Commentary

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Pharmacological intervention for dengue virus infection

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Abstract

Dengue virus (DENV) infection has a considerable health impact in tropical and subtropical countries worldwide. Escalation of infection rates greatly increases morbidity and mortality, most commonly from deaths due to dengue hemorrhagic fever and dengue shock syndrome. Although the development of an effective, long-lasting vaccine has been a major aim for control and prevention of DENV infection, the currently licensed vaccine has limitations and is less than satisfactory. Thus, there remains an important need to identify effective and tolerable medications for treatment of DENV-infected patients both in the early phase, to prevent progression to fatal outcomes, and to minimize deaths after patients develop severe complications. This review will address several specific points, including (1) approaches to identify anti-DENV medications, (2) recent advances in the development of potential compounds targeting DENV infection, (3) experience with clinical trials of regimens for DENV infection, (4) some available medications of potential for clinical trials against DENV infection, (5) reasons for unsuccessful outcomes and challenges of anti-DENV treatments, and (6) directions for developing or selecting better anti-DENV strategies. This review provides useful guidance for clinicians selecting drugs for DENV-infected patients with severe manifestations or potential fatal disease progression, and for basic researchers seeking to develop effective anti-DENV regimens.

Keywords: Dengue virus; Drug; Therapy; Cytokine

Abbreviations

DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; ADE, antibody-dependent enhancement; HCV, hepatitis C virus; HTS,
high-throughput screening; TNFα, tumor necrosis factor α; IFN, interferon; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; IL-1Ra, IL-1 receptor antagonist; RANTES, regulated on activation, normal T cell expressed and secreted; NF-κB, nuclear factor-kappaB; NS, non-structural; IVIG, intravenous immunoglobulin; IMP-α, importin α; IMP-β1, importin β1; D2R, dopamine D2 receptor; PCZ, prochlorperazine; 4-HPR, 4-hydroxyphenyl retinamide
1. Introduction

Dengue virus (DENV), a mosquito-borne flavivirus, infects 50 to 100 million people worldwide annually, and 40% of people living in tropical and subtropical areas of Asia, Africa, and the Americas are at risk of DENV infection. Although most dengue symptoms are self-limiting, nearly half a million persons die from fatal sequelae such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are associated with widespread plasma leakage and vascular pathology [1]. Current therapies are fluid replacement and supportive care; there is no specific, effective treatment [2]. In the past few years, great effort has been devoted to developing a dengue vaccine, and the first such vaccine, CYD-TDV (Dengvaxia®), was approved by a few countries in late 2015 and early 2016 [3]. Unlike vaccine prevention, drug intervention circumvents the potential immunopathological consequences of DENV infection; thus, many drug discovery programs against DENV infection and high-throughput screening (HTS) strategies have been developed. Therapeutic strategies focus on shortening the duration of DENV replication and limiting disease severity, to minimize life-threatening manifestations and the severity of major complications. However, the need for an effective anti-DENV treatment remains urgent.

2. Dengue virus, host cells, and immunopathogenesis

Four DENV serotypes (1, 2, 3, and 4) have been identified and each of them can infect humans and cause severe diseases [4]. There are 25-40% difference at the amino acid level among four DENV serotypes and are separated further into genotypes with a variation by up to ~3% [5]. DENV infects a variety of host cells, including dendritic cells, macrophages, B cells, and mast cells, with different levels of viral productivity. The term “bone-breaking fever” was generally used in Chinese medicine to describe one of the major symptoms in
DENV-infected patients. Recently, osteoclasts were found to be a target for DENV, and infected osteoclasts produced cytokines with an intensity comparable to that of DENV-infected macrophages [6]. Among the target cells, dendritic cells are the major primary targets and are responsible for induction of anti-DENV immune responses, especially during the early phase of infection [7]. To initiate DENV infection, viral particles adsorb to the cell surface, and viral envelope glycoproteins bind several possible host cell receptors [8]. Alternatively, DENV-containing immune complex can enter cells through the binding between the antibody Fc portion and the Fc receptor on the surface of immune cells [9].

After binding, DENV is endocytosed into an endosome, and the acidic environment of endosomal vesicles leads to fusion between the virus and endosomal membranes (Fig. 1). Viral RNA is then released into the cytoplasm and presented to the rough endoplasmic reticulum. The 11-kb viral positive-sense RNA genome is translated into a single polyprotein containing structural proteins, capsid (C), precursor membrane (prM), and envelope (E), and seven non-structural (NS) proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [4, 10, 11]. Then, the polyprotein precursor is cleaved by host and viral proteases such as NS2B/NS3, to form the machinery for viral RNA synthesis from a negative-stranded intermediate. The synthesized RNA genomes associate with the viral C protein to form a nucleocapsid and enter the lumen of the endoplasmic reticulum to produce immature virus particles containing viral E and prM proteins. Further maturation occurs in the trans-Golgi network, where the host protease furin cleaves between the pr protein and M protein, resulting in the release of mature virion from the cell. Successful completion of the DENV lifecycle involves many viral and cellular factors [9].

In DENV-infected patients, the viral load in plasma usually reaches a peak within 24–48 h of fever onset. Fever declines rapidly and patients typically become afebrile 5–7 days after the onset of symptoms. Unfortunately, a small number of patients progress to severe clinical
manifestations such as DHF and DSS. Although the mechanisms leading to these severe sequelae are unclear, a commonly acknowledged mechanism is antibody-dependent enhancement (ADE), which results when a pre-existing antibody from the primary infection cross-reacts with heterologous dengue serotypes during secondary infection [1, 5, 12, 13]. An interesting and plausible theory was proposed suggesting that the existence of DENV-specific maternal IgG antibodies that transfer across the placenta may account for the development of infant DHF in humans after acquiring a primary DENV infection [5]. Nevertheless, non-ADE mechanisms may also trigger development of severe sequelae [14]. Host factors, such as activation of the complement system, and viral factors, such as genetic variation of viral strains, may have roles in these processes [15]. In this regard, cytokine storm has been shown to play a major role in the development of severe complications in DENV-infected patients [16].

During the past decade, several anti-DENV therapeutic trials have been conducted but with very limited success [17]. In contrast to hepatitis C virus (HCV), also a member of the Flaviviridae family, no specific medication has been approved for treatment of DENV infection. Because of the limited understanding of pathogenesis and lack of suitable animal models, considerable challenges remain in developing effective regimens for treating DENV infection [18].

