Designing a mRNA vaccine against HFMD, key considerations

ASSOCIATE PROFESSOR JUSTIN JANG HANN CHU

Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore

Biosafety Level 3 Core Facility, Yong Loo Lin School of Medicine, National University of Singapore

Institute of Molecular and Cell Biology (IMCB), A*STAR
Research Focuses of A/Prof Justin Chu

- Molecular Virology
- Host-virus interactions
- Antimicrobial discoveries & strategies (antimicrobial, antivirals and vaccine developments)
- Human enteroviruses, HFMD
- Coronaviruses (SARS CoV-2)
- Mosquito-Borne Viruses
  - Dengue virus (DENV)
  - Chikungunya virus (CHIV)
  - Zika virus (ZIKV)
  - Mayaro virus (MYV)
HFMD

- Acute infectious disease
- Transmissible via bodily fluids
- Can affect anyone, especially
  - Young children
  - Elderly
  - Immuno-compromised individuals
- 3-5 days incubation period
- Usually mild and self-limiting
- Occasional manifestations into severe and fatal conditions
Etiological agents of HFMD

- Picornaviruses
- Coxsackieviruses (CV-A16, CV-A6, CV-A10)
- Enteroviruses (EV-A71)
- Echoviruses (E-7)

Table 1: EVs associated with HFMD

<table>
<thead>
<tr>
<th>Species</th>
<th>Associated Enterovirus serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV-A</td>
<td>CVA2, CVA4, CVA5, CVA6, CVA7, CVA8, CVA10, CVA12, CVA13, CVA16, EV-A69, EV-A71</td>
</tr>
<tr>
<td>EV-B</td>
<td>CVA9, CVB1, CVB2, CVB3, CVB4, EV-B5, E-3, E-4, E-5, E-6, E-7, E-9, E-11, E-14, E15, E16, E-18, E-19, E-21, E-30, EV-B84</td>
</tr>
<tr>
<td>EV-C</td>
<td>CVA1, CVA19, CVA21, CVA22, CVA24, EV-C99</td>
</tr>
</tbody>
</table>

Zhu et al, Current status of hand-foot-and-mouth disease (2023)
Clinical manifestations of EV-A71

- Hand, foot and mouth disease
- Severe disease
  - Aseptic meningitis
  - Brainstem encephalitis
- Death from respiratory failure
- Long-term neurological sequelae
Virology of Human Enterovirus

Adapted from Yi et al., (2016).
Epidemiology of HFMD

A: EV-A71; B: CVA16; C: CVA6; D: CVA10.

Zhu et al, Current status of hand-foot-and-mouth disease (2023)

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>1,952,435</td>
<td>56</td>
</tr>
<tr>
<td>Japan</td>
<td>358,764</td>
<td>0</td>
</tr>
<tr>
<td>Korea</td>
<td>289,700</td>
<td>0</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>358</td>
<td>0</td>
</tr>
<tr>
<td>Macau</td>
<td>3,402</td>
<td>0</td>
</tr>
<tr>
<td>Singapore</td>
<td>33,663</td>
<td>0</td>
</tr>
<tr>
<td>Vietnam</td>
<td>48,009</td>
<td>1</td>
</tr>
</tbody>
</table>

Hand, Foot and Mouth Disease Situation Update 2017. WHO. https://apps.who.int/iris/handle/10665/274106
# Molecular Epidemiology of EV-A71

(Yi et al., 2017)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>C2</td>
<td>B3</td>
<td>B4</td>
<td>C4</td>
<td>B3</td>
<td>C2</td>
</tr>
<tr>
<td>China</td>
<td>C2</td>
<td>C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hong Kong</td>
<td>C4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
<td>C4</td>
<td>B5</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>C3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
<td></td>
<td></td>
<td>B5</td>
</tr>
<tr>
<td>Singapore</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
<td></td>
<td></td>
<td>B5</td>
</tr>
<tr>
<td>Philippines</td>
<td>C2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taiwan</td>
<td>B3</td>
<td>C4</td>
<td>B4</td>
<td>C2</td>
<td>B5</td>
<td>B5</td>
</tr>
<tr>
<td>Thailand</td>
<td>C1</td>
<td></td>
<td>C4</td>
<td></td>
<td>C4</td>
<td>C1</td>
</tr>
<tr>
<td>Vietnam</td>
<td>C1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>C1</td>
<td>C4</td>
<td></td>
<td>C1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HFMD in Singapore

