**Abstract**

Dengue virus (DENV) is the most prevalent vector-borne virus that affects humans, with close to 2.5 billion people living in areas where the virus can be transmitted. Currently, there are no vaccines or antiviral medications against DENV. Sanofi Pasteur’s live-attenuated vaccine (LAV) candidate against DENV, ChimeriVax™ Dengue (CYD), has shown promising results in SE Asia and the Americas. Overall efficacy against symptomatic disease is between 55% and 65%, while reducing the hospitalization rate by 80% and protection against severe manifestation of the disease by 95% over the 25 month Active Surveillance period [1,2]. The vaccine regimen consists of three doses given over one year period to induce a balanced immune response. We are currently investigating the potential of using the RepliVax™ platform to produce a tetravalent DENV vaccine consisting of single-cycle replication vaccines. These vaccine-development-reflective defective viruses could eliminate potential replication interference among the four replication-competent viruses in the LAV, potentially allowing for a reduced dose efficacy. The four RVWNDEN candidates, with three serotypes (1, 2, and 4), were cloned into the RepliVax™ backbone, which consists of the WN virus nonstructural (NS) genes and a truncated cap (C) protein [3]. Viral RNA was in vitro transcribed and transfected into baby hamster kidney (BHK) helper cells expressing WN C protein to generate virus. Serial passaging of the transfected BHK cells was performed, and viral stocks were used to inoculate 10 day old neonatal mice. The virus was passaged an additional fourfold until a high titer was observed on the 10th day of passage. The four RVWNDEN vaccine candidates were then used to inoculate 4 week old Balb/c mice. Inoculated mice were bled at various time points, and titers of the vaccine candidates were determined. The RVWNDEN1-3 and -4 were found to be protective in the Balb/c mouse model, with the RVWNDEN2 vaccine candidate showing a high level of protection against all four serotypes of DENV. These results suggest that the RepliVax™ platform could be a promising approach for the development of a tetravalent DENV vaccine.

**Characterization and Optimization of RepliVax™ Dengue: a single cycle tetravalent vaccine candidate**

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**Overview of RepliVax™ Technology**

![Diagram](image)

**Results**

- **Conserved Envelope Stem Region Important in Viral Fusion**
  - Figure 3: Two formulations of RVWNDEN were tested for immunogenicity in mice and compared to ChimeriVax™ Dengue (CYD). Both RVWNDEN formulations showed a similar effect after receiving a 31 day booster virus inoculation, showing RVWNDEN exhibits comparable immunogenicity to CYD. The Envelope mutant of RVWNDEN showed similar PRNT titers for a virus with a wild-type Env, suggesting that the stem mutation does not affect immunogenicity.

- **RVWNDEN4 Induces Similar Immunogenicity to CYD4 in Mice**
  - Figure 4: The conserved mutation found in RVWNDEN-3 stocks is located in the stem region of the Env protein, the major protein involved in viral membrane fusion. (A) A representation of the dimeric Env protein before being exposed to a pH lower than 6.2. (B) After being exposed to low pH, Env dissociates and folds into a trimeric confirmation. The hydrophobic fusion loop then binds into the outer layer of the target membrane bilayer, followed by further rearrangement that completes viral fusion. The stem region plays an important role in the formation of the hydrophobic fusion loop(C) Color coded schematic of the domains of the Env protein, with the stem region magnified (4).

- **Expression of Env from Plasmids Containing DEN prM/E Sequences**
  - Figure 7: Immunofluorescence staining of HEK-293FT cells 72 hours post-transfection with DNA plasmids expressing DEN-4 prM/E under the CMV promoter. Staining shows that the RVWNDEN wild type and DEN-4 Env #4 mutant express Env protein. Western blot optimization is currently ongoing to quantify differences in secretion between the wild-type DEN-4 and Env #4 mutant. The prM/E genes from all four DEN serotypes will be expressed using this assay to determine if higher titers can be correlated with increased Env secretion.

- **Conclusions/Next Steps**
  - 1. **Table: RVWNDEN4 Stocks for Immunogenicity Studies and A.A. Adaptations**
    - | Virus | Titre (ffu/ml) | Volume (ml) |
    - | RVWNDEN 1 | 3.70E+07 | 12 |
    - | RVWNDEN 2 | 1.38E+06 | 10 |
    - | RVWNDEN 3 | 8.06E+06 | 10 |
    - | RVWNDEN 4 | 1.22E+07 | 30 |
  - 2. **Table: Virus RNA was isolated from viral particles infected with the virus, and the nucleic acid was sequenced for mutations after passaging.**
    - | RVWNDEN1 | RVWNDEN2 | RVWNDEN3 | RVWNDEN4 |
    - | Mutation 1 | Mutation 2 | Mutation 3 | Mutation 4 |
    - | Mutation 5 | Mutation 6 | Mutation 7 | Mutation 8 |
    - | Mutation 9 | Mutation 10 | Mutation 11 | Mutation 12 |

- **References/Acknowledgements**