Replication-Competent Viral Vectors
Farshad Guirakhoo, PhD.

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• Some examples of replication-competent vectors in clinical development
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Virus classifications
• According to the 2013 International Committee on Taxonomy of Viruses (ICTV), viruses are classified into 7 orders, 103 families, 22 subfamilies, 455 genera, about 2,827 species, and over 4,000 types yet unclassified
• This traditional ICTV classification is generally used in conjunction with Baltimore's modern classification of viruses based on their mRNA production

Baltimore classification

<table>
<thead>
<tr>
<th>Class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td></td>
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</tbody>
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Infectious diseases caused by viruses

With 15 million deaths per year, infectious diseases are responsible for one-third of all mortalities worldwide.

Vaccination is the most effective way to prevent deaths from infectious diseases

Targeted for eradication*: (Polio, Mumps, Measles, Rubella)

Eradicated: (Smallpox)

Vaccine preventable diseases now: Cancer (viral) Influenza, HA, HB, HE, YF, TBE, Rabies and many others**


Current approaches for development of viral human vaccines

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Approach</th>
<th>Example on the market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated</td>
<td>Whole killed virus</td>
<td>Influenza, Polio, Hepatitis A, Rabies</td>
</tr>
<tr>
<td>Subunit</td>
<td>Expressed and purified proteins</td>
<td>Hepatitis B, HPV</td>
</tr>
<tr>
<td>Live attenuated</td>
<td>Empirical attenuation</td>
<td>YFV, Varicella, MMR, Influenza, Smallpox</td>
</tr>
<tr>
<td>Vector Based</td>
<td>Some viral genes are replaced by protective genes from vaccine targets</td>
<td>JEV (based on YF vector)</td>
</tr>
<tr>
<td>Nucleic acid based</td>
<td>DNA or RNA is injected directly into the host using a delivery system or an adjuvant</td>
<td>None</td>
</tr>
</tbody>
</table>
Vector-based viral vaccines

- Viral vectors are composed of engineered RNA or DNA from a non-pathogenic virus carrying genes of a pathogen derived from a vaccine target.
- Safe and effective viral vectors were constructed for gene delivery, protein production, transgenic animal production, oncolytic therapy, as well as vaccines for infectious diseases and cancers.
- Numerous viral vectors (replication deficient/competent) are currently being evaluated for development of vaccines against ID including Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Cytomegalovirus (CMV), Respiratory Syncytial Virus (RSV), Dengue, and Ebola.

However, there must be a balance between safety and immunogenicity of viral vaccine vectors

Safety and efficacy of replicating and non-replicating vaccine approaches

http://www.iavireport.org/Back%20Issues/Pages/IAVI-Report-123300420-
GoToHandMultiply.aspx

Common barriers/challenges

- Qualification of new cell lines/packaging cell lines
- Size of insert (AAV 5kb, AV and Alphavirus up to 7kb, Poxvirus 50 kb)
- Genetic stability of insert
- Large scale transfection (e.g. Alphavirus)
- Anti vector immunity
  - Already present in humans (Vaccinia, HSV, Ad5, measles)
  - Induced after the first injection
- Requirement of a high dose
  - Requirement of high yield manufacturing capacity (HSV, Alphavirus)
- Requirement of multiple immunizations
- Requirement of prime-boost strategy (e.g. Adeno-MVA)
Common barriers/challenges

- Immunogenicity
  - Production of protective neutralizing antibodies and CMI
- Safety
  - Persistence in hosts (latency and reactivation in vivo)
  - Pathogenicity
  - Neurovirulence/viscerotropism
- Regulatory requirements (GMO issues)
- Environmental safety (GMO)
  - Reversion to virulence
  - Shedding from the vaccinated person
  - Natural vector (e.g., mosquito) transmission
  - Recombination with WT virus
  - In vitro, during manufacturing
  - In vivo, in host
- Differentiation of vaccine from natural virus

Replication-deficient viral vectors, characteristics & challenges

- Characteristics:
  - Composed of engineered RNA or DNA from a non-pathogenic virus carrying genes of a pathogen of a vaccine target
  - Non-pathogenic vector can also be used as a vaccine itself without carrying a foreign gene like HSV2 vector (d529) used as a vaccine for HSV
  - Vaccine is produced in vitro by transfection of viral genome into an approved cell substrate (helper cell line) providing the missing gene(s)
  - Replicate only in helper cell lines used for manufacturing
  - Generally undergo a single cycle replication in the host (human, animals)
  - The main reason, they are regarded as generally safe
- Do not generally require adjuvant

