PERSPECTIVES IN THE DEVELOPMENT OF VACCINES AGAINST FLAVIVIRUSES

Thomas P Monath MD

M. Rossman

Thomas P Monath MD
Agenda

• State of the art of flavivirus vaccines
• Second generation vaccines
  – Rational design, balancing attenuation and immunogenicity
    • Viscerotropism and neurotropism
  – Multivalent live vaccines: interference
• Third generation vaccines
• Immune correlates
• Summary of vaccine profiles
## Vaccine indications

<table>
<thead>
<tr>
<th>Virus</th>
<th>Existing vaccines?</th>
<th>Target species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow fever</td>
<td>Yes</td>
<td>Human</td>
</tr>
<tr>
<td>Dengue</td>
<td>Expt’l</td>
<td>Human</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Yes</td>
<td>Human, horse, pig</td>
</tr>
<tr>
<td>Tick-borne encephalitis</td>
<td>Yes</td>
<td>Human</td>
</tr>
<tr>
<td>West Nile</td>
<td>Yes (vet), expt’l (human)</td>
<td>Human, horse, birds (e.g. goose)</td>
</tr>
<tr>
<td>Kyasanur Forest disease</td>
<td>Yes</td>
<td>Human</td>
</tr>
<tr>
<td>Murray Valley encephalitis</td>
<td>No</td>
<td>Human</td>
</tr>
<tr>
<td>St. Louis encephalitis</td>
<td>No</td>
<td>Human</td>
</tr>
<tr>
<td>Rocio</td>
<td>Defunct</td>
<td>Human</td>
</tr>
<tr>
<td>Louping ill</td>
<td>Defunct</td>
<td>Sheep, human</td>
</tr>
</tbody>
</table>
Flavivirus Vaccines- an Era of Progress

• New dengue vaccines are in advanced clinical development, one in Phase 3
  – Prospects for licensure as early as 2013-14
• New, vaccines against JE introduced in 2009-10
  – Inactivated, purified, alum adsorbed (Ixiaro®)
  – Live vectored, single dose (Imojev®)
• Vaccines against WN in Phase 2
• New vaccines against TBE and YF are in development
• Many are recombinant, rationally designed using infectious clone technology
• Third generation vaccine technology is advancing and showing promise
# Factors that facilitate vaccine development

<table>
<thead>
<tr>
<th>Factor</th>
<th>YF, JE, WN, TBE</th>
<th>DEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established immune correlate (seroprotection)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Animal model (immunocompetent, disease similar to humans)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Single serotype</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No immunopathology</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No chronic infection, immune evasion</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>High inapparent:apparent infection ratio or natural disease generally self-limited</td>
<td>Yes (except YF)</td>
<td>Yes</td>
</tr>
<tr>
<td>Vaccine/Infection</td>
<td>Time to Approval or Wide-Scale Use</td>
<td>Years</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>YF FNV (1931)</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>YF 17D (1936)</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>JE mouse (1954)</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>TBE Austria (1971)</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>JE SA14-14-2 (1981)</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>JE SA14-14-2 inactiv (2001)</td>
<td></td>
<td>8</td>
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<tr>
<td>JE ChimeriVax (1997)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>WN ChimeriVax (2000)</td>
<td></td>
<td>7 (vet)</td>
</tr>
<tr>
<td>DENGUE (1971)</td>
<td></td>
<td>?43</td>
</tr>
<tr>
<td>Vaccine Types</td>
<td>YF</td>
<td>DEN</td>
</tr>
<tr>
<td>-----------------------------------</td>
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<td>-----</td>
</tr>
<tr>
<td>(+) investigational, + commercial (v) veterinary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live, attenuated</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Live, flavi vector</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Defective, single cycle</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Non flavi vector</td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Inactivated</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Subunit E or EDIII</td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Recombinant VLP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>(+)</td>
<td></td>
</tr>
</tbody>
</table>
Evolution of Live Flavivirus Vaccines

1930s-1980s
- Yellow fever FNV (Dengue mouse brain)
- Yellow fever 17D
- DEN LAV (WRAIR)
- DEN LAV (Mahidol)

