Dengue Antiviral Development: A Continuing Journey

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Abstract

Dengue fever is a leading cause of illness and mortality in the tropics and subtropics. There are no therapeutics currently available and a recently approved vaccine is not very efficacious demanding an urgent need to develop an effective antiviral. The path to successful dengue drug development depends on availability of relevant preclinical testing models and better understanding of dengue pathogenesis. In recent years, efforts to develop dengue therapeutics have focused on both repurposing approved drugs as well as discovery of new chemical entities that act via virus or host targeted mechanisms. Here, we discuss the various innovative approaches, their outcome, and the lessons gleaned from the development efforts.

Keywords

Drug repurposing · Alpha glucosidase inhibitors · Host targets · FDG imaging · Dengue biomarker

22.1 Introduction

Dengue fever is the most prevalent mosquito-borne viral disease globally. It is endemic in more than 100 countries, and causes an estimated 400 million infections and 25,000 deaths every year [5]. As these numbers have been rising steadily over the past decades, developing efficacious antiviral agents and vaccine is imperative to control the disease burden. Current treatment guidelines rely entirely on supportive care and aggressive monitoring [23]. Recently, a tetravalent vaccine was licensed in several dengue-endemic countries, which is a considerable breakthrough [63]. Its efficacy, however, varies widely hence there remains a strong need to develop effective therapeutic modalities for dengue virus (DENV) infections [27].

Since the early 2000s, several compounds have been tested in early proof-of-concept trials but, none was able to demonstrate clinical efficacy. In this chapter, we discuss the progress that has been made and the main challenges faced in dengue therapeutics research and attempt to draw a parallel to the development of other antivirals like oseltamivir.

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22.2 Candidate Drugs

Consideration of the nature of the disease and the impacted demographics is essential for drug development – and defining a Target Product Profile (TPP) is a key step [30]. Dengue fever (DF) results in an acute self-limiting disease, with a small proportion of patients, mostly children, developing constellations of life threatening complications referred to as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF mostly affects low-income, tropical countries. Consequently, the ideal drug should have an excellent safety profile not to surpass the low risk posed by uncomplicated DF. In addition, it should be an oral drug, require minimum frequency dosing with total course of therapy not to exceed 5 days duration given the acute nature of the disease, and produce measurable clinical benefits. It should be easily manufactured and distributed at affordable cost, and be able to withstand temperatures and high humidity prevalent in tropical regions. These general TPP goals should be kept in mind but they should not stand in the way of new innovations such as prophylactic drugs or biologics in the form of potent therapeutic antibodies that target the viral envelope protein to block its infectivity.

22.2.1 Repurposed Agents

Starting from scratch, drug development is a long and expensive process, and for dengue the experiences of Novartis pharmaceutical company which formed the first major industrial effort to develop a drug that could be used as defined in the TPP has been reviewed [34]. The lack of any compounds from these ventures and the urgent need for affordable anti-dengue drugs has resulted in several attempts at repurposing drugs that were developed for other indications. The main advantage of this approach is that it has a relatively short development path as the initial safety data in animals and humans are already established. In fact, all seven compounds evaluated in dengue clinical trials to date were repurposed. These agents were either already approved for different indications or were discontinued for further development due to reasons other than acute safety and tolerability. There have been 5 completed and published and 2 ongoing randomized clinical trials for anti-dengue agents since the early 2000s.

22.2.1.1 Chloroquine

Tested by the Oxford University Clinical Research Unit in Vietnam (OUCRU), chloroquine is a 4-amino-quinoline derivative with lysosomotropic and weak base properties (Trial Identifier: ISRCTN38002730). It has evidence of in vitro and in vivo (aotus monkey) anti-viral activity that is thought to be mediated by disrupting endosomal fusion and viral maturation [21]. This oral drug is cheap and widely available, with a strong safety profile. The clinical trial for this drug monitored time to resolution of viremia and NS1 antigenemia as primary measures of drug efficacy. There was no observed difference in efficacy between the drug and placebo, but chloroquine was associated with a higher incidence of adverse events [61].

