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# An open-label, non-randomized study investigating the safety and efficacy of smallpox vaccine, LC16, as post-exposure prophylaxis for mpox

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**ABSTRACT**

Mpox is an acute exanthematous disease caused by the monkeypox virus. Since May 2022, it has spread as a community-acquired infection, mainly in Europe and the United States, and urgent measures to prevent this infection were also required in Japan. In this study, we investigated the post-exposure prophylaxis of mpox and safety after inoculating the smallpox vaccine. Participants in close contact with patients with mpox were inoculated with "Freeze-dried cell culture Smallpox Vaccine LC16," within 14 days after close contact. Six cases were registered, and all the participants were inoculated. No mpox symptoms or related complications were observed in the participants for 21 days after the close contact. Adverse events due to inoculation, such as rash, fever, lymphadenopathy, and local reaction at the inoculation site (comprising erythema, swelling, induration, and pain) were observed in the participants; however, all inoculation-related events were non-severe and non-serious, and the participants recovered during the 28-day observation period. The findings of this study suggest that inoculation with LC16 is an effective post-exposure prophylaxis in individuals who had close contact with patients with mpox. Further large-scale studies are warranted to validate these findings.

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Mpox, a zoonosis caused by monkeypox virus (MPXV) that belongs to the genus Orthopoxvirus, results in a smallpox-like disease in humans. The mpox epidemic identified mainly in Europe and the United States of America in May 2022 has been confirmed as the largest outbreak of mpox to date.[1] In July 2022, the World Health Organization (WHO) declared the mpox epidemic a public health emergency of international concern. The global trend of the mpox outbreak provided by the WHO showed that 86,838 cases and 112 deaths in 110 countries were confirmed by April 4, 2023.[1]

The incubation period of mpox is 5–21 days, and its symptoms include fever, chills, headache, sore throat, lymphadenopathy, and myalgia. Rashes of various sizes appear on the face, whole body, hands, and legs in 1–5 days, followed by healing in 2–4 weeks after onset.[2,3] The case-fatality rate of mpox is 0–11%,[4] and there is a severe disease risk, especially in immunocompromised patients. Lymphadenopathy occurs in up to 90% of the patients and is a clinical feature distinguishing mpox from smallpox.[2,3] The comorbidities of mpox include pneumonia, encephalitis, and ophthalmia, which occur mostly in children, immunocompromised individuals, and pregnant women.[5,6] MPXV infection can occur through close contact, including skin-to-skin contact with the rash, body fluids, or scabs, and intimate contact including oral, anal, and genital (labial, vaginal, penal, and testicular) contact with individuals infected with MPXV.[7,8] Several smallpox vaccines have been recommended because of their cross-protective immunity between orthopoxviruses, including MPXV.

There are three types of vaccines: ACAM2000, the Modified Vaccinia Ankara – Bavarian Nordic (MVA-BN), and LC16, employed for preventing mpox, known to be available worldwide based on WHO interim guidance.[9] In 2015, the ACAM2000 was approved by the United States Food and Drug Administration (FDA) for smallpox and mpox and was available as an mpox vaccine in the United States of America until 2019. ACAM2000 is a second-generation vaccine, and its efficacy against mpox and smallpox viruses has been tested in animal studies and clinical trials;[10,11] however, being a replicating vaccine, it is not recommended for use in immunocompromised individuals, such as people living with human immunodeficiency virus (HIV) infection, pregnant women, and patients with skin diseases, because it can cause serious side effects.[12] The MVA-BN is a live, non-replicating, third-generation vaccine with demonstrated efficacy and safety in individuals living with HIV infection or those with atopic dermatitis in several clinical trials.[13,14] In 2019, the MVA-BN vaccine was approved for mpox in Canada and licensed by the FDA for smallpox and mpox prevention in high-risk individuals aged ≥18 years in the United States of America; then, in 2022, the Emergency Use Authorization allowed the MVA-BN dose to be administered intradermally.[15,16]