- Occurrence in Singapore
- Yearly outbreaks
  - Major outbreaks every 2-3 years
- Number of reported cases
  - 2015: 28216
  - 2016: 42154
  - 2017: 33710
  - 2018: 40217
  - 2019: 5013
  - 2020: 1133
  - 2021: 1043
  - 2022: 4098

Photos courtesy of KK Women's and Children's hospital
HFMD in Singapore

HFMD symptoms usually start with mild fever
(The Straits’ Times; 30 Aug 2016)

Sending a sick kid to school
(The Straits’ Times; 3 Jul 2016)

Weekly cases of hand, foot and mouth disease hit four-year high
(The Straits’ Times; 20 May 2016)

Child caught HFMD? It could cost family $1200
(The Straits’ Times; 6 May 2014)
Public Education
Screening For Hand, Foot and Mouth Disease

A Guide for Pre-schools

Health Promotion Board (2008)
HFMD Warrior program with childcare centres in Singapore

Help Us in the Fight Against HFMD

So What is HFMD ???
Hand Foot Mouth Disease, HFMD is a nasty viral disease affecting thousands of children yearly in Singapore.

Who are we and what are we doing?
We are a group of researchers from National University of Singapore and we are one of the frontiers in the fight against HFMD.

And we need your helping hand in the combat!!

• Educate
• Sharing
• Collaborative Research

IRB approval NUS1334; NUS2628 and CIRB 2012/448/E
HFMD Warrior Program with childcare centres in Singapore

Epidemiological surveillance of HFMD in community: 2013–Current
Epidemiological surveillance of HFMD in paediatric patients and in community: 2013–2018


# Current Status of HMFD Vaccine

<table>
<thead>
<tr>
<th>Organizations</th>
<th>Sinovac Biotech Co., Ltd</th>
<th>Beijing Vigoo Biological Co., Ltd</th>
<th>Chinese Academy of Medical Sciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV-A71 Strain</td>
<td>H07 (C4)</td>
<td>FY (C4)</td>
<td>M01 (C4)</td>
</tr>
<tr>
<td>Inactivation Technique</td>
<td>Formalin</td>
<td>Formalin</td>
<td>Formalin</td>
</tr>
<tr>
<td>Cell Substrate</td>
<td>Vero cells</td>
<td>Vero cells</td>
<td>Human diploid KMB-17 cells</td>
</tr>
<tr>
<td>Dosage</td>
<td>400 U, two-dose</td>
<td>320 U, two-dose</td>
<td>100 U, two-dose</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>Aluminium hydroxide</td>
<td>Aluminium hydroxide</td>
<td>Aluminium hydroxide</td>
</tr>
<tr>
<td>Population Target</td>
<td>Children (6-35 month)</td>
<td>Children (6-35 month)</td>
<td>Children (6-71 month)</td>
</tr>
<tr>
<td>Enrollment</td>
<td>10,077</td>
<td>10,245</td>
<td>12,000</td>
</tr>
<tr>
<td>Efficacy</td>
<td>94.8%</td>
<td>90%</td>
<td>97.4%</td>
</tr>
<tr>
<td>Effective against</td>
<td>EV-A71 (B1-B4, C1-C5)</td>
<td>EV-A71 (B1-B4, C1-C5)</td>
<td>EV-A71 (B1-B4, C1-C5)</td>
</tr>
<tr>
<td>Approval Date</td>
<td>December 2015</td>
<td>December 2016</td>
<td>December 2015</td>
</tr>
<tr>
<td>References</td>
<td>NCT01507857</td>
<td>NCT01508247</td>
<td>NCT01569581</td>
</tr>
</tbody>
</table>

- Currently, only **monovalent vaccines** are available.
- These vaccines are only available in China.
  - Vaccine developed in China are based on the **C4 sub-genotype** of EV-71.

