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Progress on the Research and development of Dengue Vaccine

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ABSTRACT

Dengue fever is an infectious disease transmitted by Aedes mosquitoes caused by the dengue virus, and there are four serotypes. Each serotype acts as an independent infectious agent. Dengue illness can range from mild illness to life-threatening bleeding. Dengue fever is a growing health concern for up to a third of the world's population. Currently, there is no effective anti-dengue drug, and the treatment of severe dengue relies on intravenous fluid management and pain relief drugs. Despite decades of trying, the world already has a dengue vaccine licensed in many countries, but restrictions and conditions on its use have prevented its use. An effective dengue vaccine should be a quadrivalent vaccine that can protect against all four serotypes of dengue virus. Although inactivated vaccines are relatively safe, due to the disadvantages of low immune response, poor endurance, and prone to ADE, no significant progress has been made so far. The attenuated live vaccine has the advantages of strong immune response similar to wild strains, including cellular and humoral immune response, and immune persistence. It is the focus of current development and has made great progress. A review of live attenuated vaccines.

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Key Words: Dengue viruses, Dengue attenuate live vaccines, Dengue chimeric tetravalent vaccines, Clinical trails

Abbreviations:

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Introduction

Dengue fever, a neglected tropical disease and the most common mosquito-borne disease, has caused a serious global disease burden[1-2]. WHO estimated that there were 100 million cases of dengue infection in the world every year from 2000 to 2013[3]. In the past 10 years, outbreaks of dengue fever have occurred in many countries around the world, such as the outbreak in Nepal in 2019 [4] and the outbreak in China from 2012 to 2015[5]. Dengue virus is transmitted through the human-mosquito-human cycle, with Aedes aegypti as the main vector and Aedes albopictus as the secondary vector[6]. Aedes mosquitoes were originally found in tropical and subtropical regions, but now they are found almost all over the continent[7]. Many complex factors interact to accelerate the outbreak of dengue fever, including climate [8-9] (rainfall, temperature and humidity, etc.), traffic, population density[9-10], extreme poverty and inadequate sanitation facilities [11], etc. Vaccination is considered the most effective strategy for controlling infectious diseases. Vaccination has long been considered a necessary foundation for a multipronged approach to reducing the global burden of dengue, but developing a safe and effective dengue vaccine has been difficult. For 75 years, scientists and product developers have been trying to design and develop safe and effective dengue vaccine candidates, but the challenges are enormous and daunting [12-13]. Although many different approaches are being explored, only live attenuated virus vaccines have been licensed or reached advanced clinical development [14]. Live attenuated vaccines have strong immune responses similar to wild strains, including cellular and humoral immune responses, and immune persistence. They are the focus of current development and have made great progress. A review of live virus vaccines.

Cell passage attenuated live vaccine (PDK cell passage strain quadruple vaccine)

Traditional attenuated vaccines are mainly passaged in sensitive animals or on sensitive cells to weaken or lose the pathogenicity of the virus while retaining good immunogenicity, thereby obtaining stable mutant attenuated strains. Some biological markers are considered to be the characteristics of DENV attenuation, such as weakened or no pathogenicity in the brain of suckling mice, smaller plaques, temperature sensitivity, loss of cytopathy, and antibody production in injected monkeys but no virus development. blood disease etc.

In the 1980s, scholars at the University of Hawaii in the United States[15-17]obtained a clone PDK35-TD3 from the DENV type 4 strain H241 by the terminal dilution method. Plaques cannot be formed, and the plaques formed on rhesus monkey lung cells (FRhL cells) are medium in size and sensitive to temperature (38.5°C). In LLC-MK2 cell lines and primary dog kidney cells (PDK cells)has better stability.The above--mentioned PDK35-TD3 strain was expanded and cultured in FRhL cells to obtain two virus species,among which

PDK35-TD3FRhL p3 can produce a higher titer of neutralizing antibodies in monkeys, and its neurotoxicity to suckling mice is significantly lower than that of its parents. Strain H241. In a phase I clinical trial, after 5 yellow fever immunized volunteers were inoculated with PDK35-TD3FRhL p3, only 2 subjects produced neutralizing antibodies, and all subjects had adverse reactions. A virus with a phenotype change was isolated during the hyperemia period, indicating that the stability of the strain was poor, and the research on PDK35-TD3FRhL p3 was therefore terminated.

