OPEN

Clinical & Translational Immunology (2014) 3, e28; doi:10.1038/cti.2014.24

www.nature.com/cti

REVIEW

EV71 vaccine, an invaluable gift for children

Zhenglun Liang and Junzhi Wang

Enterovirus 71 (EV71) is a major pathogen for severe hand, foot and mouth disease (HFMD). Development of vaccines against EV71 would be the most effective approach to prevent the EV71 outbreak. Research and development (R&D) of EV71 vaccine was carried out in several Asian countries. Currently three companies in mainland China have completed Phase III clinical trials of inactivated EV71 whole-virus vaccines, whereas the other two companies have completed Phase I clinical trials separately in Taiwan and in Singapore. Results from those clinical trials have indicated high safety and immunogenicity of EV71 vaccine. Protective efficacies were over 90% on EV71-associated HFMD and over 80% on other EV71-associated diseases. In this paper, we summarize the results from three EV71 vaccine Phase III clinical trials and discuss the challenges of incorporating EV71 vaccine into Expanded Program on Immunization (EPI) in countries with EV71 epidemics.
Clinical & Translational Immunology (2014) **3,** e28; doi:10.1038/cti.2014.24; published online 31 October 2014

Hand, foot and mouth disease (HFMD) is a common illness mostly seen in children under 5 years old. Severe neurologic symptoms of HFMD in young children are often associated with enterovirus 71 (EV71), which makes EV71 infection a serious public health problem in the Western Pacific region countries.[1] From 2008 to 2012, 7.2 million cases of HFMD had been reported in mainland China, of which 2457 (0.03%) were fatal.[2] So, the rapid development of EV71 vaccines is driven by an overwhelming public health need and strong market demand in these countries. There are several types of EV71 vaccines currently under R&D, including inactivated whole-virus vaccines, recombinant vaccines and peptide vaccines.[3] Development of inactivated whole-virus EV71 vaccines progressed most rapidly, inspired by the previous experiences in developing inactivated polio vaccine and inactivated hepatitis A virus vaccines. Three companies in mainland China, including Beijing Vigoo Biological Co., Ltd. (Vigoo), Sinovac Biotech Co., Ltd. (Sinovac), and the Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming Institute) have completed their Phase III clinical trials of inactivated EV71 vaccines in more than 30 000 infants and children. Their results have shown that the EV71 vaccines have good safety in infants and children, which can prevent over 90% of EV71-associated HFMD and 80% of EV71-associated diseases.[4–6] As the current research and application of EV71 vaccines are mainly in Asian countries, there are challenges associated with the vaccine availability, affordability and the unification of vaccine standards to incorporate EV71 vaccine into Expanded Program on Immunization (EPI) in other developing countries. This paper reviews the results from those EV71 Phase III clinical trials and discusses the key issues in applying EV71 vaccines in EV71 epidemic countries.

PHASE III CLINICAL TRIALS SHOWED THAT EV71 VACCINES WERE SAFE AND EFFECTIVE

EV71 vaccines developed by Vigoo, Sinovac and Kunming Institute are all inactivated whole-virus alum-adjuvant vaccines which separately use isolated C4 genotype virus as vaccine strain.[7] The nucleotide difference and amino-acid difference of EV71 vaccine strain between three manufacturers are 97.3–99.7%, and 98.3–99.7%, respectively. Vigoo and Sinovac respectively used the bioreactor and cell factory all of which based on *Vero cell* substrate, whereas Kunming used Human *Diploid Cell (KMB-17)* and the bioreactor.[3,8] National Institutes for Food and Drug Control developed a national standard for EV71 vaccine antigen (Approval No: 20100023).[9] Vaccine doses in all three companies are measured as U unit according to the EV71 antigen standard. Based on those Phase I and II clinical trial results, vaccines containing 100 U per dose (Kunming Institute), 320 U per dose (Vigoo), and 400 U per dose (Sinovac) were used in the Phase III trials for efficacy and batch consistency evaluations. The immunization procedure is in accordance to the 0d and 28d two-vaccination procedure.[10–14]

Phase III clinical trials to evaluate the efficacy of EV71 vaccines were carried out in Jiangsu province (vaccines from Vigoo and Sinovac) and Guangxi province (vaccine from Kunming Institute) separately between February and March 2012 (Clinical Trials.gov number: NCT01507857, NCT01508247, NCT01569581).[4–6] In clinical trials of Sinovac and Vigoo,[4,5] 10 077 (vaccine group: 5044, placebo group: 5033) and 10 245 (5120; 5125) infants and children aged 6–35 months were enrolled, respectively. These participants were boosted on day 28 and received voluntary surveillance for incidence of HFMD/herpes angina in a 1-year follow up. In the clinical trial of Kunming Institute, 12 000 (vaccine group: 6000; placebo group: 6000) infants and children aged 6–71 months were enrolled. These participants also received a second dose of vaccine and followed with a follow-up for two peak epidemic seasons (Table 1).