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The "Freeze-dried cell culture smallpox vaccine LC16m8 strain [KMB]" (LC16), manufactured by KM Biologics, is a third-generation vaccine approved in 1975 in Japan for preventing smallpox. LC16 was created by combining the low-temperature passage acclimation of rabbit-primary kidney cells with plaque cloning from a Lister Original (LO) strain that contributed to smallpox eradication. Previous studies have confirmed that the LC16 strain has remarkably attenuated the central nervous system pathogenicity and skin proliferation while retaining neutralizing antibody-inducing ability similar to that of LO strains.[17,18] In several studies on the efficacy and safety of the LC16 vaccine, the induction of neutralizing antibodies against MPXV was confirmed, and no serious adverse events related to the LC16 vaccine were observed.[19–21] In Japan, 50000 children were inoculated with the LC16 vaccine from 1973 to 1974, and no side effects were observed in 10,578 children who could be followed up for clinical symptoms.[22]

Among these three types of vaccines, LC16 has been approved for children in Japan. On the other hand, MVA-BN has obtained emergency use authorized for children in the United States, and ACAM2000 should not be used in children. Based on these studies, the WHO recommended the LC16 vaccine for post-exposure prophylaxis and primary prophylaxis for pre-exposure against high-risk persons with occupational exposure to individuals infected with mpox.[9] Furthermore, the WHO guidelines on inoculation against mpox recommended appropriate second- or third-generation vaccines, including LC16, within 4 days after exposure to MPXV or up to 14 days in the absence of symptoms to prevent infection or reduce the symptom.[9]

Given this background, investigating the efficacy of post-exposure prophylaxis and safety of the inoculation with LC16 is very significant and imperative for controlling the mpox outbreak in Japan. Therefore, we aimed to evaluate the efficacy of post-exposure prophylaxis and the safety of the single dose with LC16 in a specified clinical study targeting individuals who were in close contact with mpox patients but did not develop mpox. In Japan, routine vaccination with LC16 for preventing smallpox has been discontinued since 1976. In this study, which had begun enrolling subjects in June 2022, we did not set restrictions on past vaccination histories as a criterion for participation.

This open-label, non-randomized study was conducted among individuals in close contact with mpox infected patients in the National Centre for Global Health and Medicine (NCGM) in Tokyo, Japan, from July to December 2022 (Certified Review Board of NCGM approval No. NCGM-C-004504-02, Japan Clinical Trial Registry No.: jRCTs031220137). This study was conducted following the guiding principles of the Declaration of Helsinki[23] and the Clinical Research Act.[24]

The primary endpoint was to investigate the incidence of mpox onset until 21 days after close contact by assessing participants' condition using symptoms such as fever, headache, rash, and lymphadenopathy. The secondary endpoints were to evaluate the severity of mpox onset by calculating the percentage of participants with intensive care unit admission, recumbency, minimum ambulation, death, and complications caused by mpox (secondary skin infections, bronchopneumonia, sepsis, encephalitis, and keratitis). The safety endpoints were to assess the side effects of LC16 and other adverse events detected 28 days after inoculation, including rash, fever, lymphadenopathy, headache, sore throat, and local adverse events at the inoculation site (i.e., erythema, swelling, induration, and pain). Participants recorded their body temperature, presence or absence of systematic symptoms, adverse events, and complications daily up to day 21 and day 28 after inoculation in their diary. The investigator, sub-investigator, or clinical research coordinator contacted participants by e-mail to investigate the incidence of mpox, the side effect of the vaccine, and other adverse events on days 7, 14, 21, and 28 (visits 2, 3, 4, and 5). Additionally, the investigator or sub-investigator examined participants on day 21 after the close contact to investigate the onset of mpox. The definition of mpox onset was based on the notification criteria stipulated in the Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases.[25] Furthermore, the investigator or sub-investigator checked local skin reactions at the inoculation site of participants ("take"), which indicated successful inoculation on days 10 to 14 after inoculation.

