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Targeting the Achilles Heel of Mosquito-Borne Viruses for Antiviral Therapy

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ABSTRACT: Mosquito-borne viruses encompass a wide range of pathogens, such as dengue and Zika viruses, that often cocirculate geographically. These viruses affect hundreds of millions of people worldwide, yet no clinically approved therapy is currently available for treating these viral infections. Thus, innovative therapies, especially inhibitors with broad antiviral activities against all these viruses, are urgently needed. While traditional therapeutic strategies mainly focus on inhibiting viral replication in a "one lock, one key" manner (e.g., viral protease and polymerase inhibitors), inhibitors targeting virions have recently emerged as a promising approach to achieve broad antiviral activities. Within this approach, Lipid Envelope Antiviral Disruption (LEAD) molecules were shown to broadly inhibit mosquito-borne viruses and other lipid membrane-enveloped viruses. Several LEAD molecules have been demonstrated to act against viral membranes *in vitro*, some of which have even shown *in vivo* efficacy to treat mosquito-borne viral infections. This therapeutic potential is further enhanced by molecular engineering to improve the inhibitors' pharmacological properties, laying the foundation for the LEAD antiviral strategy to be explored for possible treatment of mosquito-borne viral infections.

Mosquito-borne viral infections, such as dengue, Zika, yellow fever, Japanese encephalitis, West Nile, and chikungunya viruses, are major global health challenges.[1] They affect hundreds of millions of people worldwide and are increasingly prevalent due to the geographical expansion of mosquito vectors.[2] Currently, there are no approved therapies to treat mosquito-borne viral infections. Although a few clinical compounds (that were previously developed for other indications) were repurposed and tested in human clinical trials,[3] none showed efficacy in infected patients.[4] As a result, today's standard treatment remains supportive care and is focused on reducing symptoms as opposed to directly eliminating the virus and blocking disease development. Thus, new antiviral strategies are urgently needed to meet this huge unmet medical need.

THERAPEUTIC CHALLENGES

The development of antiviral therapies for treating mosquito-borne viral infections encounters several challenges. First, most of these infections are acute, leading to a short time window for therapy.[5,6] The majority of infected individuals are asymptomatic. For symptomatic patients, at the onset of clinical symptoms, their viremia levels have already peaked and started to decline.[7] Since viremia usually diminishes to undetectable levels within 10 days after the onset of symptoms, any delay in treatment will decrease the antiviral effect in patients. Second, many mosquito-borne viruses often cocirculate in the same geographical regions, and the viral infections present similar clinical symptoms in patients.[8] If a drug is virus-type specific and does not inhibit other mosquito-borne viruses, a diagnostic test should be performed before the treatment could start. The time required for diagnosis would delay the treatment and diminish the time window of therapy. Thus, a therapeutic strategy that could be used to develop an inhibitor against all mosquito-borne viruses would be advantageous, both in terms of overcoming challenges associated with differential diagnosis and the versatility of using a single drug for treating multiple, currently intractable viruses. Third, since many mosquito-borne viruses are neurotrophic and often cause neurological disorders, it is ideal if an inhibitor can cross the blood-brain barrier (BBB) and efficiently suppress viral replication in the brain. These neurological manifestations were highlighted by the public health crisis precipitated by West Nile virus and more recently Zika virus-mediated congenital diseases in babies born to infected mothers during pregancy.[9,10] The Zika infection in pregnant women was associated with a wide range of congenital deformations, including microcephaly, in newborn infants.[11] Additional neurological manifestations have been reported in children and adults for other mosquito-borne viral infections.[12,13] Altogether, the above challenges highlight the importance of developing antiviral drugs that (i) can inhibit all cocirculating, mosquito-borne viruses and (ii) can also cross BBB to inhibit viral infection in the brain.