22.2.1.2 Prednisolone

Prednisolone is a corticosteroid with potent anti-inflammatory activity. Some non-randomized trials have reported possible benefits of corticosteroids as a rescue treatment for severe DF, but this remains controversial [47]. It is, however, universally accepted that inflammatory factors play a crucial role in the pathogenesis of DSS. On this ground, the OUCRU in Vietnam conducted a Randomized Controlled Trial (RCT) to assess the safety of corticosteroids and outcome in viremia but was not powered to measure its therapeutic efficacy, although clinical outcomes such as DSS, ICU admission, and bleeding were measured (Trial Identifier: ISRCTN39575233). The safety profiles were similar in both groups but no clinical benefit was detected [57].

22.2.1.3 Balapiravir

A prodrug of nucleoside analogue 4′-azidocytidine, balapiravir was originally developed as a therapeutic agent against hepatitis C virus (HCV), another flavivirus. Balapiravir development against HCV was halted after evidence of excessive toxicity upon prolonged exposure. Due to the known similarities between DENV and HCV RNA-dependent RNA polymerases, this drug was evaluated as an anti-dengue agent by OUCRU in Vietnam (Trial identifier: NCT01096576). The phase II study monitored time to fever clearance, viremia and NS1 antigenemia to measure the drug efficacy. The results showed no significant difference between control and treated arms [14, 41].

22.2.1.4 Ribavirin

Ribavirin is a drug commonly used to treat HCV and also sometimes used as a broad-spectrum antiviral for RNA viruses. It has RNA-dependent RNA polymerase inhibitory activity, but its anti-flaviviral activity is believed to be mediated by intracellular GTP depletion. Ribavirin is used in conjunction with interferon-α against HCV as they show synergistic effect. Ribavirin was evaluated against DENV infection in combination with traditional Chinese medicine by the Guangzhou 8th People's Hospital, but the results have not been made publicly available yet (Trial identifier: NCT01973855).

22.2.1.5 Celgosivir

Celgosivir is another drug that was originally developed for HCV. It is an inhibitor of α-glucosidase, a host enzyme necessary for glycosylation of viral coat proteins that aids in proper protein folding. It was tested in a RCT as an anti-dengue agent by Duke-National University of Singapore (Duke-NUS)/Singapore General Hospital (SGH) in Singapore using fever and viremia reduction as measures of drug efficacy. No statistically significant difference was seen between control and treatment groups, but celgosivir was found to have a good safety profile in dengue patients [36]. Extended pharmacokinetics studies and alternative regimen testing in mouse models of dengue infection predicted that increased exposure may impact efficacy [55, 70]. To test this possibility a small pharmaceutical company 60° Pharma has partnered with Singapore General Hospital to test an altered regimen that will result in an almost 300% increase in exposure with only a modest increase in dose. The clinical trial is scheduled to start recruiting in 2017 (Trial Identifier: NCT02569827).

22.2.1.6 Lovastatin

Lovastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, widely used for its lipid lowering properties. Among their other properties, statin drugs improve endothelial function and stabilize lipid membranes, two mechanisms known to be disrupted in DF pathogenesis. On this ground, a clinical trial was conducted in Vietnam by OUCRU to assess the safety of lovastatin as an anti-DENV agent. The safety outcome was measured clinically by trending liver and muscle injury serum markers, two commonly encountered side effects of lovastatin. Lovastatin was reported to be a safe and tolerable treatment in dengue patients, but there was no evidence of beneficial effect in terms of clinical progress or viremia reduction [71].

22.2.1.7 Ivermectin

This anti-parasitic agent commonly used to treat nematode infections, scabies, and lice has been shown to have anti-viral activities by inhibiting host nuclear import receptors importin-α and importin-β [64]. These receptors are necessary for DENV nonstructural protein 5 (NS5) migration to the nucleus for efficient replication. Nevertheless the precise mechanism by which ivermectin inhibits dengue is still unclear because the nuclear localization of NS5 is not required for viral RNA replication [59]. On the positive side, the drug has been identified as a potent inhibitor

of the related flavivirus, zika virus in recent screens with FDA approved drugs [2, 74]. A clinical trial as a treatment for dengue fever with ivermectin is being carried out by the Mahidol University in collaboration with the Ministry of Health of Thailand (clinical identifier: NCT02045069). Preliminary results suggest reduction in serum NS1 antigenemia and body temperature despite the lack of detectable difference in viremia levels [1].