---

He et al, *From Monovalent to Multivalent Vaccines, the Exploration for Potential Preventive Strategies Against Hand, Foot, and Mouth Disease (HFMD)* (2020)

## Current Status of HMFD Vaccine

<table>
<thead>
<tr>
<th>Organizations</th>
<th>National Health Research Institutes (Taiwan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV-A71 Strain</td>
<td>E59 (B4)</td>
</tr>
<tr>
<td>Inactivation Technique</td>
<td>Formalin</td>
</tr>
<tr>
<td>Cell Substrate</td>
<td>Vero Cells</td>
</tr>
<tr>
<td>Dosage</td>
<td>0.5ml (2.5ug virus), Two-doses</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>Aluminium Phosphate</td>
</tr>
<tr>
<td>Population Target</td>
<td>young children aged 2 months to 5 years</td>
</tr>
<tr>
<td>Enrollment</td>
<td>3061</td>
</tr>
<tr>
<td>Effective Against</td>
<td>EV-A71 (B5, C4a, C4b, and C5)</td>
</tr>
<tr>
<td>Efficacy</td>
<td>96.8%</td>
</tr>
<tr>
<td>References</td>
<td>NCT03865238</td>
</tr>
</tbody>
</table>

- In Taiwan, a vaccine was developed using inactivated vaccine based on the EV-A71 B4 serotype.
- Demonstrated cross-neutralizing antibodies against various EV-A71 subtypes, including B5, C4a, C4b, and C5.
- MVC collaborated with the Pasteur Institute in Vietnam to conduct a multinational and multicenter Phase 3 clinical trial.
## Vaccine Approaches

<table>
<thead>
<tr>
<th>Vaccine Approach</th>
<th>Reference</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated-Bivalent Vaccine EV71:CVA16</td>
<td>Fan et al 2020</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Inactivated-Bivalent Vaccine CVA6:CVA10</td>
<td>Zhang et al 2018</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Inactivated-Trivalent Vaccine EV71:CVA16:CVA6</td>
<td>Caine et al 2015</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Inactivated-Trivalent Vaccine CVA6:CVA10:CVA16</td>
<td>Lim et al 2018</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Virus like particle Vaccine EV71-VLP:CVA6-VLP:CVA10-VLP</td>
<td>Zhang et al 2018</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>
Strategies and Challenges for mRNA HMFD vaccine design

- Monovalent HFMD vaccines targeting the currently circulating strains within their own epidemiological regions. However, this approach can lead to an epidemiological shift in HFMD viruses, potentially making other HFMD viruses dominant in circulation.

- This shift may result from the selective pressure imposed by vaccination on specific strains, leading to changes in the viral population dynamics.

- It underscores the importance of considering broader and more comprehensive vaccine strategies, such as multivalent vaccines, to address the evolving nature of HFMD viruses.

Confers protection against the current circulating genotype and serotypes
Strategies and Challenges for mRNA HMFD vaccine design

- The human enterovirus genome evolves at a rate of 1% to 2% mutation per year.
- This is particularly important due to the potential for inter-typic and intra-typic recombination and the emergence of new strains with increased virulence.
- To address this challenge, there is a need to include representative strains for each Enterovirus serotype.
- To determine the effectiveness of such multivalent vaccines, multinational efficacy trials will be essential. These trials will help assess whether the vaccines can provide broad protection against the various divergent epidemic viruses that may arise.

Unpredictability and the emergence of potentially new variant slows the development of multivalent vaccine

Zhu et al, Current status of hand-foot-and-mouth disease (2023)
Zhang et al, Hand-Foot-and-Mouth Disease-Associated Enterovirus and the Development of Multivalent HFMD Vaccines (2022)
Liu et al (2016) reported that the tetravalent EVA71/CVA16/CVA10/CVA6 vaccine exhibited obvious differences in inducing and production of neutralizing antibodies against all 4 viruses in a mouse model.