ACHILLES HEEL OF MOSQUITO-BORNE VIRUSES

It has long been known that mosquito-borne viruses possess a lipid envelope coating, which is essential for the structural integrity of individual virus particles (i.e., virions).[14] This common structural feature is intriguing because the viral lipid envelope coating is derived from cellular membranes. Hence,

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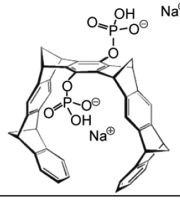
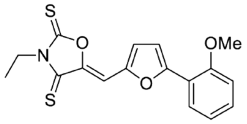
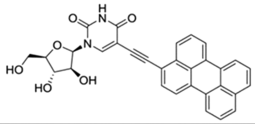
Table 1. Comparison of Exemplary Antiviral Agents That Affect the Lipid Membrane Properties of Enveloped Viruses[a]

Drug Class	Molecular Examples	Proposed Mechanism	Efficacy Range	Animal Model Testing
Molecular Tweezers	CLR01 	-Disrupts lipid raft-enriched lipid membranes *via* changes in membrane ordering. -Selectivity arises from lipid composition.	5 μM to 50 μM	-Safely administered in uninfected mice with high tolerability. -Crossed blood-brain barrier and can remain in brain tissue for up to 72 hrs.
Photosensitizers	JL122 	-Oxidizes unsaturated phospholipids to change membrane fluidity. -Selectivity arises from membrane repair capacity of mammalian cell membranes.	2 nM to 500 nM	-Virus pretreatment prevented Rift Valley fever virus-induced mortality in mice. -Delayed death with treatment starting 1 hr post-exposure.
Rigid Amphipathic Fusion Inhibitors	aUY11 	-Prevents virus-host cell fusion by positive curvature effects. -Can also oxidize unsaturated phospholipids to change membrane fluidity.	2 nM to 500 nM	Not reported.
Amphipathic Peptides	C5A (18-mer) SWLRDIWDWICEVLSDFK	-Permeabilizes lipid membranes with high partition coefficient. -Destabilizes lipid membranes independent of membrane curvature.	500 nM to 5 μM	-Topical administration protected mice from vaginal HIV transmission. -Vaginal application protected non-human primates from simian-HIV transmission.
	AH (27-mer) SGSWLRDVWDWICTVLT DFKTWLQSKL	-Causes membrane lysis after a critical density of pores is formed. -Selectively forms pores in highly curved membranes.	10 nM to 1 μM	-Protected against ZIKV-induced mortality in mice with treatment starting 3 days post-infection. -Crossed BBB to inhibit ZIKV infection in brain.

[a]ZIKV: Zika virus. Molecular structures are not drawn to scale.

the lipid envelope may represent an Achilles heel of mosquito-borne viruses if inhibitors could be designed to selectively destroy viral membranes without affecting cellular membranes. Such viral membrane disruptors are expected to have high barriers for the emergence of drug resistance. Toward this goal, a number of groups have shown that destabilizing the lipid envelope coating reduces viral infectivity *in vitro*. Inhibition can arise from disruption of virion morphology or from more subtle changes in viral membrane fluidity that hinder virus-host cell fusion.[15,16] While a wide range of membrane-disruptive compounds can interact with lipid envelopes and abrogate viral infectivity based on membrane destabilization, it has proven challenging to identify drug candidates that impair viral membranes while leaving cellular membranes unharmed.

INHIBITORS OF VIRION MEMBRANE

In recent years, several promising classes of inhibitors have been identified that target viral membranes by exploiting the differences between virus particles and mammalian cells, such as membrane repair capacity and geometrical dimensions (Table 1). In the course of studying the CLR01 molecule (that was originally designed as a "molecular tweezer" to bind lysine and arginine residues to block HIV-amyloid complex formation), it was found that micromolar concentrations of CLR01 also destabilize the lipid envelope surrounding HIV, hepatitis C, and herpes simplex virus particles.[17] It was later shown that CLR01 is active against Zika and Ebola viruses as well.[18] Notably, CLR01 exhibits inhibitory activity in human seminal fluid (as it was originally aimed at inhibiting HIV transmission in semen) but not in serum and displays low toxicity *in vitro* and *in vivo*.[19] These findings have led it to be considered as a topical microbicide candidate. The antiviral selectivity of CLR01 is speculated to arise from the compositional differences between host cell membranes and virion membranes (the latter often has a greater predominance of raft-like components due to the virus budding site[20]) as well as the membrane repair capacity of mammalian cells but not viruses.

