Advances in dengue virus vaccines and therapeutic monoclonal antibodies

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Abstract. The prevalence of dengue fever (DF) in many countries has become a global burden. The pathogen, dengue virus (DENV) contains four serotypes, and oftentimes the antibody-dependent enhancement (ADE) due to the insufficient and imbalanced host immunity leads to severe dengue symptoms in heterologous secondary infections, posing a major difficulty ahead of modern medicine. Two technical routes, vaccine and monoclonal antibody (mAb) have been widely explored and testified at various levels. Tetravalent vaccines, especially chimeric live-attenuated viruses (LAV), have been proven to induce a relatively well-rounded immune response, while other categories including virus-like particle (VLP) are also of high potential. Therapeutic mAbs also have been shown to target epitopes that can be cross-neutralizing, covering not only individual structural and non-structural proteins but also quaternary conformation of virion surface. Undoubtedly limitations of previous research have directed the refinement of vector design, efficacy assessment, and other processes, although several challenges still exist today.

Keywords: Dengue virus; antibody-dependent enhancement; vaccine; monoclonal antibody.

1. Introduction

Dengue fever (DF) is an infectious disease propagated by mosquitoes (mostly *Aedes aegypti*) that is prevalent in tropical and subtropical regions. It can be classified based on the different extents of syndromes. At first time the patient would only develop mild DF with high fever, headache, nausea, rash, pain of muscles, joints, or eyes, etc., which is usually self-limiting and could be recovered within ten days. However, it would be more severe if the patient were infected a second time by another serotype. In this case, it would lead to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) with rapid breathing, restlessness, pale and cold skin, severe abdominal pain, persistent vomiting, etc. [1, 2]. According to a data report presented by PAHO/WHO, in 2022 there were over 2.8 million diagnosed cases with 1,290 deaths in the Americas [3]. In 2019 there were 22,407 reported cases in China despite well-rounded medical conditions and protections, and historically the southern Guangdong and Hainan provinces were more badly affected than others [4]. The urbanization, frequent international trade, and communications allow it to spread over one hundred countries and become a global issue. Dengue virus (DENV) belongs to the single-stranded positive RNA Flavivirus family with four serotypes, namely DENV1 to DENV4. They share 65-70% homogeneity in amino acid sequence, of which the difference is minimal in distinct genotypes within each serotype [5].

Although there is no remedy for treating DF, various types of vaccines and monoclonal antibodies (mAbs) have been explored against DENV and tested in cell cultures, animal models, and clinical trials. The early attempts of making DENV vaccine included consecutive passage of cell cultures to make live-attenuated viruses (LAV) [6]; however, it may still contain the capability of causing a disease. The idea of mAbs dates back to the 1980s when those from mice were first screened against DENV-3 [7]. Thanks to the revolutionary development in structural biology, especially the technology of cryo-EM, the researchers associated with the field of protein science were able to gain deeper insight into mechanism clarification, drug discovery as well as other aspects. Protein science plays a vital part in vaccine and mAb design since the precise epitope and the interaction pattern could be simulated, verified, and further modified.

The major challenge of confrontation of the disease comes from the phenomenon named antibody-dependent enhancement (ADE). Generally, the primary infection of one certain serotype would result

in lifelong protection against that serotype. However, during the secondary infection of another serotype, the virus would be more effective in the entry process due to the pre-existing weakly cross-neutralizing antibodies, followed by amplification of the infection and inflammation signals [5,8]. Nonetheless, previous research also shed light on strategies for effectively reducing ADE without significantly impeding the efficacy of the products either by developing a tetravalent vaccine or altering the Fc region of the antibody.

This paper will introduce the structural features of DENV, including its genomic information, spatial organization, and the mechanism of DENV infection. Then several examples of the advancement of DENV vaccines as well as therapeutic mAbs will be focused on, including design, effectiveness, and safety issues. Finally, we will look at a series of challenges and propose several future perspectives.