22.2.1.8 Ketotifen

Ketotifen is a non-competitive anti-histamine and a mast cell stabilizer generally used to treat atopic conditions like asthma and allergic rhinitis. It is being evaluated as an anti-dengue agent based on preclinical data suggesting that mast cell degranulation is an important mediator of DF pathogenesis [16, 22]. A clinical trial (NCT026773840) is currently being conducted by Duke-NUS, SGH, and the National University Hospital in Singapore to assess its clinical safety and efficacy.

22.3 DENV Specific Drug Development

Typically, new drug discovery involves identifying a target; developing an assay to assess activity against the target; high-throughput screening of potential candidate compounds; and chemically modifying the successful candidates to optimize activity, pharmacokinetics and toxicology in in vitro and in animal models of infection. Once a lead candidate is identified, manufacturing methods are adapted to produce the drug candidate in sufficient quantities to be tested in clinical trials [54].

Usually, the target is either a viral or host protein required for entry, proteolytic processing of the newly translated polyprotein, RNA replication, viral genome packaging and virion release from infected cells. DENV proteome comprises of 3 structural proteins: Capsid (C), premembrane (prM), and envelope (E) proteins; and 7 nonstructural (NS) proteins involved in protein processing and viral replication: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The replication apparatus, especially NS3 and NS5 have been the most intensively investigated targets in dengue drug development [37]. Commonly studied host targets include α-glucosidase, inosine monophosphate dehydrogenase, and dihydroorotate dehydrogenase among others [7].

These endeavors require large amounts of investments and carry high financial risks. Like many other neglected tropical diseases, dengue has been understudied consequently. To date, no candidate drug developed specifically for dengue has had positive results in a phase II human trial. This section highlights the considerable milestones achieved in this field and the promising projects underway.

NITD-008, a nucleoside inhibitor that targets viral RNA-dependent RNA polymerase (RdRp) is a potent antiviral developed by the Novartis Institute of Tropical Diseases in Singapore. It has remarkable nanomolar efficacy against all four DENV serotypes and other Flaviviruses in vitro and *in vivo* [76]. Toxicology studies conducted in animals revealed significant renal toxicity hence further development of this candidate was halted. Nonetheless, NITD-008 is still considered the standard of preclinical efficacy in dengue drug development and widely used to benchmark new drug development.

Recently, Emergent BioSolutions Inc. developed UV-4B, an antiviral agent that shows in vitro and in vivo activity against DENV. The proposed mechanism of action is through the inhibition of the enzymatic activities of host endoplasmic reticulum α-glucosidases. Viruses require these cellular enzymes for proper processing of their proteins. Since this is a host targeted mechanism of action, it is anticipated that development of viral resistance to UV-4B is less likely to occur than with directly acting antiviral agents [44, 66, 67]. This hypothesis was tested in vitro where DENV-infected cells treated with 38 cycles of UV-4B showed no drug-induced resistance. In vivo,

UV-4B efficacy was maintained through 5 DENV passages in a mouse model [45].

An investigational new drug application has been opened for UV-4B based on preclinical safety and efficacy data. A phase 1 clinical trial completed in 2016 documented good tolerability and no serious adverse events after administration of single doses of UV-4B ranging between 3 and 1000 mg (NCT02061358). The pharmacokinetics data showed low inter-individual variability and linearity over a broad dose range. Another phase 1 clinical study has been initiated to determine the safety and pharmacokinetics of UV-4B administered orally as multiple ascending doses to healthy volunteers (NCT02696291). UV-4B is also being evaluated in preclinical studies for the treatment of influenza as an additional indication [66, 67].

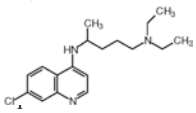
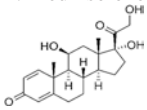
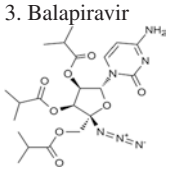
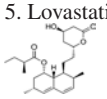
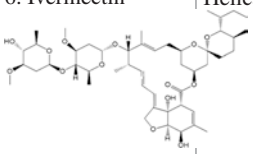
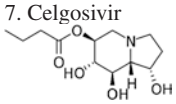
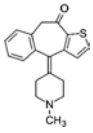
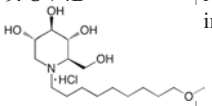
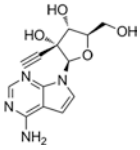
Siga Technologies reported the development of the inhibitor ST-148, which acts by inducing structural rigidity. In a nonlethal model of DENV infection in AG129 mice, ST-148 reduced viremia and viral load and lowered cytokine levels in the plasma [6].

