A Multivalent VLP mRNA vaccine for HFMD
Vaccine Design and Preclinical Development Update

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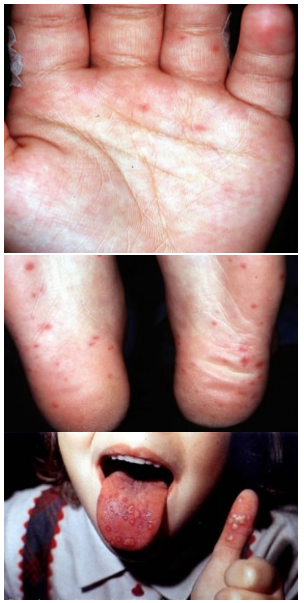
HFMD

- 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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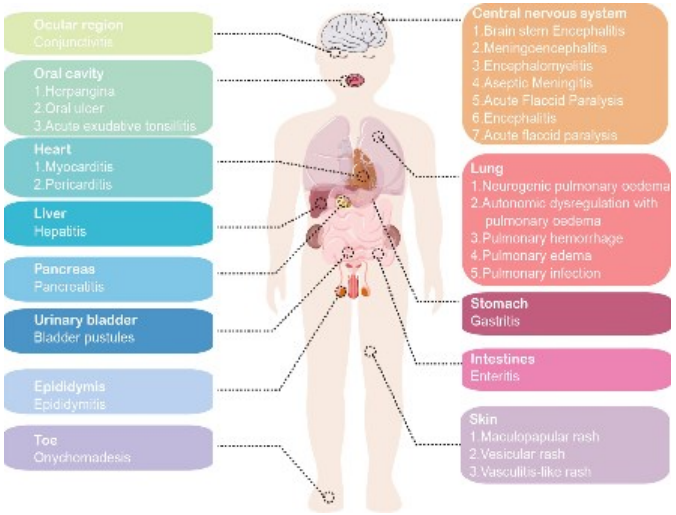
Clinical manifestations of EV-A71

- 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)

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Etiological agents of HFMD

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

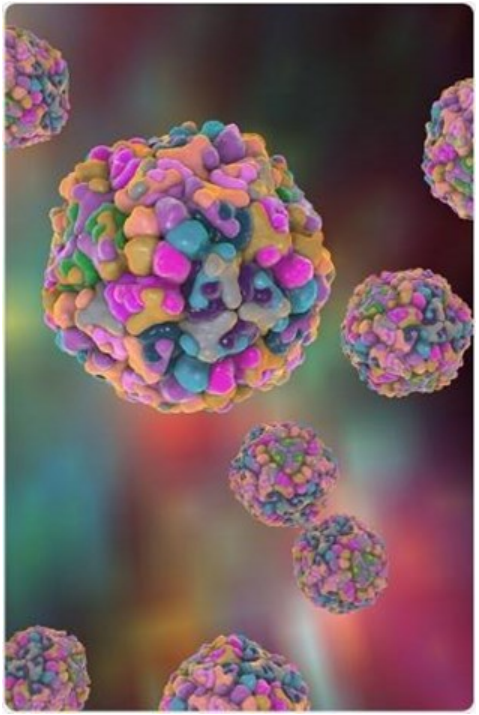

Table 1 EVs associated with HFMD

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)

