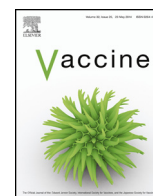




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Recent advances in the study of live attenuated cell-cultured smallpox vaccine LC16m8



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ABSTRACT

LC16m8 is a live, attenuated, cell-cultured smallpox vaccine that was developed and licensed in Japan in the 1970s, but was not used in the campaign to eradicate smallpox. In the early 2000s, the potential threat of bioterrorism led to reconsideration of the need for a smallpox vaccine. Subsequently, LC16m8 production was restarted in Japan in 2002, requiring re-evaluation of its safety and efficacy. Approximately 50,000 children in the 1970s and about 3500 healthy adults in the 2000s were vaccinated with LC16m8 in Japan, and 153 adults have been vaccinated with LC16m8 or Dryvax in phase I/II clinical trials in the USA. These studies confirmed the safety and efficacy of LC16m8, while several studies in animal models have shown that LC16m8 protects the host against viral challenge. The World Health Organization Strategic Advisory Group of Experts on Immunization recommended LC16m8, together with ACAM2000, as a stockpile vaccine in 2013. In addition, LC16m8 is expected to be a viable alternative to first-generation smallpox vaccines to prevent human monkeypox.

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1. Introduction

LC16m8 is a live, attenuated, cell-cultured smallpox vaccine developed in Japan in the 1970s to generate a vaccine strain with a safer profile than first-generation vaccines, such as the Lister and Dryvax strains, which have rare but severe adverse effects [1]. A clinical study of 10,578 children, among approximately 50,000 children vaccinated with LC16m8, showed no severe adverse events and comparable immunogenicity and efficacy with first-generation vaccines [2].

LC16m8 is a promising vaccine because of its lower virulence and replication competency. A frameshift mutation in B5R, a major extracellular envelope virion (EEV, also called EV) antigen, results in expression of a truncated protein, which attenuates the virulence of the strain [3]. LC16m8 was licensed in 1975, but was not used in the smallpox eradication program because routine smallpox

vaccinations were halted in 1976. After the terrorist attacks in the USA in 2001, vaccine production was recommenced in Japan and government stockpiles have been established to prepare for possible bioterrorism [4,5]. The safety and efficacy of LC16m8 has also been explored. Thus, in Japan, vaccination with LC16m8 has been evaluated in 3221 subjects [6] and 268 subjects [7] since 2002, and in the USA, a phase I/II clinical trial was conducted in 153 subjects [8]. Moreover, several experiments have been performed in animal models to examine the safety and efficacy of LC16m8 [3,9–14].

A review on LC16m8 was published by Kenner et al. in 2006, describing the development of LC16m8, its molecular characterization, the then-current knowledge, and comparison to other smallpox vaccines [15]. Here, we describe advances in LC16m8 research since then, including our own clinical studies, animal experiments, long-term effects, and formation of antibodies to B5 protein, since LC16m8 is a B5R-mutant strain. We also discuss possible clinical applications of LC16m8 as a human monkeypox vaccine.

2. Clinical studies

A unique feature of smallpox vaccine research is that safer vaccines are sought by assessing strain immunogenicity in the absence of an endemic outbreak [16–18]. Clear correlations of surrogate markers with protection against smallpox were limited until the

Abbreviations: EEV, extracellular enveloped virion; PRNT, plaque reduction neutralization titer; GMT, geometric mean titers; IMV, intracellular mature virion; PFU, plaque forming unit; WR, Western Reserve; MVA, Modified vaccinia Ankara; SCID, severe combined immunodeficient; LD, lethal dose; SAGE, Strategic Advisory Group of Experts.