Participants in this study were those judged to be close contacts in the active epidemiological survey by the public health center in the domicile of patients diagnosed with mpox based on the criteria of epidemiological survey issued by the Ministry of Health, Labor and Welfare (MHLW).[26] Table 1 shows the definition of close contact according to the risk level of infection by contact situation. Participants with a "high" or "middle" risk level were eligible for post-exposure prophylaxis in this study. Written informed consent was obtained from participants within 14 days of close contact

**Table 1.** Definition of close contact according to the risk level of infection by contact situation.[1,27]

| | | Contact with patients with mpox | | | | |
|---|---|---|---|---|---|---|
| | | Contact with mucus membrane including wounds | Family members or roommates who are eating and sleeping together | Contact with normal skin | History of contact within 1 meter [c)] | History of contact over 1 meter |
| Wearing PPE or using Infection Preventative Measures | No | high[a)] | high[b)] | middle[a)] | middle | low |
| | Yes | - | - | - | low | low |

Definition of close contact according to risk level of infection by contact situation.[1,28]

[a)]Including contact with rodents in mpox endemic countries.

[b)]Including sharing bedding and towels and contact with bedding and clothes with body fluids of confirmed cases during cleaning and laundry.

[c)]Determine the infectivity comprehensively based on individual circumstances, such as the surrounding environment and contact status, including contact time and the presence or absence of conversation.

Abbreviation: PPE = Personal Protective Equipment.

with patients with mpox. Even if participants consented to participate in the study but not for inoculation, we registered them as the non-inoculated group. Participants' age at informed consent was at least 1 year. Patients were excluded from the study if they had significant immune dysfunction, fever, severe acute illness, or generalized skin infection; had an anaphylactic reaction to a component of the study vaccine; were taking corticosteroids or immunosuppressants; or were pregnant.

The test vaccine used in this study was "Freeze-dried cell culture smallpox vaccine LC16 [KMB]." The test vaccine is a live vaccinia virus (strain LC16m8) obtained by proliferation in primary rabbit kidney cells that have not been previously infected with any transmissible diseases. The obtained virus was diluted, dispensed with a stabilizer, and lyophilized.[27] We dissolved the test vaccine in 0.5 mL of the attached solvent (water for injection with 20 vol% glycerin, containing $2.5 \times 10^7$ PFU of the live vaccine per 0.5 mL solvent), and dipped the designated bifurcated needle into the vaccine diluent; then, puncture was performed five times for the primary inoculation and 10 times for reinoculation. LC16 was administered as a single dose, and participants were followed up until day 28 after inoculation. If mpox developed, participants were followed up until recovery.

All statistical analyses were performed using SAS® software, version 9.4 (Cary, NC, USA), and R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). The efficacy analysis included all eligible participants with efficacy endpoint data (Full Analysis Set, FAS). The primary analysis was performed on participants in the FAS who were inoculated within 4 days after the close contact. Based on a systematic review of the epidemiology of mpox by Beer et al.,[28] which was the most useful reference at the planning stage, we set the expected incidence of infection to 7.4% for non-inoculated participants in this study. Further, using the Bayesian approach, we assumed an uninformative prior distribution of inoculated participants and considered that this vaccine was effective if the posterior probability, estimated as the probability that the incidence rate of the inoculated participants is lower than 7.4%, exceeded 90%. At least 33 participants were required to determine whether their posterior probability exceeds 90%. The study population for safety endpoint analysis was defined as enrolled participants, excluding non-inoculated participants.

For the primary analysis, we calculated point estimates of the percentage of mpox onset and corresponding 90% and 95% confidence intervals, and the probability that the incidence rates among inoculated participants was lower than 7.4% (posterior probability). The same statistical analysis methods described above were also applied to the inoculated participants in FAS. Secondary endpoints were listed only because of the small number of cases enrolled.

Adverse event incidences in the inoculated participants were collected from the case report form recorded by the investigator and sub-investigator and calculated by each adverse event in the safety analysis set. For each adverse event, the investigator and sub-investigators evaluated the severity, seriousness of causal relationship with the LC16 vaccine, and outcome. The severity of each adverse event was judged following the guidelines for industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials[29] and recorded as "mild:" without interfering with activities of daily living; "moderate:" interfering with activities of daily living; and "severe:" hinder the performance of activities daily living. The seriousness of each adverse event was defined following the ICH (International Conference on Harmonization of Technical Requirements for Pharmaceutical for Human Use) E2A guideline.[30]

Six persons judged to be in close contact with the patients with mpox were examined in the NCGM; all participants met eligibility criteria at the time of providing the informed consent. All the participants agreed to be inoculated with LC16; no participant disagreed to be inoculated with LC16. The inoculation was performed on the participants' left triceps. We followed up on their systemic condition, including mpox onset, incidence of the side effect, and any other adverse events on days 7, 14, 21, and 28 after inoculation.