3. Approaches to identifying anti-DENV compounds and their limitations

The introduction of several HTS approaches has greatly enhanced the likelihood of identifying potential anti-DENV compounds [19]. Two target-based screening approaches are commonly used, namely structure-based and enzymatic activity–based screening assays. Characterization of the crystal structures of viral E protein and several DENV enzymes, such as viral protease, viral methyltransferase, and RNA-dependent RNA polymerase, has greatly
improved development of effective anti-DENV compounds, based on structure-based approaches [20-22]. By combining chemical libraries and docking programs with the known crystal structures of DENV, potential chemical interactions between the compounds and DENV components can be analyzed. One example is the identification of DENV E protein inhibitor [23]. The weakness of this approach is that the presence of cellular and/or viral factors may interfere with the predicted chemical interactions between the compound and viral protein. Thus, the identified compound may have high binding affinity but weak inhibitory potential toward viral targets. The enzyme activity–based approach is used to functionally identify compounds that actively inhibit the enzyme activity of viral components. The success of this approach is hampered by several common factors, such as uptake of the compound by infected cells and the presence of cellular factors interfering with the interaction between the compound and viral target, as well as availability of the viral factor for the compound [24].

As compared with target-based HTS, cell-based HTS appears to be more compatible in the setting of virus-infected host cells. Different readouts, such as viral protein expression, viral RNA replication, viral progeny production, viral cytopathic effect, and cellular functions like ATP production, can be used to measure the inhibitory effects of compounds. After identification of a potential compound, antiviral mechanisms, cellular targets, and the interaction between the compound and viral factor can be characterized. This approach has been successful in identifying several potential compounds that target viral NS4B protein [25], host adenosine nucleoside [26], and dihydroorotate dehydrogenase [27].

Another form of cell-based screening was developed by using an image-based high-content assay. Although this assay requires additional labor and more-sophisticated equipment and data analysis, localization of the compound and potential interacting molecules, as well as morphological changes in host cells, can be clearly seen [28].
weakness of this assay is its inability to detect the potential cytotoxicity of the compound while visualizing images. In addition, the only accountable indicator is cell number, which is relatively unreliable in determining drug effects [28].

4. Recent progress in developing compounds with potential for clinical evaluation

For DENV to successfully infect host cells and replicate, both structural and NS proteins that preserve enzymatic activity are essential. In addition, certain host factors are crucial for the virus to complete the infectious process and produce viral progeny. It is therefore reasonable to target and inhibit any of these crucial components of the virus or host cell in order to develop a successful treatment for DENV infection. Although many compounds preserve anti-DENV activity in vitro and in vivo, ClinicalTrials.gov shows that none of these specifically developed and DENV infection-targeted compounds has entered clinical trials for dengue treatment. Here, we briefly summarize several recently developed compounds that deserve clinical attention. We will discuss several characteristics of these reported compounds (the structures are shown in Table 1), to highlight their clinical potential. First, in addition to cell-based studies, the anti-DENV effects of compounds must be verified in vivo. Second, therapeutic concentrations must be as low as possible and have a wide therapeutic range. Third, the safety of the compounds must be confirmed in animals. Because there have been many excellent reviews on related topics during the past few years, we searched Medline by using a combination of the keywords "dengue", "mice", and "drug" for articles that were published during 2012–2016 and reported potential therapeutic compounds for DENV infection.

4.1. Compounds inhibiting viral entry

A small molecule, ST-148 or
3-amino-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[3,2-e] pyridine-2-carboxamide, inhibited replication of all DENV serotypes by interacting with the viral capsid protein [29]. In AG129 mice receiving ST-148, 50 mg/kg orally three times a day for 3 consecutive days, there were significant reductions in mean viral titers of plasma and several major organs. There were also nonsignificant reductions in the levels of some cytokines and chemokines, such as tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6) but not interferon-gamma (IFN-γ) [29]. ST-148 was later shown to interfere with the assembly and disassembly of DENV nucleocapsid, thus interrupting release of viral RNA from incoming nucleocapsids and formation of virus particles [30].

4.2. Compounds targeting viral NS proteins

The DENV NS3 protein closely interacts with its cofactor NS2B protein and mediates protease activity to cleave viral polyprotein and proceed with virus maturation and replication. In addition, viral NS3 protein preserves RNA helicase, nucleoside, and RNA triphosphatase activities, which are essential for viral replication. HTS assays showed that the benzoxazole inhibitor ST-610 inhibited replication of all four DENV serotypes by suppressing DENV NS3 helicase RNA unwinding activity [31]. Both the Ames assay and Muta-Chromo plate assay excluded the mutagenic potential of this compound. The drug was well tolerated in mice. Intraperitoneal injection of 3, 30, and 100 mg/kg ST-610 twice daily into DENV-infected AG129 mice significantly reduced viral loads in spleen and liver and viremia in plasma [31].

Targeting the DENV NS4B protein as an approach to inhibit DENV production has been reviewed elsewhere [32]. In a cell-based DENV-2 replicon assay, the authors examined a compound library and identified a spiropyrazolopyridone compound that potently inhibited replication of DENV-2 and -3 with a 50% effective concentration of 10 to 80 nM. However,
the compound did not affect DENV-1 or -4. A derivative of this compound, designated compound 14a, effectively reduced viremia in DENV-2–infected AG129 mice when given orally at a dose of 100 mg/kg twice daily, even when treatment was started 10 or 24 h after viral infection. A mutation at amino acid 63 of DENV-2 NS4B conferred resistance to compound inhibition, which suggests that the molecular target of this compound is on viral NS4B [33].

4.3. Compounds targeting host factors

Suppression of host factors required for viral production is another strategy to combat DENV infection. Development of potential nucleoside inhibitors for DENV infection therapeutics is aided by experience with HCV drug discovery, because of the similarities between these viruses. However, none of the developed adenosine analogs, many of which are potent anti-HCV compounds, met required effectiveness and safety profiles in preclinical studies of DENV infection [34].