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1970s [16–18]. Therefore, a vaccine was evaluated by comparing the immunological responses of the target vaccine with first-generation vaccine(s) and by assessing the protection conferred in animal challenge models [16–18]. Three clinical studies, including one post-marketing surveillance study of LC16m8, have been performed in the United States and Japan since 2002. In Japan, clinical and immunological responses to LC16m8 were evaluated in 3221 healthy Self-Defense Force personnel aged 18–55 years, including 1529 vaccinia-naïve subjects and 1692 who had previously been vaccinated with smallpox vaccine [6]. LC16m8 was administered using a bifurcated needle. Vaccinia-naïve subjects received 5 punctures and previously vaccinated individuals received 10 punctures. The major outcomes were the frequency of adverse events up to 30 days post-vaccination, the proportion of major cutaneous reactions, and the plaque reduction neutralization titer (PRNT) in sera.

No severe adverse events and no abnormal ECG findings or symptomatic heart disease were observed in the study [6]. Two mild cases of allergic dermatitis and erythema multiforme, respectively, were suspected to have been caused by vaccination. Two other minor adverse events of pain from a swollen axillary lymph node and groin pain caused by a bacterial infection were also suspected to be related to vaccination. Majority of adverse events consisted of swelling in the axillary lymph nodes and low-grade fever at rates of 15.5% and 2.6% for primary vaccinees (vaccinia-naïve subjects) and 3.5% and 1.4% for revaccinees (previously vaccinated individuals), respectively. Adverse events occurred earlier in revaccinees than in naïve subjects, with onset of fever at 5.8 and 2.0 days and swelling of axillary lymph nodes at 5.6 and 3.2 days (means) in naïve subjects and revaccinees, respectively.

The outcomes of the study included high levels of major cutaneous reactions (“take”) and seroconversion [6], with proportions of major cutaneous reactions of 94.4% and 86.6% in vaccinia-naïve subjects and revaccinees, respectively. There were no significant differences in these proportions among age groups of revaccinees with different vaccination histories (variations in the dose and strain of routine smallpox vaccine vaccination prior to 1976). The rate of seroconversion based on the level of neutralizing antibodies raised against Dryvax was 90.2% in primary recipients, and the effective boost rate was 60.0% in revaccinees, with no significant difference in the level of PRN geometric mean titers (GMTs) in post-vaccination serum between the two groups.

A further study in 268 Self-Defense Force personnel was conducted in 2005 as a post-marketing study [7]. The subjects were aged 19–52 years, 196 were vaccinia-naïve, 71 had been previously vaccinated with smallpox vaccine(s), and 1 had an unknown vaccination history. The number of punctures was identical to that used in the previous study [6]. No severe adverse events occurred [7] and the proportions of major cutaneous reactions were 94.4% and 81.7% in naïve and previously vaccinated individuals, respectively. Seroconversion based on PRNT was found in 84.2% of naïve individuals at 1 month post-vaccination.

Kennedy et al. [8] compared the safety and immunogenicity of LC16m8 with those of the Dryvax strain in a randomized, double-blind, multicenter phase I/II clinical trial in 153 vaccinia-naïve adults aged 18–34 years in the USA. Dryvax or LC16m8 was administered using a bifurcated needle via 15-puncture intraepidermal inoculation. The primary endpoints were the anti-intracellular mature virus (IMV) PRNT 30 days after vaccination and the rate of vaccine-attributable adverse events. Secondary endpoints were the rate of major cutaneous reactions, lesion size at the vaccination site, anti-EEV PRNT, cell-mediated immune responses, viral persistence, and viremia after vaccination. There were no clinically significant abnormalities including cardiac toxicity, and the local and systemic reactions after vaccination were similar for both strains [8]. The major local reactions included axillary lymph node tenderness (Dryvax, 50%; LC16m8, 48%), tenderness at the

vaccination site (Dryvax, 46%; LC16m8, 42%), and swollen axillary lymph nodes (Dryvax, 46%; LC16m8, 37%). The rates of swollen axillary lymph nodes and rash were higher in the US study (37% and 2%) than in the Japanese trial (16% and 1%) [6]. This difference may be related to the number of skin punctures, to different vaccine lots, and to the number, training, and experience of observers [8].

