Japanese encephalitis — the prospects for new treatments

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Abstract

Japanese encephalitis is a mosquito-borne encephalitis that occurs in Asia and is caused by Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*. Although many flaviviruses can cause encephalitis, JEV causes particularly severe neurological manifestations. Despite substantial advances in our understanding of Japanese encephalitis from *in vitro* and animal models, studies of pathogenesis and treatment in humans are lagging behind. Few mechanistic studies have been conducted in humans and only four clinical trials of therapies for Japanese encephalitis have taken place in the past 10 years, despite an estimated incidence of 69,000 cases per year. Previous trials for Japanese encephalitis might have been too small to detect important benefits of potential treatments. Furthermore, the number of patients needed in a single study to reach statistical significance might be greater than the total number of patients with confirmed Japanese encephalitis ever randomly assigned in a treatment trial. Many potential treatment targets exist for Japanese encephalitis, and pathogenesis and virological studies have uncovered mechanisms by which these drugs could work. In this Review, we summarize the epidemiology, clinical features, prevention and treatment of Japanese encephalitis, and focus on potential new therapeutic strategies that are based on repurposing existing compounds that are already suitable for human use and could be trialled without delay. We use our newly improved understanding of Japanese encephalitis pathogenesis to posit potential treatments, and outline some of the many challenges that remain in tackling the disease in humans.

Key points

- Japanese encephalitis is a severe disease caused by Japanese encephalitis virus, genus *Flavivirus*, family *Flaviviridae*, which is endemic to most of rural Asia, and for which no specific treatment exists

- Japanese encephalitis causes loss of more disability-adjusted life years than does any other arthropod-borne virus, owing to the frequent neurological sequelae of the condition

- Pathogenesis studies indicate that inhibition of viral replication, viral spread, and the host response are needed in combination for optimal therapy

- Animal models and *in vitro* experiments highlight a number of compounds that are potentially suitable for treatment of Japanese encephalitis in humans that could be tested without delay

- The minimum clinically significant treatment effect has probably been under-estimated, and previous clinical trials of Japanese encephalitis have been too small; larger, pragmatic trials are needed

Introduction

Japanese encephalitis is the most commonly diagnosed epidemic encephalitis in the world, and is found throughout South and Southeast Asia, encompassing an area delimited by Pakistan to the West, the Philippines and Japan to the East, and the Australian Torres Strait Islands in the South. The most comprehensive estimate of incidence within the past decade suggests that 69,000 cases of Japanese encephalitis occur every year[1]. However, other estimates vary widely — from 50,000 to 175,000 cases per year — and in reality this number can fluctuate with cycles of transmission and is likely to be an underestimate. Japanese encephalitis is thought to cause the loss of 709,000 disability-adjusted life years annually[2], making Japanese encephalitis the foremost arboviral disease of man by this measure, ranking above dengue in the 2004 WHO global burden of disease report[3], the last time that Japanese encephalitis featured as an individual diagnosis in the report.

Japanese encephalitis is caused by Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*, family *Flaviviridae*, which includes dengue, West Nile, and Zika viruses[4]. Although several members of the genus *Flavivirus* cause encephalitis, JEV is remarkable in the high likelihood and severity of neurological disease that it elicits; by contrast, systemic features such as haemorrhage and jaundice are infrequently described for JEV. Over the past decade, many advances have been made in our understanding of the biology of the virus, yet in the same period only four treatment trials have been conducted into therapies for Japanese encephalitis. To date, no trials on Japanese encephalitis have translated into improvements in treatment; however, in total, only 381 patients with proven Japanese encephalitis have been randomly assigned in treatment trials. Nevertheless, *in vitro* and animal model studies have revealed many drugs that have activity against JEV, which could be suitable for use in humans. Studies of pathogenesis and virology have uncovered the mechanisms by which these drugs might work and identified potential new strategies for Japanese encephalitis treatment, often based on repurposing existing compounds. Here, we review basic biological and clinical aspects of the disease, and discuss new advances in our understanding of Japanese encephalitis that have highlighted compounds with anti-JEV activity *in vitro* and in animal models, and could be used in humans. In addition, we discuss some tentative sample size calculations for Japanese encephalitis treatment trials, using a clinical scoring system specifically developed for measurement of outcome from encephalitis in field settings such as those were JE is endemic. In this way, we aim to stimulate new activity in the field to improve treatments for individuals with Japanese encephalitis. Although vaccines for Japanese encephalitis are available, the disease will never be eradicated owing

to its zoonotic cycle. Moreover, its propensity to cause unpredictable outbreaks in unexpected places make vaccination planning challenging, underscoring the need to develop treatments for this devastating condition.

[H1] Virology and epidemiology

JEV is a single-stranded, positive sense RNA virus[5] (FIG. 1) — features that it shares in common with other flaviviruses. JEV circulates between wild wading birds in Asia and is transmitted by mosquitoes of the genus *Culex*. Seminal epidemiological studies in Japan in the mid-20th century established pigs as amplifying hosts for JEV[6,7]. However, the dominance of pigs as amplifying hosts has been challenged, as countries such as Bangladesh that have predominantly Muslim populations and very little pig farming still have an appreciable burden of Japanese encephalitis in humans[8]. In 2017, a complete JEV genome was identified by unbiased RNA sequencing in a patient co-infected and clinically diagnosed with yellow fever in Cunene province, Angola[9], raising the possibility that the geographic range of JEV might be greater than previously thought. Non-vector transmission of JEV between pigs via respiratory secretions has also been described under experimental conditions[10], although the relevance of this process to human disease is unclear.

In endemic areas, Japanese encephalitis mostly affects children; however, cases are observed in adults when JEV is introduced into areas previously considered non-endemic[11,12], or when non-immune adults are introduced into Japanese encephalitis endemic areas[13,14]. Japanese encephalitis is mostly a rural disease[12], although urban and peri-urban transmission is also reported[15,16]. Historically, two patterns of JEV transmission were recognised: seasonal epidemic transmission that peaks in late summer in temperate regions (23–43º N), and year-round low-level endemic transmission in tropical regions (1–13º N)[17]. In reality, outbreaks do occur in tropical regions and between 13–23º N[18], but cases occur at all times of year and are often under-recognised[19]. The incidence of Japanese encephalitis varies from 0.003 per 100,000 people in areas with established high quality vaccination programs, such as Japan and Korea, to 3.7 per 100,000 people in areas such as Cambodia, Indonesia and Malaysia[1], but these numbers are probably under-estimates.

