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Zika virus vaccine: Progresses and challenges

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SUMMARY

The explosive emergence of Zika virus has inspired a global effort to develop vaccines. Zika virus, which is a flavivirus primarily transmitted by mosquitoes, can cause devastating congenital syndrome in fetuses of pregnant women, including microcephaly, craniofacial disproportion, spasticity, ocular abnormalities, and miscarriage. In adults, Zika infection has been linked to the autoimmune disorder Guillain-Barré syndrome. Thus, despite the current waning in newly reported Zika infections, an efficacious vaccine is urgently needed to help limit the emergence of another detrimental epidemic. Here we summarize the current status of the Zika vaccine pipeline and highlight the challenges for clinical efficacy trials.

MAIN TEXT

Zika virus (ZIKV) is a mosquito-borne flavivirus that has recently caused global epidemics. Many other flaviviruses, including the four serotypes of dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV), also cause global outbreaks and epidemics, posing constant threats to public health. ZIKV was first isolated from a sentinel rhesus macaque in the Zika Forest of Uganda in 1947. Outside Africa, ZIKV was isolated for the first time from *Aedes aegypti* mosquitoes in 1966 in Malaysia. Before 2007, ZIKV had silently circulated between primates and mosquitoes in forests in Africa and Southeast Asia without causing detectable outbreaks and severe human disease. However, ZIKV caused an epidemic on Yap Island, Micronesia in 2007, spawned large epidemics in French Polynesia and other regions of the South Pacific in 2013–14, and arrived in the Americas in 2014, causing >700,000 cases of autochthonous infection (Ikejezie et al., 2017). Importantly, during the recent epidemics, ZIKV infections were shown to cause devastating congenital Zika syndromes (CZS, including microcephaly, congenital malformation, and fetal demise) in about 6–11% of the fetuses from infected pregnant women (Hoen et al., 2018). In adults, Zika infections may cause Guillain-Barré syndrome (GBS), an autoimmune disease caused by the immune system attacking the peripheral nerves, leading to a rapid onset of muscle weakness and even

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paralysis (Dos Santos et al., 2016). The CZS and GBS potential of ZIKV was unexpected because the virus had only been associated with mild disease before 2013. Due to the explosive epidemics and teratogenic potential, the WHO declared ZIKV to be a Public Health Emergency of International Concern in February 2016. Since then, intensive global efforts have been made to understand ZIKV biology and to develop countermeasures at an unprecedented pace, leading to the establishment of a promising vaccine portfolio.

Licensed flavivirus vaccines

The feasibility to develop a safe and efficacious ZIKV vaccine is supported by the availability of licensed vaccines for other four flaviviruses: YFV (live-attenuated), TBEV (inactivated), JEV (both inactivated and live-attenuated), and DENV (chimeric live-attenuated). Lessons learned from these licensed flavivirus vaccines should be useful to guide the development of a ZIKV vaccine. Due to the four serotypes of DENV (30–35% amino acid variation among serotypes), the development of a tetravalent DENV vaccine has proven challenging. An effective DENV vaccine needs to induce long-lasting immune protection against all four serotypes. Otherwise, a vaccine recipient with incomplete immunization may become sensitized to life-threatening dengue hemorrhagic fever or dengue shock syndrome upon subsequent DENV infections. This challenge was reflected by the results of phase 2b/3 trials of Dengvaxia with an average efficacy of 30–61%. Unfortunately, Dengvaxia seems to increase the risk of hospitalization over time in children <9 years of age who were seronegative at the time of vaccination, possibly through vaccine-induced antibody enhancement. In contrast to DENV, ZIKV exists as two genetic lineages (African and Asian) with <5% amino acid variation among the different strains. Immune serum from ZIKV-infected humans and animals was shown to equivalently neutralize various ZIKV strains from both lineages, demonstrating ZIKV as a single serotype (Dowd et al., 2016a). In support of this idea, serum from non-human primates immunized with an inactivated ZIKV vaccine, a DNA subunit vaccine, or an adenovirus-based subunit vaccine were shown to equally cross-neutralize ZIKV strains from Brazil, Puerto Rico, the Philippines, Thailand, and Uganda (Abbink et al., 2017).