The proportions of major cutaneous reactions were 100% in the 125 LC16m8 vaccine recipients and 86% in the 28 Dryvax recipients [8]. In the 4 subjects without a major cutaneous reaction to Dryvax, the vaccine was administered at the same location on the same day. The subjects were not seroconverted, suggesting a problem with vaccine reconstitution. The average size of the major cutaneous reaction was larger with Dryvax than with LC16m8, but LC16m8 induced robust humoral and cell-mediated immunity [8]. The anti-IMV PRNT was assessed against three poxviruses: vaccinia, monkeypox, and variola. Sera from LC16m8 vaccinees had antibody titers >1:40 against all these viruses. The mean GMT PRNT was higher for Dryvax than for LC16m8, but cellular immune responses induced by LC16m8 were comparable to those induced by Dryvax. LC16m8-induced interferon- γ (IFN- γ) measured by ELISPOT was lower than that induced by Dryvax, but LC16m8-induced lymphoproliferation was higher in comparison to Dryvax [8].

These three studies [6–8] indicate that LC16m8 can be safely administered and has comparable efficacy to Dryvax (Table 1). The number of punctures is a potential cause for the differences in the rates of weak and mild adverse events between the studies conducted in the US [8] and Japan [6], as suggested by Kennedy et al. [8].

3. Experiments in animal models

Animal model challenge studies are critically important for evaluation of smallpox vaccines, since it is impossible to evaluate these vaccines in endemic controls [16–19]. Several studies of the safety and efficacy of LC16m8 have been conducted in mouse [3,9,11,12,14], rabbit [3,11], and non-human primates [10,13].

The protective efficacy of LC16m8 was compared with those of its parental strain LC16mO and the grandparental Lister strain in BALB/c mice vaccinated subcutaneously with a single dose of the vaccine or PBS [9]. At 21 days after vaccination, the animals were challenged with 10^6 PFU of pathogenic Western Reserve (WR) strain of vaccinia virus. Clinical signs, survival rate, body-weight loss, and histopathology of the nasal tissue were examined and sera from pre- and 14 days post-challenge were tested for anti-vaccinia and anti-B5 antibody by ELISA, for neutralizing antibody against IMV by PRN assay, and for anti-EEV antibody production by comet inhibition activity. The survival rate of LC16m8-vaccinated animals was 100%, similar to the LC16mO and Lister strains, while PBS-treated control mice died within 9 days after infection, and had clinical symptoms and body-weight loss. Vaccinated mice exhibited only slight, transient body-weight loss after WR challenge and this did not differ between vaccine strains.

The protective efficacy of LC16m8 against lethal WR challenge was also examined in a comparative study with the Dryvax strain in BALB/cByJ mice [12]. Outcomes include humoral immunity (anti-IMV antibody, immunoglobulin isotype, anti-A33 specific antibody, anti-B5 specific antibody, and anti-IMV and -EEV neutralization), cell-mediated immunity (interferon- γ), and protection against lethal WR challenge. Sera from LC16m8- and Dryvax-vaccinated mice had similar antibody production against vaccinia and neutralizing antibodies for IMV, and similar cell-mediated immunity (IFN- γ production). Sera from LC16m8-vaccinated mice contained anti-B5 antibody, but at much lower levels than in sera from Dryvax-vaccinated mice. Sera from both groups exhibited anti-EEV neutralizing activity *in vitro*. However, possible effects of

Table 1
Summary of three clinical studies of smallpox vaccine LC16m8.