1990s-2000s
- DEN 1Δ30, 4Δ30 (NIH)
- YF chimeras (Acambis)
- DEN2 chimeras (CDC)
- DEN4 Δ30 chimera (NIH)
- Ad5 vector (GenPhar)
- Measles (Inst Pasteur)

2010
- Single cycle (Flavi and alphavirus)
  (UTMB, U Queensland, Carolina Vaccine Inst)

Empirical

Vector from established vaccine strain

1st generation

Rational design

2nd generation

3rd generation
Evolution of Inactivated Flavivirus Vaccines

First generation: Crude suspensions
1930s-1940s

Mouse brain
JE (Russia, Japan)

Chick embryo
JE (USA)

Tissue or Primary cells

Wild-type virus

Second generation: Purified virus
1950s-1970s,

Primary hamster kidney
JE (China)

Mouse brain, purified
JE (Japan)

Third generation: purified (adjuvanted)
1980s

Chick embryo cell (alum)
TBE (Austria)

1990s-2010

Cell line

Vero cell
JE (Japan, China)
JE SA14-14-2 alum (US → Austria)
YF alum (US)
Dengue (US)

Attenuated virus
## New Flavivirus Vaccines in Clinic

<table>
<thead>
<tr>
<th>Indication</th>
<th>Developer</th>
<th>Type</th>
<th>Stage</th>
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<tbody>
<tr>
<td>Yellow fever</td>
<td>Xcellerex</td>
<td>Inactivated</td>
<td>Phase I</td>
</tr>
<tr>
<td>JE</td>
<td>Sanofi Pasteur</td>
<td><strong>Live, recombinant</strong></td>
<td>Approval</td>
</tr>
<tr>
<td>Dengue</td>
<td>Sanofi Pasteur</td>
<td><strong>Live, recombinant</strong></td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>GSK</td>
<td><strong>Live, empirical</strong></td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>(NIH) Biologicals E, Butantan</td>
<td><strong>Live, recombinant</strong></td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Inviragen</td>
<td><strong>Live, recombinant</strong></td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Hawaii Biotech/Merck</td>
<td>Subunit</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>US Navy</td>
<td>DNA</td>
<td>Phase I</td>
</tr>
<tr>
<td>West Nile</td>
<td>Sanofi Pasteur</td>
<td><strong>Live, recombinant</strong></td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Hawaii Biotech/Merck</td>
<td>Subunit</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>NIAID/VRC</td>
<td>DNA</td>
<td>Phase I</td>
</tr>
<tr>
<td>TBE</td>
<td>NIH</td>
<td><strong>Live, recombinant</strong></td>
<td>Phase I</td>
</tr>
</tbody>
</table>
First Generation Live Vaccines

• Empirical passage, altered host range
  – Principles established by Pasteur (serial passage of rabies in rabbit brain)
• Tissue substrates (YF, JE) or primary cells (JE, DEN)
• Facilitated by animal model to assess attenuation (YF, JE)
• Dengue relied on in vitro markers (ts, plaque size), sometimes proved fallible
Live vaccines
The ‘See-saw Dilemma’
Empirical Passage

YF (French)
42
FNV
Mouse brain
134

FNV
Under-attenuated
Encephalitis 2-4%

YF (Asibi)
Chick embryo
114
176

YF (Asibi)
Chick embryo
114
176

JE (SA14)
PHK cells
100

SA14-5-3
(over-attenuated)

SA14-14-2
No recognized SAEs

Encephalitis 0.8/10^5
Hepatitis 0.4/10^5

Attenuation, NHP

Viscero
Neuro
### Viscerotropism and Neurotropism are Distinct Phenotypes

<table>
<thead>
<tr>
<th>YF Strain</th>
<th>Mouse (weanling)</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC (AST)</td>
<td>IC</td>
</tr>
<tr>
<td>Parent (wild-type)</td>
<td>Death, encephalitis (7-8 days)</td>
<td>Death, high viremia, hepatitis</td>
</tr>
<tr>
<td>FNV</td>
<td>Death, encephalitis (4-6 days)</td>
<td>Death, encephalitis</td>
</tr>
<tr>
<td>17D</td>
<td>Death, encephalitis (9-11 days)</td>
<td>Survival/mild encephalitis</td>
</tr>
</tbody>
</table>
Attenuation in incidence of disease following YF or JE vaccine vs. wt virus