Current Zika vaccine pipeline

Table 1 summarizes the overall pipeline of ZIKV vaccine progress. Vaccine candidates using distinct technologies are being pursued at different stages of development, some of which have already advanced to clinical phase I/II trials. The vaccine candidates belong to three general categories: (i) inactivated vaccine, (ii) subunit vaccine, and (iii) live-attenuated vaccine. Recently, three phase I clinical studies have been published to demonstrate the safety and immunogenicity of inactivated vaccines (Modjarrad et al., 2017) and DNA subunit vaccines (Gaudinski et al., 2017; Tebas et al., 2017). The following sections focus on reviewing the published preclinical and clinical results of different vaccine platforms, particularly on the recent phase I trial data.

(I) Inactivated vaccine

Purified formalin-inactivated ZIKV vaccine (ZPIV; derived from Puerto Rico strain PRVABC59) was shown to protect against ZIKV infection in mice and non-human primates

(Abbink et al., 2016). In clinical phase I trials, human volunteers were given two doses of 5 μg of ZPIV intramuscularly on days 1 and 29. ZPIV caused only mild to moderate adverse events with local pain (60%) or tenderness (47%) at the injection site, fatigue (43%), headache (39%), and malaise (22%). On day 57, 92% and 77% of the vaccine recipients developed neutralizing antibody titers of ≥1:10 and ≥1:100 (measured by a microneutralization assay), respectively. Adoptive transfer of purified IgG from vaccinated people to mice protected against ZIKV challenge when the neutralizing titers reached >1:100 in recipient mice (Modjarrad et al., 2017).

(II) Subunit vaccines

Subunit vaccines express two virion surface glycoproteins of prM (membrane) and E (envelope) using DNA, mRNA, or viral vectors (*e.g.*, adenovirus, measles virus, vesicular stomatitis virus, and modified vaccinia virus). Alternatively, recombinant E protein or purified virus-like particles (formed by prM and E proteins) could be used directly as subunit vaccines. Many of these subunit vaccine candidates have shown efficacy in protecting mice and/or non-human primates from ZIKV infection (Abbink et al., 2016; Dowd et al., 2016b; Larocca et al., 2016; Pardi et al., 2017; Richner et al., 2017b), among which two-dose preconception immunization of female mice with mRNA vaccine protected maternal-to-fetal ZIKV transmission during pregnancy (Richner et al., 2017a).

Two clinical phase I studies have been recently published on DNA subunit vaccines. Gaudinski *et al.* tested two DNA vaccine candidates: plasmid VRC5288 (ZIKV and JEV chimeric prM-E to increase the secretion level of empty virus-like particles) and plasmid VRC5283 (wild-type ZIKV prM-E). The prM-E sequence of ZIKV was derived from a French Polynesia strain H/HF/2013. Human volunteers were immunized with three 4-mg doses of DNA vaccine via the intramuscular route. Four different administering regimens were compared: single-dose needle injection, split-dose needle injection, single-dose needle-free injection, or split-dose needle-free injection. Both vaccine candidates were safe and well tolerated with local pain and tenderness (46–80%) at the injection site, headache (22–33%), and malaise (27–38%). Among the tested regimens, vaccination at weeks 0, 4, and 8 via split-dose needle-free injection generated the highest immunogenicity: 100% of the recipients developed neutralizing titers of ≥1:100 with geometric mean titer of 304 (measured by a reporter replicon particle assay). The DNA subunit vaccine also elicited a significant T-cell response (Gaudinski et al., 2017). Based on these results, VRC5283 has advanced to phase II efficacy trial of vaccination at 0, 4, and 8 weeks via needle-free delivery (ClinicalTrials.gov identifier NCT03110770).