Study	Saito [6]	Nishiyama [7] ^a	Kennedy [8]
Country	Japan	Japan	USA
Study design	Clinical study, open-label	Post-marketing surveillance	Clinical trial phase I/II, randomized, double-blind
Race	Asian (100%)	Asian (100%)	Native American (1%), Asian (6%), African American (2%), Pacific Islander (1%), Caucasian (88%), Multiracial (2%)
Vaccine	LC16m8	LC16m8	LC16m8
Vaccination status	Naive	Naive	Naive
Number of participants	1529	196	125
Number of punctures	5	5	15
Major cutaneous reaction ("take") (%)	94.4	94.4	86 ^b
Serious adverse events	No	No	No
Weak or mild adverse events (%) ^c	22.4	27.0	68
Swollen axillary lymph nodes (%)	15.5	25.5	46
Swollen cervical lymph nodes (%)	0.4	- ^d	-
Fever (%)	2.6	1.0 ^e	-
		Vaccinated	Dryvax
		71	28
		10	15
		81.7	86 ^b
		No	No
		5.6	68
		2.8	46
		-	-
		2.8 ^e	-

Three studies are summarized based on data from references [6–8].

^a The vaccination history of one participant in the Nishiyama study was unknown.

^b See text.

^c Rate for local reactogenicity in the Kennedy 2011 study.

^d Not described.

^e Fever was recorded for two participants for both Naive and Vaccinated groups.

revertant B5 protein detected at low levels by western blot analysis could not be excluded [12].

The protective efficacy of a B5-deletion strain has also been compared to that of MVA and Dryvax in BALB/c mice challenged with virulent WR [3]. Vaccine strains were administered intramuscularly and WR challenge was given intranasally. The protective efficacy of the B5-deficient strain was similar to that of Dryvax and superior to that of Modified vaccinia Ankara (MVA), based on survival rate and loss of body weight. The B5-deficient strain also induced a neutralizing antibody response against WR. Results in severe combined immunodeficiency (SCID) mice also showed that LC16m8 and B5-deficient strains could be used safely in these immunodeficient animals.

The safety of LC16m8 vaccination with Lister and LC16mO strains has also been compared in suckling mice, SCID mice, and cyclosporin-A-treated mice [14]. Suckling mice received intracerebral inoculations and SCID mice were inoculated intraperitoneally. The survival rates of suckling mice after intracerebral inoculation with 10^{3.3} PFU vaccine were 10% with both Lister and LC16mO strains and 70% with LC16m8 in an observation period of 21 days. The mean survival times were 6.3, 6.1, and 17.1 days for the Lister, LC16mO, and LC16m8 strains, respectively. The survival rates of SCID mice were 0% for Lister and LC16mO strains, and 100% for LC16m8, with mean survival of 30.8, 24.5, and >120 days, respectively. Cyclosporin-A-treated mice developed severe vaccinia-related symptoms, including pock formation and rash, whereas LC16m8-inoculated mice displayed no clinical symptoms.

Empig et al. evaluated the efficacy of LC16m8 in protection against orthopoxvirus challenge in mice and rabbits [11], as reviewed by Kenner [15]. In rabbits, the protective efficacy of LC16m8 and Dryvax against rabbitpox virus was examined. This animal model was used to evaluate the anti-EEV efficacy of LC16m8 because rabbitpox virus produces high levels of EEV. LC16m8 and Dryvax gave similar protection against rabbitpox virus, whereas non-vaccinated animals did not survive lethal doses of the virus. Anti-IMV neutralizing antibodies were significantly higher in sera from LC16m8-vaccinated rabbits than in those vaccinated with Dryvax. Anti-EEV neutralizing antibodies in sera from LC16m8-vaccinated rabbits were lower than those in Dryvax-vaccinated rabbits, but the difference was not significant.