Unlike in pigs, no onward transmission occurs from humans because viraemia is insufficient to be infectious to the mosquito vector, making humans a dead-end host for JEV. The vast

majority of JEV infection in humans is asymptomatic or causes an illness so mild as to not present to health facilities. An estimated 0.1–1% of JEV infections culminate in encephalitis[20,21], although, given the estimated annual number of cases (~69,000[1]) and the size of the population at risk (2–3 billion people), the actual risk could be lower. After exposure, neutralizing antibody develops[22], and disease is not seen in adult populations in endemic areas, implying that immunity is lifelong. Episodic re-exposure of individuals to JEV probably also aids maintenance of life-long immunity. Consequently, childhood residence in areas with an established Japanese encephalitis endemic results in near universal exposure and almost all adults in these regions are JEV seropositive[23]. Immunity is determined by subclinical exposure, not by age, although cases can also be observed in old age as immunity wanes[24].

[H1] Clinical features

JEV infection begins as an undifferentiated febrile illness. This might be the only manifestation[25], although it is unknown what proportion of JEV infection in humans causes self-limiting illness without involvement of the CNS. Patients with encephalitis have usually had other non-specific features such as coryza, diarrhoea or rigors. After 3 or 4 days, this is followed by the onset of acute encephalitis syndrome, with clouding of consciousness, headache, vomiting and often seizures. Focal neurological signs are variable, and can reflect the anatomical sites of damage. Parkinsonian features, including mask-like blank staring faces (FIG. 2), and tremors reflect involvement of the basal ganglia[26]; other movement disorders include lip-smacking, bruxism, choreoathetosis and hemiballismus. Poliomyelitis-like flaccid paralysis indicates damage of anterior horn cells in the spinal cord[27], and cranial nerve signs include facial palsies, ptosis and abnormalities of eye movements. Obvious generalized tonic–clonic seizures can occur, and subtle motor seizures can also be observed in some instances, especially in advanced disease[28]. Features outside the brain have also been described, including pulmonary oedema (which can be neurogenic as a result of brainstem involvement)[13], hepatomegaly, splenomegaly, modestly raised liver enzymes and thrombocytopaenia[29]. When Japanese encephalitis occurs in adults, similar signs are seen[30].

JEV has occasionally been associated with Guillain Barré syndrome (GBS), which provides an interesting parallel with Zika virus-related disease in South America[31,32]. Patients with JEV-associated GBS have shown electrophysiological evidence of demyelination and, occasionally, axonal damage, and inflammatory infiltrates of lymphocytes and monocytes

have also been observed in a fatal case[31]. A good response to steroids and intravenous immunoglobulin was described in one individual[32], but the use of plasmapheresis has not been reported. JEV can present with flaccid paralysis[27] — as can other flaviviruses, particularly West Nile virus[33] — but this feature arises from the distinct mechanism of involvement of the anterior horn cells of the spinal column (in a similar manner to poliomyelitis), damage to which can be seen histologically at post mortem[34]. GBS has also been reported in other flavivirus infections, such as dengue[35] and West Nile virus[36]. In the single case of GBS attributed to West Nile virus, neuropathy was predominantly demyelinating but an axonal component was also present, so anterior horn cell infection is difficult to completely rule out in this instance[36]. Immune mediated transverse myelitis[37], acute disseminated encephalomyelitis[38] and N-methyl-D-aspartate (NMDA) receptor encephalitis[39] have also been reported following JEV infection. Although JEV can impair neurogenesis in experimental systems[40], the few reports that exist of human infection in pregnancy describe either abortion (with virus isolated from fetal CNS in one case) or normal delivery[41,42]. Infection of pregnant sows also causes abortion and can be a major threat to pig farming[43]. JEV has not been associated with microcephaly as Zika virus has in South America. The mechanisms behind this difference are as yet unknown, but the fact that Japanese encephalitis predominantly affects children in endemic areas might provide one explanation.

JEV is one of many causes of an acute encephalitis syndrome in which patients present with impaired consciousness; as such, diagnosis of Japanese encephalitis requires laboratory confirmation. Abnormalities of routine peripheral blood tests of patients with Japanese encephalitis are non-specific; neutrophilia is common and hyponatraemia can be present. Analysis of cerebrospinal fluid (CSF) typically shows a lymphocytic pleocytosis, but CSF can be acellular in some cases[28,44,45]. Diagnosis of Japanese encephalitis is confirmed by demonstration of JEV-specific IgM in CSF by ELISA, which is present in nearly all patients by day 7 of illness[46]. Repeat lumbar puncture can be necessary if early testing is negative and the diagnosis needs to be confirmed; in practice, during outbreaks, this confirmation is rarely needed if many patients have already tested positive. If CSF is unavailable, serum testing should be interpreted with caution in areas co-endemic for other flaviviruses, such as dengue virus, and neutralisation assays for both JEV and co-circulating viruses might be necessary. Viral nucleic acids and infectious virus can also be found in CSF but much less frequently than JEV-specific IgM [47,48]. Unlike many other members of the *Flavivirus* genus, JEV is very infrequently isolated or detected by reverse transcription PCR (RT-PCR) from blood during acute illness, although it has been isolated from clot-derived white blood cells after co-culture with healthy donor peripheral blood mononuclear cells (PBMCs)[49]. JEV also

cannot be detected in urine by RT-PCR[50]. Many imaging findings are described in patients with Japanese encephalitis[51], but diagnostic utility has only been assessed for hypodense lesions in the thalami on CT (FIG. 2). The sensitivity of this finding is only 22% in a Japanese encephalitis endemic area, and thalamic hypodensities are also found in most other flaviviral encephalitides — as such, these features must be interpreted in the geographical context[52].

The reported outcomes of Japanese encephalitis vary widely, with mortality ranging from around 5% to 50% [14,19,24,28,29,42,44,53-85]; the neurological sequelae observed are dependent in part on the duration of follow up[66]. The average mortality in studies published over the past 30 years is 18% (95% CI 14–21%; FIG. 3a). Over the same period, the proportion of individuals who survive but do not experience a full neurological recovery is 44% (95% CI 35–53%), meaning that just over half of patients who develop clinical Japanese encephalitis do not recover completely.

[H3] Latency. Although Japanese encephalitis is a typical acute infection, with a clinical course lasting 2–3 weeks[12], some evidence shows that virus can persist in the nervous system[86] and PBMCs[87] for 4–8 months after illness onset. This phenomenon seems to be rare in individuals with Japanese encephalitis, with few published reports in humans. There is some evidence for latent JEV infection in mice[88], and West Nile virus RNA has been identified in urine of individuals infected with this pathogen many years after initial illness[89], so the phenomenon might not be confined to JEV. JEV-infected microglial and neuronal mouse cell lines can produce infectious virus for many weeks[90,91], and human monocytes have also been shown to produce JEV for weeks[92], providing a possible cellular basis for persistent infection. However, the consequences of this finding in living patients remains unknown, and persistent infection does not seem to be a common issue in clinical practice.