Participant backgrounds were as follows. The median age ± standard deviation (SD) of the six participants was 42 ± 6.5 years (range, 33–49 years); all of the participants were men. Of the six participants, one had a history of previous smallpox inoculation ('reinoculated participant') and the remaining five had no history of smallpox inoculation ('primary-inoculated participants'). Participants' comorbidities included HIV infection ($n = 2$, 33.3%), dyslipidemia ($n = 2$, 33.3%), diabetes mellitus ($n = 2$, 33.3%), hyperuricemia ($n = 1$, 16.7%), keloids ($n = 1$, 16.7%), hypertension ($n = 1$, 16.7%), and history of pneumonia ($n = 1$, 16.7%). Concomitant medications were used by three participants for comorbidities. This study was set up to exclude participants with obvious immunodeficiency diseases. The two participants with HIV infection had been appropriately treated and were without immunodeficiency at the time of inoculation with LC16; therefore, they were judged to be eligible to receive LC16. The close contact situations were contacts with mucous membranes, including wounds ($n = 1$, 16.7%) and contact as a family member or cohabitant under the same roof ($n = 6$, 100%). In this study, we have not collected information on the presence or absence of sexual contact. Five participants (83.3%) were inoculated within 4 days and one participant (16.7%) within 5–14 days from close contact. Throughout the study, there were no cases with unscheduled medical examination, deviation from protocol, or discontinuation.

As shown in Table 2, the participants were inoculated within 4 days ($n = 5$) after the close contact had no incidence of mpox until day 21 following the close contact. In the primary endpoint analysis, the incidence of mpox until day 21 after the close contact with 90% and 95% CIs in participants inoculated within 4 days after close contact was 0% (90% CI: 0.0–45.1, 95% CI: 0.0–52.2). The posterior probability of mpox onset in <7.4% of the participants inoculated within 4 days was 36.5%. Moreover, all the inoculated participants ($n = 6$), including one inoculated within 5–14 days after the close contact, had no incidence of mpox until day 21 after the close contact. The percentage of mpox onset until day 21 with 90% and 95% CI in all the inoculated participants was 0% (90% CI: 0.0–39.3, 95% CI: 0.0–45.9). The posterior probability of mpox

**Table 2.** Summary of primary and secondary endpoints.

| | |
|---|---|
| Percentage of mpox onset until day 21 in participants inoculated within 4 days after close contact | |
| Number of evaluated participants | 5 |
| Percentage of participants with mpox onset in evaluated participants (%) | 0 |
| 90% CI[a)] [LCL, UCL] | [0.0, 45.1] |
| 95% CI[a)] [LCL, UCL] | [0.0, 52.2] |
| Posterior probability (%)[b)] | 36.5 |
| Percentage of mpox onset until day 21 in all of the participants inoculated within 14 days after close contact | |
| Number of evaluated participants | 6 |
| Percentage of participants with mpox onset in evaluated participants (%) | 0 |
| 90% CI[a)] [LCL, UCL] | [0.0, 39.3] |
| 95% CI[a)] [LCL, UCL] | [0.0, 45.9] |
| Posterior probability (%)[b)] | 41.0 |
| Symptom related to mpox onset for 21 days after close contact in inoculated participants, n (%) | |
| Number of evaluated participants | 6 |
| Fever | 0 (0) |
| Headache | 0 (0) |
| Rash | 0 (0) |
| Lymphadenopathy | 0 (0) |
| Systematic condition for 28 days after inoculation, n (%) | |
| Number of evaluated participants | 6 |
| Conduct activities as usual | 6 (100) |
| Minimum ambulation | 0 |
| Recumbency | 0 |
| Admission of intensive care unit | 0 |
| Death | 0 |
| Complications related to mpox onset observed for 28 days after inoculation, n (%) | |
| Number of evaluated participants | 6 |
| Secondary skin infection | 0 (0) |
| Bronchopneumonia | 0 (0) |
| Sepsis | 0 (0) |
| Encephalitis | 0 (0) |
| Keratitis | 0 (0) |
| Local skin reaction at the inoculation site of inoculated participants on days 10 to 14 after inoculation, n (%) | |
| Number of evaluated participants | 6 |
| "Take" (successful inoculation) | 6 (100) |
| No reaction (unsuccessful inoculation) | 6 (0) |

Abbreviations: CI = Confidence Interval; LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; FAS = fill analysis set; *n* = number of participants in intervention group; %, percentage based on evaluated participants.