In non-human primates, the efficacy of LC16m8 against monkeypox virus in cynomolgus monkeys was compared with that of the Lister strain [10]. Vaccine or PBS was administered by the multiple puncture method with a bifurcated needle in macaques, similar to the vaccination procedure for humans. For virulent monkeypox virus challenge, two models were used: at 5 weeks post vaccination, monkeypox virus was administered intranasally (Liberia strain) or subcutaneously (Zr-599 strain). The proportions and sizes of major cutaneous reactions were assessed after immunization. In the intranasal challenge model, LC16m8- and Lister-vaccinated animals had no clinical symptoms, whereas non-vaccinated animals developed typical symptoms [10]. In the subcutaneous challenge model, LC16m8-vaccinated animals had no clinical symptoms, but exhibited an ulcer at the vaccination site. Lister-vaccinated animals were protected against virulent challenge, while control animals developed typical symptoms and died.

The safety and efficacy of LC16m8 and Dryvax strains were compared in cynomolgus macaques administered with vaccine or saline, and injected with monkeypox virus (Zaire 79 strain) 60 days later [13]. The animals were protected by both vaccines. To investigate the mechanism underlying the local containment of vaccinia-induced skin lesions, animals were depleted of host B cells or T cells before vaccination with Dryvax or LC16m8 [13]. B-cell depletion had no effect on the size of skin lesions in animals vaccinated with either vaccine, whereas T-cell depletion caused progressive vaccinia only in Dryvax-vaccinated animals.

These results indicate that LC16m8 is safer than Dryvax for use in immunocompromised individuals.

Thus, studies in three animal models (mouse, rabbit, and macaque, Table 2) have shown that LC16m8 protects the host against viral challenges [3,9–13] and has a good safety profile, including low neurotoxicity, consistent with findings of studies conducted in the 1970s [1]. Notably, LC16m8 can be administered to immunodeficient animals without severe adverse events, which is markedly different from the first-generation vaccine (Table 3) [3,13,14].

4. Long-term immunity conferred by LC16m8

Long-term immunity is a prerequisite for a good vaccine. Previous reports have demonstrated the decades-long efficacy of anti-smallpox vaccination [20–23]. Routine smallpox vaccination was conducted until 1976 in Japan and conferred long-term immunity, as confirmed by recent studies on anti-vaccinia antibodies and neutralizing antibodies [6–7,24].

In a study of the effect of LC16m8 for up to 7 months post-vaccination in humans [7], GMT was decreased, but the sero-conversion rate in primary vaccinees based on PRNT was still 75% at 7 months. Cell-mediated immunity was evaluated up to 180 days post-vaccination for 9 Dryvax vaccinees and 38 LC16m8 vaccinees in a US study [8], and lymphocyte proliferative responses were observed in 97% of LC16m8 and 89% of Dryvax vaccinees. Five LC16m8 vaccinees were negative for IFN- γ at 180 days post-vaccination, whereas all Dryvax vaccinees were positive.

In a comparison of the long-term effects of LC16m8 and the Lister strain in BALB/c mice [25,26], both groups of vaccinated mice were protected when challenged with a lethal amount of virulent WR after 1 year, whereas non-vaccinated mice died. An evaluation of serum antibody profiles by protein array 1.5 years after vaccination with LC16m8 [27] showed that antibody titers and neutralizing activities were substantially lower in comparison to 30-day sera, but that the LC16m8-vaccinated serum possessed antibodies common to Lister-vaccinated serum.

5. LC16m8 and formation of antibodies to B5 protein

B5 is a major antigen of EEV and antibodies against B5 are important for EEV neutralization [28,29]. B5 is a 42-kDa glycosylated type I membrane protein located in the EEV membrane [30,31]. After translation, B5 is transferred to the Golgi network and is internalized to the plasma membrane of the infected cell and exposed at the cell surface [31–33]. B-cell epitopes of anti-B5 monoclonal antibodies are localized to amino acid residues 56–84 and 254–275 [34]. LC16m8 has a point mutation in the B5R sequence, resulting in truncation to amino acids 1–91 of the original protein [35]. Given that this mutant lacks a C-terminal transmembrane domain, it is unlikely to be localized in the EEV [10]. In LC16m8-infected cultured cells, the truncated B5R product is recovered in cell extracts or supernatants [3,12]. Anti-truncated B5 antibodies are produced in mice upon vaccination with LC16m8 [12]. With regard to whether LC16m8 vaccination can induce the production of antibodies against the B5 protein in humans, Johnson et al. reported that the anti-B5 antibody did not increase upon vaccination of vaccinia-naïve individuals, as determined by ELISA [36]. The neutralizing ability of sera from LC16m8-vaccinated humans was lower than that of sera from Dryvax-vaccinated humans, based on 30% EEV plaque reduction activity [8]. This is consistent with the results of Johnson et al. [36].

