Designing a mRNA vaccine against HFMD, key considerations

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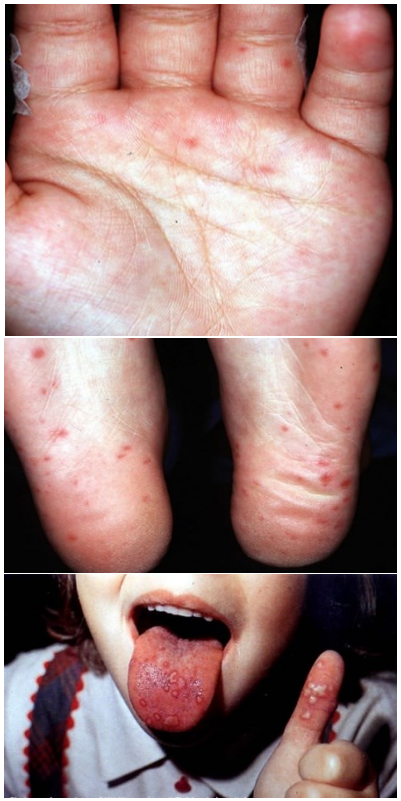
Research Focuses of A/Prof Justin Chu

- ✓ 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

Watson, L. (2015)



Health Promotion Board (2015)