Neutralizing antibody titers were (TCID\textsubscript{50}) 1/708 for EV-A71, 1/22 for CVA16, 1/16 for CVA10, and 1/100 for CVA6

Exact mechanisms underlying this result are still not precisely known. (Immune biases or immune interference?)

Potential strategies could include adjusting the vaccine dose or incorporating adjuvants to enhance the immunogenicity of weaker antigens, thereby ensuring a more balanced and effective immune response.
Strategies and Challenges for mRNA HMFD vaccine design

Prior immune exposure can enhance pathology in the enteroviruses infection?

- Elmastour et al (2016) link the increased pathology of secondary coxsackievirus infections to enhancement of infection by antibody to the coxsackievirus.
- Antibody Dependent Enhancement (ADE)?

Explore to include a suitable animal model to evaluate the safety and efficacy of HFMD multivalent vaccine

- The immunogenicity of many vaccines varies between non-human primates and mice.

Strategies and Challenges for mRNA HMFD vaccine design

- The widespread use of EV-A71 vaccines can influence the natural transmission of the wild EV-A71 virus and potentially alter its epidemiological characteristics.
- In the short term, there will likely be a significant decrease in HFMD infections. Yet, in the long term, there could be a shift in the epidemiology of HFMD towards CVA16 or other HFMD viruses that may cause more severe disease.
- Gathering and integrating research on highly effective vaccines will lay the groundwork for enhancing vaccine design and development.

Collection and Integrated Research of Different Epidemiological Characteristics, Spatial Clusters and Periodical Incidence Information of HMFD for vaccine design improvement

Zhu et al, Current status of hand-foot-and-mouth disease (2023)
Peng et al, Epidemiological and aetiological characteristics of hand, foot, and mouth disease in Sichuan Province, China, 2011–2017 (2020)
Vaccine Clinical Trial Involving Young Children

Well-informed Consent

- Parental consent is needed by ensuring they are well informed about the type of vaccine that would be testing on their children.
- Transparent communication & safeguarding the welfare of participating children.

Establishment and Awareness of Potential Side Effects

- For clinical trial to be conducted in children, side effects have to be established in clinical phase I trial before moving on to children due to unknown effect of the vaccine.
- This is also due to immature development of immune system in which side effect would often magnify.
- Combination of adult and children could be considered in phase I trial to allow researcher to know about the side effect and dosage between adult vs children.
Absence of broad cross-neutralizing activity of current vaccine makes it challenging to develop a multivalent vaccine. The unpredictability and emergence of new viral variants further slow the development process. A suitable animal model is needed to evaluate the safety and efficacy of the HFMD multivalent vaccine.

To improve vaccine design, considerations include:

1. Establishing a Clinical Trial framework.
2. Exploring the exact mechanisms to reduce adverse outcomes.
3. Developing multivalent vaccines through the combination of effective monovalent vaccines or by constructing chimeric vaccines with different virus serotypes.
4. Collecting and integrating research on different epidemiological characteristics, spatial clusters, and periodic incidence of HFMD for vaccine design improvement.
5. Only conferring protection against the current circulating genotype.

In summary, the development of a better HFMD vaccine requires a comprehensive understanding of current challenges and innovative strategies to address them.
Collaborators in Asia:
Dr Frederic Bard, IMCB A*STAR, Singapore
A/P Vincent Chow, NUS, Singapore
A/P Sylvie Alonso, NUS, Singapore
A/P Chong Chia Yin and Dr Natalie Tan, KKH, Singapore
Prof Leo Yee Sin, NCID, Singapore
A/P Chan Yoke Fun, University Malaya, Malaysia
Prof Ruby Jih-Jin Tsai, Kaohsiung Medical University Hospital, Taiwan
Prof Wang Jen-Ren, National Cheng Kung University, Taiwan
Prof Robert Wang, Chang Geng University, Taiwan
Prof Qin Cheng-Feng, Beijing Institute of Microbiology and Epidemiology, China
Prof Kinh Nguyen Van & Associate Professor Nguyen Vu Trung from Hanoi Medical University, Vietnam
Thank you

- Justin Chu’s email: miccjh@nus.edu.sg