The Needs and Development of Enterovirus 71 Vaccine
--from Clinical Point of View

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The Need of EV71 Vaccine

The Development of EV71 Vaccine
**Enterovirus 71**

**TAXONOMY**
- ssRNA positive-strand viruses
- Family: *Picornaviridae*
- Genus: *Enterovirus*
- Members: Polio, Echo, Coxackie, rhinovirus

**EVOLUTIONS of VIRUS**
- **Genotype A**: The EV71 prototype strain, BrCr-CA-70, the sole strain of A isolated in 1970
- **Genotype B**: 1972~1988 strains in the United States, and circulate in the other part of the world
- **Genotype C**: contains strains isolated in 1985 or later in the United States, Canada, Australia, and China.

Hand Foot Mouth Disease may also be induced by other Enteroviruses, such as Coxackie A, B viruses.

Mostly are self-limited within one-week disease course.
**EV71 Infection - Neurological Diseases**

- Aseptic meningitis
- Poliomyelitis-like paralysis
- Brainstem encephalitis
- Neurogenic pulmonary edema
- Cerebellar ataxia

- **EV71 infection Morbidity Rate**: 1.5~6.42 (per 1000 people)
- **EV71 infection Mortality Rate**: 0.003~0.2 (per 1000 people)

Indirectly Calculated from Taiwan Epidemiology Bulletin, May 21, Vol 29, No.10, 2013
Trends of Enterovirus during 2007~2012
Occurrence of EV Infections with Severe Complications During 2007~2012

Figure 3. Occurrence of cases of EV infections with severe complications during 2007-2012

Note: The number within the parenthesis represents the number of fatal cases.
Serotypes of Enterovirus infection with severe complications in Taiwan

Data from Taiwan CDC
## Age-Dependent disease severity-EV71

### Table 2. Relative Attack, Case Severity, and Fatality Rates of Hand, Foot and Mouth Disease Disease During 2010a

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Relative Attack Rateb</th>
<th>Relative Case Severity Ratec</th>
<th>Relative Case Fatality Rate(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.5</td>
<td>1.50</td>
<td>5.17</td>
<td>25.23</td>
</tr>
<tr>
<td>0.6–&lt;1</td>
<td>17.54</td>
<td>4.64</td>
<td>19.01</td>
</tr>
<tr>
<td>1–&lt;3</td>
<td>19.97</td>
<td>3.67</td>
<td>9.89</td>
</tr>
<tr>
<td>3–&lt;6</td>
<td>8.95</td>
<td>1.74</td>
<td>3.16</td>
</tr>
<tr>
<td>6–&lt;10(^e)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥10</td>
<td>0.02</td>
<td>0.95</td>
<td>0.57</td>
</tr>
</tbody>
</table>

# Threats of EV71 diseases

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Case Number</th>
<th>Deaths</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>Malaysia</td>
<td>2628</td>
<td>29</td>
<td>B3</td>
</tr>
<tr>
<td>1997</td>
<td>Singapore</td>
<td>39</td>
<td>0</td>
<td>B3, B4, C1, C2</td>
</tr>
<tr>
<td>1998</td>
<td>Taiwan</td>
<td>469</td>
<td>78</td>
<td>B4, C2, C4</td>
</tr>
<tr>
<td>2000</td>
<td>Singapore</td>
<td>81</td>
<td>4</td>
<td>B4</td>
</tr>
<tr>
<td>2000</td>
<td>Taiwan</td>
<td>291</td>
<td>25</td>
<td>B4</td>
</tr>
<tr>
<td>2001</td>
<td>Taiwan</td>
<td>393</td>
<td>27</td>
<td>B4</td>
</tr>
<tr>
<td>2003</td>
<td>Japan</td>
<td>110</td>
<td>0</td>
<td>B4, B5, C2, C4</td>
</tr>
<tr>
<td>2000 - 2003</td>
<td>Malaysia</td>
<td>277</td>
<td>4</td>
<td>B4, B5, C1</td>
</tr>
<tr>
<td>2005</td>
<td>Vietnam</td>
<td>173</td>
<td>3</td>
<td>C5</td>
</tr>
<tr>
<td>2006</td>
<td>Brunei</td>
<td>34</td>
<td>2</td>
<td>B5</td>
</tr>
<tr>
<td>2008</td>
<td>Taiwan</td>
<td>373</td>
<td>14</td>
<td>B5, C4, C5</td>
</tr>
<tr>
<td>2008</td>
<td>China</td>
<td>610</td>
<td>27</td>
<td>C4</td>
</tr>
<tr>
<td>2008</td>
<td>Thialand</td>
<td>23</td>
<td>1</td>
<td>C4</td>
</tr>
<tr>
<td>2009</td>
<td>China</td>
<td>512</td>
<td>-</td>
<td>C4</td>
</tr>
<tr>
<td>2011</td>
<td>China</td>
<td>499</td>
<td>10</td>
<td>C4</td>
</tr>
<tr>
<td>2012</td>
<td>Taiwan</td>
<td>153</td>
<td>2</td>
<td>B5</td>
</tr>
</tbody>
</table>
Estimated need-Birth rate