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Etiological agents of HFMD

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

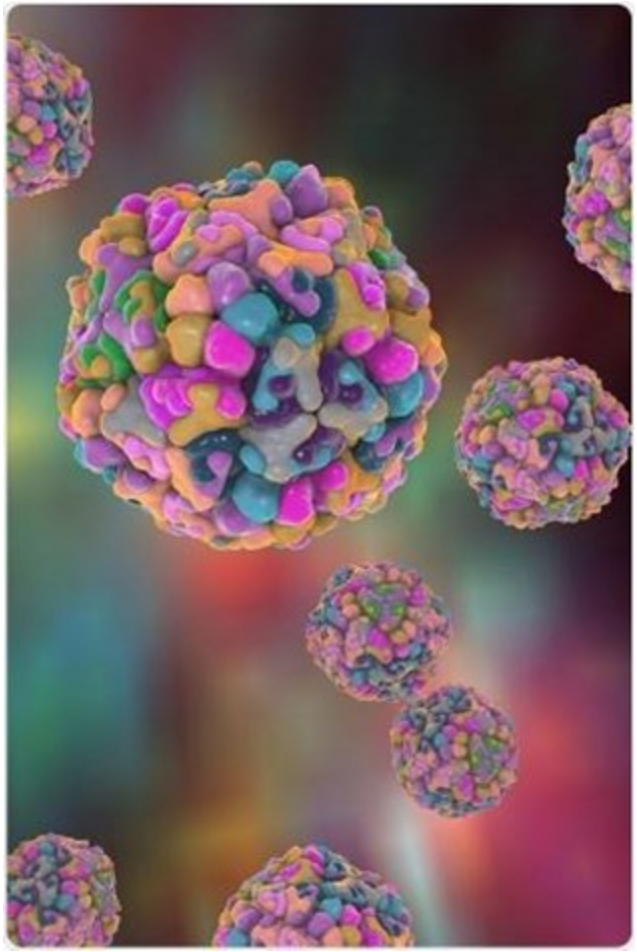


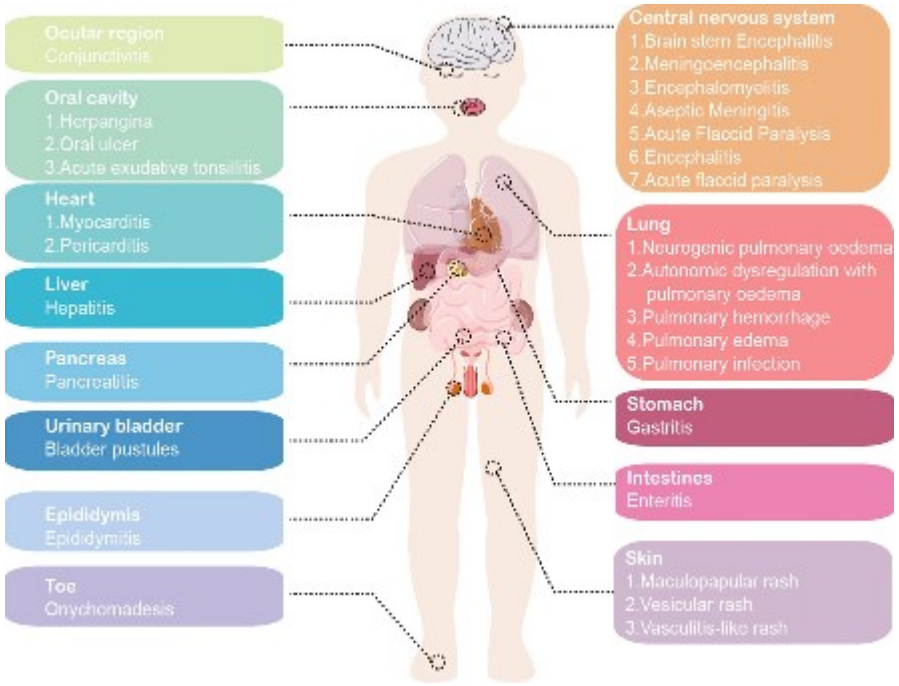
Table 1 EVs associated with HFMD

Species	Associated Enterovirus serotypes
EV-A	CVA2, CVA4, CVA5, CVA6, CVA7, CVA8, CVA10, CVA12,CVA13, CVA16
	EV-A69, EV-A71
EV-B	CVA9, CVB1, CVB2, CVB3, CVB4, CV-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
EV-C	CVA1, CVA19, CVA21, CVA22, CVA24, EV-C99

