Hilleman Laboratories
MSD · Wellcome Trust Joint Venture

Summary of key scientific and technical consideration to advance HFMD vaccine development

Pei-Yin Lim, PhD

Summary of key scientific and technical consideration to advance HFMD vaccine development

1. Hand Foot and Mouth Disease (HFMD) vaccine – a multivalent vaccine
    - Selection of viruses to be included in the HFMD vaccine.
    - Selection of sequences used for the mRNA vaccine.
2. Selection of viral protein(s) to be encoded in the mRNA vaccine
3. Animal models and limitations
4. Immune/vaccine interference
5. WHO reference material
6. Safety and efficacy of mRNA vaccine
7. Storage/transport conditions and stability of the vaccine
8. Clinical trials

Enterovirus 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. J Biomed Sci 2023

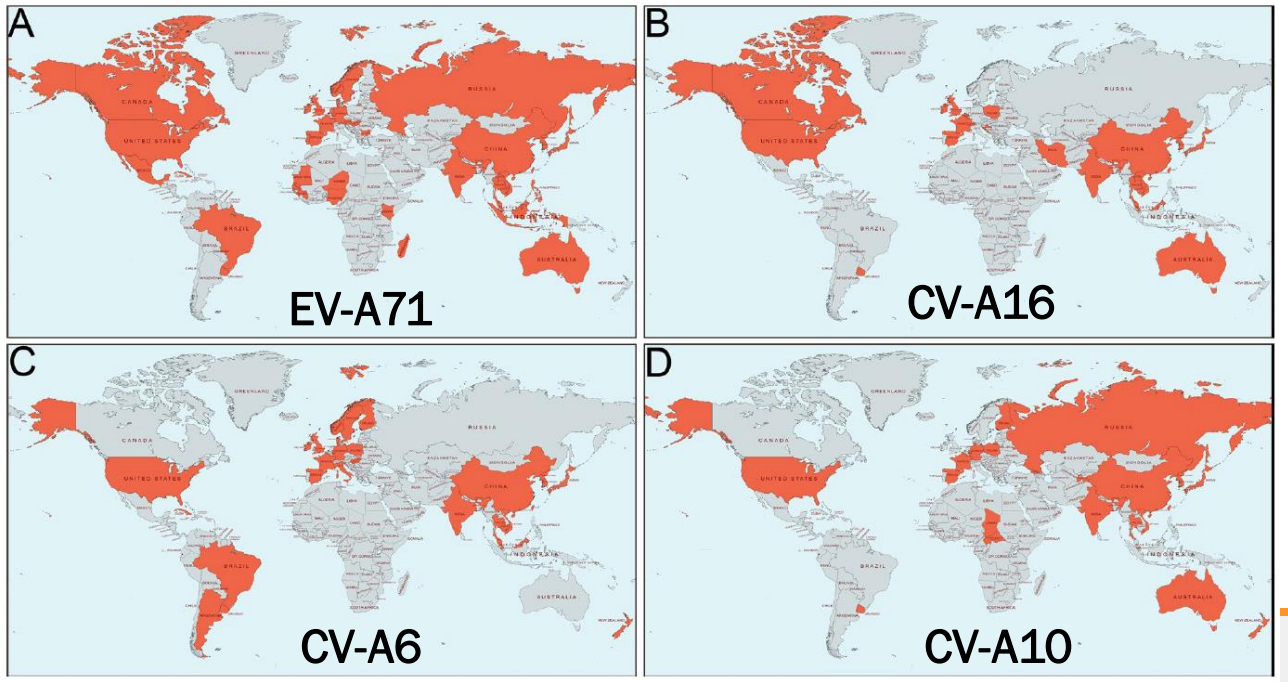
Picornaviridae Family

Enterovirus Genus

Primary causative agents of HFMD:

EV-A71, CV-A16, CV-A6 and CV-A10

Global distribution of patients with HFMD



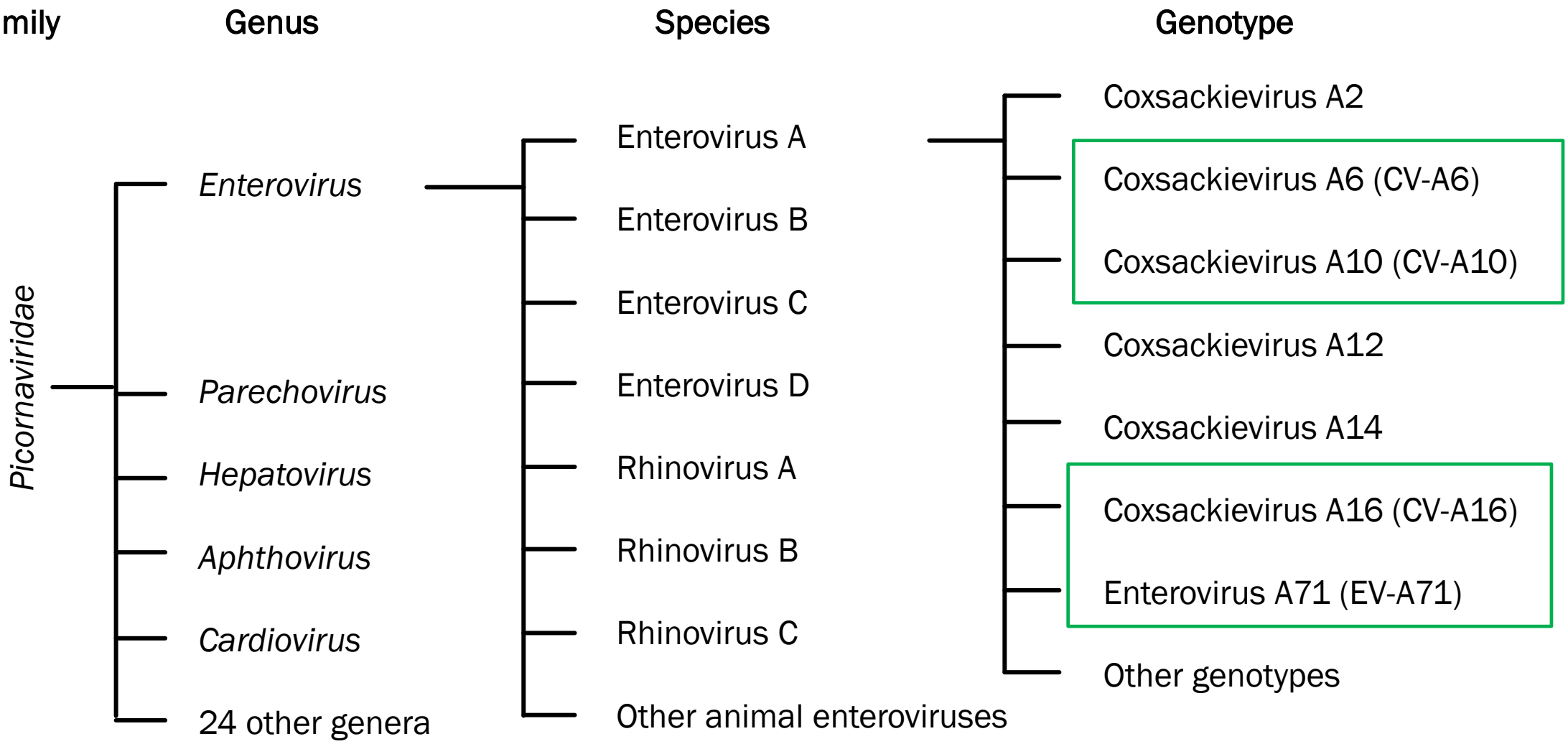
Zhu, et al. J Biomed Sci 2023

The HFMD vaccine development landscape focuses on EV-A71

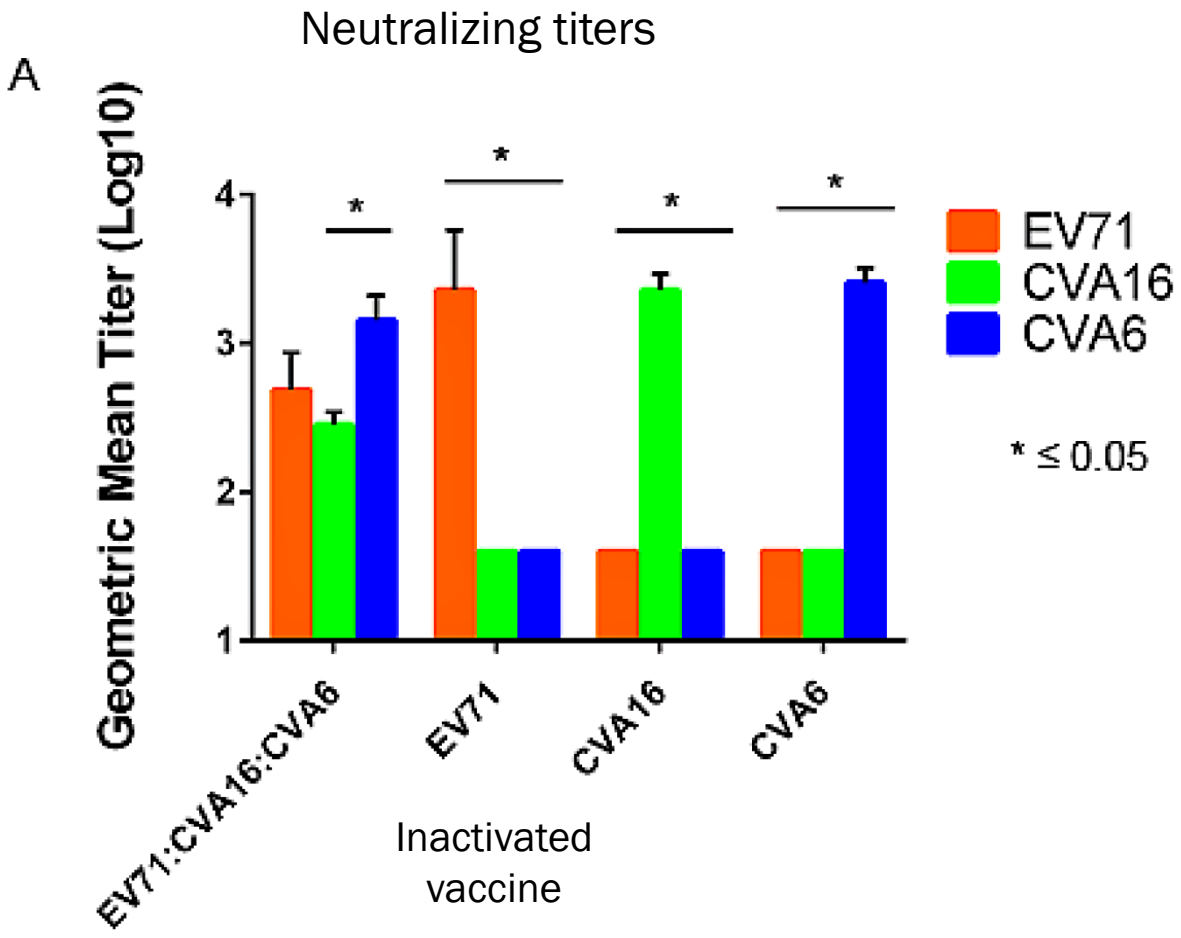
	Sinovac	Chinese Academy of Medical Sciences	Beijing Vigoo	Enimmune	Medigen	Takeda (Inviragen)	Sentinext Therapeutics	inno.N	Sinovac
Stage	Licensed	Licensed	Licensed	Phase III	Phase III	Phase I	Phase I	Phase I	Phase I/II
Virus	**EV-A71**	**EV-A71**	**EV-A71**	**EV-A71**	**EV-A71**	**EV-A71**	**EV-A71**	EV-A71/CV-A16 (bivalent)	EV-A71/CV-A16 (bivalent)
Sub-genogroup	C4	C4	C4	B4	B4	B2	B3		
Technology	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (binary ethylenimine)	Virus-like Particles (VLP)	inactivated whole virus	inactivated whole virus (formalin)
Cell Substrate	Vero cells	KMB-17 cells	Vero cells	Vero cells	Vero cells	Vero cells	Baculovirus	Vero cells	Vero cells
Adjuvant	aluminum hydroxide	aluminum hydroxide	aluminum hydroxide	aluminum hydroxide	aluminum phosphate	aluminum hydroxide	aluminum hydroxide		
Dosing schedule	2 doses, 28 days apart	2 doses, 28 days apart	2 doses, 28 days apart	2 doses, 28 days apart	2-3 doses: days 1 and 57, plus day 366 for under 2s	2 doses, 28 days apart	2 doses, 28 days apart	3 doses, 28 days apart	2 doses, 1 month apart
Route of administration	IM	IM	IM		IM	IM	IM		
Efficacy	94.7% year one 95.1% year two	97.40%	90.0% year one 94.8% year two		100%				
Target population	6 mo - 6 years	6 mo – 6 years	6 mo - 3 years	2 mo - 6 years	2 mo - 6 years				
Registration and target countries	China (licensed 2015)	China (licensed 2015)	China (licensed 2016)	(Taiwan and Vietnam)	(Taiwan and Vietnam) stated intention to market across ASEAN countries		(Malaysia and Australia)	(Korea)	China
Clinical trial number					NCT05099029	NCT01376479		NCT04182932 NCT04637919	NCT06063057

