The Dengue Fever vaccine: How it can help against Zika
Objectives

• Epidemiology of Dengue and Zika
• Clinical disease manifestations of Dengue and Zika
• Vaccine Development for Dengue
• Vaccine Development for Zika
# World's Deadliest Animals

## Deaths per Year

<table>
<thead>
<tr>
<th>Animal</th>
<th>Deaths per Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito</td>
<td>725,000</td>
</tr>
<tr>
<td>Freshwater snail</td>
<td>110,000</td>
</tr>
<tr>
<td>Ascaris roundworm</td>
<td>60,000</td>
</tr>
<tr>
<td>Venomous snake</td>
<td>50,000</td>
</tr>
<tr>
<td>Rabid dog</td>
<td>40,000</td>
</tr>
<tr>
<td>Assassin bug</td>
<td>12,000</td>
</tr>
<tr>
<td>Tsetse fly</td>
<td>9,000</td>
</tr>
<tr>
<td>Tapeworm</td>
<td>2,000</td>
</tr>
<tr>
<td>Crocodile</td>
<td>1,000</td>
</tr>
<tr>
<td>Hippo</td>
<td>500</td>
</tr>
<tr>
<td>Elephant</td>
<td>100</td>
</tr>
<tr>
<td>Lion</td>
<td>100</td>
</tr>
<tr>
<td>Wolf</td>
<td>10</td>
</tr>
<tr>
<td>Shark</td>
<td>10</td>
</tr>
</tbody>
</table>

Source: Data Vizco

---

*Images: Top: Mosquito on human skin, Bottom: Hippopotamus and man in the wild.*
Deaths and lost productivity Worldwide due to Infectious Disease
Transmission

• Factors
  • Initiation and maintenance of epidemic

  1) Strain of virus
  • Strains vary in virulence, duration of viremia

  2) Density, behavior and competence of the vector
  • Rainy season
  • Increased transmission due to prolonged vector survival, shortens extrinsic incubation period

  3) Susceptibility of human populations
  • Host factors

  4) Introduction of virus into susceptible community
Dengue

- *Aedes aegypti, Aedes albopictus*

Zika

- *Aedes aegypti, Aedes albopictus*
Estimated range of *Aedes aegypti* and *Aedes albopictus* in the United States, 2016*

*Aedes aegypti* mosquitoes are more likely to spread viruses like Zika, dengue, chikungunya than other types of mosquitoes such as *Aedes albopictus* mosquitoes.
Emerging Infections

Emergence as a 2-step process

1) Introduction of an agent into a new host population
   - New infection
   - Variant of existing infection

2) establishment and dissemination within a new host ("adoption")
   - Variety of factors associated with "spread"

Vector borne diseases

• Spread supported/facilitated by:
  – Global trade
  – Ineffectiveness of vector control
    • Biochemical
      – Resistance issues
    • Removal of breeding grounds
    • Biologic targeting of mosquitoes
  – Urban crowding/living conditions
  – Poorly designed irrigation and water storage
  – Poor waste disposal
  – Increasing in global travel
  – Deforestation and habitat destruction
  – global warming?
Dengue and Zika

- Flavivirus
  - West Nile
  - Dengue
  - Yellow Fever
  - Tick-borne Encephalitis Virus (TBE)
  - Saint Louis Encephalitis (SLE)
  - Japanese Encephalitis Virus (JEV)
Dengue
Risk and Incidence

2 billion persons live in tropics/subtropics
- 40% of world’s population at risk

Most rapidly spreading mosquito-borne virus in the world
- 1950s annual case reports to WHO totaled 900
  - By 2005 annual case reports in 60 countries

Annually:
- 120 million travel annually to these areas.
- 50-100 million cases dengue fever annually
- 250-500,000 cases Dengue Hemorrhagic Fever
- Approximately 20,000 deaths, but limited knowledge from many corners of the globe.
Epidemic Dengue Hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS)

- Emerged over 50 years ago in Southeast Asia
- Emerged in 1981 in the Americas
- Emerged in 1989 in Southern Asia
- Since post-WWII
  - Incidence of DHF/DSS has increased 500 fold
  - One of the leading causes of pediatric morbidity and mortality in Southeast Asia
Dengue incidence

Worldwide incidence of Dengue

Average number of DF and DHF cases reported to WHO

Dengue as an emerging disease in the Americas
Trends in Incidence in Dengue Fever among Hospitalized Patients in U.S.