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

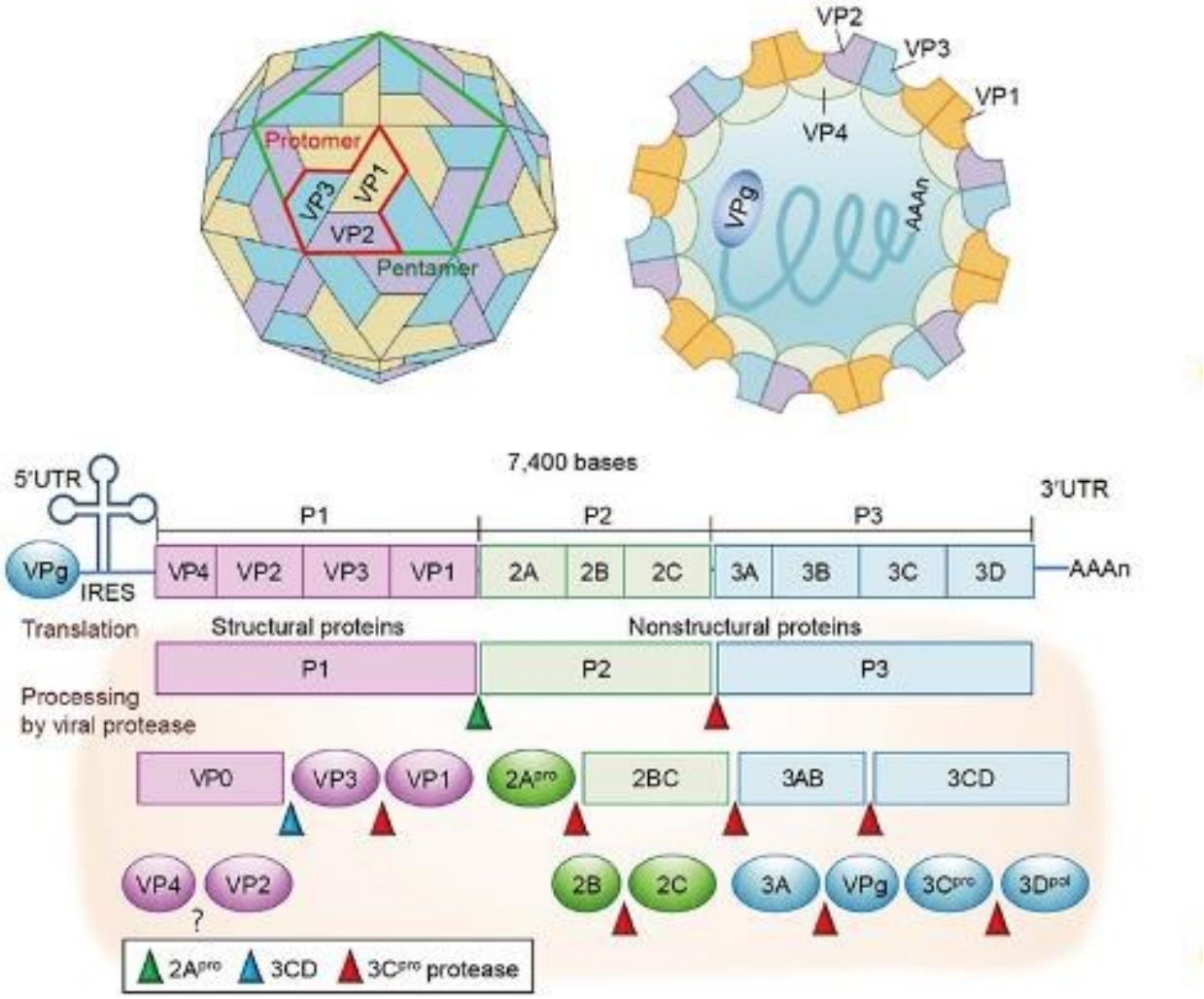
Clinical manifestations of EV-A71

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



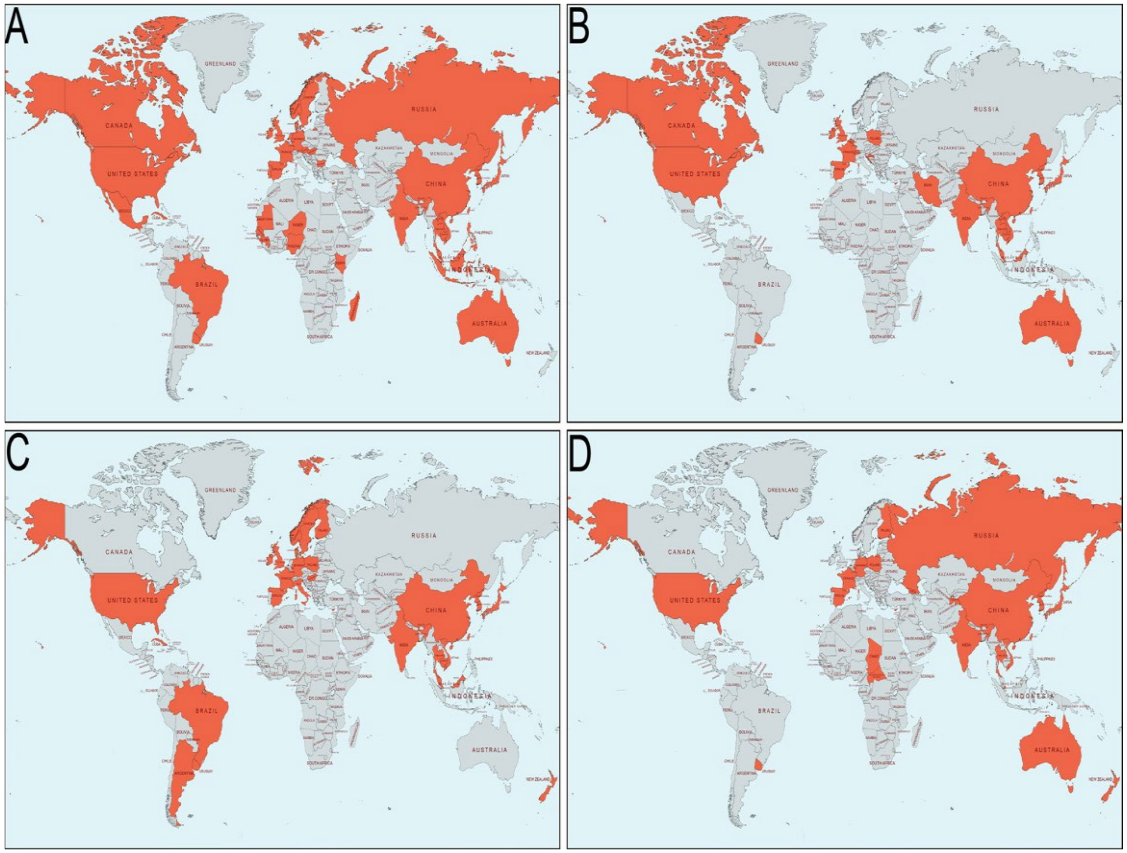
Zhu *et al, Current status of hand-foot-and-mouth disease* (2023)

Virology of Human Enterovirus



Adapted from Yi *et al.*, (2016).

