Medical Countermeasures against Smallpox and Botulism

HIROYUKI YOKOTE

The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
1. Briefing of KAKETSUKEN
2. Attenuated Smallpox Vaccine, LC16m8
3. Botulism Antitoxin, Tetravalent (Type A, B, E and F)
Mission
Contribute to human health and the prevention and treatment of illness and infectious diseases through the development and supply of biomedical products.
KAKETSUKEN
(The Chemo-Sero-Therapeutic Research Institute)

◆ Type of Organization
  ➢ Japanese General Incorporated Foundation

◆ Founding
  ➢ founded in Kumamoto, Japan in December 1945

◆ Total employees
  ➢ 1,826 employees (April 2013)

◆ Sales

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of Products</th>
<th>Major Products</th>
<th>Sales (FY2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Vaccine Antitoxins</td>
<td>15</td>
<td>Flu, DPT-IPV, JE, HB, HA, Rabies, Smallpox etc.</td>
<td>US$ 216M</td>
</tr>
<tr>
<td>Veterinary Vaccine</td>
<td>48</td>
<td>Flu, JE, Rabies, ACME, ART2 etc.</td>
<td>US$ 37M</td>
</tr>
<tr>
<td>Blood Plasma Products</td>
<td>20</td>
<td>Albumin, Bolheal, IVIG etc.</td>
<td>US$ 133M</td>
</tr>
</tbody>
</table>

Total: US$ 408M
1. Briefing of KAKETSUKEN
2. Attenuated Smallpox Vaccine, LC16m8
3. Botulism Antitoxin, Tetravalent (Type A, B, E and F)
Smallpox

・Pathogen: Variola virus

・Infectious Route: Host is only humans
   Aerial infection, droplet infection, contagious infection in humans

・Incubation Period: 7-14 days (usually 12-14 days)

・Symptoms: 1. Rash to emerge on face 2-4 days after fever and muscle pain
  2. Rash to spread from face to arms and legs and then to the hands and feet
  3. Synchronous progress of symptoms
     Papules - Vesicles - Pustules

・Fatal rate: 30% or more (variola major)
Why now smallpox?

• Ken Alibek, a former deputy director of the Soviet Union's civilian bioweapons program reported that the USSR successfully achieved mass production of smallpox virus and application of the virus to bombs and intercontinental ballistic missiles after 1980. They also established their capacity to manufacture many tons of smallpox virus per year.

• It is concerned that smallpox virus and mass-production technology are transferred to the third persons/countries due to declined financial support.

JAMA 1999; 281: 2127-2137
## Smallpox Vaccine

<table>
<thead>
<tr>
<th>Generation</th>
<th>Vaccine/Strain</th>
<th>Production</th>
<th>Virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st})</td>
<td>Lister, EM63, Dryvax (NYCBH), Ikeda, Dairen1</td>
<td>Calf Lymph</td>
<td>Strong</td>
</tr>
<tr>
<td>2(^{nd})</td>
<td>ACAM2000 (NYCBH)</td>
<td>Cell culture</td>
<td>Strong</td>
</tr>
<tr>
<td>3(^{rd})</td>
<td>LC16m8 MVA</td>
<td>Cell culture</td>
<td>Weak</td>
</tr>
</tbody>
</table>
On January 13, 2009, a healthy service member aged 20 years received a primary smallpox vaccination (ACAM2000 [Acambis, Inc., Cambridge, Massachusetts]) in accordance with the U.S. Department of Defense smallpox vaccination policy.

On January 28, after transfer to a U.S. Navy tertiary-care facility, he was diagnosed with AML M0.

The results of the diagnostic testing combined with the patient's medical history met the PV level 1 case definition as defined by the Brighton Collaboration and the confirmed case definition as described by CDC surveillance guidelines.

The patient described in this report received VIGIV in the amount originally estimated to treat 30 persons.
LC16m8 derived from the parent strain, Lister/Elstree through 53 serial passages in primary rabbit kidney cells, resulting in a temperature-sensitive, small-plaque phenotype. The primary mechanism of attenuation is due to an one-base deletion in $B5R$, a membrane protein gene.

LC16m8 was widely used in Japan in the 1970s, including 90,000 children, with no serious adverse events.

The safety and efficacy of LC16m8 were proven through detailed observation of approx. 40,000 subjects in the clinical research.

LC16m8 is a smallpox vaccine licensed in Japan in 1975.
### Background of LC16m8 Clinical Research

Refer to Hirayama M.: Research Summary of an Attenuated LC16m8 Smallpox Vaccine by the Smallpox Vaccine Research Committee (in 1973~75).