Figure. National estimates of dengue yearly incidence rates and 95% exact binomial confidence intervals (error bars), calculated by using data from the National Inpatient Sample, United States, 2000–2007. The trend (dotted line) is based on a logistic regression model fit by using generalized estimating equations. Note that the trend is curvilinear in the incidence rate, yet linear in the log odds of the incidence rate.
"New" Clinical Dengue Classifications

The WHO Classification (2009)

Symptomatic dengue infection

Dengue -/+ warning signs

Probable dengue
Live in/travel to dengue endemic area.
Fever and 2 of the following:
- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Any warning sign

and
- Supportive serology;
or
- Occurrence at the same location and time as other confirmed dengue cases

Warning signs**

- Abdominal pain and tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement > 2 cm
- Laboratory: increase in hematocrit concurrent with rapid decrease in platelet count

**requiring strict observation and medical intervention

Severe dengue

Any of the followings:
- Severe plasma leakage leading to shock or respiratory distress.
- Severe bleeding as evaluated by clinicians.
- Severe organ involvement
  - Liver (AST, ALT ≥ 1000)
  - CNS: impaired consciousness
  - Heart and other organs

- CID 2011:53 (15 September) - Srikitkhachorn et al
Clinical Disease Caused by Dengue Viruses

Dengue fever (DF):
- Fever
- Headache
- Retro-orbital pain
- Myalgia
- Arthralgia
- Hemorrhage
- Rash
- Leukopenia
- Neutropenia
- Elevated ALT / AST
- Viremia
- Serum antibodies

Severe dengue:
- Hemorrhagic Fever (DHF)
  - Fever (2-7 days)
  - Thrombocytopenia
  - Petechial rash
  - Bruising
  - Bleeding
  - Coagulopathy
  - Vascular leakage
- Pleural effusion
- Ascites
- Hemoconcentration
- Shock Syndrome (DSS):
  - Hypotension
  - Shock

Based on WHO 1975, 1997
Clinical Disease

- Classic disease
  - Incubation period 3-14 days (average 4-7)
  - May have asymptomatic disease or mild febrile illness

- 3 phases
  - 1) Febrile Phase
  - 2) Critical Phase
  - 3) Recovery Phase
Clinical

- Risk factors for severe disease
  - AB blood group
  - Race
  - Young age
  - Viral strain
  - Female sex
  - High BMI
  - Genetic variants of Human Leukocyte Antigen (HLA)
  - Possibly chronic disease: Sickle cell disease, diabetes, asthma

- Factors that Decrease risk of severe disease
  - Race
  - Malnutrition
  - Polymorphisms in Fcγ and Vitamin D receptor
Immunopathogenesis of Severe Disease

Antibody dependent Enhancement (ADE)

• After initial infection antibodies remain cross reactive with other serotypes

• Non-neutralizing antibodies could then mediate an increased uptake of virus into monocyte/macrophage

• Leading to increased viral replication, immune activation and cytokine release

Zika
The Beginning


Dr. George Dick
Countries and territories showing historical time-line of Zika virus spread (1947 - 2016)

- Senegal
- Pakistan
- Burkina Faso
- Côte D’Ivoire
- Cameroon
- Sierra Leone
- Gabon
- Indonesia
- Malaysia
- Nigeria
- Costa Rica
- Cambodia

World Health Organization

The Robert Larner, M.D.
College of Medicine

25
Zika Fever in Central and South America

As of January 16, 2016, the HealthMap digital surveillance system has detected a total of 9,920 confirmed* Zika fever cases in the region. A total of 76 media alerts originating from more than 15 countries were used to develop this monthly epicurve. (Source: www.healthmap.org/zika)

*In the event that a media alert included case counts for both confirmed and suspected cases, only confirmed cases were included. The true number of Zika fever cases is likely to be significantly higher.