← Currently mostly monovalent with multi-valent on horizon

Classification of the virus family *Picornaviridae*



No cross protection between EV-A71, CV-A16, CV-A6 and CV-A10



Caine et. al., Viruses 2015

- Mice were vaccinated with inactivated vaccine (trivalent or mono-valent)
- Serum from vaccinated mouse were used tested for neutralizing activities against EV-A71, CV-A16, and CV-A6

Each genotypes consists of multiple genogroups

Genotype	Genogroups/subgenogroups
Enterovirus A71 (EV-A71)	A, B0-B5, C1-6, D, E, F, G, H
Coxsackievirus A16 (CV-A16)	A, B, C, D
Coxsackievirus A6 (CV-A6)	A, B1-B3, C1-C2, D1-D3
Coxsackievirus A10 (CV-A10)	A, B, C, D, E, F, G

Is there cross-protection among genogroups/subgenogroups?

EV-A71 vaccines cross-protect against various genogroups

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Stage	Licensed	Licensed	Licensed	Phase III	Phase III	Phase I	Phase I	Phase I	Phase I/II
Virus	EV-A71	EV-A71	EV-A71	EV-A71	EV-A71	EV-A71	EV-A71	EV-A71/CV-A16 (bivalent)	EV-A71/CV-A16 (bivalent)
Sub-genogroup	**C4**	**C4**	**C4**	**B4**	**B4**	**B2**	**B5**		
Technology	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (formalin)	inactivated whole virus (binary ethylenimine)	Virus-like Particles (VLP)	inactivated whole virus	inactivated whole virus (formalin)
Cross neutralization (sub-genogroup)	**A, B0, B1, B2, B3, B4, C1, C2, C4, C5**	**A, B0, B1, B2, B3, B4, C1, C2, C4, C5**	**A, B0, B1, B2, B3, B4, C1, C2, C4, C5**	**N/A**	**B5, C4a, C4b, C5**	**B2, B4, B5, C4**	**B3, B4, C2, C4**	**N/A**	**N/A**
Reference for cross neutralization (DOI)	Liu et. al., Viruses 2021	Liu et. al., Viruses 2021	Liu et. al., Viruses 2021	N/A	Huang et. al., Vaccine 2019	Tambyah et. al, Vaccine 2019	Salmons et. al., Vaccine 2018 Lim et. al. Vaccine 2015	N/A	N/A

Evidence of cross-protection among genogroups for EV-A71 with various efficiency

Genogroups	Member/description
A	BrCr prototype, identified in a patient with encephalitis in California in 1696
B	B0-B5
C	C1-C6
D	discovered in India from patients with acute flaccid paralysis
E	discovered in Africa
F	discovered in Madagascar
G	discovered in India from patients with acute flaccid paralysis
H	discovered in sewage samples in Pakistan

- EV-A71 vaccines cross-neutralized various genogroups. (Liu et. al., Viruses 2021)
- Serum sample from rats challenged with various genogroups of EV-A71 cross-neutralized various genogroups of EV-A71. (Liu et. al., Viruses 2021)
- Broad cross-neutralization between EV-A71 genogroup B, C, E, F (Volle et. al., J Gen Virol 2023)
  - Monoclonal antibodies, human serum against B and C to neutralize virus from genogroup E and F (Africa)

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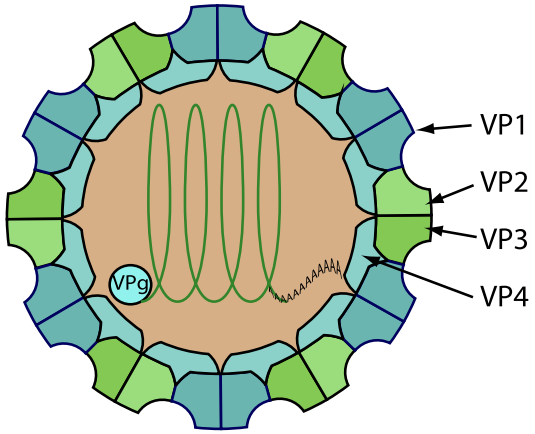
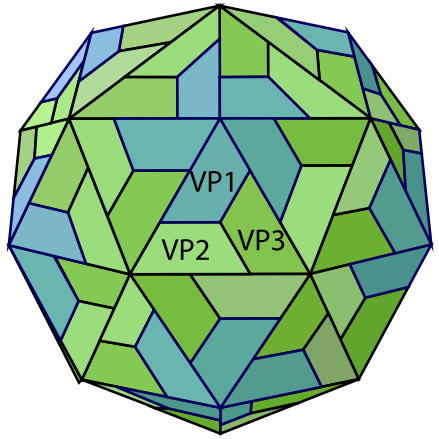
    - Selection of viruses to be included in the HFMD vaccine.
    - Selection of sequences used for the mRNA vaccine.
    - No cross-protection between EV-A71, CV-A10, CV-A6 and CV-A16.
    - Cross-protections among EV-A71 genogroups, efficiency varies.
    - Ensure that the chosen sequence will provide cross-neutralizing antibodies among the genogroups.
2. Selection of viral protein(s) to be encoded in the mRNA vaccine

