Zika Vaccine Development

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Zika virus (ZIKV), an emerging arthropod-borne flavivirus.The situation prompted scientists to increase research on antivirals and vaccines against the virus. These efforts are still ongoing as the pathogenesis and immune evasion mechanisms of ZIKV have not yet been fully elucidated. Understanding the viral disease mechanism will provide a better landscape to develop prophylactic and therapeutic strategies against ZIKV. Currently, no specific vaccines or drugs have been approved for ZIKV. However, some are undergoing clinical trials. Notably, different platforms have been evaluated for the design of vaccines, including DNA, mRNA, viral vectors, virus-like particles (VLPs), inactivated virus, live attenuated virus, peptide and protein-based vaccines, passive immunizations by using monoclonal antibodies (MAbs), and vaccines that target vector-derived antigens. These vaccines have been shown to induce specific humoral and cellular immune responses and reduce viremia and viral RNA titers, both in vitro and in vivo.

Zika virus | Zika vaccines | DNA vaccines

1. Introduction

Zika virus (ZIKV) is an arthropod-borne virus with the genus Flavivirus of the *Flaviviridae* family of enveloped RNA viruses. ZIKV has an ~11 kb positive sense RNA genome. Translation of viral RNA in the cytoplasm generates a polyprotein that is cleaved into three structural proteins (capsid (C), pre-membrane/membrane (prM/M), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [1]. ZIKV buds into the lumen of the endoplasmic reticulum as an immature virion composed of 60 icosahedrically arranged prM-E heterotrimers [2]. The ZIKV E protein is composed of three ectodomains (DI, DII, and DIII) and is the primary target of neutralizing antibodies (nAbs) [3]. ZIKV was first isolated from a non-human primate in 1947 in Uganda [4], and ZIKV infections in humans were sporadic for half a century before emerging in the Pacific and the Americas [5]. ZIKV is usually transmitted by the bite of infected *Aedes aegypti* mosquitoes, but sexual and vertical transmission has also been reported [6][7]. About two billion people worldwide live in tropical and sub-tropical regions with suitable environmental conditions for ZIKV [8], and increased globalization continues to raise the risk for disease spread.

The clinical presentation of Zika fever is nonspecific.The outbreak of ZIKV threatens public health worldwide, especially as it can cause devastating congenital syndrome in the fetuses of pregnant women [9], including microcephaly, craniofacial disproportion, spasticity, ocular abnormalities, and miscarriage. In adults, Zika infection has been linked to the autoimmune disorder Guillain–Barré syndrome [10]. Prevention of congenital Zika syndrome

(CZS) is the primary goal for immunization, and the vaccine must provide protection against intrauterine transmission for use during pregnancy and in women of childbearing age [11].

Since 2016, a number of candidates using multiple vaccine platforms have been developed and have shown promising results in preclinical testing [12]. Candidate ZIKV vaccines that are currently in phase I/II clinical trials include live virus vaccines, inactivated, subunit-based, viral vector vaccines, DNA–RNA vaccines, and even vaccines based on mosquito salivary antigens [13]. One or more pivotal Phase III trials is normally used to demonstrate safety and efficacy [14]. The next step would be Phase III field efficacy trials, which would not be possible since its peak in early 2016 [15], as the incidence of Zika virus (ZIKV) cases has declined [16]. The rapid progress in vaccine development demonstrates the capacity of governments, public health organizations and the scientific community to respond to the threat of a pandemic [17]. Despite low levels of transmission during recent years, ZIKV has become endemic in the Americas and the potential for large Zika outbreaks remains real [18]. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority [5], It is important for vaccine researchers to continue developing and improving Zika vaccines, so that a potential vaccine is ready for deployment and clinical efficacy trials when the next ZIKV outbreak occurs [12].