Scholars at Mahidol University in Thailand continuously passaged the four serotypes of dengue viruses in PDK cells (serotypes 1, 2 and 4) and primary green monkey kidney cells (PGMK) (serotype 3), and obtained Attenuated strains of four serotypes (DEN-1PDK13, DEN-2PDK53, DEN-3PGMK30/F3 and D-4PDK48). Mahidol University combined the above-mentioned attenuated strains into monovalent, bivalent, trivalent and quadrivalent vaccines for trials in susceptible adults, and found that the monovalent vaccines of the four serotypes could induce good immune effects There were no abnormal reactions among the test subjects. Bivalent (DEN 2+4, DEN 1+4, and DEN 1+2) and trivalent vaccines (DEN 1+2+4) can also induce the corresponding bivalent and trivalent antibodies, but the results of quadrivalent vaccines are not ideal[18]. The quadrivalent vaccine was inoculated to 59 Thai adult volunteers. After 1 dose, all subjects produced antibodies against DEN-3, but most (47/59) subjects detected DEN-3. 3 viremia, while viremia against other serotypes was rare. After the first dose of inoculation, 58% of the subjects produced antibodies to three or more serotypes, and 35% of the subjects produced antibodies against four serotypes. Six months after the second dose, the above proportions Respectively increased to 76% and 71%, the subjects did not appear adverse reactions [19]. Children aged 5 to 12 were inoculated with two quadrivalent vaccines in different ratios. After one immunization, the subjects developed symptoms such as fever, headache, and rash. The seroconversion rates of the two quadrivalent vaccines in the subjects were 90% and 79%[20]. Phase 1b clinical trial [21] found that all subjects developed dengue-like syndrome after vaccination, and 7 out of 10 subjects developed DEN-3 viremia. Later, in the research experiment on the reactogenicity of the DEN-3 vaccine strain, all volunteers (15/15) had adverse reactions[22], and the experiment was terminated because the virulence and immunity of the quadrivalent vaccine did not reach a balance.

Researchers at the Walter Reed Army Research Institute in the United States[23] passaged four serotypes of DENV in PDK cells, and obtained four vaccine candidate strains: DEN-1PDK20, DEN-2PDK50,

DEN-3PDK20 and DEN-4PDK 20. In the Phase 1 clinical trial, the above-mentioned four strains of viruses were combined into 16 groups (1-16) of different quadrivalent vaccines in different doses, and the results showed that 7 groups (2, 5, 10, 11, 13, 14, 15) Can cause positive conversion of antibodies against three or more serotypes (⩾ 75% of subjects have positive conversion), among which the positive conversion rates of DEN-1, 2, and 3 antibodies are relatively high (69%, 78% in sequence) %, 69%), while the DEN-4 antibody positive conversion rate was low, only 38%, and 3 of the above 7 groups (13, 14, 15) showed better immunity and weaker adverse reactions , but there is no group that can make all subjects produce antibodies against the four serotypes. Later studies found that insufficient attenuation of DEN-1PDK20 was the main cause of adverse reactions [24], while over-attenuation of DEN-4PDK20 resulted in poor immunity. Through further screening, a higher generation of DEN-1PDK27 was used to replace DEN-1PDK20, and a lower generation of DEN-4PDK6 was used to replace DEN-4PDK20. The monkey test showed that the toxicity of DEN-1PDK27 to monkeys was reduced. (Low level of viremia) while maintaining a good level of antibody response, while DEN-4PDK6 significantly increased the level of antibody while maintaining a low level of virulence[25]. In Phase II clinical trials, DEN-1PDK27 and DEN-4PDK6 strains were combined with the original DEN-2 PDK50 and DEN-3PDK20 to form a new quadrivalent vaccine (No. F17). Two groups of quadrivalent vaccines (F13 and F14) compatible with the original virus were compared and observed in DEN antibody-negative adults. The positive conversion rates of the four serotypes of DEN antibodies were 63% for the former, 36% and 40% for the latter [26].