In a second DNA vaccine phase I study, Yebas *et al.* tested a DNA subunit vaccine (GLS-5700) containing a consensus sequence of prM-E from pre-2016 human ZIKV strains. Human volunteers received three doses of either 1 mg or 2 mg of DNA via intradermal injection followed by electroporation at weeks 0, 4, and 12. No serious adverse events were found: Approximately 50% of the vaccinated individuals had injection-site pain, redness, swelling, and itching. By week 14, 62% individuals that received the vaccine developed neutralizing titers between 1:18 to 1:317 (measured by a microneutralization assay on Vero cells). Besides humoral response, the DNA vaccine also elicited T cell activation. Adoptive

transfer of vaccinated human serum protected 92% of the A129 mice when challenged with a lethal dose of ZIKV. Surprisingly, serum from five vaccinated people with no neutralizing titer (but with positive antibodies in an ELISA assay) protected the A129 mice in an adoptive transfer experiment (Tebas et al., 2017). These data suggest that mouse protection was independent of the neutralizing titer derived from vaccinated humans. The molecular mechanism that contributed to this independency remains to be determined.

Although the inactivated vaccine and DNA subunit vaccines showed promising immunogenicity in the clinical phase I trials, the longevity of protective immunity remains to be followed in humans. In non-human primates, two immunizations with inactivated vaccine or one immunization with the adenovirus-based subunit vaccine afforded protection against ZIKV challenge at one year post-vaccination, whereas two immunizations with a DNA subunit vaccine resulted in reduced protective efficacy and declined neutralizing antibody titers to sub-protective levels at 1 year (Abbink et al., 2017).

(III) Live-attenuated vaccine

Two approaches have been taken to generate live-attenuated ZIKV vaccines: (i) Engineering attenuating mutations in an authentic ZIKV isolate or (ii) generating a chimeric flavivirus using DENV, JEV, or YFV to express ZIKV prM-E genes. For approach (i), Shan *et al.* developed two live-attenuated vaccine candidates containing a 10-nucleotide (ZIKV-3′UTR-Δ10) or 20-nucleotide (ZIKV-3′UTR-Δ20) deletion within the 3′UTR of the ZIKV genome from a pre-epidemic Cambodian strain FSS13025 (Shan et al., 2017b). The Cambodian strain FSS13025 was selected because, compared with epidemic American isolates, this strain is attenuated in neurovirulence, immune antagonism, and mosquito infectivity (Xia et al., 2018). After a single-dose vaccination, both candidates showed efficacy in mice and non-human primates, among which the 20-nucleotide deletion candidate elicited neutralizing antibody titers of >1:1,000 (measured by an mCherry ZIKV assay) in two weeks and conferred sterilizing immunity in monkeys. The 3′UTR deletion vaccines could also prevent maternal-to-fetal transmission during pregnancy as well as male reproductive tract infection in mice. Importantly, these vaccine candidates exhibited an excellent safety profile, including >1,000-fold less neurovirulence than the licensed live-attenuated vaccines YFV 17D and JEV SA14-14-2 (Shan et al., 2017a; Shan et al., 2017b). For approach (ii), chimeric DENV-2 and JEV SA14-14-2 harboring ZIKV prM-E genes were reported to protect mice from ZIKV infection after a single-dose vaccination (Li et al., 2018; Xie et al., 2017). The JEV SA14-14-2 chimeric ZIKV vaccine was also shown to protect non-human primates from ZIKV infection and maternal-to-fetal transmission in mice (Li et al., 2018). Taking a similar approach, the National Institute of Health is developing a chimeric ZIKV vaccine using a live-attenuated DENV-4 vaccine backbone (rDEN4Δ30; Table 1).