[H1] Genotype and geographic spread

JEV can be divided into five genotypes, all of which can be traced back to a common ancestor that probably emerged in the region of modern-day Indonesia and Malaysia in Southeast Asia (FIG. 5a)[93,94]. Early isolates from the 1930s to the 1950s of JEV were all genotype III. However, genotype I JEV is gradually replacing genotype III (FIG. 5b)[95], although most human cases are still genotype III (FIG. 5c)[96]. JEV genotype I is better adapted than genotype III to the mosquito vector, with a shorter extrinsic incubation time[97]; but this adaptation comes at the cost of a narrower host range[96]. Climate might also have an

effect on genotype distribution, as genotypes Ia, II and IV tend to be found in tropical Asia, and genotypes Ib and III in temperate Asia[94] — although genotype III has also been isolated in many tropical areas.

JEV continues to spread and reach new populations. For example, JEV reached the Australian Torres Strait islands in 1995[11]; molecular characterisation of the virus in this setting revealed that it belonged to genotype II[98]. Rare Japanese encephalitis cases continued to occur in this area over the next few years, including one on the Australian mainland[99], where JEV was also isolated from mosquitoes[100]. Since this time, Japanese encephalitis cases in this region have abated, and although there have been subsequent intermittent isolations of JEV, no human cases of Japanese encephalitis have been reported in Australia or the outer islands since 1998[101]. Why JEV has not become more firmly established in Northern Australia and the outer islands is not clear, as the ecological conditions are similar to many regions where JEV is endemic. The presence of mosquitoes that have a tendency to feed on vertebrates that do not become highly viraemic following JEV infection is one potential explanation[101].

Many regions of the world also have conditions that seem to be appropriate for JEV, yet no circulation of the virus. The related flaviviruses St Louis encephalitis virus and West Nile virus both circulate in the USA, which has given rise to concern around the potential for introduction of JEV in this region[102]. Parts of Southern Europe also have suitable conditions for JEV, and nucleic acid sequences that seem to correspond to parts of the JEV genome have been detected in birds[103] and mosquitoes[104] in Italy. However, the sequences identified were very short (only 167 and 215 base pairs), full length viral genome or infectious virus has not been isolated, and no evidence of human infection has been reported[105]. Consequently, emergence of JEV in Italy seems improbable, thus far. West Nile and Usutu viruses circulate in the same region, so ongoing flavivirus surveillance could be expected to detect JEV if it does emerge. JEV has always been thought to be exclusively transmitted by mosquitoes, which affects its potential geographic range. However, the detection of vector free transmission via respiratory secretions in pigs[10] indicates that a JEV reservoir could be maintained in temperate locations with short mosquito seasons. How JEV is maintained in more Northern, temperate endemic areas is not known; JEV might persist over-winter in mosquitoes, or in lizards, frogs or snakes, or could be re-introduced in migrating birds[101]. The ecology and geographic expansion of JEV is reviewed in more detail elseswhere[101].

[H1] Japanese encephalitis vaccination

Several different vaccines have been used to control Japanese encephalitis since the 1950s[106] (TABLE 1, reviewed in ref 69). Early isolates of JEV, which were the foundation of vaccine development, were all genotype III. Randomised controlled trials have shown the efficacy of vaccination against Japanese encephalitis, and showed that neutralising antibody correlated with protection[53]. Early Japanese encephalitis vaccines were mostly derived from mouse brain, but this manufacturing method has now been replaced owing to safety concerns and relatively poor immunogenicity in humans [106]. The Japanese encephalitis vaccines that are most commonly used today are all based on the attenuated strain SA14-14-2 that was derived from JEV SA14 strain (which itself has fairly low pathogenicity[107]) grown from a pool of *Culex pipiens* larvae from Xi'an in 1954. The live attenuated Japanese encephalitis vaccine SA14-14-2 generates neutralising antibody against wild genotype III strains (Beijing-1 and SA14) in humans[108-111] but has not been tested against genotype I strains. Inactivated, adjuvanted SA14-14-2 (marketed in Europe and North America as IXIARO and in Australasia as JE-SPECT) induces neutralising antibody to JEV genotypes I–IV and gives similar seroconversion rates for genotype I and III viruses[112,113], although titres against genotype I tend to be lower than those for genotype III. In the past few years, a new vaccine has been developed based on an Indian genotype III strain, Kolar-821564XY (TABLE 1). This Japanese encephalitis vaccine, JENVAC, also induces neutralising antibody against genotypes I–IV[114]. Whether protection induced by a genotype III vaccine will be as durable for genotype I remains to be seen, as genotype I disease has been reported to occur after incomplete genotype III vaccination[115]. Moreover, JEV genotype V is also emerging[116], although cases in humans are rare. Current vaccines might not be as protective against genotype V as other genotypes, although this notion is based on a small sample thus far[117].

Vaccine use has resulted in a decrease in Japanese encephalitis incidence in many Asian countries[118,119], but estimates suggest that 80% of cases still occur in areas with established Japanese encephalitis vaccination programs[1]. Why so many cases occur despite vaccination is not clear; possible explanations are a lack of vaccine coverage, a lack of vaccine effectiveness, or presence of waning immunity in areas where JEV circulation is intermittent. In 2006, India — which, along with China, accounts for much of Japanese encephalitis disease burden — introduced the live Japanese encephalitis vaccine SA14-14-2, and a small case control study in Lucknow (Uttar Pradesh, North India) showed a 6-month vaccine efficacy of 94.5%[120]. However, post-marketing studies suggest a lower efficacy[121], and although cases reported to the National Vector Borne Disease Control Program of India (NVBDCP) decreased after vaccine introduction, they have since begun to rise again

(http://nvbdcp.gov.in/je-new.html). Several sources have documented lower seroconversion rates to Japanese encephalitis vaccine SA14-14-2 in India, from 58% to 74%[114,121,122], compared with studies undertaken outside India. However, clear examples can be found of circumstances where serum concentrations of neutralising antibody to contemporary strains have waned to undetectable levels in most of the population, yet a sustained reduction has been observed in the incidence of Japanese encephalitis[123]. Consistent with these findings is the observation that priming of memory B cells in mice is protective in the absence of serum neutralising antibody[124]. What accounts for the low seroconversion rate of Japanese encephalitis vaccine SA14-14-2 in India and whether this finding has clinical importance remain unknown. Interference by dengue virus in India has been hypothesized to interfere with SA14-14-2 immunogenicity[122].

Whatever the reasons for the continued existence of Japanese encephalitis cases in endemic areas — whether vaccine failure or failure to administer vaccine — Japanese encephalitis remains a major problem in Asia. Although efforts to prevent Japanese encephalitis undoubtedly need to be strengthened, the continued occurrence of clinical disease despite vaccination campaigns highlights the need to develop new treatments and to improve the care of the patients who contract the disease.

