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Review

The dengue vaccine pipeline: Implications for the future of dengue control

Lauren M. Schwartz[a,c,*], M. Elizabeth Halloran[b,c,d], Anna P. Durbin[e], Ira M. Longini Jr.[d,f]

[a] Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA, USA
[b] Department of Biostatistics, School of Public Health, University of Washington, Seattle, WA, USA
[c] Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[d] Center for Inference and Dynamics of Infectious Diseases, Seattle, WA, USA
[e] Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[f] Department of Biostatistics, College of Public Health and Health Professions and College of Medicine, University of Florida, Gainesville, FL, USA

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ABSTRACT

Dengue has become the most rapidly expanding mosquito-borne infectious disease on the planet, surpassing malaria and infecting at least 390 million people per year. There is no effective treatment for dengue illness other than supportive care, especially for severe cases. Symptoms can be mild or life-threatening as in dengue hemorrhagic fever and dengue shock syndrome. Vector control has been only partially successful in decreasing dengue transmission. The potential use of safe and effective tetravalent dengue vaccines is an attractive addition to prevent disease or minimize the possibility of epidemics. There are currently no licensed dengue vaccines. This review summarizes the current status of all dengue vaccine candidates in clinical evaluation. Currently five candidate vaccines are in human clinical trials. One has completed two Phase III trials, two are in Phase II trials, and three are in Phase I testing.

1. Introduction

Dengue virus (DENV) is a mosquito-borne flavivirus that infects at least 390 million people per year [1]. It is estimated that nearly half the world's population is at risk for dengue infection [1]. A recent report from the Pan American Health Organization points out that reported dengue cases in the America rose by a factor of five in the last ten years [2]. The primary mosquito vector for dengue, *Aedes aegypti*, continues to spread widely and into new habitats due to increased urbanization and climate change. The less efficient vector *A. albopictus* is also rapidly expanding its habitat [3]. Dengue has become the most rapidly expanding mosquito-borne infectious disease on the planet, surpassing malaria.

Dengue infection and illness are caused by four distinct DENV serotypes that cross-react immunologically. Infection with a particular serotype is believed to result in life-long immunity to that serotype and cross-protection to the other serotypes for up to two years [4]. People who have had a single primary infection have been observed to have a higher risk of severe dengue including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) upon a second infection, a phenomenon often attributed to antibody enhancement [5]. Infants with waning maternal dengue antibodies have been observed to be at higher risk of DHF and DSS compared to infants with no maternal dengue antibodies [6]. There is no specific effective antiviral treatment for dengue illness other than supportive care, especially for severe cases. Good case management of severe dengue cases can greatly reduce the death rate. The only current means for dengue control are various forms of vector control. However, vector control has been only partially successful in reducing dengue disease burden [7,8]. More effective vector control measures such as integrated vector control, the use of Wolbachia infection in mosquitoes [9], or genetically modified mosquitoes [10] could eventually prove effective, but implementation of these methods is probably years into the future. Against this backdrop of an expanding dengue pandemic and no effective means to mitigate spread, the potential use of safe and effective tetravalent dengue vaccines is a very attractive addition to dengue control. Even if only partially effective, the use of dengue vaccines could be highly beneficial in blunting dengue epidemics, and for increasing population-level immunity to the level where vector control could be more effective.

Dengue vaccines could have beneficial individual-level effects by reducing the probability of infection given exposure to an infected mosquito, i.e., vaccine efficacy (VE) for susceptibility to

* Corresponding author at: Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, M2-A885, Seattle, WA 98109, USA. Tel.: +1 2066677775.
E-mail address: laurenms@uw.edu (L.M. Schwartz).

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Table 1
Summary of dengue vaccine candidates in clinical development.