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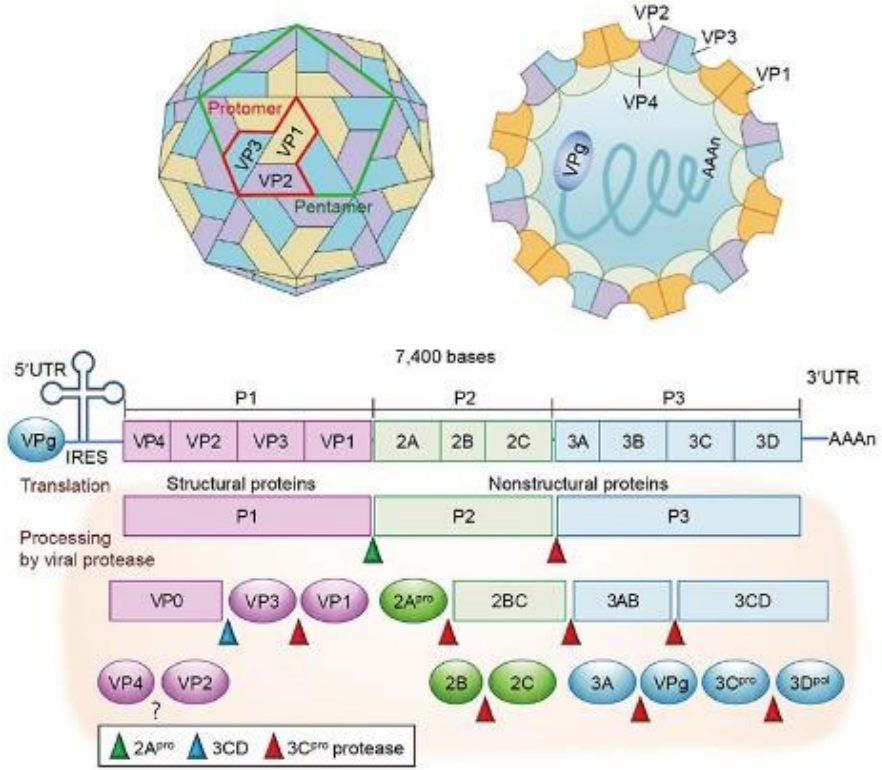
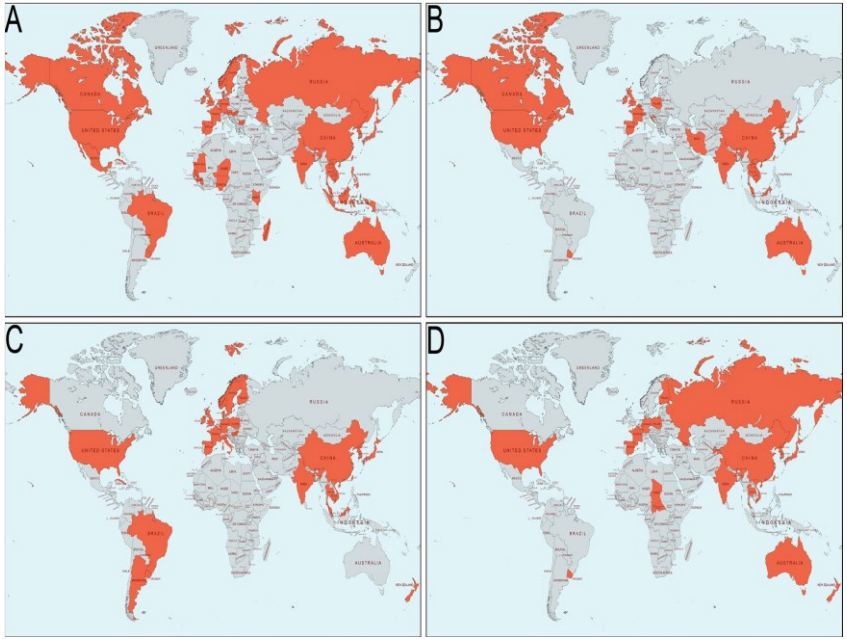
Virology of Human Enterovirus