More than 30 000 infants and children were immunized with vaccines made by these companies. Safety data have shown that the most common adverse reaction was fever less than Grade 3. The fever incidence rate in vaccine groups was 35.2, 41.6 and 34.7%, respectively

National Institutes for Food and Drug Control, Beijing, China
Correspondence: Dr J Wang, National Institutes for Food and Drug Control, No.2, TiantanXili, Beijing 100050, China.
E-mail: wangjz@nifdc.org.cn
Received 30 April 2014; revised 9 September 2014; accepted 9 September 2014

Table 1 The immunological results of the current phase III clinical trial

Manufacturers	Clinical trials gov no.	Subjects	Treatment	GMT and positive rate						Vaccine efficacy	
				Pre-vaccination	Post-vaccination					EV71-associated HFMD	EV71-associated cases
					1 Dose	2 Dose	3 Dose	8 Month	13 Month		
Sinovac[4]	NCT01507857	Infants (6–35 month)	Vaccine (400 U/person/dose)	7.5, 15%	—	98.8%, 165.8	99.3%, 88.8	99.2%, 92.1	—	97.5% (6 month), 94.8% (12 month)	89.3% (6 month), 88.0% (12 month)
			Placebo control (aluminum adjuvant)	8.2, 18%	—	19.6%, 8.9	28.1%, 12.5	34.7%, 13.2	—		
Vigoo[5]	NCT01508247	Infants (6–35 month)	Vaccine (320 U/person/dose)	11.5 (9.9–13.3)	—	325.3 (284.8–371.7)	—	187.3 (162.4–216.0)	191.9 (170.8–215.6)	90.0% (67.1 to 96.9)	80.4% (58.2 to 90.8)
			Placebo control (aluminum adjuvant)	12.1 (10.4–14.1)	—	13 (11.1–15.2)	—	18.2 (15.1–21.8)	16.2 (13.6–19.1)		
Kunming Institute[6]	NCT01569581	Infants (6–71 month)	Vaccine (100 U/person/dose)	170.6, 100%	—	86.1%, 106.0	—	99.3%, 88.3	—	97.3% (92.6–99.0%)	—
			Placebo control (aluminum adjuvant)	3.5%	—	3.2%, 4.5	—	30.4%	—		

Abbreviations: EV71, enterovirus 71; GMT, geometric mean titers; HFMD, Hand, foot and mouth disease.

for Vigoo, Sinovac and Kunming trials, whereas it was 33.9, 35.2 and 35.1% respectively for the correspondent placebo groups. The rate of severe adverse events in vaccine groups was 2.2, 1.2 and 1.1% respectively, whereas in placebo groups it was 2.6, 1.5 and 2.1% respectively in those three trials. Severe adverse events incidence rate was similar for vaccines generated using the diploid vaccine technology and the Vero vaccine process. There were no vaccine-associated severe diseases and immune-pathological responses during the surveillance period, suggesting a good tolerability of the inactivated EV71 vaccines.

In Vigoo's study,[5] the incidence rate for EV71-associated diseases was 0.16% (8/4907) in the vaccine group and 0.83% (41/4939) in the placebo group. Protective efficacy for EV71-associated diseases was 80.4%. The incidence rate for EV71-associated HFMD was 0.03% (3/4907) in the vaccine group and 0.16% (30/4939) in the placebo group, indicating a 90% efficacy for EV71-associated HFMD. In Sinovac's study,[4] the incidence rate for EV71-associated diseases was 0.3% (13/5041) in the vaccine group and 2.1% (106/5028) in the placebo group. Efficacy for EV71-associated diseases was 89.3%. No incidence of EV71-associated HFMD and severe HFMD was observed in the vaccine group. The incidence rates for EV71-associated HFMD and EV71-associated severe HFMD in the placebo group were 0.48 (24/5028) and 0.16% (8/5028), respectively. The rates in preventing hospitalization for EV71-associated HFMD and EV71-associated severe HFMD were both 100%. In Kunming's study,[6] the incidence rates for EV71-associated HFMD were 0.07% (4/5481) in the vaccine group and 0.83% (151/5499) in the placebo group. Efficacy on the EV71-associated HFMD was 97.4%. All three clinical trials have shown high efficacies of EV71 vaccines in preventing EV71-associated HFMD/HA, EV71-associated hospitalization and severe cases of HFMD. Vaccine immune correlation analysis indicated that the optimal protection against EV71-associated disease was achieved when EV71 neutralizing antibody titers were 1:16 and 1:32.

