RECOMMENDATION FOR AN EMERGENCY USE LISTING OF "Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" (LC16m8 vaccine) Submitted by KM Biologics, Japan

Abstract

The freeze-dried cell culture smallpox vaccine, "LC16 (KMB)" (LC16m8 vaccine) is manufactured by KM Biologics, a Japanese vaccine manufacturer, by growing live attenuated vaccinia virus (LC16m8 strain) in primary rabbit kidney cells, diluting the obtained virus solution, and dispensing the solution with a stabilizer. This product uses a bovine blood-derived ingredient (serum), bovine milk-derived ingredients (lactalbumin and casein), and porcine-derived ingredients (trypsin, peptone, and enzyme) in early steps (e.g., master and working seeds) of the manufacturing process.

Each vaccine dose contains an estimated potency of not less than 1.5~1.8 x 10^5 PFU.

The EUL application was submitted on 23 August 2024 and accepted for evaluation on 30 August 2024. The applicant submitted a dossier based on the format and content of the International Conference of Harmonization (ICH) Common Technical Document (CTD).

The data reviewed were based on non-clinical and clinical data from published literatures and reports from studies that became publicly available after the approval of smallpox vaccine LC16; the data served to evaluate the efficacy and safety of smallpox vaccine LC16m8. According to the data presented, only one study in humans connected LC16m8 with protection again mpox virus, and the immune response in this study was generated in 26 subjects. However, LC16m8 produced "take" in primary and secondary vaccinated participants, and "take" is also used to measure response. On the important question of protection against clade 1b, Applicant provided NHP data that suggested protection of LC16m8 against clade 1 mpox virus. The applicant should provide acceptable Risk Management Plan (RMP) appropriate for LMIC environment.

"LC16 (KMB)" (LC16m8 vaccine) is indicated for active immunization to prevent mpox disease and the vaccine is given by multiple puncture vaccination using bifurcated needle. It is given as a one dose vaccine from one year of age.

Pharmaceuticals and Medical Devices Agency (PMDA) of the Ministry of Health Labour and Welfare (MHLW) of Japan is the reference National Regulatory Authority for the listing of this vaccine.

This report was prepared by the product evaluation group (PEG) to be discussed by the technical advisory group for emergency use listing (TAG-EUL).

1 Introduction

1.1 Background

In July 2022, the WHO Director-General (DG) declared the mpox outbreak as a Public Health Emergency of International Concern (PHEIC) under the International Health Regulations (2005) (IHR). In May 2023 the PHEIC status was removed as significant progress was made in controlling the outbreak globally. On 14 August 2024, the DG, determined that the upsurge of mpox in the Democratic Republic of the Congo and a growing number of countries in Africa constitutes a PHEIC under IHR. The DG stated that: "The emergence of a new clade of mpox, its rapid spread in eastern DRC, and the reporting of cases in several neighboring countries are very worrying. On top of outbreaks of other mpox clades in DRC and other countries in Africa, it's clear that a coordinated international response is needed to stop these outbreaks and save lives."

Following this second declaration of a PHEIC for mpox, the Director-General triggered the process for the EUL of mpox vaccines, to accelerate access for lower-income countries which have not yet issued their own national regulatory approval. EUL also enables partners including Gavi and UNICEF to procure vaccines for distribution.

The WHO department of Regulation and Prequalification (RPQ) through the Vaccine assessment team (VAX) of the Prequalification Unit (PQ) issued a called for submission of Expression of Interest (EoI) for mpox vaccine manufacturers. Evaluation of vaccine candidates forms a major part of WHO's effort to curtail the spread of mpox.

1.2 mpox vaccines

The WHO classifies smallpox and other O*rthopoxvirus* vaccines into three generations. The first-generation vaccines include the Lister strain vaccine and Dryvax (New York City Board of Health [NYCBH] strain) manufactured by Wyeth Laboratories, Inc. These vaccines were manufactured and used during the WHO's Smallpox Eradication Programme and they contributed to the eradication of the disease. The second-generation vaccines are produced using cell culture with a virus strain isolated from the first-generation vaccine strains or their vaccine stocks by a plaque cloning technique. The third generation include vaccines prepared in cell culture with an attenuated virus strain developed during the late phase of the Smallpox Eradication Programme.

The basis for this repurposing is the data, suggesting that immunity acquired through exposure to a virus of the *Orthopoxvirus* genus, almost completely responds to the other virus of the same genus (such as smallpox virus, MPXV, and vaccinia virus [strain for smallpox vaccine]), and thus vaccination against smallpox is considered to provide cross-immunity to these viruses.

The current mpox public health emergency underscores the need for the availability of more vaccines for use against the mpox disease outbreak in some member states of the African continent, as the strategy for combating this has a public health and a vaccine component. The vaccine component involves the careful review and discussions of available vaccine data to assess their value in protecting against mpox disease and a potential recommendation of use based on a careful benefit - risk approach.

Vaccines that could exert protective immunity after a single dose are preferred.

1.2.1 "LC16 (KMB)" vaccine

This freeze-dried cell culture smallpox vaccine, "LC16 (KMB)" (LC16m8 vaccine) is manufactured by growing live vaccinia virus (LC16m8 strain) in primary rabbit kidney cells (free of infectious agents), by diluting the obtained virus solution, and by dispensing the solution with a stabilizer. This product uses a bovine blood-derived ingredient (serum), bovine milk-derived ingredients (lactalbumin and casein), and swine-derived ingredients (trypsin, peptone, and enzyme) in its early steps of the manufacturing process (e.g., preparation of virus seed banks).

LC16m8 vaccine is a freeze-dried smallpox vaccine prepared in cell culture, initially approved for the indication of "prevention of smallpox". The vaccine is given by multiple puncture vaccination (by scarification) using a bifurcated needle. It is a one dose vaccine given from one year of age. In August 2022, PMDA extended the indication to include "prevention of mpox".

Each dose contains an estimated potency of not less than 1.5 - 1.8 x 10^5 PFU.

Since LC16m8 vaccine has never been tested for efficacy against smallpox (e.g. variola virus) in clinical trials, the protective efficacy was evaluated in various animal studies using mouse, rabbit and monkeypox models. In 2009, results of two clinical trials conducted in healthy subjects were published. The first study was performed by the Japanese Self Defense Forces between 2002 and 2005 in 3468 vaccinia naïve and previously vaccinated adults aged 18 to 55 years. In another phase I/II clinical trial performed in the US, the safety and immunogenicity of LC16m8 vaccine were compared with Dryvax in healthy vaccinia-naïve subjects 18 to 34 years of age. Results of these studies are also published and detailed in section 3.3.

The Chiba Serum Institute in Japan received an unconditional license in 1975 in Japan for manufacturing the vaccines of the LC16m8 strain for the indication of "prevention of smallpox". In 1975 Chiba Serum Institute produced the vaccine in a frozen formulation, and in 1980 a lyophilized formulation was developed. The routine vaccination against smallpox in Japan halted in 1976; and Chiba Serum Institute ended the production of the vaccine accordingly, without distributing the product in the market. The license and all product-related rights of LC16m8 vaccine were transferred to the Chemo-Sero Therapeutic Research Institute (hereafter, "Kaketsuken") in September 2002, and thereafter by KM Biologics in July 2018 due to the transfer of pharmaceutical business from Kaketsuken. Manufactured vaccine lots have been maintained as part of the national stockpile.