2. Principles of DENV infection

2.1. Genomic and structural organization of DENV

DENV is close to a spherical shape with 40-50 nm in size. The DENV genome comprises three structural proteins and seven non-structural proteins which are capsuled in a polycistronic single-stranded RNA (~10.7kb). On the 5' and 3' ends are the regulatory sequences [8]. The polyprotein is translated in rough ER and then cleaved by self or host proteases (NS2B-NS3 or signal peptidase respectively). The viral RNA is enclosed by capsid (C) proteins and further wrapped by a bilayer membrane with a coated protein shell (envelope). The envelope (E) protein contains a transmembrane (TM) domain and three ectodomains with β-barrel named EDI, EDII, and EDIII, in which EDI is associated with EDII and EDIII via hinge regions [8]. The E protein has different organizations corresponding with separate states of the virus. First, in immature virions the E proteins adopt a trimeric, spiky appearance due to the association with precursor membrane (prM) protein; then cleavage of prM by furin in Golgi apparatus triggers rearrangement of E proteins to become antiparallel dimeric arrangement during viral maturation and the virion surface becomes more smooth [8-10]. The surface E protein also contains various epitopes not only in the natural immune response but also in mAb design. The non-structural proteins have various intracellular functions including RNA replication and modification, blockage of host cell immune signaling pathways, protein cleavage, etc [8].

2.2. Mechanism of DENV infection in the host cell

At the beginning of the primary infection process, the virion is engulfed by the host cell via receptor-mediated, clathrin-dependent endocytosis [8]. EDIII contains an immunoglobulin (Ig) like domain and is responsible for receptor binding. The acidic condition inside the late endosome induces a conformational change of E protein from a 'head-to-tail' dimer to a 'face-to-face' trimer, in which protruding formation of the fusion loop leads to membrane curvature and fusion, allowing for RNA release into the host cytoplasm [11]. During the replication of the viral RNA, the negative strand is first synthesized, acting as the template for the amplification of the positive strand [5]. The virus makes use of host rough ER and Golgi for directing protein production and virion assembly.

2.3. ADE effect during secondary infection: the original antigenic sin

Generally, the first infection of any DENV serotype would lead to relatively mild syndromes with long-term specific protection. However, a second encounter with another serotype would result in more serious cases mainly because the self-antibodies produced during the first strike can be highly cross-reactive but less effective in neutralizing the other serotypes. Another explanation is that at the moment of the second strike, the concentrations of self-antibodies are below the threshold to efficiently neutralize the virion [5]. The ADE is accomplished when the immunocomplex formed by virion and cross-reactive antibody (the affinity is not strong enough to block all the epitopes and functional components on the viral surface) activates monocytes and macrophages via Fc receptor,

followed by endocytosis and viral activation. Since those antigen-presenting cells would circulate to the lymph node to trigger the adaptive immune response, it allows for the diffusion and acceleration of the infection towards multiple organs [2]. It was shown that the memory B cells were responsible for the ADE effect, and somatic hypermutation (SHM) was lowered upon the secondary infection [5]. The original antigenic sin lies in here because SHM is fundamental to affinity maturation of self-antibody production as well as B cell activation and it can be weakened by cross-reactive memory B cells. Furthermore, the memory T cells also contribute to the inflammation by secreting a series of cytokines, which together with macrophages and mast cells may cause plasma leakage and vascular endothelial damage. Meanwhile, the cross-reactive T cell receptor is also deficient in recognizing and eliminating infected cells during the secondary strike, thus providing less efficiency in resolving ADE [9].

3. Development of DENV vaccines

3.1. Strategies of DENV vaccine design

Given that DF has become a global threat in the past decades, numerous efforts have been devoted to vaccine development. Generally, the DENV vaccine can be divided into two mainstreams: replicate and non-replicate vaccines. Replicate vaccines contain live-attenuated viruses (LAV) with lower capability of eliciting symptoms but still being able to replicate *in vivo*, which can be achieved by consecutive passage in cell culture, directed mutagenesis, and chimeric with other types or subtypes of viruses. Non-replicate vaccines include inactivated virus, recombinant subunit vaccine (e.g., the ectodomain of E protein, E80), DNA vaccine (expression of viral proteins in APCs and activate immune response via MHC-I pathway), virus vectored vaccine, and virus-like particle (VLP) [8].

