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Freeze-dried live attenuated smallpox vaccine prepared in cell culture "LC16-KAKETSUKEN": Post-marketing surveillance study on safety and efficacy compliant with Good Clinical Practice

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ABSTRACT

Background: In Japan, production of smallpox vaccine LC16m8 (named LC16-KAKETSUKEN) was restarted and was determined to be maintained as a national stockpile in March 2002.
Objective: To conduct a post-marketing surveillance study of the vaccination of freeze-dried live attenuated smallpox vaccine prepared in cell culture LC16-KAKETSUKEN using attenuated vaccinia strain LC16m8. The study complied with Good Clinical Practice, focusing on a comparison between primary vaccinees and re-vaccinees.
Method: 268 personnel (261 males and 7 females) of the Japan Ground Self-Defense Force were inoculated with LC16-KAKETSUKEN and thereafter adverse events and efficacy were evaluated.
Results: Among 268 vaccinee participants, the following vaccinees showed adverse events, none serious: 53 of 196 primary vaccinees (without previous smallpox vaccination), 4 of 71 re-vaccinees (with previous smallpox vaccination) and 1 vaccinee with unknown previous vaccination history. A breakdown of adverse events observed in this study (total 268 vaccinees) showed the following minor or mild adverse events: 52 (19.4%) swelling of axillary lymph node, 4 (1.5%) fever, 2 (0.7%) fatigue, 1 (0.4%) of rash, 14 (5.2%) erythema at the inoculation site, 1 (0.4%) swelling at the inoculation site and 1 (0.4%) autoinoculation. The incidence of adverse events for primary vaccinees (53/196; 27.0%) was significantly higher than for re-vaccinees (4/71; 5.6%). However, the proportion of vaccine take was significantly higher for primary vaccinees (185/196; 94.4%) than for re-vaccinees (58/71; 81.7%). Although the proportion of vaccine take of re-vaccinees was significantly lower than for primary vaccinees due to preexisting immunity by previous vaccination, no significant difference was found in neutralizing antibody titers between primary vaccinees and re-vaccinees at 1, 4 and 7 months after LC16-KAKETSUKEN vaccination.
Conclusion: The present post-marketing surveillance study compliant with Good Clinical Practice demonstrated the efficacy and safety of the smallpox vaccine LC16-KAKETSUKEN in an adult population. LC16-KAKETSUKEN is the sole currently available licensed smallpox vaccine for both adult and pediatric populations.

1. Introduction

In the early 1970s, Hashizume et al. [1,2], then at the Chiba Serum Institute (CSI), Chiba, Japan, developed an attenuated tissue-cultured smallpox vaccine, LC16m8, from the Lister (Elstree) original strain that was used worldwide in the World Health Organization (WHO) smallpox eradication program [1,2]. LC16m8 was vaccinated into more than 50,000 children in Japan during 1973–1974, without showing problematic adverse events, such as postvaccinal encephalitis, progressive vaccinia, and skin complications, including autoinoculation, postvaccinal exanthems and eczema vaccinatum. Based on these studies, the vaccine strain LC16m8 was licensed in 1975 in Japan, and its freeze-dried vaccine preparation was also licensed in 1980. Because of the success of

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the smallpox eradication program, and followed by the declaration of its success by WHO in 1980, a regular vaccination program was ended in 1976 and smallpox vaccination was legally abolished in 1980 in Japan. Therefore, the LC16m8 strain is unlikely to show its effectiveness against endemic smallpox. However, the situation surrounding smallpox vaccination has changed dramatically after the incidents of September 2001 in the U.S.A., because concern about bioterrorism has risen. Among many pathogens, variola virus is one of the most feared.

Under such a situation, serious attempts have been made, both at home and abroad, to restart the development of smallpox vaccines with lower virulence compared with conventional vaccines. A vaccinia ACAM1000 clone established using cell cultures from the Dryvax vaccine may induce myocarditis [3,4]. Modified vaccinia virus Ankara and NYVAC (modified Copenhagen strain) replication-incompetent viruses are certainly safer, but may require high vaccine doses, multiple doses or boosting with replication-competent vaccines [5,6]. LC16m8 vaccine has drawn renewed attention as a promising vaccine against bioterrorism because it is a highly attenuated live vaccine prepared in cell cultures. Of note, a 2013 WHO Strategic Advisory Group of Experts Meeting on Immunization recommended both licensed ACAM2000 (2nd generation vaccine) and LC16m8 (3rd generation vaccine) as preferred WHO stockpile vaccines [7].