- 2-dose design for each newborn

China: 16M
Taiwan: 200,000
Vietnam: 1.52M
Thailand: 760,000
Malaysia: 600,000
Singapore: 30,000
The Need of Enterovirus 71 Vaccine

- No effective antiviral drug

- Follow the successful experience of the poliomyelitis control program  

- Current EV71 vaccines do not provide cross-protection  
  against all circulating genetic lineages of EV71.  

- Consequently, it may be necessary to develop multivalent vaccines  
  to ensure that protection is provided against all EV71 strains.  
The Need of EV71 Vaccine

The Development of EV71 Vaccine
### Formalin-inactivated EV71 Whole-virus Vaccine Candidates Currently Being Tested in Clinical Trials

<table>
<thead>
<tr>
<th>Company</th>
<th>Country</th>
<th>Adjuvant</th>
<th>Virus genotype</th>
<th>Cell</th>
<th>Registration status</th>
<th>Clinical Trial.gov No.</th>
<th>Licensing dose (EV71 antigen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC</td>
<td>Taiwan</td>
<td>AlPO₄</td>
<td>B4</td>
<td>Vero</td>
<td>Phase II</td>
<td>NCT02200237</td>
<td>1.25, 2.5, 5μg (by total protein)</td>
</tr>
<tr>
<td>Adimmune</td>
<td>Taiwan</td>
<td>AlPO₄</td>
<td>B4</td>
<td>Vero</td>
<td>Phase II</td>
<td>N.A.</td>
<td>0.25, 0.5, 1, 2, 5μg (by total protein)</td>
</tr>
<tr>
<td>Sinovac</td>
<td>China</td>
<td>Al(OH)₃</td>
<td>C4</td>
<td>Vero</td>
<td>NDA</td>
<td>NCT01507857</td>
<td>400 KU(1μg) (by ELISA)</td>
</tr>
<tr>
<td>Vigoo</td>
<td>China</td>
<td>Al(OH)₃</td>
<td>C4</td>
<td>Vero</td>
<td>NDA</td>
<td>NCT01508247</td>
<td>320 U(0.5μg) (by ELISA)</td>
</tr>
<tr>
<td>CAMS</td>
<td>China</td>
<td>Al(OH)₃</td>
<td>C4</td>
<td>KMB-17</td>
<td>NDA</td>
<td>NCT01569581</td>
<td>100 EU(N/A) (by ELISA)</td>
</tr>
<tr>
<td>Takeda (Inviragen)</td>
<td>Japan (Singapore)</td>
<td>Al(OH)₃</td>
<td>B2</td>
<td>Vero</td>
<td>Phase I</td>
<td>NCT01376479</td>
<td>0.3 and 3μg (by DAFIA)</td>
</tr>
</tbody>
</table>
Evolution of EV71vac®

Initiation of R&D by CDC

- Virus isolate (E59) from a fatal case in Taiwan
- Establishment of virus and Vero cell banks
- Establishment of roller bottle process using serum-contained medium

Tech-transferred to MVC

- Contract-manufacture to NHRI VC
- Carry forward to Phase II clinical trial

Tech-transferred to NHRI

- Establishment of master/working virus seeds and Vero master/working cell banks
- Improvement of roller bottle process and serum-free medium was applied
- Conduct a Phase I clinical trial
B4