- Challenges:
  - Have not produced desired immune responses on their own
  - Moderate immunogenicity due to limited antigenic dissemination in the host
  - Require generally a high dose for induction of protective immunity
  - Vector immunity generally limits use of multiple inoculations
  - Insufficient expression of transgene in the host could lower its efficacy and duration of immunity
  - Genetic stability and generation of replication-competent viruses during manufacturing needs to be monitored
  - Potential limitation in size of transgene
  - Yield in a qualified cell substrate may not be sufficient for a cost effective product
### Replication-deficient viral vectors, some examples

<table>
<thead>
<tr>
<th>Viral Vector</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno (e.g., HIV, malaria, TB, FMDV, flu, Ebola)</td>
<td>- Produces an immune response</td>
</tr>
<tr>
<td>Adeno associated Virus (e.g., HIV)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>Alphavirus</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- VEE (infectious diseases, colon, prostate, and breast cancer)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>- SFV (e.g., HIV, HPV therapeutic)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- Sindbis (e.g., flu HA)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>flavivirus</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>- Replikun (Kunjin based vaccine, cancer, HIV, SIV)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- RV1 (e.g., HIV)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>- HSV1 (e.g., HIV)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- HSV2 (e.g., dl529)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>Lentivirus (e.g., melanoma, HIV therapeutics)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>Poxvirus</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>- Orthopox virus</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- MVA (e.g., HIV, TB, Measles, HPV, RSV, breast, colorectal [TroVax, 5T4], lung [MUC1, 122] and prostate cancer)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- NYVAC (e.g., HIV, HSV1)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- SFV (e.g., HIV)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>- HSV1 (e.g., HIV)</td>
<td>- Can be used for cancer therapy</td>
</tr>
<tr>
<td>- HSV2 (e.g., dl529)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>LCMV (e.g., HCMV)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>Polio virus (e.g., HIV, cancer)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>Sendai virus (e.g., dM-gp120 HIV)</td>
<td>- Can be used for gene therapy</td>
</tr>
<tr>
<td>VSV (e.g., dG protein vector for HIV)</td>
<td>- Can be used for gene therapy</td>
</tr>
</tbody>
</table>

### Characteristics:
- Composed of engineered RNA or DNA from a non-pathogenic virus carrying genes of a pathogen of a vaccine target
- Produced in vitro by transfection into an approved cell substrate
- No need for a helper cell line
- Undergo multiple rounds of replication in the host (e.g., human or animals) (in this case the human becomes the production factory for the virus)
- Safety can be increased by mutation or deletion mostly in virulent genes
- High level of induction of antibody and T cell responses in the host mimicking natural infections
- Require medium dose for immunization
- Do not require adjuvant
- Manufacturing is cost effective
- Could be effective as single dose vaccines

### Challenges:
- Genetic stability could be an issue if size of transgenes is too large
- Genetic stability not an issue in case of chimeric viruses
- Vector immunity could hamper multiple immunizations
- Balance between safety (attenuation) and immunogenicity (replication)*
- Genetic stability during virus passages undergoing multiple rounds of genome replication during manufacturing process needs to be monitored

* Live attenuated HIV vaccines are not safe (e.g., "Sydney blood bank cohort")
8 Accidental infections with HIV delta nef eventually developed low level of viremia (Nature 340, 863, 1992; Science 270, 988, 1995; Science 267, 1820)
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Rationale for development of replication-competent viral vector based vaccines

• Cost effective manufacturing
• Low dose requirement for seroconversion
  – (Sometimes 1000-fold less than a non-replicating vector)
• Immunogenicity similar to natural infection
• High level of antigen dissemination in the host
• Induction of both humoral and cellular immune responses
• Th1 type of response in the host
• Anti vector immunity not a significant problem for chimeric viruses
• No need for adjuvant, self adjuvanted
• No evidence of genetic instability during manufacturing or in humans
• Potential as a single dose vaccine
• Booster maybe required (1-10 years depending on the type of vector)
• Regulatory pathway similar to other live viral vaccines with the exception of requirement for filing a GMO dossier