**YELLOW FEVER**

- **YF wild-type**: 143 cases/1000 persons infected
- **YF 17D**: 0.01 cases/1000 persons infected

**DECREASE IN VIRULENCE**

- **14,300-fold**

**JAPANESE ENCEPHALITIS**

- **JE wild-type**: 7 cases/1000 persons infected
- **JE SA14-14-2**: 0 cases/1000 persons infected

- **>10^6-fold**
Immunogenicity of two live vaccines

Yellow fever 17D

Monath T & Chen L, unpub. 2010

JE SA14-14-2

Sohn et al Vaccine 1999;17:2259
Immunogenicity of inactivated vs. live first generation JE vaccine

- Live, SA14-14-2
  - Sohn et al Vaccine 1999;17:2259
  - Seroconversion: 96%

- Inactivated, SA14-14-2 (Ixiaro®)
  - 1 dose
  - Seroconversion: 96%
  - 2 doses
  - Seroconversion: 96%
  - 3 doses
  - Seroconversion: 94%

- Inactivated, JE-VAX®
  - Intercell AG Ixiaro®
  - Prescribing information

GMT (PRNT_{50})

0  50  100  150  200  250  300
Learnings No. 1

• Residual neurotropism (vicerotropism) are a feature of all live vaccines
  – Host and viral factors determine disease expression
  – There is a small percentage of the human population with genetically determined susceptibility to severe flavivirus infection

• Immunogenicity and attenuation are correlated
  – Reflected in dose response and nonclinical biomarkers

• A single dose of live vaccine is as immunogenic as multiple doses of inactivated antigen
  – Adjuvant effect, innate immunity, replication/antigenic mass
Second Generation Live Vaccines

• Rational design enabled by
  – infectious clone technology
  – an understanding of genome structure-function relationships
  – sequencing for QC

• Two general approaches:
  – Site directed mutagenesis or deletion
  – Chimeric constructs
  – These approaches are often combined

• Can take advantage of existing attenuated vaccines (e.g. YF, JE, DEN-2)
  – As ‘body parts’ in the chimeric constructs
  – As guides to construction
    • e.g. use of wild-type DEN strains as prM-E donors (experience with DEN-2 PDK-53 vaccine)
Second Generation Live Vaccines

Chimeric Vaccine strategy

– Heterologous vector

- Vector can be an existing vaccine
  - Provides a benchmark phenotype for nonclinical and clinical evaluation
- ?less interference between constructs with different donor gene serotypes
- Heterologous T cell epitopes, fewer cross-reactive CTLs where these may be undesirable (e.g. dengue)
Development of NIH Dengue Vaccines

**DEN1 Candidates**
- Under-attenuated
  - DEN1
  - DEN4
  - Under-attenuated
  - D1
  - DEN4
  - Over-attenuated
  - DEN1
  - DEN4
  - Over-attenuated
  - D1
  - DEN4
  - Under-attenuated
  - DEN1
  - Vaccine candidate

**DEN3 Candidates**
- Under-attenuated
  - DEN3
  - 30
  - D3
  - DEN4
  - Over-attenuated
  - DEN3
  - D3
  - DEN4 3’NCR
  - Vaccine candidate
  - DEN3
  - 30
  - Vaccine candidate
  - DEN3
  - 30,31
Learnings No. 2

• Construction is relatively easy, assessing biology is not

• Imperfect knowledge of
  – Molecular determinants of virulence (virulence is multigenic)
  – Epitope composition

• Achieving the right balance of attenuation and immunogenicity is challenging

• Genetic instability of RNA viruses remains an issue for live vaccines
YF 17D as a Vector

- Long history of use, approved in all countries
- Powerful immunogen (innate immune activation, self-adjuvanting)
- Single injection, low dose requirement
- Rapid onset of immunity (10 days)
- Durable immunity (≈life-long)
- No anti-vector immunity (prM-E replaced)
- Rare SAEs, but steps could be taken to dial in additional safety features
Yellow fever 17D; strong innate response drives adaptive immunity, balanced Th1/2