3.2. Recent advancements in DENV vaccines

Of all the vaccine candidates, two of them have been approved for clinical use under restrictions. Dengvaxia (CYD-TDV) is a three-dose tetravalent chimeric LAV vaccine derived from yellow fever (YF) vaccine 17D vector with the replacement of DENV1-4 prM and E homologs. It is the first vaccine licensed in 20 countries. The other one, TAK003, uses the backbone of attenuated DENV2 via PDK-53 cell culture and replaces the prM and E genes with other serotypes [12,13]. They were verified to be effective against all four serotypes from previous clinical trials; however, the protections were imbalanced. The dominant serotype-specific antibodies generated were against DENV4 and DENV2 respectively [12]. Moreover, Dengvaxia was reported to increase hospitalization rate in children under 9 years old and DENV naïve recipients [8]. In case of safety concerns, the baseline DENV serostatus can be examined by enzyme-linked immunosorbent assay (ELISA) of DENV1-4 NS1 IgG from both non-acute and acute dengue samples to exclude naïve subjects for guidance [14]. Recent data analysis of former phase II and phase III clinical trials suggested that the incidence rate of DHF in TAK003-treated (0.07%) was lower than that of the placebo group (0.25%) in 3 years after two doses and relative risk of hospitalization caused by virologically confirmed dengue (VCD) was 0.14 for DENV-positive subjects and 0.23 for DENV-negative ones [15], indicating that TAK003 had better performance in safety issues. Besides neutralizing antibody titers, activation of auxiliary CD4+ and cytotoxicity CD8+ T cells is another crucial determinant in eliciting immunity against DENV. It is believed Dengvaxia is less competent because the T cell response mainly targets DENV NS proteins and using the YF17D backbone lacks self-NS proteins. The TAK003 with DENV2 backbone would induce effector T cells against DENV2 which are also cross-reactive for other serotypes, corresponding with its efficacy and safety concerns [13].

TV003 is another tetravalent LAV candidate that is created by the deletion of a fragment in the 3’UTR region. A phase I clinical trial reported that participants generally showed mild, self-limiting adverse events including rash, headache, fatigue, and so on after dose 1 and dose 2. Viremia was detected higher in the DENV-naïve subgroup than DENV-experienced subgroup after dose 1 (97.2% versus 48.8% in any DENV), but the latter had higher seropositivity rates against all four DENV

serotypes 28 days post the first injection. Nonetheless, TV003 showed lasting protection in the majority of the participants. The seropositive rates against DENV1-4 in the DENV-naïve subgroup one year after dose 2 were 80%, 100%, 64%, and 80% respectively. All of the DENV-experienced subjects and over three-quarters of DENV-naïve subjects acquired tri- or tetravalent DENV immunity one year after the dose 2 [16]. Another phase 2 trial in Brazil showed that viremia was induced in over 80% of the participants, yet none of them belonged to DENV2. Although 88% of the participants developed tri- or tetravalent humoral response after a single dose, and seroconversion rates were similar among different DENVs (between 82% and 94%), the antibody titers varied greatly, with DENV2 being the highest and almost four-fold higher than DENV3. Nonetheless, the production of IFN-γ, an indicator of CD8+ T cell response, was detected in 94% of vaccinees of Butantan-DV, an analog of TV003 [17]. Further efforts could be made to reduce the imbalance of humoral immunity due to multiple possible reasons such as the prevalent viral genotype in the region of study, discrimination of immunogenicity in the intrinsic DENVs, and artificial vaccine design.

The VLP is an artificial platform with viral antigen assembly based on real conformations. Recently, an attempt at self-adjuvanted multivalent vaccine based on the novel VLP design has been proposed. This design utilizes an adenovirus vector encoding DENV2,3,4 capsid, prM, and E proteins with sequence modifications to facilitate processing and secretion processes. Electron microscopy results showed that components of DENV VLPs were able to self-assemble under experimental conditions. The epitopes on ED1 and ED2 were verified by antibody binding tests. In addition, the integration of α-galactosylceramide (α-GalCer), an adjuvant to the VLP construct resulted in significant NK cell activation *in* vitro, together with humoral and cytotoxic T cell responses in mice, enhancing the immunogenicity of this vaccine. Still, there were limitations including a lack of homogeneity in sample size and elasticity [18]. Another study suggested an approach to overcoming the low yield of DENV2 VLPs by substituting the stem and TM regions in the C terminal of the E protein with those of vesicular stomatitis virus (VSV) proteins. The recombinant products exhibited a higher secretion level and normal functionalities as wild-type DENV2 via a series of tests [19]. The innovation of VLP is to create a non-replicating, 'programmable' protein-based complex that structurally resembles a virion, and this research sheds further light on future clinical trials of refined products [18,19].