Vaccine candidate	Manufacturer	Vaccine type	Mechanism of attenuation or inactivation	Clinical phase	References
CYD	Sanofi Pasteur	Live attenuated	Yellow fever vaccine backbone, premembrane and envelope proteins from wildtype dengue virus	III	[11–34]
DENVax	Takeda	Live attenuated	Wildtype DEN2 strain attenuated in primary dog kidney cells and further attenuated by mutation in NS3 gene	II	[35–43]
TV003/TV005	NIAID and Butantan Institute	Live attenuated	Wildtype strains with genetic mutations	II	[44–59]
TDENV PIV	GSK and WRAIR	Purified inactivated	Formalin inactivated	I	[60–63]
V180	Merck	Recombinant subunit	Wildtype premembrane and truncated envelope protein via expression in the *Drosophila* S2 cell expression system	I	[64,65]
D1ME100	NMRC	DNA	Premembrane and envelope proteins of DENV1 are expressed under control of the human cytomegalovirus promoter/enhancer of the plasmid vector VR1012	I	[66,67]

infection, reducing the probability of clinical disease given infection or the probability of severe disease, i.e., VE for disease progression, or reducing the probability that an infected vaccinated person will transmit virus to a mosquito that bites him or her, i.e., VE for direct transmission. In addition, with increasing vaccine coverage in a population, vaccines could reduce the overall transmission in the entire community, even to unvaccinated people, and thus have indirect or herd effects. All of these individual-level and community-level vaccine effects need to be taken into account when assessing the potential effectiveness and impact of dengue vaccines. In this paper, we summarize properties of the dengue vaccine candidates that are currently in some stage of clinical development with vaccine trials in phases I–III (Table 1). We note that only one vaccine has made it to double-blinded, placebo-control, phase III vaccine trials, the Sanofi Pasteur tetravalent chimeric yellow-fever dengue (CYD) vaccine, as summarized below.

2. Overview of vaccines in clinical development

2.1. CYD (Sanofi Pasteur)

Sanofi Pasteur's CYD vaccine is a live attenuated tetravalent chimeric vaccine. For each of the four dengue serotypes the premembrane and envelope proteins from a wild type dengue virus are substituted into the yellow fever (YF) 17D vaccine backbone [11]. The first CYD clinical trial in healthy adults, which only assessed the serotype 2 vaccine strain, found a high dose ($5 \log_{10}$ plaque forming units (PFU)) elicited a strong neutralizing antibody response to DENV2. Participants previously given YF vaccine seroconverted to all 4 dengue serotypes [12]. This multivalent neutralizing antibody response was further observed in a phase IIa study in Australian adults. To safely mimic the dengue endemic target population, participants were vaccinated with YF vaccine or monovalent DENV1 or DENV2 vaccines one year before vaccinating with one dose of tetravalent CYD. In flavivirus-naïve participants, no participant seroconverted to DENV1 by day 28 and only ~22% had seroconverted to DENV2 (compared to ~60% who seroconverted to DENV3 and ~70% who seroconverted to DENV4). The pre-existing flavivirus immunity increased neutralizing antibody response to all four serotypes compared to flavivirus-naïve participants [13].

The first phase I study in children was conducted in the dengue non-endemic region of Mexico City. Children aged 2 to 17 years received three doses at 0, 3.5 and 12 months. Seropositivity rates after the first dose were lowest for DEN1 and DEN2 [14]. A phase I trial conducted in the Philippines, where both dengue and Japanese encephalitis are endemic, compared the immunogenicity of three doses of CYD at 0, 3.5 and 12 months to only two doses of CYD at 3.5 and 12 months. 85% of participants were seropositive to all four serotypes regardless of the dosing schedule [15]. Early studies in flavivirus-naïve adults compared a 0, 4, and 12–15 month dosing schedule to a 2-dose schedule at 4 and 12–15 months. In the three-dose group all participants seroconverted to all four serotypes, while in the two dose group 92% seroconverted to DENV1 and 100% seroconverted to DENV2–4 [16]. To limit viral interference and subsequently increase immunogenicity and balance the immune response in naïve populations, Sanofi moved forward with a 0, 6, and 12 month dosing schedule.

