Status of research and development of vaccines for enterovirus 71

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A B S T R A C T

Although outbreaks of Hand, Foot, and Mouth Disease (HFMD) in young children have long been recognized worldwide, the occurrence of rare life-threatening neurological, respiratory, and cardiac complications has propelled this common condition into the spotlight as a major public health problem in the affected countries. Various enteroviruses cause HFMD, but the severe complications have been mostly associated with enterovirus 71 (EV71). Medical treatment is supportive and measures to interrupt transmission have been challenging to implement. Preventive vaccines could have an important clinical impact, especially among children younger than 3 years old who are most susceptible to the neurological complications. Several groups in the highly affected Asia-Pacific region are working towards vaccines against EV71 and some candidates have progressed to late-stage clinical trials with two vaccines recently reported to have been approved by the regulatory authorities in China. This report summarizes current issues and progress in the development of vaccines against EV71.

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Enterovirus 71 (EV71) – a single-stranded RNA virus of the Picornavirus family – is one of the most common causes of Hand, Foot and Mouth Disease (HFMD) outbreaks. It may be accompanied by rare but life-threatening neurological and cardiopulmonary complications. Other enteroviruses cause HFMD as well but in a more limited distribution. The largest outbreaks typically have been traced to either Coxackievirus A16 (CA16) or enterovirus 71 (EV71) \cite{1,2}. Although CA16 is more prevalent, EV71 is more neurotropic – associated with severe neurological manifestations and death. EV71 was first isolated in 1969 in the United States from a child who died of encephalitis \cite{3}. Since that time, outbreaks have erupted sporadically across the world. From the late 1990s onwards, however, the largest EV71 epidemics have occurred almost exclusively in the Asia-Pacific region, including Taiwan, mainland China, Hong Kong, Malaysia, Vietnam, Singapore, and Thailand \cite{2,4,5}. A recent study from the Chinese National Enhanced Surveillance System, characterizing the epidemiology of HFMD, found that, from 2008 to 2012, more than 7.2 million cases of HFMD were reported. Between 2010 and 2012, there were 1.2 cases per 1000 person-years and 500–900 deaths each year \cite{6}. Children less than 5 years old had the highest risk of disease, and age-specific incidence and mortality rate was highest in the 12 to 23 month age-group (38.2 cases per 1000 and 1.5 deaths per 100,000 in 2012). Similar to other affected countries, incidence was lowest among infants younger than 6 months, older children (age 5–14 years) and adults (age ≥ 15 years). These data are generally consistent with other studies that find about 50–80% of children are seropositive for EV71 by the time they reach 5 years of age \cite{7,8,9}.

EV71 is highly contagious and transmitted through close contact with bodily fluids such as nasopharyngeal secretions and stool. Children from age 6 months to 5 years are at highest risk of infection. The disease usually presents with fever, oral lesions (that can sometimes be severe), and a papulovesicular or maculopapular exanthem on the palms of the hands, soles of the feet, buttocks, knees, and elbows. The illness is usually self-limiting but can be associated with more serious complications. Brainstem encephalitis is considered a hallmark of severe HFMD, although a spectrum of neurological manifestations ranging from aseptic meningitis to acute flaccid paralysis have been observed. Further progression to respiratory distress, pulmonary edema, and cardiorespiratory failure can often be fatal. Early recognition of cases at high risk of developing serious systemic disease is one of the clinical challenges of this condition. Some warning signs and risk predictors of progressing to severe disease have been described in studies conducted during outbreaks, including severity and duration of fever, vomiting, lethargy, agitation or irritability \cite{10}. More specific neurological signs become more prominent with increasing severity and have been used in some studies to guide the management of cases according to the stage of disease severity \cite{11}. This is important as close monitoring of those with signs of neurological
involvement is critical to patient treatment, clinical management and prognosis.

Even if the patient recovers, there remains a high likelihood of chronic neurological sequelae and, in the case of children, severe developmental deficiencies. Although smaller-scale outbreaks of HFMD are well-recognized in pediatric populations, it is the larger outbreaks with serious neurological, respiratory and cardiac manifestations and occasional fatalities that have galvanized serious concern within both lay and public health communities. Such outbreaks are often associated with a high rate of hospitalization, placing pressure on already stressed and under-resourced local healthcare systems in affected countries. A recent cost-burden study in China estimates that the annual economic burden of mild EV71-associated HFMD is about $161–323 million. This estimate does not account for the costs associated with severe and fatal illness, long-term sequelae and cases managed outside of the healthcare system [12].

