

REVIEW

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Dengue: an update on treatment options

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Dengue is the most important mosquito-borne viral pathogen globally, with approximately 100 million cases of acute dengue annually. Infection can result in severe, life-threatening disease. Currently, there is no effective vaccine or licensed antiviral. Management is primarily supportive with fluids. Direct antiviral therapies that reduce dengue severity could be useful although these would need to inhibit all four viral serotypes effectively. This review focuses on the interventions that currently considered the gold standard in case management as well as exploratory therapies that have been studied in clinical trials. Although antiviral drug and therapeutic antibodies for dengue remain a work in progress, these studies have produced some promising results and may have the potential to be future drugs.

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Dengue virus (DENV) is the most prevalent mosquito-borne viral hemorrhagic fever. DENV is entrenched in over 100 countries in the tropics [1]. Endemic virus transmission, coupled with frequent cyclical epidemics, puts almost half of the world's population at risk of infection each year [2–4]. Both DENV and its mosquito vectors, principally *Aedes aegypti*, are also encroaching into nonendemic areas due to increasing international travel and rapid urbanization with poor public health infrastructure. Recent trends in global warming could further exacerbate the spread of dengue vectors both northward and southward [5,6]. Besides causing mortality, the cost of acute illness to society is considerably resulting from loss of productivity to costs arising from medical care [7,8].

DENV belongs to the *Flaviviridae* family. There are four antigenically distinct serotypes of this virus (DENV-1, 2, 3 and 4) [9]. A fifth serotype has been suggested although data demonstrating its antigenic distinctness from the other four DENVs remain to be reported in the literature [10]. The virus is enveloped and possesses a single-stranded positive-sense RNA genome [11]. The genome is translated as a single polypeptide, which is then cleaved by host and viral proteases into three structural proteins that collectively package the RNA genome: capsid (C), premembrane (prM), membrane (M) and envelope (E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The NS proteins encode enzymatic functions necessary for RNA replication and evasion of host immune responses.

Overview of dengue pathology

In susceptible human hosts, DENV is thought to infect Langerhans cells at the site of mosquito inoculation, which then migrates through the lymphatics. There, the infection then spreads to other cells of hematopoietic lineages, including monocytes and macrophages, before further systemic

KEYWORDS

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• flavivirus • therapeutic
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dissemination [12–14]. Viremia becomes apparent 1–2 days prior to the onset of symptoms, peaks during days 1–2 of fever and declines over next 3–5 days until resolution of fever [9,11,15]. DENV infection stimulates both innate and acquired immunity. Innate immune response, such as complement and type I interferon (IFN), functions as the first line of defense against DENV [16,17]. Acquired immune response involves serotype-specific CD4⁺ and CD8⁺ T cells, which can result in lysis of DENV-infected cells and production of cytokines such as IFN- γ , TNF- α and lymphotoxin [9,18,19]. Antibody response to DENV is primarily targeted at prM, E and NS1 viral proteins [20–22]. Antibodies may neutralize DENV by blocking virus attachment to cells or by blocking viral fusion with cellular membranes [23,24]. Conversely, if antibody titers are below the threshold necessary for DENV neutralization, they may opsonize DENV and facilitate viral entry into cells that express Fc γ receptors more efficiently. The result is an increase in virus replication and higher risk of severe dengue, a phenomenon termed antibody-dependent enhancement (ADE) of infection [25–28].

• Clinical presentation

Approximately a quarter of dengue infections are symptomatic. In those who develop symptomatic infection, there is a wide spectrum of presentation and often with unpredictable clinical evolution and outcomes [15,29].

The symptomatic phase of dengue is summarized in the latest WHO guidelines and is divided into three phases: the febrile phase, critical phase and recovery phase [15]. After an incubation period of 4–10 days, the febrile phase begins abruptly and lasts for 2–7 days. It is characterized by acute fever and constitutional symptoms which could be debilitating (including chills, malaise, headache, arthralgia, myalgia, retro-orbital pain, anorexia, nausea, vomiting, lethargy and rash). Thrombocytopenia, leukopenia and variable rise in hematocrit (which indicates vascular leak) develop. Dehydration is commonly due to pyrexia, anorexia and vomiting [15,18].

The critical phase coincides with fever deferescence and viremia resolution, lasting approximately 24–48 h. There is associated capillary leakage, plasma volume loss and, if untreated or improperly managed, shock [7,15]. It is hypothesized that clinical complications are virus-driven immunological responses [30–37].

If the patient survives the critical phase, recovery phase begins after 48–72 h. Patient's symptoms improve, and hemodynamic status stabilizes with reabsorption of extravasated fluid, along with rapid improvement in white cell and platelet counts. However, if excessive fluid therapy has been administered, patient may develop ascites and respiratory distress from massive pleural effusion, pulmonary edema or congestive heart failure [15,38].

• Rationale for dengue therapy

Currently, treatment for dengue is largely supportive. There is no licensed therapeutic drug. Several clinical trials of therapeutic interventions against acute dengue have been conducted and several more are in progress. These therapies target the virus, host immune responses or host factors required by DENV to complete replication cycle. Some clinical studies reported higher viremia levels (>tenfold) in patients with severe (dengue hemorrhagic fever [DHF]/dengue shock syndrome [DSS]) compared with nonsevere (dengue fever) during early infection [30,39–41]. This observed trend has led to the hypothesis that reducing viremia by antiviral therapy in the early phase of dengue infection may reduce severity of clinically apparent disease [30,42,43].