4. Progress in DENV therapeutic mAbs

4.1. Protein targets for DENV mAb design

Apart from precautionary vaccines, there is also an urgent need for strong neutralizing antibodies for pharmaceuticals. Undoubtedly mAb is prioritized since polyantibody repertoire of the natural immune response would be highly likely to induce ADE. The first class is serotype-specific mAbs whose targets are mainly conserved amino acids that belong to the specific serotype of the E protein (mainly EDIII). The second type is cross-reactive mAbs which targets the common structure as well as the conserved amino acids mainly in EDII and/or EDIII domains. A novel strategy of mAb design focuses on the common quaternary structure of E dimer/trimer, and several mAbs exhibited cross-protection against DENV1-4. Modification of the Fc epitopes could reduce binding with the Fc receptor, thus alleviating ADE. It is also used to increase the stability of the mAb [5].

4.2. Recent discoveries of DENV mAbs

In a relationship study of pre-membrane (prM) protein cleavage and flavivirus infection, the researchers isolated and investigated two anti-prM mAbs named prM12 and prM13 from mice inoculated with DENV2. The neutralization ability was negatively correlated with prM cleavage efficiency. K26 and E28 of DENV2 were the epitopes responsible for antibody binding. Cryo-EM single particle reconstruction revealed two modes of attachment pattern of immature DENV-mAb binding, with prM12 Fabs contacting the prM at a steeper angle. *In vivo* experiments showed that the mAbs were likely to induce ADE, but the modification of aglycosyl (N297Q) in Fc effectively

abrogated Fcγ receptor binding and promoted survival in mice. This research indicated that blockage of DENV can be achieved by targeting circulating immature virions. It also provided further insight that partially mature virion is probably involved in DENV pathogenesis independent of ADE [20].

The membrane-anchored, dimeric DENV NS1 protein is believed to have multiple functions, including engagement in viral replication inside the host cell, activation of macrophages, and complement leading to vascular damage in a hexameric, secreted form. To make matters worse, it could cross-react with self-proteins to exacerbate the syndromes. Nevertheless, it is believed to be excluded from ADE; hence it is a putative drug target, especially for severe dengue cases [21,22]. Recently a paper reported four human mAbs against DENV and Zika virus (ZIKV). The mAbs came from two patients with DENV2 infection. Of the four candidates, clone 8 (D25-2B11E7) showed the best results of cross-neutralization against DENV1-4 as well as ZIKV NS1 proteins, albeit with different affinities (with IC50 5.23–47.17 μg/mL against DENV1-4 and 20.21μg/mL against ZIKV 24h post-infection). The mAbs were shown to stimulate complement-mediated cell cytolysis and inhibition of viral replication. Clone 20 (D25-4D4C3) and clone 8 protected NS1-mediated endothelial leakage and massive pro-inflammatory cytokine stimulation *in vitro*. The experimental data indicated that the mAbs recognized amino acids 221–299 in the C-terminal region, yet the mechanism remains unclear [22]. Further study of structural information could be followed up to map the exact epitopes and reveal the interaction patterns of those mAbs. An *in vivo* study of another mAb 33D2 (which recognizes amino acid 112-120) in a mouse model also verified its efficacy of reducing bleeding time and skin hemorrhage, coupling with lysis of infected cells [23].