Iminosugars have potential because they competitively inhibit the host endoplasmic reticulum α-glucosidase enzyme [35], which is important for DENV replication, and have been used to treat diabetes, although their side effects limit their broad application. AG129 mice were treated intraperitoneally with anti-prM/M monoclonal antibody and then infected with DENV-2 to induce ADE-mediated DHF/DSS-like disease. The protective effects of the iminosugar UV-12, administered 1 h before virus challenge at a dose of 20–100 mg/kg three times a day for 7 days, were then examined. UV-12 protected 100% of animals (for longer than 9 days); whereas, survival was 0% among mice treated with vehicle (for less than 5 days) [36]. The findings were similar for a guinea pig model [36]. Comparable protective effects were reported for another iminosugar drug, UV-4, which, in addition to reducing mortality, also potently inhibited viremia and cytokine storm in an ADE mouse model [37]. The
DENV-protective effects of the UV-4 hydrochloride salt UV-4B were evaluated in the same ADE mouse model. The results showed that a therapeutic dose of 10–20 mg/kg administered thrice daily for 7 days significantly improved survival of animals. Encouragingly, the therapeutic effects remained when treatment was initiated 48 h after virus challenge. Further investigation confirmed that UV-4B inhibited endoplasmic reticulum α-glucosidases [38].

The nucleoside analog 2'-C-methylcytidine targeted the viral RNA polymerase and exhibited potent anti-DENV activity in a cell-based system at a half-maximal inhibitory concentration (IC$_{50}$) of 11.2 ± 0.3 µM. Anti-DENV effects were also seen in a DENV-infected suckling mice model [39].

5. **Clinical experience with drugs for DENV infection**

In addition to viral mechanisms, the overwhelming immune response and increased cytokine levels during DENV infection substantially contribute to development of DHF and/or DSS [40, 41]. Thus, reducing viral load with anti-DENV drugs and suppressing the immune response with immunomodulatory regimens are important strategies in studies of clinical therapies for DENV infection. During the past few years, the risk/benefit profiles of several drugs (the structures are shown in Table 1) have been evaluated in patients with DENV infection (the clinical data are shown in Table 2). Two recently published articles offer an excellent review of experiences with these drugs [42, 43].

5.1. **Corticosteroids**

Corticosteroids are potent immunosuppressants and were evaluated for their effectiveness against DENV infection. Patients with a platelet count lower than 50 × 10$^9$/L were allocated to receive placebo or 4 mg intravenous dexamethasone followed by 2 mg every 8 h for 24 h. In total, 100 patients were enrolled in each arm, and increase in platelet
count from day 1 to day 4 was selected as the primary endpoint. Low-dose dexamethasone treatment did not increase platelet counts in patients with DENV infection [44].

The risk/benefit relationship of low-dose (0.5 mg/kg) and high-dose (2 mg/kg) prednisolone for 3 days was studied in 225 Vietnamese patients examined within 3 days of fever onset after DENV infection. As compared with placebo, there was no increase in the duration of viremia or incidences of adverse events among patients receiving prednisolone treatment. In addition, there were no differences in development of DENV-associated fatal manifestations, like shock, between patients treated with prednisolone and those receiving placebo [45]. Subsequent biological and genetic studies analyzed the effects of prednisolone in these patients. Only 81 of the 47,231 transcripts in the whole-blood gene expression profile that manifested during DENV infection were associated with prednisolone treatment. In addition, prednisolone treatment did not affect concentrations of proinflammatory cytokines in DENV-infected patients [46].

Despite the unfavorable outcomes for corticosteroid therapy during the early phase of DENV infection, limited data from patients with severe manifestations are encouraging. A clinical trial of 149 adults with grade II DHF examined the efficacy of adjunctive corticosteroid therapy. The patients were divided into three arms, which received a short course of full-dose, continuous intravenous dexamethasone (4 mg every 6 h for 2–3 days), intermittent intravenous dexamethasone (4 mg as needed during febrile episodes), or no adjunctive corticosteroid therapy. Adjunctive corticosteroid therapy had no effect on the severity of thrombocytopenia or liver impairment. However, illness and hospital stay durations were significantly shorter in the arm receiving the short course of full-dose, continuous intravenous dexamethasone [47]. Furthermore, a 2-year-old boy with DENV infection and hemophagocytic syndrome received intravenous dexamethasone, 10 mg/m², with gradual dose tapering. This regimen resulted in sustained improvement of symptoms and
Existing evidence from a limited number of studies suggests that corticosteroids given during the early phase of DENV infection do not have significant clinical effects [49, 50]. From a clinical perspective, the main concern in administering corticosteroids is suppression of defense mechanisms needed for viral clearance, as antiviral cytokines are quickly induced in immunocompetent patients [16, 51]. Because most DENV-infected patients recover uneventfully, host immunity likely has a critical role, especially during the early stage of virus infection. Early suppression of immunity with corticosteroids may greatly reduce immune defense and therapeutic benefits.

5.2. Antimalarial drugs

The need for acidic conditions in endosomes for DENV to complete replication suggests potential therapeutic effects for the antimalarial medication chloroquine, a lysosomotropic 4-aminoquinoline derivative [52, 53]. In addition to preserving anti-DENV activity [54], chloroquine, with its immunomodulatory effects, is a well-known disease-modifying antirheumatic drug for treatment of autoimmune disorders.

A double-blind, randomized, placebo-controlled trial examined chloroquine treatment for early-phase DENV infection in 307 adults, 257 (84%) of whom had laboratory-confirmed DENV infection. Patients received a 3-day course of chloroquine (n = 153), 600 mg on days 1 and 2 and 300 mg on day 3 (the recommended therapeutic doses for treatment of susceptible *Plasmodium vivax* infection), or placebo (n = 154). The primary endpoints were time to resolution of DENV viremia and time to resolution of DENV NS1 antigenemia. Duration of DENV viremia tended to be longer among chloroquine-treated patients, but chloroquine treatment did not affect NS1 antigenemia. However, a nonsignificant decrease in the incidence of DHF was noted among patients receiving chloroquine (29 patients [23.2%])
with DHF in the chloroquine arm versus 41 [33.6%) patients in the placebo arm) [55].

Although the incidence of adverse gastrointestinal events was higher in the group receiving chloroquine, the symptoms were mild and tolerable [55]. A randomized, placebo-controlled, double-blind trial of 129 patients with DENV-related symptoms was conducted in Brazil. Among 37 patients with confirmed DENV infection, 19 received chloroquine and 18 received placebo. Treatment with 500 mg chloroquine (300 mg base) twice a day did not affect disease duration or fever severity or duration. However, pain intensity and performance of daily activities were significantly better in the group receiving chloroquine [56].

There are many possible explanations for the inadequate therapeutic effects of chloroquine for DENV infection. It may be that therapeutic concentrations of the drug were not maintained. A minimum plasma chloroquine concentration of 1 µg/mL (~3.125 µM/L), or a minimum concentration of 16 µM/L in whole blood, should be maintained throughout the period of viremia until virus titers are undetectable [57]. Although not yet known, a possibility that DENV-infected patients may metabolize drug differently should also be investigated.