- IFNα

Integrated stress response
- EIF2K4, EIF2 phosphorylation
- calreticulin, protein disulfide isomerase, etc

Adaptive response
- TH1
- TH2
- CD8
- B cell

Pulendran B Nature Reviews/Immunol 2009;9:1
Construction of Chimeric Virus

1. Full length cDNA $\rightarrow$ SP6 transcribe to RNA
2. Transfect RNA (Electroporation)
3. Grow virus in Vero cell culture
4. Envelope proteins are JE
5. Replicative ‘engine’ is YF 17D
History of ChimeriVax™-JE (Imojev®)

1989
YF infectious clone (Rice)

1991
First inter-typic chimera (DEN) (Bray, Lai)

1996
YF/JE chimera (Chambers)

1997
Acambis initiates project

2000
Phase 1 initiated

1998
PMC license

2005
Phase 3 initiated

2010
Approval Australia, Thailand
SA14-14-2 Mutations

YF 17D Mutations

Non-structural YF 17D

C  prM-E SA14-14-2

Non-structural YF 17D

NS1\textsubscript{307}

NS2\textsubscript{A}\textsubscript{61, 110, 115, 126}

NS2\textsubscript{B}\textsubscript{109}

NS3\textsubscript{485}

NS4\textsubscript{A}\textsubscript{146}

NS4\textsubscript{B}\textsubscript{95}

NS5\textsubscript{836, 900}

4 nt

5'NCR

SA14-14-2 Mutations

I

III

Stem-anchor

138

279

315

439

107

176

177

227

264

274
Attenuation of a chimeric vaccine (ChimeriVax™-JE)

YF 17D

5’ C M E NS1 NS2a NS2b NS3 NS4a NS4b NS5 3’

Nakayama

YF 17D

5’ SA14-14-2 3’

YF/JE (SA14-14-2)

JE (SA14-14-2)

YF/JE (Nakayama)

JE (Nakayama)

YF 17D

Neurovirulence weanling mice i.c.

4 log_{10} PFU

Chambers et al J Virol 1909;73:3095
Guirakhoo et al Virology 1999;257:363
Attenuation of a chimeric vaccine (ChimeriVax™-JE)

\[
\begin{array}{cccccccc}
C & M & E & NS1 & NS2a & NS2b & NS3 & NS4a & NS4b & NS5 \\
\end{array}
\]

5’ Nakayama YF 17D 3’

5’ SA_{14-14-2} YF 17D 3’

prM E

- E107 L→F
- E138 E→K
- E176 I→V
- E279 K→M
- E315 A→V
- E279 K→R

Fusion peptide

Leu 107
Reversion at 3 or more specific sites in E required for neurovirulent phenotype

- E107, E138, E176
- E107, E138, E279
- E138, E176
- E107, E176
- E439 R->K
- E315 V->A
- E279 M->K
- E176 V->K
- E138 K->E
- E107 F->L
- YF/JE (SA14-14-2)
- YF/JE (Nakayama)

Mortality %

Learnings No. 3

• Chimerization process *per se* attenuates virulence
• Chimera of two empirically derived attenuated vaccines (SA14-14-2 and YF 17D) yielded a suitable candidate
• E gene principal determinant of virulence/attenuation
  – Insertion of highly attenuated prM-E from SA14-14-2 abrogated YF 17D neurotropism
• At least 3 E gene a.a. mutations produced neuroattenuation
• At least 3 reversions required to restore neurovirulence
Learnings No. 4

• YF 17D serves as a benchmark for attenuation of new flavivirus vaccines
  – Quantitative measures in comparative studies of neurotropism

<table>
<thead>
<tr>
<th>Mouse IC</th>
<th>Monkey IC</th>
<th>Human SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Death, encephalitis</td>
<td>Standardized YF neurovirulence test (histopath scores)</td>
<td>Neurotropic adverse event incidence 0.8 per 100,000</td>
</tr>
<tr>
<td>-Dose response/survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-8 day old mouse model</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

• However, attenuation of viscerotropism is more difficult to assess
  – Monkey viremia levels used as a biomarker, but no data to show correlation with YEL-AVD
Comparative biology YF 17D and ChimeriVax™-JE