The sequence of fusion loop (FL) of EDII is highly conserved among flaviviruses (amino acids 72-76, 98-111). In a research paper, França et al. isolated three human mAbs and identified their interaction patterns. Antibodies were selected via phage display based on an FL peptide of ZIKV, and three candidates were then generated in a single-chain variable fragment (scFv) format by combining the enriched VHs with the most enriched VL. They showed cross-recognition of DENV1-4 *in vitro* [24]. Sarker et al. proposed several modifications of a scFv derived from E53 mAb against FL and bc loop since the lack of Fc fragment would abolish ADE. Substitution mutations were introduced in the complementarity-determining regions (CDRs) and the modified product was then examined *in vitro* for affinity maturation. Some of them (D31L, Y105W, and S227W) exhibited an affinity constant (KD) 100-fold lower than the original one. Structural analysis revealed that hydrogen bonds, steric effects, and hydrophobic effects of the peripheral epitope-binding area play a vital part in affinity determination [25].

Additionally, mAbs targeting conformational (quaternary) epitopes have also been widely investigated. The human mAb C10 can cross-neutralize DENV1-4 and ZIKV. Structure-function analysis demonstrated that C10 had a lower affinity for DENV2-4 because of a conformational landscape of the E protein, yet the icosahedral geometry of the virion allows both Fab arms to bind asymmetric epitopes on distinct E dimers to ensure the bivalent binding capability as a compensation [26]. In another work to clarify the DENV3-specific antigenic sites, the authors isolated several mAbs from patients. Epitope mapping was performed utilizing both loss-of-function DENV3 and gain-of-function chimeric DENV1/3 recombinant viruses. The assays suggested six groups of antibody-binding patterns, most of which are quaternary epitopes spanning different domains or subunits of the trimeric E protein [27]. These structural and functional studies would further evoke encouragement in future neutralizing antibody design of better demonstrated paratope-epitope interaction and higher affinity to effectively block viral activity (eg. entrance into the host cell) which is also a possible way of inhibiting ADE.

5. Limitations and future development

Recent years have witnessed breakthroughs in both product design and methodology, whereas a couple of challenges still exist beyond ADE. The first one is due to the different virulence of DENV1-4, as was reported that DENV-2 had a higher tendency to cause DHF; also the pathogenicity can be

altered by the host's immune response (eg. previous experience of DENV infection) [5]. This added to the difficulty of providing balanced protections against DENV1-4 within a uniform manufacture of the vaccine. The second threat is co-occurrence with other mosquito-borne diseases like Zika in many tropical, developing regions. It was also shown that previous DENV infection was able to induce ADE of ZIKV infection via cross-recognition of the fusion-loop epitope [28]. The next challenge is the heterogeneity in the clinical data, including the limitation of human volunteers. Ooi and Kalimuddin mentioned that the much too wide spectrum of clinical presentation (virus strain, age, environment, genetic information) may hinder the integration and interpretation of the data and that using LAV in the study of controlled human infection model (CHIM) may not reflect real efficacy compared with wild type virus [13]. Another limitation is the inaccuracy of the current animal models. It is said the mice used for the DENV study are usually immunodeficient (devoid of IFN-γ signaling pathway) and may not truly mimic the immune status in human [13]. Finally, the threat of DF is amplified due to urbanization and climate change with higher social burden, since expansion of the city provides habitat for the mosquito *Aedes aegypti*, and higher density of population allows for quicker spread of pandemics such as COVID-19 [2].

To overcome these difficulties, great efforts can be made in several aspects together with novel designs and ongoing clinical trials. First, a serotype information bank ('big data') can be established to distinguish homogeneity and heterogeneity from demographic statistics, predict the prevalence of diseases, and direct inoculation program. This database would also become the basis of an adaptable, personalized vaccination scheme. The second highlighting point is that a multi-layer protection against antigens in multiple viral proteins responsible for different processes would be necessary and sufficient. For example, experiments in primates showed that blockage of viral replication complex NS3-NS4B inhibited DENV1 or DENV2 infection, suggesting a practical combination therapy of small molecule inhibitors against non-structural components and mAbs against surface envelope protein complexes [29]. Targeting the bc loop in EDII is also believed to induce cross-reactive humoral immunity without significant ADE [25]. Besides, a comprehensive evaluation of balanced immunity is also essential in both proof-of-concept assays and clinical trials (eg. taking T cell response and innate immune response into consideration besides the antibody titers) [13]. Additionally, an affordable and accessible detecting method is required for timely and informative personal protection. Overall, DENV and ADE effect will be eliminated with the help of modern medicine (Fig. 1).