5.3. Doxycycline and tetracycline

In addition to their bacteriostatic properties, doxycycline and tetracycline have immunomodulatory effects [58]. Patients who developed dengue fever (DF) or DHF within 72 h after diagnosis were allocated to receive placebo (n = 34), tetracycline (n = 35), or doxycycline (n = 45). Drug dose varied in relation to patient disease status and age. Symptomatic and supportive care was provided as needed. Changes in serum levels of IL-6, IL-1β, TNF, TNF receptor 1, and IL-1 receptor antagonist (IL-1Ra) were evaluated in treated patients at the start of therapy and after 3 and 7 days of treatment. Treatment with tetracycline or doxycycline appeared to reduce cytokine levels and increase IL-1Ra levels [59]. As
compared with tetracycline, doxycycline seemed to have stronger immunomodulatory activity. Although the authors suggested that changes in cytokine levels were evidence that the drugs had potential therapeutic benefits, detailed clinical and virological data were not included in the report. Future studies should attempt to confirm the therapeutic benefits of doxycycline and tetracycline for DENV infection.

5.4. Cholesterol-lowering agents

The concept that interference with cholesterol biosynthesis might regulate DENV replication was first noted when researchers used siRNA screening to identify molecules that regulate DENV replication. The study showed that siRNA-mediated knockdown of mevalonate (diphospho) decarboxylase inhibited DENV-2 replication in an A549 DENV-2 subgenomic replicon cell line containing a Renilla luciferase cassette [60]. Later studies of AG129 mice revealed that pretreatment with one (200 mg/kg/day orally, 24 h before virus inoculation) or three doses (200 mg/kg/day orally at 72, 48, and 24 h before virus inoculation) of lovastatin increased survival rate but that only the three-dose arm showed reduced viremia as compared with untreated mice. Histopathological analysis of the livers and spleens of the animals revealed a marked decrease in extramedullary erythropoiesis foci and inflammatory infiltrate after lovastatin treatment [61].

Three hundred DENV-infected Vietnamese adults were randomized within 72 h of fever onset to receive placebo (n = 151) or 80 mg lovastatin (n = 149) for 5 days. The primary outcome was safety, and the secondary outcomes included disease progression rate, fever clearance time, plasma viremia, and quality of life. Although lovastatin treatment appeared to be safe and well tolerated, there was no beneficial effect on clinical variables or DENV viremia [62].
5.5. Intravenous immunoglobulin (IVIG)

Polyclonal plasma-derived IgG, a preparation pooled from thousands of blood donors, has long been used as replacement therapy for immunodeficiency disorders and as a treatment for autoimmune diseases with life-threatening manifestations [63]. The anti-inflammatory mechanisms of IVIG are not fully understood, but recent evidence suggesting the involvement of cellular Fc-γ receptors and Fc glycosylation improves our understanding of how this therapeutic regimen may work [64].

Because DENV-infected patients have elevated levels of platelet-associated IgG and severe thrombocytopenia, a randomized controlled trial of IVIG was conducted. The IVIG-treated arm comprised 10 patients with DF and five patients with DHF; the control arm (without IVIG treatment) included nine and seven patients with DF and DHF, respectively. Under the standard protocol, supplemental IVIG 0.4 g/kg/day was given on the second, third, and fourth days after hospitalization. IVIG treatment had no significant effect on platelet count between day 2 and day 7 [65]. Interestingly, a study of three patients with DENV infection associated with hemophagocytic syndrome suggested that combined treatment with dexamethasone and IVIG was beneficial; the patients recovered uneventfully [66].

Although IVIG has uncertain therapeutic effects, anti-D immune globulin, which is highly effective in blocking the Fc-γ receptor, offers hope in the fight against DENV infection. Patients (27 children and 20 adults) with DHF and severe thrombocytopenia (platelet count \( \leq 50,000/\text{mm}^3 \)) were allocated to receive placebo or intravenous anti-D (Rh\(\text{D}^\text{o}\)-D) immunoglobulin, 50 µg/kg (250 IU/kg). An increase in platelet count of 20,000/mm\(^3\) over baseline values after 48 h of drug administration was chosen as the indicator of response. The mean peak platelet count for the observation period was 166,400/mm\(^3\) with anti-D treatment as compared with 138,800/mm\(^3\) with placebo treatment in children; the respective values were 140,143/mm\(^3\) and 90,857/mm\(^3\) in adults. The mean maximum platelet count at 48 h was
91,500/mm$^3$ in the anti-D arm and 69,333/mm$^3$ in the placebo arm; 75% of patients in the anti-D treatment arm had an increase in platelet count ≥ 20,000/mm$^3$, as compared with 58% of those in the placebo arm. Although anti-D antibodies attached to red blood cells in Rh-positive patients, the decrease in hemoglobin in the two arms was not statistically significant [67]. Because anti-D treatment is cheaper than IVIG, it may have a therapeutic role for DENV-infected patients with thrombocytopenia.

5.6. Celgosivir

An early study reported that the alpha-glucosidase inhibitor castanospermine inhibited infections by all four DENV serotypes. Furthermore, castanospermine treatment significantly reduced mortality and increased survival among DENV-infected mice at doses of 10, 50, and 250 mg/kg of body weight per day [68]. Celgosivir (6-O-butanoyl castanospermine), a pro-drug of castanospermine, protected AG129 mice from lethal DENV infection at a dose of 50 mg/kg twice daily for 5 days. Notably, treatment was still effective when delayed by 48 h [69]. A phase 1b, randomized, double-blind, placebo-controlled, proof-of-concept trial enrolling 50 DF patients examined the therapeutic effects of celgosivir for DENV infection [70]. The patients (n = 24) received a loading dose of 400 mg celgosivir, followed by 200 mg every 12 h, for a total of nine doses, and the effects and safety were compared with those of patients receiving placebo (n = 26). As compared with placebo, celgosivir treatment was associated with nonsignificant reductions in viral load and fever burden [70]. The period of treatment was short, and there were no detectable differences in adverse events between celgosivir and placebo arms [70]. A subsequent study used an increased celgosivir dosing interval to treat DENV-infected AG129 mice. In contrast to the ineffective twice-daily dosing regimen, celgosivir given four times daily significantly reduced viremia in animals [71].
A clinical trial enrolling 72 DENV-infected patients is currently examining the effects of ceglosivir, 150 mg every 6 h, and modipafant, 50 or 100 mg every 12 h, for 5 days. Both viral load and platelet nadir were selected as primary outcome measurements. The estimated completion date of the study is August 2018 (ClinicalTrials.gov identifier: NCT02569827).