- **n= total >90,000 infants (0~13 years old, all naïve)
- Vaccination: 1-dose, ~10^8 pfu/mL delivered via scarification (2x10^5 pfu/dose)**

#### 9,538 subjects

<table>
<thead>
<tr>
<th>Efficacy evaluation (n=9,538)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Take</strong></td>
<td>9,075</td>
<td>95.2</td>
</tr>
</tbody>
</table>

#### Immunogenicity evaluation

<table>
<thead>
<tr>
<th>Vaccine Strain</th>
<th>n</th>
<th>AVE. NT (4X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC16m8</td>
<td>97</td>
<td>2.5</td>
</tr>
<tr>
<td>Lister</td>
<td>12</td>
<td>2.4</td>
</tr>
</tbody>
</table>

#### 30,466 subjects

<table>
<thead>
<tr>
<th>Efficacy evaluation (n=30,466)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Take</strong></td>
<td>29,712</td>
<td>97.5</td>
</tr>
</tbody>
</table>

#### Safety evaluation (n=9,538)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (&gt;37.5°C)</td>
<td>663</td>
<td>7.7</td>
</tr>
<tr>
<td>Satellite Vesiculation</td>
<td>28</td>
<td>0.29</td>
</tr>
<tr>
<td>Auto Inoculation</td>
<td>9</td>
<td>0.09</td>
</tr>
<tr>
<td>Postvaccinal Exanthem</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>Temporary benign febrile convulsions</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>Eczema Vaccinatum</td>
<td>1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

#### Safety evaluation (n=30,466)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satellite Vesiculation</td>
<td>97</td>
<td>0.3</td>
</tr>
<tr>
<td>Auto Inoculation</td>
<td>33</td>
<td>0.1</td>
</tr>
<tr>
<td>Temporary benign febrile convulsions</td>
<td>14</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Currently being stockpiled against Bioterror threat

Smallpox vaccination program applied to the members of Japan Self-Defense Forces to be dispatched to overseas
Clinical and Immunological Response to Attenuated Tissue-Cultured Smallpox Vaccine LC16m8

Tomoya Saito, MD, PhD
Tatsuya Fujii, MD
Yasuhiro Kanatani, MD, PhD
Masayuki Sajo, MD, PhD
Shigeru Morikawa, DVM, PhD
Hiroyuki Yokota, MS
Tsunomu Takeuchi, MD, PhD
Noriyuki Kawahara, MD, PhD

THE THREAT OF SMALLPOX bioterrorism has prompted reconsideration of the need for smallpox vaccination.1-3 Serious adverse events associated with first-generation vaccines such as the New York City Board of Health (Dryvax; Wyeth, Madison, New Jersey), Lister, and Ikeda strains have raised obstacles to vaccination campaigns in the United States.4-6 Second-generation vaccines such as ACAM2000 (Acambis, Cambridge, Massachusetts) that use a first-generation seed virus but are grown in tissue culture are also usually accompanied by a high frequency of adverse events.7-10 Developing a vaccine that is safer than first-generation vaccines yet highly immunogenic is crucial.

Context The attenuated, tissue-cultured, third-generation smallpox vaccine LC16m8 was administered to vaccinia-naive infants in Japan during the 1970s without serious adverse events. It is a good candidate for use as part of a prevention plan for bioterrorism.

Objective To assess the immunogenicity and frequency of adverse events of LC16m8 vaccine in unvaccinated and previously vaccinated adults.

Design, Setting, and Participants Between 2002 and 2005 we vaccinated and revaccinated 1529 and 1692 adults, respectively, in the Japan Self-Defense Forces with LC16m8 vaccine, given intradermally using a bifurcated needle. Vaccines were examined 10 to 14 days after vaccination to determine if they had developed a major skin reaction (“take”). Neutralizing antibody responses among 200 participants were assessed using a plaque-reduction neutralization test 30 days postvaccination. We monitored vaccinees for adverse events for 30 days postvaccination.

Main Outcome Measures Documentation of a vaccine take, presence of neutralizing antibody response, and frequency of adverse events.

Results The proportions of take in vaccinia-naive and previously vaccinated individuals were 1443 of 1529 (94.4% [95% confidence interval [CI], 93.2%-95.9%] and 1465 of 1692 (86.6% [95% CI, 85.0%-88.2%], respectively. Seroconversion or an effective booster response among the individuals with take was elicited in 37 of 41 (90.2% [95% CI, 81.2%-99.3%]) vaccinia-naive participants and in 93 of 156 (60.0% [95% CI, 52.3%-67.7%]) previously vaccinated participants. One case of allergic dermatitis and another of erythema multiforme, both of which were mild and self-limited, were suspected to be caused by vaccination. No severe adverse events were observed.