Source: CDC. Data as of 3/09/16.
Symptoms of Zika

- Conjunctivitis
- Mild fever, Headache
- Skin rash (exanthema)
- Joint pain

Symptoms normally last for 2-7 days. Only 1 in 4 people show any symptoms at all.
Zika clinical signs

Lab Findings
- Low White blood cell counts
- Low Platelet counts
- Elevated Liver Enzymes
What is ZIKA?
What is microcephaly

Infant’s head is smaller than the heads of other infants of same age and sex

Can occur as a result of congenital insult or post-natal insult
Microcephaly

Range of Microcephaly Severity
## Selected causes of microcephaly

<table>
<thead>
<tr>
<th>Isolated microcephaly (true microcephaly, microcephaly vera)</th>
<th>Neuroanatomic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive (eg, autosomal recessive primary microcephaly types 1 through 6, Amish lethal microcephaly)</td>
<td>Neural tube defects (eg, anencephaly, hydranencephaly, encephalocele)</td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>Holoprosencephaly</td>
</tr>
<tr>
<td>X-linked microcephaly</td>
<td>Atelecephaly (aprosencephaly)</td>
</tr>
<tr>
<td><strong>Chromosomal abnormalities and syndromes</strong></td>
<td>Lissencephaly</td>
</tr>
<tr>
<td>Trisomies (eg, 21, 18, 13)</td>
<td>Schizencephaly</td>
</tr>
<tr>
<td>Monosomy 1p36 deletion</td>
<td>Polymicrogyria</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td>Pachygyria (macrogyria)</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>Fetal brain disruption sequence</td>
</tr>
<tr>
<td>Williams-Beuren syndrome (7q11.23 deletion)</td>
<td><strong>Metabolic disorders</strong></td>
</tr>
<tr>
<td>Cornelia de Lange syndrome</td>
<td>Maternal diabetes mellitus</td>
</tr>
<tr>
<td>Miller-Dieker lissencephaly syndrome (17p13.3 deletion)</td>
<td>Untreated maternal phenylketonuria</td>
</tr>
<tr>
<td>Wolf-Hirschhorn syndrome (4p deletion)</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td>Cri-du-chat syndrome (5p15.2 deletion)</td>
<td>Methylmalonic aciduria</td>
</tr>
<tr>
<td>Mowat-Wilson syndrome</td>
<td>Citrullinemia</td>
</tr>
<tr>
<td>Rubinstein-Taybi syndrome</td>
<td>Neuronal ceroid lipofuscinosis</td>
</tr>
<tr>
<td>Aicardi-Goutières syndrome</td>
<td><strong>Environmental causes</strong></td>
</tr>
<tr>
<td>Cockayne syndrome</td>
<td>Congenital infection (eg, cytomegalovirus, herpes simplex virus, rubella, varicella, toxoplasmosis, human immunodeficiency virus, syphilis, enterovirus, zika virus)</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td>In utero drug or toxin exposure (eg, alcohol, tobacco, marijuana, cocaine, heroin, antineoplastic agents, antiepileptic agents, radiation, toluene)</td>
</tr>
</tbody>
</table>

Environmental causes:

- Congenital infection (eg, cytomegalovirus, herpes simplex virus, rubella, varicella, toxoplasmosis, human immunodeficiency virus, syphilis, enterovirus, zika virus)
- Meningitis
- In utero drug or toxin exposure (eg, alcohol, tobacco, marijuana, cocaine, heroin, antineoplastic agents, antiepileptic agents, radiation, toluene)
- Perinatal insult (eg, hypoglycemia, hypothyroidism, hypopituitarism, hypoadrenalinism)
- Anoxia/ischemia
Rates of suspected Microcephaly in Brazilian states by year

Euro CDC
BRIEF REPORT

Zika Virus Associated with Microcephaly

Figure 3. Electron Microscopy of Ultrathin Sections of Fetal Brain and Staining of a Flavivirus-like Particle.
Panel A shows a damaged brain cell with a cluster of dense virions located in the disrupted endoplasmic reticulum. Remains of membranes derived from different cellular compartments and filamentous structures are also seen. A magnified view of the boxed area with virions clearly visible (arrows) is shown in Panel B. Panel C shows a group of enveloped structures with a bright interior, presumably indicating viral replication (arrow). Panel D shows a negatively stained viral particle with morphologic characteristics consistent with those of Flaviviridae viruses (arrow).
(II). PATHOGENICITY AND PHYSICAL PROPERTIES

(3) Zika virus is highly neurotropic in mice and no virus has been recovered from tissues other than the brains of infected mice.

(4) Cotton-rats, guineapigs and rabbits show no clinical signs of infection after intracerebral inoculation of late passage mouse brain virus.