In recent years several groups have reported the discovery and development of NS4B inhibitors. NITD reported the activity of spiropyrazolopyridone compound NITD-618. Although, the compound showed reduced virema against DENV-2 in AG129 model of infection they were unable to identify a candidate that could show pan serotypic activity. In another screening effort, van Cleef et al. reported a new inhibitor, SDM25N, from screening the NIH Clinical Collection (NCC); a library of drug-like small molecules a stably replicating DENV serotype 2 (DENV2) subgenomic replicon. SDM25N, which restricts genomic RNA replication by – directly or indirectly – targeting the viral NS4B protein [62, 65, 72]. Janssen is also currently developing a NS4B inhibitor [43].

22.3.1 Recent Structural Discoveries

Recent advances in immunological, molecular, and structural virology have offered new ways to develop drugs more efficiently. Nuclear magnetic resonance spectrometry, X-ray crystallography, and cryo-electron microscopy have provided detailed structural data of DENV proteins. This information can be combined with molecular tools such as in silico approaches and infectious clone technology to discover new drug targets against DENV and potentially other flaviviridae [6, 33, 35, 73]. The viral processes that can be targeted include entry/fusion (E protein), translation/polyprotein processing of nonstructural (NS) proteins (NS3, NS2B-NS3 complex), replication (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), and viral packaging (Capsid) [54]. Being essential to replication and infectivity and highly conserved among flaviviruses, NS3 and NS5 have been the most popular targets of DENV antiviral development.

Most notably, this structural information has been used in studies of human antibodies isolated from convalescent patients and yielded greater understanding of the epitopes that need to be targeted for effective virus neutralization [48]. Serotype-specific and cross-reactive neutralizing monoclonal antibodies are both being explored [60]. Ab513, a monoclonal antibody that binds domain III of the E protein of all 4 DENV serotypes was engineered by Visterra (Cambridge, MA). This agent has shown promising preclinical results with the ability to lower viremia and control DHS/DSS features in humanized mouse models without enhancing infection despite the presence of cross-reactive antibodies. It is poised to enter clinical trial by early 2018.

Compound and structure	Mechanism of action	Study site	Preclinical results	Clinical results
1. 	Lysosomal fusion inhibitor	OUCRU, Ho Chi Minh City, Vietnam	Cell-based assay (U937): EC_{50}: 50 µM Aotus monkey: significant reduction in viremia [21]	No change in viremia or NS1 antigenemia
2. Prednisolone 	Anti-inflammatory activity	OUCRU, Ho Chi Minh City, Vietnam	NA	No change in hematological virological or clinical endpoints
3. Balapiravir 	Polymerase inhibitor	OUCRU, Ho Chi Minh City, Vietnam	Cell based (Huh-7) EC_{50};1.9–11 µM [41]	No change in virological and immunological endpoints [40]
4. Ribavirin 	Nucleoside analogue	Guangzhou 8th People's Hospital	Cell-based (LLC-MK2): IC_{50}: 50.9 µM [56]	Pending
5. Lovastatin 	Improving endothelial function and stabilizing lipid membranes	OUCRU, Ho Chi Minh City, Vietnam	AG129 mouse model: Increased survival at dose of 200 mg/kg/day [71]	No evidence of beneficial effect on clinical progress or DENV viremia [71]
6. Ivermectin 	Helicase inhibitor	Mahidol University/Siriraj Hospital, Thailand	Enzyme assay: IC_{50}: 0.5 µM [1]	NS1 antigenemia and fever reduction (preliminary)
7. Celgosivir 	Alpha glucosidase inhibitor	SGH/Duke-NUS, Singapore	Cell-based assay: EC_{50}: 0.2 µM AG129 mouse model: 100% survival at dose of 50 mg/kg BD for 5 days [68–70]	No statistically significant reduction of viral load or fever [36]
8. Ketotifen 	Mast cell stabilizer	SGH/Duke-NUS, Singapore	Mouse model: significant reduction of severe dengue features [51]	Pending
9. UV4B 	Alpha glucosidase inhibitor	Emergent BioSolutions, Maryland USA	AG129 mouse model: 100-fold viremia reduction at 100 mg/kg PO dosing Minimum effective dose: 10 mg/kg PO [44, 66, 67]	Phase 1a Single Ascending Dose Study in healthy volunteers showed UV-4B is well tolerated up to 1000 mg single dose
10. NITD008 	Nucleoside analogue-polymerase inhibitor	NITD, Singapore	Cell based assay (PBMC) EC_{50}: 0.16–0.85 µM AG129 mouse model: 100% survival at dose >10 mg/kg PO [76]	Excessive nephrotoxicity, development halted