Other classes of small molecules have been discovered that work as photosensitizing agents and cause oxidation of unsaturated phospholipids in viral membranes via light-dependent generation of singlet oxygen species.[16] Such alterations can decrease membrane fluidity and affect membrane ordering to hinder cell-virus fusion. For example, LJ001 is an aryl methyldiene rhodanine derivative that exhibits potent antiviral activity against Rift Valley fever, yellow fever, and West Nile viruses at a nanomolar concentration range.[21] Biophysical experiments indicated that LJ001 treatment affects membrane organization without causing permeation.[21,22] To enable *in vivo* applications, improved versions of LJ001 were designed that had greater potency, red-shifted absorption spectra, increased quantum yields, and higher bioavailability.[23] Such lead compounds, including JL122 (Table 1), were able to delay death in a lethal mouse model of Rift Valley fever virus infection.

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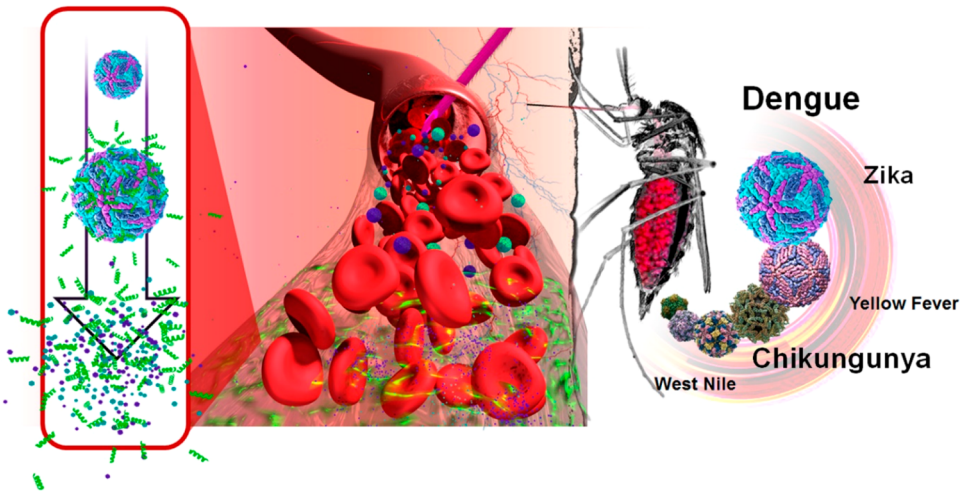

Figure 1. LEAD antiviral strategy to treat mosquito-borne viral infections. LEAD drug candidates disrupt the lipid envelope surrounding mosquito-borne virions, abrogate viral infectivity, and reduce viral load. A LEAD drug candidate, termed AH peptide, is indicated as green helices in the left panel. Blood vessels and cells are shown in the middle panel. A mosquito and a number of mosquito-borne enveloped viruses are presented in the right panel. This therapeutic effect can potentially improve disease outcomes by lowering the viral burden, as evidenced by the Zika virus case. In principle, the strategy works similarly to other types of entry inhibitors (e.g., envelope-protein-targeting antibodies), but the mechanism of action afforded by the LEAD strategy enables a single drug candidate to potentially target a wide range of enveloped viruses. In addition, the molecular properties of certain LEAD drug candidates allow them to access immune-privileged sites such as the brain.

Rigid amphipathic fusion inhibitors (RAFIs) are another class of small molecules that act against enveloped viruses and are believed to target viral membranes by inhibiting the formation of negative membrane curvature required for host cell fusion along with causing light-induced lipid oxidation.[24,25] As exemplified by aUY11 (Table 1), RAFIs exhibit nanomolar antiviral activity *in vitro*. Structure−activity relationship studies have revealed that different parts of the RAFI molecular structure are responsible for antiviral and cytotoxic activities.[26] Hence, it is possible to design RAFIs with potent antiviral activity and low cytotoxicity, although available studies are limited to *in vitro* testing.