The history of LC16m8 vaccine after its licensing in 1975 in Japan is briefly as follows. Kaketsuken started in March 2002 in Japan to manufacture national stockpile vaccine recently named LC16-KAKETSUKEN as a medical countermeasure against bioterrorism as a result of the incident of September 11, 2001. Kaketsuken is manufacturing LC16-KAKETSUKEN from the master virus seed (MVS) transferred from CSI by using the modified manufacturing procedure of CSI as described in Section 3. Its efficacy and safety was shown in mice, rabbits and monkeys [8–13]. A study on the safety and efficacy in adult populations was done by using the vaccine manufactured by CSI (licensed LC16m8 vaccine, lot No. Chiba 02): the LC16m8 vaccine was inoculated from 2002 into personnel of the Japan Ground Self-Defense Force (JGSDF), showing its good efficacy and safety [14].

The series of clinical studies of this post-marketing surveillance (PMS) study were done under Good Clinical Practice (GCP) compliant conditions to confirm the safety and immunogenicity of LC16-KAKETSUKEN in members of JGSDF with detailed background information and with an extended follow up of neutralizing antibody titers until 7 months after vaccination. Clinical data on adult populations in the Package Insert of LC16-KAKETSUKEN were also used.

2. Material and methods

This study was done from June 6, 2005, through to March 31, 2010, under the initiative of principal investigator Tatsuya Fujii, MD in the Department of Internal Medicine, Self-Defense Forces Central Hospital.

2.1. Vaccine and vaccination

LC16-KAKETSUKEN vaccine, a freeze-dried live attenuated smallpox vaccine prepared in cell culture, containing a suspension of greater than 1×10^8 pock-forming units (pfu)/mL of the LC16m8 strain as the only virus component, was used. The process of establishing LC16-KAKETSUKEN vaccine by modifying the method of CSI is described in Section 3. This vaccine was reconstituted with 0.5 mL of the packaged vaccine diluent (water for injection, containing 20, volume per volume % of glycerin) and 0.01 mL was inoculated into the skin. Inoculation was done by physicians who received the instructions and training of the vaccination method by using designated bifurcated needles. Pressure pricking (puncture) was done 5 times for primary vaccinees (without previous smallpox vaccination) and 10 times for re-vaccinees (with previous smallpox vaccination).

2.2. Participants

The Self-Defense Forces Central Hospital planned and organized the smallpox vaccination program for selected personnel in the JGSDF. Participants were JGSDF personnel who were scheduled for deployment in International Peacekeeping Operation activities of the United Nations Disengagement Observer Force [15], except for personnel who did not comply with the implementation guidance of smallpox vaccine inoculation of JGSDF and personnel who were described as ineligible for vaccination or as being contraindicated according to the Package Insert of the LC16-KAKETSUKEN. The following items were confirmed before vaccine inoculation: no abnormality in the blood, biochemical and urea tests, negativity of hepatitis B virus antigens and hepatitis C virus antibodies, and no infection with human immunodeficiency virus. Vaccine inoculation was contraindicated for participants who were pregnant, under immunosuppression or with eczema, and participants who receive live vaccine within 30 days. The participants were 268 volunteers who were vaccinated with LC16-KAKETSUKEN in this PMS study. All the participants were given instructions to avoid contact with pregnant women and newborn babies within one month after