(5) Monkeys develop an inapparent infection after subcutaneous inoculation with mouse brain virus. After intracerebral inoculation one of five monkeys showed a mild pyrexia, the others showed no signs of infection. Viraemia during the first week after inoculation has been found in all monkeys tested and antibody has been demonstrated by the 14th day after inoculation.

(6) Of 99 human sera tested, 6 (6.1 per cent.) have neutralized more than 100 LD_{50} of virus. Antibody has also been found in the serum of one of 15 wild monkeys tested.
Congenital ZIKV Infection
….not just microcephaly

- Microcephaly
- Brain atrophy
- Ventricular enlargement
- Intracranial calcifications
- Ocular defects
- Joint contractures

- Absence of the corpus callosum
- Agenesis of the vermis
- Thalamus absent
- Cataracts
- Hydrops fetalis
Guillain–Barré Syndrome (GBS)

Acute, immune mediated
May lead to paralysis
Roughly 25,000 cases/yr in the US
Preceding infection in previous weeks
10-30% will require mechanical ventilation
Most fully recover
Mortality rate 5%
Risk of GBS and Infectious Disease

Guillain-Barré Syndrome

*chance per million exposures*

- 1976 swine flu vaccine: 0.1
- Seasonal Flu: 17
- Zika virus: 240
- Campylobacter jejuni: 250-650
- Cytomegalovirus: 600-2,200

Results:

• 98% of the 42 patients with GBS had anti-ZIKV IgM or IgG, and 100% had neutralizing antibody against ZIKV compared with 56% of 98 patients in control group (p<0.0001)

• 88% of 42 patients with GBS reported symptoms of ZIKV infection 6 days before onset of neurological symptoms.

• Estimated rate of GBS with ZIKV infection = 1/5000
FIG 9 Temporal association between cases of Zika fever (blue columns) and GBS (red line) during the French Polynesian outbreak.
Guillain–Barré Syndrome Associated with Zika Virus Infection in Colombia

Beatriz Parra, Ph.D., Jairo Lizarazo, M.D., Jorge A. Jiménez-Arango, M.D., Andrés F. Zea-Vera, M.D., Ph.D., Guillermo González-Manrique, M.D., José Vargas, M.D., Jorge A. Angarita, M.D., Gonzalo Zuñiga, M.D., Reydmar Lopez-Gonzalez, M.D., Cindy L. Beltran, M.D., Karen H. Rizcala, M.D., Maria T. Morales, M.D., Oscar Pacheco, M.D., Martha L. Ospina, M.D., Anupama Kumar, M.B., B.S., David R. Cornblath, M.D., Laura S. Muñoz, M.D., Lyda Osorio, M.D., Ph.D., Paula Barreras, M.D., and Carlos A. Pardo, M.D.
68 patients with GBS

• 66 (97%) with previous symptoms c/w Zika
  • 42 patients with +PCR
    • Urine/CSF
  • ?increased risk/link between Zika and GBS with previous Dengue infection
Figure. Detection of Zika virus in blood and urine specimens of 6 patients by using real-time reverse transcription PCR with primers/probe 1086/1152c/1107-Cy5 (11) New Caledonia, 2014. A) Patient 1; B) Patient 2; C) Patient 3; D) Patient 4; E) Patient 5; F) Patient 6. Triangles indicate urine samples and squares indicate serum samples. The cutoff cycle threshold (Ct) value is 38.5, as previously reported (11) and is indicated by horizontal lines. Black symbols indicate amplifications with Ct < 38.5, gray symbols indicate amplifications with Ct ≥38.5, and white symbols indicate negative amplifications. Onset of disease (day 0) was defined retrospectively after questioning patients about initial symptoms. Dashed lines indicate a period >2 days without a sample being obtained. Arrows indicate onset of rash.
First Reported Case of Sexually Transmitted Zika Virus

Probable Non-Vector-borne Transmission of Zika Virus, Colorado, USA

BD Foy, RB Tesh et al.

- American scientist contacted Zika virus infection in Senegal in 2008 and transmitted virus to his wife after his return home
- Sexual contact implicated as transmission route
The algorithm below will help you determine whether or not to test your patient for Zika virus infection. For information on which test to use, see CDC’s interim guidance.

**If your patient is**
- Experiencing or has recently experienced symptoms of Zika*
- An asymptomatic pregnant woman

**Ask the following questions**

Does the patient live in or has the patient recently traveled to an area with Zika?