This review focuses on the clinical intervention that is widely and currently considered the gold standard in case management as well as those that have been tested in clinical trials as treatment for dengue. However, while development in dengue therapeutics is in progress, a number of practical challenges are already apparent. Patients may present to healthcare settings too late in their illness to benefit from antiviral therapy. Differentiating dengue from other febrile diseases is clinically difficult and diagnostic tests are usually lacking in resource-limited settings. Identifying patients at risk of severe disease and therefore most likely to benefit from antiviral treatment remains challenging. Furthermore, as viremia declines rapidly after illness onset, antiviral treatment must be fast acting. Dengue therapeutics development must therefore be coupled with improved diagnostic and prognostic tools in order to translate research into clinical practice.

• Supportive therapies during acute dengue

Fluid therapy

Sensible fluid resuscitation is of paramount importance in the management of patients with

Table 1. Summary of clinical trials that assessed fluid resuscitation for the treatment of dengue shock syndrome.

Study (year)	Design	Setting	Patients	Participants (n)	Fluid	Intervention	Conclusion	Ref.
Dung <i>et al.</i> (1999)	Double-blind, randomized, controlled trial	Vietnam	Pediatrics ICU patients aged 5–15 years with DSS	50	NaCl 0.9%; dextran 7; 3% gelatin; Ringer's lactate	Study fluid was administered for the first 72 h	Colloids may be superior to crystalloids, with more rapid restoration of hematocrit and cardiovascular stability	[47]
Ngo <i>et al.</i> (2001)	Double-blind, randomized trial	Vietnam	Pediatric ICU patients (aged 1–15 years) with DSS without severe hemorrhage	222	Dextran 70; 3% gelatin; Ringer's lactate; NaCl 0.9%	Study fluid was administered for the first 1 h, followed by Ringer's lactate (WHO guideline*)	No clear advantage to using any of the fluids in cardiovascular recovery time. Colloids were more likely to benefit patients severe shock	[44]
Wills <i>et al.</i> (2005)	Double-blind, randomized trial	Vietnam	Pediatric ICU patients (aged 2–15 years) with DSS	383 with moderately severe DSS; 129 with severe DSS	Moderately severe DSS group received crystalloid (Ringer's lactate) vs colloid (6% dextran 70 or 6% hydroxyethyl starch). Severe DSS group received dextran 70 vs starch	Study fluid was administered for the first 2 h, followed by Ringer's lactate*. Patients with persistent shock after administration of study fluid were given rescue colloids	Requirement for rescue colloid was similar for the different regimens. Significantly, more recipients in the dextran group had coagulopathy and fluid overload than starch. Colloids performed similarly in severe DSS. Ringer's lactate should be used in children with moderately severe DSS	[46]
Kalayanarooj (2008)	Randomized, single-blinded trial	Thailand	Pediatric DHF patients (aged 8.6 ± 3.9 years) who initially received crystalloid, but failed to improve or developed fluid overload	104	Colloids – 10% hydroxyethyl starch vs 10% dextran 40	Doses of study colloids were given in boluses when indicated at clinician's discretion	Both colloids were equally effective with no difference in complications with regard to fluid overload, renal functions and blood coagulation	[48]

*By WHO guideline 1997.

*Abstract only.

*Clinical diagnosis according to the WHO 1999 guideline.

DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome.

Table 2. Summary of clinical trials that assessed prophylactic blood product transfusions in dengue patients.

Study (year)	Design	Setting	Patients	Participants (n)	Treatment	Conclusion	Favor transfusion?	Ref.
Sellahewa <i>et al.</i> (2008)	Randomized, double-blind-controlled trial	Sri Lanka	Dengue adult patients with platelet count <40,000/mm ³ without bleeding	109	Prophylactic FFP	FFP may contribute to an increased platelet count in the first 12 h, but no difference was observed at 24–48 h	No	[54]
Assir <i>et al.</i> (2013)	Randomized, nonblinded trial	Pakistan	Adult dengue patients with platelet count <30,000/ μ l without bleeding or with mild bleeding	87	Prophylactic platelets	No benefit in the prevention or resolution of bleeding. 7% developed severe side effect	No	[55]
Lye <i>et al.</i> (2009)	Nonrandomized, retrospective cohort study	Singapore	Adult patients with severe thrombocytopenia (<20 $\times$ 10 ³ / μ l) without clinical bleeding	266	Prophylactic platelets	No benefit	No	[52]
FFP: Fresh frozen plasma.								

severe dengue, and a clinical algorithm has been published in detail in the latest WHO guidelines [15]. The goal is to prevent the complications of vascular leakage and hypovolemic shock.