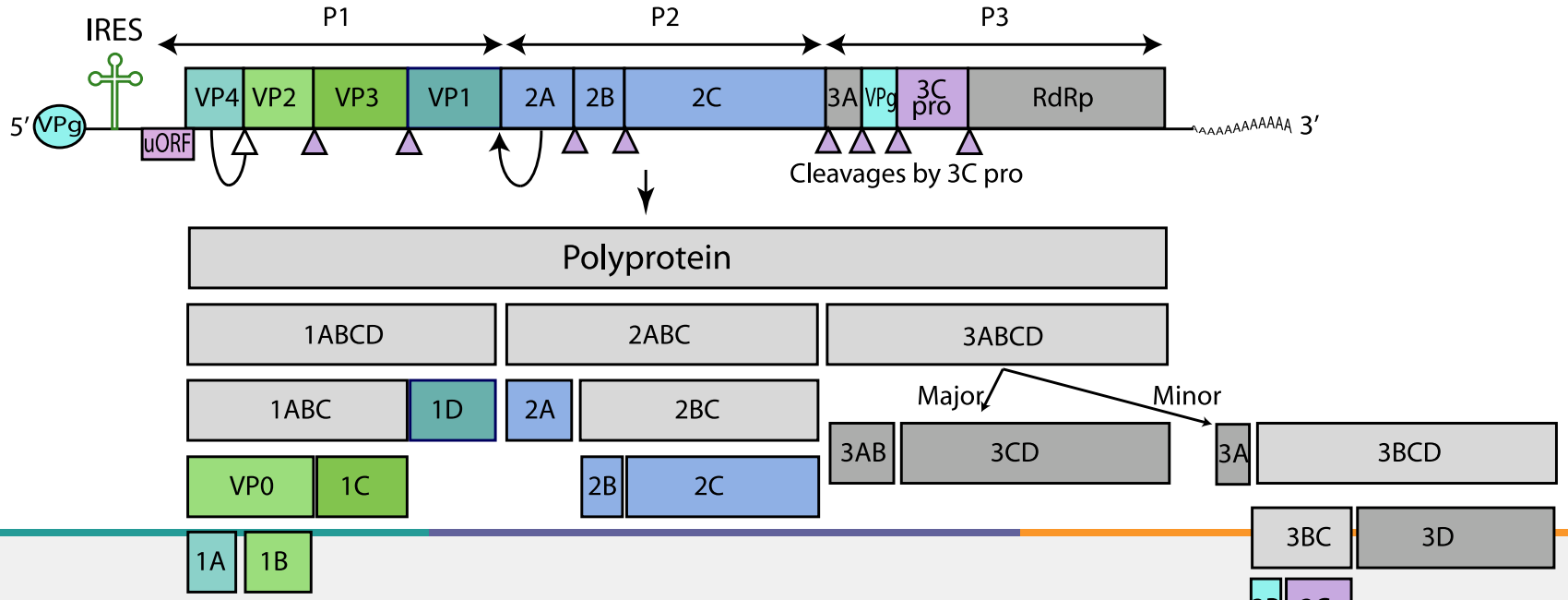
Viral particle and viral genome



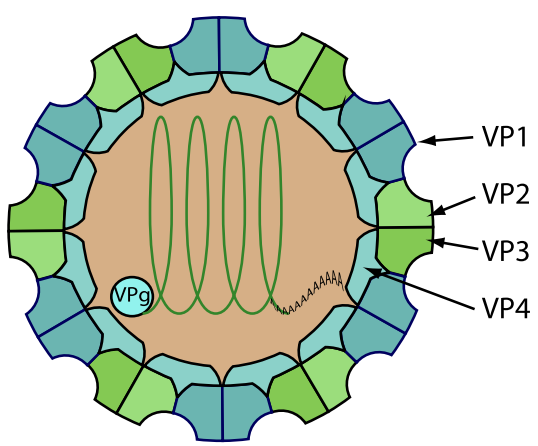


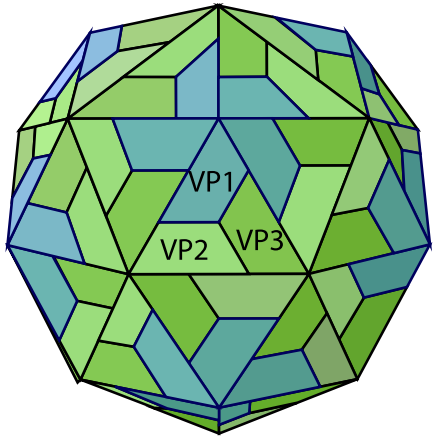
- Non-enveloped, icosahedral capsid
- Positive strand RNA viral genome
- Capsid consists of 60 protomers, each consisting of 4 polypeptides, VP1, VP2, VP3 and VP4

Viral proteins are translated into one polyprotein, and then cleaved into many proteins