[a)]Percentages of participants with mpox onset for 21 days after inoculation with 90% and 95% confidence intervals in participants inoculated within 4 days and in all participants inoculated within 14 days after the close contact were calculated by Clopper–Pearson method.

[b)]Posterior probability Pr (p_E<p_S|x, n) was calculated by assuming the uninformative prior distribution of Beta (1,1) for the prior distribution in the percentage of participants with mpox onset in the intervention group.

onset in <7.4% of all inoculated participants was 41.0%. Fever, headache, rash, and lymphadenopathy were observed within day 21 after the close contact in several patients; however, these symptoms were not severe and were measured as the post-inoculation systemic side effects by the investigators (refer to Table 3). No minimum ambulation, recumbency, intensive care unit admission, death, and mpox complications (i.e., lymphadenopathy, secondary skin infections, bronchopneumonia, sepsis, encephalitis, and keratitis) were observed in all of the inoculated participants. One case of bronchopneumonia was detected 7 days following the inoculation because of SARS-CoV-2 infection. The local skin reaction at the inoculation site ("take") could be detected on days 10 to 14 after the inoculation in all participants, which meant that the inoculation was definitely successful.

Adverse events observed in this study are shown in Table 3. Adverse events related to or definitely related to the inoculation were observed in all participants between 1 and 17 days after inoculation. The most observed adverse event was rashes, with an incidence of 83.3%. Rashes at the inoculation site were observed in five participants (83.3%) between 1- and 8-days (mean ± SD: 4.0 ± 3.0) after the inoculation and continued for 6–22 days (mean ± SD: 14.0 ± 6.4) until symptoms disappeared. Rashes on the back were observed in one participant (16.7%) and judged as non-related to the inoculation. The other adverse event observed at the inoculation site was pruritus in one participant (16.7%), which was different from that observed in other participants who developed rashes at the inoculation site. According to the participants' diary, these adverse events at the inoculation site were accompanied by erythema, swelling, induration, and pain.

The systematic adverse events were fever (incidence percentage in all participants: 33.3%, moderate fever: 16.7%, mild fever: 33.3%), lymphadenopathy (33.3%; axilla at the inoculation site: 16.7%, cervical at the inoculation site: 16.7%), headache (16.7%), malaise (16.7%), pruritus on the back (16.7%), and shoulder pain at the inoculation site (16.7%). Sore throat was not observed during the observation period. These adverse events were considered non-serious and related or definitely related to the inoculation. Moderate fever interfering with daily living was observed in one participant, but this event was non-serious, and the participant recovered within 4 days after the inoculation. On the 10th day after the recovery of moderate fever (17 days after inoculation), another episode of mild fever was

**Table 3.** Summary of adverse events detected within 28 days after the inoculation.

| | Number of participants (%) | Severity[a)] | Seriousness[b)] | Post-inoculation day of onset | Duration day | Causal relationship with the inoculation |
|---|---|---|---|---|---|---|
| Adverse event | | | | | | |
| Rash | 5 (83.3) | | | | | |
| Inoculation site | 5* (83.3) | Mild | Non-serious | 4.0 ± 3.0, [1, 8][c)] | 14.0 ± 6.4, [6, 22][d)e)] | Related or definitely -related |
| Primary-inoculation | 4 | | | 3.0 ± 2.3 [1, 5][c)] | 16.0 ± 5.4 [11, 22][d)e)] | |
| Reinoculation | 1 | | | 8 | 6 | |
| Back | 1* (16.7) | Mild | Non-serious | 3 | 13 | Non-related |
| Fever | 2 (33.3) | | | | | |
| | 1 (16.7) | Moderate | Non-serious | 4 | 4 | Related or definitely -related |
| | 2 (33.3) | Mild | Non-serious | 11.5 ± 7.8, [6, 17][c)] | 2.0 ± 1.4, [1, 3][d)] | Related or definitely -related |
| Lymphadenopathy | 2 (33.3) | | | | | |
| Axilla in inoculation site | 1 (16.7) | Mild | Non-serious | 9 | 3 | Related or definitely -related |
| Cervical in inoculation site | 1 (16.7) | Mild | Non-serious | 5 | 6 | Related or definitely -related |
| Headache | 1 (16.7) | Mild | Non-serious | 13 | 1 | Related or definitely -related |
| Malaise | 1 (16.7) | Mild | Non-serious | 6 | 1 | Related or definitely -related |
| Pruritus | 2 (33.3) | | | | | |
| Back | 1 (16.7) | Mild | Non-serious | 5 | 1 | Related or definitely -related |
| Inoculation site | 1 (16.7) | Mild | Non-serious | 8 | 12 | Related or definitely -related |
| Shoulder pain in inoculation site | 1 (16.7) | Mild | Non-serious | 8 | 4 | Related or definitely -related |
| COVID-19 | 1* (16.7) | Mild | Serious | 0 | 14 | Non-related |