**YES**
- **Test for Zika**

**NO**
- Has the patient had unprotected sex with a partner who has lived in or traveled to an area with Zika?

**NO**
- **Do Not Test for Zika**

**YES**
Zika testing

• Serum
  • Antibody testing and PCR
    • IgM lasts up to 12 weeks (present by D4)
      • Cross-reactivity with other flaviviruses
  • Urine
    • Submitted alongside serum samples
  • CSF
  • Amniotic fluid
  • Tissue
  • Saliva
    • Alternative if blood cannot be collected
“Newer” recommendations

• Men
  • Wait 6 months from last possible exposure before trying to conceive with partner

• Women
  • Wait 8 weeks from last possible exposure before trying to conceive

• Pregnant women
  • PCR screening of blood and urine up to 14 days after last possible exposure
  • If evaluated 2-12 weeks after travel
    • IgM and PCR

CDC.gov. 2016
The Dengue Fever vaccine: How it can help against Zika

Sean Diehl, Ph.D.
Assistant Professor

Kristen Pierce, M.D.
Associate Professor

Medicine-Infectious Disease
The world’s most dangerous animals

Source: Gates Notes, 4/25/2014
How Vaccines Work

General Rule: The more similar a vaccine is to the disease-causing form of the organism, the better the immune response to the vaccine

Introduce the immune system to a pathogen in a “controlled” environment

Cause the immune system to remember the pathogen and to respond to it

Enable the immune system to effectively clear the pathogen to prevent disease
Arms of the Immune system

Front line defense
Innate immune cells

Dispatchers/coordinators
Antigen-presenting cells

Decision makers/archivists
T cells – Generals / commanders / admirals
B cells – fighter pilots
• make antibodies = missiles/bombs

Vaccines engage the immune system like an emergency preparedness drill to be ready for the real threat.
# Vaccine Types

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide</td>
<td>Typhoid</td>
<td>MMR, Flumist, YFV, JEV, Dengue, (Zika)</td>
</tr>
<tr>
<td>Subunit</td>
<td>Tetanus, HepB Pneumococcal</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Ebola, Zika (experimental)</td>
<td></td>
</tr>
<tr>
<td>Virus-like particle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated</td>
<td>HPV</td>
<td></td>
</tr>
<tr>
<td>Live attenuated</td>
<td>FLU shot</td>
<td></td>
</tr>
</tbody>
</table>

- **Reactogenicity:**
  - Polysaccharide: MAX
  - Subunit: MIN
  - DNA: MIN
  - Virus-like particle: MIN
  - Inactivated: MIN
  - Live attenuated: MIN

- **Pathogen - likeness:**
  - Polysaccharide: MAX
  - Subunit: MIN
  - DNA: MIN
  - Virus-like particle: MIN
  - Inactivated: MIN
  - Live attenuated: MIN

- **Ability to induce memory engage immune system:**
  - Polysaccharide: MAX
  - Subunit: MIN
  - DNA: MIN
  - Virus-like particle: MIN
  - Inactivated: MIN
  - Live attenuated: MIN
Why develop a live attenuated vaccine?

Live attenuated vaccines have been successful for other flaviviruses: yellow fever and Japanese encephalitis virus.

Highly immunogenic, requiring only one dose.

Expected to induce lifelong immunity.

Can be very economical to produce and can be manufactured locally in endemic countries.

Induces both humoral and cellular immune responses.
Challenges to dengue vaccine development

• Four serotypes cause disease
• Cannot predict circulation patterns
• Usually the 2\textsuperscript{nd} DIFFERENT dengue serotype is the culprit
• Need to avoid interference between viruses in vaccine
• Attenuation in a specific way
• Engaging the whole immune system (not just antibodies)
Dengue circulation patterns are unpredictable
Dengue is caused by any of four distinct dengue viruses:

- Dengue-1
- Dengue-2
- Dengue-3
- Dengue-4

Outside of virus (structural):

- Copying
- Decoy factors

Blueprint protection
Where do these vaccine candidates come from?
DENGUE 1 Western Pacific.

1974

From the serum of a Chinese traveler to Nauru, reporting mild dengue at the Pacific Biomedical Research Center in Honolulu, Hawaii
DENGUE 2

New Guinea C¹:
- 1944: Mild disease

Tonga²:
- 1974: Outbreak of mild disease, 17% infection rate.
- 1975: Severe disease, high attack rate.