Epidemiology of HFMD



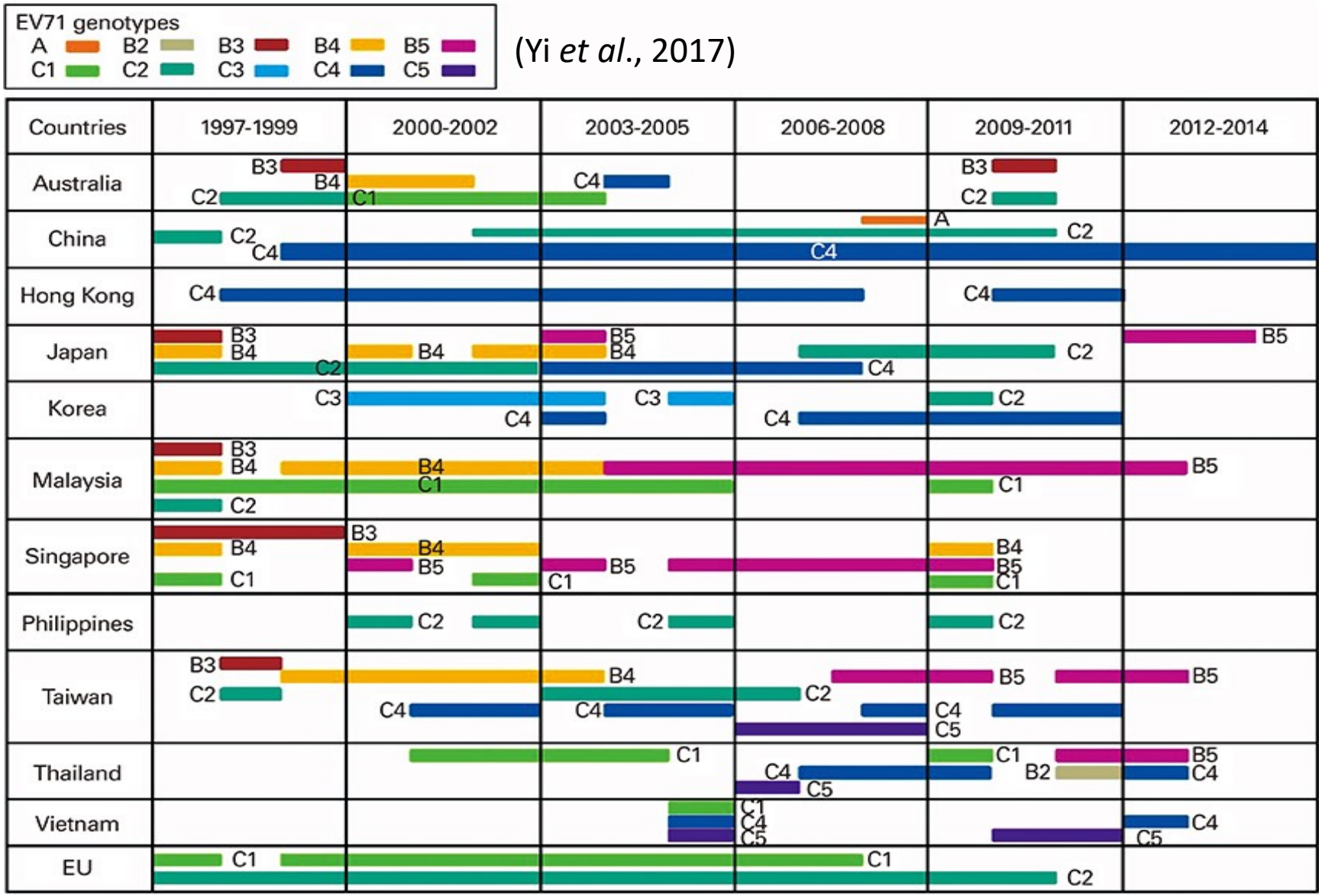
A: EV-A71; B: CVA16; C: CVA6; D: CVA10.