Abbreviation: Asterisk means one participant with reinoculation was included in counting; %, percentage based on the inoculated participants ($n = 6$).
[a)]Severity was classified as follows: "mild," without interfering daily living activities; "moderate," interfere with daily living activities; "severe," hinder performing daily living activities."[29]
[b)]Serious adverse event was corresponding to the following medical occurrence: (1) results in death or disease leading to death, (2) requires inpatient hospitalization or prolongation of existing hospitalization, (3) results in persistent or significant disability, (4) serious disease in accordance with (1) – (3), (5) congenital anomaly/birth defect.[30]
[c)]Post-inoculation duration of the rash at the inoculation site and the mild fever showed mean day ± standard deviation [Min, Max].
[d)]Duration of the rash at the inoculation site and the mild fever showed mean day ± standard deviation [Min, Max].
[e)]In one participant, temporary improvement of the rash for 2 days was observed in the duration.

observed in the same participant. Both moderate and mild fevers were considered definitely related to the inoculation because they were observed during the incubation period of mpox.

One serious adverse event was reported in one participant resulting in hospital admission for COVID-19. The COVID-19 incidence happened on the same day of the inoculation. Given the incubation period of COVID-19 between 1 and 14 days (5 days on average), this infection would have preceded the inoculation; hence, it was judged as having a non-causal relationship with the inoculation.

The duration of the adverse event, such as rash and pruritus, developed at the inoculation site, was 6–22 days, whereas that of the systemic adverse event, such as fever, headache, malaise, lymphadenopathy, pruritus on the back, and shoulder pain related or definitely related to the inoculation, was 1–6 days. Participants recovered from all adverse events without any sequelae within 28 days after the inoculation.

In addition, the results of the incidence and duration of the local adverse events at the inoculation site collected from the participants' diaries are shown in Table 4. All local adverse events developed between 1 and 10 days after the inoculation. Erythema and swelling occurred in all participants for 7–18 days (mean ± SD: 12.8 ± 4.1) and 6–17 days (mean ± SD: 9.0 ± 4.4), respectively, whereas induration and pain were observed in three of six participants (50%) for 1–9 days (mean ± SD: 5.3 ± 4.0) and 1–6 days (mean ± SD: 3.3 ± 2.5), respectively.

As shown in Tables 3 and 4, only rash at the inoculation site, erythema, and swelling related to the inoculation were observed in the reinoculated participant. In the reinoculated participant, the mean post-inoculation days of the rash,

**Table 4.** Local adverse event at the inoculation site within 28 days after the inoculation.

| Local adverse event | Number of participants (%) | Post-inoculation day of onset[a)] | Duration day[b)] |
|---|---|---|---|
| Erythema | 6* (100) | 4.8 ± 2.9, [1, 8] | 12.8 ± 4.1, [7, 18] |
| Primary-inoculation | 5 | 4.2 ± 2.8 [1, 8] | 14.0 ± 3.2, [11, 18][c)] |
| Reinoculation | 1 | 8 | 7 |
| Swelling | 6* (100) | 5.8 ± 2.4, [2, 9] | 9.0 ± 4.4, [6, 17] |
| Primary-inoculation | 5 | 5.2 ± 2.0 [2, 7] | 9.6 ± 4.6 [6, 17][d)] |
| Reinoculation | 1 | 9 | 6 |
| Induration | 3 (50.0) | 4.0 ± 3.6, [1, 8] | 5.3 ± 4.0, [1, 9] |
| Pain | 3 (50.0) | 6.3 ± 4.7, [1, 10] | 3.3 ± 2.5, [1, 6] |