A 1-year follow up was conducted for the trials of Sinovac and Vigoo on a subgroup of participants who were seronegative at baselines. The follow-up results showed that the positive rates for neutralizing antibody in both groups were 99% ($>$1:8) and 92% ($>$1:16), respectively, 1 year after the vaccination. The anti-EV71 neutralizing antibody geometric mean titers were 191.2 and 92.1.

Vaccine batch consistency was also evaluated at the same time for the vaccines from those three manufacturers (Clinical Trials.gov number: NCT01636245, NCT01508247, NCT01569581). In Sinovac's study,[15] 1400 participants aged 6–59 months were vaccinated with either vaccines from three different batches or placebo from one batch following the 0d/28d procedure. Twenty-eight days after the second vaccination, the positive rate for neutralizing antibody was 99.7–100%, and neutralizing antibody geometric mean titers were 140.3, 141.5 and 146.6 for those three batches respectively, indicating good batch consistency. In Vigoo's study,[5] 56 days after the first dose of vaccine, the ratio for neutralizing antibody geometric mean titers was 0.5–2.0, in agreement with pre-protocol consistency criteria. These results indicated stable immunogenicity of vaccines from consecutive batches.

Despite the differences in seed strains, manufacture processes and dosages, these Phase III trials, conducted in various regions of mainland China, have indicated satisfactory safety and efficacies against EV71-related diseases and EV71-associated HFMD, with good batch consistency and persistent immunogenicity, suggesting the potential of getting approved for market entry.

KEY POINTS FOR EV71 VACCINE APPLICATION

R&D characteristics of EV71 vaccine and its application

EV71 Phase III clinical trials have indicated the efficacy of vaccines and thus their likely importance as a foundational tool in the prevention of severe HFMD outbreaks. R&D of EV71 vaccine was led by pharmaceutical companies in developing countries, which lacked the experience of major pharmaceutical companies in establishing new vaccine standards and promoting vaccines in various countries and regions. In addition, EV71 vaccine belongs to the first group of vaccines used in preventing infectious diseases prevalent in developing countries in Asia.[16] As a result, there will be challenges associated with the approvals in different countries and the appropriate application on targeted populations. There is a strong need for cooperation between vaccine development companies, non-profit organizations, governments and WHO to solve issues such as vaccine availability and affordability, and the standardization of vaccine quality control and evaluation. However, the key issue on that developing country induces EV71 vaccine into EPI depends on his own immune planning policy for HFMD. As WHO guideline recommended,[1] existing approaches to HFMD control include establishing and strengthening surveillance, providing assistance to kindergartens, and so on. These specific prevention and control measures have their own benefits and limitations. Furthermore, reliable surveillance data and information on the impact of prevention methods are important in determining how a vaccine should be applied and how it should be evaluated. For example, HFMD can be caused by a variety of enteroviruses. How to persuade the public to accept that HFMD is still epidemic even after EV71 vaccination is the key point for promoting the application of vaccines. Therefore, except strengthening the research and monitoring of HFMD pathogenic spectrum, the R&D of the EV71–CA16 combined vaccine should be strengthened for providing tools to effectively prevent HFMD.[17]

Technical problems to be solved

The cross-protection effect of EV71 vaccines. EV71 is currently divided into four genotypes (A, B, C and D genotypes) and is further divided into 12 sub-genotypes. C4 and B5 were the two pandemic strains in recent years. The key factor affecting vaccine effectiveness is whether vaccine derived from a specific strain with single genotype can protect against the other prevalent viruses. Serum from people vaccinated with C4 vaccine showed good cross-neutralization and protection effect against various sub-genotypes of EV71 virus (B4, B5, C2, C5).[18] However, serum from those vaccinated with Taiwan B4 strain was unable to neutralize the C2 strain.[19] Hence, research should focus on the cross-protection effect of vaccines against various pandemic virus strains and the monitoring changes of EV71 epidemic strains.