Table 2
Animal model challenge studies of smallpox vaccine LC16m8.

Study	Morikawa [9]	Kidokoro [3]	Empig [11]	Meseda [12]	Empig [11]	Saijo [10]	Gordon [13]
Host animal	BALB/c mouse	BALB/c mouse	A/NCR mouse	BALB/cBYJ mouse	Rabbit	Cynomolgus macaque	Cynomolgus macaque
Vaccine	LC16m8, LC16mO, Lister	LC16m8, LC16m8 derivatives, Dryvax, MVA Intramuscular	LC16m8, Dryvax	LC16m8, Dryvax	LC16m8, Dryvax	LC16m8, Lister	LC16m8, Dryvax
Administration route	Subcutaneous	Intranasal	Scarfication	Scarfication	Bifurcated needle scarification	Bifurcated needle scarification	Bifurcated needle scarification
Administration dose (PFU)	10 ⁵ , 10 ⁷	10 ⁴ –10 ⁶	2 × 10 ⁵	10 ⁶	2 × 10 ⁵	>1 × 10 ⁸	2.5 × 10 ⁵
Challenge Virus	Vaccinia WR strain	Vaccinia WR strain	Ectromelia	Vaccinia WR strain	Rabbitpox	Monkeypox Liberia strain	Monkeypox Zaire strain
Inoculation route	Intranasal	Intranasal	Aerosol	Intranasal	Intradermal	Intranasal	Intravenous
Inoculation dose	10 ⁶ PFU	10 ⁶ PFU	1–2 × LD ₅₀	100 × LD ₅₀ (3.2 × 10 ⁶ PFU), 250 LD ₅₀ (8 × 10 ⁶ PFU)	1 × LD100 (200 PFU), 5 × LD100 (1000 PFU)	10 ⁶ PFU	5 × 10 ⁷ PFU

Animal model challenge studies are summarized based on data from references [3,9–13]. PFU, plaque forming unit; WR, Western Reserve; MVA, Modified vaccinia Ankara; LD, lethal dose.

Table 3
Studies of smallpox vaccine LC16m8 in immunodeficient animals.

Study	Kidokoro [3]	Yokote [14]			Gordon [13]
Immunodeficient animal	SCID mouse	Suckling mouse	SCID mouse	Cyclosporin-A treated mouse	T-cell, B-cell depleted rhesus macaque
Vaccine	LC16m8, LC16m8 derivatives, Dryvax, MVA	LC16m8, LC16mO, Lister	LC16m8, LC16mO, Lister	LC16m8, Lister	LC16m8, Dryvax
Administration route	Intraperitoneal	Intracerebral	Intraperitoneal	Intraperitoneal	Scarification
Administration dose (PFU)	10^3 – 10^7	$10^{3.3}$ – $10^{5.3}$ (LC16m8), $10^{1.3}$ – $10^{3.3}$ (LC16mO, Lister)	$10^{5.3}$	10^7	2.5×10^5

Immunodeficient animal studies are summarized based on data from references [3,13,14].

SCID mouse, severe combined immunodeficient mouse; MVA, Modified vaccinia Ankara; PFU, plaque forming unit.