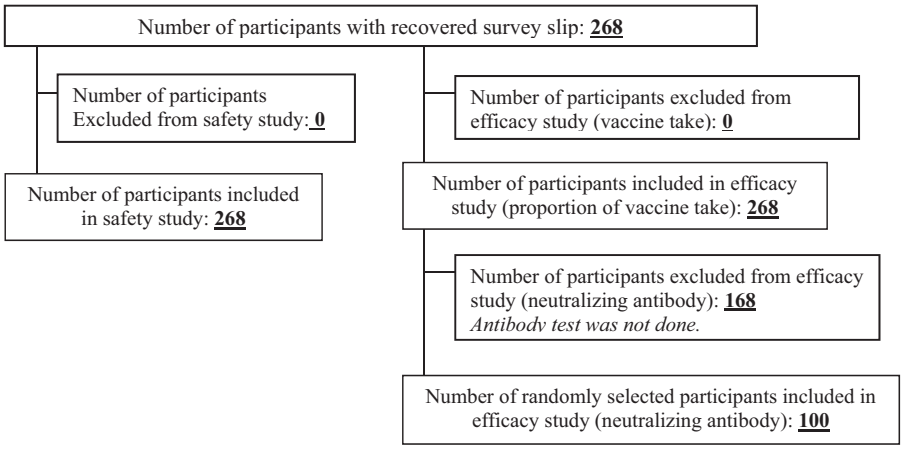

Fig. 1. Flow of participants through the study. This figure shows the flow of participants in safety and efficacy studies. Efficacy was evaluated by the proportion of vaccine take of LC16-KAKETSUKEN and by neutralizing antibody titers in the serum after vaccination with the vaccine. The 100 participants included in efficacy study were selected randomly from 268 participants.

inoculation. All the participants were distributed individually with adhesive tape for the inoculation site.

Fig. 1 shows the flow of participants through the study. The total number of participants was 268: 261 males and 7 females. The average age was 29.2 (19–52): 9 were born before 1961, 43 were born from 1962 to 1968, 57 were born from 1969 to1975 and 159 were born after 1976 (see also Table 3). Because smallpox vaccination continued until 1976 in Japan, participants born before 1976 had a high possibility to have had previous vaccination (re-vaccinees). The discrimination between primary vaccinees and re-vaccinees was done by taking into account the age and the presence or absence of a vaccination scar. Among 268 vaccinee participants, 71 (26.5%) were judged to be re-vaccinees, 196 (73.1%) to be primary vaccinees and 1 (0.4%) was unknown. An examination for allergy showed that 10 (3.7%) experienced some sort of allergic history, such as pollen allergy. No participants with immune deficiency were found and most of them had a history of receiving vaccination for hepatitis A, B and other-non-hepatitis vaccines.

2.3. Blood pressure and clinical laboratory test results

Immediately before and one month after inoculation, the following tests were done and the obtained data were investigated and analyzed: blood pressure, blood tests (numbers of leukocytes and red cells, hemoglobin value, hematocrit value, number of blood platelets), biochemical tests (total cholesterol, whole protein, albumin, urea nitrogen, uric acid, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, lactase dehydrogenase, alkaline phosphatase, C-reactive protein, blood glucose and urinalysis (protein, glucose)).

2.4. Vaccine take, examination of adverse events and local findings

At 10–14 days after inoculation, physicians who had received the required training engaged judged the vaccine take and examined participants for the presence of adverse events and local findings.

2.5. Neutralizing antibody assay

Neutralizing antibody assay was done for blood samples collected 1, 4 and 7 months after inoculation from 100 participants selected randomly. Neutralizing antibody titers against Lister vaccinia virus strain were assessed by the plaque reduction neutralization test assay [14,16]. The neutralizing antibody titer was defined as the reciprocal of the dilution level resulting in a 50% reduction of total plaques formed by Lister vaccinia virus strain with no treatment by vaccinated sera. Vaccinees showing 4 or more in the ratio of maximal post-inoculation titers to pre-inoculation titers were judged as a positive conversion.

2.6. Data analysis

Significant differences in the frequency of adverse events and vaccine take between primary vaccinees and re-vaccinees were determined using the Steel test and the Wilcoxon rank sum test. The Fisher exact probability test and the Steel test were used to compare geometric mean titers of neutralizing antibodies. Statistical analyses were done using SAS/Base/Stat, version 8.0.2. or the updated version.

2.7. Ethical notice

This study was done according to the guidelines of clinical studies and was approved by the institutional review board of the JGSDF Central Hospital (No. 16-004; August 30, 2004: No. 18-022; December 21, 2006).

3. Results

3.1. Improvement of vaccine production process

The amount of MVS of the LC16m8 strain transferred from CSI was limited and therefore we started to produce LC16-KAKETSUKEN by a procedure modified as follows. The number of passages from MVS to vaccine product was extended from 2 to 3 to make production more efficient, but this modification resulted in generating some revertant mutants that form slightly larger plaques than plaques formed by the original MVS. Because the contents of these revertant mutants in the primary passage preparation affected their contents in the final products, the method was modified to produce a primary passage preparation that contains a smaller amount of revertant mutants to a level similar to that of the original MVS.