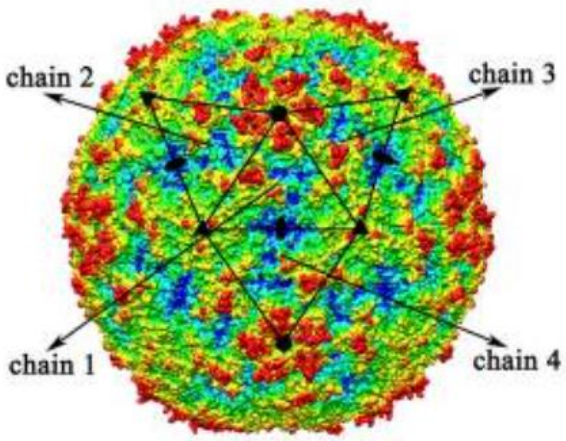


Cryo-EM structures of enteroviruses have been extensively studied

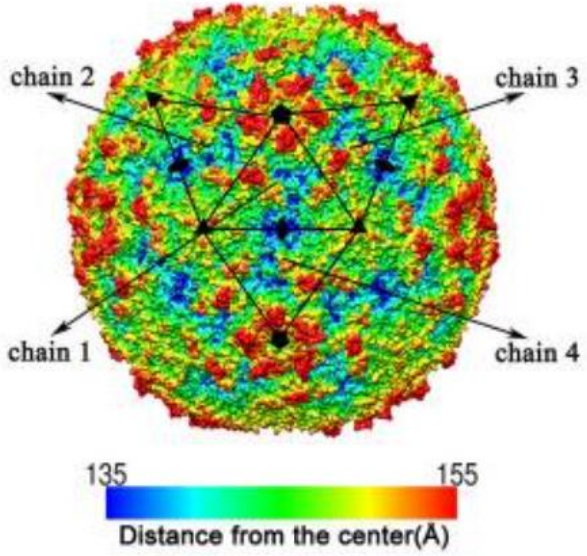




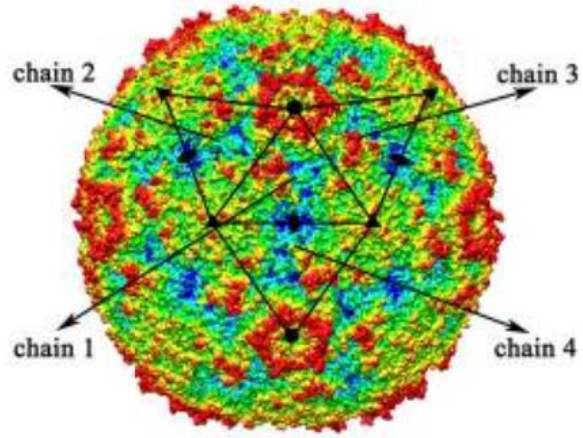
EV-A71



CV-A16



CV-A10



Reviewed by Wang et. al., Sci Rep 2021 https://viralzone.expasy.org/97

Conformational epitopes involving VP1, VP2, and VP3 are important for vaccine design

