Zika and Other Emerging Viruses: Aiming at the Right Target

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The Zika emergency calls for urgent countermeasures. Recently, Barrows et al. (2016) and Xu et al. (2016) conducted in vitro anti-ZIKV screens to identify potential therapeutics. The off-label use of drugs that may protect against Zika virus-induced brain damage has, however, to be balanced with their risk during pregnancy.

Emerging and neglected (or in the case of Zika, "forgotten") viruses claim the scene with an apparently accelerating speed; examples include (but are not limited to) the Hendra virus (1994), the West Nile virus (1999), the SARS and MERS coronaviruses (2002 and 2012), H1N1 pandemic influenza (2009), and the Ebola virus (2014). At the occasion of most outbreaks, authorities announce public health emergencies. Each time neither the scientific community nor the pharmaceutical industry is ready to conquer the problem. Outbreak after outbreak, the world is taken by surprise and left without vaccines or potent antivirals. At the time that a highly pathogenic virus (re-) emerges, years will be needed, if not a decade, before vaccines or drugs will become available, even with accelerated procedures. The recent re-emergence of the Zika virus (ZIKV) is no exception to this. This time, the pathogen hits mostly the most vulnerable, i.e., the human fetus (Lessler et al., 2016). Until safe and efficient ZIKV vaccines become available, mankind is, given the lack of specific antivirals, blunted of any countermeasures to prevent or treat infections with this virus.

In an attempt to bridge this gap, two groups recently conducted an in vitro anti-ZIKV screen using a set of ~800 FDA-approved drugs (Barrows et al., 2016) or, in addition to this collection, another ~5,600 drug-like molecules with known biological activity (Xu et al., 2016). Barrows and colleagues employed an imaging-based approach in which compound-pretreated human hepatoma cells were infected with a ZIKV isolate from the current outbreak. Infected cultures were fixed 24 hr after infection, and the fraction of cells expressing ZIKV antigens was quantified. Using this approach, 24 molecules (hit rate 6%) were reported to inhibit ZIKV replication. The antiviral activity of a smaller subset of hits was confirmed in cells derived from pathophysiologically relevant tissues, such as the CNS and amniotic tissue.

Xu et al. (2016) established their screenings assay based on the previous observation that ZIKV of the African lineage induces activation of effector caspases and subsequent cell death within 4 hr post infection of glioblastoma cells. In an orthogonal screen, 116 hits (hit rate ~2%) were shown to protect human neuronal progenitor cells against the virus. The activity of 35 of these molecules was confirmed in three different cell lines, including astrocytes. This set of 35 inhibitors was divided into (i) those that affect ZIKV replication or (ii) agents that protect infected cells from death.

Among their 24 hits, the Barrow team identified mycophenolic acid (MPA, the active component of the immunosuppressive drug Cellcept), the antiparasitic drug ivermectin (which we reported earlier to inhibit in vitro flavivirus replication; Mastrangelo et al., 2012), and mefloquine, an inhibitor of autophagy. Other molecules identified in this study were antibiotics with an unknown mechanism of action (such as daptomycin), the proteasome inhibitor bortezomib, and the antidepressant sertaline. Some of these drugs affect nucleoside metabolism (MPA depletes dGTP and GTP pools), DNA synthesis, and cellular signaling. Such molecules may serve as good chemical probes to aid in understanding the biology of ZIKV replication (Figure 1). The observed effects were already quantified 24 hr after infection, a timespan during which roughly one to two full viral replication cycles can take place. Typically longer assays allow for a more stringent measure of potential antiviral activity and selectivity. For almost all molecules, the in vitro antiviral activity was accompanied with a marked cell loss (Barrows et al., 2016), pointing toward rather non-selective antiviral effects. Molecules that exhibited the most pronounced activity in these assays, such as MPA and bortezomid, belong to pregnancy category D drugs that are strictly contraindicated. Furthermore, it is also reasonable to assume that treatment with the immunosuppressive drug MPA may prolong, rather than shorten, virus persistence (Driggers et al., 2016). Moreover, depending on the concentration that was used, a compound such as the antibiotic daptomycin also resulted in an unwanted, proviral effect.