[H1] Pathogenesis and therapeutic targets

[H3] Pathogenesis. Despite substantial advances in the past few years, much remains to be learned about the pathogenesis of Japanese encephalitis (FIG. 6a). Studies in mouse models have characterised the initial pathogenesis of JEV infection: after peripheral inoculation with JEV, a round of replication occurs in the local lymph nodes[125], and virus can be found peripherally in monocytes and some T cells[126]. The initial replication is then followed by viraemia and the spread of infection to the CNS (FIG. 6b). JEV replicates well in monocytes[92,127] and dendritic cell lineages[127-130] from humans and mice, although replication in human neuronal cell lines is much more efficient[92]. JEV probably infects cells of the macrophage and dendritic cell lineage in the skin — in an analogous fashion to dengue virus[131] — with the infected cells then carried to local lymph nodes from where viraemia and then CNS infection can occur, if replication is sufficient[125]. The incubation period of JEV in mice is around 5 days, whereas the incubation period after intranasal inoculation of JEV in macaques is 7–10 days[132]. In humans, the incubation period is often described as 5–15 days; however, published data describing the incubation period in humans are scant, and they suggest a median of around 8.4 days, similar to the macaque model[133].

Spread of JEV to the CNS can be prevented by neutralising antibody, which alone is sufficient to prevent encephalitis[134] (FIG. 6a). The importance of antibody in protection from JEV infection is highlighted by the observation that mice lacking B cells are extremely sensitive to JEV infection[135], and priming[136] or adoptive transfer[124] of JEV immune memory B cells is protective even in the absence of pre-challenge neutralising antibody.

[H3] Entry into the CNS. The mechanism by which JEV enters into the brain remains elusive. The observations of peripheral replication and viraemia in animal models, along with the distribution of lesions in the brain[125,137], strongly suggest that JEV enters the CNS from the blood. JEV antigen is detected in the vascular endothelium in vivo[137-139], but whether this observation reflects actual replication is unknown: a 'Trojan horse' mechanism of CNS entry via an infected leucocyte cannot be discounted completely [49,87,126].

The effect of JEV infection on the blood–brain barrier (BBB) has been studied mostly in mouse models of Japanese Encephalitis. JEV is not a natural pathogen of mice, which are in general resistant to disease, unless JEV is inoculated when the mice are very young, or via the intracerebral route. Although there is substantial variation between laboratories in this regard, using a model where JEV is pathogenic and produces encephalitis in the infected mice, BBB breakdown can be readily demonstrated,[140]. The mechanism is uncertain, with some evidence it may be induced by soluble mediators, possibly interleukin (IL)-8[141]. However, BBB breakdown seems to be a consequence rather than the cause of JEV infection of the brain[142] and, although it contributes to pathology, is not absolutely required for virus entry into the CNS.

[H3] CNS pathology and inflammation. Although JEV can replicate in many cell types *in vitro,* inside the CNS its principle target cells are neurons [137-139,143]. In the macaque model of Japanese encephalitis, JEV antigen is also found in microglia and occasionally in blood-derived macrophages[143]. Marked inflammation is present in the brain of patients with Japanese encephalitis — a phenomenon that might be amenable to treatment. Inflammatory infiltrates in the brains of humans infected with JEV have not been very well characterised, but are dominated by T cells and, to a lesser extent, by monocytes and macrophages[137].

Reports of the composition of T cells in inflammatory infiltrates in the brains of people with Japanese encephalitis have been inconsistent. $CD4^+$ T cells predominantly were observed at post mortem in seven children who died from Japanese encephalitis[138], but $CD8^+$ T cells

were most prevalent in two adult fatal cases[144]. $CD4^+$ T cells were also found to be enriched in the CSF of 15 children infected with JEV [145], although the small number of cases makes it difficult to draw a firm conclusion that the T cell infiltrates in adults and children are different. Histologically, fatal cases of Japanese encephalitis in humans are characterised by perivascular cuffing and vascular leakage, sometimes with small haemorrhages, and small areas of well demarcated ('punched out') focal necrosis[137,138,146]. Viral antigen is found in inflamed areas of brain, but is also seen in non-inflamed areas of brain[132,146]. Because JEV spreads to the brain via the blood, and viraemia is so transient and hard to demonstrate in humans, it is likely these non-inflamed brain regions represent areas that are affected by JEV later in the disease process by contiguous or axonal spread of the virus [139], which suggests that a therapy that blocks viral spread might limit damage to the CNS. Alternatively, this may represent viral protein spread by axonal transport, with no active replication in the non-inflamed areas. Ultimately, neurons probably die through a combination of direct viral-mediated damage, immune-mediated damage (see later in the article) and apoptosis, which together give rise to the clinical symptoms of the condition. Features of raised intracranial pressure are reported in both clinical and pathological studies of Japanese ecephalitis[28,138,139], indicating that measures to reduce this pressure might also be beneficial.

Several lines of evidence support a pathological role for CNS inflammation in patients with Japanese encephalitis. In animal models of Japanese encephalitis, the BBB breaks down, which results in a large influx of monocytes and lymphocytes into the brain[147-149]. Consistent with pathological findings in fatal cases of Japanese encephalitis in humans and in animal models of the disease, high levels of inflammatory mediators can be detected in CSF of patients infected with JEV, and increasing concentrations of TNFα and IL-6 correlate with poor outcome in these individuals [150,151]. Blockade of TNF with Etanercept reduces inflammation and improves survival in JEV-infected mice[152], and manipulation of pro-inflammatory responses by other means — such as genetic ablation of toll-like receptor (TLR)4 — protects mice against the lethal effects of Japanese encephalitis[147]. Generally, mice that survive acute JEV infection in these models (typically mice that are not affected or have recovered approximately three weeks into the experiment) show strong type I interferon (IFN) responses, which result in a greater restriction of viral replication, less uncontrolled inflammation in the CNS, and enhanced resistance to JEV. However, treatment with IFNα did not affect outcome from Japanese encephalitis when it was tested in a clinical trial[59].