Table 1
Summary of adverse events.

	Previous smallpox vaccination history			Total
	No	Yes	Unknown	
Number of participants	196	71	1	268
Number of participants with adverse events	53	4	1	58
Number of adverse events	71	5	1	77
Incidence of adverse events[a]	27.0%	5.6%	100%	21.6%
Type of adverse event	Incidence of each adverse event (%)			
Autoinoculation following vaccination[b]	0	1(1.4)	0	1(0.4)
Swelling of axillary lymph node	50(25.5)	2(2.8)	0	52(19.4)
Rash	0	0	1(100.0)	1(0.4)
Systemic disorders				
Fatigue[b]	2(1.0)	0	0	2(0.7)
Fever	2(1.0)	2(2.8)	0	4(1.5)
Local disorders at inoculation site				
Erythema at inoculation site[b]	14(7.1)	0	0	14(5.2)
Swelling at inoculation site[b]	1(0.5)	0	0	1(0.4)
Complications associated with vaccination[b]	2(1.0)	0	0	2(0.8)

Coding by MedDRA Ver.12.1.
[a] Number of participants with adverse events/number of participants × 100.
[b] Adverse events unpredictable from PRECAUTIONS.

3.2. Incidence of adverse events

Table 1 summarizes the adverse events, and Table 2 shows adverse events for each background of vaccinees. Among 268 vaccinee participants, the following vaccinees showed adverse events: 53 of 196 primary vaccinees, 4 of 71 re-vaccinee and 1 vaccinee participant with unknown previous vaccination history. Swelling of the axillary lymph node was observed for 50 of 196 primary vaccinees and 2 of 71 re-vaccinees. Thus, primary vaccinees showed a higher incidence of adverse events, including lymphoadenopathy, than re-vaccinees. The breakdown of adverse events of a total 58 of 268 vaccinees was: 52 vaccinees with swelling of the axillary lymph node, 4 vaccinees with fever, 2 vaccinees with fatigue, 1 vaccinee with a rash, 14 vaccinees with erythema at the inoculation site, 1 vaccinee with swelling at the inoculation site and 1 vaccinee with autoinoculation. Complications with vaccination were observed for 2 primary vaccinees. One complication was allergic dermatitis that was not eczema vaccinatum and the vaccinee recovered without supportive care. The other case was a rash unrelated to the vaccination.

All the adverse events were mild and no serious events (related to cardiovascular disorder, encephalitis, accessory vaccinia or satellite lesions, or progressive vaccinia) were observed. Electrocardiography examination was done for all participants, who also received both chest X-ray and electrocardiogram examinations. Adverse events related to cardiovascular disorder were not observed, except for 2 vaccinees with electrocardiography examination (1st degree atrioventricular block). Both of these vaccinees were re-vaccinees; one of them had no special past and present disorder history, while the other had a history of allergy history. However, their disorder was mild and therefore a causality between this disorder and the vaccination was denied. Adverse events related to encephalitis or those related to accessory vaccinia and progressive vaccinia was not observed. No subject died during this research.

3.3. Blood pressure and clinical tests (data not shown)

Some vaccinees showed changes in total cholesterol, glutamic-pyruvic transaminase and number of leukocytes before and after vaccination. However, all changes were considered to be due to changes in daily habit or transiently catching a cold, and were judged to be not due to vaccination. Abnormality of blood pressure accompanied by vaccination was also not observed.

3.4. Vaccine take

Table 3 shows the examination of vaccine take for each background of vaccinees. All 268 vaccinees were analyzed. Vaccine take was positive for 244 (91.0%) vaccinees and was negative for 24 (9.0%) vaccinees. Primary vaccinees showed a statistically higher proportion of vaccine take (94.4%; 185/196) than that shown by re-vaccinees (81.7%; 58/71) (Odds ratio at 95% confidential interval = 0.265; 0.113–0.624). The diameter of erythema was significantly larger ($P < 0.001$, Wilcoxon rank sum test) for primary vaccinees than for re-vaccinees, and the diameter of a blister was larger for primary vaccinees than for re-vaccinees without statistical significance ($P = 0.277$, Wilcoxon rank sum test) (data not shown).