Antibody
PRNT50, day 30 (human)

Viremia
Cyno mean peak (PFU/mL)
Human mean peak PFU/mL

Neurovirulence
Mouse (8d) log10 icLD50
Vicerotropism markers in inter-strain chimeras

Asibi/DEN4 prM-E: Not ill, no liver path
Asibi/17D prM-E: Not ill, mild liver path
17D/Asibi prM-E: Not ill, no liver path
Asibi: Death, hepatitis, ↑ proinflam. cytokines

Viremia Genome Copies/mL

McGee et al JID 2008;197:692
Vicerotropism markers in inter-strain chimeras

Inference of the study:

Chimerization of YF 17D by insertion of a heterologous prM-E sequence from a less hepatotropic virus (JE, dengue) will reduce likelihood of serious adverse events.
Growth of YF 17D, ChimeriVax-DEN, and wt DEN in human liver cells (HepG2)-similar in THLE-3

Brandler et al. AJTMH 2005;72:74
Immunogenicity of graded doses of ChimeriVax™-JE, human subjects

Monath et al J Infect Dis 2003;188:1213
ChimeriVax™-JE Phase 3– Statistical non-inferiority endpoints met
Efficacy Population (N=408/group)

Torresi et al. Vaccine 2010;78:7993
T cell responses in humans
ChimeriVax™-WN
WN E peptide pool stimulation

Monath et al PNAS 2006;103:10823
Learnings No. 3

- A single dose of live chimeric vaccine can provide superior immunity to multiple doses of inactivated antigen
- Rapid, durable N antibody response
- T cell responses to both the donor (E gene) and backbone
- Strain differences in donor E gene modulate antigenicity and neutralization
  - Other examples
    - YF 17D neutralization > YF Asibi
    - JE Beijing-1 > Nakayama
    - Den2 (American) > Den2 (Asian)
    - EDIII specific immunization
  - Implications for:
    - Selection of strains as vaccine candidates
    - Restricted epitope constructs (e.g. gene shuffling, EDIII)
    - Design of non-inferiority trials where two vaccines incorporate different strains
Dengue Vaccines in Development

<table>
<thead>
<tr>
<th>Phase 3 (monovalent)</th>
<th>Phase 3 (tetravalent)</th>
<th>Phase 2</th>
<th>Phase 1</th>
<th>Phase 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>sanofi pasteur (Acambis) ChimeriVax</td>
<td>GSK (WRAIR) LAV (empiric)</td>
<td>NIAID LAV (molec)+Chimeric</td>
<td>Inviragen (CDC) LAV+Chimeric</td>
<td>Merck (Hawaii BioTech) Subunit</td>
</tr>
</tbody>
</table>
Live Dengue Vaccine Candidates

Sanofi Pasteur

YF 17D

DEN1

YF 17D

DEN2

YF 17D

DEN3

YF 17D

DEN4

NIH

DEN1 Δ30

DEN2 Δ30

DEN3 Δ30,31

DEN4 Δ30-200,201

Inviragen

DEN2 PDK53

DEN3 PDK53

DEN4 PDK53

Alternate DEN 3 and 4 candidates under evaluation

Attenuated (Mahidol)
sanofi pasteur ChimeriVax™-DEN
Unmodified dengue prM-E

PUO359 (Thailand, human, 1980)

PUO218 (Thailand, human, 1980)

PaH881/88 (Thailand, human, 1988)

1228 (Indonesia, human, 1978)
Monovalent ChimeriVax™-DEN2, clinical data

**Viremia**
- ChV-DEN2 3 log: 1.6 log
- ChV-DEN2 5 log: 1.8 log
- YF-VAX® 5 log: 2.1 log

**N antibody**
- ChV DEN2 (3 log): 100%*
- ChV DEN2 (5 log): 100%

* Seroconversion rate

Guirakhoo et al. Hum Vacc 2006;2:60 and Acambis Protocol H-050-001
Viremia and antibody in human subjects, tetravalent ChimeriVax™-DEN (4 \(\log_{10}\) PFU of each virus) or YF-VAX®