[H3] Adaptive immunity and immunopathogenesis. Although the role of antibodies in protection against Japanese encephalitis is well established, the role of T cells, both in protection and pathology, is less well established. Evidence for a protective role by T cells in mouse models of Japanese encephalitis is contradictory, with some investigators finding no role for T cells[136], some finding a partially protective role[135,153] and still others finding complete protection[154,155]. In one mouse model of the disease, depletion of $CD8^+$ T cells resulted in a 100-fold increase in virus in the CNS, whereas mice that did not undergo T cell depletion had high levels of $IFN\gamma^+$ $CD8^+$ T cells in brain [135]. These pivotal experiments could explain some of the observed differences between mouse models, but — more importantly — they suggest that either anti-viral or anti-inflammatory therapies alone might not be maximally effective, and that studies of combination therapies aimed at suppressing both virus replication and pathologic host responses together should be investigated. Some evidence also supports this paradoxical role of host immunity in humans: memory T cell responses in individuals who were asymptomatic after JEV exposure were found to be predominantly $CD8^+$ and were highly cross-reactive whereas in individuals who had recovered from Japanese encephalitis T cell responses were mostly $CD4^+$ and not cross-reactive[156]. Among individuals who had recovered from Japanese encephalitis, the quality of the T cell response correlated with the clinical outcome, and responses dominated by $CD4^+$ T cells that produce TNFα alone, and not IFNγ or IL2 were associated with incomplete recovery (FIG. 7). This observation, together with the observation that most of the T cells found in brain and CSF in patients with Japanese encephalitis are $CD4^+$[138,145], supports the notion that $CD4^+$ T cells are pathogenic in this disease. Taken together, these results suggest that immunity is effective if JEV can be contained in the periphery, but once inside the CNS, prevention of virus-induced damage comes at the cost of substantial immune-mediated neuronal injury.

[H1] Management

Given the potential severity of Japanese encephalitis, and its potential to emerge in previously non-endemic areas, the lack of work done to test treatments for this disease is surprising. To date, no specific treatment for Japanese encephalitis has been shown to work. However, supportive management is beneficial in patients with Japanese encephalitis, and several complications of the disease that increase risk of death are treatable. For example, the presence of seizures, which are associated with raised intracranial pressure[28], indicates a poor prognosis, and prognosis is worse still in the presence of intractable seizures or status epilepticus[28,66]. Seizure activity, which can be subtle, should be actively

sought out and aggressively treated, and some observational evidence supports the use of routine sedation to improve outcome[60]. Despite the fact that a clinical trial of high dose (0.6 mg/kg) dexamethasone given to all patients with suspected Japanese encephalitis did not show a benefit[56], agents such as corticosteroids or mannitol are nevertheless commonly administered to patients with Japanese encephalitis who have intractable seizures or other signs of raised intracranial pressure. Although the trial of dexamethasone in patients with Japanese encephalitis showed this treatment to be safe, several prominent examples of commonly used treatments for other disorders have been shown to be harmful when studied systematically — such as bolus fluids in children with shock in a malaria endemic area[157]. This finding further underlines the need for more definitive evidence to validate the use of common supportive therapies in Japanese encephalitis.

Other complications, particularly those associated with immobility such as pressure sores and contractures, should be prevented by good nursing care. However, this care often falls to the family in some areas endemic for Japanese encephalitis. Care should be taken to maintain fluid balance and avoid both under-hydration and over-hydration in patients with the disease[44,60]. In unconscious patients, clinicians should be vigilant for aspiration pneumonia, which should be treated upon early clinical suspicion. In practice, many patients who present with fever and altered consciousness are treated with broad spectrum antibiotics, as uncertainty often exists regarding the presence of bacterial infection such as meningitis or brain abscess. However, routine antibiotics are not necessary in Japanese encephalitis, so if the diagnosis of Japanese encephalitis is secure or strongly suspected, and CSF analysis or imaging does not suggest bacterial infection, then antibiotics need not be used.

[H3] Clinical trials in Japanese encephalitis. To date, few randomised clinical trials have tested treatments for Japanese encephalitis (TABLE 2). Replication of JEV is inhibited *in vitro* by type I interferon and ribavirin, but neither agent, when tested alone, altered the outcome in patients with Japanese encephalitis[59,62]. High dose dexamethasone did not affect the outcome from Japanese encephalitis (see previous discussion)[56], but this trial was small and underpowered. A preliminary study of intravenous immunoglobulin given for virus-neutralising and anti-inflammatory effects showed that this approach was safe and feasible, but the study was also not powered to detect an improvement in outcome[45]. However, the study showed enhancement of the antibody response to JEV from intravenous immunoglobulin treatment, at least in some individuals, a well described phenomenon in animal models of Japanese encephalitis[158]. Lastly, two studies of minocycline — one in acute encephalitis syndrome of any cause (hypothesised to improve BBB integrity) and one

in Japanese encephalitis — recruited only 29 and 44 patients with Japanese encephalitis respectively and so, again, were underpowered[159,160].

[H1] Existing compounds as therapies

None of the trials on Japanese encephalitis to date have demonstrated a benefit; however, many approaches remain untested. Numerous compounds are available that have anti-JEV activity (reviewed elsewhere[161]), including several that have already been used in humans for other indications[162] (TABLE 3). All of the compounds in TABLE 3 that have direct evidence of anti-JEV activity — except for arctigenin[163] and clinidipine[164] — have been used in clinical trials in children, or are in routine use in children.

Most of the therapies for Japanese encephalitis that were previously tested in mouse models were administered at the time of or shortly after JEV infection[165,166]. The exceptions are an anti-JEV monoclonal antibody, which was partially protective when given on day 5 after infection, at which time JEV was detectable in brain[134]; minocycline, which was effective after symptom onset[167]; and etanercept (a recombinant fusion protein of TNF receptor to IgG1), which was administered at days 3 and 5 after infection[152] (TABLE 3). Fenofibrate was only effective if administered several days before JEV infection[168] and so is unsuitable for clinical testing — although useful mechanistic information about the disease might be learned from this agent. Pentoxifylline has been studied as an adjunctive treatment both for malaria and dengue fever because of its anti-TNF activity[169,170], but it also inhibits JEV replication *in vitro* and has protective effects in mouse models of Japanese encephalitis[171]. The pathological role of TNF in Japanese encephalitis is further supported by the protective effect of direct TNF blockade with etanercept, as well observations that link high levels of TNF with poor outcome in humans with the disease[150,156]. Although etanercept improved survival of JEV infected mice and reduced neruoinflammation, JEV replication was marginally increased[152].

As described previously in the article, some mouse studies demonstrate that animals with intact immune systems die with uncontrolled inflammation after JEV infection, whereas those with impaired immunity — for example, depletion of $CD8^+$ T cells — die with less inflammation but a much higher viral burden[135]. This finding implies that targeting viral replication and inflammation concurrently may have a synergistic effect, which might be achieved using the available agents in TABLE 3, or using combinations of agents that have

already been tested, such as steroids, ribavirin and interferon; however, this strategy has not yet been attempted in humans.

