

Current Status of Dengue Therapeutics Research and Development

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Dengue is a significant global health problem. Even though a vaccine against dengue is now available, which is a notable achievement, its long-term protective efficacy against each of the 4 dengue virus serotypes remains to be definitively determined. Consequently, drugs directed at the viral targets or critical host mechanisms that can be used safely as prophylaxis or treatment to effectively ameliorate disease or reduce disease severity and fatalities are still needed to reduce the burden of dengue. This review will provide a brief account of the status of therapeutics research and development for dengue.

Keywords. dengue; flavivirus; dengue drug discovery; antivirals; dengue prophylaxis; dengue therapeutics.

The geographic distribution of dengue has expanded globally in the past 5 decades. This mosquito-borne acute disease is now endemic in >100 countries, with an estimated 400 million infections each year [1]. Recently, Dengvaxia (CYD-TDV), a tetravalent vaccine developed by Sanofi Pasteur that consists of genes encoding the premembrane (prM) and E proteins of dengue virus (DENV) serotypes 1–4 (DENV 1–4) inserted onto the genomic backbone of live attenuated yellow fever vaccine strain, was licensed in several dengue-endemic countries [2]. The vaccine efficacy, however, varied by age and serostatus of the vaccine recipient at baseline and by the DENV serotype causing the infection; lower efficacy was observed for DENV 1 and 2 as compared to DENV 3 and 4 [3–5]. Hence, despite the availability of a dengue vaccine, improvements in case management to reduce the risk of severe dengue are still needed. Current approaches are entirely supportive care in the form of judicious fluid replacement and close clinical monitoring during the critical phase of illness [6]. No antiviral drug has been developed despite the association between higher viremia levels and severe dengue. The current status of dengue burden and impact of various countermeasures is summarized in Figure 1.

Dengue Drug Targets

The RNA genome of DENV is translated as a single polypeptide that is then cleaved into 3 structural proteins (capsid [C], prM, and E) and 7 nonstructural (NS) proteins (NS1, NS2A, NS2B,

NS3, NS4A, NS4B, and NS5) by cellular proteases and viral serine protease, composed of NS2B and NS3 [7]. The NS proteins are essential components of replication machinery of the DENV genome. Several recent studies have also shown that their interaction with host factors lead to suppression of natural innate immune responses that may contribute to the epidemiology and pathogenesis that drive the spread of dengue [8].

Antiviral approaches explored thus far have targeted both structural and nonstructural proteins of DENV. Small molecules that target viral entry have been examined, although the most advanced intervention against virus entry is in the form of therapeutic antibodies. These are at various stages of clinical development [9–11]. The search for small-molecule inhibitors has focused on the multifunctional enzymes NS3 and NS5, the supposedly “low-hanging” antiviral targets [12, 13]. In addition, the C protein and NS4B are also being explored as drug targets [14–17]. However, no antiviral that has been developed exclusively for DENV has entered clinical trials. The only drug that is believed to directly target one of the viral proteins (NS5) that has been clinically investigated is balapiravir. This nucleoside analogue, developed by Roche Pharmaceutical originally for hepatitis C, was examined as a short-course indication against dengue because of its useful short-course safety profile [18]. This compound, however, did not meet the efficacy end point, possibly because of altered host cell kinase expression or activity during DENV infection [19].

Antiviral drug development can, however, now benefit from advances in molecular and structural virology. Structural information of the virus and several NS proteins that are critical for the virus life cycle have been determined by nuclear magnetic resonance spectroscopy, X-ray crystallography, or cryo-electron microscopy. A portrait of the important elements that can contribute to the drug discovery effort is shown in Figure 2. These high-resolution structures could be combined with molecular tools such as in silico approaches and infectious clone