Theoretically, colloids provide volume expansion over and above the actual fluid volume infused. Colloid molecules increase plasma oncotic pressure and promote retention of fluid in the intravascular compartment. The magnitude of this effect is determined by the average molecular weight of the colloid molecule and circulation retention time determines the duration of the effect [44,45]. For crystalloids, the plasma-volume-expanding capacity is related to sodium concentration. NaCl 0.9% (154 mM) in theory may perform better than Ringer’s lactate (131 mM). In addition, there are theoretical risks of worsening tissue acidosis and lactate accumulation when large volumes of Ringer’s lactate are infused [44,45]. However, Wills *et al.* compared Ringer’s lactate with two colloids and observed no significant advantage of colloid compared with lactate infusion in treating children with DSS [46]. In addition, the effects of colloid were transient, despite early rebound of hematocrit in children receiving colloids during initial resuscitation; there was no difference between the different fluid regimens in terms of outcome and severity of fluid overload 48–72 h after study infusion.

Besides the study by Wills *et al.*, three other trials have been conducted to compare the different types of fluid resuscitation regimen of DSS. **Table 1** summarizes all four such randomized controlled trials [44,46–48]. Taken collectively, there is no clear advantage to the use of colloids in terms of the overall outcome. However, colloids may be the preferred choice if blood pressure needs to be restored urgently (in those with pulse pressure <10 mmHg) as they have been shown to restore the cardiac index and hematocrit faster than crystalloids in patients with intractable shock [44,46–48]. Furthermore, the overall low (0–0.2%) mortality of the study population supports the importance of carefully titrated fluid therapy to maintain vital functions during the vascular leakage period without overfilling the intravascular space and meticulous supportive care for patients with DSS.

Blood products to reduce bleeding complications

Thrombocytopenia (platelet count below 150 $\times$ 10⁹/l) is a hallmark of DENV infection and

usually observed between days 3 and 8 following the onset of illness. Platelet count plummets in parallel with a rising hematocrit, indicative of progression to the critical phase of the disease. Platelet count reaches nadir during defervescence (days 3–6), which is then followed by gradual spontaneous recovery [15,18]. Minor bleeding (mucosal, petechiae) without hemodynamic instability is common and usually resolves spontaneously. In patients with severe thrombocytopenia (defined as $\leq 20 \times 10^9/l$), strict bed rest and avoidance of nonsteroidal anti-inflammatory drugs and intramuscular injections alone are usually sufficient to reduce the risk of severe bleeding [15].

Prophylactic platelet transfusion in patients without bleeding when platelet falls below $10\text{--}20 \times 10^9/l$ is widely practiced in sepsis [49], but is not supported by evidence in dengue management. First, a clear inverse correlation between platelet count and bleeding risk is lacking [50–53]. Rather, the two risk factors of severe bleeding are prolonged shock and normal–low hematocrit at the diagnosis of shock [50]. A summary of platelet and fresh frozen plasma transfusion trials (Table 2) shows that current data do not support prophylactic platelet transfusion given the lack of sustainable and significant benefits [52,54,55]. Furthermore, the benefits of treatment are outweighed by significant risks including fluid overload, transfusion-associated lung injury, blood-borne infections and allergic reactions.

When major bleeding occurs, it usually arises from the gastrointestinal tract and/or vagina in adult females. Patients at risk of major bleeding include those who have: first, prolonged or refractory shock; second, renal or liver failure; third, persistent metabolic acidosis; fourth, taken nonsteroidal anti-inflammatory drugs or anticoagulants (e.g., heparin, warfarin) and fifth, preexisting peptic ulcer disease [15]. In the event of severe bleeding, timely transfusion of packed red cells, platelets and fresh frozen plasma may be lifesaving.

• Therapies targeting the host immune response

Corticosteroids

Corticosteroids have inhibitory effects on a broad range of immune responses mediated by T and B cells as well as native immune responses of phagocytes. High-dose corticosteroids are of benefit in many conditions with immune aberrancy, notably autoimmune diseases such as systemic lupus erythematosus [56]. In a double-blind

Table 3. Summary of clinical trials that assessed steroids as a treatment of dengue.

Study (year)	Design	Setting	Patients	Participants (n)	Drug	Conclusion	Favor steroids?	Ref.
Pongpanich <i>et al.</i> (1973)	Nonblinded, randomized	Thailand	Children with dengue shock	26	IV hydrocortisone	No difference in duration of shock or need for fluid replacement	No	[57]
Min <i>et al.</i> (1975)	Randomized, double-blinded	Thailand	Children with dengue shock	98	IV hydrocortisone for 3 days	Favors steroids in children >8 years. Case fatality 44% (22 of 50) in nonsteroid group, and 18.75% (9 of 48) in steroid group	Yes	[58]
Sumarmo <i>et al.</i> (1982)	Randomized, nonblinded	Indonesia	Children <10 years with dengue shock	97	IV hydrocortisone $\times 1$ dose	No difference in duration of shock or need for fluid requirement. No difference in mortality	No	[59]
Tassniyom <i>et al.</i> (1993)	Randomized, double-blinded	Thailand	Children <15 years with dengue shock	63	IV methylprednisolone $\times 1$ dose	No difference in mortality	No	[60]
Kularatne <i>et al.</i> (2009)	Randomized, placebo-controlled, double-blinded	Sri Lanka	Dengue patients aged 12–65 years with platelets $<50 \times 10^9/l$	200	IV dexamethasone for 24 h	Ineffective in increasing platelet count	No	[61]
Tam <i>et al.</i> (2012)	Randomized, placebo-controlled, double-blind	Vietnam	Dengue patients aged 5–20 years ≤ 72 h	255	Oral prednisolone for 3 days	Steroids not associated with prolonged viremia	(Underpowered)	[62]
Shashidhara <i>et al.</i> (2013)	Randomized, controlled, nonblinded	India	Adults with dengue aged >18 years	61	IV dexamethasone for 4 days	Ineffective in increasing platelet count	No	[63]