2. ZIKV DNA Vaccines

DNA vaccines can be quickly designed and manufactured at a low cost and are relatively stable and safe for adults and fetuses, avoiding virulence recovery induced by reproducible vaccines [19].A DNA-based vaccine platform using two DNA vaccine candidates, VRC5288 and VRC5283, was developed by the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA. These candidates encode the protein prM (prM) and envelope protein E (E), respectively, and have completed clinical trials (Phases I and II; these trials are registered with ClinicalTrials.gov, numbers NCT02840487, NCT02996461 and NCT03110770) [20]. The results obtained from the immunization of non-human primates provide sufficient evidence to show that VRC5283 was well tolerated and to support clinical studies of VRC5283 in regions with endemic Zika virus to assess efficacy in humans [20]. In addition, C57BL/6 and IFNAR1 (deficient for IFN receptor I) mice inoculated (intramuscularly, by jet injection or by electrophoration (EP)) with a DNA vaccine candidate expressing tandem repeated ZIKV envelope domain III (ED III × 3), showed a significant induction of humoral and cellular immunity, by measuring nAbs and IFN-γ secretion from splenocytes.A novel DNA vaccine encoding a secreted ZIKV NS1 confers rapid protection from systemic ZIKV infection in immunocompetent mice. The results show that functional NS1-specific T-cell responses are critical for protection against ZIKV infection [21]. The rapid development timeline of these vaccines further highlights the potential of the DNA vaccine platforms for meeting the challenge of developing therapies to stop the spread of potential Zika outbreaks and other emerging infectious diseases.

3. ZIKV mRNA Vaccines

mRNA vaccine technology has arisen as a simplified, flexible, and fast vaccine production platform [22]. A versatile ZIKV vaccine platform in which lipid nanoparticles encapsulate modified mRNA (mRNA-LNP) encoding ZIKV structural genes has been generated [23]. Following a prime-boost immunization of modified mRNA encoding ZIKV prM-E genes that produced virus-like particles resulted in high levels of nAbs that protected immunocompetent (C57BL/6) and immunocompromised (AG129, deficient in interferon alpha/beta/gamma receptors) mice [24]. These mRNA induced protective antibody responses against ZIKV in mice and minimized the generation of cross-reactive antibodies that enhanced DENV infection in cell culture and pathogenicity in mice [25]. Additionally, a single low dose intradermal immunization with nucleoside-modified mRNA-LNP encoding the pre-membrane and envelope (prM-E) glycoproteins of ZIKV from the 2013 outbreak elicits rapid and durable protective immunity in mice and non-human primates, therefore representing a new and promising vaccine candidate for the global fight against ZIKV [26].Moderna has advanced its Zika vaccine candidate (mRNA-1893, phase I trials are registered with ClinicalTrials.gov, numbers NCT04064905), the preclinical data have shown that vaccination with mRNA-1893 protected against Zika virus transmission during pregnancy in mice [27], and currently it is being studied in a phase II clinical trials in United States and Puerto Rico (the phase II trials are registered with ClinicalTrials.gov, numbers NCT04917861), being developed in collaboration with the Biomedical Advanced Research and Development Authority (BARDA). With the success of mRNA vaccine technology in facing the coronavirus (COVID-19) pandemic, the development of mRNA vaccine against ZIKV and other infectious diseases will be reinforced, as a promising alternative to conventional vaccine approaches [28].

4. ZIKV Viral Vector Vaccines

Adenovirus type 5 (Ad5) has been the most extensively used vector due to the robust immune response elicited [29]. Adenovirus (Ad) vectored Zika virus vaccines work by inserting a ZIKV prM-E gene expression cassette into human Ad types 4 (Ad4-prM-E) and 5 (Ad5-prM-E). Ad5-prM-E elicited both strong antibody and T-cell responses, whereas another adenoviral vector, Ad4-prM-E, did not induce any detectable anti-ZIKV antibodies, although it still induced a strong T-cell response when it presented the same antigens, in female Ifnar1$^{-/-}$ mice [30]. After a lethal ZIKV challenge in this mouse model, Ad5-prM-E provided superior protection to Ad4-prM-E vaccination.