The LC16m8 vaccine was developed more than 20 years ago. Japan had or has stockpiles of the vaccine produced in cell culture , based on the Lister derived strain LC16m8, which has revealed a take rate similar to that of Lister vaccines and did not show any neurovirulent properties in various animal models.

The strain LC16m8 is derived from strain Lister-Elstree by multiple passages in PRK cells at 30°C followed by additional plaque cloning. The highly attenuated strain seems to have the same potential to induce a protective immune response compared to the old type of vaccines as far as evaluated with old criteria of immunity to smallpox vaccine. However, according to animal models and from limited numbers of human

trials, LC16m8 shows lower neurovirulence and a decrease in side effects compared to Lister-Elstree and Ikeda strain.

The vaccine was developed and initially used in Japan and is approved by the Japanese PMDA for the indication of "prevention of smallpox and mpox". Its marketing authorization holder is KM Biologics Co., Ltd. Vaccine batches are released by National Institute of Infectious Diseases (NIID) .

The vaccine has emergency authorization in the Democratic Republic of Congo.

1.3 Emergency Use Listing

The Emergency Use Listing (EUL) is a time limited benefit-risk assessment for emergency use of vaccines, medicines and *in vitro* diagnostics during a PHEIC when limited data are available and the products are not yet ready for licensure and WHO prequalification. As the EUL is time-limited in nature, the applicant is still expected to complete the development of the product and submit application for licensure and prequalification.

The issuance of an EUL for a product reflects WHO's recommendation for emergency use following a robust scientific benefit-risk assessment. However, each WHO Member States has the sole prerogative to allow the emergency use of a product under an EUL within their country.

2 Assessment process

This vaccine is manufactured by KM Biologics and was assessed under the WHO EUL procedure based on the review of data on quality, safety, efficacy, risk management plan (RMP) and programmatic suitability performed by WHO vaccine prequalification experts and evaluators from national regulatory authorities (NRAs) from different countries and regions.

During the evaluation of the dossier, several meetings were held with the applicant as well as several rounds of questions and answers. WHO emphasizes the assessment of programmatic considerations (e.g., storage, handling and administration of the vaccine) and the Risk Management Plan (RMP) to ensure that the perspectives and concerns of regulators from different regions are considered; these may not be considered by the NRA of reference whose assessment is focused on issues related to its own jurisdiction.

3 Scientific Review

3.1 Quality Overview

In accordance with the abbreviated review, the quality assessment by WHO of Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" manufactured by KM Biologics, in Japan consisted in the evaluation of programmatic characteristics of the vaccine and its suitability for LMICs (see section 6.2 of

this report). A summary of the quality aspects of the product, based on the inputs received from the PEG and the PMDA assessment report, is provided in sections below.

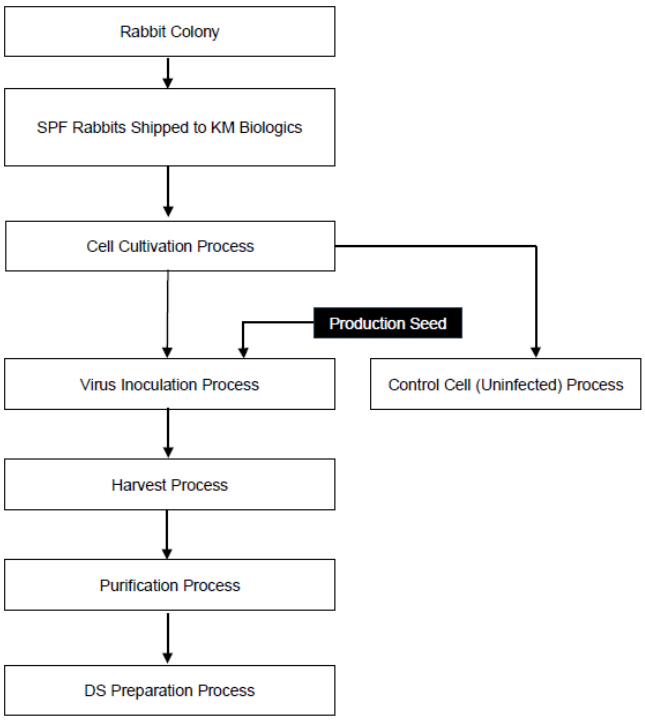
The drug substance and drug product (the vaccine) are manufactured and controlled by KM Biologics, Japan. Prior to the current PHEIC, the vaccine was licensed and used only in Japan. The PMDA conducted a recent GMP inspection to the facilities where LC16m8 is manufactured.

Smallpox vaccine LC16m8 is manufactured through the process comprising propagation of live vaccinia virus (LC16m8 strain) in the **primary rabbit kidney cells** (PRK), dilution of the obtained viral suspension, formulation of the diluted viral suspension with stabilizers, subdivision of the formulated viral suspension, and lyophilization of the subdivided viral suspension.

3.1.1 Drug Substance

The LC16m8 vaccine is an attenuated live virus vaccinia vaccine produced in cell culture. The cell substrate used for production is primary rabbit kidney (PRK) cells from the Kbl:JW strain of rabbits. Traces of gelatin, phenol red, and the antibacterial agents (streptomycin sulfate for injection and erythromycin lactobionate) could be present in the drug substance, which is a yellow, red liquid. Antibacterials are used at very early stages of the manufacturing process (e.g., seed lot system).

Production started with the seed preparation, cell cultivation, virus inoculation and cultivation, harvest, drug substance preparation, and storage of drug substance.
C16m8 Cell Substrate Preparation & Drug Substance Production



Every main step, as represented in the above diagram, is divided in several other sub steps. For example, the Cell Cultivation Process consists of 6 steps as follows:

a) Rabbit receipt and health assessment
b) Bled out and washed
c) Necropsy and kidney extraction
d) Kidney processing
e) Preparation of RK cells
f) Cell Cultivation in roller bottles

Process validation

Process validation was conducted where six batches of intermediate (IM) were used to prepare the three (3) consecutive "Conformance Lots" that were manufacture in September and December 2008.

Stability study was performed and completed on the LC16m8 drug substance using the commercial-scale of three confirmation lots, and a long-term storage test (–80°C ± 10°C, 60 months), accelerated test (–20°C ± 5°C, 12 months), and stress test (5°C ± 3°C, 7 days).

Temperature condition	State	Period	Reference
–80°C ± 10°C	Frozen	60 months	Long-term storage test
–20°C ± 5°C	Frozen	12 months	Accelerated test
5°C ± 3°C	Thawing	7 days	Stress test

Batches were tested for:

- Test for virus content (must be not less than 108 PFU/mL)
- Appearance test
- Marker test (test for the temperature-sensitivity of the virus growth)
- Marker test (test for pock morphology)
- Neurovirulence test
- Test for antibiotic content (streptomycin sulfate)
- Test for antibiotic content (erythromycin)
- Sterility test
- Mycoplasma test
- Test for mycobacterial

All mean values at 60 months for the test of virus content are $10^{8.87}$ to $10^{8.89}$ PFU/mL when stored at -80°C ± 10°C and 12 months when stored at -20°C ± 5°C. All results of quality tests performed in this stability study met the specifications, and there were no changes, which might raise concerns about stability of quality, during the storage period. In conclusion, the LC16m8 drug substance was confirmed to be stable at least under the temperature conditions and storage periods described in the table above.