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

Total Cases of HFMD under WHO Surveillance (2017)		
Country	Total	Deaths
China	1,952,435	56
Japan	358,764	0
Korea	289,700	0
Hong Kong	358	0
Macau	3,402	0
Singapore	33,663	0
Vietnam	48,009	1

Hand, Foot and Mouth Disease Situation Update 2017. WHO. https://apps.who.int/iris/handle/10665/274106

Molecular Epidemiology of EV-A71



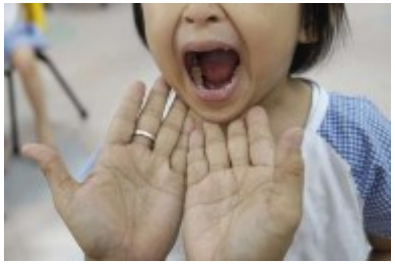
(Yi *et al.*, 2017)

HFMD in Singapore

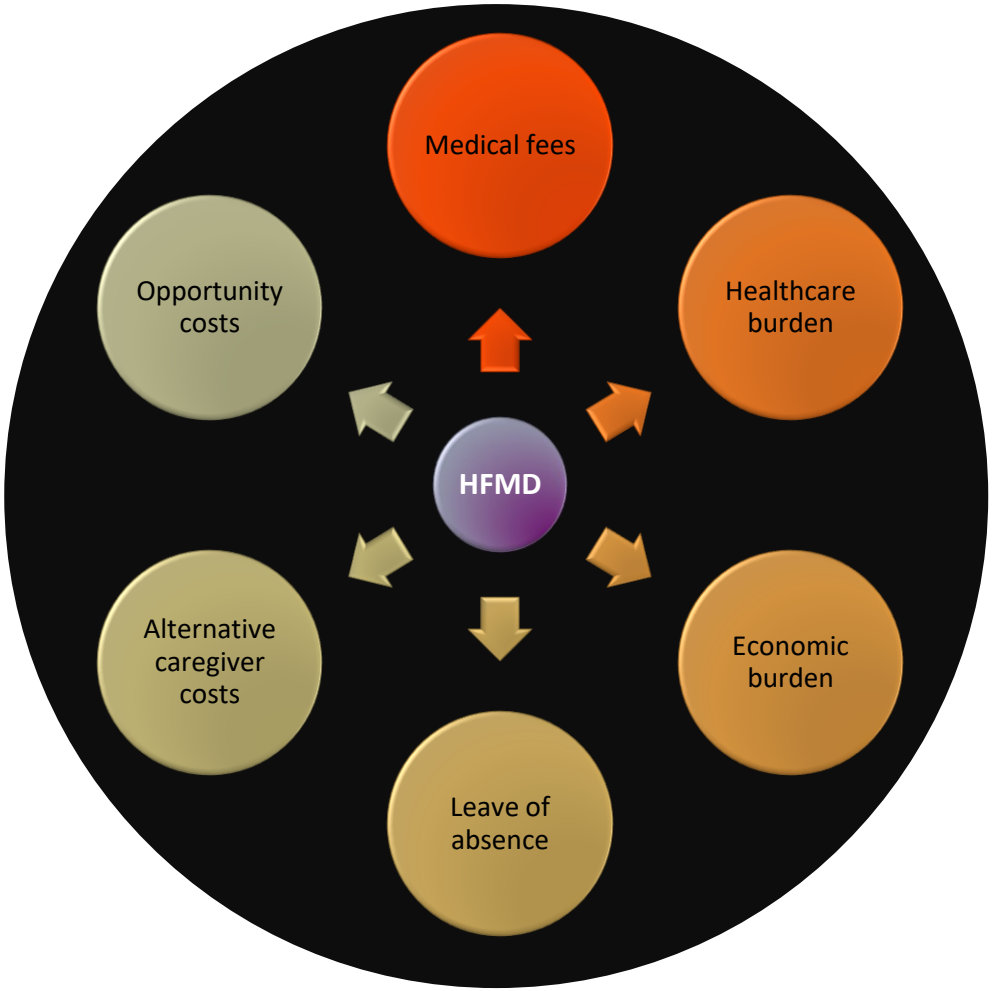
Photos courtesy of KK Women's and Children's hospital

- 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**

HFMD in Singapore



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



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

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

Child caught HFMD? It could cost family $1200
(The Straits' Times; 6 May 2014)