Examples of replication-competent viral vectors in clinical development

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Indication</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV</td>
<td>HIV, Ebola, Marburg (Chimera)*</td>
<td>VSV G replacement with functional Env. VSV—hVSG in phi, induced T cell specific response, no causing serious AEs. VSV-Ebola in Ph1 ongoing</td>
</tr>
<tr>
<td>Sendai virus</td>
<td>Influenza</td>
<td>Prima-boost with DNA resulted strong suppression of Simian2358 replication after IV challenge. Phd. with Sendai-HIV gag by IAVI and DNAVEC, ongoing with AHSV prima-boost</td>
</tr>
<tr>
<td>Measles and CDV</td>
<td>Measles, CDV</td>
<td>Measles and CDV—immunogenic in macaques, in Ph3 I by IAVI Pasture. Used by mucosal route with high dose or exchanged envelope with GH with CDV to overcome preexisting immunity. CDV react is low in humans, no human disease</td>
</tr>
<tr>
<td>YFV</td>
<td>Yellow Fever, WNV, JE, Dengue (Chimera)*</td>
<td>Not NV for mice or monkeys by IC inoculation, do not replicate in mosquitoes, lack of recombination, YF-WN approved as a horse vaccine, YF-JE approved as human vaccine, YF-DEN successfully tested in 2 Ph3 trials (4,200 subjects)</td>
</tr>
</tbody>
</table>

Selected replication-competent vectors vaccines (chimera), marketed or in late stages of developments

• VSV vector for Ebola vaccine, Ph1
• YF-based vectors
  – YF-WN (Veterinary vaccine) marketed as PreveNile™
  – YF-WN (Human vaccine) completed Ph2 in elderly
  – YF-JE (Human vaccine), marketed as Imojev™
  – YF-DEN TV (Human vaccine) completed large Ph3 efficacy trials

* IMOJEV™ is trademark of Sanofi Pasteur
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Vesicular Stomatitis Virus (VSV) as a replication-competent vector for vaccine against Ebola

- Prototype member of the genus Vesiculovirus of the family Rhhabdoviridae
- An arbovirus (arthropod borne virus) transmitted by sand fly insects to animals, causes a mild flu-like illness in humans
- Genome contains a single molecule of negative-sense RNA, 11,161 nucleotide long which encodes five major proteins
- Poorly replicates in normal human cells (possibly due to intact interferon system)
- Used as an oncolytic virus for some cancer therapy (many cancer cells have reduced interferon system thereby allowing replication of VSV and lyzing cancer cells preferentially**)

19* Has recently been used as a vector for Ebola vaccine


**: Lee et al., Nature Medicine 6, 1075-9 (2000)

Ebola virus (EBOV)

- Member of Filoviridae family contains 2 genera, Marburg virus and Ebola virus)
- Single-stranded, negative-sense RNA, about 19,000 nucleotides codes for seven structural proteins and one non-structural protein
- Highly virulent virus causes a severe, often fatal disease (50-90% fatality) in humans and other mammals
- Its natural reservoir believed to be bats, particularly fruit bats, and is primarily transmitted to animals and humans through bodily fluids
- The first disease outbreak occurred in a remote village in Central Africa, near the Ebola River, in Democratic Republic of Congo, in 1976
- The most recent outbreak occurred in March 2014 in West Africa
- On August 8, 2014, WHO declared the new EBOV outbreak as a public health emergency of international concern

This has been the largest outbreak with more cases and deaths than all others combined (19,340 cases with 7,518 deaths in the countries most impacted by the epidemic (Jan 10, 2015)

20*: (http://www.who.int/influenza/outbreaks/ebolavirus)


Construction of VSV-Ebola vector (Chimera)

- Chimeric VSV-Ebola
  - The Glycoprotein (GP) of an attenuated strain of VSV was replaced by GP of EBOV
  - GP of EBOV is the only virally expressed protein on the surface of the virion which is the target of neutralizing antibodies
  - GP of EBOV is critical for virus attachment to host cell and fusion process to release EBOV genome into the host*
  - rVSV-ZEBOV-GP chimaera was non-pathologic in primates and mice
  - Protected 100% of non-human primates given 4 w before challenge**
  - Merck MSD holds an exclusive world wide license from NewLink Genetics for development and commercialization of the chimeric vaccine
  - After successful completion of the Ph1 trial, a large randomized, controlled Phase III study to evaluate the safety and efficacy of the VSV-EBOV vaccine will be conducted
  - However, sequencing of close to 100 EBOV from patients revealed that the virus mutates as fast as seasonal influenza which may pose challenges for the development of an effective vaccine***