Poxvirus-based vectors have been extensively used as vaccine candidates [31].A single intramuscular immunization of immunocompetent mice with a ZIKV vaccine based on the ZIKV NS1 protein from a clinically proven safe, MVA vector (MVA-ZIKV-NS1 vaccine candidate) provided robust humoral and cellular responses, and afforded 100% protection against a lethal intracerebral dose of ZIKV (strain MR766) [32]. The NS1 protein itself has been shown to be a viable vaccine target, and it has been demonstrated that an attenuated recombinant vesicular stomatitis vector (rVSV)-based vaccine expressing ZIKV prM-E-NS1 as a polyprotein is a promising vaccine candidate for protection against ZIKV infection, highlighting an important role for NS1 in ZIKV-specific cellular immune responses [33].

5. ZIKV Virus-Like Particles (VLPs) Vaccines

The delivery of VLPs, based on the use of structural proteins [34], can be achieved by different approaches including DNA (e.g., NIAID/VRC) and mRNA (e.g., Moderna). The NIAID/VRC DNA vaccine encodes sequences from the French Polynesian isolate strain H/PF/2013 [19], and the Moderna mRNA vaccine encodes sequences from the Asian ZIKV strain Micronesia 2007 [24]. This VLP was generated in vitro for use as a vaccine following introduction of plasmid DNA encoding Zika structural protein (prM-E) genes into mammalian cells. There are several studies that show an efficient generation and purification of ZIKV VLPs [35]. One of these studies showed that VLPs produced in HEK293 mammalian cells using the prM and E structural proteins, when aluminum-adjuvanted, were able to induce nAbs in both mice and non-human primates and protected against ZIKV challenge [36]. Other forms of ZIKV VLPs have been also reported, featuring the co-expression of the prM-E, prM-E-NS1, C-prM-E, and NS2B/NS3 viral genes in human cells [37]. These studies show a strategy to assemble Zika VLPs by co-expressing the structural (C-prM-E) and non-structural (NS2B/NS3) proteins, showing that VLP immunizations elicited higher titers of nAbs, compared with the titers after inactivated Zika virus vaccination [38].

6. ZIKV Purified Inactivated Vaccine

Inactivated vaccines provide enhanced safety at the cost of reduced immunogenicity, although they often require multiple doses and periodic boosters [39]. Inactivated virus vaccine platforms have a long track record of safety in both pregnant women and fetuses [11][40]. Based on the expected safety profile, inactivated virus vaccines including ZPIV adjuvanted with aluminum hydroxide are a potentially favored platform for vaccinating pregnant women. Another inactivated ZPIV vaccine candidate (Takeda's TAK-426) was found to be well tolerated with an acceptable safety profile and was immunogenic in both flavivirus-naive and flavivirus-primed adults. Based on the safety and immunogenicity profiles of all TAK-426 doses assessed, the TAK-426 was selected for further clinical development [41]. A Vero cell-adapted ZIKV strain (GMZ-002) and a purified inactivated virus (PIV) vaccine were shown to present significantly increased productivity in Vero cells, and IFNAR1-blocked C57BL/6 mice administered with two doses of the PIV were fully protected against lethal challenge. This candidate elicited a robust and persistent protective immunity, therefore representing a promising vaccine candidate for ZIKV [42].

7. ZIKV Live Attenuated Vaccine

Live attenuated vaccines generally offer fast and durable immunity, but sometimes with the trade-off of reduced safety [43]. Different candidates have been generated in order to increase safety. A live attenuated vaccine candidate that contains a 10-nucleotide deletion in the 3′ untranslated region of the ZIKV genome (3′UTR 10-del ZIKV) has been shown to be highly attenuated, immunogenic, and protective in the A129 mouse model. Importantly, a single dose of this attenuated vaccine induced sterilizing immunity and a high level of nAbs, completely preventing viremia after challenge and inducing a robust T-cell response in the immunized mice. The attenuated 10-deletion ZIKV was incompetent in infecting mosquitoes after oral feeding of spiked blood meals, representing an additional safety feature for use in non-endemic regions. Collectively, a good balance between immunogenicity and safety warrant further development of this promising live attenuated ZIKV vaccine candidate [44].