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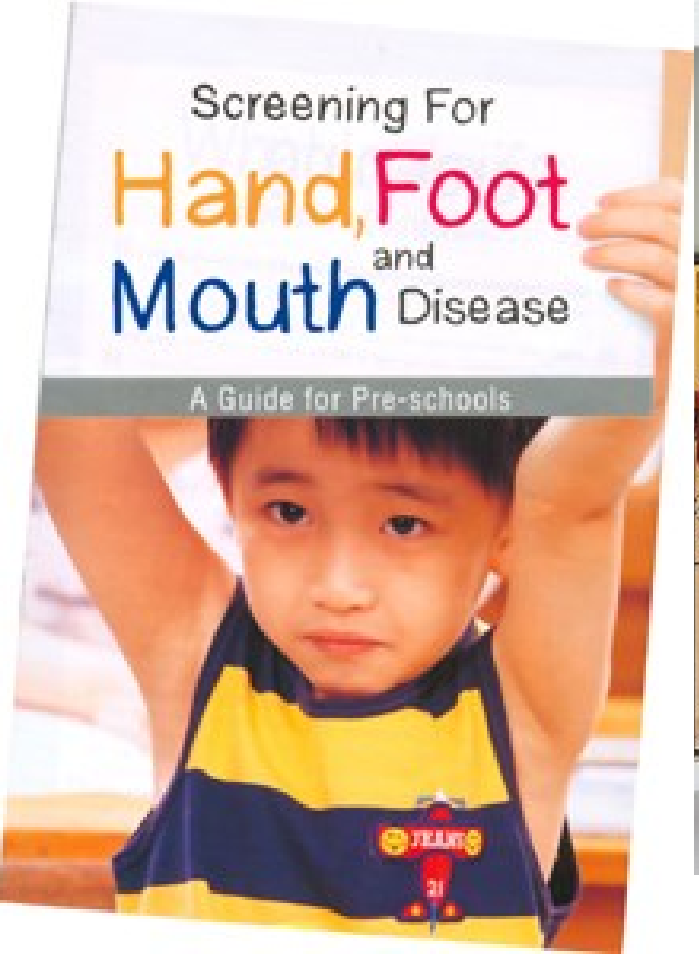
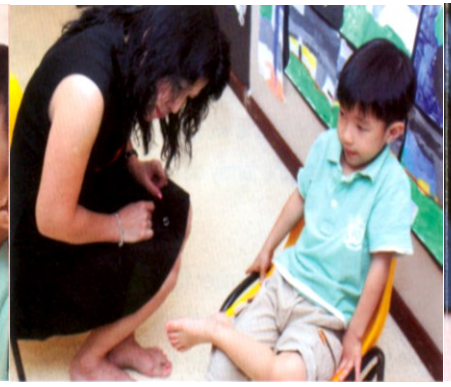
Public Education





Health Promotion Board (2008)

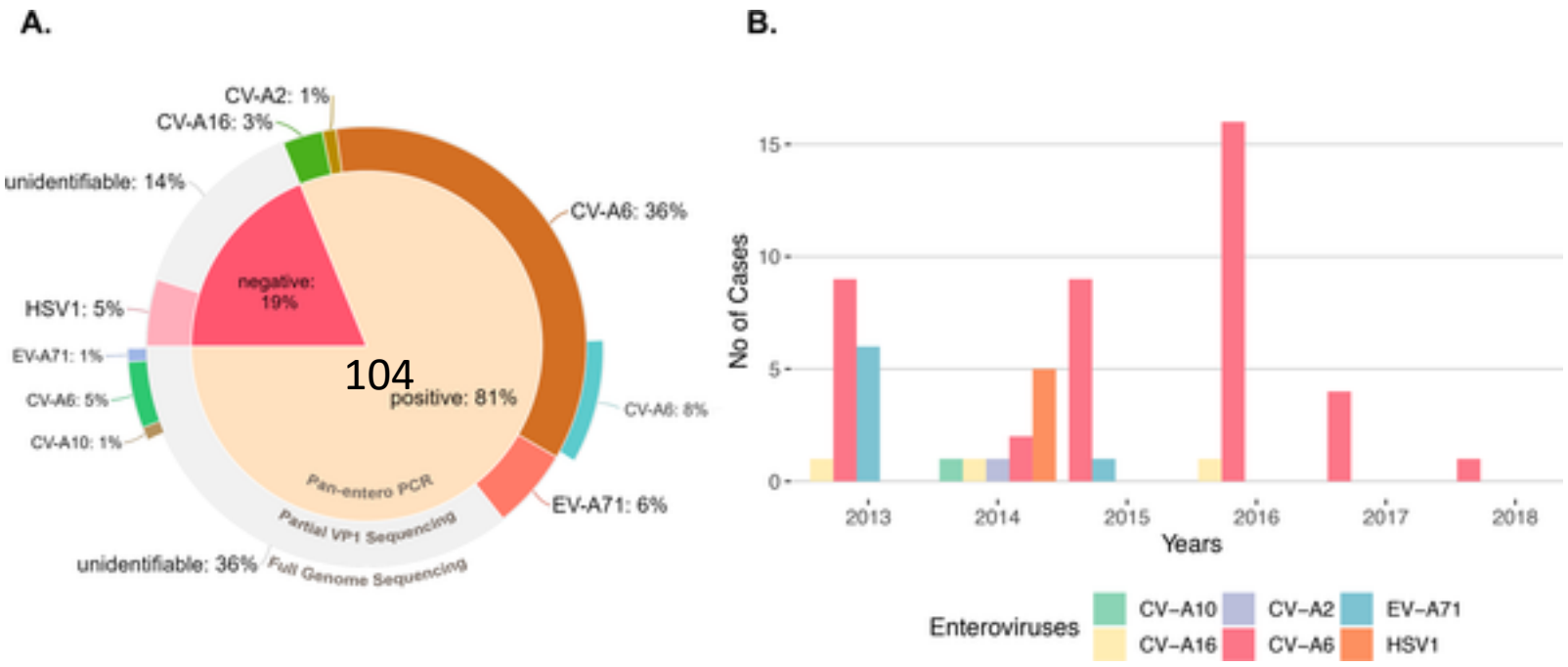
HFMD Warrior program with childcare centres in Singapore