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

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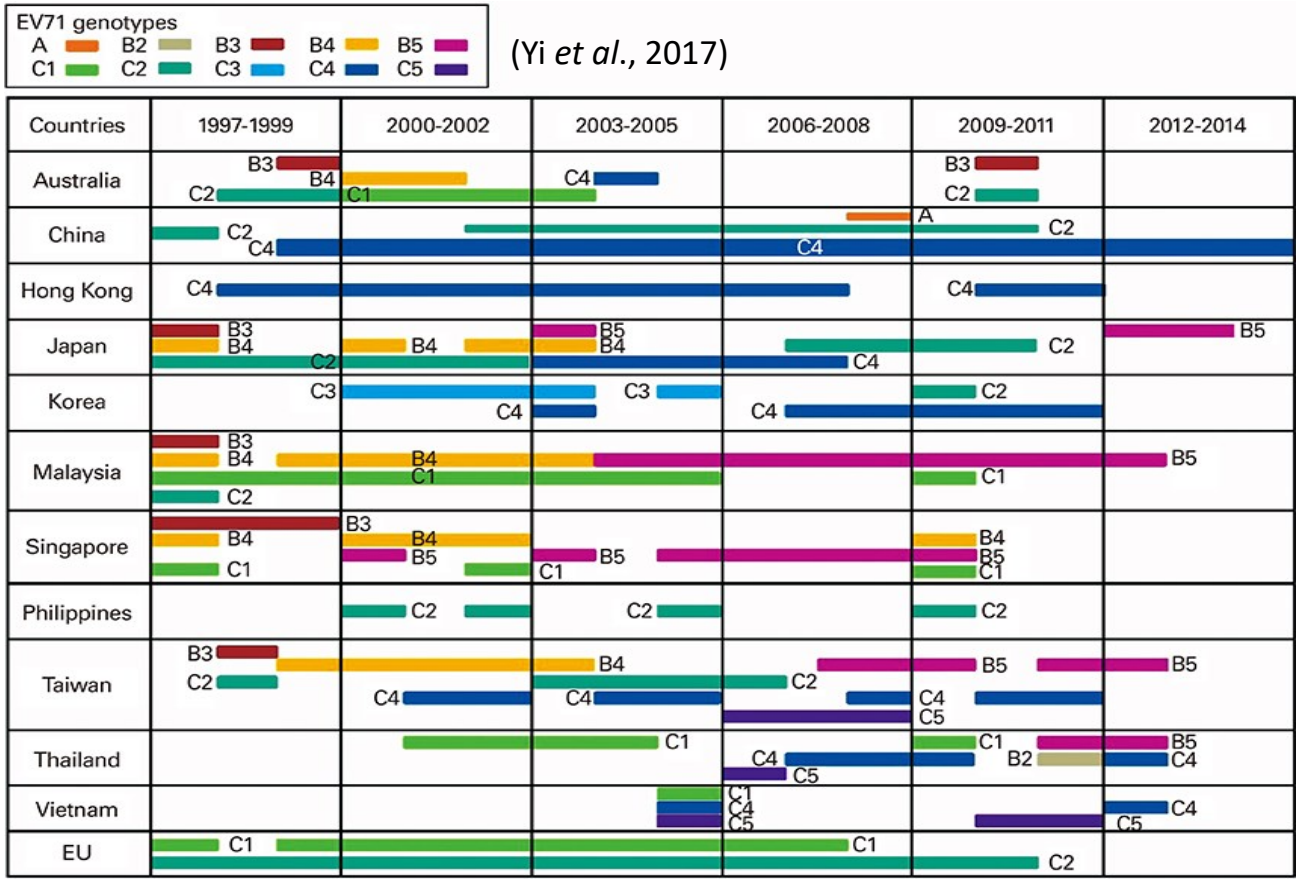
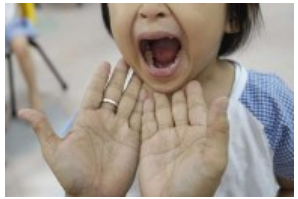
Molecular Epidemiology of EV-A71