8. ZIKV Peptide-Based Vaccines

Development of peptide therapeutics against ZIKV (peptide-based vaccines) has attracted rising attention on account of their high safety and low development cost, in comparison to small therapeutic molecules and antibody-based anti-viral drugs [45].Using characterization techniques and web-based bioinformatics servers, four peptide stretches have been identified in the E protein, being well conserved, surface exposed and predicted to have reasonable epitope binding efficiency [46].These immunoinformatic approaches represent a great tool to initiate the generation of new vaccine candidates for further in vivo validation.

9.Anti-ZIKV Monoclonal Antibodies Vaccines

The anti-E80 and anti-EDIII sera were found to potently neutralize ZIKV infection in vitro [47][48], and passive transfer of either anti-E80 or anti-EDIII sera protected recipient mice against lethal ZIKV challenge [47].Other approaches, such as high-throughput antibody isolation, have contributed to a better understanding of the B-cell responses elicited following infection and/or vaccination [49]. The isolation of potent nAbs, coupled with detailed examination of their properties at the molecular level, have provided pivotal insights related to immunogen design or, ultimately, cross-flavivirus ZIKV vaccines [50]. These findings suggest that passive vaccination with antibodies could be a useful strategy to protect against ZIKV infection.

10. Anti-ZIKV Mosquito Salivary Protein Vaccines

Saliva components are capable of changing the local immune environment, leading to an increase in flavivirus-susceptible cells at the bite site [51]. A previously undescribed salivary gland (SG) protein (termed neutrophil stimulating factor 1 (NeSt1)) was shown to stimulate neutrophils at the mosquito bite site, altering the immune microenvironment and allowing a higher level of early viral replication that triggers ZIKV pathogenesis. Based on these observations, it is possible that a vaccine against NeSt1 might protect people against severe Zika virus infection, based on immunization experiments performed in mice [52]. Another mosquito salivary protein, *A. aegypti* bacteria-responsive protein 1(AgBR1), could induce inflammatory responses at the bite site. It has been described that passive immunization with AgBR1 antiserum and active immunization with recombinant AgBR1 protein adjuvanted with aluminum hydroxide partially protected mice from a mosquito-borne ZIKV infection [53][54], suggesting that AgBR1 may be used as another target for vaccine development against ZIKV. Further research showed that passive immunization with a combination of AgBR1- and NeSt1-sera enhanced survival and reduced the viral burden in blood, thereby protecting mice from mosquito-borne ZIKV infection [55]. There are also many other mosquito salivary proteins that could promote ZIKV transmission, such as LTRIN by its interaction with the lymphotoxin β receptor or AaVA-1 activating autophagy pathways [56][57]. Taken together, these findings suggest that targeting a combination of mosquito saliva proteins could be an interesting approach for vaccine development to help prevent mosquito-borne ZIKV infection.

11. Conclusions

In summary, there is a lot that is still unknown about ZIKV, and the occurrence of more prominent epidemics caused by other pathogenic microorganisms has slowed down the advance of ZIKV-related research. The cellular and animal models available have shed light on how the virus acts, why it acquired a more aggressive nature in the recent epidemics and how to potentially combat it more effectively, but there is still a lot of progress to be made in the development of effective vaccines, and both organoid cultures and nanotechnology compounds can pave the way to achieve them.