Abbreviation: Asterisk means one participant with reinoculation was included in counting; %, percentage based on the inoculated participants ($n = 6$).
[a)]Post-inoculation day of each local adverse event showed mean day ± standard deviation [Min, Max].
[b)]Duration of each local adverse event showed mean day ± standard deviation [Min, Max].
[c)]In two participants, temporary improvement of erythema was observed for 2 days.
[d)]In one participant, temporary improvement of the swelling was observed for 2 days.

erythema, and swelling were 8, 8, and 9 days, and the durations were 6, 7, and 6 days, respectively. In contrast, in the primary-inoculated participants, the mean post-inoculation of rash, erythema, and swelling were 3.0 ± 2.3, 4.2 ± 2.8, and 5.2 ± 2.0 days, and the mean durations were 16.0 ± 5.4, 14.0 ± 3.2, and 9.6 ± 4.6 days, respectively.

To the best of our knowledge, this is the first study to assess efficacy for preventing mpox onset, severity, and complication and the safety of inoculation with LC16 as post-exposure prophylaxis in individuals who had close contact with patients with mpox. In this study, five participants with primary-inoculation, who underwent the LC16 inoculation within 4 days after close contact, and one with reinoculation, who underwent the LC16 inoculation within 5–14 days after close contact, were examined. As shown in Table 3, some systemic adverse events, such as fever, rash, and lymphadenopathy, appeared to overlap with symptoms of mpox. However, they were not diagnosed with MPXV infection because the rashes with pustules, anal ulcers, oral ulcers, and white moss, which are characteristic of mpox, were not seen in any of the participants.

On the other hand, this study had some limitations in verifying the preventive effect of LC16 against mpox due to the small number of participants enrolled. In Japan, the first case of mpox was reported in July 2022; an adult man who had contact with mpox patients in Europe was diagnosed with mpox after returning to Japan. Consecutively, the second case was reported in the same month. Based on the infectious situation in Japan and abroad, the Ministry of Health, Labour and Welfare in Japan approved mpox prophylaxis against LC16 in August 2022. In response to this additional approval, participant enrollment in this clinical study ended in December 2022. Between June and December 2022, from the initiation to the termination of the study, just eight mpox cases were reported in all of Japan, resulting in enrollment of only six participants with close contact with mpox patients. As described previously, at least 33 inoculated patients were required to determine whether the posterior probability exceeds 90%. However, in this study, the number of inoculated participants was only six, which was insufficient to verify the preventive effect against mpox accurately. Furthermore, this study could not collect enough information on the contact situation. Several epidemiology studies have shown that the 2022 mpox outbreak was mainly due to sexual transmission.[7,8] Although participants in this study were reported to have been in contact with mpox patients through mucous membranes, including wounds, or contacted as a family member or cohabitant under the same roof, it was unclear whether this was via sexual contact. Considering these limitations, at present, it is not possible to definitively discuss whether the prevention of mpox was due to LC16 inoculation or merely coincidental. Immunogenicity assessments, such as the measurement of neutralizing antibody titers, will be required to support the protective effect of LC16 against mpox.