21*: (Guirakhoo, F. et al. Science 325, 1571-7 (2009)
**: (Guirakhoo, F. et al. Science 332, 453-6 (2011)

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YF 17D vaccine virus as a replication competent vector

- YFV is the prototype virus within the Flaviviridae family
- Strain YF 17D was developed as a live, attenuated vaccine in 1937
- Used in >400 MM persons
- Single dose for complete immunization
- Efficacy ~100%
- Rapid immunity (<10 days)
- Lifelong immunity
- Approved for persons ≥ 9 mo
- Well tolerated, very rare serious AEs

ChimeriVax® platform*

Chimerization

- Replacing the envelope genes of the YF 17D vaccine virus with those of another Flavivirus
- The resulting live attenuated virus contains replication engine of YF 17D vaccine virus but the immunogen proteins of a vaccine of interest

ChimeriVax® is a registered trademark of Sanofi Pasteur Biologics. It was originally developed by Acambis, Inc. now part of Sanofi Pasteur

Status of ChimeriVax® vaccines

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>WN (ProveNile™)</td>
<td>Preclinical Ph 1 Ph 2 Ph 3 Registration Market</td>
</tr>
<tr>
<td>JE (IMOJEV™)</td>
<td></td>
</tr>
<tr>
<td>Dengue (Dengvaxia™)</td>
<td></td>
</tr>
<tr>
<td>WN (human)</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td></td>
</tr>
<tr>
<td>TBE</td>
<td></td>
</tr>
</tbody>
</table>
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ChimeriVax® platform
- Construction
- Preclinical and clinical studies
- ChimeriVax® JE
- ChimeriVax® WN
- ChimeriVax® Den

Genome organization
Flavivirus Genome and Polyprotein Processing
Single open reading frame 10,233 nucleotides

17D YF virus genome cloned as cDNA
Full-length cDNA = RNA
Exchange coat proteins sequences for vaccine virus (e.g. JE, WN, DEN)
Recombinant cDNA

A single dose of a LAV induces both humoral and cellular response and provides a long-term protection that outlasts standard vaccines or recombinant antigens.
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Preclinical studies of ChimeriVax® viruses

- **in vitro**
  - Genetic stability
  - Phenotypic stability
  - Biochemical and structural analysis

- **in vivo**
  - Immunogenicity in animal models
  - Viremia
  - Neurovirulence testing
  - Protection studies

- GMO related studies
  - Vector transmission
  - Reversion to virulence
  - Recombination

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YF-WN vaccine

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West Nile virus transmission cycle

- Enzootic (Maintenance/Amplification)
  - Amplifying hosts
  - Epidemic
- Incidental hosts?
- Epizootic
- Epidemic
- Amplifying hosts

- **Fever, headache**
- **Sore throat**
- **Backache**
- **Myalgia**
- **Arthralgia**
- **Fatigue**
- **Meningitis**
- **Encephalitis**
- **Death**

- **Encephalitis**
- **Death**

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Update WN human cases 1999-2013:
Outbreak in 2012 has been the country’s largest ever

Close to 40,000 WND cases since 1999 which represent an estimated 2 million infections with the virus in the US over the last 15 years.

ChimeriVax®-WN vaccine candidate (cloned)

Site-directed mutagenesis
Passage to make seed lots

ChimeriVax™WN01
Veterinary Marketed

ChimeriVax™WN02
Human vaccine Ph2

Molecular control of neurovirulence

West Nile
YF/WN chimera
Yellow fever
E mutations

Mortality (mouse) I.P. / I.C.