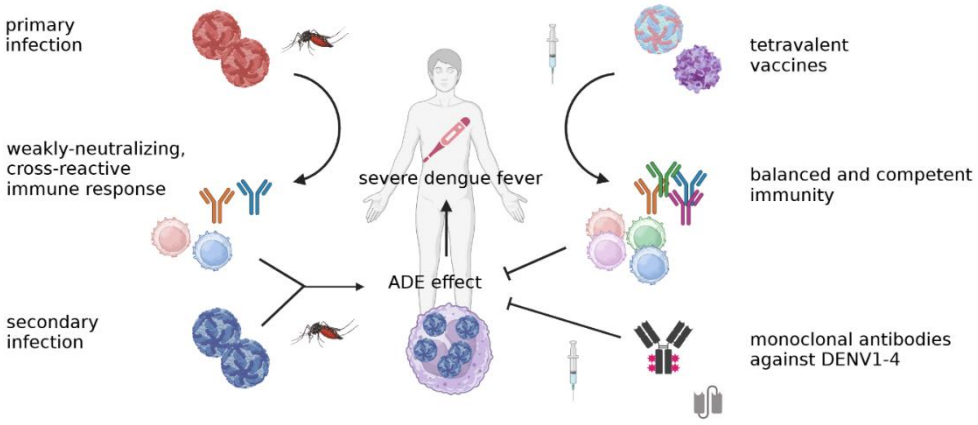

Fig 1. An overview of DENV infections and protections of vaccines or mAbs against ADE. Each color represents a specific type of DENV, self-antibody, and effector T cell. Different routes of design (LAV or VLP, modified Fc or scFv as examples) are also illustrated here.

6. Conclusions

The major goal of both DENV prevention and treatment is to impart a robust, well-rounded, and sound immune response against all four serotypes. LAV is still at the frontline of vaccines because

of the qualified immunogenicity, well-defined procedures, and success from those against other diseases. Meanwhile, the study of the VLP is also growing since it harbors the advantages of being non-replicating and fully programmable (adjuvants included), thus becoming a promising candidate for manufacture. Structural biology and protein engineering dramatically enhance the discovery of viral epitopes and drug design. The future anti-DENV strategy could be 'multiple lines' in which the attachment and invasion of virions as well as functionalities of critical viral proteins are all blocked. Only when mature DENV vaccines and mAbs are put into broad clinical use will DF no longer be a global threat to human society.

References

[1] World Health Organization, Dengue and severe dengue fact sheet. March 17, 2023. Retrieved on: August 11, 2023. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

[2] Wilder-Smith, Annelies et al. "Dengue." Lancet (London, England) vol. 393, 10169 (2019): 350-363.

[3] World Health Organization, Dengue - the Region of the Americas. July 19, 2023. Retrieved on: August 11, 2023. Retrieved from: https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON475

[4] Wu, Tiantian et al. "Dengue fever and dengue virus in the People's Republic of China." Reviews in medical virology vol. 32, 1 (2022): e2245.

[5] Sarker, Animesh et al. "Dengue virus neutralizing antibody: a review of targets, cross-reactivity, and antibody-dependent enhancement." Frontiers in immunology vol. 14 1200195. 2 Jun. 2023.

[6] Eckels, K H et al. "Isolation of a temperature-sensitive dengue-2 virus under conditions suitable for vaccine development." Infection and immunity vol. 14, 5 (1976): 1221-7.

[7] Dittmar, D et al. "Monoclonal antibodies specific for dengue virus type 3." Journal of clinical microbiology vol. 12, 1 (1980): 74-8.

[8] Khetarpal, Niyati, and Ira Khanna. "Dengue Fever: Causes, Complications, and Vaccine Strategies." Journal of immunology research vol. 2016 (2016): 6803098.

[9] Guzman, Maria G, and Eva Harris. "Dengue." Lancet (London, England) vol. 385, 9966 (2015): 453-65.

[10] Kuhn, Richard J et al. "Structure of dengue virus: implications for flavivirus organization, maturation, and fusion." Cell vol. 108, 5 (2002): 717-25.