Antibody footprint

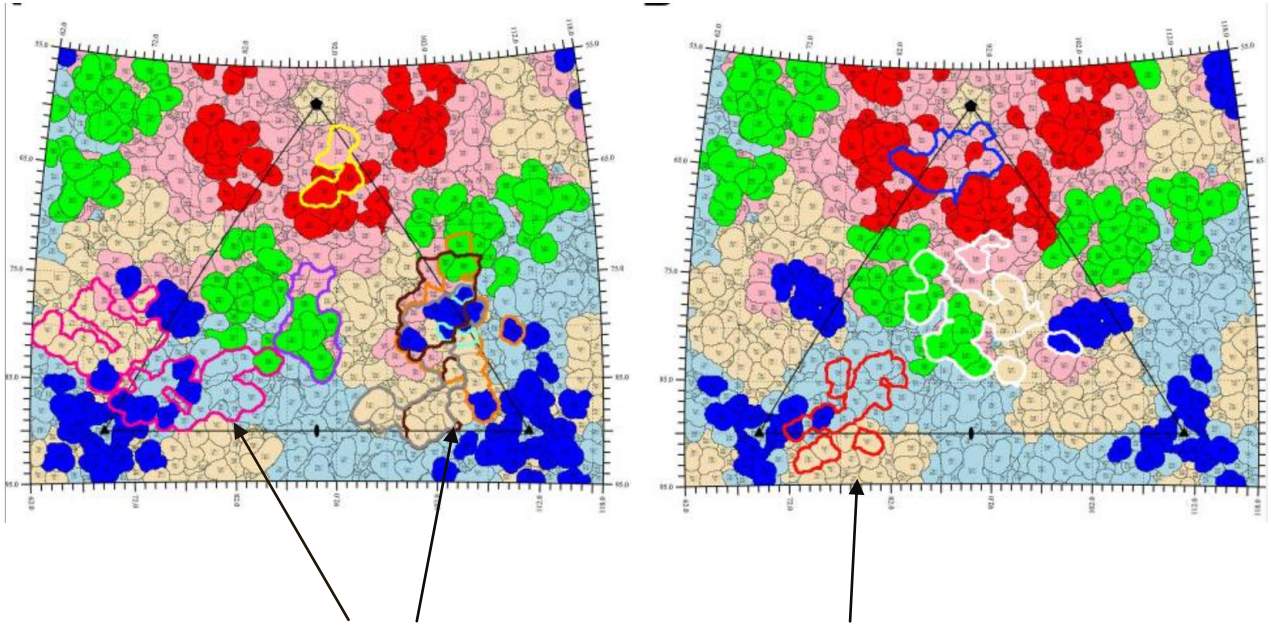
Host cell receptor footprint

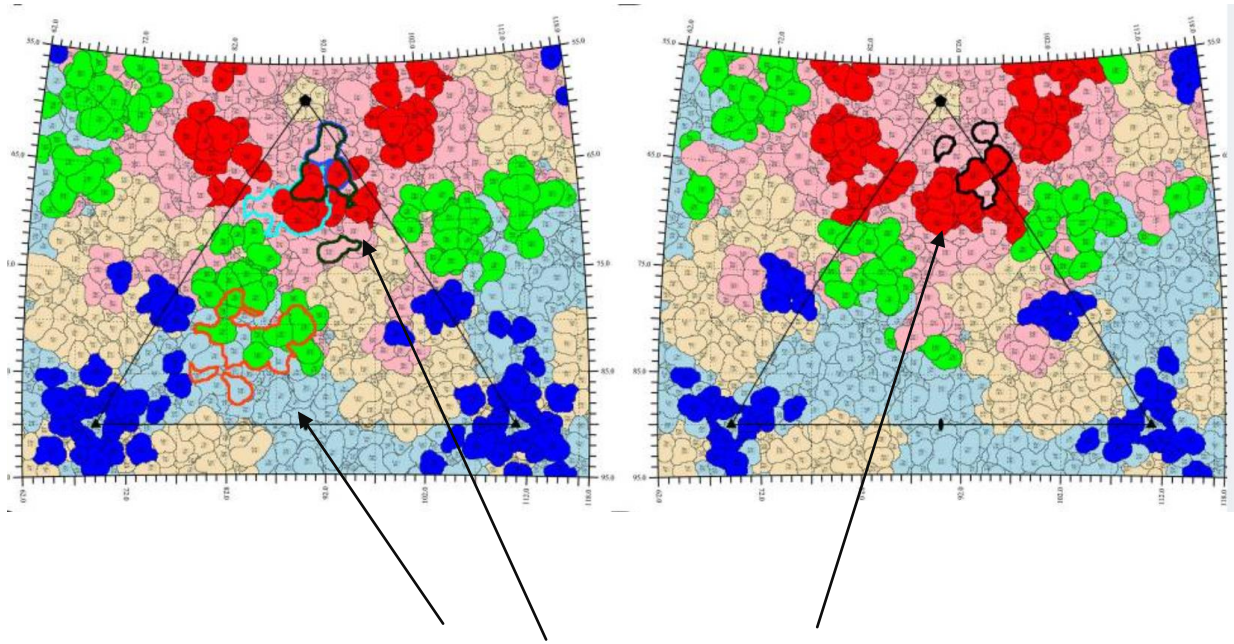
EV-A71

CV-A16

EV-A71

CV-A16





Neutralizing antibody binding sites

Receptor binding sites

Reviewed by Wang et. al., Sci Rep 2021

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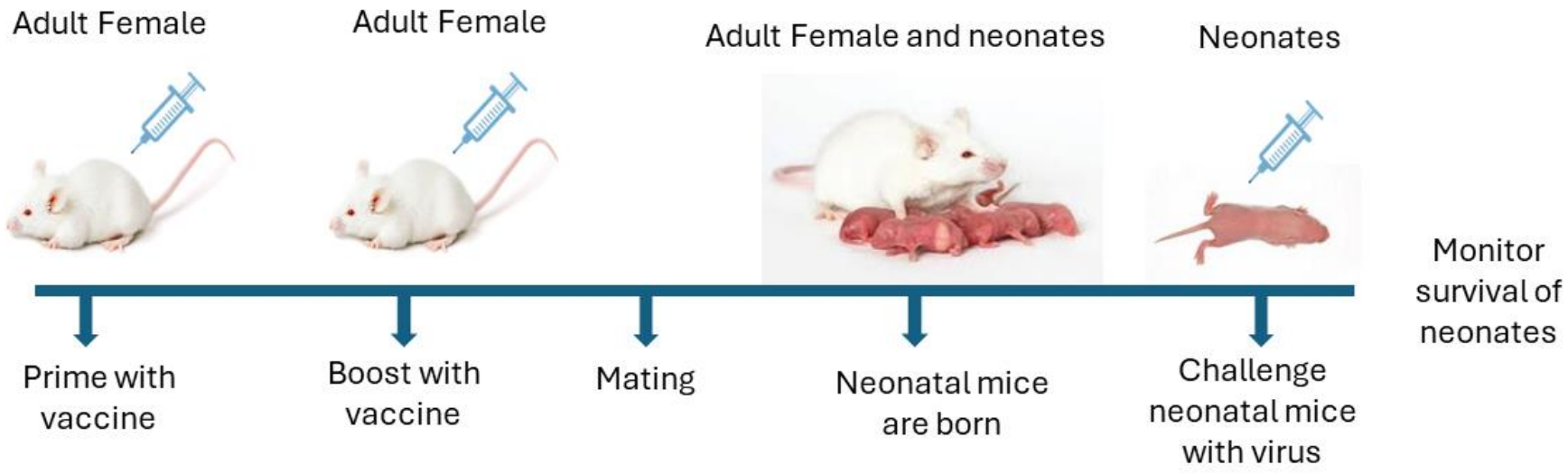
    - Selection of viruses to be included in the HFMD vaccine.
    - Selection of sequences used for the mRNA vaccine.
2. Selection of viral protein(s) to be encoded in the mRNA vaccine
    - Sequence encoding for all viral structural proteins may be needed for the mRNA vaccine
3. Animal models and limitations

Animal models for HFMD and their limitations

- Neonatal suckling mouse
  - Commonly used animal model for HFMD
  - Good challenge model because lethal infection.
  - Immune system is immature in neonatal suckling mouse
  - May required mouse-adapted enterovirus.
  - Not good for vaccine efficacy studies, mice are susceptible to infection <1 week old
  - Vaccine efficacy studies – assess transfer of protective antibodies from vaccinated parents to their offspring.



Animal models for HFMD and their limitations

- Immunodeficient mice
  - Incomplete immune system
  - Example, AG129 mice – deficient in interferon α/ß and γ receptors (innate immune response)
  - Window of susceptibility:2-6 weeks
  - May require mouse-adapted enterovirus

- Cellular receptor transgenic mice
  - Example, Human SCARB2 transgenic mice (Imura et. al., J Virol 2020)
  - hSCARB2 – cellular receptor for some of the enteroviruses
  - Age-dependent susceptibility up to 3 week post-natal, limits it used for vaccine efficacy studies
  - Susceptible to some clinical strains of enteroviruses

Animal models for HFMD and their limitations

- Mongolian Gerbil
  - Develop severe diseases (neurological symptoms) after infection with clinical isolates of EV-A71 (Yao et. al., PLoS One 2012)
  - Has been used to examine vaccine efficacy
    - Inactivated CV-A16 vaccine (Sun et. al., Emerg Microbes Infect 2022)
    - Bivalent inactivated EV-A71 and CV-A16 vaccine (Yi et. al., Biomol Ther (Seoul) 2023)

Yao et. al., PLoS One 2012

- Non-human primates
  - Cynomolgus Macaques, Rhesus, and African Green Monkey
  - Animals exhibit neurological symptoms after infection with EV-A71
  - Expensive and ethical constraints.

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2. Selection of viral protein(s) to be encoded in the mRNA vaccine
3. Animal models and limitations
    - Different animal models maybe required for different enteroviruses.
4. Immune/vaccine interference

Immune/Vaccine interference

- Immune/vaccine interference has been observed in multivalent vaccines.
  - Polio vaccine (Payne et. al., Bull World Health Organ 1960)
    - seroconversion rates ≥ 90% for monovalent
    - seroconversion rates for trivalent formulation falls to 68% (type I), 82% (type II), 43% (type 3)
  - Dengue vaccine – vaccine efficacy against different serotype is different (Thomas et. al., NPJ Vaccines 2023)

- What do we know about immune/vaccine interference for enterovirus vaccine?

Potential Immune/Vaccine interference in a multivalent HFMD vaccine

Vaccine	Antigen	Animal model	Description	Reference
Inactivated whole vaccine	EV-A71 and CV-A16	Mouse	Balance immune response	Cai et al. 2014
	EV-A71 and CV-A16	Rhesus macaques	Balance immune response.	Fan et al. 2020
	EV-A71 and CV-A16	Mongolian Gerbil	Better protective response towards EV-A71	Yi et al. 2023
	CV-A6 and CV-A10	Mouse	Balance immune response.	Zhang et al. 2018
	EV-A71, CV-A16, CV-A6	Mouse	Trivalent vaccine protected animals from lethal infection by EV-A71, CV-A16, and CV-A6. Antibody response against CV-A6 was lower in mice vaccinated with trivalent vaccine compared with monovalent.	Caine et al. 2015
	CV-A6, CV-A10, and CV-A16	Mouse	Protection was skewed towards CV-A6 and CV-A10	Lim et al. 2018
Virus-like particles	EV-A71 and CV-A16	Mouse	Balance immune response	Ku et al. 2014
	EV-A71, CV-A6, CV-A10, and CV-A16	Mouse	Balance immune response	Zhang et al. 2018