IV: Intravenous.

placebo-controlled trial, the use of corticosteroids during the early acute phase of dengue infection showed no effects on shock, plasma leakage or platelet count recovery in dengue patients. It also did not change the kinetics of dengue virological markers or plasma cytokine concentrations (Table 3) [57–63]. In summary, the use of steroids cannot be recommended [64].

Intravenous immunoglobulins

Intravenous immunoglobulins (IVIG) is an accepted treatment for idiopathic thrombocytopenia purpura (ITP). Thrombocytopenia in ITP is attributed to autoantibodies called platelet-associated IgG (PAIgG). PAIgG-coated platelets undergo accelerated clearance through Fcγ receptors expressed on mononuclear phagocytic cells. The mechanism of IVIG is probably via competitive inhibition of Fcγ receptors or ligation of inhibitory receptors [25,26,65].

A randomized, controlled study of 36 dengue patients was conducted to evaluate treatment with IVIG according to the dosage and frequency used for treatment of ITP for total of 4 days [66]. The study failed to show efficacy of IVIG in promoting platelet recovery.

• Mast cell inhibitors

Recent studies by St John *et al.* identified the role of mast cell (MC) activation in the pathogenesis of dengue-vascular leakage and hemorrhage [67,68]. During DENV infection, activated MC was shown in a mouse model to release various proteases, particularly chymase and tryptase, into the serum that results in loss of vascular integrity. Consistent with the observation in mice, the authors also showed that serum chymase levels correlated with dengue severity in patients enrolled in a prospective study. The team then showed that MC-stabilizing compounds, including cromolyn, montelukast and ketotifen, reduced vascular leakage in wild-type mice model of DENV challenge despite small (but nonsignificant) increase in mice viremia [67]. Together, these data highlight the potential of MCs as therapeutic targets to limit DENV pathology. A proof-of-concept clinical trial to test the efficacy of ketotifen to reduce the degree of vascular leakage is currently in progress in Singapore [TAMBYAH PA, ST JOHN A; PERS. COMM.].

Target profile for a dengue therapeutic

There is still an unmet need for an effective antidengue drug that can shorten the duration

of illness and prevent the development of severe complications. Ideal properties for a future dengue therapeutics are summarized in Table 4. The life cycle of DENV, drug targets and candidate drugs are summarized in Figure 1 and reviewed in detail elsewhere [69].

• Drugs targeting host factors required by DENV to complete its life cycle

Chloroquine

Chloroquine (CQ) was the first drug that underwent clinical trial with reduction of viral load as its primary end point [70]. CQ is a cheap and widely available lysosomotropic 4-aminoquinoline derivative that is well established for the treatment of malaria and inflammatory disorders such as rheumatoid arthritis and systemic lupus erythematosus. *In vitro*, CQ inhibits flaviviruses, retroviruses and coronaviruses by interfering with pH-dependent steps of viral replication. In DENV infection, CQ inhibits endosomal fusion and furin-dependent viral maturation, both of which are dependent on low-pH environment in the endosome and trans-Golgi network, respectively [70,71]. CQ also has immunomodulatory effects by suppressing release of TNF-α and IL-6, both of which may be responsible for the inflammatory complications of viral infections [72].

In a randomized, double-blind placebo-controlled trial of 307 Vietnamese adult patients hospitalized with laboratory-confirmed DENV infection, CQ-treated patients had longer duration of viremia [70]. There was also no apparent reduction in the development of DHF. Furthermore, CQ treatment was associated with a significant increase in the rate of vomiting. In a more recent small, randomized, double-blind study of 37 positive dengue patients, CQ treatment was shown to reduce the severity of pain symptoms but failed to reduce disease duration, the degree or days of fever [73].

Celgosivir

DENV assembly occurs in the endoplasmic reticulum (ER), where heterodimers of prM and E proteins localize to the luminal side of ER to form an immature particle. N-linked glycosylation of prM and E proteins is required for viral assembly and release of mature, infectious DENV particles [15,74]. DENV infection also induces ER stress, which activates the unfolded protein response machinery. The expression of *UPR* genes in turn activates various signaling

pathways that induce apoptosis [75].

Celgosivir is an alkaloid castanospermine derived from the Moreton Bay Chestnut tree [75]. Following oral administration, it readily crosses cell membranes and is converted to Cast, which inhibits the catalytic activity of ER-resident enzymes, alpha-glucosidase I and II. Both enzymes catalyze the removal of terminal glucose residue attached to N-linked glycans of newly synthesized glycoproteins. Inhibition therefore is thought to result in misfolding of E, prM and NS1 proteins during virus replication. Celgosivir could also reduce cellular apoptosis due to virus-induced ER stress [75]. *In vitro*, celgosivir inhibited all four serotypes at submicromolar concentrations [76]. Celgosivir-treated mice showed reduced viremia, robust immune response and increased survival upon lethal DENV challenge, even when treatment was delayed by 48 h after infection [75]. Celgosivir was tested in Phase I and II trials as a possible treatment for HIV and hepatitis C infection and was found to be safe [75,76]. However, the drug was not further developed because it failed to demonstrate superiority over existing treatments.