Xu et al. (2016) identified, possibly biased by their initial caspase reporter readout, the pan caspase-inhibitor emricasan as an agent that protects against ZIKV-induced apoptosis. Whereas emricasan was found not to interfere with viral replication, several inhibitors of cyclin-dependent kinases (CDKi) suppressed the expression of ZIKV proteins and viral progeny production (Figure 1). The most pronounced effect was observed with seliciclib, a known inducer of apoptosis. Another antihelmintic drug, namely niclosamide, was shown to inhibit ZIKV infection. Auspiciously, the combination of emricasan (protecting against cell death) and niclosamide (inhibiting viral replication) or a CDKi reduced both the number of infected cells and the number of cells undergoing concomitant cell death. Among these, at least niclosamide is not precluded during pregnancy.

Intriguingly, but also of concern, is that both screens, though using the same library of FDA-approved drugs, do not

report a single overlap of hits. This may possibly, to some extent, be explained by the use of different cell types and virus strains. However, molecules to be used in humans for the prevention or treatment of aggressive viral infection should exert their activity in as many cell lines/types and against as many viral isolates as possible. It should also be mentioned that the nucleoside hepatitis C virus (HCV) polymerase inhibitor sofosbuvir, which was recently reported to inhibit the in vitro replication of the ZIKV (Sacramento et al., 2016), was not identified in these screens.

Despite the proof of concept reported in these two studies, we believe that one should not engage in clinical trials for off-label use of either of the FDA-approved drugs with antiviral or neuroprotective potency for the treatment and/or prevention of ZIKV-induced disease in pregnant women. Considering that the pathogenesis of the ZIKV (a virus with a genome of only ~10,000 nt) still largely remains an unresolved enigma, how much deeper are the mysteries and the vulnerability of human fetal brain development? The fact that the best inhibitors identified in both screens target major pathways of cell homeostasis (apoptosis, cyclin-dependent kinases, proteasome) that are directly involved in (brain) development calls for extreme prudence.

The ZIKV belongs (within the family of the Flaviviridae) to the genus flavivirus, which includes well-known pathogens such as those causing dengue, yellow fever, West Nile, and Japanese encephalitis. There are many other flaviviruses that are pathogenic to man and, akin to the ZIKV, new flaviviruses may emerge in the future. There are no approved antivirals against infections with flaviviruses, despite the fact that annually an estimated 96 million people develop dengue disease (Bhatt et al., 2013). Dengue is the second most important mosquito-borne disease after malaria. In addition to the laudable effort to explore repurposing existing drugs, we should aim to develop (dengue) drugs that are also endowed with pan-

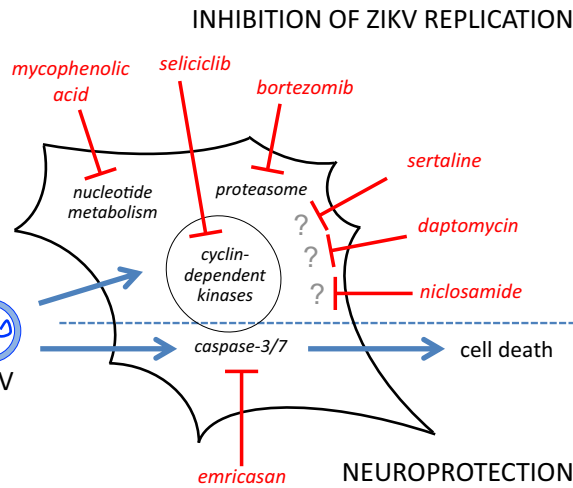
INHIBITION OF ZIKV REPLICATION

Figure 1. Repurposing of Licensed Drugs and Drug-like Small Molecules to Prevent and Treat ZIKV Infections

In two drug repurposing screens, a small selection of FDA-approved drugs and other biologically active compounds proved (i) to inhibit in vitro ZIKV replication or (ii) to protect infected cells from death. While many of these hits are strictly contraindicated during (early) gravidity, at least daptomycin and niclosamide are not precluded from use in pregnant women.

flavivirus activity. Such drug(s) could, besides being employed for the treatment and/or prophylaxis of dengue, be used for the off-label treatment of infections with other flaviviruses, i.e., those that we know today but also those that may possibly emerge in the near or more distant future. We may take the field of drug development against HCV (another member of the family of Flaviviridae) as an example. In recent years, highly potent well-tolerated combinations of all oral HCV inhibitors have been developed that allow typically >95% of patients to be cured within weeks of this chronic condition. This demonstrates the power of potent directly acting antivirals. The HCV drugs target unique viral proteins such as the viral RNA-dependent RNA polymerase, the viral NS3 protease, and the NS5A protein. Likewise, other flaviviruses encode proteins that are more or less conserved within the genus and that are perfectly druggable (Kok, 2016; Lim et al., 2013). Given sufficient efforts and investments, it should thus be equally possible to develop highly potent and pan-flavivirus inhibitors that would also offer a tool to control ZIKV and other neglected and emerging flaviviruses.