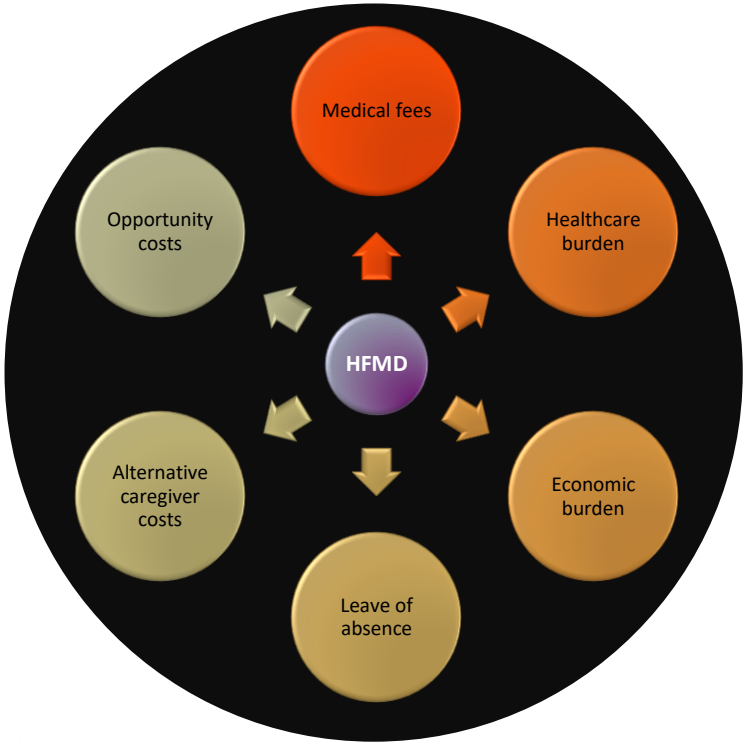
(Yi *et al.*, 2017)

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)

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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.

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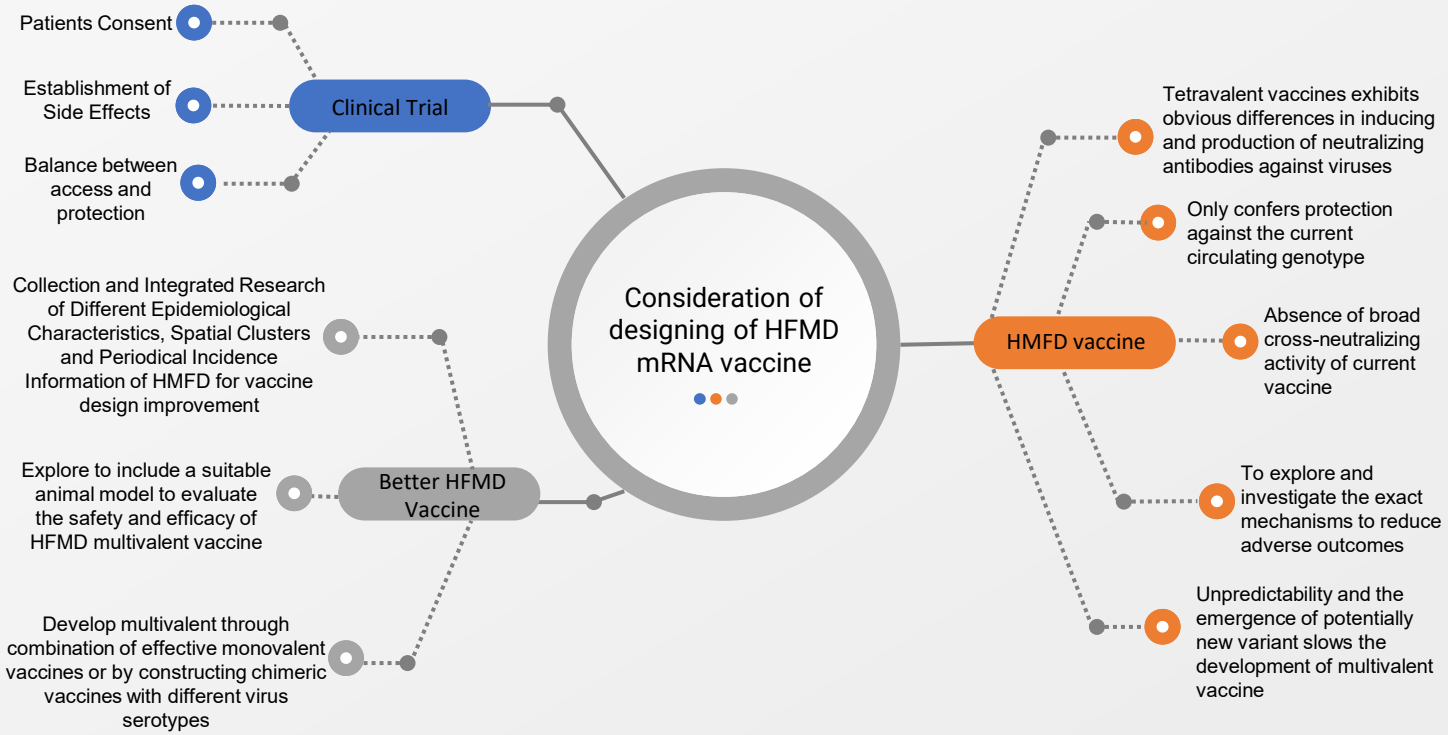
Liu *et al*, *Cross-Antigenicity between EV71 Sub-Genotypes: Implications for Vaccine Efficacy* (2021)
Huang *et al*, *Immunogenicity, safety, cross-reaction, and immune persistence of an inactivated enterovirus A71 vaccine in children aged from two months to 11 years in Taiwan* (2019)