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-vaccination</th>
<th>&gt;4-fold increased post 1\textsuperscript{st} vaccination</th>
<th>Neutralization titer against B4 (GMT)</th>
<th>&gt;4-fold increased post 2\textsuperscript{nd}-vaccination</th>
<th>Neutralization titer against B4 (GMT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;1.8</td>
<td>12/13 (92.3%)</td>
<td>210.9</td>
<td>13/13 (100%)</td>
<td>181.3</td>
</tr>
<tr>
<td></td>
<td>≥1.8</td>
<td>15/17 (88.2%)</td>
<td>606.2</td>
<td>17/17 (100%)</td>
<td>777.9</td>
</tr>
<tr>
<td>B</td>
<td>&lt;1.8</td>
<td>13/13* (100%)</td>
<td>259.8</td>
<td>13/14 (92.9%)</td>
<td>175.3</td>
</tr>
<tr>
<td></td>
<td>≥1.8</td>
<td>14/15** (93.3%)</td>
<td>1068.4</td>
<td>14/15** (93.3%)</td>
<td>993.7</td>
</tr>
</tbody>
</table>

*\textit{Nt below detection limit (<1:8) were assigned a value of 1:4 for calculation purposes.}

*One blood sample was misplaced and lost.

**One subject withdrew from the study.

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Immunogenicity-Cross Protection

# Immunogenicity-Cross Protection

<table>
<thead>
<tr>
<th>EV71 virus</th>
<th>Group</th>
<th>Pre-vaccination</th>
<th>Post 1\textsuperscript{st}-vaccination</th>
<th>Post 2\textsuperscript{nd}-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;4-fold increase Nt</td>
<td>Neutralization titer (GMT)</td>
</tr>
<tr>
<td></td>
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<td>Neutralization titer (GMT)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Neutralization titer (GMT)</td>
<td>Neutralization titer (GMT)</td>
</tr>
<tr>
<td>C2</td>
<td>A</td>
<td>&lt;1:8</td>
<td>27/30 (90.0%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1:8</td>
<td>3/30 (10.0%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>&lt;1:8</td>
<td>30/30 (100%)</td>
<td>1/28\textsuperscript{a, b} (3.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1:8</td>
<td>0/30 (0%)</td>
<td>0/0 (0%)</td>
</tr>
<tr>
<td>CA16</td>
<td>A</td>
<td>&lt;1:8</td>
<td>27/30 (90.0%)</td>
<td>7/27 (25.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1:8</td>
<td>3/30 (10.0%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>&lt;1:8</td>
<td>22/30 (73.3%)</td>
<td>3/21\textsuperscript{a} (14.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥1:8</td>
<td>8/30 (26.7%)</td>
<td>1/7\textsuperscript{a} (14.3%)</td>
</tr>
</tbody>
</table>

Nt below detection limit (<1:8) were assigned a value of 1:4 for calculation purposes.

\textsuperscript{a}One blood sample was misplaced and lost.

\textsuperscript{b}One subject withdrew from study.

doi:10.1371/journal.pone.0079783.t002
Medigen Vaccinology Corp. (MVC)

A partner of Akzo Nobel for “Flu Vaccine” BOO project in Taiwan

Vaccine business formed according to H1N1 pandemic

NT$32.4M obtained from MOEA for the development of cell-based H1N1 vaccine

MDCK & H5N1 cell technology licensed from NHRI

H5N1 Phase I study obtained approval from TFDA and NTUH.

Vaccine business spun off as Medigen Vaccinology Corp.