Indonesia

Sleman, 1978, 32% infected, mild illness

Bantu, 1977, 65% infected, severe disease

were admitted to one of three study observation. Two of these were lo-

molic hospital in Sleman, and speci-

ing. Acute stage blood samples were
tified to serotype by the comple-

sera from which virus was isolated

by inoculation of serial tenfold
groups of male mosquitoes and the pre-

urotoes was determined as described

titers were calculated by the method

were negative for dengue and CHIK infections. All but four of the confirmed patients were children under the age of 15 years, with a majority in the 5- to 9-year age group.

Clinical manifestations of 39 confirmed patients with adequate information are shown in Table 1. The majority had only fever and nonspecific constitutional symptoms. Only five patients (13%) had overt hemorrhagic manifestations, and these were mild (epistaxis and gum bleeding). Likewise, only two patients (5%) (both children) had dengue shock syndrome (DSS) during our 2-week stay, and there were no deaths.

Acute sera of patients who showed a fourfold or greater rise in dengue HI antibody between sera. In Bantul, virus was isolated from 100% of patients with dengue HI titers of ≤40, whereas in Sleman virus was isolated from only 39% of patients with HI titers of ≤40 (P < 0.001).

The comparative dengue virus isolation rates from patients classified as primary and secondary infections in the two epidemics are shown in Table 4. Virus was isolated from all primary infections in Bantul compared to only 45% in Sleman. It should be noted that none of the patients classified as having primary infections had detectable dengue HI antibody in the acute serum. Isolation rates from patients classified as having secondary infections in Bantul and Sleman were 57% and 23%, respectively. These differences for both pri-
May 1981: Generally mild disease. Distinct from the concurrent 1981 Cuba outbreak, which led to severe disease – which was DENV2.
Dengue and Zika viruses carry their own blueprints and the host cell builds new viruses.
Live attenuation strategy: editing dengue genome

Outside of virus (structural)

- Copying
- Decoy factors

- Blueprint protection

DENGUE-1

DENGUE-2

DENGUE-3

DENGUE-4
Testing individual dengue vaccine candidates to ensure a balanced immune response (antibody)
Over 400 subjects at UVM and Johns Hopkins participated in testing of safety of each component and as a mixture.
Final tetravalent vaccine formulation TV003

DENGUE-1

DENGUE-2

DENGUE-3

DENGUE-4

Dosing

$10^3$

$10^3$ or $10^4$

$10^3$

$10^3$
TV003 gives balanced immune response to all four DENV

Sanofi (CYD)

NIH (TV003)

Takeda

ANTIBODY responses

After 1 dose

TV003 gives balanced immune response to all four DENV
Will it work? Dengue human infection model

NIH (TV003) – OR – PLACEBO

DENV2 Tonga

6 months

DENV2 Tonga

Kirkpatrick, Durbin, Whitehead, Pierce, Diehl et al. (2016) Science Translational Medicine

Cover story March 2016
Will it work?

- TV003 protects against DEN2 virus in blood
- DEN2 virus gives a rash
- TV003 protects against this

Rash:
- Mild (usually unnoticed)
- Lasts 1 day
- Indicates good response

---

**Primary treatment**

<table>
<thead>
<tr>
<th>AE</th>
<th>TV003 (n = 21)</th>
<th>Placebo (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>9.5%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Pain</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Tenderness</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Induration</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Viremia</td>
<td>0.0%</td>
<td>100.0%*</td>
</tr>
<tr>
<td>Fever</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Headache</td>
<td>23.8%</td>
<td>30.0%</td>
</tr>
<tr>
<td>Rash</td>
<td>0.0%</td>
<td>80.0%*</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0.0%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>0.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Myalgia</td>
<td>4.8%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>9.5%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14.3%</td>
<td>15.0%</td>
</tr>
<tr>
<td>Nausea</td>
<td>14.3%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Photophobia</td>
<td>4.8%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Elevated PT</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Elevated PTT</td>
<td>4.8%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0.0%</td>
<td>10.0%</td>
</tr>
</tbody>
</table>

---

What’s next for dengue vaccine

• How many serotypes needed to get protection? (2, 3, all 4?)

• Protection against other serotypes (DENGUE-3)

• Safety and efficacy in field trials
  • Bangladesh
  • Thailand
  • Brazil (17,000 subjects) 3 years

• Which parts of immune system are necessary for protection?