Development of complementary vaccine platforms

Inactivated and subunit vaccines are not infectious, and usually require multiple doses and periodic boosting. In contrast, live-attenuated vaccines usually have the advantage of single dose, quick immunity, durable protection, and low cost. Since ZIKV is mostly endemic in developing countries, a vaccine with single-dose efficacy is of practical importance, particularly when immunizing populations in remote areas where multiple dosing and

periodic boosting could be challenging. The unique features of distinct vaccine platforms justify the development of complementary platforms in parallel to provide options for different populations, including pregnant women, women of child-bearing age, children, infants (with immature immune systems), healthy men, and the elderly (immune-senescence with increased risk of GBS). Specifically, non-infectious subunit and inactivated vaccines are desirable for pregnant women, immunocompromised individuals, and senior people; whereas single-dose live-attenuated vaccines may be preferred when immunizing non-pregnant healthy individuals and children (before reaching child-bearing age) living in or travelling to ZIKV-endemic areas, especially in developing countries.

Immune correlates

Neutralizing antibody titers of >1:10 have been established as correlates of protection for most licensed flavivirus vaccines in humans (Hombach et al., 2005). However, the correlates of protection for the DENV vaccine (Dengvaxia) remain elusive. For ZIKV vaccine development, the concept of neutralizing antibody titers as correlates of protection is generally supported by several lines of available evidence. (i) A strong correlation between neutralizing antibody response and protective efficacy has been consistently observed from various ZIKV vaccine platforms in both mice and non-human primates. In non-human primates, the threshold neutralizing titer required for protection was estimated to be log 2.0 to 2.1 (Abbink et al., 2017). (ii) Adoptive transfer of immune serum from vaccinated humans or monkeys to mice have approximated the minimal protective neutralizing titers to be log ≥2 (Modjarrad et al., 2017) and ≥1.77 (Abbink et al., 2016), respectively. However, as mentioned above, an independency was observed between the neutralizing titers in GLS-5700 DNA-vaccinated people and protection in human-sera-transferred mice (Tebas et al., 2017). It should be noted that the quantitative titers required for ZIKV protection may differ among mice, non-human primates, and humans. The minimal neutralizing titer required for protection in humans will be determined in the ongoing phase II efficacy trials. (iii) In DNA subunit vaccine-immunized mice, depletion of $CD4^+$ and/or $CD8^+$ T lymphocytes did not abrogate protective efficacy (Larocca et al., 2016), indicating that neutralizing antibodies represent the primary mechanism of protection, and that a T lymphocyte response may not be essential for protection. However, $CD8^+$ T lymphocytes were recently shown to play a role in reducing ZIKV burden in mice (Elong Ngono et al., 2017). Further, it is known that a cellular immune response could shape the quality and longevity of the humoral response. Therefore, vaccine optimization should also include T cell responses.

Knowledge gaps and challenges

Despite promising progresses, a number of knowledge gaps remain to be addressed for ZIKV vaccine development. First, ZIKV infection causes GBS at an incident rate of about 1 in 4,000 to 5,000 infected adults (Dos Santos et al., 2016). If viral antigen(s) are responsible for the cause of GBS disease, we need to identify the antigen(s) that elicit antibodies that attack the peripheral nerves. Once such viral antigen(s) has been defined, the vaccine candidates should be re-engineered to exclude the cross-reactive epitope(s). Unfortunately,

animal models that can recapitulate human GBS are currently not available to study the molecular details of the disease.