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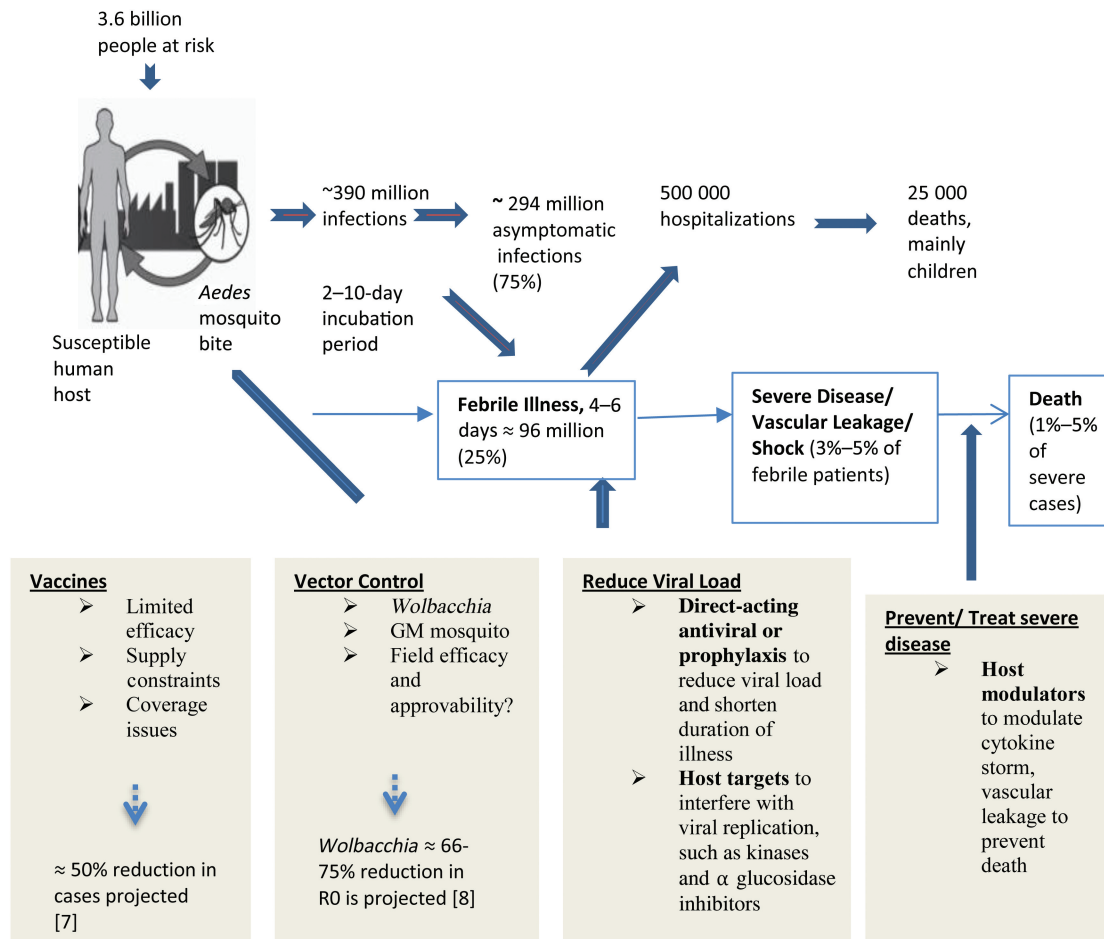


Figure 1. Schematic diagram summarizing the state of the global dengue epidemic, showing countermeasures and their impact on the total dengue burden. Abbreviations: GM, genetically modified; R0, basic reproduction number.

technology to identify new and thus hitherto unexplored drug targets for DENV and possibly other flaviviruses [12, 20].

An RNA-based approach to inhibit gene expression and serve as antivirals is another strategy that can be potentially exploited if the current limitations such as stability and mode of delivery can be adequately addressed [21].

Target Product Profile That Can Have Maximum Clinical Utility

Dengue is an acute, self-limiting disease in most instances, with a small proportion of patients progressing to severe disease manifested by increased plasma leakage, hemodynamic compromise, shock, and bleeding. If dengue is left untreated, mortality can reach as high as 30%. The acute and self-limiting nature of the disease in the majority of cases thus require that an effective antiviral should have an excellent safety profile and be active against all 4 serotypes of DENV. Ideally, an oral drug that is dissolvable would be available, because there is a large disease burden in the pediatric population. A once-daily dosing schedule would also be useful for good compliance. Pragmatically, however, dosing of up to 3 or 4 times per day may be

necessary to maintain drug levels above a minimum effective concentration, as exemplified by antivirals against other acute infections, such as acyclovir for varicella zoster and antibiotics against common acute bacterial infections [22, 23]. The use of biologics such as therapeutic antibodies may overcome the challenges faced in the field with small-molecule drugs, as human immunoglobulin G1 is known to have long half-life. These could be used as a single-dose treatment or as short-term prophylaxis for travelers from countries where dengue is not endemic.