References

1. Shan, C.; Xia, H.; Haller, S.L.; Azar, S.R.; Liu, Y.; Liu, J.; Muruato, A.E.; Chen, R.; Rossi, S.L.; Wakamiya, M.; et al. A zika virus envelope mutation preceding the 2015 epidemic enhances virulence and fitness for transmission. Proc. Natl. Acad. Sci. USA 2020, 117, 20190–20197.
2. Prasad, V.M.; Miller, A.S.; Klose, T.; Sirohi, D.; Buda, G.; Jiang, W.; Kuhn, R.J.; Rossmann, M.G. Structure of the immature zika virus at 9 a resolution. Nat. Struct Mol. Biol. 2017, 24, 184–186.
3. Zhao, H.; Fernandez, E.; Dowd, K.A.; Speer, S.D.; Platt, D.J.; Gorman, M.J.; Govero, J.; Nelson, C.A.; Pierson, T.C.; Diamond, M.S.; et al. Structural basis of zika virus-specific antibody protection. Cell 2016, 166, 1016–1027.
4. Dick, G.W.; Kitchen, S.F.; Haddow, A.J. Zika virus. I. Isolations and serological specificity. Trans. R. Soc. Trop. Med. Hyg. 1952, 46, 509–520.
5. Safadi, M.A.P.; Almeida, F.J.; de Avila Kfouri, R. Zika virus outbreak in brazil-lessons learned and perspectives for a safe and effective vaccine. Anat. Rec. (Hoboken) 2021, 304, 1194–1201.
6. Gao, D.; Lou, Y.; He, D.; Porco, T.C.; Kuang, Y.; Chowell, G.; Ruan, S. Prevention and control of zika as a mosquito-borne and sexually transmitted disease: A mathematical modeling analysis. Sci. Rep. 2016, 6, 28070.
7. Brasil, P.; Vasconcelos, Z.; Kerin, T.; Gabaglia, C.R.; Ribeiro, I.P.; Bonaldo, M.C.; Damasceno, L.; Pone, M.V.; Pone, S.; Zin, A.; et al. Zika virus vertical transmission in children with confirmed antenatal exposure. Nat. Commun. 2020, 11, 3510.
8. Messina, J.P.; Kraemer, M.U.; Brady, O.J.; Pigott, D.M.; Shearer, F.M.; Weiss, D.J.; Golding, N.; Ruktanonchai, C.W.; Gething, P.W.; Cohn, E.; et al. Mapping global environmental suitability for zika virus. Elife 2016, 5, e15272.
9. Plourde, A.R.; Bloch, E.M. A literature review of zika virus. Emerg. Infect. Dis. 2016, 22, 1185–1192.
10. Beattie, J.; Parajuli, S.; Sanger, M.; Lee, G.; Pleninger, P.; Crowley, G.; Kwon, S.; Murthy, V.; Manko, J.A.; Caplan, A.; et al. Zika virus-associated guillain-barre syndrome in a returning us

traveler. Infect. Dis. Clin. Pract. (Baltim Md.) 2018, 26, e80–e84.