Notably, vaccinees with erythema less than 10 mm in diameter had a lower proportion of vaccine take (87.5%) compared with other vaccinees with erythema 10 mm or more, all showing 100% proportion of vaccine take (Table 3). Younger vaccinees tended to show a relatively higher proportion of vaccine take than older vaccinees, probably because most younger vaccinees were primary vaccinees (Table 3). We observed no clear difference in proportion of vaccine take dependent on the history of previous disorders, current medical history, history of allergy and side effects, history of other vaccinations and usage of drugs in combination.

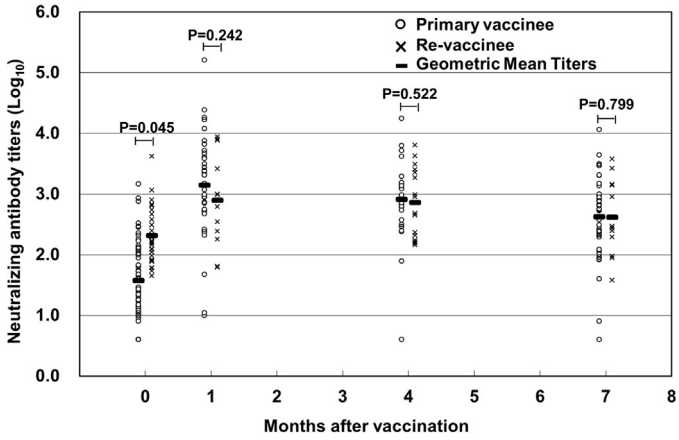

Fig. 2. Time course of neutralizing antibody titers in the serum after vaccination. The time course of neutralizing antibody titers was examined for both primary vaccinees and re-vaccinees. Of note, the titer of one primary vaccinee changed from seropositive to seronegative (<8) at 7 months after inoculation. See the method for titration in Section 2.

3.5. Level of neutralizing antibody responses

Fig. 2 and Table 4 show the results of neutralizing antibody titers and seroconversion rate, respectively. Among 268 vaccinees, the neutralization antibody titer in the serum was determined for 100 randomly selected vaccinees (Fig. 2) done as follows: 99 vaccinees before vaccination (0 month), 51 vaccinees at 1 month, 46 vaccinees at 4 months and 53 vaccinees at 7 months after vaccination. While primary vaccinees showed a statistically higher seroconversion rate at 1, 4 and 7 months after vaccination than that shown by re-vaccinees, primary vaccinees tended to show equivalent or lower antibody titers without exception at 0, 1, 4 and 7 months after vaccination compared with re-vaccinees (Fig. 2 and Table 4). However, both vaccinees kept higher antibody titers even at 7 months after vaccination than those vaccinees at 0 month (Fig. 2). Younger vaccinees tended to show a higher seroconversion rate than older vaccinees, probably because most older vaccinees still had a high neutralizing antibody titer elicited by previous vaccinations (Table 4 and Fig. 2). A statistically significant positive correlation ($P < 0.001$, Fisher exact probability test) was observed between vaccine take and seroconversion (data not shown).

4. Discussion

We showed that LC16-KAKETSUKEN is safe and effective in an adult population at a level similar to that of the previous LC16m8 vaccine (Lot No. Chiba 02) manufactured by the CSI [14]. The following points are especially noted. (1) A clinical study on LC16-KAKETSUKEN, produced by using the improved manufacturing process, was done for the first time strictly compliant with GCP under the approval of the Ministry of Health, Labor and Welfare (MHLW). (2) To evaluate the safety of LC16-KAKETSUKEN, the background of the participants was examined and was described in detail for more than 15 items, such as history of allergy and vaccination with various vaccines, including smallpox vaccine, previous diseases and existence or non-existence of immune deficiency. Such a detailed background of patients was not described in our previous clinical study [14]. (3) Neutralizing antibody titers were monitored until 7 months after LC16m8 vaccination, much longer than in the previous study [14]. A detailed comparison between

Table 2
Adverse events and vaccine take for each background of vaccinees.