Indeed, the use of antivirals as a tool to prevent infection, either in travelers or in populations living in areas with focal outbreaks, could augment public health measures currently available to prevent dengue. Besides therapeutic antibodies, small molecules administered either once daily or even at longer intervals, such as antimalarial prophylaxis, could be clinically beneficial. In this respect, the pharmacokinetic properties to prevent infection may be less demanding than that needed to rapidly reduce viremia levels in patients with dengue. A strong safety profile in a drug that broadly acts on all DENV serotypes will be necessary for good compliance. However, clinical trials

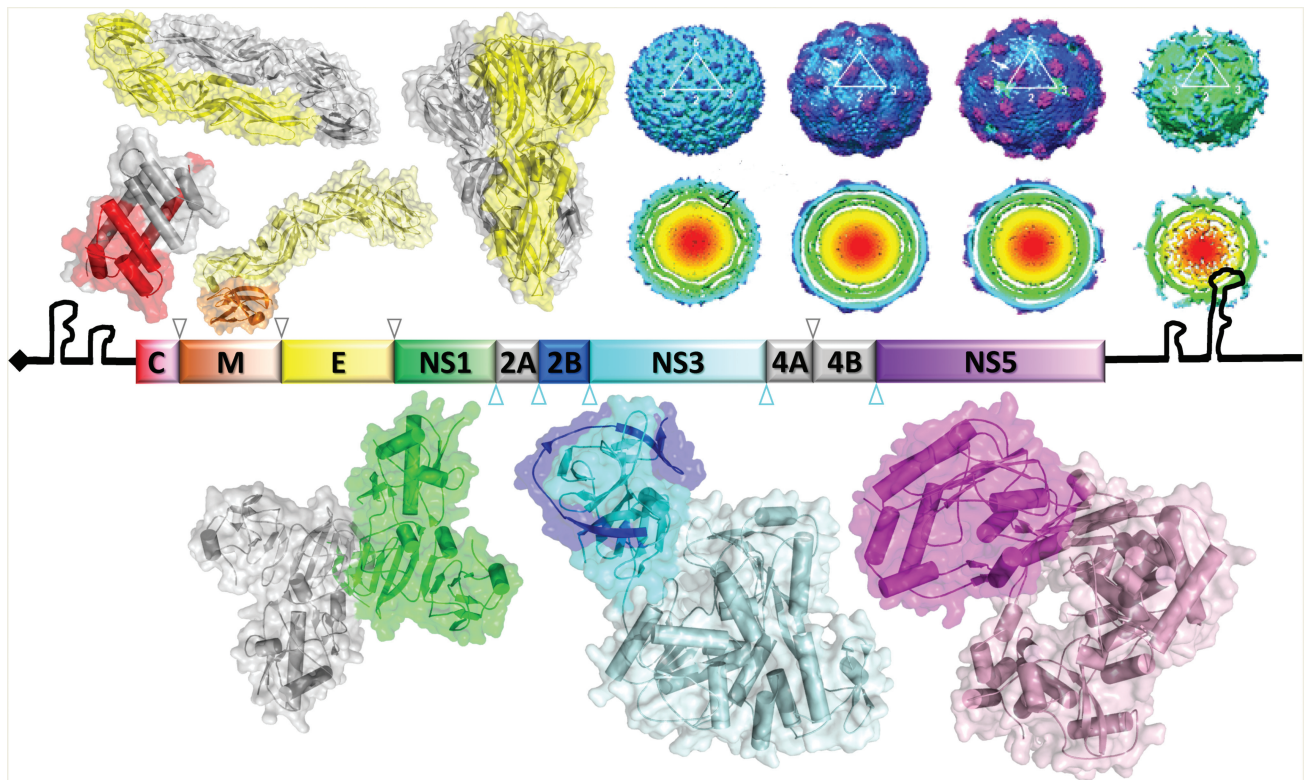


Figure 2. The dengue genome and proteome. The 5' and 3' untranslated regions and the arrangement of the genes encoding 3 structural and 7 nonstructural (NS) proteins are shown. The structures for capsid protein (C; PDB code: 1R6R), E-dimer (PDB code: 1UZG), premembrane and E (prM/E) proteins (PDB code: 3C6E), E-trimer (PDB code: 1OK8), and various images of the dengue virus virion reconstructed on the basis of cryo-electron microscopy (kindly provided by Dr Shee Mei Lok) are shown above the schematic of the genome, while the NS1 (PDB code: 4O6B), NS3 (PDB code: 2VBC), and NS5 (PDB code: 4V0R) structures are shown below. The figure was provided by Dr Dahai Luo.