11. Kim, I.J.; Blackman, M.A.; Lin, J.S. Pre-clinical pregnancy models for evaluating zika vaccines. Trop. Med. Infect. Dis. 2019, 4, 58.
12. Castanha, P.M.S.; Marques, E.T.A. A glimmer of hope: Recent updates and future challenges in zika vaccine development. Viruses 2020, 12, 1371.
13. Poland, G.A.; Ovsyannikova, I.G.; Kennedy, R.B. Zika vaccine development: Current status. Mayo. Clin. Proc. 2019, 94, 2572–2586.
14. Prakash, S. Development of covid 19 vaccine: A summarized review on global trials, efficacy, and effectiveness on variants. Diabetes. Metab. Syndr. 2022, 16, 102482.
15. Lowe, R.; Barcellos, C.; Brasil, P.; Cruz, O.G.; Honorio, N.A.; Kuper, H.; Carvalho, M.S. The zika virus epidemic in brazil: From discovery to future implications. Int. J. Environ. Res. Public Health 2018, 15, 96.
16. Vannice, K.S.; Cassetti, M.C.; Eisinger, R.W.; Hombach, J.; Knezevic, I.; Marston, H.D.; Wilder-Smith, A.; Cavaleri, M.; Krause, P.R. Demonstrating vaccine effectiveness during a waning epidemic: A who/nih meeting report on approaches to development and licensure of zika vaccine candidates. Vaccine 2019, 37, 863–868.
17. Barouch, D.H.; Thomas, S.J.; Michael, N.L. Prospects for a zika virus vaccine. Immunity 2017, 46, 176–182.
18. Sun, H.; Dickens, B.L.; Jit, M.; Cook, A.R.; Carrasco, L.R. Mapping the cryptic spread of the 2015-2016 global zika virus epidemic. BMC Med. 2020, 18, 399.
19. Dowd, K.A.; Ko, S.Y.; Morabito, K.M.; Yang, E.S.; Pelc, R.S.; DeMaso, C.R.; Castilho, L.R.; Abbink, P.; Boyd, M.; Nityanandam, R.; et al. Rapid development of a DNA vaccine for zika virus. Science 2016, 354, 237–240.
20. Gaudinski, M.R.; Houser, K.V.; Morabito, K.M.; Hu, Z.; Yamshchikov, G.; Rothwell, R.S.; Berkowitz, N.; Mendoza, F.; Saunders, J.G.; Novik, L.; et al. Safety, tolerability, and immunogenicity of two zika virus DNA vaccine candidates in healthy adults: Randomised, open-label, phase 1 clinical trials. Lancet 2018, 391, 552–562.
21. Grubor-Bauk, B.; Wijesundara, D.K.; Masavuli, M.; Abbink, P.; Peterson, R.L.; Prow, N.A.; Larocca, R.A.; Mekonnen, Z.A.; Shrestha, A.; Eyre, N.S.; et al. Ns1 DNA vaccination protects against zika infection through t cell-mediated immunity in immunocompetent mice. Sci. Adv. 2019, 5, eaax2388.
22. Schlake, T.; Thess, A.; Fotin-Mleczek, M.; Kallen, K.J. Developing mrna-vaccine technologies. RNA Biol. 2012, 9, 1319–1330.

23. VanBlargan, L.A.; Himansu, S.; Foreman, B.M.; Ebel, G.D.; Pierson, T.C.; Diamond, M.S. An mrna vaccine protects mice against multiple tick-transmitted flavivirus infections. Cell Rep. 2018, 25, 3382–3392.
24. Richner, J.M.; Himansu, S.; Dowd, K.A.; Butler, S.L.; Salazar, V.; Fox, J.M.; Julander, J.G.; Tang, W.W.; Shresta, S.; Pierson, T.C.; et al. Modified mrna vaccines protect against zika virus infection. Cell 2017, 168, 1114–1125.
25. Dai, L.; Xu, K.; Li, J.; Huang, Q.; Song, J.; Han, Y.; Zheng, T.; Gao, P.; Lu, X.; Yang, H.; et al. Protective zika vaccines engineered to eliminate enhancement of dengue infection via immunodominance switch. Nat. Immunol. 2021, 22, 958–968.
26. Pardi, N.; Hogan, M.J.; Pelc, R.S.; Muramatsu, H.; Andersen, H.; DeMaso, C.R.; Dowd, K.A.; Sutherland, L.L.; Scearce, R.M.; Parks, R.; et al. Zika virus protection by a single low-dose nucleoside-modified mrna vaccination. Nature 2017, 543, 248–251.
27. Chaudhary, N.; Weissman, D.; Whitehead, K.A. Mrna vaccines for infectious diseases: Principles, delivery and clinical translation. Nat. Rev. Drug. Discov. 2021, 20, 817–838.
28. Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. Mrna vaccines—A new era in vaccinology. Nat. Rev. Drug. Discov. 2018, 17, 261–279.
29. Shaw, A.R.; Suzuki, M. Immunology of adenoviral vectors in cancer therapy. Mol. Ther. Methods. Clin. Dev. 2019, 15, 418–429.
30. Bullard, B.L.; Corder, B.N.; Gorman, M.J.; Diamond, M.S.; Weaver, E.A. Efficacy of a T cell-biased adenovirus vector as a zika virus vaccine. Sci. Rep. 2018, 8, 18017.
31. Hazlewood, J.E.; Dumenil, T.; Le, T.T.; Slonchak, A.; Kazakoff, S.H.; Patch, A.M.; Gray, L.A.; Howley, P.M.; Liu, L.; Hayball, J.D.; et al. Injection site vaccinology of a recombinant vaccinia-based vector reveals diverse innate immune signatures. PLoS Pathog. 2021, 17, e1009215.
32. Brault, A.C.; Domi, A.; McDonald, E.M.; Talmi-Frank, D.; McCurley, N.; Basu, R.; Robinson, H.L.; Hellerstein, M.; Duggal, N.K.; Bowen, R.A.; et al. A zika vaccine targeting ns1 protein protects immunocompetent adult mice in a lethal challenge model. Sci. Rep. 2017, 7, 14769.
33. Li, A.; Yu, J.; Lu, M.; Ma, Y.; Attia, Z.; Shan, C.; Xue, M.; Liang, X.; Craig, K.; Makadiya, N.; et al. A zika virus vaccine expressing premembrane-envelope-ns1 polyprotein. Nat. Commun. 2018, 9, 3067.
34. Yan, D.; Wei, Y.Q.; Guo, H.C.; Sun, S.Q. The application of virus-like particles as vaccines and biological vehicles. Appl. Microbiol. Biotechnol. 2015, 99, 10415–10432.
35. Kuadkitkan, A.; Ramphan, S.; Worawichawong, S.; Sornjai, W.; Wikan, N.; Smith, D.R. Production of zika virus virus-like particles. Methods Mol. Biol. 2021, 2183, 183–203.