3.1.2 Drug Product

KM Biologics Co., Ltd. Kumamoto Production Center 1-6-1 Okubo, Kita-ku, Kumamoto-shi, Kumamoto 860-8568, Japan is the manufacturer of the LC16m8 vaccine. This site is responsible for the final bulk preparation, formulation (filling, lyophilization, crimping), labeling/packaging, tests on final bulk, tests on final product, tests on finished product, in-process control test and test of raw materials.

The finished product is a freeze-dried smallpox vaccine prepared in cell culture and controlled as per the Japanese Minimum Requirements for Biological Products (MRBP).

The final product is a yellowish, freeze-dried preparation. When reconstituted with the supplied diluent, it becomes a clear or slightly turbid, yellowish or reddish liquid.

Component	Content – 0.5 mL	Function
Live vaccinia virus (LC16m8 strain)	Not less than 5.0×10^7 PFU[a] Not less than 2.5×10^7 PFU[b]	Active ingredient
Sorbitol	5 w/v%	Stabilizer
Peptone	5 w/v%	Stabilizer
Phenol red	Not more than 0.002 w/v%	Coloring agent
Gelatin	Not more than 0.15 w/v%	Stabilizer
Medium 199	Remaining amount	Tonicity agent
Sodium bicarbonate	Appropriate amount	pH adjustment

a: Plaque forming unit
b: Pock forming unit

This is a live attenuated vaccine, manufactured by growing vaccinia virus (LC16m8 strain) in primary rabbit kidney cells (PRK cells).

The finished product consists of 1 vial of the final product as a freeze-dried powder containing live vaccinia virus (LC16m8 strain), the active ingredient, for ≥50 doses, and 1 vial of diluent (0.5 mL).

In cases such as well coordinated mass vaccination where a large number of persons need to be inoculated consecutively, the EUL applicant claimed that approximately more than 250 recipients can be vaccinated if the 0.5 mL of vaccine solution is prepared by reconstituting this vaccine with 0.5 mL of provided diluent and bifurcated needles for smallpox vaccination with a single collection volume of 1 ± 0.5 µL (specified value) are used.

Manufacturing

The sterility of aseptic operations in the filling step is confirmed by performing process simulation test before starting manufacture. For the manufacturing equipment to be used in the filling and lyophilization steps, media fill tests were performed when manufacturing equipment was transferred from the Chiba Serum Institute.

LC16m8 vaccine is manufactured and tested, assuring that the reconstituted vaccine vial contains at least 1.0×10^8 PFU (plaque forming unit) /mL or $1.0 \times 10^{7.7}$ PFU (pock forming unit) /mL of infectious vaccinia viruses.

Quality Control

Control tests includes airtightness, description, moisture content, foreign insoluble matter test, mass variation test, sterility test, potency (assay for plaque formation and plaque-formation assay) and thermostability tests.

The batch analysis is submitted for batches manufactured between 2004 to2020.

Test methodologies are verified or qualified (e.g., BSA, test for moisture content, foreign insoluble matter test) or validated (e.g., sterility test by MRBP; mycoplasma; plaque formation / pock formation, etc.). Testing methodology is based on MRBP and/or Japanese Pharmacopoeia.

Container closure system

For the final product vials and rubber stoppers, acceptance testing is conducted in accordance with the Test for Glass Containers for injections and Test for Rubber Closure for Aqueous Infusions in the Japanese Pharmacopoeia.

The vaccine vial is made out of borosilicate glass Type I, with a nominal capacity of 2 mL (2R), with butyl rubber stopper and closed with a cap of aluminum and type of flip off of polypropylene.

Stability

The vaccine has proven to be stable for up to 48 hours at 37°C after reconstitution. In this test, the formulation is placed at 37 ± 1°C for 4 weeks, then the potency test is performed. The potency should be “not less than 1/10 of the potency value before exposing the vaccine at this condition''. Although this test does not necessarily demonstrate the thermal stability after reconstitution, the fact is that the potency of lyophilized vaccine remains approximately 1/3 of the potency even after the heat treatment at 37 ± 1°C for 4 weeks, still above the minimum potency specification.

It was confirmed that the finished product is stable at –20°C or below for up to 143 months from the manufacturing date. Based on real time data, the recommended storage temperature is at –20°C or below this temperature. When stored at this temperature, the shelf-life of the vaccine is 10 years, from the date it is released by national control laboratory. However, vaccine vials should not be stored at –35°C or below because the rubber stopper could deteriorate or be damaged.

This vaccine can be stored at 2°C to 8°C for 2 years, without reconstitution.

Avoid exposing the drug product to light, both before and after reconstitution. Sunlight rapidly inactivated the viral active substance.

Once the vaccine is moved to refrigerated storage, it should be used without being returned to frozen storage, and within the expiration period or within 2 years after being moved to refrigerated at 2°C to 8°C.

After reconstitution with the provided vaccine diluent, the vaccine should be used during the planned immunization session, or discarded after 6 hours, whatever becomes first. However, because of the nature of this product and the prescribed handling conditions, it is recommended its use immediately after reconstitution.

Because the vaccine does not contain preservatives, caution should be exercised to prevent bacterial contamination when it is reconstituted, and rubber stopper is removed. Therefore, the vaccine solution in a vial whose stopper has been removed in a non-sterile environment should be used immediately. The solution remaining in the vial must always be disposed of without being stored again and used for the next vaccination.

Because the shelf-life of the vaccine was extended, the applicant is introducing labels affixed to the individual carton boxes that will be send to recipient countries. These labels will have QR codes printed on them. By scanning the QR code, users will be directed to a web site where the administrative notice of MHLW (English version) and the latest package insert (English version) are posted. This will allow verification and comparison of the old and new expiry date as well as access to other information. Additionally, the EUL holder will inform the vaccine-receiving country or organization about the above risk minimizing initiatives before proceeding with the export.

3.2 Non-clinical overview

For the present application, no evaluation data have been submitted. Published literature of studies on the immunogenicity of smallpox vaccine LC16m8 and vaccine efficacy have been submitted as reference data on primary pharmacodynamics.

Smallpox Vaccine Safety Is Dependent on T Cells and Not B Cells (J Infect Dis. 2011;203:1043-53)

Macaques' monkeys (unknown sex) were (depleted systemically of T or B cells) vaccinated with either Dryvax or an attenuated vaccinia vaccine and LC16m8. They percutaneously received a single dose of 2.5 × 10^5 PFU/animal of smallpox vaccine LC16m8 (n = 14), 2.5 × 10^5 PFU/animal of Dryvax (n = 4), or phosphate-buffered saline (PBS) (n = 6) with a bifurcated needle. The animals were intravenously challenged with 5 × 10^7 PFU of MPXV (Zaire 79 strain) 60 days after vaccination.

During a period from vaccination to challenge, smallpox vaccine LC16m8 and Dryvax induced comparable neutralizing antibody and cell-mediated immune responses to MPXV. In the negative control group, intravenous infection with MPXV resulted in multiple skin lesions throughout the body followed by deaths or euthanasia in all the animals within 12 days after challenge with MPXV. The number of skin lesions in smallpox vaccine LC16m8-immunized animals was greater than that in Dryvax immunized animals but was smaller than that in animals in the negative control group. In addition, in smallpox vaccine LC16m8-immunized animals and Dryvax-immunized animals, all skin lesions fully recovered and formed scabs within 12–15 days after challenge with MPXV, and no deaths occurred. The blood viral genome loads in the smallpox vaccine LC16m8 group and Dryvax group were remarkably lower than that

in the negative control group and became undetectable by 9 days after challenge with MPXV. This data identifies LC16m8 vaccine as a safer and effective alternative to ACAM2000 and Dryvax vaccines for immunocompromised individuals.