Second, whether ZIKV vaccine-induced antibodies enhance the infection of closely related DENV and vice versa. Since ZIKV, DENV, and other flaviviruses co-circulate in many geographic regions, such cross-reactive antibody-mediated enhancement poses a major challenge for ZIKV vaccine development. So far, a complex interplay has been reported between ZIKV and DENV infections. (i) DENV or WNV antibodies can enhance ZIKV replication in cell culture and mice (Bardina et al., 2017; Dejnirattisai et al., 2016), but such enhancement has not been observed in non-human primates (Pantoja et al., 2017). (ii) On the contrary, prior infection of rhesus macaques with ZIKV led to a significant enhancement of DENV-2 viremia that was accompanied by neutropenia, lympocytosis, hyperglycemia, and higher reticulocyte counts, along with the activation of pro-inflammatory monocyte subsets and release of inflammatory mediators (George et al., 2017). This result indicates that prior ZIKV infection could enhance subsequent DENV-2 infections. (iii) Among the four serotypes of DENV, a pediatric cohort study showed that the risk of severe dengue disease was the highest within a narrow range of preexisting anti-DENV antibody titers, whereas all symptomatic dengue diseases were protected at high antibody titers (Katzelnick et al., 2017), supporting cross-serotype enhancement among sequential DENV infections in humans. (iv) For ZIKV itself, sub-neutralizing levels of antibodies against ZIKV did not enhance the replication of subsequent ZIKV challenge in non-human primates, even though these antibodies exhibited enhancement effects in cell culture (Abbink et al., 2017). Besides the antibody-mediated interplays between ZIKV and DENV described above, cross-reactive T cell responses from prior DENV infection could also influence the outcome of ZIKV infection in mice and humans, suggesting a role for prior DENV immunity in shaping the T cell response to subsequent ZIKV infection (Grifoni et al., 2017; Wen et al., 2017). Furthermore, the interplay between T cell and antibody responses is important as the affinity matured, long-lived antibody response is dependent of $CD4^+$ T cells. Given the above knowledge, it is perceivable that the immune response to ZIKV vaccination and its potential impact on subsequent DENV infection will be different between individuals with and without prior flavivirus immunity. An optimal ZIKV vaccine should be designed to elicit virus type-specific, long-lasting protective antibodies with minimal cross reactivity that may enhance other flavivirus infections. For achieving this goal, studies should be pursued to define the epitopes responsible for virus-specific neutralizing antibodies.

Third, given the decline of ZIKV human cases, efficacy testing in both non-pregnant participants and pregnant women is challenging. Development of a controlled human infection model for ZIKV will accelerate many aspects of ZIKV research and countermeasure development, particularly for down-selecting vaccine candidates for clinical development, for demonstrating proof-of-concept efficacy, and for defining correlates of protection in humans. The controlled human infection model will also be invaluable to evaluate antiviral efficacy for therapeutics development. Such controlled human infection model has been successfully developed for DENV-2 and used for DENV vaccine development (Kirkpatrick et al., 2016). Regulatory approval requires careful ethics review about the risks to human volunteers and social benefits of the controlled human infection of ZIKV.

Fourth, since approximately 80% of ZIKV-infected individuals are asymptomatic and even the symptomatic individuals exhibit mild disease, a well-defined correlate of protection and mechanisms of *in utero* infection are needed for rapid and accurate assessment of vaccine efficacy. As discussed above, correlates of protection derived from the ongoing phase II clinical trials and possibly from controlled human infection model will facilitate the pre- and post-licensure studies.

Fifth, a reliable diagnostic assay for ZIKV infection is needed to support clinical trials (Balmaseda et al., 2017). Viral infection is an important endpoint to measure vaccine efficacy. During viremic period, viral RNAs can be reliably quantified using the well-established RT-PCR assays. After the viremic period, a serologic assay is required for detection of ZIKV infection. Since antibodies against viral prM and E proteins cross-react among different flaviviruses, it is critical to develop a serologic assay that could differentiate among pre-existing flavivirus antibodies, ZIKV vaccine-induced antibodies, and subsequent ZIKV infection-induced antibodies. Such virus-type-specific serologic assay will greatly facilitate vaccine efficacy trials.

Six, vaccines administered before pregnancy have been shown to prevent ZIKV maternal-to-fetal transmission in mice (Richner et al., 2017a; Shan et al., 2017a). It remains to be demonstrated if vaccines administered after pregnancy (*i.e.*, maternal vaccination) can prevent *in utero* transmission of ZIKV in animal models. It is conceivable that immune responses may differ between non-pregnant and pregnant women. This leads to a number of inter-related questions: Is the correlate of protection for ZIKV infection in non-pregnant individuals different from that for prevention of *in utero* transmission in pregnant women? Does maternal vaccination require a dose that is different from the dose of preconception vaccination in order to prevent maternal-to-fetal transmission? Does prevention of CZS require both cellular and humoral immunity in pregnant women? Considerable research is required to address these questions through better defining the biology of ZIKV in humans and through designing and analyzing vaccine clinical trials.