Several phase II studies have been conducted throughout the world in adults and children. In the dengue-naïve population of Singapore, immunogenicity data on 600 participants found that after three doses of CYD at 0, 6, and 12 months 66.5% of those vaccinated were seropositive to all four serotypes, though seroconversion rates were higher in children [17]. A study of 300 2–11 year olds in Peru with 82% of children YF seropositive at baseline found 94.1% to be seropositive to all four serotypes after the third CYD dose. The overall antibody geometric mean titer (GMT) was higher in participants who were dengue seropositive at baseline compared to participants who were dengue seronegative at baseline [18]. A trend of higher seroconversion and GMT antibody response in baseline *Flavivirus* seropositive participants has also been seen in phase II studies in Brazil [19], Malaysia [20], and Latin America (Colombia, Honduras, Mexico, Puerto Rico) [21]. A phase IIb proof-of-concept trial was conducted in 4002 Thai children aged 4–11. Children were randomized to placebo or vaccine with three doses at 0, 6, and 12 months. This was the first trial with a primary endpoint of vaccine efficacy and secondary endpoints including safety and immunogenicity. In this study the per-protocol vaccine efficacy of CYD against all serotypes was 30.2% and not statistically significant (95% confidence interval (CI): −13.4–56.6) [22]. Efficacy after at least one injection against serotypes DENV1, DENV3, and DENV4 was statistically significant (VE = 61.2%, 81.9%, 90.0%, respectively), though the trial was not designed or powered for this post-hoc analysis. Vaccine efficacy against DENV2 was not significant (VE = 3.5%, 95%CI: −59.8–40.5). The immunogenicity sub study in only 296 subjects found increased GMT (as measured by plaque reduction neutralization test (PRNT)) after the first, second and third doses for all serotypes. Investigators suggest that immunogenicity as measured by PRNT may not indicate protection, the GMTs were not high enough to protect this particular lineage of viruses, or there was an antigenic mismatch between the vaccine serotype 2 virus and the circulating DENV2 causing disease in Thailand. Further investigations showed that antigenic diversity between vaccine virus and wild type did not impact neutralization and was likely not a cause of the low efficacy [23]. Additional phase II safety and immunogenicity studies have been completed in Vietnam [24], the Philippines

[25], and India [26]. Phase II studies further examining immune interference by investigating 0, 2, and 12 month dosing schedules are ongoing [27,28].

Two large-scale phase III efficacy trials in Latin America (Brazil, Colombia, Honduras, Mexico, Puerto Rico) and Asia (Indonesia, Malaysia, Philippines, Thailand, Vietnam) have been completed [29–32]. In both studies participants were randomized 2:1 to vaccine or placebo for 3 doses at 0, 6, and 12 months with a primary outcome of vaccine efficacy against symptomatic, virologically-confirmed dengue more than 28 days after the third injection. In the study conducted in 5 countries in Asia among 10,278 children aged 2–14 years the per-protocol vaccine efficacy estimate was 56.5% (95%CI: 43.8–66.4). Two hundred and fifty dengue cases were identified in the per-protocol analysis but 595 children were identified with dengue starting after baseline. The vaccine efficacy after 3 doses for dengue hemorrhagic fever, severe dengue and hospitalized dengue was also statistically significant (88.5% (95%CI: 58.2–97.9), 80.8% (95%CI: 42.7–94.7), 67.2% (95%CI: 50.3–78.6), respectively). Similar to the phase IIb study in Thailand, while immunogenicity was high for all serotypes, the serotype specific efficacy for DENV2 in this study was not statistically significant while efficacy for serotypes 1, 3 and 4 was significant.

In the immunogenicity subset, more than 67% of participants were seropositive for any dengue serotype at baseline. In an exploratory analysis the vaccine efficacy in the baseline dengue seropositive group was 74.3% (95%CI: 53.2–86.3) and 35.5% (95%CI: −26.8–66.7) in the baseline dengue seronegative group [33]. Similarly, younger children were found to have a lower vaccine efficacy, possibly due to their limited exposure to dengue compared to older children, suggesting the vaccine boosts existing naturally-acquired immunity [34].

In the Latin American study, 20,869 children aged 9–16 years were randomized 2:1 to vaccine or placebo for 3 doses at 0, 6 and 12 months. The per-protocol vaccine efficacy estimate was 60.8% (95%CI: 52.0–68.0). In the modified per-protocol analysis for serotype-specific vaccine efficacy, all serotypes showed a statistically significant efficacy ranging from 42.3% for DEN2 to 77.7% for DENV4. In the immunogenicity subset 79% of participants were seropositive for any dengue serotype at baseline. An exploratory analysis found vaccine efficacy among children seropositive at baseline to be higher (83.7% 95%CI: 62.2–93.7) compared to children seronegative at baseline (43.2% 95%CI: −61.5–80.0) [32]. Vaccine efficacy also varied by country (77.5% in Brazil to 31.3% in Mexico) as the dominant DENV serotype circulating in countries differed (DENV4 in Brazil, DENV1 and DENV2 in Mexico).