- 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 paediatric patients and in community: 2013–2018



CV-A6 is the major etiological agent for symptomatic HFMD in Singapore, 2013–2018.

Min et al (2021) An epidemiological surveillance of hand foot and mouth disease in paediatric patients and in community: A Singapore retrospective cohort study, 2013-2018.PLoS Negl Trop Dis. 2021 Feb 10;15(2):e0008885.

Current Status of HMFD Vaccine

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

- 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)
Liu *et al, Cross-Antigenicity between EV71 Sub-Genotypes: Implications for Vaccine Efficacy* (2021)

Current Status of HMFD Vaccine

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

- 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.

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Liu *et al, Cross-Antigenicity between EV71 Sub-Genotypes: Implications for Vaccine Efficacy* (2021)
Huang *et al, Immunogenicity, safety, cross-reaction, and immune persistence of an inactivated enterovirus A71 vaccine in children aged from two months to 11 years in Taiwan* (2019)

Vaccine Approaches

Vaccine Approach	Reference	Status
Inactivated-Bivalent Vaccine EV71:CVA16	Fan et al 2020	Preclinical
Inactivated-Bivalent Vaccine CVA6:CVA10	Zhang et al 2018	Preclinical
Inactivated-Trivalent Vaccine EV71:CVA16:CVA6	Caine et al 2015	Preclinical
Inactivated-Trivalent Vaccine CVA6:CVA10:CVA16	Lim et al 2018	Preclinical
Virus like particle Vaccine EV71-VLP:CVA6-VLP:CVA10-VLP:CVA16-VLP	Zhang et al 2018	Preclinical

Strategies and Challenges for mRNA HMFD vaccine design

Confers protection against the current circulating genotype and serotypes

- 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.

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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)

Strategies and Challenges for mRNA HMFD vaccine design

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

- 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.

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Zhang *et al, Hand-Foot-and-Mouth Disease-Associated Enterovirus and the Development of Multivalent HFMD Vaccines* (2022)

Strategies and Challenges for mRNA HMFD vaccine design

Tetravalent vaccines exhibits obvious differences in inducing and production of neutralizing antibodies against viruses

- *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 ($TCID_{50}$) **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.

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Chapman NM. Prior immune exposure can protect or can enhance pathology in the enteroviruses: what predicts the outcome? Virulence. 2017 Aug 18;8(6):643-645. doi: 10.1080/21505594.2016.1269048. Epub 2016 Dec 9. PMID: 27935428; PMCID: PMC5626233.

Strategies and Challenges for mRNA HMFD vaccine design

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

- 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.

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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.