• Combination with ZIKA virus?
Flaviviruses

From Latin, *flavi* = yellow

- Yellow Fever Virus
- Dengue (Dengue 1, 2, 3, and 4)
- Japanese encephalitis (JEV)
- St Louis Encephalitis
- West Nile Virus

- Zika virus
ZIKA has a familiar structure

Key points:
- Icosahedral (soccer ball)
- Unique sugar structure – receptor binding?
- Stability
ZIKA is a stable flavivirus

Key points:

- Dengue virus loses infectivity at higher temperatures
- ZIKV retains infectivity even at 40°C
- Implications for unique transmission
ZIKV – cytopathic flavivirus

- All ZIKV strains are cytopathic
- Dengue not cytopathic

Dengue always looks like this even at high doses (cells fully intact)
Routes of ZIKA transmission

Main vector: *Ae. aegypti*
Other vectors involved:
- *Ae. africanus* (ZIKV, 1948)
- *Ae. furcifer*
- *Ae. taylori*
- *Ae. luteocephalus*
- *Ae. dalmatii*
- *Ae. opok*
- *An. coustani*
- *Mansonia uniformis*
- *Culex perifuscus*
- Others

Vector-borne

Non-vector-borne

Fig. 1 Summary of reported forms of transmission of Zika virus

# ZIKA vaccine approaches

<table>
<thead>
<tr>
<th>Type</th>
<th>Candidate</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killed virus</td>
<td>PaxVax, California</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>NewLink Genetics, Massachusetts</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>GSK, United States/Belgium</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Bharat Biotech, India</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>WRAIR/Sanofi Pasteur, United States and France</td>
<td>Phase 1: 2016–2017</td>
</tr>
<tr>
<td>Outer shell of virus</td>
<td>Protein Sciences, Connecticut</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Hawaii Biotech, Hawaii</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Bharat Biotech, India</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Replikins, Massachusetts</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Live attenuated</td>
<td>NIAID-LID/Instituto Butantan, United States/Brazil</td>
<td>Phase 1: Q4 2016</td>
</tr>
<tr>
<td></td>
<td>UTMB/Instituto Evandro Chagas, United States/Brazil</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Sanofi Pasteur, France</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Parts of ZIKA in another virus</td>
<td>Jenner Institute (chimpanzee adenovirus), UK</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Harvard University (VSV), Massachusetts</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Themis Bioscience (measles), Austria</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Parts of ZIKA as genetic pieces</td>
<td>NIAID-VRC (Biojector needle-free), United States</td>
<td>Phase 1: Q3 2016</td>
</tr>
<tr>
<td></td>
<td>Inovio Pharmaceuticals (electroporation), Pennsylvania</td>
<td>Phase 1: Q3 2016</td>
</tr>
<tr>
<td></td>
<td>GSK (RNA), United States/Belgium</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

*Table 1* Proposed Zika virus vaccine candidates/platforms

Experimental Zika viruses

- ZIKA is known to replicate less than DENV

- A genetic blueprint of ZIKA is now available

\[5' - C|prM|E|NS1|NS2A|NS2B|NS3|NS4A|NS4B|NS5 \Delta 30 3'\]
Challenges with rapid global vaccine development

• The infection has to be circulating and some go away (examples: Ebola, ZIKA?)

• ZIKA has typically been associated with small outbreaks (before this one)

• Hard to know exactly how virus behaves
Sustainability of a ZIKA vaccine

Dengue
• 390 million infections per year
• No congenital syndromes known
• Still mainly just mosquito transmission

ZIKA
• 1.62 million infections since 1947 (with 1.5M in 2015-2016)
  But…
  • Congenital microcephaly
  • Risk of Guillan-Barre
  • Unique transmission modes
Proposed dengue/ZIKA combination vaccines

Tetravalent (TV003/005)

rDEN1Δ30
rDEN2/4Δ30
rDEN3Δ30/31
rDEN4Δ30
rZIKVΔ30
rZIKV(prM/E)D2Δ30

DEN1  DEN2  DEN3  DEN4  +  ZIKV

The Robert Larner, M.D.
College of Medicine
The University of Vermont

84
The Challenge in Vaccine Development

Shift decision making to the left

20 years and $1B for Sanofi dengue vaccine
Vaccine Testing Center
University of Vermont College of Medicine

Center for Immunization Research
Anna Durbin
CIR clinic and lab teams

Stephen Whitehead

General Clinical Research Center