Conclusion Administration of an attenuated tissue-cultured smallpox vaccine (LC16m8) to healthy adults was associated with high levels of vaccine take and seroconversion in those who were vaccinia-naive and yielded an effective booster response in some previously vaccinated individuals.
## Immunogenicity Evaluation

### Take rate and PRNT

#### Take rate

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Primary Vaccinee (N=1529)</th>
<th>Re-vaccinee (N=1692)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Take (95% CI)</td>
<td>94.4 (93.2 ~ 95.9)</td>
<td>86.6 (85.0 ~ 88.2)</td>
</tr>
</tbody>
</table>

#### LC16m8-based PRNT

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Primary vaccinee (N=41)</th>
<th>Re-vaccinee (N=155)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-LC16m8 GMT (95%CI) (Pre-vaccination)</td>
<td>6.1 (4.4 ~ 8.5)</td>
<td>21.0 (17.4 ~ 25.3)</td>
</tr>
<tr>
<td>Anti-LC16m8 GMT (95%CI) (Post-vaccination)</td>
<td>112 (71.4 ~ 175.7)</td>
<td>137.3 (110.7 ~ 170.3)</td>
</tr>
<tr>
<td>% Seroconversion or effective boosting (95%CI) (Post/Pre ≥4)</td>
<td>90.2 (81.2 ~ 99.3)</td>
<td>60.0 (52.3 ~ 67.7)</td>
</tr>
</tbody>
</table>
## Minor Adverse Events

<table>
<thead>
<tr>
<th>AE</th>
<th>LC16m8 Primary (N =491)</th>
<th>LC16m8 Revaccinee (N=575)</th>
<th>ACAM2000* Primary (N =873)</th>
<th>ACAM2000* Revaccinee (N =1371)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Axillary lymph node swelling</strong></td>
<td>76 15.5</td>
<td>20 3.5</td>
<td>494 57</td>
<td>261 19</td>
</tr>
<tr>
<td><strong>Fever (&gt;37.5°C)</strong></td>
<td>13 2.6</td>
<td>8 1.4</td>
<td>276 32</td>
<td>271 20</td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td>5 1.0</td>
<td>0 0</td>
<td>433 50</td>
<td>437 32</td>
</tr>
<tr>
<td><strong>Skin itching</strong></td>
<td>4 0.8</td>
<td>3 0.5</td>
<td>804 92</td>
<td>1130 82</td>
</tr>
<tr>
<td><strong>Injection site pain</strong></td>
<td>0 0</td>
<td>0 0</td>
<td>582 67</td>
<td>505 37</td>
</tr>
</tbody>
</table>

*VRBPAC Briefing Document*
Can LC16m8, an attenuated smallpox vaccine be an effective countermeasure against bio-terrorism with smallpox virus?
# Efficacy

## Immunity Induction in a short time

### Mouse WR challenge study

<table>
<thead>
<tr>
<th>WR Challenge timing</th>
<th>Vaccination</th>
<th>Survival ratio</th>
<th>Mean survival time (days)</th>
<th>Log rank test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 2</strong> Post-immunization</td>
<td>Non-immunized</td>
<td>0/6</td>
<td>9.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>LC16m8</td>
<td>6/6</td>
<td>&gt;14</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>Lister</td>
<td>6/6</td>
<td>&gt;14</td>
<td>P=0.001</td>
</tr>
<tr>
<td><strong>Day 4</strong> Post-immunization</td>
<td>Non-immunized</td>
<td>0/6</td>
<td>7.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>LC16m8</td>
<td>6/6</td>
<td>&gt;14</td>
<td>P=0.001</td>
</tr>
<tr>
<td></td>
<td>Lister</td>
<td>6/6</td>
<td>&gt;14</td>
<td>P=0.001</td>
</tr>
</tbody>
</table>

**Design:** Mouse: BALB/c, Female

**Vaccination dose:** $2.5 \times 10^5$ PFU, single dose

**Challenge timing:** 2, 4 days P.I

**WR challenge dose:** $1 \times 10^5$ PFU, IN
Efficacy

Long Lasting Protective Immunity

Mouse WR challenge study

Design: Mouse: BALB/c, Female
Vaccination dose: 2.5x10^5PFU, single dose
Challenge timing: 3, 8, 12, 24 weeks & 1 year P.I.
WR challenge dose: 1x10^6PFU, IN

- 3 weeks P.I.
- 8 weeks P.I.
- 12 weeks P.I.
- 24 weeks P.I.
- 1 year P.I.