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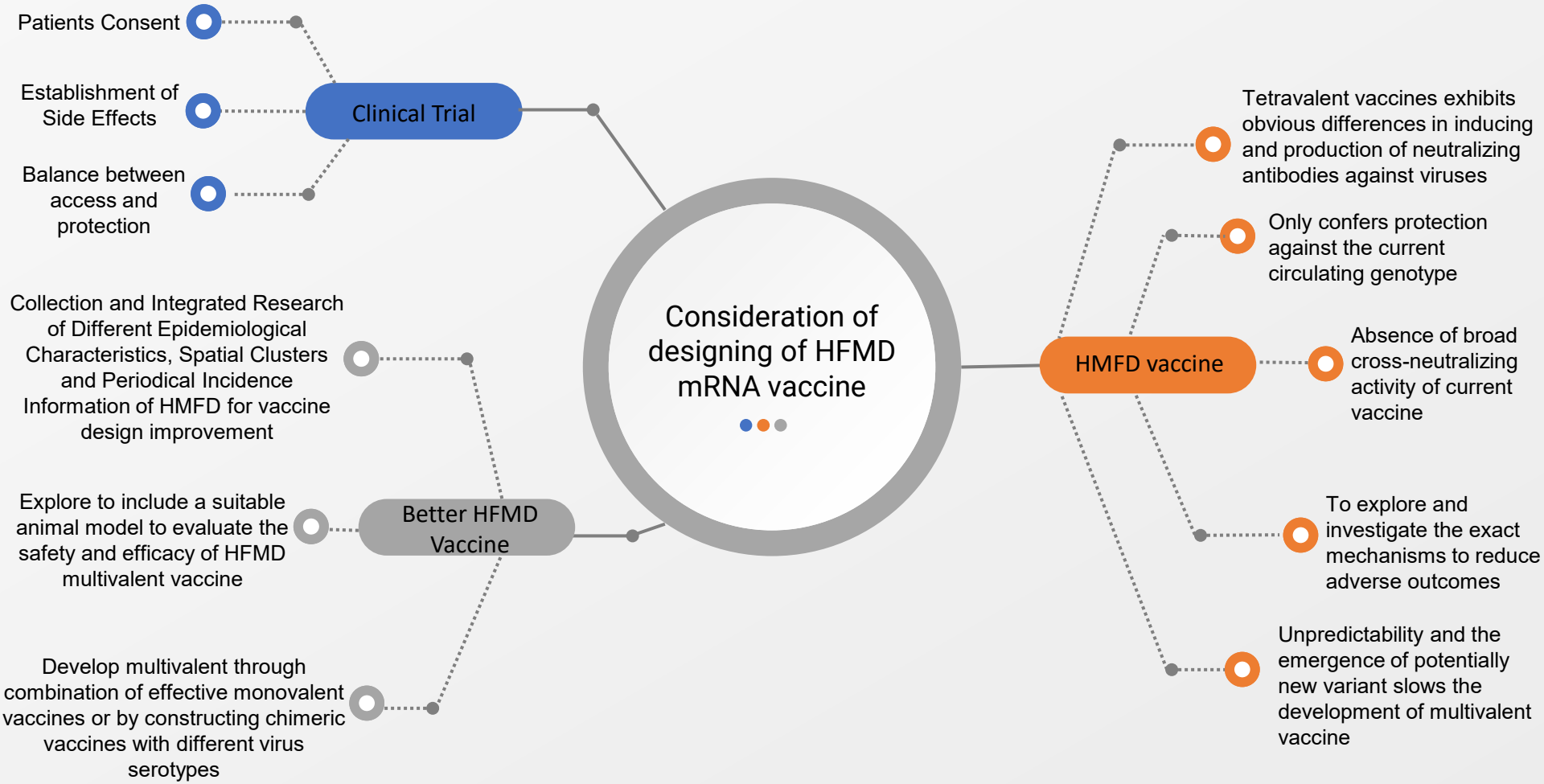
In summary


Consideration of designing of HFMD mRNA vaccine
Clinical Trial
Patients Consent
Establishment of Side Effects
Balance between access and protection
Better HFMD Vaccine
Collection and Integrated Research of Different Epidemiological Characteristics, Spatial Clusters and Periodical Incidence Information of HMFD for vaccine design improvement
Explore to include a suitable animal model to evaluate the safety and efficacy of HFMD multivalent vaccine
Develop multivalent through combination of effective monovalent vaccines or by constructing chimeric vaccines with different virus serotypes
HMFD vaccine
Tetravalent vaccines exhibits obvious differences in inducing and production of neutralizing antibodies against viruses
Only confers protection against the current circulating genotype
Absence of broad cross-neutralizing activity of current vaccine
To explore and investigate the exact mechanisms to reduce adverse outcomes
Unpredictability and the emergence of potentially new variant slows the development of multivalent vaccine

Collaborators in Asia:
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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
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Prof Kinh Nguyen Van & Associate Professor Nguyen Vu Trung from Hanoi Medical University, Vietnam

Thank you

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