5.7. Balapiravir

The recently developed nucleoside analog R1479 (4′-azidocytidine) is a potent inhibitor of the NS5B polymerase of HCV [72]. An oral form, R-1626 (balapiravir), is rapidly converted to the active compound R-1479 and was found to suppress HCV infection \textit{in vitro} and \textit{in vivo} [73]. Because DENV and HCV have a similar architecture for the RNA-dependent RNA polymerase [74], the effects of R1626 were evaluated for DENV infection. An early phase, dose-escalating, randomized placebo-controlled trial examined men with DF diagnosed within 48 h. The patients were allocated to receive placebo (n = 30) or 1500 mg (n = 10) or 3000 mg balapiravir (n = 22) orally for 5 days. As compared with placebo, balapiravir neither significantly downregulated viremia or NS1 antigenemia nor reduced fever clearance time [75]. In addition, treatment did not affect concentrations of several plasma cytokines [75]. Further analysis revealed that balapiravir was poorly metabolized to its active form in DENV-activated peripheral blood mononuclear cells, which might explain its inadequate therapeutic effects in patients with DENV infection [76].

5.8. Pentoxifylline and calcium supplementation

Pentoxifylline blunts the proinflammatory actions of TNF-α, a key mediator of DHF. A prospective, randomized and double-blind study of the effects of pentoxifylline on 55 children with DHF grade III-IV was carried out in Colombia. Patients received standard therapy for DHF or intravenous infusion of pentoxifylline at 12.5 mg/kg/day for 3
consecutive days. The study showed a significant decline in TNF-α 24 h after pentoxifylline treatment (n = 28) compared to the control group (n = 27). However, there was no benefit in outcomes measuring mortality, hospital length of stay or complications. Among the most severely ill children, the mean length of stay was statistically insignificant shorter in the treatment group [77].

A clinical trial examining the efficacy of calcium carbonate, 1.2 to 1.8 g/day, for DF patients with thrombocytopenia showed that the treatment increased the number of blood platelets in a small patient group (10 patients per treatment plus a control arm). The treatment also reduced the duration of clinical symptoms and signs of DF [78].

6. **Drugs currently undergoing clinical trials or with unreported trial outcomes**

6.1. **Ivermectin**

The DENV NS5 protein has several enzyme activities, including methyltransferase, RNA-dependent RNA polymerase, and, possibly, guanylyltransferase. Nuclear localization of NS5 is an important process in the DENV infectious cycle; thus, the association of DENV NS5 with the host cell nuclear import proteins importin α (IMP-α) and IMP-β1 is critical in viral production. An effective agent for treating scabies and HIV infection [79], ivermectin also inhibits replication of yellow fever virus with a 50% effective concentration (EC<sub>50</sub>) in the sub-nanomolar range. Ivermectin blocked DENV infection by inhibiting IMP-α/-β nuclear import and binding of IMP-α/-β1 to NS5 but had no effect on the other nuclear import pathways examined [80]. In addition, ivermectin effectively inhibited the NS3 helicase activity of DENV [81]. A trial of the effects of ivermectin in DENV infection enrolled 360 patients, and time to resolution of viremia was selected as the primary outcome measure. Patients received ivermectin 200–400 µg/kg once daily for 2 days or 3 days, or placebo. The
estimated study completion date was February 2016 (ClinicalTrials.gov identifier: NCT02045069).

6.2. Ketotifen

Because vascular leakage is an important factor in the development of fatal manifestations after DENV infection, the mast cell–stabilizing drug ketotifen was selected for evaluation as a treatment for DENV infection. The randomized, double-blind study compared the responses of DENV-infected patients given either 2 mg ketotifen twice daily or placebo (n = 55 per arm) for 5 days. The primary outcome was fluid accumulation in the pleural cavity, as determined by MRI. The secondary outcome measures included clinical symptoms such as rash, petechia, purpura, ecchymosis, epistaxis, and other signs of bleeding. The estimated date of final data collection for the primary outcome measures was July 2016 (ClinicalTrials.gov identifier: NCT02673840).

7. Drugs with clinical potential for treatment of DENV infection

In addition to the medications above, which are in the process of, or have already undergone, preliminary clinical evaluation, several other drugs have clinical potential for treatment of DENV infection. These medications have not been examined clinically but were reported to have anti-DENV effects in cell-based studies, with or without supportive evidence in animal studies. These medications are briefly discussed below.

7.1. Dopamine D2 receptor (D2R) antagonist

Prochlorperazine (PCZ), a D2R antagonist commonly used to treat nausea, vomiting, and headache, was shown to have anti-DENV activity. PCZ inhibited viral binding and viral entry through D2R- and clathrin-associated mechanisms, respectively. PCZ given orally
and/or intraperitoneally with different doses (1-8 mg/kg body weight) and dosing intervals (1- or 2-day interval) immediately or 6 h after DENV infection in a signal transducers and activators of transcription (STAT)1-deficient mouse model significantly reduced or delayed death [82]. In addition to mild adverse events such as drowsiness and headache, the major side effects from taking prochlorperazine are extrapyramidal syndrome with the presentation of acute dystonia, pseudoparkinsonism, or akathisia as well as the life-threatening condition of neuroleptic malignant syndrome [83]. As calculated, the protective effective doses for PCZ in DENV-infected mice are well below the potentially hazardous doses and in humans are clinically feasible [82].

SKI-417616 belongs to a class of tricyclic small-molecule compounds, the dihydrodibenzothiepines, and was shown to inhibit early events in the life cycle of DENV infection. Interestingly, SKI-417616 is structurally similar to antagonists of dopamine and serotonin receptors, and inhibition of D4R suppressed DENV infection [84].

7.2. 4-hydroxyphenyl retinamide (4-HPR) (fenretinide)

A synthetic retinoid derivative, 4-hydroxyphenyl retinamide (4-HPR) (fenretinide), has been clinically evaluated as a treatment for several illnesses, including cystic fibrosis, autoimmune disorders, and cancers. 4-HRP was identified by screening a library of bioactive lipids and modulators of lipid metabolism for anti-DENV activity. Twice-daily administration of oral 4-HPR, 90 mg/kg of body weight, significantly reduced viremia in DENV-infected AG129 mice lacking type I and II IFN receptors [85]. In addition, 4-HPR inhibited DENV replication, ADE infection, and ex vivo infection in peripheral blood mononuclear cells [86]. Oral 4-HPR, 20 mg/kg, given at the time of infection and then twice daily for 5 consecutive days provided 70% protection against lethal DENV ADE infection [86]. Because of its tolerable safety profile in humans, this drug might be useful for treating patients with DENV
infection.