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3. Animal models and limitations
4. Immune/vaccine interference
    - Potential immune/vaccine interference in a HFMD vaccine
    - Minimize the number of vaccine components included in multivalent vaccine, and aim for best public health impact
5. WHO reference material

WHO reference material – available for EV-A71

Product number	Description
14/140	1st International Standard for Anti EV71 Serum Human
13/238	Anti-EV71 serum LOW (WHO Reference Reagent)
18/116	WHO International Standard Enterovirus 71 (EV71) Inactivated vaccine (Geno Group C4)
18/120	WHO Reference Reagent Enterovirus 71 (EV71) Inactivated Vaccine (Geno Group C4)
18/156	WHO Reference Reagent Enterovirus 71 (EV71) Inactivated Vaccine (Geno Group B4)

- Use as controls for assay development or *in vivo* studies
- Compare the robustness of the mRNA vaccine candidate with the inactivated vaccine

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3. Animal models and limitations
4. Immune/vaccine interference
5. WHO reference material
    - Available for EV-A71, should be used as controls/reference for assay development and *in vivo* studies.
6. Safety and efficacy of mRNA vaccine

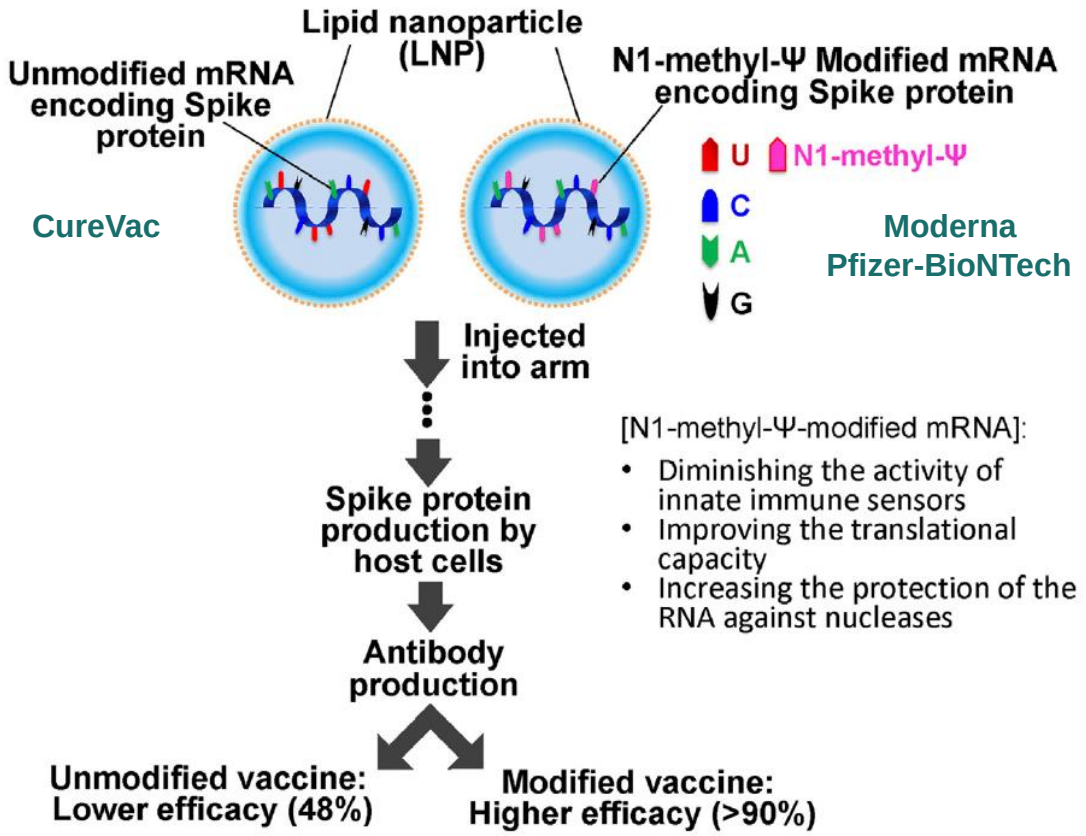
Safety of mRNA vaccine for children

- Target population of the vaccine: children aged 6 months to 2 years
- mRNA vaccine against COVID-19 (Moderna and Pfizer-BioNTech)
    - Safe in children aged 6 months to 2 years (Hause et. al., MMWR Morb Mortal Wkly Rep 2022; Hause et. al., MMWR Morb Mortal Wkly Rep 2023; Tannis et. al., MMWR Morb Mortal Wkly Rep 2023; Munoz et. al., N Engl J Med 2023; Anderson et. al., N Engl J Med 2022)

Covid-19 vaccine	Adult (µg per dose)	Children, 6 months-4 years (µg per dose)
Pfizer/BioNTech	30	3
Moderna	50	25