2.2. DENVax (Inviragen/Takeda)

The DENVax vaccine candidate contains a mixture of whole live-attenuated DENV2 and chimeric DENV1, DENV3, DENV4 based on the attenuated DENV2 backbone. These are based on the previously developed DEN-2 PDK-53 vaccine. A wild-type DEN2 strain from a symptomatic patient in Thailand was attenuated by 53 serial passages in primary dog kidney (PDK) cells. The DEN-2 PDK-53 vaccine was originally derived at Mahidol University in Bangkok, Thailand, and has been tested preclinically and clinically since the late 1980s. The current DENVax vaccine is further attenuated by a mutation in the NS3 gene. DENVax vaccine strains for serotypes 1, 3 and 4 were created by replacing the premembrane and envelope proteins of the DEN-2 PDK-53-V with genes from the respective dengue virus wild type serotype [35].

There are currently several ongoing and completed phase I and phase II trials in dengue naïve and endemic populations. Two phase I studies in the US and in a dengue non-endemic region of Colombia assessed low and high doses of DENVax and different routes of administration (subcutaneous or intradermal) in healthy adults at 0 and 3 months [36,37]. In Colombia, both formulations and administration routes were safe and induced neutralizing antibody to all serotypes [38]. Two additional phase I studies compared vaccine administration via the traditional needle-syringe mechanism, a needle-less injector, and a needle-free Pharmaject Injector [39,40]. Different formulations of the vaccine are also being evaluated [41]. An ongoing phase II study of 344 children and adults assessing the safety and immunogenicity of the vaccine in Colombia, Puerto Rico, Singapore and Thailand started in 2011 [42]. In late 2014 a phase II study in 1800 children in Asia and Latin America commenced to examine immunogenicity of 3 dosing schedules (day 0 only, 0 and 3 months, day 0 and 1 year) [43].

2.3. TV003/TV005 (NIAID)

The National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIAID) took a different approach in examining the potential vaccine strains to be incorporated into their tetravalent dengue vaccine candidate. To ensure that each serotype specific strain included in the vaccine was safe, infectious, and immunogenic, NIAID tested several monovalent vaccines with similar genetic mutations in flavivirus naïve participants. By using strains with the same genetic mutation researchers attempted to create strains that were kinetically similar. This would allow for a balanced humoral response and could reduce immune interference. The vaccine strains for serotypes 1 and 4 are based on a wild type DENV1 and DENV4 strains with a 30 nucleotide deletion in the 3′ untranslated region (rDEN1Δ30 and rDEN4Δ30). Early studies showed both monovalent vaccines to be highly immunogenic [44] with some hepatotoxicity in rDEN4Δ30 when given at a dose of $5 \log_{10}$ PFU [45,46]. This resolved when the vaccine was given at lower doses ($3 \log_{10}$ PFU–$1 \log_{10}$ PFU). Attempts were made to generate a less toxic/reactogenic form of the serotype 4 strain, such as incorporating additional mutations [47,48]. NIAID moved forward with the original monovalent vaccine at a dose of $3 \log_{10}$ PFU.

Δ30 mutations in wild type dengue serotypes 2 and 3 were attempted in animal models, but were not found to be as attenuated as serotypes 1 and 4, and other methods of attenuation were examined. The vaccine for serotype 2, rDEN2/4Δ30(ME) uses the serotype 4 vaccine strain (rDEN4Δ30) and replaces the premembrane and envelope proteins of DENV2. This method resulted in a monovalent vaccine that was safe and induced 100% seroconversion to DENV2 [49]. The serotype 3 strain, rDEN3Δ30/31, is based on DENV3 with the 30 nucleotide deletion similar to the other strains, but an additional 31 nucleotides are removed upstream of the Δ30 mutation [50]. A different dengue 3 vaccine strain which inserted the 3′ untranslated region of the dengue serotype 4 vaccine candidate into DENV3 virus was also examined, but tetravalent vaccine studies showed a vaccine with rDEN3Δ30/31 induced a broader neutralizing antibody response [51].