A Phase Ib, randomized, double-blind, placebo-controlled, proof-of-concept, single-centered trial to evaluate the safety and efficacy of celgosivir as an antidengue drug (CELADEN) was conducted in Singapore [77]. It recruited 50 PCR-confirmed adult dengue patients within the first 2 days from illness onset. Individuals were randomized to receive celgosivir for 5 days or matched placebo. Results showed that celgosivir treatment did not significantly reduce viremia, fever or pain scores in patients with dengue.

White blood cell and platelet counts as well as changes in hematocrit levels over the course of illness were not different between treated and placebo groups. Thus, the two proof-of-concept trials for inhibitors of host factors of DENV failed to show useful therapeutic efficacy.

Others (HMG-CoA-reductase inhibitors)

Inhibition of cholesterol synthesis may result in faulty viral particle assembly and protein glycosylation. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, known as statins, have cholesterol-lowering effects and established roles in cardiovascular risk reduction [78]. They also exhibit anti-inflammatory and endothelial-stabilizing effects and possible antiviral effect targeting DENV virion assembly [79]. In an AG129 model of DENV-2 infection, lovastatin increased survival rate when administered either before or after infection [78]. However, it only reduced viral load if given before DENV-2 infection [78]. A randomized controlled trial to evaluate the effects of lovastatin on adult dengue patients is in progress in Vietnam [80].

• Drugs targeting dengue proteins

Various direct-acting antiviral agents (DAAs) have been developed that target specific dengue proteins. Interested readers can refer to these excellent reviews on the latest developments, chemical structures and mechanisms of actions of individual novel DAAs and therapeutic antibodies, which will not be discussed in detail here [81–83]. Instead, we have provided broad overviews of the development of these approaches to treating dengue.

Table 4. Ideal properties of a dengue therapeutic.

Drug parameters	Ideal properties
Spectrum	Effective against all four DENV serotypes
Clinical outcome	Rapid resolution of symptoms, reduced severity
Safety	Well tolerated, minimal toxicity and does not require lab monitoring
Bioavailability	Fast acting, high volume of distribution
Pharmacokinetic	Sufficient half-life to enable infrequent (e.g., once daily) dosing to promote compliance
Route of administration	Oral rather than parenteral
Interactions	Minimal with common drugs
Target population	Adults, children, infants, pregnant women, patients with co-morbidities (renal, hepatic)
Others	Long shelf-life, stable without refrigeration to facilitate rural distribution
Cost	Affordable in dengue-endemic countries

DENV: Dengue virus.