Monitoring vaccine safety and effectiveness after market entry. Over 30 000 infants and children were enrolled in these three Phase III clinical trials. Although there were no reported severe cases of vaccine-related diseases and immune-pathological responses, safety profiles need to be further evaluated with a larger populations in Phase IV clinical trials following the market entry of the vaccine. In addition, the vaccine efficacy also requires further assessment.

Issues associated with EV71 vaccine in EPI in developing countries

Vaccine availability in developing countries. Vaccine availability includes vaccine production, registration reviews in different countries and international procurement and distribution. Although the above three Chinese companies have large production capacity, their Phase III clinical trials were not conducted in multiple international sites, and their vaccines were not registered with Food and Drug Administration (FDA) and European Medicines Agency (EMEA). Therefore, the major factor affecting vaccine availability is the vaccine registration and review in other developing countries.

Cost-effectiveness of EV71 vaccine usage in developing countries. Developing countries need to assess the cost of manufacturing EV71 vaccines and the medical cost of treating EV71-associated disease, and to develop relevant immunization programs. Studies have shown that when EV71 vaccine efficacy reaches 70% and vaccine cost is below $25 per dose, there is a good cost-effectiveness for EV71 vaccination in large populations of infants and children in China.[20]

Co-administration with other EPI vaccines. Chen *et al.*[21] have shown that the co-administration of EV71 vaccine with a pentavalent combination vaccine containing Poliovirus, Bordetella pertussis, Haemophilus influenza type B, diphtheria toxoid and tetanus toxiod vaccines (PEDIACEL, Sanofi Pasteur, Toronto, Ont, Canada) in mice didn't affect the antibody response of the pentavalent vaccine. According to the EPI immunization program, infants and children in the ages of 6 months or above need multiple vaccinations. Although different inactivated vaccines can simultaneously produce immunogenicity, further studies on the cross-interactions between EV71 vaccine and other EPI vaccines are needed in future clinical trials.

Unification of vaccine quality control standards and internationalization of reference standards. The unification of vaccine quality control standards will help vaccine registration reviews and applications in other countries. The EV71 antigen quantification standard has been used to unify quality control standards and vaccine dosages for vaccines from those three Chinese manufacturers, which accelerated the registration review and market entry in China.[9] Vaccines from those companies were registered and the clinical trials were finished at the same time. However, the quality control standard and the dosage unit are different in other countries, which might affect vaccine registration and application in those countries. Hence there is an urgent need to establish WHO guidelines on EV71 vaccines to accelerate the vaccine application process.

CONCLUSION

'EV71 vaccine is an invaluable gift for children in the Asia-Pacific regions and worldwide'.[22] The success of clinical trials on inactivated EV71 vaccines has established them as an effective tool to be used in the fight to control escalating epidemics of EV71 infection-associated severe HFMD. The development of EV71 vaccines was led by companies in Asia, indicating an improvement in vaccine R&D and evaluation in these Asian countries. Nevertheless, technical and policy challenges remain for market entry approvals and applications in other countries with large targeted populations of infants and children. The challenges include vaccine availability, the feasibility of incorporating EV71 vaccine into EPI, and the unification of vaccine standards. Therefore, international cooperation is especially important for the promotion of EV71 vaccine in developing countries, and WHO should lead the effort to establish guidelines on EV71 vaccine quality control and evaluation. The good news is that National Institutes for Food and Drug Control and National Institute for Biological Standards and Control are jointly developing WHO neutralizing antibody standards for EV71 vaccines, which might provide a way to standardize the vaccine quality control and evaluation. However, only Japanese encephalitis (JE) vaccine from Chengdu institute in China currently has passed WHO pre-qualification. It still has a long way to introduce the cost-effective EV71 vaccine globally.