to evaluate such therapy could be challenging to conduct, as they will require treatment of large number of volunteers over long periods, coupled with active surveillance for febrile illness and DENVs.

Therapeutic Development Landscape

Several therapeutic trials performed in Asia and South America that used antivirals or disease modulators have been described since early 2000. Unfortunately, interpretations of results of these early trials are confounded by lack of information on patient demographic characteristics, dengue severity at recruitment, and defined end point measurements [24–30].

Because the pathway to discovery of new small-molecule drugs take a long time to reach the clinic, dengue researchers have taken advantage of the cost-saving and time-saving benefits of drug repurposing [13]. The most recent proof-of-concept clinical trials for dengue have been performed using repurposed or off-patent drugs, namely chloroquine, prednisolone, balapiravir, celsogivir, and lovastatin (Table 1). These trials have all used the conventional double-blinded, randomized, placebo-controlled design with clearly defined primary end points. The drugs were found to be safe in patients with acute dengue, but all of these compounds failed to meet a priori-defined trial end points [18, 31–34].

Two other trials (involving ivermectin and ketotifen) are currently recruiting in Thailand and Singapore, respectively (clinical trials identifiers NCT02045069 and NCT026773840, respectively). Interestingly, the preliminary findings from the phase 2 ivermectin study suggests a reduction in serum NS1 levels and body temperature with high-dose ivermectin, despite no detectable difference in viremia levels (as measured by real-time quantitative polymerase chain reaction [qPCR]) [35].

Although all of the clinical trials thus far have failed to meet their primary efficacy end points, they have provided unique insights into dengue viremia and NS1 antigenemia. This new information is useful for clarifying efficacy end points for future trials.

Lessons Learned From Using Fever and Viremia as a Primary End Points in Clinical Trials

The rationale of using fever and viral load reduction in these trials stemmed from earlier observational studies that showed a positive correlation between viremia level and disease severity [36, 37]. These observations, together with the known profile of patients with DENV viremia led to the hypothesis that early treatment within 48–72 hours of fever onset with an effective anti-DENV drug could potentially lower the viral load and

Table 1. List of Clinical Studies on Dengue Therapeutics

Compound	Rationale	Study Site(s)	Study Drug Characteristics	Subject Characteristics	Primary End Point(s)	Results	Reference
Chloroquine	Widely used antimalarial drug presumed to interfere with virus entry mechanism by inhibiting fusion between virus and host membrane	OUCRU, Ho Chi Minh City, Vietnam	Placebo vs chloroquine (600 mg on d 1, 600 mg on d 2, 300 mg on d 3)	Age, >18 y; trial size, 307 (154 received placebo, 153 received chloroquine)	Laboratory: time to resolution of viremia, time to resolution of NS1 antigenemia	No change in viremia and NS1 antigenemia	[31]
Prednisolone	Antiinflammatory properties, publication of studies supporting modulation of the function of endothelial glycocalyx	OUCRU	Placebo or prednisolone (0.5 mg/kg or 2 mg/kg once daily for 3 d)	Age, 5–20 y; trial size, 225 (75 received placebo, 75 received prednisolone 0.5 mg/kg, 75 received prednisolone 2 mg/kg)	Clinical: safety; laboratory: virological log reduction	Not powered for efficacy; no change in hematological, virological, or clinical end points	[32]
Balapiravir	Presumed to be an NS5 nucleoside inhibitor developed for HCV by Roche	OUCRU	Placebo vs balapiravir (1500 mg or 3000 mg twice daily for 5 d)	Age, 18–65 y; trial size, 64 (32 placebo recipients, 10 balapiravir 1500 mg recipients, 22 balapiravir 3000 mg recipients)	Laboratory: viral log AUC from first dose to study d 7, time to first viremia level of <1000 copies/mL, time to resolution of NS1 antigenemia	No change in virological and immunological end points	[18]
Celgosivir	Inhibitor of ER-associated α glucosidase	SGH/Duke-NUS, Singapore	Placebo vs celgosivir	Age, 21–65 y; trial size, 50 (26 placebo recipients, 24 celgosivir recipients)	Clinical: fever reduction; laboratory: virological log reduction	No statistically significant reduction of viral load or fever	[33]
Lovastatin	Cholesterol synthesis inhibitor thought to limit membrane mobilization required for viral RNA replication complex assembly	OUCRU	Placebo vs lovastatin (80 mg once daily for 5 d)	Age, >18 y; trial size, 300 (149 placebo recipients, 151 lovastatin recipients)	Clinical: safety and tolerability	Not powered to address efficacy; no evidence of beneficial effect on any clinical manifestations or DENV viremia	[34]