Lastly, a theoretic basis supports a benefit for some other drugs in Japanese encephalitis and thus these agents merit testing, at least in an animal model of the disease (TABLE 3). Knockout of *Tlr4* protects mice against the lethal effects of Japanese encephalitis, and so this receptor represents a potential therapeutic target[147]. TLR4 is antagonised by eritoran, which has been used in phase III trials for sepsis and has also been shown to protect mice in a model of influenza[172]. Recruitment of inflammatory monocytes into the brain can be prevented by blockade of the interaction between integrin α4β1 and vascular cell adhesion protein 1, which represents the mechanism of action of the multiple sclerosis treatment natalizumab. Blockade of this pathway also partly protects mice from lethal West Nile infection[173]. Inhibition of c-Jun N terminal kinase (JNK1; also known as mitogen-activated protein kinase 8) reduced neuroinflammation, viral load and mortality in a mouse model of Japanese encephalitis[174]; consequently, JNK1 is a potential drug target in humans, but different compounds would be required to those used in mice. High IL-6 levels in CSF correlate with mortality in humans[151], so the anti-IL6 antibody tocilizumab might also have therapeutic potential in patients with Japanese encephalitis.

[H1] Considerations for new treatment trials

Most treatment trials in patients with Japanese encephalitis have been designed to show an absolute reduction in mortality or improvement in outcome of around 20–25%, which translates into a relative reduction in mortality (or poor outcome) of greater than 50% in most cases — a large effect. The sample sizes of humans with Japanese encephalitis have been modest, ranging from 44 to 153 patients per study, with an overall total of only 381 patients with confirmed Japanese encephalitis ever to be recruited into randomised treatment trials (TABLE 3).

An alternative approach that could enable the detection of a smaller effect size than in existing trials, would be to use a linear outcome measure, such as an outcome score, instead of a categorical good/bad outcome. Whilst many scoring systems exist to measure neurological disability and outcome after various types of neurological injury, few have been developed specifically for encephalitis, and fewer still have been validated in the setting where Japanese encephalitis occurs. The Liverpool outcome score (LOS) is a simple numerical scoring system that has been developed and applied to patients with Japanese

encephalitis as well as other forms of encephalitis across different age groups and cultures[63,64,156,175] . This score classifies outcomes as complete recovery (V), recovery with sequelae but with the (age appropriate) ability to live independently (IV), recovery with disability precluding independent living (III), fully dependent for daily activities (II) and death (I). The score is easy to use and is suitable for use in the field, an important consideration for Japanese encephalitis trials. An increase of one point on this scale represents a clinically meaningful improvement.

Four studies have been conducted using the LOS[63,64,156,175], we estimate that 38% of patients in these studies have a poor outcome, defined as death or neurological sequelae sufficiently bad to preclude independent living (assuming an 18% mortality (FIG. 4) in those studies that recruited only follow up cases). An average improvement of one point on the LOS scale would result in a reduction in the proportion of patients with poor outcome to 27%. For a trial to have 80% power to show this difference at 5% significance level would require 569 patients to be recruited (assuming 10% loss to follow up). Treating the LOS as a continuous variable (which, strictly speaking, it is not) could allow the sample size to be reduced to 356. Although this number is likely an under-estimate, due to the ordinal categorical nature of the LOS (a patient cannot have an outcome score of 3.5), it is highly likely that the number of patients needed to show clinically meaningful benefit would reduce from 569 (the number needed to show improvement in a categorical outcome measure). The real figure will lie somewhere in the range 356 to 569, but all scenarios would still require approximately the same number of patients, or more, with confirmed Japanese encephalitis in a single treatment trial than have ever been randomised.

The choice of follow-up time point also requires careful consideration; an end-point reached during the in-patient stay may have less loss to follow up, as trial participants are relatively “captive” in fixed locations, but follow-up of three to six months gives a more accurate picture of the real clinical outcome[66], though is challenging in the rural settings where JE occurs. Assessment of combinations of therapies, including supportive measures, perhaps using factorial design, will make trial design more complex still. Consequently, it is easy to see how the existing studies on treatment of Japanese encephalitis have not been sufficient to answer key questions and improve patient outcomes.

[H1] Conclusion

Japanese encephalitis remains a devastating disease with no specific treatment other than best supportive care. Despite the availability of vaccines, 69,000 cases of the disease are estimated to occur every year in Asia, and this figure probably represents an under-estimate. However, developments in our understanding of Japanese encephalitis within the past few years point to several potential avenues of treatment. Several compounds that are already approved for human use could be tested without delay. Such trials should be larger than previous studies, and will almost certainly need to be multi-centre. Recruitment of individuals with Japanese encephalitis can be challenging as many other conditions mimic the disease and outbreaks are unpredictable and can occur in remote and resource-limited settings. For a successful trial, sufficient infrastructure for good quality diagnostics is needed to ensure confirmation of every case. Given the apparently paradoxical role of the immune system in Japanese encephalitis, with evidence of both protective and pathological elements, strong consideration should be given to trials of combination therapy that test treatment regimens comprising both anti-inflammatory and anti-viral drugs. Some potential treatments, such as a JEV-specific monoclonal antibody, might provide a clinical proof of principle — if successful —to develop treatments for other arboviral encephalitides, and could speed up the development of treatments for other emerging flaviviruses of the future.

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Figure legends

Figure 1 | JEV Structure. a | The structure of the Japanese encephalitis virus (JEV) virion solved by cryo-electron microscopy (from Wang *et al.*[178], with permission). The JEV virion is ~50 nm in diameter and contains a central nucleocapsid core of viral RNA and core (capsid) protein. In the mature virion, this core is surrounded by a lipid bilayer envelope in which the viral membrane and envelope proteins are embedded[4]. **b** | The structure of JEV solved by X-ray crystallography of the envelope protein (from Luca *et al.*[177], with permission). As with other flaviviruses, the outer surface of the mature JEV virion is smooth and consists of 180 copies of envelope and membrane protein, with the envelope proteins arranged as 90 head-to-tail homodimers in an icosahedron, symmetry T = 3[177,178]. **c** | JEV genome organization. The JEV genome is 11 kb of single-stranded positive sense RNA, comprising a single 10.7 kb open reading frame (ORF) flanked by 5' and 3' untranslated regions, which function in replication. At the 5' end of the ORF are the core (C, encoding the capsid), pre-membrane (prM) and envelope (E) proteins, which together make up the viral particle and are referred to as structural proteins. The remaining non-structural (NS) proteins are expressed during replication.

Figure 2 | Features of Japanese encephalitis. a | The typical blank staring facial expression of an individual with Japanese encephalitis is shown. Reproduced from Solomon *et al.* 2000[179] with permission. **b** | CT scan in an individual with Japanese encephalitis**.** Bilateral thalamic hypodensity in a 7-year-old child in Ho Chi Minh City, Vietnam is illustrated (white arrows). In this setting, this finding has a sensitivity of 22% and specificity of 100% for a diagnosis of Japanese encephalitis[52]. However, this feature is also seen in other flaviviral encephalitides, so interpretation needs to take account of the local epidemiology. (Reproduced from Dung et al. 2009 figure 2a[52] with permission.)

