goal, rilpivirine analogs previously synthesized during lead optimization could be tested against ZIKV. Because HIV-1 and ZIKV may have different structure-and-activity (SAR) relationships, compounds with better potency for ZIKV may be identified from the rilpivirine analogs. Meanwhile, efforts could be made to solve the co-crystal structure of compound-RdRp complex. The structural information could be used for rational design, as previously shown for dengue RdRp inhibitors.13 In addition, compounds with improved potency could also be tested in a pregnant mouse model for prevention of maternal-to-fetal viral transmission.14 Collectively, the study by Sariyer et al.7 reinforces the potential of drug repurposing for therapeutic development against emerging and re-emerging pathogens.

REFERENCES
1. Aliota, M.T., Bassit, L., Bradrick, S.S., Cox, B., Garcia-Blanco, M.A., Gavegnano, C., Friedrich, T.C., Golos, T.G., Griffin, D.E., Haddow, A.D., et al. (2017). Zika in the Americas, year 2: What have we learned? What gaps remain? A report from the Global Virus Network. Antiviral Res. 144, 223–246.
2. Sariyer, I.K., Gordon, J., Burdo, T.H., Wolleho, H., Gianti, E., Donadoni, M., Bellizzi, A., Cicalese, S., Loomis, R., Robinson, J.A., et al. (2019). Suppression of Zika virus infection in the brain by the antiretroviral drug rilpivirine. Mol. Ther. 27, this issue, 2067–2079.
3. Shan, C., Xie, X., Barretti, A.D., Garcia-Blanco, M.A., Tesh, R.R., Vasconcelos, P.F., Vazalaks, N., Weaver, S.C., and Shi, P.Y. (2016). Zika Virus: Diagnosis, Therapeutics, and Vaccine. ACS Infect. Dis. 2, 170–172.
4. Barrows, N.L, Campos, R.K., Powell, S.T., Prasanth, K.R., Schott-Lerner, G., Soto-Acosta, R., Galarza-Muñoz, G., McGrath, E.L., Urbazab-Garza, R., Gao, J., et al. (2016). A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection. Cell Host Microbe 20, 259–270.
5. Xu, M., Lee, E.M., Wen, Z., Cheng, Y., Huang, W.K., Qian, X., Tcw, J., Koznetzova, J., Ogden, S.C., Hammack, C., et al. (2016). Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. Nat. Med. 22, 1101–1107.
6. Tang, H., Hammack, C., Ogden, S.C., Wen, Z., Qian, X., Li, Y., Yao, B., Shin, J., Zhang, F., Lee, E.M., et al. (2016). Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. Cell Stem Cell 18, 587–590.
7. Zhu, Z., Gorman, M.I., McKenzie, L.D., Chai, J.N., Hubert, C.G., Prager, B.C., Fernandez, E., Richner, J.M., Zhang, R., Shan, C., et al. (2017). Zika virus has oncolytic activity against glioblastoma stem cells. J. Exp. Med. 214, 2843–2857.
8. Chen, Q., Wu, J., Ye, Q., Ma, F., Zhu, Q., Wu, Y., Shan, C., Xie, X., Li, D., Zhan, X., et al. (2018). Treatment of Human Glioblastoma with a Live Attenuated Zika Virus Vaccine Candidate. MBio 9, e01683-18.
9. Ferreira, A.C., Zavarcha-du-Valle, C., Reis, P.A., Barbosa-Lima, G., Vieira, Y.R., Mattos, M., Silva, P.P., Sacramento, C., de Castro Faria Neto, H.C., Campanati, L., et al. (2017). Sofosbuvir protects Zika virus-infected mice from mortality, preventing short- and long-term sequelae. Sci. Rep. 7, 9409.
10. Deng, Y.Q., Zhang, N.N., Li, C.F., Tian, M., Hao, J.N., Xie, X.P., Shi, P.Y., and Qin, C.F. (2016). Adenosine Analog NTID008 Is a Potent Inhibitor of Zika Virus. Open Forum Infect. Dis. 3, 06175.
11. Zou, J., and Shi, P.Y. (2019). Strategies for Zika drug discovery. Curr. Opin. Virol. 35, 19–26.

12. Azijn, H., Tirry, I., Vingerhoets, J., de Béthune, M.P., Kraus, G., Boven, K., Jochmans, D., Van Craenenbroeck, E., Picchio, G., and Rimsky, L.T. (2010). TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. Antimicrob. Agents Chemother. 54, 718–727.

13. Noble, C.G., Lim, S.P., Arora, R., Yokokawa, F., Nilar, S., Seh, C.C., Wright, S.K., Benson, T.E., Smith, P.W., and Shi, P.Y. (2016). A Conserved Pocket in the Dengue Virus Polymerase Identified through Fragment-based Screening. J. Biol. Chem. 291, 8541–8548.

14. Richner, J.M., Jagger, B.W., Shan, C., Fontes, C.R., Dowd, K.A., Cao, B., Himansu, S., Caine, E.A., Nunes, B.T.D., Medeiros, D.B.A., et al. (2017). Vaccine mediated protection against Zika virus induced congenital disease. Cell 170, 273–283.e12.

15. Duan, W., Song, H., Wang, H., Chai, Y., Su, C., Qi, J., Shi, Y., and Gao, G.F. (2017). The crystal structure of Zika virus NS5 reveals conserved drug targets. EMBO J. 36, 919–933.