LC16m8, a Highly Attenuated Vaccinia Virus Vaccine Lacking Expression of the Membrane Protein B5R, Protects Monkeys from Monkeypox (J Virol. 2006; 80: 5179-88).

In this study, Cynomolgus monkeys percutaneously received a single dose of at least 1×10^8 PFU/mL of smallpox vaccine LC16m8 (3 females per group), at least 1×10^8 PFU/mL of Lister strain vaccine (2 or 3 females per group), or a mock-up vaccine as negative control (2 females or 1 male and female each per group) with a bifurcated needle. Five weeks after vaccination, smallpox vaccine LC16m8-immunized animals, Lister strain vaccine-immunized animals, and mock-immunized animals were intranasally or subcutaneously inoculated with 1×10^6 PFU of MPXV Liberia strain or 1×10^6 PFU of Zr-599 strain.

During a period from vaccination to challenge with MPXV, smallpox vaccine LC16m8 and Lister strain vaccine induced anti-vaccinia virus IgG antibodies and neutralizing antibody responses to MPXV. Vaccinia virus (VV) antigen-specific IgG became detectable by IgG-ELISA in monkeys immunized with Lister or LC16m8 within 2 weeks postimmunization. The time courses and levels of IgG response determined by ELISA were similar for monkeys immunized with LC16m8 and those immunized with Lister. IgG reactive to VV antigens became detectable by IgG-ELISA in the IN-Naïve and SC-Naıve groups within 2 weeks after MPXV challenge. The levels of neutralizing antibody to MPXV were tested before and after challenge with MPXV. At the time of challenge with MPXV, neutralizing antibody was detected in the monkeys immunized with LC16m8 or Lister. The titers were not increased after the challenge. Neutralizing antibody was demonstrated in both of the animals in the IN-Naıve group and in one of the two animals in the SC-Naïve group.

After the intranasal inoculation with MPXV, viremia occurred in the negative control group but did not occur in the smallpox vaccine LC16m8 group or Lister group. At clinical observation after challenge with MPXV, papulovesicles and rhinorrhoea were observed in the negative control group, but no monkeypox-related lesions were observed in the smallpox vaccine LC16m8 group or Lister group. At histopathological examination 3 weeks after challenge with MPXV, lesions in the skin, lungs, pancreas, thymus, tonsil, and lymph nodes were observed in the negative control group, but no monkeypox-related lesions were observed in the smallpox vaccine LC16m8 group or Lister group.

After the subcutaneous inoculation with MPXV, the blood viral genome load in the smallpox vaccine LC16m8 group was higher than that in the Lister group but was lower than that in the negative control group, with a shorter period of detection. At clinical observation after challenge with MPXV, many severe monkeypox related lesions such as papulovesicular were observed in mock-immunized animals, and thus these animals were euthanized. In the smallpox vaccine LC16m8 group, on the other hand, lesions limited to the site of MPXV inoculation (erythema, papulovesicular, and ulcer) were observed. In the Lister group, no monkeypox-related lesions were observed. At histopathological examination 3 weeks after exposure to MPXV, monkeypox-related lesions in the lymph nodes, thymus, tonsil, lungs, trachea, stomach, small intestine, colon, rectum, liver, bladder, uterus, ovaries, and skins were observed in the negative control group, but no monkeypox-related lesions were observed in the smallpox vaccine LC16m8 group except for skin lesions at the site of MPXV inoculation. In the Lister group, no monkeypox-related lesions were

observed. These results indicate that LC16m8 prevents lethal monkeypox in monkeys, and they suggest that LC16m8 may induce protective immunity against mpox.

A Single Vaccination of Nonhuman Primates with Highly Attenuated Smallpox Vaccine, LC16m8, Provides Long-term Protection against Monkeypox (Jpn J Infect Dis. 2017; 70:408-15)

Cynomolgus monkeys percutaneously received a single dose of at least 1×10^8 PFU/mL of smallpox vaccine LC16m8 (3 males per group), at least 1×10^8 PFU/mL of Lister strain vaccine (2 males per group), or a mock-up vaccine as negative control (3 males and 1 female per group) with a bifurcated needle. Smallpox vaccine LC16m8-immunized animals, Lister strain vaccine-immunized animals, and mock-immunized animals were subcutaneously inoculated with 10^6 PFU of MPXV (Zr-599 strain) 6 or 12 months after vaccination.

During a period from vaccination to challenge with MPXV, smallpox vaccine LC16m8 and Lister strain vaccine induced anti-vaccinia virus IgG antibodies. The anti-vaccinia virus IgG antibody titer in smallpox vaccine LC16m8-immunized animals was lower than that in Lister strain vaccine-immunized animals 6 and 12 months after vaccination but increased immediately after challenge with MPXV.

After challenge with MPXV, several papulovesicular lesions, as well as ulcerative lesions at the site of MPXV inoculation were observed in the negative control group. In the smallpox vaccine LC16m8 group, on the other hand, only ulcerative lesions at the site of MPXV inoculation were observed. None of such skin lesions were observed in the Lister group. At histopathological examination 3 weeks after challenge with MPXV, monkeypox-related lesions were observed in many organs of the lymphatic, respiratory, gastrointestinal, and urogenital systems in mock-immunized animals, but none of such lesions were observed in the smallpox vaccine LC16m8 group or Lister group.

Dose-Range Finding Study of LC16m8TM in the Intratracheal Monkeypox Challenge Model in Cynomolgus Macaques - Southern Research Institute Study No.: SR11-002F Final Report, February 20, 2012.

In this study the immunogenicity of different doses of smallpox vaccine LC16m8™ and its ability to confer protection against lethal respiratory exposure with $5x10^6$ PFU MPXV via the intratracheal route compared to its counterpart vaccine ACAM2000™ in cynomolgus macaques were investigated. Fifty-four (54) cynomolgus macaques were assigned to 9 groups of 6 animals each. On Study Day 0, Groups 1-5 were vaccinated with decreasing doses of LC16m8™ ranging from $1.5x10^6$ - $2.5x10^3$ PFU by scarification. Group 6, Group 7 and Group 8 were vaccinated with $2.5x10^5$, $2.5x10^4$ and $2.5x10^3$ PFU ACAM2000™, respectively. Group 9 received vaccine diluent only and served as the negative controls. On Study Day 60, all macaques were challenged by IT route with $5x10^6$ PFU MPXV and subsequently monitored for 28 days post-challenge.

Doses of $1.5x10^6$ (Group 1), $6.2x10^5$ (Group 2), $2.5x10^5$ (Group 3), $2.5x10^4$ (Group 4) and $2.5x10^3$ (Group 5) PFU LC16m8™ administered by scarification provided 83%, 100%, 83%, 0 and 17% protection, respectively, in cynomolgus macaques challenged with 5x106 PFU MPXV via IT route. ACAM2000™ when similarly administered at $2.5x10^5$ (Group 6), $2.5x10^4$ (Group 7) and $2.5x10^3$ (Group 8) PFU conferred 100%, 83% and 50% protection, respectively against the same challenge dose of MPXV.