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



https://cdn.who.int/media/docs/default-source/immunization/mrna-ttp/november-2023/7_chu_hfmd.pdf?sfvrsn=bd31c083_1

02

Background

- HFMD has been reported worldwide with a dramatic increase in the Western Pacific in the last two decades.
- The only approved vaccines are whole inactivated virus vaccines with limited distribution and targeting only EV-A71, just one out of the many HFMD-associated serotypes of enteroviruses.
- Unfortunately, the scope of protection of EV-A71 vaccines is limited to its subgenotypes, hence a multivalent vaccine is necessary to control the transmission since there are, to-date, 39 HFMD-associated enteroviruses across three species: *Enterovirus A* to *C*[1]. Current dominant serotypes are CV-A6, CV-A10, CV-A16 and EV-A71.
- Prevalent serotypes differ across geographical locations and change over time – flexibility in formulation adjustments in response to changes in serotype prevalence is essential for the success of a HFMD vaccine.
- mRNA vaccine platform is well established which makes it easy and faster (compared to traditional inactivated vaccines) to scale up vaccine production for clinical testing and actual adoption.

[1] Zhu et al. Current status of hand-foot-and-mouth Disease. Journal of Biomedical Science (2023) 30:15

mRNA Vaccine for HFMD

01

Disease target and therapeutic goal

- Multiple serotypes of human enteroviruses can cause hand, foot and mouth disease (HFMD).
- Our aim is to develop a **multivalent vaccine** that can confer protection against dominant circulating serotypes with the flexibility to change vaccine formulation based on disease trends.

Key technology

- mRNA vaccine technology: vaccine is a codon-optimized version of selected protein coding regions of virus genome and delivered using lipid nanoparticles. After delivery, the mRNA will be translated and processed to form virus-like particles (VLP) as the antigen. The mRNA vaccine technology makes the vaccine easier to produce and offers the flexibility needed for changes to be made to the vaccine formulation, as required, in the future. With VLP as the final product of the mRNA, this design ensures most if not all epitopes of an intact virus particle will be presented without the risk of incidental infections, making it a safe and robust vaccine that can be administered to children and immunocompromised individuals.

Potential for commercialisation and potential market

- Our vaccine technology is based on VLP.
- Potential licensing partners include pharmaceutical companies that have established mRNA vaccine production infrastructure: Pfizer, Moderna, MSD Wellcome Trust Hilleman Laboratories, Walvax Biotechnology, WuXi Biologics.
- Potential market: HFMD is endemic in most of Asia Pacific and outbreaks had been reported worldwide. There are whole inactivated vaccines against only EV-A71 approved by China Food and Drug Administration (CAMS, Vigoo and Sinovac) as well as one from Medigen (Taiwan). For all other types of vaccines, they are still in the preclinical development stage and there is no mRNA vaccine published yet.