Groundbreaking for Factory at Hsinchu

EV71 cell technology & Ph-I results licensed from NHRI

EV71 Phase II obtained approval from TFDA

EV71 vaccine

2014.10

2014.01

2013.06

2012.10

2012.08

2010.08

2009.12

2009.09

2006

TideCell system

Clinical trial network

Cell-based technology

H1N1

SBC

Cell-based technology

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A partner of Akzo Nobel for “Flu Vaccine” BOO project in Taiwan
Core Capability

Cell-based technology
- Cell culture
- Virus propagation
- Process development
- Assay development

CGMP manufacturing
- Single use system
- Quality assurance
- Quality control
- Process validation
- Antigen/biologics production
- Fill & Finish

Vaccine development
- Clinical trials
- Product registration
- ICH and GCP experience

CONFIDENTIAL
Production systems

- Roller bottles & bioreactors (Tide-cell & Biostat)
The “Tide” Principle

Fresh and conditioned air are exchanged efficiently by “Tide motion” through 0.22 um filter

A relative movement between culture medium and matrix ensures a sufficient supply of nutrient and oxygen

- Low cell stress (No shear force)
- Minimum bubble induced cell damages
- High cell density growth
- High yields

Cells reside at the matrix carrier are not disturbed by the tide motion of the medium
Productivity

20,000x Roller Bottles in One 100 L Matrix Vessel

20,000
Applications of Cell-Based Technology

MVC’s Cell-Culture Technology

- Mock-up vaccine (H5N1/H7N9)
- EV71 vaccines, Dengue
- Seasonal flu vaccines (H1N1, H3N2, B)
- Other antigens for emergency production
- Biologics and protein drugs
Developing Products

**EV71 vaccine**

*Preventive vaccine against EV71-associated disease*
- **Product name:** EV71vac
- Vaccine strain: EV71 B4 subtype
- Product Type: inactivated, whole virus with adjuvant
- Stage: Phase II

**Pandemic flu vaccine**

*Preventive vaccine against pandemic: AT-101*, AT-301, AT-501
- **Product name:** AT-301
  - Vaccine strain: H5N1 RG-14
  - Product Type: inactivated, whole virus with adjuvant
  - Stage: Phase I completed.
- **Product name:** AT-501
  - Vaccine strain: H7N9 RG-268
  - Product Type: inactivated, whole virus with/without adjuvant
  - Stage: Phase I/II

* H1N1 became one of the seasonal flu strains since 2010.
Factory

- Cell-based technology
- Multifunctions
- PIC/S GMP standard

Jan-7-2015
Functions

Multifunction & Compact

• Floor area: 2740m²
• Main structure (4F+1B):
  • 1F: Drug substance (single-use system)
    ✓ Viral antigen
    ✓ Biologics
  • 3F: Drug product (Vials/PFS)
  • 4F: QC & RD
  • Others: Warehouse/QA/Office
Clean Utility: BOSCH, Pharmatec

Purified Water Generation Unit PW1100H

(WFI) Multiple-Effect Distillation Unit 500-S4

Pure Steam Generator PSG350
Filling capacity

BAUSCH+STROBEL, SFM5110

The flexible SFM 5110 can process various kinds of glass containers supplied in nests

<table>
<thead>
<tr>
<th>Capacity</th>
<th>Prefilled Syringe</th>
<th>Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 shift</td>
<td>14,490,000/ yr</td>
<td>5,071,500/ yr</td>
</tr>
<tr>
<td>2 shifts</td>
<td>28,980,000/ yr</td>
<td>10,143,000/ yr</td>
</tr>
<tr>
<td>3 shifts</td>
<td>43,470,000/ yr</td>
<td>15,214,500/ yr</td>
</tr>
</tbody>
</table>

* 1 shift: 6hr/day, 21days/month, 11.5months/yr
## EV71vac-Phase II Study Design

<table>
<thead>
<tr>
<th>Part</th>
<th>Age</th>
<th>Number of Subjects</th>
<th>Total No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>6 -&lt;12yr</td>
<td>LD (1.25µg)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD (2.5µg)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HD (5µg)</td>
<td>45</td>
</tr>
<tr>
<td>2b</td>
<td>2 - &lt;6yr</td>
<td>Vaccine 30</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaccine 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo 10</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>6m -&lt;2yr</td>
<td>Two dosages will be decided by DSMB. The subjects will be randomized to receive either one of the two dosages or placebo in a ratio of 2:2:1.</td>
<td>100</td>
</tr>
<tr>
<td>2d</td>
<td>2m -&lt;6m</td>
<td>Two dosages will be decided by DSMB. The subjects will be randomized to receive either one of the two dosages or placebo in a ratio of 2:2:1.</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>365</td>
</tr>
</tbody>
</table>
THANK YOU!

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