The results demonstrate that LC16m8™ when administered to cynomolgus macaques at doses of $1.5x10^6$, $6.2x10^5$ and $2.5x10^5$ PFU provided the same level of protection (100 - 83%) against lethal MPXV disease compared to ACAM2000™ given at $2.5x10^5$ (standard clinical dose) and $2.5x10^4$ PFU.

PMDA summarised from the non-clinical data that Smallpox vaccine LC16m8 induces cross-immunity (humoral and cell-mediated immune responses) to MPXV, which belongs to the *Orthopoxvirus* genus as with vaccinia virus, thereby inhibiting MPXV replication and preventing monkeypox. The immunogenicity and challenge studies in monkeys showed that smallpox vaccine LC16m8 was immunogenic against MPXV (induction of neutralizing antibodies and cell-medicated immune response), prevented viremia after MPXV infection, and prevented the onset of monkeypox-related lesions, although its effects tended to be weaker than those of the first-generation smallpox vaccines such as Lister strain vaccine and Dryvax. Based on the above study results and from a pharmacological viewpoint, PMDA considers it reasonable to expect smallpox vaccine LC16m8 to induce cross-immunity to MPXV and thereby prevent monkeypox in humans as well.

Comments: Animal studies showed that LC16m8 induced durable immune response in normal and immunocompromised animals, and protected animal following challenge of lethal doses of mpox virus even after 12 months of vaccination. Some of the data provided also demonstrate that LC16m8 when administered to cynomolgus macaques at doses of $1.5x10^6$, $6.2x10^5$ and $2.5x10^5$ PFU provided the same level of protection (100 - 83%) against lethal MPXV disease compared to ACAM2000™ given at $2.5x10^5$ (standard clinical dose) and $2.5x10^4$ PFU.

3.3 Clinical Overview

Listing of clinical studies for the efficacy and safety evaluation

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Clinical research	KA01	Module 5.3.5.2	To evaluate the effect and safety of the attenuated smallpox vaccine in humans.	Open-label	Multiple puncture technique of LC16mO or LC16m8 strain vaccines	53,000 vaccinated approximately (LC16mO: 3,000, LC16m8: 50,000) 11,407 clinically evaluated (LC16mO: 829, LC16m8: 10,578)	Healthy pediatric subjects	Single-dose treatment	Complete; Published literature
Clinical research	KA02	Module 5.3.5.2	To assess the immunogenicity and safety of LC16m8 vaccine in unvaccinated and previously vaccinated adults.	Open-label	Inoculation with approximately 4 μL of vaccine containing a suspension of greater than 1×10^8 pfu/mL of the LC16m8 strain.	3468 enrolled, 3221 vaccinated (primary vaccinee: 1529, revaccinee: 1692)	Healthy adult subjects	Single-dose treatment	Complete; Published literature
post-marketing surveillance study	KA03	Module 5.3.6	To conduct a post-marketing surveillance study of the vaccination of freeze-dried live attenuated vaccinia strain LC16m8.	Open-label	Multiple puncture technique of freeze-dried live attenuated smallpox vaccine prepared in cell culture "LC16-KAKETSUKEN"	268 vaccinated and analyzed for efficacy and safety.	Healthy adult subjects	Single-dose treatment	Complete; Published literature
Phase I/II clinical study	KA04	Module 5.3.5.1	To assess safety, clinical responses, and immunogenicity of LC16m8 vaccine in healthy vaccinia-naïve volunteers	Randomized, multi-center, double-blinded, Dryvax-controlled study	LC16m8, administered by standard scarification method at an approximate dose of not less than 10^8 pfu/mL	154 vaccinated (Dryvax: 29, LC16m8: 125)	Healthy adult subjects	Single-dose treatment	Complete; Clinical Study Report and Published literature

PMDA reviewed published literature and reports from studies that became publicly available after the approval of smallpox vaccine LC16m8 because the data served to evaluate the efficacy and safety of smallpox vaccine LC16m8.

Safety and immunogenicity of LC16m8, an attenuated smallpox vaccine in vaccinia-naive adults (J Infect Dis. 2011;204:1395-402)

This phase I/II randomized double-blind comparative study was conducted in healthy adults aged 18 to 34 years without prior smallpox vaccination in the U.S. in 2004 and 2005, to evaluate the safety of smallpox vaccine LC16m8 versus Dryvax and compare the neutralizing antibody titers against viruses of the Orthopoxvirus genus including MPXV 30 days after vaccination (Day 30). A single dose of either vaccine 0.02 µL (1×10^8 PFU/mL) was administered percutaneously (through 15 punctures) with a bifurcated needle. A total of 125 subjects (81 men and 44 women) received smallpox vaccine LC16m8, and all showed a "take (major cutaneous reaction)" at the administration site between Days 6 and 12. Both vaccines achieved antivaccinia, antivariola, and antimonkeypox neutralizing antibody titers 1:40, although the mean plaque reduction neutralization titer of LC16m8 at day 30 after vaccination was significantly lower than Dryvax for anti-NYCBH vaccinia (P, 0.01), antimonkeypox (P, 0.001), and antivariola (P, 0.001). In 26 subjects randomly extracted from those receiving smallpox vaccine LC16m8, the geometric mean titer (GMT) of neutralizing antibodies against MPXV on Day 30 was 112 (95% confidence interval [CI], 82-307). This shows that smallpox vaccine LC16m8 induced neutralizing antibodies against not only various strains of vaccinia virus (NYCBH, Lister, and LC16m8 strains) but also against MPXV. LC16m8 also produced robust cellular immune responses that trended higher than Dryvax for lymphoproliferation (P = 0.06), but lower for IFN-c ELISPOT (P = 0.02).

As for the safety, 82% (102 of 125) of subjects receiving smallpox vaccine LC16m8 experienced at least 1 local reactogenicity event, and the incidence of local reactogenicity events was as follows: feeling hot, 36% (45 of 125); vaccination site tenderness, 42% (52 of 125); limited arm movement, 12% (15 of 125); axillary lymphadenopathy, 37% (46 of 125); axillary lymph node tenderness, 48% (60 of 125); rash, 2% (3 of 125); satellite lesions, 2) 2% (3 of 125). Systemic reactogenicity events including pyrexia occurred in 74% of the subjects receiving smallpox vaccine LC16m8. No subjects experienced serious adverse events related to the vaccination, such as postvaccinal encephalitis/encephalopathy, dermal complication, and myocarditis/pericarditis. The important finding of this study was that LC16m8 generates neutralizing antibody titers to multiple poxviruses, including vaccinia, monkeypox, and variola major, and broad T-cell responses, indicating that LC16m8 may have efficacy in protecting individuals from smallpox.