As enteroviruses are RNA viruses, they are prone to a high rate of mutation and recombination. EV71 can be classified into three genotypes – A, B, and C – and 11 genetic subtypes. Genetic analyses often show genotypes changing over time through genetic divergence and recombination. The recent outbreaks in mainland China, Taiwan and Vietnam have been dominated by subtypes C4 and C5 in contrast to B4 and B5 in Malaysia and Singapore. Surveillance efforts in Europe have found genotype B and subtypes C1, C2, and C4 to be most common in circulation. No subtype-specific sequences related to increased virulence have yet been identified. From a vaccinology perspective, however, animal studies indicate that there is broad cross-neutralization across EV71 subtypes. Currently, no clinical feature or rapid diagnostic test can distinguish between EV71 and the other less virulent enteroviruses, or reliably predict which child will progress to severe HFMD. Confirmatory diagnosis, based on tissue culture and virus isolation, does not adequately meet the needs of urgent clinical decision-making. Antibody capture and PCR assays are more available now and allow for more rapid diagnosis, but are not yet widely used due to cost constraints and concerns about sufficiently high test performance standards (i.e., sensitivity and specificity). Serology can also be used for EV71 diagnosis but, given the need for acute and convalescent sera, this diagnostic method may not be helpful in clinical or public health management of the disease. Treatment is mostly supportive as there are no targeted therapies available. Although recent outbreaks of severe HFMD have been largely limited to the Asia-Pacific region, HFMD caused by EV71 or CVA16 have been shown to be globally distributed [13], with seroprevalence rates rising sharply in children 5 years and above [14]. Overall these data suggest that preventive interventions targeted towards infants and children between 6 months and 5 years old will have the greatest global impact on HFMD disease burden and complications. A vaccine would particularly benefit children younger than 3 years who are the most susceptible to neurological complications [15].

1. Biological feasibility for vaccine development

Seroprevalence studies suggest that maternally-derived antibodies, like most other infectious diseases, play an important role in neonatal and early immunity, protecting infants from EV71 disease during the first months of life but wane after 6 months of age to a nadir at 7–12 months [16]. The epidemiologic correlation between maternal antibody titers and risk of HFMD suggests definable correlates of protection that can be induced by vaccines. Additionally, the availability and success of vaccination campaigns against another enterovirus, poliovirus, provides strong evidence for the feasibility of developing a vaccine against EV71. In fact, following a large-scale epidemic in Bulgaria in 1975, an inactivated whole virus EV71 vaccine candidate was developed and produced in the former Soviet Union, using a similar manufacturing process as that for the inactivated poliovirus vaccine. The vaccine was tested in 1–4 year old children and found to be safe and immunogenic [17] but was not evaluated for clinical efficacy as no subsequent outbreaks occurred in Bulgaria. Following the emergence of EV71-associated HFMD as a serious public health problem in the Asia-Pacific region, several groups in mainland China, Taiwan, and Singapore, are pursuing the development of an EV71 vaccine. Three vaccine manufacturers from mainland China have completed Phase 3 efficacy trials and applied for licensure approval from the China Food and Drug Administration in the end of 2014. All three have developed vaccines based on the inactivated whole virus approach, similar to the polio vaccine. Large, multi-center trials, Phase 3 trials (each enrolling more than 10,000 children), in either 6–35 month or 6–71 month age groups have shown similar safety and efficacy profiles, achieving more than 90% protection from EV71-associated HFMD.