Thomas et al.[27]optimized the four serotype viruses in the original F17 (named F17/Pre) by transfecting rhesus monkey lung diploid cells with RNA to improve the purity of the virus, and continuously transfected them on the cells. Passed for 3 generations and reconstructed into a new quadrivalent vaccine strain. The new quadrivalent vaccine was firstly tested in phase II clinical trial on 86 antibody-negative volunteers in the United States. The virus titer used in the test was 104-105 PFU/ml, and the DEN-4 virus was divided into high virus titer (F17) and low virus titer F19 (that is, the dose of DEN-4 virus was only 1/10 of that of F17).) two groups, the results of vaccine reactivity was transient mild to moderate, no significant difference between the groups. Vaccine antibody response in each group, the seroconversion rate for all 4 types of antibodies was 37.5%-40.0% for 1 dose and 60%-66.7% for 2 doses, with no significant difference. Later, phase II clinical trials were conducted in Thailand[28] and Puerto Rico[29], and the results showed that the reactivity of

the vaccine was only mild to moderate transient reactions and low-level viremia; For those who were formerly antibody-positive, the antibody level increased rapidly after the first dose, and the positive rate increased from 80% to 89% to 97.1% to 100%, and there was no further increase after the second dose; The positive conversion rate for each type of antibody was only 20% to 43.3%, and it could reach 78% to 97% after 2 doses of vaccination (interval 6 months). The level is higher than that of F19 group. The above results show that the newly screened and compatible quadrivalent live attenuated vaccine can achieve positive conversion to all four types of antibodies for those who are positive for antibodies before immunization, and no serious adverse reactions have occurred. However, vaccinating two doses of pre-immune antibody-negative persons can achieve at least 75% positive conversion of all four types of antibodies. The results of continuous long-term follow-up observation of children and infant volunteers who were inoculated with two doses of F17/Pre showed that [30]within 1 to 3 years after inoculation, the levels of the four types of antibodies in most volunteers decreased. And 1 infant developed DENV-2 infection 2.7 years after the second dose of F17/Pre. One year after the second dose of F17/Pre inoculated in the children group, a dose of F17/Pre was given as a booster immunization, and the positive conversion rate for the four types of antibodies was 83.3%-100% 30 days after the booster immunization. In the next 2 years, the positive conversion rates of DEN-1, 2, and 3 antibodies all decreased. It shows that the vaccine can achieve a good short-term immune response, but the persistence of the immune effect is poor, and further research is needed.

Chimeric virus live attenuated vaccine

1.DENVax chimera live vaccine

DENVax uses the infectious clone of DEN-2PDK53-V as the vector, and replaces the PreM/E region of DEN-2PDK53 with the PreM/E region of DEN-1, DEN-3 and DEN-4, respectively. The parental strain 16681 of DEN-2PDK53 was isolated from a patient with dengue hemorrhagic fever in Bangkok in 1964, tested with monkey kidney BSC-1 cells (multiple times), LLC-MK2 cells (6 times), macaques (1 time), After the Ambona giant mosquito (2 times) was subcultured, it was continuously subcultured in PDK cells for 53 times, and finally the attenuated DEN-2PDK53 strain was obtained. Experiments on 10 volunteers showed that the strain has high safety and good health. immunogenicity, neutralizing antibodies persist for up to two years [31]. Using the DEN-2PDK-53 infectious clone as a vector, the PreM/E genes of DEN-1 16007, DEN-3 16562 and DEN-4 1036 strains were replaced by the corresponding regions of the vectors to successfully construct chimeric virus strains of three serotypes. That is, type