7.3. p38 MAPK inhibitor

The clinical experience with p38 MAPK inhibitors in patients of autoimmune arthritis appeared to be not satisfactory because of limited therapeutic efficacy and potential adverse events such as liver toxicity, serious infection, gastrointestinal disorder, and central nervous system disorder [87]. While improvement of the regimens is ongoing, the expansion of other therapeutic indications, including DENV infection, was preliminarily tested in cell-based systems and animals. The p38 MAPK inhibitor SB203580 inhibited production of several pro-inflammatory cytokines such as TNF-α, IL-8, and regulated on activation, normal T cell expressed and secreted (RANTES) in immortalized and primary cells infected by DENV [88]. SB203580 given orally protected against intestinal leakage and improved several hematological parameters and inflammation in AG129 mice infected by DENV [88]. SB203580 might also attenuate liver injury and improve thrombocytopenia by downregulating proinflammatory cytokines and chemokines, including RANTES and IP-10, and apoptosis-associated molecules like the caspase-3, -8, and -9 proteins [89]. These results suggest that p38 inhibition is a potential therapeutic approach for combating DENV infection.

7.4. Bortezomib

Bortezomib, a proteasome inhibitor, was approved in the United States for treating relapsed multiple myeloma and mantle cell lymphoma and was reported to inhibit DENV egress [90]. Importantly, bortezomib inhibited infection of primary monocytes by all four DENV serotypes at concentrations of 25–50 nM [90]. In C57BL/6 mice infected with DENV-2, bortezomib, given in a single dose of 1 mg/kg (a dose comparable to that given for treatment of multiple myeloma) at 6 h post-infection significantly reduced viral load and the
severity of thrombocytopenia and plasma leakage, as compared with control mice [90].

7.5. Extract of Cissampelos pareira Linn.

An alcoholic extract of Cissampelos pareira Linn. (Cipa extract) potently inhibited replication of the four DENV serotypes and TNF-α production. In a limited drug toxicity analysis, Cipa extract did not affect platelet count or viability of red blood cells in an AG129 mouse model. There was no detectable toxicity in Wistar rats at a dose of 2 g/kg body weight for up to 1 week. The evidence suggests that this extract might be clinically useful in dengue-endemic, resource-poor countries like India and some African countries [91].

7.6. Dasatinib and leflunomide

Other drugs, such as leflunomide and dasatinib, have potential as treatments for DENV infection, but existing evidence is limited to cell-based assays. Dihydroorotate dehydrogenase is a rate-limiting enzyme in pyrimidine synthesis, and the increased need for proliferating cells makes it a potential target for anti-DENV treatment [92]. Leflunomide, a dihydroorotate dehydrogenase inhibitor and commonly prescribed immunomodulatory drug in the treatment of rheumatoid arthritis and seronegative spondyloarthropathy [93], inhibits replication of many viruses, including polyomavirus [94], ganciclovir-resistant cytomegalovirus [95], herpes simplex virus, and HIV [96, 97]. Leflunomide also exhibited anti-DENV activity in cell-based assays [98]. Dasatinib is a Fyn tyrosine kinase inhibitor and has been used for anticancer treatment. Dasatinib inhibited DENV infection by suppressing RNA replication [99].

8. Challenges remain

Over the past few decades, many compounds were found to inhibit DENV production in
vitro and in vivo, and through HTS assays, many more compounds are emerging. However, these compounds are far from entering the market. Several drugs that were clinically repurposed and evaluated to treat patients with DENV infection have not been very successful. Clearly, challenges remain in introducing effective anti-DENV regimens to market.

8.1. Challenges of early treatment: timing and dosing

Most DENV-infected patients exhibit nonspecific symptoms and signs after infection, which makes early intervention very challenging. The critical issue of early treatment in DENV infection is reflected in animal studies of celgosivir. When the drug was given on day 0 of DENV infection, it effectively reduced viral load; however, the therapeutic effects disappeared when the drug was given on day 3 after DENV infection, which suggests that plasma viral load is strongly associated with the therapeutic efficacy of anti-DENV treatment [71]. Once a patient is in severe viremia, the standard therapeutic dose may be ineffective. Evidence suggests that aside from dose escalation, increasing the frequency of drug administration might be beneficial against DENV infection [71].

8.2. Identifying the checkpoint for disease exacerbation

Most DENV-infected patients recover uneventfully within 1-2 weeks after infection, and only a small number develop fatal manifestations like DHF and DSS. Identifying the turning point for the transition from DF to DHF or DSS is therefore essential. Controversy remains regarding the association between viral load and disease severity in patients with DENV infection. An early study of 54 children with secondary DENV-3 infection suggested an association between DHF development and higher mean plasma viremia [100]. However, an analysis of a cohort of DENV-infected children in New Delhi found no association between
viral load and disease severity; however, high viral load appeared to be associated with prolonged thrombocytopenia and delayed recovery [101]. Whether plasma viral load is a useful marker for the risk of severe complications remains to be determined. Even if it is, no viral level cut-off for predicting disease exacerbation has been determined.

In the setting of type I and II IFN receptor knockout, mice infected by a non–mouse-adapted low-passage DENV-3 clinical isolate, DV3P12/08, developed lethal systemic manifestations with cytokine storm and rapid death. Pathological analysis revealed vascular leakage, extensive hemorrhage, and organ damage [102]. High concentrations of TNF-α, IL-6, and MCP-1 were detected in serum. Treatment with an anti–TNF-α neutralizing antibody extended survival and reduced liver damage in the animals; however, virus production was unaffected by treatment. Neutralization with an anti–TNF-α antibody also effectively reduced serum levels of IL-6, MCP-1, and IFN-γ. Similarly, moderate therapeutic effects and prolonged survival were observed after blocking IL-6. The results of this animal study suggest that, as compared with viral load, cytokine storm may be a more important factor in the development of severe clinical manifestations [102]. Whether the level of a single cytokine, like TNF-α, or those of a few cytokines can serve as a marker of disease progression is a topic worthy of investigation.