Abbreviations: AUC, area under the curve; DENV, dengue virus; ER, endoplasmic reticulum; HCV, hepatitis C virus; NUS, National University of Singapore; OUCRU, Oxford University Clinical Research Unit in Vietnam; SGH Singapore General Hospital.

reduce dengue severity. In reality, however, this approach poses several challenges and limitations in field sites. Patient reporting of fever duration can be highly unreliable in dating the onset of illness. As with management of most acute febrile illnesses, individuals with dengue fever often take a wait-and-see approach with home rest and self-medication, deferring seeing a physician until later stages of illness. In most instances, the stage of peak viremia level would have passed by the time they present to the clinics or get enrolled into a clinical trial. By comparison, the first studies in the clinical development of oseltamivir as an anti-influenza drug started with human challenge trials, where the onset of infection could be clearly defined [38].

DENV detection and quantification using real-time qPCR has become the method of choice in the past 20 years. This method measures RNAemia, rather than quantifies infectious viruses. RNA copy number can exceed infectious viral titers by 2–5 logs. However, direct measurement of infectious viruses is technically difficult because some clinical isolates grow poorly in cell cultures. Moreover, not all unpassaged DENVs form consistent plaques, and hence estimating the number of infectious viral particles in clinical serum samples by using a plaque assay is inherently inaccurate. The most sensitive biological assay available for measuring unpassaged infectious DENV is the mosquito inoculation technique, but the technique is hard to

master and requires an insectary, which is not available in most diagnostic virology laboratories [39].

Besides difficulty in the timing of patient enrollment into trials and limitations in viremia measurements, there is also a wide variation in the rate of viral clearance, which is influenced by factors such as DENV serotype and primary versus secondary infection. These factors thus collectively contribute to the large standard deviation often observed in viremia measurements stratified by day from fever onset. Statistical considerations for sample size must thus take into account this expected variability in viremia levels.

DENV NS1 antigen detection is often used to diagnose dengue in patients early, for enrollment into clinical trials [33, 34], and it may have a role in dengue pathogenesis [40, 41]. Its usefulness as a reliable therapeutic efficacy end point through time-to-clearance monitoring, however, is uncertain. A major problem is that the level of NS1 and the duration in which this antigen can be detected in serum differ significantly between DENV serotypes, as well as primary and secondary dengue cases [42]. Nevertheless the recent surge in structural and mechanistic studies of NS1 suggests that more-quantitative NS1 tests whose findings may correlate with disease status, perhaps by using a second host dependent biomarker, may provide reliable end points for application of a therapeutic intervention [40, 41, 43, 44].

Utility of Animal Models for Dengue Drug Efficacy Study

No animal model exists that is capable of approximating human disease [45, 46]. Among the many small-animal models developed, the AG129 mouse, which is deficient in types I and II interferon receptors, has been the most widely used for pathogenesis and immunity studies. It is also the most widely used model to evaluate dengue vaccine and antivirals [47, 48]. The 2 most recent clinical trials of celgosivir and lovastatin were extensively evaluated using this model. Although both compounds showed reduction in viremia levels and increased survival rates in treated mice [49–51], neither compound met efficacy end point in clinical trials. A contributory factor to this disparity between laboratory animal and clinical outcome could be due to the time of dosing. Typically, drug dosing in animals begins soon after viremia onset, whereas in patients with dengue, viremia is mostly in the declining phase by the time they are enrolled into any trial. Dosing regimens in animal studies should thus only be initiated at or after the point of peak viremia level. Consequently, the use of a nonlethal viremia AG129 model could be more useful to inform appropriate dosing for human trials [52].

