# Chapter 1

# INTRODUCTION

## 1.1 Background

Dengue virus (DENV) is the etiology of the most common arthropod-borne viral disease globally. It is also considered as one of the most important emerging infectious diseases in the world. World Health Organization (WHO) ranked dengue as the fastest spreading vector-borne viral disease carrying epidemic potential as it has been found that dengue incidence has elevated 30-fold since the past five decades (World Health Organization, 2012). Moreover, it has been estimated that there are at least 390 million DENV infections annually, of which an estimated 96 million manifest clinically with acute illness. Moreover, dengue is endemic in more than 128 countries, putting at least 3.9 billion people to be at risk of infection each year. (Bhatt et al., 2013 ; Brady et al., 2012). DENV, is a positive strand RNA virus belonging to *Flavivirus* family that is transmitted through the bite of blood-feeding mosquitoes, namely *Aedes aegypti* and, to a lesser extent, *Ae. albopictus* (Srikiatkhachorn, 2009 ; Tuiskunen Bäck & Lundkvist, 2013**).** Moreover, DENV consists of four antigenically related but immunologically distinct serotypes, namely DENV 1, DENV 2, DENV 3, and DENV 4. These serotypes are found circulating worldwide. Furthermore, infection from one serotype gives rise to life-long monotypic immunity that offers protection against that serotype only but not against the remaining three serotypes. Instead, antibodies that cross react but not neutralize the remaining three serotypes may paradoxically enhance infection to increase the risk of severe dengue during secondary infection (Tuiskunen Bäck & Lundkvist, 2013 ; Guzmán and Kouri, 2002; Leong et al., 2008). Without proper treatment the case fatality may exceed 20%. Additionally, specific licensed antiviral therapy for this treatment remains absent to this date hence the treatment relies only on supportive treatment (Alejandria, 2015; Whitehorn & Simmons, 2011). Vector control has been the only strategy to tackle the spread of infection although the results have not been satisfactory due to rural-urban migration,

rapid population growth, unplanned urbanisation, and insecticide-resistant mosquitoes (Gubler, 2002 ; World Health Organization, 2012). In spite of the fact that there have been numerous dengue vaccine candidates being in clinical trials, a dengue vaccine that is safe and effective regardless of prior DENV infection has not been discovered yet due to the complexity of dengue epidemiology, pathogenesis and population immunity which pose considerable challenges during the development of vaccine (Wichmann et al., 2017).

Live-attenuated vaccine (LAV) which arguably is the most potent form of vaccine, has been challenging to develop, especially since any dengue vaccine is expected to induce a balanced immune response against all four serotypes. Additionally, any vaccine needs to be safe and effective in preventing infection or development of clinically symptomatic dengue (Dar & Ghosh, 2015). Attenuation phenotype indicated by increase in replication rate, small plaque size, and increase in type I-interferon response are crucial factors for the development of LAV (Goh et al., 2016). Serial passaging has been used as an approach to produce attenuation phenotype in LAVs against two flaviviruses, which are yellow fever virus (YF17D) and dengue virus (DENV, PDK53) (Theiler & Smith, 1937 ; Yoksan, Bhamarpravanti & Halstead, 1986). However, this approach is time consuming. A laboratory in the Programme in Emerging Infectious Diseases in Duke-NUS Medical School has recently developed a rapid method in generating attenuated viruses as vaccine candidates. The laboratory cultures DENV in conditions known to increase the genome mutation rate. Mutations were introduced randomly by infecting DENV clinical isolates into Vero cells containing 5-fluorouracil (5-FU). Using such an approach, they identified viral variants that induces type-I interferon (IFN) expression by sorting for infected Huh-7 cells expressing green fluorescence protein (GFP) under the control of IFNβ-promoter – high GFP signal would contain genetic variants of DENV that induce type-I IFN robustly. Full viral genome sequencing would then be used to identify such DENV variants, which would then be rescued through infectious clone construction.

Besides generating new viral variants, the laboratory has also been active in defining the attenuating mutation of DENV2-PDK53 strain, which is currently under clinical development by

Takeda Vaccines. The laboratory has now defined which of the previously identified mutations, in nucleotide position (nt) 57 (C to T, 5'UTR), 2579 (G to A, NS1), and 5270 (Glutamic Acid to Glutamic Acid/Valine, NS3) contributed mechanistically to DENV attenuation (Butrapet et al., 2000 & Kinney et al., 1997). Therefore, this study also includes one PDK53 mutation (G2579A, NS1) to investigate whether DENV2 PDK53 mutation could also attenuate DENV3.

## 1.2 Research Objective

It is known that the conventional method for LAV is through serial passaging which requires a long time. Hence, using reverse genetic approach, this study aims to construct DENV3 infectious clones carrying the mutations identified from previous studies together with the PDK53 NS1 mutation. Using Gibson assembly to incorporate the fragmented virus, we are able to determine if mutations elsewhere in the genome would derive a virus with attenuated phenotype.

## 1.3 Hypothesis

Mutations in the viral non-structural proteins can contribute to attenuated phenotype of DENV characterized by increased in viral replication and IFN response as well as produces small plaque size.