These results were anticipated from the experience with LAV

Viremia

Day after inoculation

Day 30

N antibody Day 30

* Seroconversion rate
Interference between dengue serotypes in NIAID dengue vaccine tetravalent mixture

1 dose, antibody to homologous virus, 30 days

Monovalent (separate trials)

<table>
<thead>
<tr>
<th>PRNT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>DEN1Δ30</th>
<th>DEN2/Δ30</th>
<th>DEN3-3'Δ4Δ30</th>
<th>DEN4Δ30</th>
</tr>
</thead>
<tbody>
<tr>
<td>512</td>
<td>95%*</td>
<td>100%</td>
<td>80%</td>
<td>93%</td>
</tr>
<tr>
<td>256</td>
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<td>128</td>
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<td>16</td>
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<td>4</td>
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</table>

Tetravalent

<table>
<thead>
<tr>
<th>PRNT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>DEN1Δ30</th>
<th>DEN2/Δ30</th>
<th>DEN3-3'Δ4Δ30</th>
<th>DEN4Δ30</th>
</tr>
</thead>
<tbody>
<tr>
<td>512</td>
<td>60%*</td>
<td>40%</td>
<td>45%</td>
<td>95%</td>
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<td>256</td>
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<td>128</td>
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<td>64</td>
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<td>32</td>
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<td>16</td>
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<td>8</td>
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<tr>
<td>4</td>
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</tr>
</tbody>
</table>

* Seroconversion rate

Learnings No. 5
Interference

• High rates of seroconversion and high antibody levels when a single dose of monovalent vaccine (titer 3-4 logs) given to seronegative recipients
• When 4 vaccines mixed, antibody production lower and some serotypes predominate while others are missed
• Interference can be modified somewhat by increasing dose of less active components
• Same set of observations were historically seen for OPV
Mitigating Interference

**Live flavivectors**
- Multiple doses (interval allowing subsidence of innate and cross-reactive immunity)
- Balanced dose formulation
- Separate anatomical sites
- Heterologous pre-immunity (e.g. YF)
- Shuffled E gene

**Other approaches**
- Single live vector, tetravalent E or EDIII (Ad5, measles)
- Single cycle vector, tetravalent or monovalent mixture
- Inactivated viruses
- Subunit antigens
- DNA
- Prime-boost strategies
Multiple Dose Schedule (Live Vaccines)

- Heterologous cross-protection (Sabin) 6 mos.
- Interference (innate immunity)
- Interference, adaptive cross-reactive immunity
- Incomplete take
- ‘Missing’ serotypes take
- wt DEN exposure
- ?Risk of ADE
ChimeriVax™ Tetravalent
Seroconversion after 3 doses at long intervals (0, 4-6, 9-12 mo.), flavivirus naïve adults

![Graph showing seroconversion for different serotypes and all serotypes combined.]
ChimeriVax™ Tetravalent GMT after 3 doses in flavivirus naïve adults
Multiple dose requirement

• **Schedule issues**
  – Endemic market
    • Compliance, schedule different from EPI, potential for ADE
  – Travelers and military require rapid immunization

• **Original antigenic sin**
  – Secondary responses (on boosting) *may be* poor quality (cross-reactive) antibody
  – Vaccine should induce primary response to all serotypes on first dose
Subunit Vaccines
(Merck/Hawaii)

**Status, positives**
- Truncated E protein secreted from stably transformed Drosophila cells
- Alum or Iscomatrix® adjuvant
- Multiple dose schedule at short intervals
- No interference, tetravalent antibody responses in NHP
- No prM antibodies (ADE)
- Thermostable, liquid

**Challenges**
- Durability
- Non-neutralizing antibody, ADE
- CD8+ T cell responses
- High dose requirement
- Dose sparing requires investigational adjuvant
- COGs
- Prime-boost strategy required?

Note: Preclinical stage EDIII fusion protein vaccine development (Cuba)
Next Generation Vaccines

• **Major goals:**
  – Improved safety
  – Reduce interference (dengue)
  – Short interval boosting
  – Set immune response to all four dengue antigens on first dose
  – Durable response, strong T cell memory

• **Current approaches:**
  – Single cycle flavivirus
  – Heterologous defective or live vectors (adeno, alpha, measles)
  – DNA launch (single round infectious particles)
  – Inactivated virus with appropriate adjuvant
  – Recombinant VLPs

• **Status:**
  – Early stage, preclinical
Why Do We Need Next Gen Dengue Vaccines?