100% / 100%
0% / 100%
0% / 54%
0% / 0%
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Preclinical safety and efficacy

- Not neurovirulent for mice and monkeys by IC inoculations
- Not infectious for mosquitoes
- A single dose protected mice against IP challenge with 1000 LD_{50} wt WN
- Protected hamsters with a single dose against IP challenge with wt WN virus*
- Protected rhesus monkeys with a single dose against viremia, illness, and death after IC challenge with 5.3 log_{10} PFU wt WN virus**

**: Arroyo et al., J Virol, 2004

Summary Ph1 and Ph2 clinical trials of ChimeriVax®-WN02

- Ph1: Randomized, double-blind, placebo-controlled, in healthy volunteers aged 18–40 years
  - Well tolerated and highly immunogenic (100% seroconversion on D21 and >97% at 12M)
  - Induced a transient low viremia
- Ph2: Different age cohorts, including the elderly
  - Part 1: 18-40 years old to select the best dose for the elderly
  - Part 2: >64 years old
  - Well tolerated, the AE profile was similar between the dose groups
  - Highly immunogenic (>96% seroconversion) in all age groups including the elderly
  - Induced a transient low viremia

YF-JE vaccine

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Japanese Encephalitis

- Mosquito-borne (Culex spp) flaviviral infection
- ~3 billion people live in endemic regions
- Leading cause of childhood encephalitis in Asia
  - >50,000 cases per year
  - >10,000 deaths
  - Up to 50% of survivors have neurological sequelae
- Vaccine-preventable disease
- Need for an improved JE vaccine
  - Limited number of producers for existing vaccines
  - Multiple immunizations needed for inactivated vaccines

IMOJEV™: a new single dose live attenuated vaccine for Japanese Encephalitis

- First ever recombinant live vaccine licensed in human
- Constructed from two widely used LAVs:
  - YFV strain 17D
  - JEV strain SA14-14-2
- Grown in Vero serum-free
- No preservative or adjuvant
- Single dose for primary immunization
  - ≥4 log PFU in 0.5 mL per injection

* IMOJEV™ is the trade name of JE-CV which was originally developed as ChimeriVax™-JE by Acambis, now part of Sanofi Pasteur.
IMOJEV™: nonclinical safety studies
- Neuroinvasiveness (IP inoculation)
  - Not neuroinvasive (mice, hamsters, monkeys)
- Neurovirulence (IC inoculation)
  - Less neurovirulent than YF-170 vaccine virus (mice, monkeys)
- Viremia (IC or SC)
  - Low, transient viremia (monkeys)
- Toxicology (SC)
  - No adverse clinical signs and no toxicological findings (monkeys)
  - No organ dysfunction and no histopathological lesions (monkeys)
- Biodistribution (SC)
  - No shedding, and no virus detected in any organs (monkeys)
- Environmental risks
  - Genomic stability in vitro (Vero) and in vivo (monkeys)
  - No natural recombination (artificial recombination with WT virus reduced virulence of WT virus)
  - Does not infect mosquitoes by oral route

IMOJEV™: clinical development
- Adults
  - 9 clinical studies in Australia and USA
    - 2 Phase I/II trials
    - 5 Phase II trials
    - 2 Phase III trials
    - More than 2,450 adult subjects received IMOJEV™
    - Phase III studies in comparison to JE-VAX® and placebo
      - Mouse brain derived JE vaccines (e.g., JE-VAX®) were standard of care
        at the time of development
- Pediatric populations
  - Naive children aged 2 to 10 years in India
  - Previously immunized children as of 2 years of age in Thailand
  - Naive infants/toddlers as of 9m in India, Taiwan and S. Korea
    and 12m in Thailand and The Philippines
  - More than 12,000 pediatric subjects received IMOJEV™
    - Long-term follow-up to 5 years after single dose of IMOJEV™

Conclusions of clinical studies IMOJEV™
- A single dose of IMOJEV shows protective immune response
  - From 14 days after vaccination in adults
  - From 28 days after vaccination in pediatric populations
- In adults, data up to 5 years after a single dose of IMOJEV support
  the persistence of the protective immune response
    - Considered clinically sufficient for not recommending a booster administration
    up to at least 5 years after IMOJEV vaccination
- In adults, the overall safety profile of a single dose of IMOJEV is similar
  to that of three doses of the inactivated MBDV JE vaccine, with a trend
  for a better safety at injection site and quite similar to that of a placebo
- In pediatric populations, data up to 60 months after a single dose of IMOJEV
  support the persistence of the protective immune response*
    - Administration of a booster dose 12 to 24 months post primary single dose
      insures high level (90%) and long term seroprotection (>10 years)
- IMOJEV dossier for registration was submitted is 2009 in Asia

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Dengue disease: a major public health concern