9. Future directions

The mechanisms leading to DHF and DSS are largely unclear. An important aim of ongoing DENV research should be the detection of “checkpoint signals” that identify patients likely to develop fatal manifestations after DENV infection. In addition, there is an urgent need for a more humanized small-animal model of DENV infection. Such a model would serve as a good tool for studying the pathogenesis of DENV infection and identifying critical checkpoints. Such animal models would also be useful for testing the efficacy and safety of
newly identified compounds and hastening drug development and clinical evaluation. Encouragingly, a recently introduced mouse model with specific deletion of \( \text{Ifn-\alpha} \) receptor gene expression on subsets of murine myeloid cells was immunocompetent and susceptible to DENV infection \textit{in vivo} \([103]\). In these animals, the ADE effects could be induced with features simulating several characteristics observed in humans such as plasma leakage, liver injury, thrombocytopenia and high serum cytokine levels \([103]\).

Therapeutically, while early introduction of drugs requires more sensitive and more accurate diagnostic tools, appropriate dosing and/or increasing the frequency of drug administration is also important. Among the medications discussed above, introduction of corticosteroids in the early phase of DENV infection appears unwise, given that such treatment also suppresses antiviral immunity, which is critical in combating the virus. Immunomodulatory medications may also have limited help during the early phase of viral infection. However, corticosteroids and immunomodulatory drugs may well be introduced to patients after they develop cytokine storm or life-threatening manifestations. In contrast, antiviral regimens can be used from the time of diagnosis of DENV infection until resolution of the illness. Clinical studies should evaluate existing regimens for the various phases of DENV infection (Fig. 2).

Viral load and immune reaction are two important factors contributing to fatal manifestations; thus, strategies that simultaneously target these factors should be investigated. Patients might benefit from treatment with a combination of antiviral drugs and immunomodulatory medications, in accordance with clinical manifestations. Although the side effects of medications are always a concern, treatment for DENV infection typically continues for days or, occasionally, weeks. Thus, risk/benefit assessment should be flexible when evaluating anti-DENV regimens.
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Conflicts of Interest

The authors have no conflicts of interests related to this study.

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Dis 2010;4:e785.


[89] Sreekanth GP, Chuncharunee A, Sirimontaporn A, Panaampon J, Noisakran S,


Figure legend

Figure 1. After binding to viral receptor, DENV is endocytosed into endosome and the acidic environment of endosomal vesicles leads to fusion between virus and the endosomal membrane. Viral RNA is then released into the cytoplasm. The viral RNA genome is translated into a single polyprotein containing structural proteins, capsid (C), precursor membrane (prM), and envelope (E), and 7 nonstructural (NS) proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Subsequently, viral RNA synthesis proceeds. The synthesized RNA genomes associate with other viral components and enter the endoplasmic reticulum lumen to produce immature virus particles. After maturation in the trans-Golgi network and the aid by the host protease furin, mature virion is released from the cell.

Figure 2. Levels of strength for drug-of-choice along courses of DENV infection. DENV infection results in various clinical manifestations and most of the infected patients recover spontaneously in weeks. Some DENV-infected patients may progress to develop catastrophic manifestations such as hemorrhage fever and shock. It is suggested that drugs or not-yet-approved compounds preserving different effects may be prescribed or clinically evaluated according to the status of the patients. The well-recognized anti-DENV regimens are currently lacking. Celgosivir after adjustment of dosing intervals and the repurposed drug like prochlorperazine may be of potential as useful anti-DENV medications. Some beneficial
but statistically insignificant therapeutic effects were observed with chloroquine treatment.

Medications such as corticosteroids, IVIG and immunomodulatory drugs may only be helpful when they are prescribed in patients presenting symptoms and signs of overwhelming cytokine reaction. The intensity of individual drugs for different status of DENV infection is given. NSAID, nonsteroidal anti-inflammatory drug; IVIG, intravenous immunoglobulin.
**Figure 2**

### Dengue fever

**Cardinal manifestations:**
- Fever, headache, skin maculopapular rash, myalgia, brakebone pain, weakness, mild leukopenia, mild thrombocytopenia, elevations of serum aminotransferase

<table>
<thead>
<tr>
<th>Supportive care</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid supplement</td>
<td>++</td>
</tr>
<tr>
<td>NSAID</td>
<td>++</td>
</tr>
</tbody>
</table>

| Anti-viral regimens (to be developed and evaluated clinically) | +++ |

| Corticosteroid | ++ |
| Chloroquine | +++ |
| IVIG or anti-D (Rh0-D) immunoglobulin | ++ |
| Immunomodulatory drugs (e.g. doxycycline and tetracycline) | ++ |

### Hemorrhagic fever or shock

**Cardinal manifestations:**
- Severe thrombocytopenia, severe proteinuria, markedly elevated serum aminotransferase, bleeding tendency, hemorrhage, signs of plasma leakage or shock

<table>
<thead>
<tr>
<th>Supportive care</th>
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<tbody>
<tr>
<td>Fluid supplement</td>
<td>+++</td>
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<tr>
<td>NSAID</td>
<td>+</td>
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</tbody>
</table>

| Anti-viral regimens (to be developed and evaluated clinically) | +++ |

| Corticosteroid | ++ |
| Chloroquine | +++ |
| IVIG or anti-D (Rh0-D) immunoglobulin | ++ |
| Immunomodulatory drugs (e.g. doxycycline and tetracycline) | ++ |
Table 1. Chemical structures of compounds or drugs potential for therapeutics in DENV infection

<table>
<thead>
<tr>
<th>ST-148</th>
<th>ST-610</th>
<th>Spiropyrazolopyridone (14a)</th>
<th>UV-12</th>
<th>UV-4</th>
<th>UV-4B</th>
</tr>
</thead>
<tbody>
<tr>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

- 2-C-methylcytidine
- Prednisolone
- Dexamethasone
- Chloroquine
- Doxycycline
- Tetracycline

- Lovastatin
- Celgosivir
- Balapiravir
- Pentoxifylline
- Ivermectin
- Ketotifen

- Prochlorperazine
- 4-hydroxyphenyl retinamide (fenretinide)
- Dihydrodibenzothiepine (SKI-417616)
- Bortezomib
- Dasatinib
- Leflunomide
Table 2. Clinical experience with drugs targeting DENV infection