Comparison of PRNT Titers Using Different Plaquing Viruses at Day 30 After Vaccination in a Subset of Dryvax and LC16m8 Volunteers Tested at All Laboratories

PRNT assay virus	Dryvax	LC16m8	Dryvax/LC16m8 ratio	*P* value
Number of titers	23	26	...	
Anti-Dryvax (Focus)				
Geometric mean	919	329	2.8	<.001
95% CI	565–1493	228–474	1.6–5.0	
Anti-LC16m8 (KKT)				
Geometric mean	471	733	0.6	.24
95% CI	301–739	400–1343	.3–1.4	
Anti-Lister (KKT)				
Geometric mean	7686	17 523	0.4	.09
95% CI	3687–16 026	9104–33 725	.2–1.1	
Anti-monkeypox (JNIID)				
Geometric mean	368	112	3.3	<.001
95% CI	225–597	82–307	1.7–6.3	
Anti-NYCBH (JNIID)				
Geometric mean	482	158	3.0	.01
95% CI	287–810	82–307	1.3–7.0	
Anti-Lister (JNIID)				
Geometric mean	298	207	1.4	.29
95% CI	176–504	129–332	.7–2.9	

Per protocol cohort excludes 4 volunteers who had anti-Dryvax PRNT (Focus) titers ≥40 at baseline and 2 volunteers who had missing anti-Dryvax PRNT (Focus) titer data at day 30. Plaque viruses included Dryvax (NYCBH–vaccine-derived virus), NYCBH (Japan vaccine–derived seed), Lister, LC16m8, and monkeypox. PRNT tests were performed at 3 different laboratories: anti-Dryvax assay at Focus, Inc; anti-NYCBH at JNIID (Tokyo, Japan); anti-Lister vaccinia at both JNIID and KKT (Kumamoto, Japan); anti-LC16m8 vaccinia at KKT; and the antimonkeypox at JNIID. These assays were performed on sera obtained on day 30 after vaccination. *P* values were calculated using Wilcoxon rank-sum test.

Abbreviations: CI, confidence interval; JNIID, Japanese National Institute of Infectious Diseases; KKT, Kaketsuken; NYCBH, New York City Board of Health; PRNT, plaque reduction neutralization titers.

Freeze-dried live attenuated smallpox vaccine prepared in cell culture LC16-KAKETSUKEN (Vaccine. 2015;33:6120-7).

A use-results survey was conducted to evaluate the safety of smallpox vaccine LC16m8 in 268 members of Japan Self-Defence Force who were aged 19 to 52 years (261 men and 7 women) at a single center in Japan from 2005 to 2010. A single dose of smallpox vaccine LC16m8 at 0.01 mL (at least 1×10^8 PFU/mL) was administered percutaneously with a bifurcated needle. The number of punctures was 5 times in primary vaccinees (without prior smallpox vaccination) and 10 times in previously vaccinated individuals.

A “take” was observed in 94.4% (185 of 196) of the primary vaccinees and 81.7% (58 of 71) of the previously vaccinated individuals.

A total of 268 individuals received smallpox vaccine LC16m8. Adverse events occurred in 27.0% (53 of 196) of the primary vaccinees, 5.6% (4 of 71) of the previously vaccinated individuals, and 100% (1 of 1) of the individual with unknown vaccination history. The following adverse events were observed in this survey: axillary lymphadenopathy in 52 individuals (19.4%), pyrexia in 4 (1.5%), malaise in 2 (0.7%), rash in 1 (0.4%), vaccination site erythema in 14 (5.2%), vaccination site swelling in 1 (0.4%), and autoinoculation in 1 (0.4%). All of them were non-serious. Dermal complication associated with vaccination occurred in 2 primary vaccinees: one experienced dermatitis allergic but not eczema vaccinatum (outcome, resolved), and the other experienced rash unrelated to vaccination. Electrocardiography revealed mild atrioventricular block first degree in 2 individuals, but both events were considered unrelated to vaccination. After vaccination, laboratory test abnormal was observed in several individuals, but all events were considered unrelated to vaccination. No individuals died or had

serious adverse events (e.g., cardiovascular diseases, encephalitis, satellite lesions, and progressive vaccinia) during the survey period.

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.

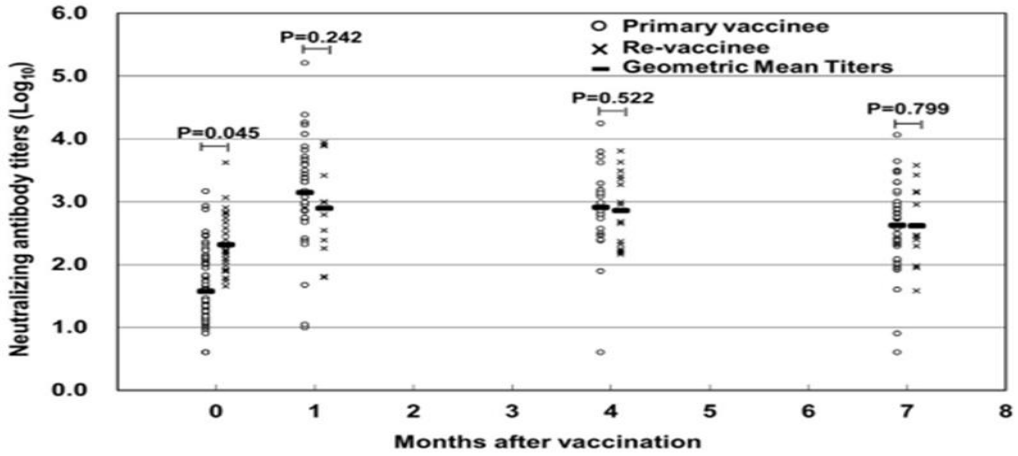


Fig. 2. Time course of neutralizing antibody titers in the serum after vaccination. The time course of neutralizing antibody titers was examined for both primary vaccinees and re-vaccinees. Of note, the titer of one primary vaccinee changed from seropositive to seronegative (<8) at 7 months after inoculation.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

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.

The above figure and table 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. In this study primary vaccinees showed a statistically higher seroconversion rate at 1, 4 and 7 months after vaccination than that shown by re-vaccinees. . 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 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).

Clinical and Immunological Response to Attenuated Tissue-Cultured Smallpox Vaccine LC16m8 (JAMA. 2009;301:1025-33)

Clinical research was conducted to evaluate the safety of smallpox vaccine LC16m8 in healthy members of Japan Self-Defence Force who were aged 18 to 55 years with no skin lesions at a single center from 2002 to 2005. A single dose of smallpox vaccine LC16m8 at approximately 4 µL (at least 1×10^8 PFU/mL) was administered percutaneously with a bifurcated needle. The number of punctures was 5 times in primary vaccinees (without prior smallpox vaccination) and 10 times in previously vaccinated participants. Of the 3,468 participants enrolled, 3,221 (92.8%) were vaccinated. Of the 3,221 vaccinated participants, 1,529 (47.5%) were primary vaccinees and 3,168 (98.4%) were men.

Comparison of Prevaccination and Postvaccination Geometric Mean Titers (GMTs) and Percentage Seroconversion or Effective Boosting of Plaque-Reduction Neutralizing Titers to LC16m8 Vaccine, by Age

	GMT (95% CI)[a]				Seroconversion or Effective Boosting	
Age Group[b]	Prevaccination	*P* Value[c]	Postvaccination	*P* Value[c]	No./Total (% [95% CI])	*P* Value[d]
A (n = 41)[d]	6.1 (4.4-8.5)	<.001	112.0 (71.4-175.7)	.40	37/41 (90.2 [81.2-99.3])	<.001
B-D (n = 155)	21.0 (17.4-25.3)		137.3 (110.7-170.3)		93/155 (60.0 [52.3-67.7])	
B (n = 47)	14.4 (9.5-21.7)	.27[e]	188.2 (119.6-296.1)	.24[e]	36/47 (76.6 [64.5-88.7])	.18[e]
C (n = 45)	19.4 (13.6-27.6)	.04[f]	131.9 (88.9-195.7)	.50[f]	28/45 (62.2 [48.1-76.4])	.12[f]
D (n = 63)	29.5 (23.5-37.1)	.003[g]	111.6 (82.2-151.5)	.05[g]	29/63 (46.0 [33.7-58.3])	.002[g]

Abbreviation: CI, confidence interval.
[a] GMT calculated as probit transform of the dilution ratio of serum (no unit).
[b] Serum samples were taken from second-round vaccinees.
[c] By χ^2 test.
[d] Four individuals without vaccination take are excluded.
[e] For comparison of B and C.
[f] For comparison of C and D.
[g] For comparison of B and D.