- Residual risk for second gen dengue vaccines
  - Still early in development
  - Efficacy not established
  - Safety
    - Rash, ALT, neutropenia
    - Rare AEs?
    - ADE, severe dengue?

- Requirement for multiple doses

- Large market opportunity with room for multiple products
Challenges for Next Gen Vaccines

• Clinical development
  – Increasingly difficult after 2\textsuperscript{nd} gen vaccine(s) approved due to ethical issues (placebo controlled trials) and decreasing incidence at established sites

• Regulatory path
  – Licensure based on non-inferiority (seroconversion, GMT) to licensed product (likely to be a live 2\textsuperscript{nd} gen vaccine)

• Showing marketing advantage, differentiation and label claims
Next Generation Live Flavivirus Vaccines

Attributes

• Single cycle ‘pseudoinfectious’ virus or live heterologous vectors
• Potentially higher safety, no progressive infection
• In vivo expression of immunogenic subviral prM-E particles and NS1 with native conformation of epitopes
• Memory, durability, Th1 responses should resemble live vaccines

Questions

• Sufficient antigenic mass and immunogenicity?
• Activation of innate immunity, durability?
• Interference?
RepliVAX™

• Single-cycle flavivirus with most of C gene deleted
• Modified to reduce potential for recombination (mutations in cyclization sequence of 5’ fragment)
• Packaging Vero cell line with non-cytopathic VEE replicon expressing C in trans
• High yields obtained in packaging cell culture
• Particles are infectious, but undergo only a single round of replication in the host
• prM-E and NS1 expressed, SVPs produced
• Not neurovirulent
• Immunogenic in several species
Immunogenicity for Hamsters after a single dose
(historical, not head-to-head)

**RepliVAX™-WN**

- 5.3 log IP
- 4.6 log IP
- 4.6 log SC
- 5.3 log SC

**Widman et al Vaccine 2009;27:5550**

**ChimeriVax™-WN02**

- 5.0 log SC
- GMT 3880

**Acambis IND BB#11241**
Non-human Primates Immunized with a single SC dose of live, chimeric or single cycle WN vaccines
(Historical, not head to head)

<table>
<thead>
<tr>
<th>PRNT50 Day 28-30</th>
<th>ChimeriVax™-WN</th>
<th>RepliVAX™-WN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyno 5 log</td>
<td>Baboon 5 log</td>
</tr>
<tr>
<td>Seroconversion</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Rhesus 4 log</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Rhesus 6 log</td>
<td></td>
</tr>
</tbody>
</table>

3735 (GMT)

Live Heterologous Vectors

- Large foreign gene carrying capacity
- Multiple foreign genes, tetravalent dengue constructs
  - Avoid interference seen with mixed monovalent vectors
- *Theoretical* improved design (EDIII based vectors)
  - Eliminate cross-reactive epitopes on prM and EDI and II (ADE)

![Diagram of viral epitopes]

- Improved safety
  - Single cycle vectors (e.g. Ad5, alpha replicon)
  - Live vectors based on licensed vaccines (measles)

Wahala et al PLoS Pathog 2010;6:e1000821
Ad5 Vectored Vaccine

• GenPhar (US)
  – Defective Ad5 with full-length E genes of DEN serotypes (sequence 2,4,1,3)
  – NHP developed high antibody titers (PRNT\textsubscript{50} 10^3) to all 4 serotypes, balanced response
  – Booster response (2 months) in face of anti-Ad5 aby
  – Protected against \textit{wt} DEN challenge

• Int’l Centre Genetic Engineering & Biotechnology (India)
  – Ad5 with DIII of 4 serotypes prime, DNA boost
  – Mice developed moderate N antibody responses to 4 serotypes
  – No effect of anti-vector immunity

Khanam et al Vaccine 2009;27:6011
Measles as a Vector
Inst. Pasteur; Themis

- Vector safe and immunogenic for infants (long clinical experience), N titers ≈1000
- Manufactured at very large scale, low cost
- Tetravalent, single DEN vector (no interference)
- “Serotype-specific” neutralization domains (EDIII)

Brandler et al. Vaccine 2010;28:6730
Measles as a Vector

• Questions/challenges
  – EDIII antigen target (human EDIII contains cross-reactive epitopes and contributes little to neutralization in natural dengue (Wahala, 2009; Midgley, 2010)
  – Immunogenicity sufficient, seroprotection level achieved?
  – Anti-vector immunity?
  – Dose requirements different from measles?