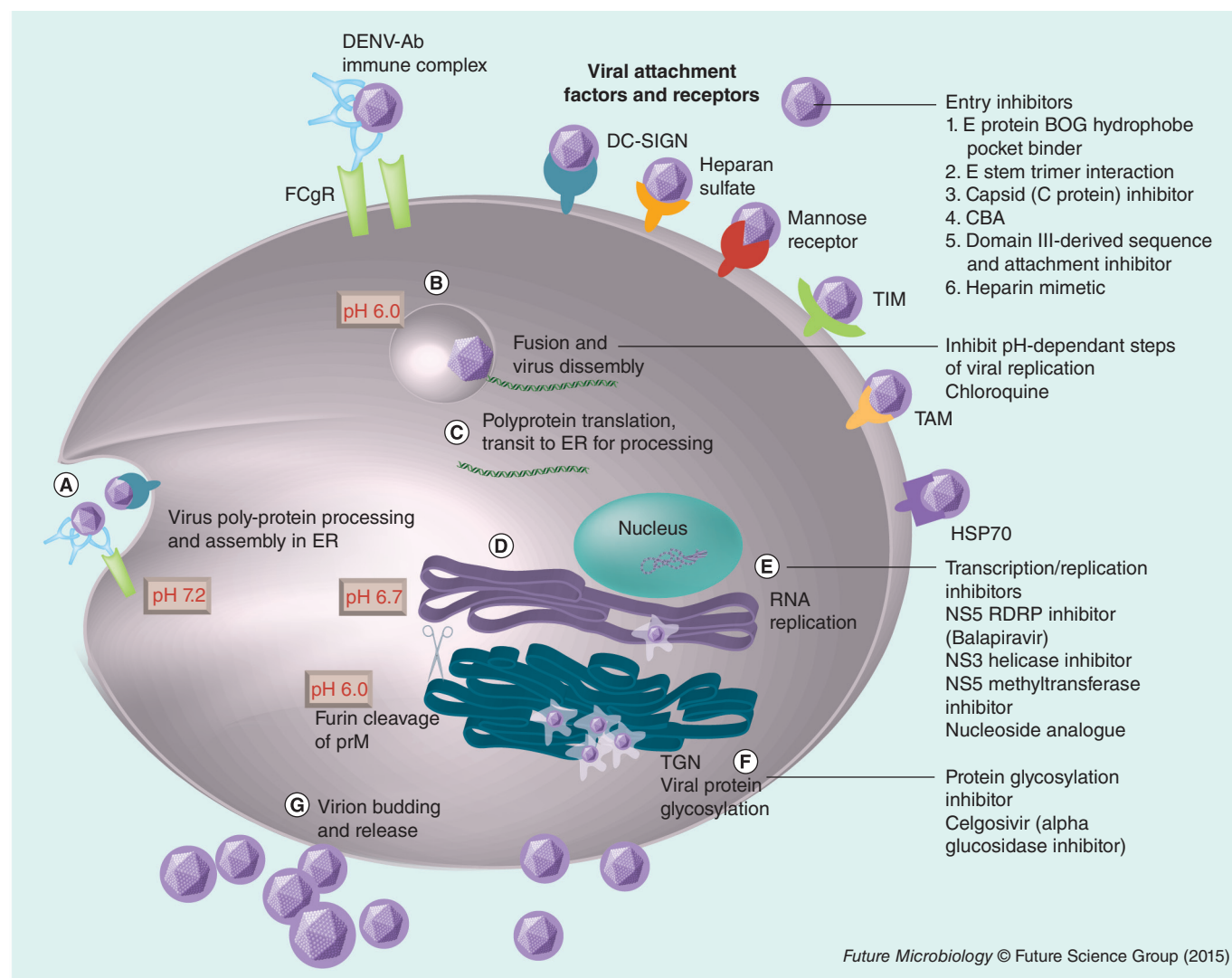


Figure 1. Schematic view of dengue virus replication cycle. (A) Virion binds to receptors on plasma membrane and enters the cell via receptor-mediated or Fc-receptor-mediated phagocytosis if DENV is opsonized to cross-reactive or subneutralizing levels of antibodies. (B) Within the acidic endosomal compartment, the E protein undergoes conformational change to mediate fusion of the viral membrane with the endosome membrane, releasing the positive-stranded genomic RNA into the cytoplasm. (C) Genomic RNA is translated into polyprotein. (D) Polyprotein is cleaved into individual proteins on the endoplasmic reticulum (ER) membranes by viral and cellular proteases. (E) RNA replication occurs on the ER membranes in association with the viral replication complex composed of NS1, NS2A, NS2B, NS3, NS4A and NS5. Viral assembly produces immature viral particles. (F) Immature viral particles (shown as spiky virus particles) are transported through the secretory pathway. Lower pH in the trans-Golgi network activates the host protease, furin to produce mature DENV particles (shown as smooth virus particles). (G) Release of mature virus into the extracellular space. Boxes represent target sites of antiviral drugs or compounds. The bold red numbers refer to the pH values of the respective compartments.

Entry/fusion inhibitors

Entry of DENV into the host cell is mediated mainly by E protein, which contains three domains (DI, DII, DIII) [84]. In the mature DENV particle, E protein is organized into 90 homodimers with the fusion loop concealed inside the dimer interface. The first step of virus entry is the binding of viral E protein to several

types of cellular receptors and/or attachment factors (e.g., DC-SIGN, heparan sulfate, mannose receptor, CD14, GRP78, laminin receptor and TAM proteins) [85]. Following binding, virus becomes internalized via clathrin-mediated endocytosis [85]. Inside the cell, the acidic pH environment induces conformational change and rearrangements of the E protein to expose

and insert the fusion loop into the endosomal membrane, followed by membrane fusion pore formation to allow viral RNA to enter the cytoplasm. Membrane fusion inhibitors bind to various portions of the structural envelope and C proteins have been developed and recently reviewed [85]. Their future use awaits further optimization, animal and clinical studies [82].

Replication & transcription inhibitors

RNA-dependent RNA polymerase inhibitor (balapiravir)

NS5 is the largest and most conserved nonstructural protein in DENV as well as other flaviviruses. NS5 contains two enzymatic domains: first, the N-terminal methyltransferase (NS5-MTase) domain and second, the C-terminal RNA-dependent RNA polymerase (RdRP) domain (NS5-pol). Balapiravir is a product of a nucleoside analog (4'-azidocytine) called R1479, which was initially developed for the treatment of chronic HCV infection [86]. This drug inhibits viral NS5 protein, thereby reducing RdRP-dependent RNA synthesis. It is not licensed for use in HCV due to bone marrow suppression that arose with long-term treatment given with pegylated IFN and ribavirin [87]. Dengue and hepatitis C share similar RdRP architecture. A randomized, double-blind placebo-controlled trial was thus conducted to study the effect of a 5-day course of balapiravir prescribed during the first 48 h of illness onset in adult dengue patients [86]. Although the drug was well tolerated, it failed to reduce either viremia, NS1 antigenemia or fever clearance time. Plasma cytokine profile and whole blood transcriptional profile were not significantly different between treatment groups. In conclusion, this trial did not support balapiravir as a candidate drug.

MTase inhibitors

The NS5-MTase domain catalyzes RNA cap methylation at both the N7 position of the guanine cap and the 2'O position of the first nucleotide of the newly synthesized positive-strand RNA [88]. It can further methylate internal adenosine of the viral RNA genome at the ribose 2'-OH position. N7 methylation of RNA cap is essential for efficient translation, as mutations of MTase that abolish N7 methylation are lethal for flaviviral replication [88]. New compounds (e.g., 'compound 10') are being developed that selectively bind and inhibit MTase. At present, suitable compounds are still being screened for further testing [89].