Aside from small molecule-based antivirals, peptide-based antivirals, such as the 18-mer C5A peptide (derived from the N-terminal region of hepatitis C virus NS5A protein), have been reported to inhibit a wide range of enveloped viruses *in vitro* (Table 1).[27−29] The C5A is a promising antiviral peptide that has been tested as a topical microbicide to prevent vaginal HIV transmission *ex vivo* in humanized mice[30] and in nonhuman primates.[31]

Until recently, most efforts were focused on progressing membrane-disruptive compounds toward topical microbicide applications due to the challenges of translating their *in vitro* antiviral activities to *in vivo* animal models. To overcome this challenge, we developed a new therapeutic strategy called Lipid Envelope Antiviral Disruption (LEAD) to thwart mosquito-borne viral infections (Figure 1). The rationale of the LEAD approach is that therapeutic reduction of the concentration of infectious virions can mitigate viral spread and ameliorate disease symptoms, and the challenge lies in identifying drug candidates that can accomplish this task *in vivo*. As Zika virus infection is associated with viral replication in the brain, we focused on engineering an amphipathic, α-helical (AH) peptide that works selectively against virion membranes and can cross the BBB when administered systemically (Table 1). The AH peptide is derived from the first 27 amino acids of hepatitis C virus NS5A protein and shares a common region of amino acid sequence with the C5A peptide described above (Table 1). Remarkably, treatment of Zika virus-infected mice with the AH peptide prevented morbidity and mortality even when the therapy started on day 3 postinfection.[32] Biophysical evidence showed that the AH peptide can form pores in highly curved membranes of virion dimensions; in contrast, the AH peptide has negligible effect on much larger, and hence lower curvature, mammalian cell membranes.[33,34] This discrimination supports a high degree of selectivity for targeting a common membrane structural element shared by all mosquito-borne enveloped viruses, including dengue, Zika, yellow fever, West Nile, and chikungunya viruses.[32]

In order to improve the pharmacological properties of AH peptide, conventional L-amino acids were replaced by D-amino acids that are more resistant to proteolytic degradation.[35] This design choice enabled an improved pharmacokinetic profile, with a relatively long circulation half-time, high bioavailability, and BBB-crossing activity to achieve therapeutic concentrations in the brain.[32] Surprisingly, even though the lipid membrane is an achiral target, the D-amino acid version of AH peptide exhibited greater rupture potency and distinct membrane-curvature-sensing activity against model liposomes, as compared to the L-amino acid version.[32]

In addition to controlling systemic infection, the engineered AH peptide is able to cross the BBB to reduce viral loads in the brain and protect against Zika virus-induced brain injury.[32] The ability to inhibit viral infection in the brain is particularly important because the BBB can remain intact after neuroinvasion, and other classes of therapeutic molecules such as antibodies typically cannot cross the intact BBB.[36] These findings indicate that the AH peptide can potentially be used to treat neurotropic infections through both systemic and neurological routes. The AH peptide should be tested against other mosquito-borne enveloped viruses in animal models. Taken together, the aforementioned progress highlights the molecular engineering of LEAD drug candidates as an exciting frontier at the convergence of infectious diseases, biochemistry, biophysics, and engineering.