1 DEN-2/1, type 3 DEN-2/3, and type 4 DEN-2/4 strains.

The genotype and phenotype character--istics of the three chimeric viruses and their mother strain DEN-2PDK53 were studied, and the results showed that the four types of viruses all had the same three main attenuating gene loci (5NCR -57-T, NS1-53-ASP,NS3-250-Val)[32].The virulence of the four strains of viruses to the brain of ICR suckling mice was significantly reduced, and the DEN-2/3 and DEN-2/4 chimeric viruses were not pathogenic to ICR suckling mice; the four strains of viruses formed on LLC-MK2 cells. Small plaques are sensitive to temperature when cultured in mammalian cells, and the replication of viruses in mosquito cells is limited, and the infection in Aedes aegypti is greatly weakened and has no transmission ability; at the same time, immunized animals produce good antibody levels, which are effective for Dengue virus-sensitive mice lacking interferon receptors AG129 were intraperitoneally immunized with a monovalent virus with a titer of 40-640, and immunized with a quadrivalent mixed virus with a dose or two doses of 80-620 and 320-2650 respectively. [33]. The four types of viruses were purified by plaque six times, and one plaque virus (P7) was screened to establish the pre-master seed bank (P8) and the pre-master seed bank (P8). A comprehensive study of the whole gene sequence and phenotypic characteristics of the seeds showed that the seed viruses of the four serotypes all maintained attenuation characteristics such as attenuation sites, small spots, and temperature sensitivity [34].

Three (3:3:3:3, 3:3:5:5, 5:5:5:5) quadrivalent vaccines with different ratios of the four types of viruses (IgPFU) were used to immunize monkeys subcutaneously. Both produced low-level transient viremia of DEN-2, neutralizing antibodies were produced after 1 or 2 doses, and the high-dose two vaccines with high doses produced high antibody positive conversion rates to the 4 types after 2 doses (87.5% to 100%), after being challenged with the wild strain, the animals in the 5:5:5:5 group were protected against all four types of viruses, and no viremia occurred[35].

The Phase I clinical trial conducted observations on antibody-negative adults at intervals of 30 days and 90 days in Colombia and the United States. The vaccine was divided into high-dose group and low-dose group, that is, the virus titers (lgPFU/ml) of the four types were 4:4: 5:5 and 3:3:4:5, which were divided into subcutaneous (sc) and intradermal (id) vaccination groups. The results showed that in terms of safety, there was no significant difference between the groups, and only transient mild adverse events occurred. response, low viremia. In terms of antibody response, the secondary results showed that the high-dose group had a higher positive conversion rate for 4 serotypes, and the positive conversion rate

for ⩾ 3 serotypes after 2 doses was 80%, and the positive conversion rate for all 4 serotypes was 80%. 46% to 80%. Which for D1, D2, D3, D4 type were 100%, 94% ~ 100%, 82% ~ 100%, 46% ~ 78%. The sc and id groups showed no significant difference [36-38]. In phase II clinical trials, 2 doses of high-concentration virus vaccines were used in 4 age groups in 4 countries where dengue fever is prevalent (results are still being sorted out). In addition, the vaccine can induce cellular immunity against DENV non-structural protein (NS) after inoculation in humans [38].

2.Tetravalent chimera live attenuated vaccine with YD-17 as the backbone (CTD-TDV, Dengvaxia)