Similar to other research groups, monovalent vaccines were evaluated when administered to participants with preexisting heterotypic DENV antibodies, induced by other monovalent dengue vaccines, as a surrogate for naturally acquired infection. The heterotypic vaccine induced a broad neutralizing antibody response to all four serotypes [52]. Different monovalent vaccine candidates were combined into five different tetravalent admixtures (TV001–TV005) to assess immunogenicity and safety of a single tetravalent dose. Each component was given at a dose of $3 \log_{10}$ PFU as a single subcutaneous dose with the exception of the DENV2 component of TV005 which was given at a dose of $4 \log_{10}$ PFU. TV003, which includes the vaccine strains previously discussed, elicited the most balanced and broad antibody response with 45% of participants seroconverting to all four serotypes after only 1 dose. Seroconversion rates were high for DENV1, DENV3, and DENV4 serotypes (85–100%) but lowest for DENV2 (50%) [51]. In that trial,

seroconversion was defined to study day 42 but it was noted that antibody titers in some vaccine recipients continued to increase after study day 42 and for that reason, seroconversion rates were monitored out to study day 90 in future trials. Sixty two percent of those vaccinated with TV003 developed a mild, transient, maculopapular rash. The rash was asymptomatic in all of those who developed a rash [53].

In a follow-on trial, the safety and immunogenicity of TV003 was compared with that of a second formulation containing a 10-fold higher dose of the rDEN2/4Δ30 component, TV005 [53]. A single dose of TV003 induced seroconversion rates to DENV1–4 of 92%, 76%, 97%, and 100%. Seventy-four percent of TV003 recipients mounted a tetravalent response. A single dose of TV005 induced seroconversion rates to DENV1–4 of 92%, 97%, 97%, and 97% and an overall tetravalent response in 90% of recipients. In both TV003 and TV005 vaccinated groups African Americans had a lower tetravalent seroconversion rate (53%) compared to non-African Americans (86%).

A new serotype 2 vaccine strain, rDEN2 Δ30-7169, is also being examined to find a vaccine strain with increased immunogenicity when part of the tetravalent vaccine [54–56]. A phase I study examining the durability of the antibody response of TV003 to 12 months is currently being conducted in dengue naïve adults [57]. Participants will be challenged with a second dose of vaccine at 12 months to evaluate the protection induced by the vaccine against infection with the vaccine given 12 months later. A phase II study in Thailand started in early 2015 to examine safety and immune response of two doses of TV003 at 0 and 6 months in adults and children [58]. TV005 is currently being evaluated in a phase II age de-escalation trial in Thailand. In collaboration with the Butantan Institute, a phase II study of TV003 in Brazil started in October 2013. Safety and immunogenicity of one dose (liquid or lyophilized) of TV003 in dengue naïve and dengue primed individuals will be evaluated with results completed by December 2018 [59].

2.4. TDENV PIV (GSK)

TDEN PIV is a tetravalent purified inactivated vaccine currently being evaluated jointly by GSK and Walter Reed Army Institute of Research (WRAIR). A phase I study of high and low doses in flavivirus naïve adults has already been conducted in the US [60]. Additionally, GSK is testing TDEN PIV with several adjuvants used in other vaccines. Aluminum hydroxide, AS01E, and AS03B have already been assessed as adjuvants with GSK's hepatitis B, malaria, and pandemic influenza vaccines, respectively. A recent phase I trial examined the safety and immunogenicity of TDEN PIV with these adjuvants at different doses in the US [61] and another trial, scheduled to end December 2016, is examining the vaccine in Puerto Rican adults, a dengue primed population [62]. A prime-boost strategy with TDEN PIV and a live attenuated dengue vaccine is also under evaluation in a phase II trial [63].

2.5. V180 (Merck)

V180 is a recombinant subunit vaccine based on the dengue wild type premembrane and truncated envelope protein (DEN-80E) via expression in the *Drosophila* S2 cell expression system [64]. The current phase I trial, scheduled to end January 2015, examines V180 at low, medium, and high doses in healthy adults at 0, 1 and 2 months. The vaccine is being assessed without an adjuvant, and with adjuvants ISOCOMATRIX and alhydrogel [65].