[11] Zonetti, Luiz F C et al. "Molecular Aspects of the Dengue Virus Infection Process: A Review." Protein and peptide letters vol. 25, 8 (2018): 712-719.

[12] Pintado Silva, Jessica, and Ana Fernandez-Sesma. "Challenges on the development of a dengue vaccine: a comprehensive review of the state of the art." The Journal of general virology vol. 104, 3 (2023): 001831.

[13] Ooi, Eng Eong, and Shirin Kalimuddin. "Insights into dengue immunity from vaccine trials." Science translational medicine vol. 15,704 (2023): eadh3067.

[14] Dai, Yu-Ching et al. "Identification of prior dengue-naïve Dengvaxia recipients with an increased risk for symptomatic dengue during fever surveillance in the Philippines." Frontiers in immunology vol. 14 1202055. 24 Jul. 2023.

[15] Patel, Sanjay S et al. "Clinical Safety Experience of TAK-003 for Dengue Fever: A New Tetravalent Live Attenuated Vaccine Candidate." Clinical infectious diseases: an official publication of the Infectious Diseases Society of America vol. 76, 3 (2023): e1350-e1359.

[16] Russell, Kevin L et al. "A phase I randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and immunogenicity of a live-attenuated quadrivalent dengue vaccine in flavivirus-naïve and flavivirus-experienced healthy adults." Human vaccines & immunotherapeutics vol. 18, 5 (2022): 2046960.

[17] Kallas, Esper G et al. "Safety and immunogenicity of the tetravalent, live-attenuated dengue vaccine Butantan-DV in adults in Brazil: a two-step, double-blind, randomised placebo-controlled phase 2 trial." The Lancet. Infectious diseases vol. 20, 7 (2020): 839-850.

[18] Collett, Simon et al. "Development of virus-like particles with inbuilt immunostimulatory properties as vaccine candidates." Frontiers in microbiology vol. 14 1065609. 7 Jun. 2023.

[19] Veena Rani, N et al. “Efficient generation and characterization of chimeric dengue viral-like particles.” Biochemical and biophysical research communications vol. 654 (2023): 10-17.

[20] A Dowd, Kimberly et al. “prM-reactive antibodies reveal a role for partially mature virions in dengue virus pathogenesis.” Proceedings of the National Academy of Sciences of the United States of America vol. 120, 3 (2023): e2218899120.

[21] Tan, Brandon E K et al. “Identification of Key Residues in Dengue Virus NS1 Protein That Are Essential for Its Secretion.” Viruses vol. 15, 5 1102. 30 Apr. 2023.

[22] Sootichote, Rochanawan et al. “Potential Protective Effect of Dengue NS1 Human Monoclonal Antibodies against Dengue and Zika Virus Infections.” Biomedicines vol. 11, 1 227. 16 Jan. 2023.

[23] Tien, Sen-Mao et al. “Therapeutic efficacy of humanized monoclonal antibodies targeting dengue virus nonstructural protein 1 in the mouse model.” PLoS pathogens vol. 18, 4 e1010469. 29 Apr. 2022.

[24] França, Renato Kaylan Alves de Oliveira et al. “New Anti-Flavivirus Fusion Loop Human Antibodies with Zika Virus-Neutralizing Potential.” International journal of molecular sciences vol. 23, 14 7805. 15 Jul. 2022.

[25] Sarker, Animesh et al. “Structure-guided affinity maturation of a single-chain variable fragment antibody against the Fu-bc epitope of the dengue virus envelope protein.” The Journal of biological chemistry vol. 298, 4 (2022): 101772.

[26] Sharma, Arvind et al. “The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses.” Cell vol. 184, 25 (2021): 6052-6066.e18.

[27] Young, Ellen et al. “Identification of Dengue Virus Serotype 3 Specific Antigenic Sites Targeted by Neutralizing Human Antibodies.” Cell host & microbe vol. 27, 5 (2020): 710-724.e7.

[28] Dejnirattisai, Wanwisa et al. “Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus.” Nature immunology vol. 17, 9 (2016): 1102-8.

[29] Goethals, Olivia et al. “Blocking NS3-NS4B interaction inhibits dengue virus in non-human primates.” Nature vol. 615, 7953 (2023): 678-686.