The proportions of "take" in vaccinia-naive and previously vaccinated individuals were 1443 of 1529 (94.4% [95% confidence interval {CI}, 93.2%-95.9%] and 1465 of 1692 (86.6% [95% CI, 85.0%-88.2%]), respectively. Seroconversion or an effective booster response among the individuals with take was elicited in 37 of 41 (90.2% [95% CI, 81.2%-99.3%]) vaccinia-naive participants and in 93 of 155 (60.0% [95% CI, 52.3%-67.7%]) previously vaccinated participants.

No serious adverse events (e.g., autoinoculation/inoculation by exposure to vaccinated person, eczema vaccinatum, progressive vaccinia, generalised vaccinia, encephalitis, and symptomatic myocarditis) were reported during the follow-up period of 10 to 14 days after administration of smallpox vaccine LC16m8. Adverse events occurred in 4 participants within 30 days after vaccination, and the events in 2 of them were suspected to be severe. One of the 2 participants was a male primary vaccinee aged 26 years. He experienced rash 3 days after vaccination (Day 3), which spread from the extremities to the trunk, resulting in hospitalization on Day 20. The rash was diagnosed as dermatitis allergic, and its causal relationship to vaccination was not ruled out. The other participant was a male primary vaccinee aged 29 years. He experienced rash on the trunk on Day 10 and was given a diagnosis of erythema multiforme.

Neither electrocardiogram abnormal nor symptoms of heart disorders were reported during the administration of smallpox vaccine LC16m8. Serum troponin T was measured in 347 participants to confirm whether they had asymptomatic myocarditis. In all of them, serum troponin T levels measured before and after vaccination were below the detection limit (0.01 ng/mL). Medical records of 1,066 participants (491 primary vaccinees and 575 previously vaccinated participants) were surveyed retrospectively for mild adverse events. The survey identified 148 adverse events; the most common event was axillary lymphadenopathy, accounting for 65% (96 events) of the identified events.

PMDA's conclusion: Smallpox vaccine LC16m8 containing vaccinia virus (LC16m8) as the active ingredient induces cross-immunity between smallpox and monkeypox and its immunogenicity is not considered to be affected significantly by ethnic differences. Smallpox vaccine LC16m8 probably prevents the onset of monkeypox in the Japanese population, because (a) the vaccine induces a neutralizing antibody response against MPXV in the Japanese population and (b) in the non-clinical studies, the vaccine induced a

neutralizing antibody response against MPXV through cross-immunity and showed a preventive effect against monkeypox.

Comments: According to the data presented, only one study connected LC16m8 with protection again mpox virus, and the immune response in this study was generated in 26 subjects. However, LC16m8 produced "take" in primary and secondary vaccinated participants, and take is also used as the measure response. The more important question is if this vaccine can protect against clade 1b which is the prevalent virus in the current outbreak. Another important issue is the indication. Initially, the package insert has no age indication. Publications suggest that the vaccine was given to children from 1 year of age but limited details of the results were available since these studies were conducted in the early 1970s. Post deployment studies are proposed in the RMP to generate this vital data especially the effectiveness of this vaccine in the context of the current PHEIC. This post EUL study can also be used to study the impact of the vaccine in infants.

Safety Overview

Smallpox vaccine LC16m8 was administered to approximately 50,000 children in 1973 and 1974 by the National Smallpox Vaccination Research Committee (nationwide research network for understanding health hazards related to smallpox vaccination and selection or development of vaccines with less adverse reactions), and no concerning adverse reactions were reported. Of these children, 10,578 were subjected to detailed clinical observation. Among them, 9,538 were vaccinated in 1974, with 95.1% (9,075 of 9,538) having a "take." Of the 9,075 children, 8,544 were observed for at least 14 days. Of the 8,544 children, 7.8% (663) experienced pyrexia of ≥37.5°C between 4 and 14 days after vaccination. In 85% of those with pyrexia, fever persisted for ≤2 days (Clinical virology. 1975;3:269-79). Furthermore, an electroencephalography study for central nervous system complications raised no relevant concerns (Clinical virology. 1975;3:269-79, Japanese journal of paediatrics. 1976;29:1409-12). In the foreign phase I/II study in healthy adults without prior smallpox vaccination conducted in the U.S., no serious adverse events (e.g., postvaccinal encephalitis/encephalopathy, dermal complication, and myocarditis/pericarditis) occurred.

In Japan, through a smallpox vaccination program initiated in 2002 as a crisis management measure, smallpox vaccine LC16m8 has been administered to the limited population (mainly healthy adults belonging to the Japan Self-Defense Force). According to the reports from (a) the clinical research in 3,221 participants who received smallpox vaccine LC16m8 between 2002 and 2005 (1,529 primary vaccinees and 1,692 previously vaccinated individuals) and (b) the use-results survey on smallpox vaccine LC16m8 from 2005 to 2010, no serious adverse events occurred, such as postvaccinal encephalitis/encephalopathy and dermal complications (problematic events with traditional vaccine strains including the Lister strain) or myocarditis/pericarditis (problematic events with the NYCBH strain used in Dryvax etc. in the U.S.); further, the incidences of local adverse reactions at the administration site and systemic adverse reactions (e.g., pyrexia and headache) were similar to those reported by the survey of the National Smallpox Vaccination Research Committee conducted in children in 1973 and 1974. However, the clinical research (JAMA. 2009;301:1025-33) reported severe cutaneous symptoms for which a causal relationship to smallpox vaccine LC16m8 could not be ruled out (dermatitis allergic and erythema multiforme in 1 participant each). As for post-vaccination transmission of live vaccinia virus strains, the use results survey of smallpox vaccine LC16m8 identified autoinoculation in 1 vaccinee after the market launch. The event was mild in severity with unknown transmitted site.

In order to raise caution, confirmed pregnant women are classified as "persons ineligible for vaccination" in the package insert for smallpox vaccine LC16m8. No data are available on the effect of vaccination in women of childbearing potential. The safety of smallpox vaccine LC16m8 has not been established in children born to women who received smallpox vaccine LC16m8 before being pregnant or in children breastfed by women who received the vaccine. As with other live vaccines, the "Precautions Concerning Vaccination" section of the package insert for smallpox vaccine LC16m8 will include precautionary statements about (a) contraception in women of childbearing potential before and after vaccination and (b) use in breast-feeding women.