36. Vang, L.; Morello, C.S.; Mendy, J.; Thompson, D.; Manayani, D.; Guenther, B.; Julander, J.; Sanford, D.; Jain, A.; Patel, A.; et al. Zika virus-like particle vaccine protects ag129 mice and rhesus macaques against zika virus. PLoS Negl. Trop. Dis. 2021, 15, e0009195.

37. Nambala, P.; Su, W.C. Role of zika virus prm protein in viral pathogenicity and use in vaccine development. Front Microbiol. 2018, 9, 1797.

38. Boigard, H.; Alimova, A.; Martin, G.R.; Katz, A.; Gottlieb, P.; Galarza, J.M. Zika virus-like particle (vlp) based vaccine. PLoS Negl. Trop. Dis. 2017, 11, e0005608.

39. Zhang, Y.; Zeng, G.; Pan, H.; Li, C.; Hu, Y.; Chu, K.; Han, W.; Chen, Z.; Tang, R.; Yin, W.; et al. Safety, tolerability, and immunogenicity of an inactivated sars-cov-2 vaccine in healthy adults aged 18-59 years: A randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect. Dis. 2021, 21, 181–192.

40. Shields, K.E.; Lyerly, A.D. Exclusion of pregnant women from industry-sponsored clinical trials. Obstet. Gynecol. 2013, 122, 1077–1081.

41. Han, H.H.; Diaz, C.; Acosta, C.J.; Liu, M.; Borkowski, A. Safety and immunogenicity of a purified inactivated zika virus vaccine candidate in healthy adults: An observer-blind, randomised, phase 1 trial. Lancet Infect. Dis. 2021, 21, 1282–1292.

42. Oh, H.S.; Yoon, J.W.; Lee, S.; Kim, S.O.; Hong, S.P. A purified inactivated vaccine derived from vero cell-adapted zika virus elicits protection in mice. Virology 2021, 560, 124–130.

43. Pollard, A.J.; Bijker, E.M. A guide to vaccinology: From basic principles to new developments. Nat. Rev. Immunol. 2021, 21, 83–100.

44. Shan, C.; Muruato, A.E.; Nunes, B.T.D.; Luo, H.; Xie, X.; Medeiros, D.B.A.; Wakamiya, M.; Tesh, R.B.; Barrett, A.D.; Wang, T.; et al. A live-attenuated zika virus vaccine candidate induces sterilizing immunity in mouse models. Nat. Med. 2017, 23, 763–767.