Nonhuman primates are natural hosts to DENV, with the capability to develop viremia, but they do not manifest the disease and its complications. Although several newer nonhuman primate models have been developed that can capture different aspects of dengue manifestations, their utility is limited by scarce laboratory expertise and cost [22].

For the reasons highlighted above, there is a case for a DENV human infection model that mimics some aspects of natural infection to be developed. Besides being cost saving in the long run, the DENV human infection model has the potential to change the way early phase therapeutic drug trials are conducted and evaluated by allowing for controlled timing of infection and treatment. It can also provide valuable opportunities for optimal pharmacokinetic studies [53]. This work is currently being performed at the State University of New York Upstate Medical University (Syracuse) and John Hopkins University (Baltimore) [54, 55].

Future of Monoclonal Antibodies as Therapeutics Against Dengue

Major advances in our understanding of the structure the DENV virion have been made in the fields of X-ray crystallography and cryo-electron microscopy in the last decade [56–60]. Studies of human monoclonal antibodies isolated from convalescent patients with dengue have led to a greater understanding of the epitopes that need to be targeted for effective virus neutralization. Both serotype-specific and cross-reactive neutralizing monoclonal antibodies are being explored for therapeutic application. The most advanced candidate, Ab513, developed by Visterra (Cambridge, Massachusetts), was engineered to bind domain III of the E protein of all 4 DENV serotypes. This antibody has been shown to bind and neutralize multiple

genotypes within each of the 4 serotypes. This antibody also appears to neutralize DENV in target cells that express Fc gamma receptor, such as monocytes, and demonstrates in vivo efficacy despite the presence of cross-reactive antibodies that would otherwise enhance infection [61, 62]. This antibody is poised to enter clinical trials by early 2017 [10, 11, 63].

While Ab513 targets a linear epitope, more-recent discoveries of potent broadly neutralizing antibodies against the quaternary E protein dimer epitope (EDE) by other groups could also have huge therapeutic potential. These antibodies bind across E proteins and act by inhibiting the conformational changes that occur during viral fusion with endosomal membranes. Structural information derived from such studies also has important implications in the future design of new therapeutics and next-generation dengue vaccine development. [64, 65] Management of severe acute viral infections occasionally involved the use of pooled human serum immunoglobulins [66, 67]. The use of intravenous immunoglobulins has, however, not been carefully explored for the treatment of severe dengue, given its antiinflammatory properties. However, the risk of antibody-dependent enhancement could pose some concerns on the use of pooled polyclonal preparation as it may contain subneutralizing levels of antibodies and, paradoxically, enhance infection instead [68–70].

SUMMARY/CONCLUSION

Dengue is the most important epidemic infectious diseases caused by flaviviruses this century, causing immense public health problems with significant morbidity and mortality, particularly in resource-poor countries [71].

A vaccine that is not completely protective and vector-control measures that lack sustainable outcomes even in a highly organized/urbanized area such as Singapore demands that approaches such as antiviral discovery and development remain in the forefront of research. Although no antiviral agent has yet been found to be effective against acute dengue in proof-of-concept trials, the therapeutic development pipeline still contains several compounds and biologics that would soon be evaluated clinically. While significant challenges still exist in the dengue research community in bringing a dengue compound through the entire development process, we are optimistic that there is enough momentum and concerted effort currently in academia, industry, and governmental and charitable organizations to advance and facilitate therapeutic development. The costs and benefits of developing an antiviral drug that can coexist with vaccines are not known at this stage. However, the recurrence of yellow fever outbreaks despite the availability of safe vaccine [72–74] should serve as a reminder and a motivation to capitalize on current momentum in antiviral development against DENV and related flaviviruses, such as Zika virus [75]. Targets such as the DENV protease and polymerase are being captured in the act of carrying out their essential

enzymatic activities, and these can contribute enormously to the development of designer compounds that could be potent inhibitors. The goal of finding a cure for dengue in the next decade is highly feasible, judging from the success of potent directly acting antivirals against the Flaviviridae family member hepatitis C virus.

Notes

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