Enterovirus 71 (EV71)

Fondation Merieux Conference -
Asia Pacific Vaccinology 2015
Bangkok, Thailand
30.11.2015 – 3.12.2015

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Enterovirus 71 (EV71)

Electron Micrograph

Crystal Structure

Schematic Diagram
Genome Structure and Encoded Proteins of EV71

Coding Region: polyprotein

- VP0 (VP4 + VP2)
- VP3
- VP1
- 2A
- 3AB
- 2BC
- 3CD
- 2B
- 3A
- 2C
- 3B (VPg)
- 3C
- 3D
Disease and Public Health Burden due to EV71

An important emerging viral disease of children.

Besides causing HFMD.

- Non-specific febrile illness
- “Common cold”
- Exanthema
- Herpangina
- Acute pharyngitis
- Infantile pneumonitis
- Infantile diarrhoea
- Aseptic meningitis
- Acute flaccid paralysis
- Acute Encephalitis
- Acute encephalomyelitis
- +/- pulmonary oedema

Outbreaks of HFMD with neuro-invasion and fatalities.

Asia-Pacific region:
- 1998, 2000/2001 - Taiwan
- 1999 - Perth Australia

China:
- 2008: 488,955 HFMD, 126 Fatalities.
- 2009: 1,155,525 HFMD, 353 Fatalities.
- 2010: 1.7 million HFMD, 905 Fatalities.
Disease Manifestations of EV71

Dermatophilic (Coxsackie virus A16-like):
- HFMD
- Exanthemata
- Enanthem - Herpangina

Neurotrophilic (Poliovirus-like):
- Neck Stiffness (Meninges)
- Acute Paresis/Flaccid Paralysis (Pyramidal nervous system)
- Ataxia, Tremor (Extra-Pyramidal nervous system)
- Acute neurogenic pulmonary oedema (Autonomic system)
Issues, Challenges and Solutions

Animal Model of EV71 Neuro-Infection, Disease and Pathology

EV71 Vaccine
What are the Current Published Animal Models?

**Monkey Models: *Macaca fascicularis*, Rhesus**

- **Intra-spinal Inoculation:**

- **Intravenous Inoculation:**

- **Intracerebral, Intravenous, Respiratory and Oral Routes:**
**What are the Current Published Animal Models?**

### Small Animals: Mouse Models

**Immuno-competent mice** *(BALB/c, C3H/HeN, ICR)*: Intracranial, Intravenous, Intraperitoneal, Oral.


(Age < 14 days, myositis, need > 10^7 pfu/ml)

**Immuno-compromised mice:**

NOD/SCID, A129, AG129 mice.

**Transgenic Mice** *(PSGL-1, SCARB2).*


**Mouse-adaptation in newborn mice:**

**Intracerebral adaptation.**
**Intra-skeletal muscle adaptation.**

Mouse-adaptation in immuno-compromised mice.
None of the EV71 strains is able to replicate in in-vitro culture mouse cells.

Current Published Animal Models

- Lack “Face Validity” (i.e., lack full resemblance to human symptoms). (Fulminant Pulmonary Edema)
- Lack “Construct Validity” (i.e., lack similarity to the underlying cause of the human disease). (Myositis)

None of the EV71 strains is able to replicate in in-vitro culture mouse cells.

TLL mouse model of EV71 infection

EV71:TLLm and EV71:TLLmv - Strains that are able to replicate efficiently in in-vitro culture mouse cells.
Public Health Perspective

- Urgent Need for an Effective and Efficacious Vaccine to Control and Prevent Hand Foot and Mouth Diseases and associated neurological complications.

**EV71 Vaccines Under Research and Development**

- cDNA vaccine.
- Subunit vaccine (VP1 peptide).
- Virus-like particle (VLP)
- **Inactivated enterovirus 71.**
- **Live attenuated enterovirus 71.**
Development of Injectable Inactivated EV71 vaccines
(Based on Salk Inactivated Poliovirus Vaccine Model)
Inactivated EV71 Vaccine Development

1975 Bulgaria, Europe.

Febrile illness: 705 reported cases.

545 (77.3%) cases of aseptic meningitis

149 (21.1%) cases of paralytic disease.

44 fatalities.

(Chumakov et al., Arch Virol 1979; 60: 329-40).
Disease Burden

Outbreak of HFMD and Fatalities in China

2008: China CDC & Office of WHO in China
Cases of HFMD: 488,955
Fatalities: **126**

Cases of HFMD: 1,155,525
Fatalities: **353**

Cases of HFMD: 1.7 million
Severe neurological complication: 27,000
Fatalities: **905**
Development of Injectable Inactivated EV71 vaccines
(based on Salk Inactivated Poliovirus Vaccine Model)

Parental (Injectable) Inactivated EV71 vaccines at various stages of development and clinical trails:

- **Beijing Vigoo, China National Biotech Group (China), EV71-C4 strain.**
- **Sinovac (China), EV71-C4 strain.**
- **Institute of Medical Biology (CAMS) (China), EV71-C4 strain.**
- **National Health Research Institute (Taiwan), EV71-B4 strain.**
- **Inviragen (Singapore), EV71-B2 strain.**
Phase III Clinical Trials (Summary)

<table>
<thead>
<tr>
<th>Vaccine Producer</th>
<th>Beijing Vigoo Biological</th>
<th>Sinovac Biotech</th>
<th>Institute of Medical Biology, CAMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Population</td>
<td>10,245</td>
<td>10,007</td>
<td>12,000</td>
</tr>
<tr>
<td>Age (month)</td>
<td>6 - 35</td>
<td>6 - 35</td>
<td>6 - 71</td>
</tr>
<tr>
<td>Dosing (unit –U)</td>
<td>320</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>Immunization Schedule (day)</td>
<td>0, 28</td>
<td>0, 28</td>
<td>0, 28</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>Alum</td>
<td>Alum</td>
<td>Alum</td>
</tr>
<tr>
<td>Efficacy (against EV71-associated HFMD)</td>
<td>90.0%</td>
<td>94.8%</td>
<td>97.3% - 97.4%</td>
</tr>
</tbody>
</table>
Development of Live Attenuated Oral EV71 Vaccine
(Based on Oral SABIN Poliovirus Vaccine Model)
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(Based on Oral SABIN Poliovirus Vaccine Model)

Hashimoto I. and Hagiwara A.