45. Karwal, P.; Vats, I.D.; Sinha, N.; Singhal, A.; Sehgal, T.; Kumari, P. Therapeutic applications of peptides against zika virus: A review. Curr. Med. Chem. 2020, 27, 3906–3923.

46. Dey, S.; Nandy, A.; Basak, S.C.; Nandy, P.; Das, S. A bioinformatics approach to designing a zika virus vaccine. Comput. Biol. Chem. 2017, 68, 143–152.

47. Qu, P.; Zhang, W.; Li, D.; Zhang, C.; Liu, Q.; Zhang, X.; Wang, X.; Dai, W.; Xu, Y.; Leng, Q.; et al. Insect cell-produced recombinant protein subunit vaccines protect against zika virus infection. Antiviral. Res. 2018, 154, 97–103.

48. Zhang, W.; Qu, P.; Li, D.; Zhang, C.; Liu, Q.; Zou, G.; Dupont-Rouzeyrol, M.; Lavillette, D.; Jin, X.; Yin, F.; et al. Yeast-produced subunit protein vaccine elicits broadly neutralizing antibodies that protect mice against zika virus lethal infection. Antiviral. Res. 2019, 170, 104578.

49. Utset, H.A.; Guthmiller, J.J.; Wilson, P.C. Bridging the b cell gap: Novel technologies to study antigen-specific human b cell responses. Vaccines 2021, 9, 711.
50. Dussupt, V.; Modjarrad, K.; Krebs, S.J. Landscape of monoclonal antibodies targeting zika and dengue: Therapeutic solutions and critical insights for vaccine development. Front Immunol. 2020, 11, 621043.
51. Demarta-Gatsi, C.; Mecheri, S. Vector saliva controlled inflammatory response of the host may represent the achilles heel during pathogen transmission. J. Venom. Anim. Toxins Incl. Trop. Dis. 2021, 27, e20200155.
52. Hastings, A.K.; Uraki, R.; Gaitsch, H.; Dhaliwal, K.; Stanley, S.; Sproch, H.; Williamson, E.; MacNeil, T.; Marin-Lopez, A.; Hwang, J.; et al. Aedes aegypti nest1 protein enhances zika virus pathogenesis by activating neutrophils. J. Virol. 2019, 93, e00395-19.
53. Uraki, R.; Hastings, A.K.; Marin-Lopez, A.; Sumida, T.; Takahashi, T.; Grover, J.R.; Iwasaki, A.; Hafler, D.A.; Montgomery, R.R.; Fikrig, E. Aedes aegypti agbr1 antibodies modulate early zika virus infection of mice. Nat. Microbiol. 2019, 4, 948–955.
54. Wang, Y.; Marin-Lopez, A.; Jiang, J.; Ledizet, M.; Fikrig, E. Vaccination with aedes aegypti agbr1 delays lethal mosquito-borne zika virus infection in mice. Vaccines 2020, 8, 145.
55. Marin-Lopez, A.; Wang, Y.; Jiang, J.; Ledizet, M.; Fikrig, E. Agbr1 and nest1 antisera protect mice from aedes aegypti-borne zika infection. Vaccine 2021, 39, 1675–1679.
56. Jin, L.; Guo, X.; Shen, C.; Hao, X.; Sun, P.; Li, P.; Xu, T.; Hu, C.; Rose, O.; Zhou, H.; et al. Salivary factor ltrin from aedes aegypti facilitates the transmission of zika virus by interfering with the lymphotoxin-beta receptor. Nat. Immunol. 2018, 19, 342–353.
57. Sun, P.; Nie, K.; Zhu, Y.; Liu, Y.; Wu, P.; Liu, Z.; Du, S.; Fan, H.; Chen, C.H.; Zhang, R.; et al. A mosquito salivary protein promotes flavivirus transmission by activation of autophagy. Nat. Commun. 2020, 11, 260.