Likewise, there are several virus families (with an RNA genome) to which either highly pathogenic viruses or viruses that cause highly debilitating con-

ditions belong. For most of them, there are no available vaccines to prevent, nor drugs to treat, these infections. These include, for example, corona- (e.g., SARS and MERS), filo- (Ebola and Marburg), alpha- (Chikungunya, VEEV), paramyxo- (Nipah, Hendra), entero- (EV68, EV71), bunya- (Crimean Congo hemorrhagic fever, Rift Valley fever, hanta), and arenaviruses (Lassa). As it stands, the world is not prepared to prevent or combat the next emerging virus. To quote Dr. M. Chan, WHO director-general at the 66th WHO Health Assembly, "Any new disease that is emerging faster than our understanding is never under control," (World Health Organization, 2013). The development of pan-genus or pan-family antivirals will help us to prepare for the control of novel emerging viral diseases (Debing et al., 2015). Each of the virus families listed above encodes unique proteins that can be targeted by small molecule inhibitors. Mankind can be prepared for the expected unexpected, but we should start right now with such efforts and aim for the right viral-specific target.

REFERENCES

Barrows, N.J., Campos, R.K., Powell, S.T., Prasanth, K.R., Schott-Lerner, G., Soto-Acosta, R., Galarza-Muñoz, G., McGrath, E.L., Urrabaz-Garza, R., Gao, J., et al. (2016). Cell Host Microbe 20, 259–270.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., et al. (2013). Nature 496, 504–507.

Debing, Y., Neyts, J., and Delang, L. (2015). Curr. Opin. Infect. Dis. 28, 596–602.

Driggers, R.W., Ho, C.-Y., Korhonen, E.M., Kuivanen, S., Jääskeläinen, A.J., Smura, T., Rosenberg, A., Hill, D.A., DeBiasi, R.L., Vezina, G., et al. (2016). N. Engl. J. Med. 374, 2142–2151.

Kok, W.M. (2016). Expert Opin. Drug Discov. 11, 433–445.

Lessler, J., Chaisson, L.H., Kucirka, L.M., Bi, Q., Grantz, K., Salje, H., Carcelen, A.C., Ott, C.T., Sheffield, J.S., Ferguson, N.M., et al. (2016). Science *353*, aaf8160.

Lim, S.P., Wang, Q.-Y., Noble, C.G., Chen, Y.-L., Dong, H., Zou, B., Yokokawa, F., Nilar, S., Smith, P., Beer, D., et al. (2013). Antiviral Res. *100*, 500–519.

Mastrangelo, E., Pezzullo, M., De Burghgraeve, T., Kaptein, S., Pastorino, B., Dallmeier, K., de Lamball-erie, X., Neyts, J., Hanson, A.M., Frick, D.N., et al. (2012). J. Antimicrob. Chemother. *67*, 1884–1894.

Sacramento, C.Q., de Melo, G.R., Rocha, N., Hoelz, L.V.B., Mesquita, M., de Freitas, C.S., Fintelman-Rodrigues, N., Marttorelli, A., Ferreira, A.C., Barbosa-Lima, G., et al. (2016). bioRxiv. http://dx.doi.org/10.1101/061671.

World Health Organization. (2013). Sixty-sixth World Health Assembly closes with concern over new global health threat. World Health Assembly, http://www.who.int/mediacentre/news/releases/2013/world_health_assembly_20130527/en/.

Xu, M., Lee, E.M., Wen, Z., Cheng, Y., Huang, W.-K., Qian, X., Tcw, J., Kouznetsova, J., Ogden, S.C., Hammack, C., et al. (2016). Nat. Med., Published online August 29, 2016. http://dx.doi.org/10.1038/nm.4184.