Over the past 2.5 years, the collective efforts from academia, industry, and government have established a promising pipeline of ZIKV vaccine development. Although the number of human cases has dropped significantly since 2017, a vaccine that can prevent CZS remains urgently needed. We need to keep the momentum to bring safe and efficacious ZIKV vaccines to licensure.

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Table 1

Zika Vaccines in Development

Strategy	Antigen form	Candidate name	Sponsor	Clinical trial dose regimen	Phase I	Phase II	Reference
Whole inactivated virus	Formalin-Inactivated virus	ZPIV	WRAIR/BIDMC	2-dose (days 1, 29)	NCT02963909 NCT02952833 NCT02937233 NCT03008122	-	(Abbink et al., 2016)
		PIZV/TAV-426	TAKEDA	2-dose (days 1, 29)	NCT03343626	-	
		Zika virus vaccine (strain MR 766)	Bharat Biotech International	-	-	-	(Sumathy et al., 2017)
Subunit	DNA	GLS-5700	GeneOne Life Science, Inc./ Inovio Pharmaceuticals	3-dose (weeks 0, 4, 12)	NCT02809443 NCT02887482	-	(Tebas et al., 2017)
		VRC-ZKADNA085-00-VP (VRC5288)	VRC/NIAID	2~3-dose (weeks 0, 8; 0, 12; 0, 4, 8; or 0, 4, 20)	NCT02840487	-	(Dowd et al., 2016b)
		VRC-ZKADNA090-00-VP (VRC5283)	VRC/NIAID	3-dose (weeks 0, 4, 8)	NCT02996461	NCT03110770	(Dowd et al., 2016b)
	mRNA	mRNA-1325	Moderna Therapeutics	-	NCT03014089	-	(Richner et al., 2017b)
		mRNA-LNP	University of Pennsylvania	-	-	-	(Pardi et al., 2017)
	Viral vector	MV-Zika	Themis Bioscience	1~2-dose (day 0 or 0, 28)	NCT02996890	-	
		RhAd52–prM-Env	BIDMC	-	-	-	(Abbink et al., 2016)
		AdC7-M/E	BILS	-	-	-	(Xu et al., 2018)
	E protein	Plant-produced ZIKV E (PzE)	Arizona State University	-	-	-	(Yang et al., 2018)
		E protein from S2 insect cell	University of Hawaii	-	-	-	(To et al., 2018)

Strategy	Antigen form	Candidate name	Sponsor	Clinical trial dose regimen	Phase I	Phase II	Reference
	Peptide	AGS-v	NIH	2-dose (days 0 and 21)	NCT03055000	-	
	VLP	Zika virus-like particles	TechnoVax, Inc	-	-	-	(Boigard et al., 2017)
Live attenuated	Attenuated Zika virus	ZIKV-3′UTR-Δ10 and ZIKV-3′UTR-Δ20	UTMB/PAHO/IEC	-	-	-	(Shan et al., 2017b)
	Chimeric flavivirus	rDEN4Δ30 chimera	NIAID/NIH	-	-	-	
		JEV SA14-14-2-chimera (ChinZIKV)	BIME	-	-	-	(Li et al., 2018)
		YFV 17D chimera	Sanofi	-	-	-	

Notes: WRAIR, Walter Reed Army Institute of Research; BIDMC, Beth Israel Deaconess Medical Center; NIAID, National Institutes of Allergy and Infectious Diseases; VRC, Vaccine Research Center; NIH, National Institutes of Health; UTMB, University of Texas Medical Branch; PAHO, Pan American Health Organization; IEC, Evandro Chagas Institute, Brazil; BILS, Beijing Institutes of Life Science, Chinese Academy of Sciences; BIME, Beijing Institute of Microbiology and Epidemiology; symbols “-”, data unavailable.