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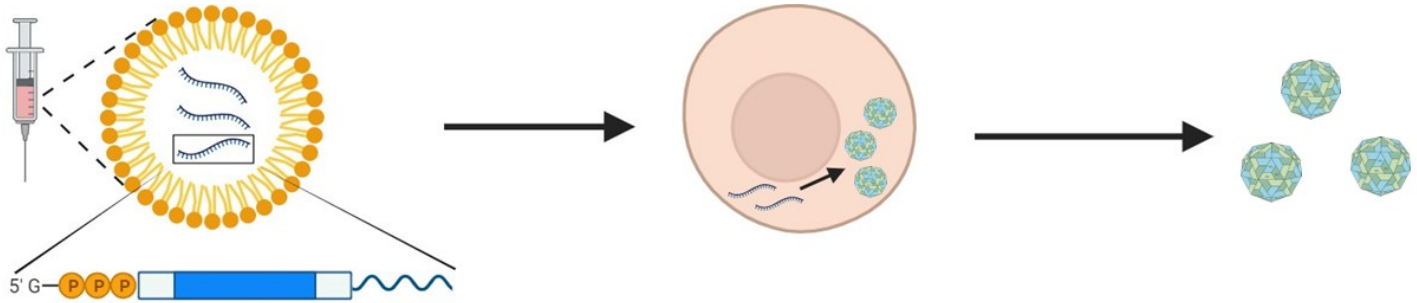
VLP mRNA vaccine

- We propose a VLP mRNA vaccine that can be produced using standard mRNA vaccine chemistry, making it cheaper and easier to scale up production with existing production infrastructure.
- Upon delivery of the mRNA packaged in LNP, the mRNA will be translated to produce a polyprotein which will be autoproteolytically processed into viral capsid proteins that will assemble into virus-like particles in the cells.
- Using VLP as the antigen has two major benefits
  - Antigenic epitopes are highly similar to intact virus particles
  - No risk of infection even in individuals with weakened immune system.



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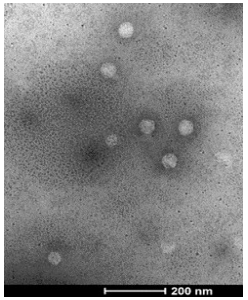
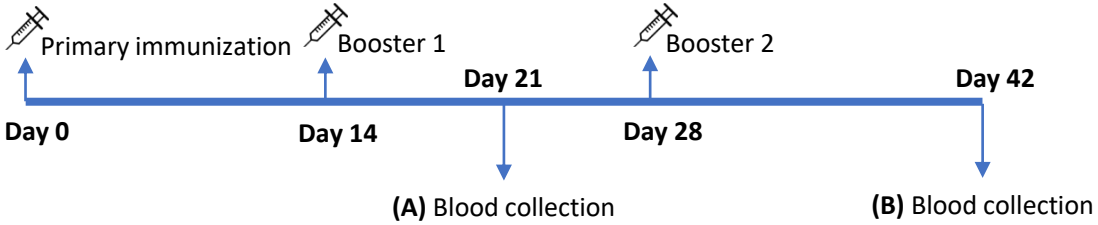
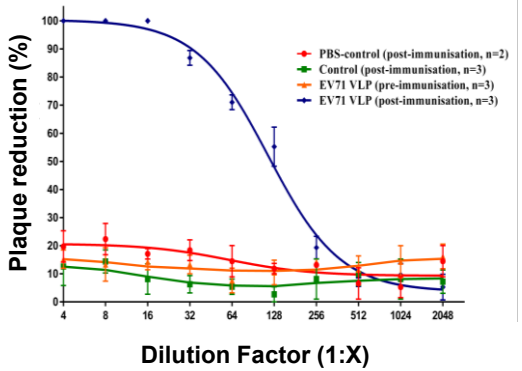
VLP mRNA vaccine pilot test







- *In vitro* expression and preclinical evaluation of EV-A71 mRNA vaccines in mice

In vitro (cell-based)

In vivo (mouse)

mRNA expression evaluation

6 months

IACUC Approved

Inoculation of mRNA-LNPs in mice and nAb titre determination

06

Research goals

Research Goals

Establish a surveillance network in multiple countries around Asia Pacific to determine dominant serotypes as well as to detect any potential outbreaks and emergence of new dominant strains/strains of interest.

Evaluate vaccines in animal models (mouse and non-human primates) to analyze the induction of protective immunity against predominant serotypes.

Synthesis and modification of pharmaceutical grade mRNA vaccines for evaluation in animal models.

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