22.4 The Clinical – Preclinical Gap

As discussed above, many compounds have shown good efficacy in preclinical studies. However, none was proven to be superior to placebo in clinical setting. The disparity between preclinical and clinical results is an inherent entity of therapeutics research. Common reasons include the nonspecific and wide spectrum of disease manifestations, the limitations of existing model organisms, and the lack of a biological marker that correlates with clinical results. A factor that makes this even more pertinent for dengue therapeutics, specifically, is the limited financial resources available for research. The recent clinical and preclinical progress achieved gives an insightful perspective on what should be future research directions.

22.4.1 Clinical Manifestation

Dengue fever has extremely nonspecific manifestations making it difficult to study in clinical settings. Contrary to preclinical setting where the time of infection is controlled, patients are recruited into clinical trials when they experience symptoms and present to their healthcare provider. Fever is virtually the only symptom clinicians base they suspicion on when screening for dengue. As with most other febrile illnesses, dengue patients do not present until later in the course of disease. Unless there is an ongoing outbreak, dengue might not be the first impression of the healthcare provider. This presents an additional challenge to clinical trial design and recruitment as administering treatment later in the course of illness when the viremia level has already past the peak can dilute any potential benefit of the therapeutic agent under investigation.

Moreover, the vagueness of early symptoms is a source of inaccuracy based on patient-reported onset of illness/fever. This is a known unreliable measure, but there is usually no better alternative.

22.4.2 Representative Model Organism

DENV is not known to be naturally pathogenic in any species other than humans. This makes it very challenging to obtain representative animal models for research. Efforts have been made to identify the specific characteristics that make us vulnerable to DENV. Multiple animals and DENV strains have been genetically engineered to replicate the identified characteristics, but, to this day, none of the models are able to accurately mimic a human DENV infection.

22.4.3 Nonhuman Primates

Nonhuman primates develop viremia and neutralizing antibody response to DENV infection but do not develop symptoms of DF [75]. They have been used to study antibody-dependent enhancement of infection (ADE) and to test candidate vaccines and antivirals efficacy by assessing viremia levels [12, 13, 24, 25]. As we discuss below, viremia is a suboptimal measure of treatment response. Some nonhuman primate models that express features of human DF have been identified; but their use is hindered by the prohibitive cost and accessibility.

22.4.4 Mouse Models

DENV does not replicate well in rodent cells. To overcome this, extensive work has been done to develop DENV mouse models. The first successful suckling mouse, was developed by serial passage DENV in mice brain, increasing the virus adaptability to mice cells [50]. Intracranial inoculation resulted in DENV encephalitis and paralysis. This model has been used by to evaluate antivirals and vaccines but the altered tropism of the virus makes the relevance to humans questionable [9].

Inoculation with high DENV titers in immunocompetent mice can induce disease comparable to human DF. Different groups have reported hepatic T cell invasion, localized hemorrhage,

thrombocytopenia and detectable viral load in serum, spleen, liver, and brain, vascular leakage; all of which are characteristic of human DF/DHS/DSS [9, 10, 12, 13, 52]. This model is useful to gain insight on the pathogenesis of DF but the high viral titer required to induce symptoms is not representative of the infection process in humans; making it inadequate for antiviral development studies.