Elements that may affect the safety and efficacy of mRNA vaccine

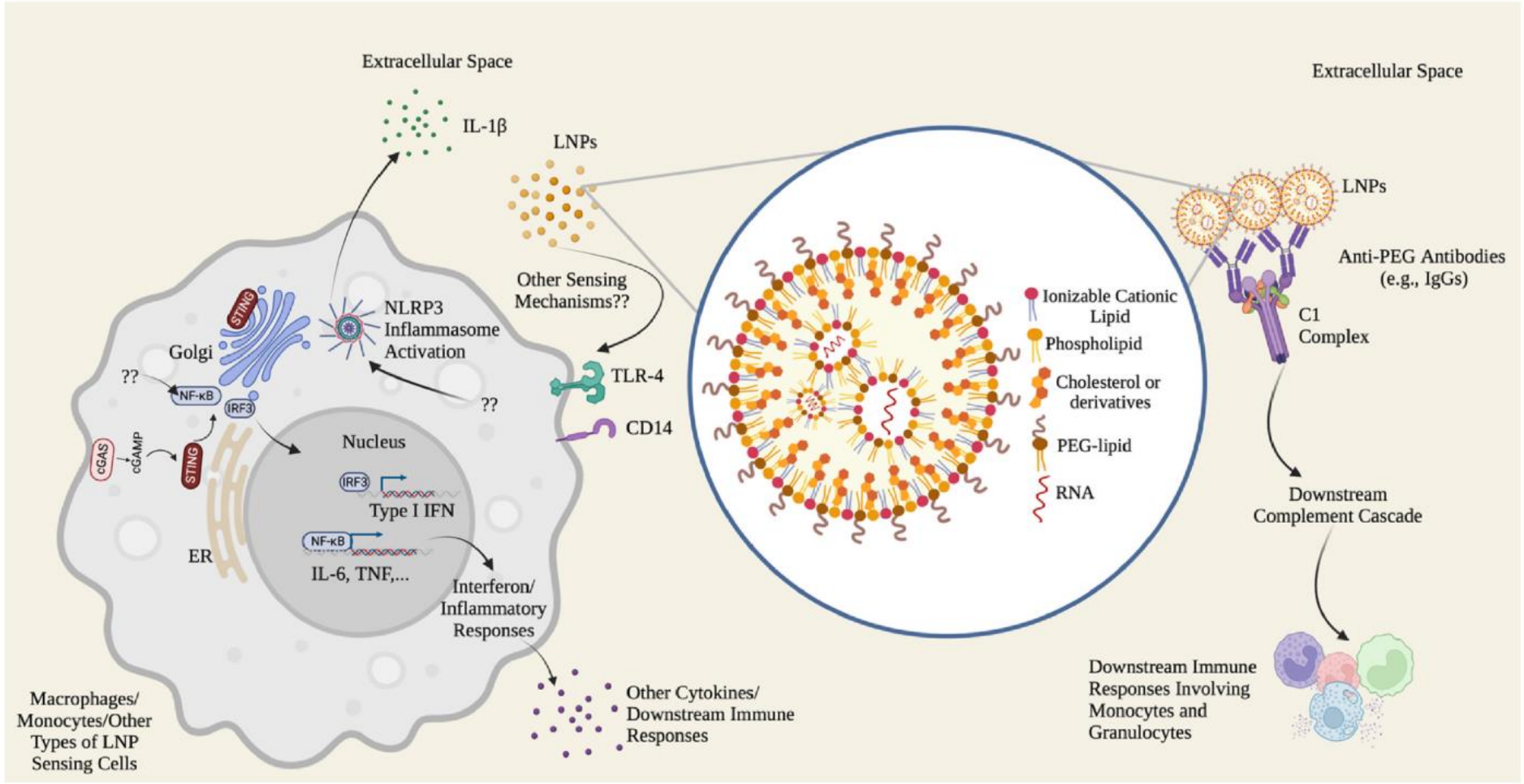
- Modified nucleotides (e.g. pseudouridine)
  - Enhance RNA stability and reduce anti-RNA immune response, thereby increase translation.
  - Incorporated in mRNA vaccine against COVID-19 (Moderna and Pfizer-BioNTech).
  - May affect vaccine efficacy
  - May affect secondary structure of RNA (Internal Ribosomal Entry Site – IRES; Untranslated Region - UTRs)



Morais et. al., Front Cell Dev Biol 2021

Elements that may affect the safety and efficacy of mRNA vaccine

- Lipid nanoparticles (LNPs) formulation



- 4 components
- Immune response
- Critical chosen LNP formulation is safe and effective, especially in children as young as 6 months

Chen and Blakney. Curr Opin Biotechnol 2024

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3. Animal models and limitations
4. Immune/vaccine interference
5. WHO reference material
6. Safety and efficacy of mRNA vaccine – ensure safety especially for children aged ≥ 6 months
7. Storage/transport conditions and stability of the vaccine

Storage/transport condition and stability of the mRNA vaccine

- Current problem with mRNA vaccine: Extreme storage and transport conditions.

	PFizer/-BioNTech COVID-19 vaccine	Moderna COVID-19 vaccine	Ideal
Long term storage condition	– 90°C to – 60°C,	– 50°C to –15°C	2-8°C
Transport condition	– 90°C to – 60°C,	– 50°C to –15°C	2-8°C
Short term storage condition	2-8°C for 10 weeks	2-8°C for 30 days	2-8°C

- HFMD Vaccine will be used in low- and middle-income countries (LMICs), important that the vaccine does not require extreme storage/transport conditions, to ease vaccine distribution and uptake.

Potential approaches to improve on storage/transport conditions

- LNP formulations
  - Evaluate novel LNP formulations or nanoparticles that can be stored/transported at nonfrozen state.
  - Potential risks: (1) Studies are at early stage, safety and biodistribution data is not available; (2) patent and freedom to operate; (3) sourcing of GMP-grade material for manufacturing (timeline)

- Excipients/lyophilization
  - Addition of excipients and/or lyophilizing the mRNA-LNP.
  - Example: addition of calcium-phosphate minerals into lyophilized mRNA-LNP is stable for 6 months at 25°C (Choe et. al., Acta Biomater 2024)
  - Potential risks: (1) Studies are at early stage, safety data is not available; (2) patent and freedom to operate; (3) sourcing of GMP-grade material for manufacturing (timeline)

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5. WHO reference material
6. Safety and efficacy of mRNA vaccine
7. Storage/transport conditions and stability of the vaccine – avoid extreme storage/transport conditions
8. Clinical trials

Clinical trial

- Factors that contribute to the complexity of the clinical trial:
    - Multivalent vaccine – evaluation of different ratios of each vaccine components and dosages to ensure a balance immune response
    - Target population – children as young as 6 months.
    - Preclinical studies are performed in mouse model (most likely), and mice lie
    - Correlates of protection is not available.
- Step-wise approach:
    1. Safety and immunogenicity in healthy adults (schedule and dose-escalation)
    2. Step-wise age de-escalation (healthy adults> adolescence > children (aged 6 to 14) > younger children aged 6 months to 5 years)
    3. Dose review by age group

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6. Safety and efficacy of mRNA vaccine
7. Storage/transport conditions and stability of the vaccine
8. Clinical trials – complex and required step-wise approach

Overview of Consortium Member Institutions

DISCOVERY & PRECLINICAL

- National University of Singapore (NUS)
- Chulalongkorn University (Chula)
- Agency for Science, Technology and Research (A*STAR)
- Hilleman Laboratories

CMC DEVELOPMENT

- Genome Institute of Singapore, GIS (A*STAR)
- Bioprocessing Technology Institute, BTI (A*STAR)
- Hilleman Laboratories

GMP PRODUCTION

- Bioprocessing Technology Institute, BTI (A*STAR)
- Hilleman Laboratories

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138567, Singapore

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