1 A Guide to clinical management and public health response for hand, foot and mouth disease (HFMD) 2011. Available from http://www.wpro.who.int/emerging_diseases/documents/HFMDGuidance/en/.
2 Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu JT *et al.* Hand, foot and mouth disease in China, 2008–12: an epidemiological study. *Lancet Infect Dis* 2014; **14**: 308–318.
3 Liang ZL, Mao QY, Wang YP, Zhu FC, Li JX, Yao X *et al.* Progress on the research and development of inactivated EV71 whole-virus vaccines. *Hum Vaccin Immunother* 2013; **9**: 1701–1705.
4 Zhu F, Xu W, Xia J, Liang Z, Liu Y, Zhang X *et al.* Efficacy, safety and immunogenicity of an enterovirus 71 vaccine in China. *New Engl J Med* 2014; **370**: 818–828.
5 Zhu FC, Meng FY, Li JX, Li XL, Mao QY, Tao H *et al.* Efficacy, safety and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 2024–2032.
6 Li R, Liu L, Mo Z, Wang X, Xia J, Liang Z *et al.* An inactivated enterovirus 71 vaccine in healthy children. *New Engl J Med* 2014; **370**: 829–837.
7 Mao Q, Li N, Yu X, Yao X, Li F, Lu F *et al.* Antigenicity, animal protective effect and genetic characteristics of candidate vaccine strains of enterovirus 71. *Arch Virol* 2012; **157**: 37–41.
8 Liang ZL, Mao Q, Gao F, Wang J. Progress on the research and development of human enterovirus 71 (EV71) vaccines. *Front Med* 2013; **7**: 111–121.
9 Liang ZL, Mao QY, Gao Q, Li X, Dong C, Yu X *et al.* Establishing China's national standards of antigen content and neutralizing antibody responses for evaluation of enterovirus 71 (EV71) vaccines. *Vaccine* 2011; **29**: 9668–9674.
10 Li YP, Liang ZL, Gao Q, Huang LR, Mao QY, Wen SQ *et al.* Safety and immunogenicity of a novel human Enterovirus 71 (EV71) vaccine: a randomized, placebo-controlled, double-blind, Phase I clinical trial. *Vaccine* 2012; **30**: 3295–3303.
11 Li YP, Liang ZL, Xia JL, Wu JY, Wang L, Song LF *et al.* Immunogenicity, safety and immune persistence of a novel inactivated human enterovirus 71 vaccine: a phase II, randomized, double-blind, placebo-controlled Trial. *J Infect Dis* 2014; **209**: 46–55.
12 Meng FY, Li JX, Li XL, Chu K, Zhang YT, Ji H *et al.* Tolerability and immunogenicity of an inactivated enterovirus 71 vaccine in Chinese healthy adults and children: an open label, phase 1 clinical trial. *Hum Vaccin Immunother* 2012; **8**: 668–674.
13 Zhu FC, Liang ZL, Li XL, GE HM, Meng FY, Mao QY *et al.* Immunogenicity and safety of an enterovirus 71 vaccine in healthy Chinese children and infants: a randomised, double-blind, placebo-controlled phase 2 clinical trial. *Lancet* 2013; **381**: 1037–1045.
14 Liu L, Zhang Y, Wang J, Zhao H, Jiang L, Che Y *et al.* Study of the integrated immune response induced by an inactivated EV71 vaccine. *PLoS ONE* 2013; **8**: e54451.
15 Hu YM, Wang X, Wang JZ, Wang L, Zhang YJ, Chang L *et al.* Immunogenicity, safety and lot consistency of a novel inactivated enterovirus 71 vaccine in Chinese children aged 6 to 59 months. *Clin Vaccine Immunol* 2013; **20**: 1805–1811.
16 Xu J, Qian Y, Wang S, Serrano JM, Li W, Huang Z *et al.* EV71: an emerging infectious disease vaccine target in the Far East? *Vaccine* 2010; **28**: 3516–3521.
17 Cai Y, Ku Z, Liu Q, Leng Q, Huang Z. A combination vaccine comprising of inactivated enterovirus 71 and coxsackievirus A16 elicits balanced protective immunity against both viruses. *Vaccine* 2014; **32**: 2406–2412.
18 Mao Q, Cheng T, Zhu F, Li J, Wang Y, Li Y *et al.* The cross-neutralizing activity of enterovirus 71 subgenotype c4 vaccines in healthy Chinese infants and children. *PLoS ONE* 2013; **8**: e79599.
19 Chou AH, Liu CC, Chang JY, Jiang R, Hsieh YC, Tsao A *et al.* Formalin-inactivated EV71 vaccine candidate induced cross-neutralizing antibody against subgenotypes B1, B4, B5 and C4A in adult volunteers. *PLoS ONE* 2013; **8**: e79783.
20 Lee BY, Wateska AR, Bailey RR, Tai JH, Bacon KM, Smith KJ *et al.* Forecasting the economic value of an Enterovirus 71 (EV71) vaccine. *Vaccine* 2010; **28**: 7731–7736.
21 Chen CW, Lee YP, Wang YF, Yu CK. Formaldehyde-inactivated human enterovirus 71 vaccine is compatible for co-immunization with a commercial pentavalent vaccine. *Vaccine* 2011; **29**: 2772–2776.
22 McMinn PC. Enterovirus vaccines for an emerging cause of brain-stem encephalitis. *New Engl J Med* 2014; **370**: 792–794.