2.6. D1ME100 (NMRC)

D1ME^{100} is a monovalent plasmid DNA vaccine currently being examined at the Naval Medical Research Center (NMRC). The premembrane and envelope proteins of DENV1 are being expressed under control of the human cytomegalovirus promoter/enhancer of the plasmid vector VR1012 [66]. The first phase I proof-of-concept trial assessed the vaccine in flavivirus naïve adults via intramuscular injection using a needle free Biojector 2000 at 0, 1 and 5 months at high and low doses [67]. The vaccine was safe but there was no neutralizing antibody response to the low dose, and only 42% of participants with the high dose produced an antibody response that was not sustained long term. Future studies of this DNA vaccine include using alternate delivery methods, a prime boost mechanism, novel adjuvants or unique platforms for DNA vaccines.

3. Discussion

We have described the dengue vaccine pipeline that includes vaccines in phases I–III in clinical development. The dengue vaccine constructs in some phase of clinical trials are live attenuated chimeric, sub-unit proteins, purified inactivated, and plasmid DNA. Other constructs such as virus-vectored, DNA and virus-like particles are still being preclinically tested.

There are several phase I–IIa studies examining vaccines for safety and immunogenicity. NIAID has tested several monovalent vaccines individually and in combination to produce a highly immunogenic vaccine with little viral interference [52,53]. Novel adjuvants and routes of administration are also being assessed in inactivated and recombinant subunit vaccines [61,62,65]. Results of these early phase I studies are still unknown. As we learn more about the importance of dengue immunogenicity at baseline in efficacy trials, the importance of examining immunogenicity in dengue-naïve and dengue-primed individuals is clear.

Importantly, there is no established correlate of protection for an effective dengue vaccine. Currently immunogenicity studies to assess neutralizing antibodies for all four serotypes are required to move forward with a vaccine candidate. Cellular immunity is less often studied and could be equally important. Early studies of CYD demonstrated strong antibody response to all serotypes, but in large phase IIb and phase III trials DENV2 serotype specific vaccine efficacy was not significant [22,31]. It is unclear why the vaccine was not efficacious against DENV2 despite its ability to induce neutralizing antibody. Studies are currently ongoing to investigate the quality of the antibody elicited as well as the role of cytotoxic T cell response in protection. Of note, the vast majority of CD8 T cell epitopes for dengue are contained in the non-structural (NS) proteins [68], which are lacking in CYD. Novel methods for evaluating and characterizing immunogenicity are needed to better predict efficacy [69].

Because of the magnitude of the dengue problem worldwide, more than one of these vaccine candidates will be needed to ensure an adequate vaccine supply in the long run. As of this date, only the Sanofi CYD vaccine has made it through phase III trials. This vaccine has been shown to be safe and has different levels of efficacy against the four serotypes, with highest efficacy against serotypes 3 and 4. In addition, it has been shown to have very high efficacy against severe clinical disease and hospitalization due to dengue [31,33]. This vaccine has also been shown to work better in people with some prior dengue immunity, and to be less efficacious in those with no prior dengue immunity. Mathematical modeling has shown that a vaccine with suitable characteristics could be quite effective in reducing overall dengue illness levels in populations that receive such a vaccine over time by routine vaccination of children with a single catch-up campaign in older children and possibly adults [70,71]. Much more work needs to be done to determine how dengue vaccines, once licensed, could be deployed, but it is clear that dengue vaccines will soon join vector control as a means for dengue control.

Conflict of interest statement

MEH and IML receive research support from the Dengue Vaccine Initiative. Both are members of the Dengue Modeling Consortium with Sanofi Pasteur for which they have received travel money. Both are involved with the design and implementation of Phase IV evaluation of the Sanofi Pasteur CYD vaccine. Johns Hopkins has a contract with NIH to study vaccines, including dengue vaccines that are developed by the NIH. The contract is: NIH Contract No. HHSN272200900010 C "Operation of a Facility for the Study of Infectious Agents, Vaccines and Antimicrobials in Adult and Pediatric Human Subjects".

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