Regarding safety, no serious adverse events were reported because of LC16 inoculation in participants with close contact. This finding is similar to the results of previous studies in healthy adults.[19–21] All systemic adverse events related to or definitely related to inoculation occurred in the primary-inoculated participants but not in the reinoculated participant (Table 3). Moreover, induration and pain at the inoculation sites were observed only in the primary-inoculated participants (Table 4). These results are consistent with those of previous studies showing a higher incidence of adverse events in the primary-inoculated participants compared with the reinoculated participants.[19,20] In addition, Tables 3 and 4 show the post-inoculation day of the appearance of the rash at the inoculation site. Erythema and swelling at the inoculation site tended to appear earlier in the primary-inoculated participants than in the reinoculated participant. This finding contradicts the results of a previous study in which healthy adults were inoculated with LC16; swollen lymph nodes and fever post-inoculation appeared significantly earlier in the reinoculated participants than in the primary-inoculated participants.[20] Besides, the duration shown in Table 4 indicates that erythema and swelling at the inoculation site were comparatively longer than those of induration and pain; in contrast, these symptoms resolved earlier in the reinoculated participant than in the primary-inoculated participants. However, only one reinoculated participant was enrolled in this study, and therefore, it is challenging to definitively discuss the progress of skin reaction and recovery duration after the LC16 inoculation in the reinoculated participant compared to those in primary-inoculated participants. Furthermore, exact conclusions and comparisons between our results and those of previous studies are difficult because of differences in the sample size, especially because of the extremely limited sample size of this study and the participants' backgrounds. Despite these limitations, our results indicate the characteristic onset of adverse events in participants who had close contact with patients with mpox. Regarding the long-lasting 22-day rash observed in a primary-inoculated participant in this study, a previous study evaluating the safety of the LC16 inoculation in 120 healthy adults who had not received the LC16 vaccine reported that the scab at the inoculation site withdrew around 28 days post-inoculation.[21] In another study of LC16 inoculation in 569 adults who had not received the LC16 vaccine identified erythema at the inoculation site in 444 cases; adverse events were followed up to day 14 after inoculation, and 192 subjects had erythema at day 14 with a mean diameter of 12 mm (SD, 7.1 mm).[20] Based on these precedents, it might be possible for the rash at the inoculation site to last for several weeks in a primary-vaccinated participant.

There were three participants with comorbidities and anamnesis: one with HIV infection, dyslipidemia, diabetes mellitus, hyperuricemia, and pneumonia; one with keloids, hypertension, dyslipidemia, and diabetes mellitus; and one with HIV infection. These participants had several adverse events related to LC16 inoculation similar to other participants but did not show any severity or seriousness, which indicates that LC16 could be safely used despite certain comorbidities. The WHO guidance described that MVA-BN would be preferentially used in severely immunocompromised patients, but the use of LC16 is contraindicated in the patients with severe immunodeficiency and medical treatment resulting in immunosuppression.[9] In this study, two participants inoculated with LC16, who infected with HIV but not

immunodeficient by appropriate treatment, showed no serious adverse events as described above, implying that LC16 might be safely used in HIV infected patients as far as appropriate treatment control has been done. However, this suggestion is based on only two participants, and there are caveats to the applicability of LC16 vaccine to populations at need, such as immunocompromised individuals.

Owing to the additional indication of the LC16 vaccine for mpox prophylaxis, the number of punctures during inoculation was changed to 15 for both primary-inoculated and reinoculated participants. The safety and efficacy of 15 punctures for primary-inoculated participants have been confirmed in a clinical trial in the United States;[21] the incidence of axillary lymphadenopathy in primary-inoculated participants was 36% (45/125), which was higher than that in other studies, in which participants were inoculated with five punctures.[19,20] The increased number of punctures supports immunization but may increase the occurrence and severity of adverse events. In this study, safety assessments of the inoculation with 15 punctures could not be performed due to sample size limitation, but it should be investigated in future studies.

The results of this study indicate that LC16 has potential to be effective for post-exposure prophylaxis in the case of inoculation within 4 days and 14 days after close contact, regardless of previous inoculation for smallpox. In the three types of vaccines for mpox prophylaxis, namely, ACAM2000, MVA-BN, LC16, only LC16 has been authorized for children in Japan. Under the spread of mpox infection, LC16 might be available for post-exposure prophylaxis of children even if the number of cases in which children are close-contacts with mpox patient in the home will increase. However, this study did not have sufficient statistical power to determine efficacy owing to the limitations of sample size, lack of control groups such as a non-inoculated participant group, and lack of any virological and immunological testing. Thereby, the absence of mpox in the inoculated participants observed herein is still suggestive, making it difficult to draw definite conclusions regarding the efficacy of the LC16 vaccine. To validate the definitive efficacy and safety of LC16 inoculation, more studies with larger sample size, inclusion of comparative groups, detailed information on contact situation, and evaluation of immunogenicity after the inoculation are required.

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