Background of vaccinees	Primary vaccinees			Re-vaccinees		
	No. of vaccines	No. of adverse events	Rate of adverse events (%)	No. of vaccines	No. of adverse events	Rate of adverse events (%)
Total	196	53	27.0	71	4	5.6
Gender						
Male	190	53	27.9	70	4	5.7
Female	6	0	0	1	0	0
Age at vaccination with LC16-K[a] vaccine (year)						
≥50	0	0	0	1	0	0
40–49	6	1	16.7	20	0	0
30–39	31	5	16.1	50	4	8.0
20–29	158	46	29.1	0	0	0
<20	1	1	100	0	0	0
Body weight						
<65 kg	51	19	37.3	13	1	7.7
65–70 kg	47	16	34.0	7	1	14.3
70–75 kg	42	8	19.0	17	2	11.8
≥75 kg	40	10	25.0	17	0	0
Unknown	16	0	0	17	0	0
History of previous disorder						
No	195	53	27.2	71	4	5.6
Yes	1	0	0	0	0	0
Current medical history						
No	195	53	27.2	68	3	4.4
Yes	1	0	0	3	1	33.3
History of allergy and side effects						
No	188	52	27.7	69	4	5.8
Yes	8	1	12.5	2	0	0
Breakdown of allergy and side effects						
Drugs	1	1	100	1	0	0
Others	7	0	0	1	0	0
Breakdown of vaccination history						
Hepatitis A	195	52	26.7	71	4	5.6
Hepatitis B	195	52	26.7	71	4	5.6
Rabies	195	52	26.7	71	4	5.6
Tetanus toxoid	178	52	29.2	54	4	7.4
Polio	193	53	27.5	68	4	5.9
Abdominal typhus	192	51	26.6	69	4	5.8
Cerebral meningitis	190	51	26.8	69	4	5.8
Yellow fever	53	21	39.6	26	2	7.7
Japanese encephalitis	39	14	35.9	13	2	15.4
Drugs used in combination						
Allopurinol	0	0	0	1	0	0
Vaccine take of LC16-K[a].						
No	11	1	9.1	13	1	7.7
Yes	185	52	28.1	58	3	5.2
Local findings of LC16-K[a] vaccine take						
Blister	88	48	54.5	12	1	8.3
Ulcer	12	6	50.0	0	0	0
Crust	29	14	48.3	10	1	10.0
Swelling	15	9	60.0	3	0	0
Others	14	4	28.6	3	0	0
Unknown	79	0	0	36	1	2.8
Breakdown of LC16-K[a] vaccine take – erythema						
<10 mm	6	1	16.7	2	0	0
10–30 mm	54	27	50.0	10	2	20.0
≥30 mm	25	14	56.0	1	0	0
Unknown	3	0	0	1	0	0
Breakdown of LC16-K[a] vaccine take – blister						
≤5 mm	34	13	38.2	6	0	0
6 mm	4	3	75.0	0	0	0
7 mm	4	3	75.0	3	1	33.3
8 mm	14	9	64.3	1	0	0
≥9 mm	20	15	75.0	1	0	0
Unknown	12	5	41.7	1	0	0

[a] LC16-KAKETSUKEN.

participants with and without previous vaccination of smallpox vaccine confirmed the previous results [14]. Based on these results, Kaketsuken could describe the present results of adults, along with existing clinical data collected from a pediatric population, in the Package Insert of LC16-KAKETSUKEN under the approval of MHLW. In the package insert, the summary of this PMS study (162 words) was newly added, including explanation about participants, and the results of vaccine take and antibody titers, as well as adverse events.

All studies on humans done hitherto, including this study, support the safety of LC16m8 vaccine, both in children [17] and adults [14]. Also, animal studies on monkeys, rabbits and mice [2,8,10,12,13] support the safety and protective efficacy of LC16m8 vaccine. Notably, LC16m8 vaccine and its genetically modified

Table 3
Proportion of vaccine take for each background of vaccinees.