Figure 3 | Clinical outcome of Japanese encephalitis. Forest plots showing mortality (part **a**) and proportion of patients with neurological sequelae (part **b**) from studies of individuals with Japanese encephalitis published in the past 30 years.

Figure 4 | Origin and genotype spread of JEV in Southeast Asia. a | Japanese encephalitis virus (JEV) probably originated in the region of Indonesia and Malaysia before spreading across Asia. Adapted from Solomon *et al.* 2003[93] with permission. **b |** Relative proportion of JEV genotypes I and III isolated from any source (humans, other mammals, birds and mosquitoes) according to decade of collection **c** | Number of JEV isolates from

humans over time. Adapted from Han *et al.* 2014[96] with permission. Blue dotted line represents JEV genotype I; red solid line represents JEV genotype III.

Figure 5 | Overview of the pathogenesis of Japanese encephalitis. a | After inoculation into the skin, virus is thought to replicate in Langerhans dendritic cells (as observed for dengue virus[131]) and/or in keratinocytes (as observed for West Nile virus). Japanese encephalitis virus (JEV) is then carried to the local lymph node where further replication takes place[125]. Viraemia occurs and the virus crosses the blood-brain barrier and enters the CNS. Major outstanding questions (indicated by "?") include: which are the true target cells for JEV in the skin; is there any role for the $CD8^+$ T cell or antibody response in the early stages before viraemia; is there any mechanism other than viraemia to gain entry to the CNS; how does JEV get across the blood-brain barrier; and which immune components are protective or pathogenic once inside the brain? Circled minus symbols indicate potential immune effects that restrict viral replication and/or spread. Modified from Turtle *et al.* 2012[176] with permission. **b** | Viral load of JEV after peripheral subcutaneous inoculation with $10^{1.5}$ LD_{50} of JEV Beijing strain into mice. The data show that viral replication is controlled at the site of inoculation and in the blood, but once virus enters the CNS unrestricted multiplication occurs[125].

Figure 6 | $CD4^+$ T cell responses in patients recovered from Japanese encephalitis. a | Overall, IFNγ responses are modestly smaller in patients with incomplete recovery from Japanese encephalitis and residual neurological disability (poor outcome) than in those who recover completely (good outcome). **b** | TNFα responses are not significantly different between individuals who have a poor outcome and those who have a good outcome. **c** | The balance of cytokines produced by $CD4^+$ T cells is different between patients who have a poor or a good outcome, with responses in the poor outcome group dominated by TNFα single producing cells, whereas responses in the good outcome group are biased towards $CD4^+$ T cells that make IFNγ, IL2 and TNFα (so called "polyfunctional" T cells). Reproduced from Turtle *at al.* 2016[156].

Description	Type	Virus strain	Common name	Country of origin, or Manufacturer/developer
Early vaccines, no longer in use				
Mouse brain	Inactivated	Nakayama	BIKEN	Japan, BIKEN
Mouse brain	Inactivated	Nakayama	Green Cross	Korea, Green Cross
Mouse brain	Inactivated	Beijing-I	NA	Japan
Primary hamster kidney	Inactivated	P3	NA	China
Currently available vaccines				
Vero cell	Inactivated	P3	NA	China
Primary hamster kidney	Live attenuated	SAI4-14-2	NA	Chengdu Biological Products, China
Vero cell	Inactivated	Beijing-I	JEBIKV	Japan, BIKEN
Vero cell	Inactivated	Beijing-I	ENCEVAC®	Japan, Kaketsuken
Vero cell	Inactivated	SA14-14-2	1C51, IXIARO	Intercell, Valneva
Vero cell	Inactivated	Kolar-82I 564XY	JENVAC	Bharat Biotech Int. ltd, India
Yellow fever 1 7D recombinant vectored	Live attenuated	SA14-14-2 (Envelope)	Imojev, Chimerivax JE	Acambis, Sanofi Pasteur

Table 1. Vaccines against Japanese encephalitis. The early, mouse-brain-derived vaccines against Japanese encephalitis are no longer in use owing to concerns regarding adverse events including acute disseminated encephalomyelitis (ADEM) — although the incidence of ADEM after vaccination was never accurately estimated. The virus strains used in vaccine development are all genotype III. Of the currently used inactivated vaccines for Japanese encephalitis, most are made for domestic markets and not sold internationally. The exception is IXIARO (known as JE-SPECT in Australia and New Zealand), which is licensed in Europe, North America and many other countries. IXIARO is the principle vaccine used for prevention of Japanese encephalitis in travelers. In areas endemic for Japanese encephalitis, the most commonly used vaccine now is the Chinese live attenuated vaccine, SA14-14-2, which has been studied extensively in China and other countries, and which was WHO pre-qualified in 2013. IXIARO is made from inactivated Japanese encephalitis virus (JEV) SA14-14-2, which was chosen for ease of production as it does not require containment level 3 facilities. Imojev, the first recombinant vaccine licensed, is based on the yellow fever 17D vaccine with the pre-M and envelope genes replaced by those of JEV SA14-14-2. The same technology was used to develop the first licensed dengue vaccine, Dengvaxia.

Study	Drug schedule tested	Number of patients randomly assigned to treatment	Number with confirmed Japanese Encephalitis	Primary endpoint	Outcome	Intervention of benefit?
Hoke et al. 1992 (Ref. 56)	Dexamethasone 0.6 mg/kg followed by 0.2 mg/kg q6h for 5 days	**65**	**55**	Death at 25 days	Dexamthasone: 6 of 24 died; placebo: 8 of 30 died	**No**
Solomon et al. 2003 (Ref. 59)	Interferon alpha-2a 106 U/m^2 body surface area for 7 days	**112**	**87**	Death or severe sequelae at discharge and 3 months	Interferon: 16 of 57 poor outcome*; placebo 13 of 50 poor outcome.	**No**
Kumar et aI. 2009 (Ref. 62)	Ribavirin 10 mg/kg daily for 7 days	**153**	**153**	Early death (in hospital)	Ribavirin: 19 of 70 died; placebo: 21 of 83 died	**No**
Rayamajhi et al. 2015 (Ref. 45)	Intravenous immunoglobulin (IummunoRel) 400 mg/kg daily for 5 days	**22**	**13**	Feasilbity - not powered for a clinical endpoint	IVIG: 5 of 9 recovered completely, 1 died; placebo: 2 of 9 recovered completely, 2 died.	**No**
Kumar et al. 2016 (Ref. 159)	Minocycline 5 mg/kg daily followed by 2.5 mg/kg daily (age <12) or 200 mg followed by 100 mg q12h for 7 days	**281**	**29**	Death at three months	Minocycline: 2 of 13 died; placebo 5 of 14 died (JEV+ only)	**No**
Singh et al. 2016 (Ref. 160)	Minocycline 5-6 mg/kg daily in two divided doses for 10 days	**44**	**44**	Not stated	Minocycline: 2 of 22 died; placebo 5 of 22 died	**No**
Total number of patients randomly assigned to treatment		**677**	**381**	**-**	**-**	**-**

Table 2 | Randomized clinical trials of treatments for Japanese encephalitis. Five studies have tested treatments for Japanese encephalitis and one study[159] tested minocycline for acute encephalitis syndrome and included patients with Japanese encephalitis. Minocycline has also been studied in patients with Japanese encephalitis alone, though this study was underpowered[160]. None of these studies showed a detectable benefit on any clinical outcome measure. *Poor outcome is death or severe neurological sequelae.