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism Aimed targets</th>
<th>Trial designs</th>
<th>Therapeutics</th>
<th>Patients No.</th>
<th>Targeted endpoints</th>
<th>Therapeutic results</th>
<th>Remark</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroid as prednisolone (Pred) or dexamethasone (Dex)</td>
<td>Pan-Immuno suppressant</td>
<td>DF Random, DB, PC</td>
<td>Dex (4 mg than 2 mg Q8h) or Plb for 24 h</td>
<td>100/arm</td>
<td>Degree of mean platelet count rise</td>
<td>No significant changes in mean platelet count</td>
<td>No effect</td>
<td>43</td>
</tr>
<tr>
<td>Grade II DHF</td>
<td>Retro-spective analysis</td>
<td>DF Random, PB, PC</td>
<td>Pred (0.5 or 2 mg/kg) or Plb for 3 days</td>
<td>75/arm</td>
<td>Rates of DSS, ICU admission, clinical bleeding… etc</td>
<td>No significant changes in all measured parameters</td>
<td>No effect on plasma cytokines</td>
<td>44</td>
</tr>
<tr>
<td>Chloroquine (CQ)</td>
<td>Inhibits viral replication</td>
<td>DF Random, DB, PC</td>
<td>600 mg (day 1 and 2), 300 mg (day 3) or Plb</td>
<td>CQ:153</td>
<td>Time to resolution of viraemia, viral NS1 antigenaemia… etc</td>
<td>Significant increase in platelet count and clinical improvement</td>
<td>No severe AE</td>
<td>55</td>
</tr>
<tr>
<td>Doxycycline (Dox) or tetracycline (Tet)</td>
<td>Immune modulation</td>
<td>DF and DHF Random</td>
<td>Doses depends on age and status of the patients</td>
<td>Tet:35,Dox:45,Control:34</td>
<td>Changes of cytokine levels</td>
<td>No beneficial effect on clinical or virologic parameters</td>
<td>Lower cholesterol levels with Lov treatment</td>
<td>58</td>
</tr>
<tr>
<td>Lovastatin (Lov)</td>
<td>Inhibits viral replication</td>
<td>DF Random, DB, PC</td>
<td>80 mg for 5 days or Plb</td>
<td>Lov:149, Plb:151</td>
<td>Disease progression; fever clearance time; plasma viremia; life quality and safety… etc</td>
<td>No significant changes in measured parameters</td>
<td>No effect</td>
<td>61</td>
</tr>
<tr>
<td>IVIG</td>
<td>Immune modulation</td>
<td>DF and DHF Random, controlled</td>
<td>0.4 g/kg/day at 2nd, 3rd and 4th day after admission</td>
<td>IVIG:15, Control:16</td>
<td>Platelet count and levels of platelet-associated IgG</td>
<td>No changes of targeted parameters</td>
<td>No effect</td>
<td>64</td>
</tr>
<tr>
<td>anti-D (Rh-D) immunoglobulin</td>
<td>Immune modulation</td>
<td>DHF Random, PC</td>
<td>50 μg/kg (250 IU/kg)</td>
<td>anti-D:25, Plb:22</td>
<td>Platelet counts and safety</td>
<td>Significant increase of platelet count</td>
<td>No severe AE</td>
<td>66</td>
</tr>
<tr>
<td>Celgosivir (Ccl)</td>
<td>Inhibits viral replication</td>
<td>DF Random, DB, PC</td>
<td>Loading 400 mg, then 200 mg Q12h (total 9 doses)</td>
<td>Ccl:24, Plb:26</td>
<td>Viral load, fever burden and safety</td>
<td>No significant changes in targeted parameters</td>
<td>No severe AE</td>
<td>69</td>
</tr>
<tr>
<td>Balapiravir (Bal)</td>
<td>Inhibits viral replication</td>
<td>DF Random, PC</td>
<td>1500 mg, 3000 mg or Plb for 5 days</td>
<td>1500 mg:10, 3000 mg:22, Plb:30</td>
<td>Viremia, NS1 antigenemia, fever clearance time… etc</td>
<td>No significant changes in targeted parameters</td>
<td>No severe AE</td>
<td>74</td>
</tr>
<tr>
<td>Pentoxifylline (Pent)</td>
<td>Immune modulation</td>
<td>Grade III-IV DNF Prospective Random, DB.</td>
<td>12.5 mg/kg/day for 3 days</td>
<td>Pent:28, Control:27</td>
<td>Mortality, length of hospital stay or complications</td>
<td>Insignificant shorter of mean length of hospital stay</td>
<td>No significant effect</td>
<td>76</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>stabilize platelet</td>
<td>DF Random, control</td>
<td>1.2 to 1.8 g/day</td>
<td>10/arm</td>
<td>Platelet count; clinical outcome</td>
<td>Significant increase in platelet count and clinical improvement</td>
<td>Limited patient number</td>
<td>77</td>
</tr>
</tbody>
</table>

DB, double-blinded; PB, partially blinded; Random, randomization; PC, placebo-controlled; Placebo: Plb; Gp, group; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; IL, interleukin; TNF, tumor necrosis factor; IL-1Ra: interleukin-1 receptor antagonist; NS1: non-structural protein1; ICU, intensive care unit; AE, adverse event

Remark:
- Dex 4 mg than 2 mg for 5 days or 600 mg (day 1 and 2), 300 mg (day 3) or Plb
- No Dex (Gp 3)
- Plb for 3 days
- Doxycycline (Dox) reduced IL-6, IL-1β, and TNF but increased IL-1Ra
- Study favors continuous but short course of Dex
- Improvement in pain intensity and daily activity
Appropriate choosing or testing drugs for anti-DENV treatment: Levels of strength for drug-of-choice along courses of DENV infection

**Dengue fever**

**Cardinal manifestations:**
- Fever, headache, skin maculopapular rash, myalgia, brakebone pain, weakness, mild leukopenia, mild thrombocytopenia, elevations of serum aminotransferase

**Supportive care**
- Fluid supplement: ++
- NSAID: ++

**Anti-viral regimens**
- (to be developed and evaluated clinically): +++

**Corticosteroid:**
- ++

**Chloroquine:**
- ++++

**IVIG or anti-D (Rh0-D) immunoglobulin:**
- ++

**Immunomodulatory drugs (e.g., doxycycline and tetracycline):**
- ++

**Hemorrhagic fever or shock**

**Cardinal manifestations:**
- Severe thrombocytopenia, severe proteinuria, markedly elevated serum aminotransferase, bleeding tendency, hemorrhage, signs of plasma leakage or shock

**Supportive care**
- Fluid supplement: +++
- NSAID: +

**Anti-viral regimens**
- (to be developed and evaluated clinically): +++

**Corticosteroid**
- ++

**Chloroquine**
- ++++

**IVIG or anti-D (Rh0-D) immunoglobulin**
- ++

**Immunomodulatory drugs (e.g., doxycycline and tetracycline):**
- ++