Background of vaccinees	Vaccine take (number)		Proportion of vaccine take (%)	Odds ratio (95% confidential interval) or comparative test[a,b,c]
	Yes	No		
Total	244	24	91.0	(0.876–0.945)
Gender				
Male	238	23	91.2	0.580 (0.067–5.027)
Female	6	1	85.7	
Age at vaccination with LC16-K[d] vaccine (year)				
≥50	1	0	100.0	Steel test
40–49	20	6	76.9	≤29 vs ≥50 <0.001 (<0.001–>999.999) $p = 0.994$
30–39	72	10	87.8	≤29 vs 40–49 5.663 (1.781–18.003) $\mathbf{p = 0.004}$
20–29	150	8	94.9	≤29 vs 30–39 2.622 (0.993–6.924) $p = 0.129$
<20	1	0	100.0	Wilcoxon rank sum test $Z = -2.989$, $\mathbf{p = 0.003}$
Body weight				
<65 kg	63	1	98.4	Wilcoxon rank sum test $Z = 1.567$, $p = 0.117$
65–70 kg	49	5	90.7	
70–75 kg	54	6	90.0	
≥75 kg	52	5	91.2	
History of previous disorder				
No	243	24	91.0	>999.999 (<0.001–>999.999)
Yes	1	0	100.0	
Current medical history				
No	240	24	90.9	>999.999 (<0.001–>999.999)
Yes	4	0	100.0	
History of allergy/side effects				
No	234	24	90.1	>999.999 (<0.001–>999.999)
Yes	10	0	100.0	
Breakdown of allergy/side effects				
Drugs	2	0	100.0	–
Foods	0	0	–	
Breakdown of vaccination history				
Hepatitis A	243	24	91.0	
Hepatitis B	243	24	91.0	
Rabies	243	24	91.0	
Tetanus toxoid	216	17	92.7	
Polio	239	23	91.2	–
Abdominal typhus	239	23	91.2	
Cerebral meningitis	238	22	91.5	
Yellow fever	72	8	90.0	
Japanese encephalitis	48	5	90.6	
History of previous smallpox vaccination				
No	185	11	94.4	
Yes	58	13	81.7	**0.265 (0.113–0.624)**
Unknown	1	0	100.0	
Drugs used in combination				
No	243	24	91.0	>999.999 (<0.00–>999.999)
Yes (Allopurinol)	1	0	100.0	
Local findings of LC16-K[d] vaccine take				
Blister	100	0	100.0	
Ulcer	12	0	100.0	
Crust	40	0	100.0	–
Swelling	19	0	100.0	
Others	15	2	88.2	
Breakdown of LC16-K[d] vaccine take/erythema				
<10 mm	7	1	87.5	
10–30 mm	64	0	100.0	–
≥30 mm	26	0	100.0	
Breakdown of LC16-K[d] vaccine take/blister				
≤5 mm	40	0	100.0	
6 mm	4	0	100.0	
7 mm	7	0	100.0	–
8 mm	15	0	100.0	
≥9 mm	21	0	100.0	

The bold value signifies that the *P* value is under 0.05 or 0.01 and the 95% confidential interval of Odds ration is under 1.0.

[a] The calculation of odds ratio or comparative test was not done for unspecified items, such as "unknown" and "others".

[b] The Steel test was used as a multiple comparative technique.

[c] Odds ratio (95% lower limit of confidence interval–95% upper limit of confidence interval).

[d] LC16-KAKETSUKEN.

virus induced no serious adverse events in immunodeficient animals [2,13,18]. The protective ability of LC16m8 vaccine against lethal challenge with the highly pathogenic vaccinia WR virus accompanied by induction of neutralizing antibodies to WR was shown for mice [12,16]. LC16m8 also protected monkeys from monkeypox [8,13]. These animal studies provide supportive evidence for the safety and efficacy of LC16m8 vaccine, including LC16-KAKETSUKEN, in humans.

Table 4
Time course of seroconversion rate depending on smallpox vaccination history and age at vaccination.