Nucleoside analog (NITD008)

An adenosine analog NITD008 was developed that exhibits antiviral effect via inhibition of DENV RdRP and termination of RNA chain synthesis [90]. *In vitro* studies showed that NITD008 reduced DENV 1–4 replication. DENV-infected mice treated with NITD008 demonstrated reductions in viral load, proinflammatory cytokines and mortality. However, this drug also showed significant side effects during preclinical toxicology studies in mice, and therefore not further developed for clinical trials [90].

Helicase inhibitors

Helicase inhibitors work by inhibiting DENV NS3 unwinding activity during RNA replication [91]. Recently, a novel small-molecule helicase inhibitor (ST-610) was identified. This compound potently and selectively inhibited all four serotypes of DENV *in vitro* [92]. In mice, it was well tolerated and effective in reducing viremia. However, this drug has poor oral bioavailability and requires parenteral administration, hence limiting its use especially in resource-limited settings.

Protease inhibitors

The DENV NS3 is a serine protease in association with NS2B as a cofactor. During DENV replication, correct co- and post-translational processing of the polypeptide gives rise to the structural and nonstructural proteins and is therefore essential for virus replication [93]. These steps require multiple proteases derived from the host (e.g., furin) and virus (NS2B/NS3). Viral proteases are proven antiviral targets as demonstrated by the highly effective HIV-1 and HCV protease inhibitors [93]. DENV 1–4 NS3 protease shares 63–74% sequence similarity, suggesting that although it is plausible to develop effective protease inhibitors against all four DENV serotypes, the barrier to resistance could be low. Several potential protease inhibitors have been identified (including 166347, ARDP006, thyrothricin, compound 23i and compound 7n), but their development is still in its infancy and to date no drugs have been evaluated *in vivo* [82].

NS4B inhibitor

NS4B is a transmembrane protein without identified enzymatic activity but is required for the formation and anchorage of the active viral

replication complex onto ER membranes [94]. NS4B also interferes with IFN- α/β signaling to promote virus propagation [95]. Several groups have identified compounds that inhibit DENV replication by targeting NS4B. NITD-618 was one of the compounds that was identified and demonstrated to be active against all four DENV serotypes [96]. However, NITD618 is highly lipophilic, which resulted in poor pharmacokinetic properties that hindered its testing *in vivo* [82]. Lycorine, a plant alkaloid, was another NS4B inhibitor with ability to inhibit WNV, YFV and DENV 1–2 replication [97,98]. Another compound, SDM25N, was shown to be active against DENV 2 *in vitro* [99]. The efficacy, safety and ultimate clinical utility of NS4B inhibitors await further clinical research.

Therapeutic antibodies

Therapeutic human serum polyclonal antibodies (IgG), prepared from pools of plasma obtained from multiple healthy blood donors, is assumed to contain wide array of antibodies that would be present in normal human serum and against common viral pathogens [100]. However, the disadvantage of polyclonal antibodies includes batch-to-batch variations, risk of blood-borne pathogens and multiple side effects especially allergic reactions [101]. Furthermore, batches must be screened to ensure neutralization activity against all four DENV serotypes [21]. Therapeutic monoclonal antibodies (mAbs), in contrast, can be produced in high quantities, specificity and consistency with significantly reduced adverse events associated with polyclonal IgGs [83].

Antibodies targeting the lateral ridge or A-strand of E protein EDIII can potentially neutralize some but rarely all four serotypes [102]. An A-strand-specific mAb named 4E11 was redesigned through computational chemistry to neutralize all four serotypes *in vitro* and in mouse model of DENV challenge [103]. More recently, the team further developed this humanized antibody by using a combination of computational protein chemistry and X-ray crystallography to further improve this antibody for therapeutic application [104]. This antibody is currently undergoing preclinical development.

Alternatively, mAbs isolated from convalescent dengue patients that neutralize all DENVs with remarkable potency may also have therapeutic potential. Unlike mouse-derived antibodies,

human mAbs mostly bind quaternary epitopes. de Alwis *et al.* identified a potent serotype-specific mAb that binds the hinge region between EDI and EDII [105]. Teoh *et al.* identified a potent serotype-specific mAb HM14c10 that binds a discontinuous epitope spanning adjacent surfaces of E-protein dimers on DENV-1 [106]. Fibriansah *et al.* showed that a highly potent mAb against DENV-3 binds the functionally important domains across DI–III proteins [107]. The same group also identified a DENV2-specific human mAb 2D22 that binds across E proteins and blocks the E-protein structural reorganization required for virus fusion [108]. Dejnirattisai *et al.* identified human mAbs that bind epitopes that bridge across two E proteins (called envelope dimer epitopes or EDE) [109]. Interestingly, these antibodies show pan-serotype neutralizing activity and could be useful therapeutically.