Humanized mice developed by transplanting human hematopoietic stem cells into severe combined immunodeficient mice have been shown to be susceptible to low passage DENV. They exhibit DF-like feature such as fever, rash and thrombocytopenia, but not DHS/DSS [4]. Kuruvilla and colleagues reported that humanized mice can develop fever and viremia for up to 21 days and produce human anti-DENV Ig-M and Ig-G capable of neutralizing DENV. There was, however, no significant cellular immune response induced [32]. Several groups have tried to replicate a human like T-cell response in humanized mice, but there still has not been any model capable of mimicking severe dengue symptoms, or human-like immune response; limiting their use in therapeutics research [9].

Immunocompromised mice have been the most widely used model thus far. By knocking out key genes involved in immune response, infectible mice models have been engineered. The most established in therapeutics research is the type I and II interferon receptors deficient AG129. This model is susceptible to both mouse-adapted and clinical DENV infection. Concurrently, efforts were also being put into identifying a DENV strain that could successfully infect mice. D2S10 was developed by alternate passaging of PL046, a clinical isolate, between AG129 mice and C3/C6 mosquito cells. This DENV strain was able to cause lethal infection in mice without neurological deficits and; most importantly, D2S10 caused vascular leakage making it very pertinent to study human DF. Of note, celgosivir and lovastatin were proven to be efficacious in AG129 mice, with increased survival and decreased viremia. In clinical trials, however, no significant effect was observed [36, 68, 69, 71].

22.4.5 Need for Human Infection Model

Although there has been reports of certain DENV strains causing some features of DHS and DSS in certain mouse models, the pathogenesis remains substantially different from that in humans [58]. The ideal way around this obstacle is the use of a human infection model (HIM). HIM was an important contributor to the development of oseltamivir. The illness caused by influenza virus infection in healthy young adults is short lived and of mild severity; the use of a HIM was hence accessible to the developers of oseltamivir [28]. That enabled them to control the timing between infection and initiation of therapy, one of the most critical challenges in anti-dengue clinical trials [37]. Perhaps more importantly, it also allowed them to quickly down-select candidate compounds [19]. This was crucial to a remarkably fast drug development; oseltamivir was approved for treatment of influenza only 7 years after the search for an orally available neuraminidase inhibitor was initiated [54].

HIM has been used to study several aspects of dengue before. For instance, Startler and colleagues studied the process of DENV induced plasma leakage in healthy individuals [53]. In 2011, Gunther et al. used HIM to study the immune response to DENV in previously vaccinated people [26]. More recently, Kirkpatrick and colleagues proposed HIM to validate vaccines before moving onto large scale clinical trials [31]. Even though HIM would revolutionize dengue research, there are still strong reservations about its use. DF is typically not associated with mortality in healthy adults, but has a potential for high morbidity requiring hospitalization [19]. Another limitation of HIM is the fact that there is no possibility to access tissue samples for pathological analysis, limiting HIM studies to a descriptive nature [9].

22.5 Objectively Measurable Endpoints

22.5.1 Viremia

Since the earliest dengue studies, viremia as a biomarker for dengue disease has been a universally accepted paradigm [49, 54]. This concept was popularized by the availability of sensitive methods to detect and quantify viral particles in clinical samples. In fact, all trials for candidate antiviral drugs and vaccines have reduction of viremia as one of their primary clinical endpoints, if not the only endpoint. However, it has become evident that measured viremia does not always correlate with clinical outcome. For instance, during secondary infections, measured viremia is often markedly lower than primary despite the higher likelihood of developing severe disease. One of the possible explanations for this phenomenon is intrinsic to the viremia assay methodology itself.

22.5.1.1 qPCR

In the 7 clinical trials mentioned above, viremia was measured using quantitative polymerase chain reaction assay (qPCR). This method detects and quantifies viral RNA in serum, but it does not reflect the amount infective viruses present. RNA copies can exceed infectious viral units by 2–5 logs. This effect has been described for other viral infections as well and was attributed to a probable large quantity of cells infected by defective proviruses, masking the absolute viremia [20].