6. Discussion

Since 2002, repeated evaluations of LC16m8 have consistently demonstrated its excellent safety profile [6–8]. No severe adverse events attributable to the vaccine were reported in studies conducted in the 1970s [1] or in the three relatively recent studies presented here [6–8]. These results indicate that LC16m8 is safer than first-generation smallpox vaccines, although the absence of rare adverse events cannot be conclusively confirmed because of the relatively small number of vaccinated subjects. Furthermore, LC16m8 can be safely administered to immunodeficient animals [3,13,14]; however, the safety of the administration of LC16m8 to immunocompromised patients has yet to be evaluated.

Vaccination with LC16m8 produces a high proportion of major cutaneous reactions [6–8], which is consistent with studies performed in the 1970s [1]. LC16m8 vaccination stimulates the production of neutralizing antibodies against orthopoxviruses, vaccinia, monkeypox, and variola viruses in human sera [8] and demonstrates host protection in animal-model challenge studies [3,9–13]. As experiments conducted in animal models are critical for research on smallpox vaccines [16–19], a comprehensive evaluation of clinical studies, *in vitro* experiments, and animal model studies suggests that the efficacy of LC16m8 is similar to that of the first-generation smallpox vaccines.

The LC16m8 vaccine induces both humoral and cellular immunity [3,6–14]. The efficacy of this vaccine has been debated because LC16m8 lacks an intact B5 protein [37]. However, LC16m8 has been shown to clearly protect the host in challenge studies conducted in mice [9,11,12] and other animal models [10,11,13]; recent reports suggest that even B5-deleted viruses can confer protection against virulent virus challenges in mice [3,38]. A protein array examination [39] of the immunogenicity of sera after vaccination with smallpox vaccine revealed the induction of multiple antibodies in a redundant manner [40–46]. This redundancy may account for LC16m8-conferred protection, which is similar to that observed in other vaccinia strains [40–46]; however, the role and importance of the truncated B5 protein in LC16m8-conferred protection remains unknown.

The WHO Strategic Advisory Group of Experts (SAGE) on Immunization have recommended LC16m8, together with ACAM2000, as a stockpile vaccine in 2013 [47]. To select the vaccine strain for stockpiling, SAGE conducted a systematic review of vaccines, which included the number of doses that should be stockpiled and the population to be targeted in case of smallpox re-emergence or as a preventive strategy [48]. SAGE made its recommendations based on expert consultations regarding the safety, immunogenicity, and efficacy of the vaccine and the practical properties that may be advantageous in the event of a smallpox outbreak, including lyophilized storage, administration via bifurcated needles, and production of major cutaneous reactions.

LC16m8 has been shown to confer protective effects against monkeypox in macaques [10,13], and sera from LC16m8-vaccinated humans exhibit anti-monkeypox neutralizing abilities [8]; these findings raise the possibility of the use of LC16m8 against human monkeypox. Surveys conducted in the Democratic Republic of the Congo revealed that a lack of smallpox vaccination caused a 5.2-fold higher risk of human monkeypox in non-vaccinated individuals [49]. Thus, LC16m8 is a viable alternative to first-generation smallpox vaccines to prevent human monkeypox, at least in high-risk populations [49]; however, its clinical efficacy against human monkeypox is yet to be evaluated.

7. Conclusions

Compared to first-generation smallpox vaccines, LC16m8 has a safer profile and similar efficacy. LC16m8 also has advantageous properties for practical use, such as possible administration with bifurcated needles. Vaccination with LC16m8 produces a high proportion of visible major cutaneous reactions. They are indicators of successful vaccination and the production of immunity. By using an established freeze-drying production method, LC16m8 can be easily stocked and administered, and the success of vaccination can be evaluated. This balance between safety and efficacy makes this vaccine valuable for use in the event of a smallpox outbreak. Further studies are expected to evaluate the use of LC16m8 against human monkeypox and in immunocompromised populations.

Conflict of interest

H. Y. is employed by the Chemo-Sero Therapeutic Research Institute. All other authors report no potential conflicts of interest.

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