YF-17D is an attenuated live vaccine strain for the prevention of yellow fever (YF). In 1989, Rice et al. [39] constructed an infectious clone of this strain. At present, using this infectious clone as a vector, multiple viruses including Japanese encephalitis[40]and DENV have been successfully constructed.chimeric virus. Guirakhoo et al[41,42] used YF-17D infectious clone as a vector, chimerized DEN-1 (PUO359 strain, isolated in Thailand in 1980), DEN-2 (PUO-218, isolated in Thailand), DEN-3 (PaH881/88 strain, isolated in Thailand in 1988) and DEN-4 (1228 strains, isolated in Indonesia in 1978) PrM/E gene, respectively constructed YF/DEN1, YF/DEN2, YF/DEN3 and YF/DEN DEN4 four strains of chimeric viruses were tested in mice and monkeys. The YF/DEN2 monovalent virus has no pathogenicity in the brain of 4-week-old mice, only produces very low viremia in rhesus monkeys, and produces high-titer neutralizing antibodies after 30 days. After 60 days, DEN-2 wild virus All monkeys in the inoculation group had no viremia, while the control group had viremia lasting for 5 days, indicating that the pathogenicity of the chimera was significantly weaker than that of YF-17D, and it had a good immune protection[41].Afterwards,YF/DEN1,YF/DEN 3 and YF/DEN4 monovalent vaccines of three serotypes were inoculated into the brains of mice aged 3 to 4 weeks, and there was no neurotoxicity. All rhesus monkeys subcutaneously inoculated with the first dose of monovalent vaccines All produced viremia, and the level and duration of viremia were similar to those of YF-17D, but lower than those caused by the three types of DEN parent strains.

The YF/DEN2 monovalent vaccine was the first to conduct a phase I clinical trial in the United States on flavivirus antibody-negative healthy adults, and the results showed[43] that the YF/DEN2 chimeric virus was as safe as YF-17D, with only a low level of transient viremia. 100% and 92.3% of volunteers who had not received YF-17D immunization in the past produced antibodies to dengue type 2 wild strain (16681 strain) respectively. However, the level of neutralizing antibody against

dengue type 2 virus in the YF-17D immunized group was higher than that in the non-immunized YF-17D group. This shows that immunization with YF-17D can make the immune effect of YF/DEN2 stronger, with a wider range of protection and a longer duration. Morrison et al[44] combined the above four types of monovalent chimeric viruses to construct a tetravalent chimeric vaccine, each containing 5.0 lg of PFU virus, and observed YF antibody-negative adult volunteers in the United States. Three doses were inoculated in 12-15 months, and the second group was inoculated with 2 doses with an interval of 8-11 months. As a result, all the volunteers in the first group produced antibodies against the four serotypes of DENV international standard strains, and the second group 92.3% to 100% antibody positive conversion, and the seroconversion rate and GMTs increased with the number of vaccinations increased.

The adverse reactions of this group of vaccines were relatively mild. After the first dose of vaccination, 78.9% of the subjects developed low viremia, and serotype 4 was the main one. After the second dose of vaccination, 13.3% of the subjects had After the third dose of vaccination, only one subject developed serotype 3 viremia. Later, the quadrivalent vaccine was evaluated in different age groups in the Philippines, an endemic area of dengue fever, and Mexico, a non-endemic area[45,46]. Volunteers were divided into four groups according to age (2-5 years old, 6-11 years old, , 12-17 years old, 18-45 years old) were inoculated with three doses of the vaccine. The results showed that the safety of the vaccine was consistent with previous observations, and no serious adverse reactions occurred. The ratio of adverse reactions after the second dose and the third dose was It is mild after the first dose of vaccination, and the adverse reactions of children after vaccination are weaker than those of adults.

The level of antibody response in the endemic area is higher than that in the non-endemic area. The positive conversion rate for types 1-4 is 88%-100% in the former, and 77%-92% in the latter. Adults (100%) are higher than other age groups (88% to 100%). In another trial conducted in Australian adult volunteers, the volunteers had been vaccinated with traditional live attenuated DEN-1 and DEN-2 vaccines before inoculation with this group of quadrivalent vaccines. Antibodies against four serotypes of DENV were produced after one dose of quadrivalent vaccine without adverse reactions [47].