Background of vaccinees	Seroconversion rate (%)[a]			Maximum seroconversion rate after vaccination[b]	Steel test[c]				
	After vaccination (1 month)	After vaccination (4 month)	After vaccination (7 month)		Compared with 0 month			Compared with 1 month	
					0 month vs 1 month	0 month vs 4 month	0 month vs 7 month	1 month vs 4 month	1 month vs 7 month
Total	72.5 (37/51)	71.7 (33/46)	62.3 (33/53)	72.7 (72/99)	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**	*p* = 0.994	*p* = 0.432
History of previous smallpox vaccination									
No	84.2 (32/38)	89.3 (25/28)	75.0 (30/40)	86.8 (59/68)	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*p* = 0.783	*p* = 0.506
Yes	33.3 (4/12)	44.4 (8/18)	16.7 (2/12)	40.0 (12/30)	***P* = 0.003**	***P* < 0.001**	*P* = 0.066	*p* = 0.768	*p* = 0.546
Unknown	100.0 (1/1)	– (0/0)	100.0 (1/1)	100.0 (1/1)					
Fisher exact probability test (No vs Yes)	***P* = 0.002**	***P* = 0.002**	***P* = 0.001**	***P* < 0.001**					
Age at vaccination with LC16-KAKETSUKEN (year)									
≥50	– (0/0)	– (0/0)	– (0/0)	– (0/0)	Test im.[b]	Test im.[b]	Test im.[b]	*p* = 0.312	*p* = 0.432
40–49	66.7 (4/6)	28.6 (2/7)	33.3 (2/6)	46.2 (6/13)	***P* = 0.004**	*P* = 0.129	*P* = 0.089	*p* = 0.312	*p* = 0.432
30–39	40.0 (6/15)	60.0 (9/15)	35.3 (6/17)	50.0 (16/32)	***P* = 0.001**	***P* < 0.001**	***P* = 0.001**	*p* = 0.451	*p* = 0.948
20–29	89.7 (26/29)	91.7 (22/24)	82.8 (24/29)	92.5 (49/53)	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*p* = 0.957	*p* = 0.672
<20	100.0 (1/1)	– (0/0)	100.0 (1/1)	100.0 (1/1)	Test im.[b]	Test im.[b]	Test im.[b]	Test im.[b]	Test im.[b]
Steel test									
≤29 vs ≥50	Test im.[d]	Test im.[d]	Test im.[d]	Test im.[d]					
≤29 vs 40–49	*P* = 0.251	***P* = 0.001**	***P* = 0.022**	***P* < 0.001**					
≤29 vs 30–39	***P* = 0.001**	***P* = 0.036**	***P* = 0.002**	***P* < 0.001**					

The bold value signifies that the *P* value is under 0.05 or 0.01 and the 95% confidential interval of Odds ration is under 1.0.

[a] Antibody titer after vaccination/before vaccination (0 month) ≥4 was considered as positive seroconversion.

[b] Portion of the participants who seroconverted at one time point at least after vaccination.

[c] Steel test was conducted to compare the seroconvertion rate between the indicated time points.

[d] Test-im.: Test was impossible.

In the past, two severe cases of adverse events possibly caused by vaccination with LC16m8 vaccine clinically were reported [14]. One was a 26-year-old male primary vaccinee who experienced rash onset on day 3 after vaccination. The patient was hospitalized for 20 days after vaccination. A skin biopsy from the rash was consistent with allergic dermatitis, which did not disprove a causal relationship with vaccination. The other case was 29-year-old male primary vaccinee who developed a rash on his trunk on day 10 after vaccination and was diagnosed with erythema multiform.

One of the major concerns with adult smallpox vaccination has been myopericarditis observed in the United States vaccination program [19]. Inflammatory cardiac disease was recognized in adult recipients of Dryvax and ACAM2000 vaccine in the United States, but a study since then [14] and this PMS study showed no serious abnormality by electrocardiography examination.

In January 2011, based on the research findings of this PMS study, the section of "Precautions for Use" in the Package Insert for LC16-KAKETSUKEN was modified under the approval of MHLW to ensure appropriate use for not only pediatric populations, but also for adult populations. Notably, according to the Package Insert and Product Information for ACAM2000 and IMVANEX (smallpox vaccine derived from MVA strain, Bavarian Nordic), vaccination of pediatric populations is not authorized by national regulatory authorities now. Therefore, LC16-KAKETSUKEN is the sole currently available smallpox vaccine for both adult and pediatric populations.

5. Conclusion

From the safety and efficacy results of this PMS study that complied with GCP, smallpox vaccine LC16-KAKETSUKEN was confirmed to be a highly useful and excellent vaccine for both primary vaccinees and re-vaccinees as judged from the absence of serious adverse events, proportion of vaccine take and increase in neutralizing antibody titers.

Conflicts of interest

TF received funds to their hospital from Kaketsuken to support their work in the PMS study and he did not receive any direct funds. YS and HY are employees of Kaketsuken. YN, YK and SH declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.09.067.

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