- Non-immunized
- LC16m8
- Lister
The licensed smallpox vaccine, ACAM2000, is a cell culture derivative of Dryvax. Both ACAM2000 and Dryvax are administered by skin scarification and can cause progressive vaccinia, with skin lesions that disseminate to distal sites. We have investigated the immunologic basis of the containment of vaccinia in the skin with the goal to identify safer vaccines for smallpox. Macaques were depleted systemically of T or B cells and vaccinated with either Dryvax or an attenuated vaccinia vaccine, LC16m8. B cell depletion did not affect the size of skin lesions induced by either vaccine. However, while depletion of both CD4+ and CD8+ T cells had no adverse effects on LC16m8-vaccinated animals, it caused progressive vaccinia in macaques immunized with Dryvax. As both Dryvax and LC16m8 vaccines protect healthy macaques from a lethal monkeypox intravenous challenge, our data identify LC16m8 as a safer and effective alternative to ACAM2000 and Dryvax vaccines for immunocompromised individuals.
Objective:

To compare the safety of LC16m8 to that of NYCBH (Dryvax) in immunologically compromised and healthy macaques.

Study design:

Group 1

α-CD4 D-7
α-CD8 D-4

LC16m8, D0

Group 2

α-CD4 D-7
α-CD8 D-4

Dryvax(NYCBH), D0

Sacrifice

Group 3

LC16m8, D0

Sacrifice

Group 4

Dryvax(NYCBH), D0

Sacrifice
Safety

Applicable to a Wide Range of People

Immunocompromised Monkey Study

LC16m8

Dryvax®(NYCBH)

D0  D10  D16  D27

Image size w-4cmXh-3cm

Dryvax® (NYCBH) -aCD4/CD8tx

D0  D10  D16  D27

Dryvax® (NYCBH) -aCD4/CD8tx

LC16m8αCD4/8

DryvaxαCD4/8

Size of Primary Lesions (Area cm²)

Days post vaccination
Safety
Applicable to a Wide Range of People
Immunocompromised Monkey Study

LC16m8
αCD4 αCD8, Day 14

Vaccination site
(Between the scapula)

Lesion Dissemination
(Right axillary skin)

Dryvax (NYCBH)
αCD4 αCD8, Day 14
Answer

It is greatly expected that LC16m8 will be an effective medical countermeasure against bio-terrorism with smallpox virus.
Production of LC16m8 Manufacturing Facility

LC Building
KAKETSUKEN recommends LC16m8 to the government as a national stockpile vaccine for five reasons as the following.

- LC16m8 has a high level of efficacy comparable to that of NYCBH (ACAM2000) as well as a high level of safety comparable to that of MVA.
- Since LC16m8 is a freeze-dried vaccine, it maintains high stability for a long time.
- Only a single shot is effective. (Bifurcated needle delivery)
- The inoculation method is easy.
- Visual recognition of scab makes “Take” judgment easy.
1. Briefing of KAKETSUKEN
2. Attenuated Smallpox Vaccine, LC16m8
3. Botulism Antitoxin, Tetravalent (Type A, B, E and F)
Non-proprietary name: Freeze-dried botulism antitoxin

Brand name: Freeze-dried botulism antitoxin for injection “KAKETSUKEN”

Components
Active ingredients: Botulism antitoxin (horse immunoglobulin)
Multivalent (dissolve in 20ml of the diluent)
- Type A antitoxin No less than 500 U/mL
- Type B antitoxin No less than 500 U/mL
- Type E antitoxin No less than 500 U/mL
- Type F antitoxin No less than 200 U/mL

Storage: Store with protection from light at below 10°C.

Shelf-life: 10 years
Botulism Antitoxin (Multivalent)

【Effect/Efficacy】
・Treatment and prevention of botulism
・Botulism antitoxin (type E) is used when it is apparent that the poisoning is caused by type E botulism toxin.

【Dosage and Administration】

<Treatment>
It depends on the symptoms, but the usual method of immunization is to administer 20~40 mL intramuscularly (subcutaneously) or intravenously. Or administer intravenously diluted in saline solution for intravenous infusion as early as possible.
If symptoms are still not reduced, additionally administer 20 mL or more at an interval of 3~4 hours.

<Prevention>
To those who has eaten food suspected of being infected with botulinum, administer 5~10 mL intramuscularly (subcutaneously) or intravenously as early as possible.
KAKETSUKEN has technology, confidence, experience based on our long history of vaccine manufacturer in Japan.

Attenuated Smallpox Vaccine, LC16m8 has been stockpiled in Japan, and it is suitable as a countermeasure against bioterrorism because of the following features; the efficacy, safety, stability, administration by the bifurcated needle, and it produces a major cutaneous reaction “take.”

Botulism Antitoxin (types A, B, E & F) has been stockpiled in Japan, and it is suitable as a countermeasure against bioterrorism because of the efficacy, safety and stability.

KAKETSUKEN is confident that we can contribute to the establishment of countermeasures against bioterrorism in Asian countries by supplying smallpox vaccine and botulism antitoxin.
Thank you very much for your attention!

Contact information:
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International Strategy Department,
KAKETSUKEN