Despite the demonstration of efficacy in these trials, an EV71 vaccine still runs into the challenge of preventing and controlling not just EV71-associated HFMD but all-cause clinical HFMD. As expected from previous preclinical studies, the vaccines that have been developed, thus far do not demonstrate any evidence of cross-protection across enterovirus species. As it happens, EV71 did not turn out to be the dominant cause of the HFMD outbreaks and that in two of the trials, and only a small proportion of the total HFMD cases were caused by EV71 when confirmed by laboratory diagnosis. Of the 1704 cases of HFMD diagnosed clinically over 12 months of follow-up in one of the Phase 3 trials, only 36 (2.1%) were confirmed as being associated with EV71 [18]. The remainder of etiologies included 33.9% (577 episodes) positive for CA16, 34.5% (588 episodes) positive for other enteroviruses and 29.5% (503 episodes) not associated with any enterovirus. Thus, for every episode of EV71, there were 11 episodes of CA16. In contrast, in another trial, of the 594 cases of clinical HFMD, 155 (26%) were lab-confirmed EV71. Although the vaccine was not cross-reactive against other subtypes, vaccine efficacy against clinical HFMD was 49% [39–56] [19]. All three vaccines were immunogenic, achieving geometric mean antibody titers of 165.8 (Sinovac; 400 U/2-dose), 170.6 (Chinese Academy Medical Sciences; 100 U/2-dose), and 325.3 (Beijing Vigoo; 320 U/2-dose) and inducing seroconversion in >90% of subjects [18–20]. Although neutralizing antibody titers waned through 8 months of follow-up post-boost, they remained stable between 8 and 14 months, were still higher than at baseline and were significantly higher than titers in the placebo group.

Formulating vaccination policy will be difficult, given the challenges in justifying clinical benefit in an HFMD epidemic where EV71 is not the dominant strain. Careful thought and preparation will be required to help parents and the public understand that while such vaccines might prevent severe or fatal HFMD, they may offer no protection against HFMD caused by other enteroviruses. The results also underline the importance of CA16 in the HFMD disease burden: thus an effective HFMD vaccine might have to at least be bivalent. Additional constraints on EV71 vaccine development include the lack of an appropriate animal model, the need for more knowledge on virus transmission, epidemiology and disease burden, and an assessment of health care burden and costs for the prevention, treatment and control of an HFMD epidemic.

2. Technical and regulatory assessment

EV71 vaccines have been developed and tested outside the traditional drug and vaccine pipeline, which normally involves a mature regulatory infrastructure that has experience not only in the development and enforcement of quality control standards, but also in the conduct and review of clinical trial data and the drafting of
policy on the introduction and use of novel vaccines. Mainland China, Taiwan and Singapore have more limited experience in these areas, although there are ongoing efforts, particularly in mainland China by the Chinese Drug Administration, to develop reference standards in a transparent manner and harmonize local, regional and international quality control requirements for EV71 vaccines. Recently, the WHO ECBS approved a joint initiative between the National Institutes for Food and Drug Control and National Institute for Biological Standards and Control to develop quantitative standards for detecting EV71 neutralizing antibodies and antigens with the purpose of developing candidate vaccines. Similar to polio vaccines, neutralizing antibody titers may serve as a good correlate of protection for EV71 vaccines. Two of the Phase 3 trials discussed previously provided evidence that neutralizing antibodies correlated with protection at levels titers of 1:16 to 1:32. Furthermore, experience from polio vaccines also show that there is variability in the induction of neutralizing antibody responses that result from the several antigenic formats following the production of inactivated whole virus. Therefore the development of reference reagents and establishment of harmonized assays that measure the consistency and potency of the vaccines are critical for the assessment of these vaccines not only in China but also by regulatory authorities in other regions.

Although detailed cost-effectiveness analyses are not widely available, one study has suggested that routine EV71 vaccination in China, with a background incidence of 0.04%, may be cost-effective if vaccine efficacy is >70%, and the vaccine costs $25 or less, or if vaccine efficacy is >50% and the vaccine costs $10 or less [21]. Higher infection incidence and vaccine efficacy were observed in the cited Phase 3 efficacy trials. It is estimated that, based on rates and outcomes observed in these trials, immunization would be cost-effective. Policies on the use of these vaccines will also need to consider if and how the EV71 vaccine would be introduced into a government’s national immunization program as well as catch-up campaigns. In addition, given the epidemic nature of the condition, considerations will also have to be made for the use of these vaccines in outbreak settings and how their effectiveness would be subsequently evaluated.

The use of an EV71 vaccine may impact natural transmission of wild EV71 virus and change its epidemiological characteristics [22]. Surveillance and monitoring will be required to determine disease rates in the aftermath of vaccine deployment. Evaluation of EV71 epidemiology and pathogenicity will also have to take place in the context of HFMD as a whole in addition to HFMD specifically caused by EV71. Therefore, the laboratory-based surveillance system will have to be strengthened to adequately monitor prevalent strains and strain variation. Some groups are already moving toward the next step of vaccine development for HFMD, multivalent and combination vaccines. The latter may be a viable approach to broadening coverage with the inclusion of multiple enterovirus species that cause HFMD. This strategy is being pursued by several groups, some of whom are developing bivalent HFMD vaccines with either the inactivated whole viruses or virus like particles (VLPs). However this will require additional characterization of the disease burden, pathogenic spectrum and virological and molecular features of additional enteroviral targets. Although the legacy of the polio vaccines may provide lessons for combination vaccine approaches, there will be additional technical and regulatory requirements that will need to be taken into account.