- Transmitted from mosquitoes to humans
- World's fastest growing vector-borne disease
- Endemic in > 100 countries
  - 2.5 billion people currently at risk, rising to 5 or 6 billion people by 2085 (assuming climate change and population growth)
- Each year:
  - Close to 400 million infections per year
  - 96 million manifestation (any level of clinical or subclinical severity)*
  - 2 million severe disease (90% children)
  - 25,000 deaths
- Increasing geographic spread and burden of disease
  - Large parts of Europe, West and Central Africa, and South America face the threat of outbreaks**

Dengue virus and its vector

- Dengue virus is a Flavivirus
- 4 antigenically distinct viruses DEN-1, DEN-2, DEN-3, DEN-4
- Single stranded +RNA

* Virus is transmitted to humans by Aedes mosquitoes, especially A. aegypti
** Stagnant water is the main habitat for mosquitoes
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**Summary phase 1 trial YF-Den2 chimera***

- First in man trial of chimeric dengue
- Vaccine was well tolerated
- Safety profile consistent with that of YF-VAX***
- Induced low levels of viremia with short durations
- Induced neutralizing antibodies against homologous and heterologous dengue 2 strains in 100% of subjects
- Low cross reactivities to other dengue serotypes
- Level of neutralizing antibodies remained high after 1 year upon a single dose
- Preimmunity to YFV did not interfere with vaccination but enhanced the level of dengue cross reactive neutralizing antibodies
- These positive data put the dengue program on a fast track

**Phase 2 trial of TV YF-Den vaccine was however, disappointing**

- 4002 children in Thailand were inoculated with Tetravalent vaccine
- The overall efficacy was 30% and not statistically significant to that of control
- The reason for lower efficacy was mainly due to YF-DEN 2 chimera
- Nevertheless multiple Ph3 trials around the globe were planned to allow for precision in efficacy
Comparison of two Ph3 efficacy trials of YF-DEN tetravalent vaccine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ph3 in Asia*</th>
<th>Ph3 in Latin America**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study sites</td>
<td>Indonesia, Malaysia, The Philippines, Thailand, Vietnam</td>
<td>Brazil, Colombia, Honduras, Mexico, Puerto Rico (US)</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>16,175</td>
<td>20,809</td>
</tr>
<tr>
<td>Age range (Year)</td>
<td>2-14</td>
<td>9-16</td>
</tr>
<tr>
<td>No of doses</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Primary objectives: safety &amp; efficacy in preventing dengue disease</td>
<td>Safe and 51% efficacious against DF 92% efficacy against severe disease DHF/DSS</td>
<td>Safe and 61% efficacious against DF 95% efficacy against severe disease DHF/DSS</td>
</tr>
<tr>
<td>Additional objectives: efficacy against 4 serotypes</td>
<td>50% against DEN1 35% against DEN2 70% against DEN3 75% against DEN4</td>
<td>50% against DEN1 65% against DEN2 75% against DEN3 78% against DEN4</td>
</tr>
<tr>
<td>Pre-immunity factor</td>
<td>Efficacy higher in older population: 74% in 12-14 y old 60% in 6-11 y old 34% in 2-5 y old</td>
<td>Efficacy higher in seropositive subjects: 86% in sero+ 42% in sero-</td>
</tr>
</tbody>
</table>


Summary of YF-Den TV vaccine
- First successful construction of the first chimeric in 1999
- So far close to 40,000 subjects (ages from 9 m-60 years) have been inoculated with the vaccine virus
  - Includes >7000 in phase 2 and > 30,000 in phase 3 trials
- No SAE to vaccination
- Low level of viremia (Serotype 4 and 3 most frequently detected)
- Viremia lower in pre-immune subjects or after the 2nd or the 3rd dose
- Balance immune response to all serotypes with trend for higher efficacy in older population

Summary: replication-competent vectors
- Replication competent viral vaccines are licensed for human use and more anticipated entering into clinical trials in future
- VSV-Ebola (Merck) is the most advanced Ebola vaccine completing the Ph1 trial (J&J and GSK are entering clinic with replication-deficient vectors)
- YF-based vectored vaccines are the world’s only licensed replication-competent human vaccines
  - Single dose YF-WN chimaera (Prevenir® horse vaccine), marketed
  - Single dose YF-WN chimaera (Human vaccine) completed Ph2 in elderly subjects
  - Single dose YF-JE (Imojev™ human vaccine), marketed
  - Tetravalent YF-DEN1-4 successfully completed efficacy trials and expected to be marketed in 2015/16

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Replication Competent Viral Vectors
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