3．Quadruple Live Vaccine with Nucleotide Deletion in 3′ UTR (UTR△30)

The 3′ non-coding region (3′ UTR) of dengue virus can form a stable secondary structure and play an important role in the replication and assembly of the virus. If this region is deleted or mutated, it will change the secondary structure of genomic RNA. The structure will seriously affect the

pathogenicity of the virus. Researchers from the NIH Institute of Metamorphosis and Infectious Diseases deleted 30 nucleotides of 3′ UTR172～143nt of the DEN-4814669 strain and obtained a restored virus (rDEN-4△30). Compared with its mother strain, the incidence of viremia in monkeys was less, the virus titer was lower, and the time was shorter, but all experimental monkeys produced DEN-4 neutralizing antibodies, and they were challenged with DEN-4 mother strain 42 days after infection. None of the monkeys developed viremia, which indicated that rDEN-4△30 reached a good balance between attenuation and immune effects[48]. The vaccine made of rDEN-4△30 was used to inoculate 20 adult volunteers aged 18 to 45 with 105 PFU of the virus. Biting volunteers has no ability to spread. At the same time, the neutralizing antibodies of all volunteers have increased by ≥ 4 times, and the average neutralizing antibody titer is as high as 1:580 [49.50]. Later, a phase II clinical trial was conducted, in which 50 volunteers were inoculated with 103 PFU/ml virus, the results also showed low reactogenicity, low viremia, and the average highest virus titer was only 0.5-0.7 lg PFU/ml. ml, at the same time 93% of the volunteers produced DEN-4 antibody seroconversion[51]

Summary and Prospect

Dengue vaccine research has been going on for decades, and the vaccines being studied include live attenuated vaccines, chimera live vaccines, purified inactivated vaccines, DNA vaccines, recombinant subunit vaccines and virus-like particle vaccines, but the progress is the fastest and the data Only the four categories mentioned above have entered or completed phase II to III clinical trials at most. Among them, Dengvaxia, the YF-17D/dengue chimera quadruple vaccine developed by Sanofi Pasteur, has completed phase III clinical trials for tens of thousands of people in more than 10 countries including Asia and South America, and obtained comprehensive data. The results show that , In the first 2 years of observation, only mild transient reactions occurred in the vaccination, and no serious cases of ADE occurred. After immunization, those who are positive for flavivirus antibodies will increase rapidly after 1 dose of vaccination, and the positive conversion rate for all DEN 1-4 types after 2 doses can reach more than 90%.

However, for those who are negative for the antibody, the seroconversion rate of all four types is less than 30% after one dose of vaccination, 70% to 80% after two doses, and more than 90% after three doses. Moreover, the protective effect of the vaccine is poor, with a total protective rate of only about 60%, especially for type 2, which is 9.2% to 40%, and the protective effect for severe cases such as dengue hemorrhagic fever is relatively low Well, it can reach 90%, and the effective rate of reducing hospitalization is also high, reaching 67% to

80%. However, the antibody level decreased significantly 3 years after immunization, the vaccine is prone to ADE in young children, the number of hospitalized severe cases increased, the protection rate decreased (16.7%), and the relative risk (RR) increased (4.95). Therefore, although the vaccine has been approved by the WHO and has obtained application licenses in four countries, relevant experts expressed concerns and doubts about its safety and effective control in dengue fever endemic areas[52-54]

In conclusion, the dengue fever vaccine has shown promising prospects after many failures. Currently, in addition to expanding the observation of the safety, effectiveness and immune persistence of the vaccine in endemic areas, especially the possibility of ADE, the focus of research is on the protective mechanism of the vaccine. In particular, the protective effect of cellular immunity on the effectiveness of vaccines, including the function and immune protection of different gene protein components in cellular immunity. Establish in vitro and in vivo detection techniques to predict the effectiveness of vaccines on humans; strengthen the genotype analysis of current circulating virus strains in dengue endemic areas and their matching with vaccine strain virus genotypes. In addition, some experts suggested that immunized volunteers should be challenged with live attenuated dengue vaccine strains that cause mild infections in humans, so as to initially assess and predict the effectiveness of the vaccine on humans, and the research is currently in progress[55,56].

Competing interests

The authors declare all financial and non-financial competing interests.

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