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CONCLUSIONS AND OUTLOOK

The LEAD strategy provides a conceptual framework to develop antiviral drugs that selectively target virion membranes (an "Achilles heel") and inhibit mosquito-borne enveloped viruses. In recent years, there has been significant progress in identifying LEAD drug candidates that inhibit multiple mosquito-borne enveloped viruses *in vitro*. Aided by molecular engineering, further testing in animal models has validated the treatment potential of several drug candidates, including preventing vaginal transmission of sexually transmitted viruses and protecting against lethal infections caused by mosquito-borne viruses. In addition to evaluating treatment outcomes, recent progress with the engineered AH peptide has offered evidence that the LEAD strategy can reduce viral loads and inflammation levels *in vivo*. With numerous application possibilities, it will be important to identify which LEAD drug candidates work optimally against particular mosquito-borne viral infections. While mosquito-borne enveloped viruses have similar virion structures, disease progression can vary according to the virus type, including the extent of viral burden in blood and in different tissues. It will be important to continue characterizing the pharmacokinetic properties of LEAD drug candidates in order to understand how long they stay in the body as well as where they go in the body and in what concentrations. The demonstrated ability of certain LEAD drug candidates to cross the BBB is particularly significant and could help to address the longstanding challenge of treating viral infections in the brain. Another important goal will be to evaluate the toxicokinetic properties of LEAD drug candidates in animal models. Ultimately, further evaluation of dose-dependent effects in animal models is warranted for assessing treatment efficacy and safety as part of the next stage of preclinical testing. With the potential of a single drug candidate working against multiple mosquito-borne viral infections, there is strong merit to further consider the LEAD strategy. These efforts could lead to the development of antiviral inhibitors that work against cocirculating, mosquito-borne viruses.

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Notes

The authors declare the following competing financial interest(s): J.A.J. and N.-J.C. are named coinventors on patent applications related to antiviral peptide technologies.

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REFERENCES

(1) Weaver, S. C., and Reisen, W. K. (2010) Present and future arboviral threats. *Antiviral Res. 85*, 328−345.
(2) Weaver, S. C. (2013) Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. *Trends Microbiol. 21*, 360−363.
(3) Lim, S. P., Wang, Q.-Y., Noble, C. G., Chen, Y.-L., Dong, H., Zou, B., Yokokawa, F., Nilar, S., Smith, P., and Beer, D. (2013) Ten years of dengue drug discovery: progress and prospects. *Antiviral Res. 100*, 500−519.
(4) Xie, X., Zou, J., Shan, C., and Shi, P.-Y. (2017) Small molecules and antibodies for Zika therapy. *J. Infect. Dis. 216*, S945−S950.