Key words: enterovirus/virulence/temperature-sensitive mutant/fingerprinting

Isolation of a Temperature-sensitive Strain of Enterovirus 71 with Reduced Neurovirulence for Monkeys

An Attenuated Strain of Enterovirus 71 Belonging to Genotype A Showed a Broad Spectrum of Antigenicity with Attenuated Neurovirulence in Cynomolgus Monkeys

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Development of Oral Combined Live Attenuated Vaccine against HFMD

“18 Years (1998 – 2015) JOURNEY”
HFMD in Malaysia

May 1997; Sibu, Sarawak.

July 1997; FT, Peninsular Malaysia.

Number of cases of HFMD?

41 deaths reported (29 Sarawak, 12 Peninsular Malaysia).

Virus: EV71, CA16.
HFMD in Taiwan (1998)

129,106 reported cases (estimated 10% of total cases).

405 hospitalised for severe disease (80% <= 5 years old).

78 fatalities.

Serious complications: Encephalitis, Encephalitis and pulmonary oedema or haemorrhage, Aseptic meningitis, Myocarditis with encephalitis, Acute flaccid paralysis.

65 (83%) died of Encephalitis with pulmonary oedema or haemorrhage.
Challenges of EV71 Vaccine

Challenges inherent in the viruses causing HFMD:

- HFMD due to a number of enteroviruses, especially CA16 and EV71.
- Genetic and Antigenic Diversity of EV71.
- EV71 undergoes “rapid” Genetic Mutation and Antigenic Drift in the last 16 years.

Challenges inherent in inactivated vaccine:

- Need for parental administration.
- High cost.
- Need for adjuvant with its associated side-effects.
- Reduced “Robustness/Quality” of neutralizing antibodies due to structural changes during the process of inactivation.
- Do not induce gut immunity. Thus, do not prevent silent EV71 infections in intestinal mucosa that lead to transient carriers and shedding of the virus.
- Public Health Perspective - ? Feasibility of Mass Vaccination
Development of Live Attenuated Oral EV71 Vaccine
(Based on Oral SABIN Poliovirus Vaccine Model)

Phenotypic and Genetic
STABILITY and REVERSION
Searching for the

STABLE TEMPERATURE SENSITIVE

EV71 Strain
Mutation Rates

$3 \times 10^{-3}$ to $2 \times 10^{-6}$ ($1.6 \times 10^{-4}$)
Enterovirus Entry, Replication and Morphogenesis

Genome Replication:

5’NC – “cloverleaf”, 3’NC
2BC, 2B, 2C; 3AB, 3A, 3B; 3CD, 3C, 3D
Host cellular protein (Reticulon 3)

Translation (Viral Protein Synthesis):

5’NC-IRES, 2A;
host cellular protein initiation complex, host cellular ribosomes

Attachment, Uncoating and Assembly:

VP1, VP2, VP3; VP1, VP2, VP3, VP4;
VP0, VP1, VP3, VPg, 2C, RNA
Candidate Attenuated EV71 strains: EV71:TLLα, EV71:TLLβ or EV71:TLLγ strains
Candidate Attenuated EV71 strains: 
EV71:TLLα or EV71:TLLβ strains
Phenotypic Stability
Genetic Stability
EV71:TLLβP20

[Stable Cold-Adapted Temperature-Sensitive/Conditional Lethal]

(Patent: PCT/SG2013/000027)
Safety and Immunogenicity: Monkey’s Study

1 monkey: normal control

3 monkeys (2889M, 0791M and 2891F): $10^7$ CCID$_{50}$ of EV71: TLLβP20

3 monkeys (2890M, 2202F and 2247M): $10^8$ CCID$_{50}$ of EV71: TLLβP20
Need to Extend Protection against: other Genotypes of EV71 and CA16
Chimeric Enteroviruses

**EV71:TLLeC5** (PCT/SG2015/050106)

**TLLeCA16** (PCT/SG2015/050106)
Final Candidate Vaccine

An Oral Combined Live Attenuated Vaccine
(EV71:TLLβP20, EV71:TLLeC5, and TLLeCA16)
against Hand Foot and Mouth Disease
due to EV71 and CA16
Future Prospect

Formalin-Inactivated EV71 Vaccine

Implementation of a 2-dose regimen in China with a third booster dose.

Issues Need to be Considered:

- Multinational Efficacy Trials.
- Harmonization and Standardization of Antigen (Virus Strain).
- Co-ordinated Quality Control Reagents and Immunoassays.
- Co-administration of EV71 vaccine with the commercial paediatric pentavalent vaccine.
- A global surveillance: Circulating genotypes/subgenotypes or new serotype emerging from EV71 and CA16.
- HFMD due to CA16 virus.
Future Prospect

An Oral Combined Live Attenuated Vaccine (EV71:TLLβP20, EV71:TLLeC5, and TLLeCA16) against Hand Foot and Mouth Disease due to EV71 and CA16
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- **Public Health Perspective - ? Feasibility of Mass Vaccination**
Acknowledgement

Virologists and Molecular Biologists
Thank You