While therapeutic antibodies hold much promise, they have only been studied *in vitro* and in animal models. Molecular mechanisms that operate and potentially influence the efficacy outcome have been reviewed elsewhere [83]. While these may have to be considered when translating antibodies to therapeutic use, it would nonetheless be interesting to see the progress of this form of therapeutics into the clinical space as a treatment for an acute viral disease like dengue.

Conclusion

Despite the large number of candidate compounds that show antiviral effects against DENV *in vitro*, few have been evaluated in clinical trials. Although the optimal choice of fluids in acute dengue is still debatable, meticulous fluid therapy with close monitoring is the only established standard of care that ameliorates complications due to vascular leakage, shock and organ failure. Drug discovery is hindered by the lack of a reliable animal model of DENV infection and the long expensive process of new molecules discovery. It is hoped that efforts to sustain and perhaps even accelerate the translation of bench-based findings into therapeutic trials will continue in the coming years, especially since a fully preventative dengue vaccine remains challenging [112–114].

Future perspective

While an effective therapeutic against dengue remains elusive, the number of translational

EXECUTIVE SUMMARY

Spectrum of dengue infection

- Dengue is an acute, systemic viral infection caused by one of four dengue serotypes in the family *Flaviviridae*, transmitted by *Aedes* mosquitoes.
- Dengue occurs primarily in the tropics.
- Dengue presents with wide clinical spectrum, ranging from subclinical infection, or may cause mild dengue fever (DF), to severe and even fatal dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).
- Death is usually primarily by plasma leakage, leading to shock, organ failure and hemorrhage.
- Three phases of disease exist – febrile phase, critical phase and recovery phase.
- There is no licensed drug or highly effective vaccine available at present.
- Clinical assessment (history, physical examination and vital signs) and laboratory tests (complete blood count and hematocrit) are required to assess disease phase and severity.

Dengue treatment: supportive therapy during acute infection

- Judicious therapy is currently the cornerstone of managing acute dengue to prevent complications due to plasma leakage, shock, organ failure and bleeding. However, excessive fluid can result in fluid overload.
- There is no clear advantage to the use of colloids over crystalloids in outcome. Colloids may be preferred in severe (pulse pressure ≤ 10 mmHg) or refractory shock to quickly improve hemodynamic status.
- Blood product transfusion is indicated in patients with major hemorrhage.
- Thrombocytopenia is common in dengue during the febrile phase, but overt bleeding is uncommon. Prophylactic platelet or fresh frozen plasma transfusion for severe thrombocytopenia in hemodynamically stable patients is not beneficial and not recommended.

Dengue treatment: drugs targeting host immune response

- There is no evidence to support the use of intravenous immunoglobulins or corticosteroids in dengue and are not recommended.
- Mast cells may be implicated in severe dengue via release of chymase and tryptase into the serum, causing vascular leakage. So far, macrophage-stabilizing compounds have showed promising results in improving outcomes in mouse models. Its therapeutic use in humans awaits clinical trials.

Drugs targeting host factors required by dengue virus to complete its life cycle

- Celgosivir, an alpha-glucosidase inhibitor, effectively inhibits dengue virus (DENV) 1–4 *in vitro*. In Phase Ib clinical trial, it failed to demonstrate viral load reduction and clinical benefit.
- Chloroquine, a lysosomotropic 4-amino-quinoline derivative, inhibits dengue through pH-dependent steps of viral replication cycle and has ability to suppress cytokine-mediated inflammatory responses. No clinical benefit was observed based on two randomized, double-blind studies of dengue patients.
- Cholesterol-lowering drugs (statins) may exhibit anti-inflammatory and endothelial-stabilizing effects and possible antiviral effect targeting DENV assembly. Lovastatin demonstrated survival benefits in mouse model of DENV infection. Its use in humans awaits clinical trials.

Drugs targeting dengue proteins or direct-acting antiviral agents

- Drugs that target critical stages of dengue replication cycle (entry, fusion, translation, genome replication, protein synthesis and egress) have been extensively studied. Most of these drugs have not been studied in animal models or humans.
- Balapiravir acts via inhibition of NS5/RdRP-dependent RNA synthesis. It is the only direct-acting antiviral agent (DAA) that entered clinical trial. However, treatment with balapiravir failed to reduce NS1 antigenemia, viral load and fever clearance time in dengue patients.
- NS4B inhibitors act via inhibition of viral replication and prevents viral downregulation of interferon response.
- Therapeutic mAbs that potently neutralize DENV by binding to the quaternary structures of envelope proteins without antibody-dependent enhancement could potentially be used against homologous serotypes.
- To date, no licensed dengue DAAs are currently available.

studies that have been carried out is encouraging. These efforts are long overdue against a disease that results in significant global health burden. As new studies are being developed, downstream studies necessary for clinical translation of any therapeutic should also be actively considered. Since the great majority of patients with dengue infection recover without progression to DHF/DSS, studies to demonstrate clinical benefits of therapy to prevent severe disease would therefore require recruitment of a very large number of patients. In this context, perhaps there is an urgent need to revisit human challenge studies that could serve as proof-of-concept clinical trials to complement therapeutic development. Such trials can provide clear and definitive efficacy signals

and guide the design of larger trials. Such an approach would prevent prolonged and expensive clinical trials that often reach dead ends due to insufficiently robust efficacy. Proposition for human challenge studies has been discussed elsewhere [110,111].

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