(5) Simmons, C. P., Wolbers, M., Nguyen, M. N., Whitehorn, J., Shi, P. Y., Young, P., Petric, R., Farrar, J., and Wills, B. (2012) Therapeutics for dengue: recommendations for design and conduct of early-phase clinical trials. *PLoS Neglected Trop. Dis. 6*, e1752.
(6) Boldescu, V., Behnam, M. A., Vasilakis, N., and Klein, C. D. (2017) Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond. *Nat. Rev. Drug Discovery 16*, 565−586.
(7) Vaughn, D. W., Green, S., Kalayanarooj, S., Innis, B. L., Nimmannitya, S., Suntayakorn, S., Endy, T. P., Raengsakulrach, B., Rothman, A. L., and Ennis, F. A. (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J. Infect. Dis. 181*, 2−9.
(8) Carrillo-Hernández, M. Y., Ruiz-Saenz, J., Villamizar, L. J., Gómez-Rangel, S. Y., and Martínez-Gutierrez, M. (2018) Co-circulation and simultaneous co-infection of dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border. *BMC Infect. Dis. 18*, 61.
(9) Shan, C., Xie, X., Barrett, A. D., Garcia-Blanco, M. A., Tesh, R. B., Vasconcelos, P. F. d. C., Vasilakis, N., Weaver, S. C., and Shi, P.-Y. (2016) Zika virus: diagnosis, therapeutics, and vaccine. *ACS Infect. Dis. 2*, 170−172.
(10) Brasil, P., Pereira, J. P., Jr, Moreira, M. E., Ribeiro Nogueira, R. M., Damasceno, L., Wakimoto, M., Rabello, R. S., Valderramos, S. G., Halai, U.-A., and Salles, T. S. (2016) Zika virus infection in pregnant women in Rio de Janeiro. *N. Engl. J. Med. 375*, 2321−2334.
(11) Mlakar, J., Korva, M., Tul, N., Popović, M., Poljšak-Prijatelj, M., Mraz, J., Kolenc, M., Resman Rus, K., Vesnaver Vipotnik, T., and Fabjan Vodušek, V. (2016) Zika virus associated with microcephaly. *N. Engl. J. Med. 374*, 951−958.
(12) Burt, F. J., Rolph, M. S., Rulli, N. E., Mahalingam, S., and Heise, M. T. (2012) Chikungunya: a re-emerging virus. *Lancet 379*, 662−671.
(13) Carod-Artal, F. J., Wichmann, O., Farrar, J., and Gascón, J. (2013) Neurological complications of dengue virus infection. *Lancet Neurol. 12*, 906−919.
(14) Welsch, S., Müller, B., and Kräusslich, H.-G. (2007) More than one door−budding of enveloped viruses through cellular membranes. *FEBS Lett. 581*, 2089−2097.
(15) Badani, H., Garry, R. F., and Wimley, W. C. (2014) Peptide entry inhibitors of enveloped viruses: the importance of interfacial hydrophobicity. *Biochim. Biophys. Acta, Biomembr. 1838* (9), 2180−2197.
(16) Vigant, F., Santos, N. C., and Lee, B. (2015) Broad-spectrum antivirals against viral fusion. *Nat. Rev. Microbiol. 13*, 426−437.
(17) Lump, E., Castellano, L. M., Meier, C., Seeliger, J., Erwin, N., Sperlich, B., Stürzel, C. M., Usmani, S., Hammond, R. M., and von Einem, J. (2015) A molecular tweezer antagonizes seminal amyloids and HIV infection. *eLife 4*, e05397.
(18) Röcker, A. E., Müller, J. A., Dietzel, E., Harms, M., Krüger, F., Heid, C., Sowislok, A., Riber, C. F., Kupke, A., and Lippold, S. (2018) The molecular tweezer CLR01 inhibits Ebola and Zika virus infection. *Antiviral Res. 152*, 26−35.
(19) Attar, A., Chan, W.-T. C., Klärner, F.-G., Schrader, T., and Bitan, G. (2014) Safety and pharmacological characterization of the molecular tweezer CLR01−a broad-spectrum inhibitor of amyloid proteins' toxicity. *BMC Pharmacol. Toxicol. 15*, 23.