• So far, only CD46-IFNAR mouse data available
  – Dengue N antibody titers following 2 doses are very low
    • Mice lack IFN α/β receptors
  – However, previous work with measles-HIV (monkeys) is encouraging
**Alphavirus Replicon Construct**

**Manufacturing Process**

Replicon and RNA or DNA helpers are introduced into certified VERO cells by electroporation.

Virus-like replicon particles (VRP) harvested after 18-24 hours incubation (*Single cycle production*)

**Replicon RNA**

- Packaging Signal
- 26S promoter

**Split Helper**

- DEN prM-E
- Capsid
- glycoprotein

RNA or DNA expression systems; transformed cells

Single-cycle vaccine particles, containing the replicon RNA
Leaning No. 6

Many factors determine vaccine immunogenicity

- Attenuation, replication, antigenic mass
- Anatomical/cell tropism, antigen duration
- Antigenic structure, conformation
- HLA and cytokine/cytokine receptor gene polymorphisms
- Innate immunity and specific pathways activated
- Previous immunity to related viruses and original antigenic sin
- Interference and vaccine interactions
How much immunity is required?

- What is the level of antibodies corresponding to protection?
- Established for JE and TBE vaccines only \((\text{PRNT}_{50} \geq 10)\)
- Seroprotection for viscerotropic viruses (YF, DEN) may be 2 to 10-fold higher
- Dengue problematic because of cross-reactive epitopes and difficulty determining homotypic and heterotypic responses
  - Dengue infections occur in subjects with N antibody to the infecting serotype and strain
- Regulatory issues: use of immune correlates for vaccines where field efficacy cannot be shown (WN, YF)
Neutralization following natural infection with dengue is associated with PRNT$_{50}$ titers $>100$

Kraus et al J Clin Micro 2007;45:3777

Wahala et al Virology 2009;392:103
Cross-reactive N antibody does not protect against DF

Reference strain  Patient’s isolate

Endy et al JID 2004;189:990
## Immune correlates

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Virus</th>
<th>Correlate of protection</th>
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<tbody>
<tr>
<td></td>
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<td>PRNT</td>
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<tr>
<td>Encephalitis</td>
<td>Japanese encephalitis</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Tick-borne encephalitis</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Poliomyelitis</td>
<td>10</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Yellow fever</td>
<td>20-40</td>
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<tr>
<td></td>
<td>Hepatitis A</td>
<td>20</td>
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<tr>
<td>Febrile rash</td>
<td>Dengue</td>
<td>?&gt;100</td>
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<td></td>
<td>Measles</td>
<td>120</td>
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<tr>
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<td>Smallpox</td>
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## Vaccine Profiles

<table>
<thead>
<tr>
<th></th>
<th>Safety</th>
<th>Immune-genicity</th>
<th>Durability</th>
<th>T cells</th>
<th>Single dose</th>
<th>No interference</th>
<th>No adjuvant</th>
<th>Low COGS</th>
<th>Thermostable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Live, flavivector</strong></td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++*</td>
<td>+++</td>
<td>+++</td>
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</tr>
<tr>
<td><strong>RepliVAX®</strong></td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Live, non flavivector</strong></td>
<td>+++</td>
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<tr>
<td><strong>Inactivated</strong></td>
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<tr>
<td><strong>Subunit</strong></td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>
| **DNA (incl. single round)** | +++ | EP*** | +++ | +++ | ++ | +++ | +++ | +++ | +++ | ** Except dengue**
| **May be compromised by anti-vector immunity**
| **Electroporation**
Thank you!