22.5.1.2 Plaque Assay

A more representative assay would be direct culturing of the clinical samples. However, this is extremely challenging because clinical isolates have widely varying abilities to grow in vitro. Unpassaged viruses, for instance, are known to be less able to infect consistently in vitro despite their potential infectivity in vivo. This makes culture and plaque assays inherently inaccurate. Mosquito inoculation can be used to account for unpassaged DENV, but this technique requires an insectary and highly skilled personnel making it inaccessible to most diagnostic virology laboratories [15].

22.5.2 NS1 Antigenemia

Serum NS1 levels rise early in the infection course making it a good diagnostic tool. However, NS1 antigenemia varies considerably by DENV serotypes and primary versus secondary infection [18, 68, 69]. There is also evidence that it may be involved in DF pathogenesis [3, 39]. Structural and in vivo mice studies suggest that, if combined with a host dependent biomarker, NS1 could be a reliable biomarker and clinical endpoint measure for therapeutic trials [40]. Further work is needed to confirm this.

22.5.3 Host Biomarkers: Cytokine, Endothelial Activation Markers, Cells, Biochemical Markers

It is believed that patients with DHS/DSS experience a "cytokine storm," causing their clinical symptoms. Trending these cytokines may, consequently, serve as a good prognostic biomarker. Identified markers that correlate with disease progress include interleukin-10, complements C3a and C5a, and macrophage migration inhibitory factor [11, 29, 38].

The correlation between blood cellular components and dengue severity has been extensively studied, platelet and red blood cell counts in particular. Thrombocytopenia is an established hallmark of severe dengue disease [17, 23]. A study conducted in Thailand was able to predict more than 97% subsequent severe disease development using an algorithm based on white blood cell counts, percent monocytes, platelet count, and hematocrit information obtained in the first 72 h of disease [46]. A similar study conducted in

Vietnam proposed a prognostic scoring system based on the platelet count, history of vomiting, blood aspartate aminotransferase level, and NS1 rapid test status [42]. This scoring system had a sensitivity of 87% and specificity of 88%; with a negative predictive value of 99% amongst their study participants.

Biochemical markers like liver enzymes, nitric oxide, and lipids are also known to be deranged in severe disease, and have been studied as biomarkers and potential clinical endpoints. Several endothelial activation markers, including angiopoietin, von Willebrand factor, and VEGF, have also been associated with disease severity [29].

All these host biomarkers are used at varying degrees in dengue research. They all have a common short coming, however; poor specificity. There are many factors that can be responsible for host biomarkers changes and over-relying on them can result in false interpretations [29]. Nonetheless, cautiously combining them with DENV biomarkers greatly improves their prognostic power, making a case for their use in therapeutics trials [37].

22.5.4 18F-Fluorodeoxyglucose Imaging

In addition to developing optimal infection models, identifying new measurable endpoints that are consistent and comparable between animal models and humans would greatly help narrow the aforementioned preclinical-clinical gap. A team of researchers in Singapore is working on identifying new and more representative DF biomarkers by building upon the known pathogenesis. DENV particles preferentially replicate and accumulate in tissues that are not readily accessible. The ability to monitor tissues specifically instead of relying on systemic circulating markers offers a novel way to make early and robust inferences about the course of illness. 18F-Fluorodeoxyglucose uptake monitoring in dengue-infected mice has identified specific inflammation patterns in the intestines that strongly correlate with progression of disease and treatment response [8]. A human proof-of-concept trial is currently recruiting dengue infected subjects in Singapore to validate these findings in human.

22.6 Conclusion

Dengue fever imposes a significant global burden and low-income tropical countries are more heavily impacted. Efforts to develop vaccines and treatment modalities have been very slow until the 2000s. For the most part, this was due to the lack of available resources allocated to the cause. Over the past two decades, more governmental, academic, and private agencies have devoted funds to dengue research; which resulted in considerable advances, most notably a tetravalent vaccine licensed in most endemic countries. Nonetheless, the road to relieving the dengue burden is still long. The available vaccine's efficacy is not ideal, making it imperative to have effective therapeutic options. Dengue antiviral drug development endeavors with both repurposed and de novo agents have not yet yielded effective products in clinical trials but have bolstered the understanding of dengue pathogenesis and laid up the ground for further projects. Recent technological advances and innovative approaches may be offering faster, targeted, and more cost-efficient models to develop and assess new drugs.

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