Compound	Evidence and comments	References
Compounds protective against Japanese encephalitis in animal models		

Anti-JEV monoclonal antibody (503)	82% of mice protected with 200 µg/mouse antibody given 5 days post infection, when virus is detected in the brain.	Kimura-Kuroda & Yasui 1988 (Ref. 134)
Rosmarinic acid	80% of mice protected by 25 mg/kg from day 1 post-infection.	Swarup et al. 2007 (Ref. 166)
Minocycline	70% of mice protected at 90 mg/kg/day from 6 days post-infection, inhibited JEV replication in vivo	Mishra et al. 2008 (Ref. 167)
Arctigenin	100% of mice protected by 20 mg/kg/day from day 1 post-infection. Only used at phase I in pancreatic cancer, no data in children.	Swarup et al. 2008 (Ref. 163)
Pentoxifylline	50% of mice protected at 100 mg/kg/day given immediately after JEV infection, 100% protection at >200 mg/kg/day; inhibited JEV replication in vitro	Sebastian et al. 2009 (Ref. 171)
Fenofibrate	80% of mice protected by 100 mg/kg/day from day 4 pre-infection to day 9 post-infection	Sehgal et al. 2012 (Ref. 168)
Nitazoxanide	70% of mice protected at 75 mg/kg/day from 1 day post-infection, 90% protection at 100 mg/kg/day; inhibited replication JEV in vitro	Shi et al. 2014 (Ref. 165)
Etanercept	80% survival with 100µg etanercept (30% without) on days 3 & 5 post-infection, reduced CNS inflammation, reduced production of inflammatry mediators in vitro	Ye et al. 2014 (Ref. 152)
Compounds with anti-JEV activity in vitro		
Ivermectin	Inhibited JE, yellow fever, dengue & West Nile virus replication in vitro	Mastrangelo et al. 2012 (Ref. 162)
Niclosamide	Inhibited JEV replication in vitro, also inhibits Zika virus replication in human neural progenitor cells	Fang et al. 2013 (Ref. 164)
Cilnidipine	Inhibited JEV replication in vitro, no data in children	Fang et al. 2013 (Ref. 164)
Compounds with theoretical activity or indirect evidence		
Eritoran	TLR4 antagonist: TLR4 KO reduces JE lethality in mice, eritoran protects mice against lethal influenza	Han et al. (Ref 147)
Tocilizumab	Anti-IL6 antibody: IL6 levels in CSF correlate with mortality in JE in humans	Winter et al. (Ref 151)
Natalizumab	Anti-VLA4 antibody: protective in a mouse model of WNV	Getts et al. (Ref 173)
PGL5001	c-Jun N terminal kinase (JNK) inhibitor: JNK inhibition by a different compound protective in a mouse model of JEV	Ye et al. 2016 (Ref. 174)

Table 3 | Compounds suitable for human use that have animal model, in vitro, and theoretical anti-JEV activity. Compounds with evidence for anti-JEV activity in mouse models are shown in the top panel and with anti-JEV activity (inhibition of replication) in cell culture are shown in the middle panel. All these compounds except arctigenin and cilnidipine have been used in children. Pentoxifylline and nitazoxanide also inhibit JEV replication *in vitro*. Ivermectin inhibits flavivirus NS3 helicase activity[162], and inhibited JEV production *in vitro* with EC_{50} 0.3 µM. Niclosamide and cilnidipine inhibited JEV induced cytopathic effect and replication[164], EC_{50} 5.8 and 6.52 µM, respectively. The bottom panel shows compounds with theoretical benefit in Japanese encephalitis. Eritoran (synthetic TLR4 antagonist), tocilizumab (anti-IL6 antibody), natalizumab (anti-VLA4 antibody) and PGL5001 (c-Jun N

terminal kinase (JNK) inhibitor) all have indirect evidence for benefit; their anti-JEV potential is hypothetical and requires testing in animal models.

Feature	Stage			
	V	IV	III	II
History from child, parents and/or carers				
Speech	The same as other children of this age	*NA*	Reduced	Not speaking or communicating
Feeding	The same as other children	*NA*	Occasionally needs help	Always needs more help
Can the child be left alone?	Too young or yes	*NA*	Yes briefly in familiar environment	No
Behavior	Normal	Gets angry easily, other behavioral problems	*NA*	Severely abnormal
Recognition (other than the main carer)	Too young or yes		Some	None
School/work, home activity if too young	Back to normal at school/work, or if too young able to do the same tasks at home	Not doing as well, not able to do the same tasks as before	Dropped a school grade, no longer attending school/work, not able to do anything at home	*NA*
Seizures in the last 2 months	No seizures and not on antiepileptic drugs	Seizure free on antiepileptic drugs	Has had seizures	Seizures most days
Ability to dress	The same as other children the same age	*NA*	Occasionally needs extra help	Always needs more help than other children of the same age
Urinary and fecal continence	The same as other children the same age	*NA*	*NA*	Needs more help or is incontinent of bowel or bladder
Hearing	Normal	Reduced in one or both ears	Cannot hear at all	*NA*
Observation of the child				
Sitting	Too young or can sit independently	*NA*	Needs help	Not at all
Able to move from sitting to standing	Too young or can walk independently	*NA*	Needs help	Not at all
Observe the child walking for 5 meters	Too young or normal	*NA*	Abnormal but independent with crutches or stick	Not able to walk
Put both hands on head - ask child to copy	Too young or normal with both hands	Abnormal with one or both hands	Unable to put one or both hands on head	*NA*
Pick up small object	Too young or normal pincer grasp both hands	*NA*	Unable with one hand or abnormal with one hand or both hands	Unable both hands

Table 4 | The Liverpool outcome score for assessing outcome from encephalitis. For each domain, the child is scored II–V according to residual neurological or behavioral deficits as detailed in the table. The final outcome score is the lowest score of any domain; For example, if an 8-year old child is normal in every respect other than not being able to be left alone, except briefly in a familiar environment, then the outcome score is III. A fatal case has a score of I. Cells marked NA represent values that are not used for the domain in question.

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