DOI: 10.1021/acsinfecdis.8b00286
ACS Infect. Dis. 2019, 5, 4−8

(20) Suomalainen, M. (2002) Lipid rafts and assembly of enveloped viruses. *Traffic 3*, 705−709.
(21) Wolf, M. C., Freiberg, A. N., Zhang, T., Akyol-Ataman, Z., Grock, A., Hong, P. W., Li, J., Watson, N. F., Fang, A. Q., and Aguilar, H. C. (2010) A broad-spectrum antiviral targeting entry of enveloped viruses. *Proc. Natl. Acad. Sci. U. S. A. 107*, 3157−3162.
(22) Hollmann, A., Gonçalves, S., Augusto, M. T., Castanho, M. A., Lee, B., and Santos, N. C. (2015) Effects of singlet oxygen generated by a broad-spectrum viral fusion inhibitor on membrane nanoarchitecture. *Nanomedicine 11*, 1163−1167.
(23) Vigant, F., Lee, J., Hollmann, A., Tanner, L. B., Ataman, Z. A., Yun, T., Shui, G., Aguilar, H. C., Zhang, D., and Meriwether, D. (2013) A mechanistic paradigm for broad-spectrum antivirals that target virus-cell fusion. *PLoS Pathog. 9*, e1003297.
(24) St. Vincent, M. R., Colpitts, C. C., Ustinov, A. V., Muqadas, M., Joyce, M. A., Barsby, N. L., Epand, R. F., Epand, R. M., Khramyshev, S. A., and Valueva, O. A. (2010) Rigid amphipathic fusion inhibitors, small molecule antiviral compounds against enveloped viruses. *Proc. Natl. Acad. Sci. U. S. A. 107*, 17339−17344.
(25) Vigant, F., Hollmann, A., Lee, J., Santos, N. C., Jung, M. E., and Lee, B. (2014) The rigid amphipathic fusion inhibitor dUY11 acts through photosensitization of viruses. *J. Virol. 88*, 1849−1853.
(26) Speerstra, S., Chistov, A. A., Proskurin, G. V., Aralov, A. V., Ulashchik, E. A., Streshnev, P. P., Shmanai, V. V., Korshun, V. A., and Schang, L. M. (2018) Antivirals acting on viral envelopes via biophysical mechanisms of action. *Antiviral Res. 149*, 164−173.
(27) Cheng, G., Montero, A., Gastaminza, P., Whitten-Bauer, C., Wieland, S. F., Isogawa, M., Fredericksen, B., Selvarajah, S., Gallay, P. A., Ghadiri, M. R., and Chisari, F. V. (2008) A virocidal amphipathic α-helical peptide that inhibits hepatitis C virus infection in vitro. *Proc. Natl. Acad. Sci. U. S. A. 105*, 3088−3093.
(28) Bobardt, M. D., Cheng, G., de Witte, L., Selvarajah, S., Chatterji, U., Sanders-Beer, B. E., Geijtenbeek, T. B., Chisari, F. V., and Gallay, P. A. (2008) Hepatitis C virus NS5A anchor peptide disrupts human immunodeficiency virus. *Proc. Natl. Acad. Sci. U. S. A. 105*, 5525−5530.
(29) De Witte, L., Bobardt, M. D., Chatterji, U., Van Loenen, F. B., Verjans, G. M., Geijtenbeek, T. B., and Gallay, P. A. (2011) HSV neutralization by the microbicidal candidate C5A. *PLoS One 6*, e18917.
(30) Denton, P. W., Othieno, F., Martinez-Torres, F., Zou, W., Krisko, J. F., Fleming, E., Zein, S., Powell, D. A., Wahl, A., and Kwak, Y. T. (2011) One percent tenofovir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. *J. Virol. 85*, 7582−7593.
(31) Veazey, R. S., Chatterji, U., Bobardt, M., Russell-Lodrigue, K. E., Li, J., Wang, X., and Gallay, P. A. (2016) C5A protects macaques from vaginal simian-human immunodeficiency virus challenge. *Antimicrob. Agents Chemother. 60*, 693−698.
(32) Jackman, J. A., Costa, V. V., Park, S. H., Real, A. L. C. V., Park, J. H., Cardozo, P. L., Ferhan, A. R., Olmo, I. G., Poreira, T. P., Bambirra, J. L., Queiroz, V. F., Queiroz-Junior, C. M., Foureaux, G., Souza, D. G., Ribeiro, F. M., Yoon, B., Wynendaele, E., De Spiegeleer, B., Teixeira, M. M., and Cho, N.-J. (2018) Therapeutic treatment of Zika virus infection using a brain-penetrating antiviral peptide. *Nat. Mater. 17*, 971−977.
(33) Jackman, J. A., Saravanan, R., Zhang, Y., Tabaei, S. R., and Cho, N.-J. (2015) Correlation between membrane partitioning and functional activity in a single lipid vesicle assay establishes design guidelines for antiviral peptides. *Small 11*, 2372−2379.
(34) Jackman, J. A., Goh, H. Z., Zhdanov, V. P., Knoll, W., and Cho, N.-J. (2016) Deciphering how pore formation causes strain-induced membrane lysis of lipid vesicles. *J. Am. Chem. Soc. 138*, 1406−1413.
(35) Hancock, R. E., and Sahl, H.-G. (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol. 24*, 1551−1557.
(36) Iwasaki, A. (2017) Immune regulation of antibody access to neuronal tissues. *Trends Mol. Med. 23*, 227−245.

DOI: 10.1021/acsinfecdis.8b00286
ACS Infect. Dis. 2019, 5, 4−8