### 3. Status of vaccine R&D activities

Various approaches have been pursued in the research development pipeline for EV71 vaccines, including live attenuated viruses, inactivated whole viruses, VLPs, and recombinant protein subunits. As described earlier, the most advanced candidates are inactivated whole virus vaccines that have leveraged previous knowledge and experience from the development and manufacturing of inactivated polio vaccines. Most of the other approaches are at the stage of early or late preclinical development. Five candidate vaccines have reached clinical evaluation, all inactivated whole viruses. The three groups in mainland China that have developed inactivated whole virus vaccines benefited from the priority placed by the Chinese government on developing an EV71–HFMD vaccine in the aftermath of the 2008 outbreaks. This has led to a vaccine development agenda that is highly coordinated among national vaccine manufacturing agencies in the most affected regions. With a fast-track provided by regulators, all three Chinese companies have been able advance rapidly from first-in-human studies to Phase 3 trials in less than 18 months and out of these, two have received regulatory approval to manufacture and market their vaccine in China. The vaccine manufacturers are also working with the regulatory authorities to develop standards that will allow for the comparison of the potency and immunogenicity of the respective vaccine candidates. Outside of mainland China, similar vaccines (e.g., inactivated EV71 virus in an alum adjuvant) are being developed in Taiwan and Singapore. The National Health Research Institute in Taiwan is producing an inactivated whole virus vaccine based on the B4 genotype vaccine strain. The vaccine entered Phase 1 clinical trials in 2010 and showed the induction of 4-fold or greater rise in neutralization antibody titers in healthy adults after the first vaccination. Inviragen of Singapore completed Phase 1 testing of a vaccine based on the B3 subtype that also demonstrated immunogenicity. No further progress has been reported on these candidates following completion of their Phase 1 trials (Table 1).

An alternate to the inactivated virus approach is the development of VLP vaccines, which offer the advantage of presenting all surface epitopes of the EV71 capsid proteins (VP0, VP1, and VP3) in their native conformations at once. Experimental animal model immunization and challenge studies have indicated that VLPs can generate protective neutralizing antibodies. Importantly, studies have also demonstrated that neutralizing antibodies induced by vaccination were cross-reactive against multiple subtypes that were not in the vaccine [23]. Most of these efforts are currently in

### Table 1

Development status of current vaccine candidates (POC = Proof of concept trial).

<table>
<thead>
<tr>
<th>Candidate Name/Identifier</th>
<th>Phase I</th>
<th>Phase II</th>
<th>POC</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>Vero cell–based EV71 inactivated EV71 C4 genotype vaccine with aluminum hydroxide/Sinovac Biotech</td>
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the preclinical stages. Live attenuated recombinant subunits, peptides and DNA approaches have been described in the literature but to our knowledge these approaches are not being pursued by vaccine developers.

4. Summary

The three formalin-inactivated EV71 vaccines tested in Phase 3 trials in China have reported a high-degree of efficacy. Two vaccines have received regulatory approval and EV71-associated HFMD could become a vaccine-controllable disease in China. As EV71 vaccine development in LMICs is aimed at protecting infants and children less than 5 years of age, the vaccines could be considered within the scope of GAVI’s mission. However, the lack of cross-species activity complicates vaccine introduction campaigns and could limit their acceptance for widespread implementation. Improved understanding of the epidemiology and transmission dynamics of EV71 specifically and other HFMD-causing enteroviruses generally is needed to power efficacy trials as well as to assess vaccine impact. The development of HFMD vaccines that cover the multiple viral etiologies through multivalent approaches is underway and could expand the clinical and public health impact of the next-generation vaccines for HFMD.

Conflict of interest

ZR has no conflict of interest to declare. MJ C is the chief scientific officer of and has shares in Sentinex Therapeutics based in Penang, Malaysia. Sentinex Therapeutics is developing a HFMD vaccine with an EV71 VLP at its core.

References