PMDA's conclusion: Based on the reported literature, the safety profile of smallpox vaccine LC16m8 remains unchanged by the addition of the new indication "prevention of monkeypox," and the safety in humans including children is acceptable. However, since severe cutaneous symptoms (dermatitis allergic and erythema multiforme) were reported after the launch of smallpox vaccine LC16m8, these symptoms should be classified as an important potential risk. Attention should be paid to information regarding the cutaneous symptoms that may be accumulated in the future, and actions should be taken as necessary. Autoinoculation after administration of smallpox vaccine LC16m8 was reported only in 1 vaccinee, but it is an important potential risk associated with the vaccine. Information regarding autoinoculation should therefore be disseminated through not only the package insert but also materials for healthcare professionals and vaccinees, to ensure full understanding about autoinoculation through explanations and instructions. PMDA accepted the applicant's proposal to include precautionary statements in the package insert regarding (a) contraception in women of childbearing potential before and after vaccination and (b) use in breast-feeding women, as with other live vaccines.

Comments: Although the method of safety assessment is not very clear, the data suggests that the vaccine is safe. There is a potential risk of autoinoculation and as the vaccine is replicating, the applicant indicated that it is contraindicated in pregnant women. The use of the vaccine in children is not definitely stated in the PMDA review, however the WHO position paper[1] suggest that LC16m8 could be used in children. The data available is only a summary and published in Japanese.
The main English reference is not a report of the study, but only a summary of what was done, as well as the data described earlier in this report. Epidemiological data from the outbreak in Africa indicates that the morbidity and mortality of Mpox is worse the younger the age group. Although the reason for this has not been studied, vaccination of this vulnerable population is an important preventive tool. Thus in the current public health emergency, perhaps the vaccine could be used and this should be accompanied by detailed RMP implications (see below).

3.4 Risk Management Plan

3.4.1 Product description

Acceptable

[1] WER9934-eng-fre.pdf (who.int)

3.4.2 Nonclinical information

Acceptable

3.4.3 Clinical information

Clinical Information: During the clinical trials no safety signal of any safety concern were identified.

a. Important identified risks:

KM Biologics	WHO	Comments
Convulsion, febrile convulsion	Convulsion, febrile convulsion	Although febrile convulsion was not reported in a drug use-results survey in 268 adults vaccinated with this product,1) febrile convulsion was reported in 3 of 10,578 children with available detailed clinical symptom data in a smallpox vaccination study in approximately 50,000 children vaccinated with this product conducted in 1974.

b. Important potential risks:

KM Biologics	WHO	Comments
Serious skin symptoms	Serious skin symptoms	Dermatitis allergic and erythema multiforme in 1 recipient each have been reported as serious skin symptoms, which are suspected of having a causal relationship to this product. Serious skin symptoms are classified as an important potential risk because of the absence of sufficient information to classify them an identified risk associated with this product.
Shock, anaphylaxis	anaphylaxis	This product contains stock solution-derived gelatin, although these events have not been reported with this product. Cases of shock and anaphylaxis (eg, urticaria, dyspnoea, lip oedema, laryngeal oedema) have been reported with administration of gelatin-containing products.
Autoinoculation (ectopic inoculation), horizontal transmission	Autoinoculation (ectopic inoculation), horizontal transmission	Autoinoculation (ectopic inoculation) with this product has been reported in 1 vaccine recipient. Although the symptoms were mild, the possibility

		of developing such an event after administration of this product cannot be ruled out. Horizontal transmission has not been reported with this product. Outside Japan, however, horizontal transmission of the virus has been reported from vaccine recipients to non-vaccine recipients after the administration of a live vaccine (injection) manufactured with a vaccinia virus strain different from the strain used in this product.
Vaccinia virus infection	Vaccinia virus infection	The pharmacological class effects described above are proposed as important potential risks. They are not specific to any subgroup, i.e. they are specific to the active substance (and its class); hence stratification by a specific formulation, indication or route of administration
	Incorrect route of drug administration	Risk minimization activities need to be implemented

c. Missing information:

KM Biologics	**WHO**	**Comments**
None	Use during pregnancy and breastfeeding.	The vaccine has not been evaluated for safety and efficacy this population. Animal studies did not reveal any evidence of impaired female fertility.
	Elderly subjects	Limited data is available.
	Individuals with organ impairment	No information available, as not studied.
	Clinically immunocompromised individuals	The safe administration of LC16m8 in these population is limited
	Safety experience in mass vaccination due to smallpox outbreak	No information available, as not studied.
	Interactions with other vaccines and concomitantly	No interactions are known for LC16 KMB

	administered immunoglobulins	
	Long term safety effectiveness	Additional activities will be needed to obtain such information. Additional activity is proposed by the applicant.

3.4.4 Pharmacovigilance Plan

a. Routine activities

The applicant proposed routine pharmacovigilance activities to collect safety information, those activities involve the following:

Individual Case Safety Reports (ICSRs)

- Signal detection:
    - Signal Evaluation of Adverse events of special interest
- Data sources for signal detection and frequency of review
- Periodic Safety Update Report (PSUR)
- Specific Adverse Reaction Follow-Up Questionnaires
- Enhanced Passive Surveillance

b. Additional pharmacovigilance activities

The applicant is not proposing any additional pharmacovigilance activity.

3.4.5 Risk minimization activities

The routine risk minimization activities are sufficient to manage the safety concerns of the medicinal product. The applicant is requested to submit the educational materials aimed at minimizing the risk of immunization errors, such as printed posters or guides that are mentioned in the RMP, in addition to providing information with the SmPC.

3.4.6 Conclusion

The risk benefit assessment is acceptable. The applicant included a partial translation of the RMP that is only intended for use in Japan. The applicant should submit an RMP to WHO that includes the modifications on the safety profile according to the WHO requirements. The applicant needs to consider additional pharmacovigilance activities to monitor and collect the safety and effectiveness data in LMIC and indicate how this information will be collected in these regions. During the assessment process, the applicant provided an updated the RMP. However, a detailed protocol for the post-EUL surveillance is expected.

The RMP should also include/address the following:

- Safety specifications detailed description
  - Identified risk: *Convulsion, febrile convulsion.*
  - Potential risks: *Serious skin symptoms, anaphylaxis, Autoinoculation (ectopic inoculation), horizontal transmission, Vaccinia virus infection, Incorrect route of drug administration.*
  - Missing information: add *long term effectiveness and safety, clinically immunocompromised individuals, elderly, infants, safety experience in mass vaccination due to smallpox outbreak, Interactions with other vaccines and concomitantly administered immunoglobulins, concomitant infections, pregnancy.*
- Pharmacovigilance plan
  - *Use in pediatric population based on the updated variant, morbidity and mortality in this vulnerable population in LMIC.*
  - *The applicant is urged to conduct additional pharmacovigilance activities (non-interventional and interventional that include effectiveness, the timeline is relevant to monitor the advances of this activity. It is necessary to explain how this information will be collected in LMIC.*
- Risk minimization activities
  - *Include the material of the educational intervention as part of the risk minimization activities.*

4 Outcome of review

4.1 Quality

Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 “KMB” vaccine was developed more than 20 years ago in Japan and used in Japan, only.

Vaccinia virus strains were used for vaccination against smallpox and these differed in their reactogenicity in man. Attenuated vaccinia virus strains such as LC16m8 (Japan) and MVA (Germany) were developed and used towards the end of the smallpox eradication campaign.

In Japan, 1.1 million doses of two old vaccines made from strain Lister-Elstree and Ikeda were stockpiled. These first-generation vaccines were produced on calf lymph between 1978 and 1981, stored at -15 to -20°C. The virus titre of the stockpiled vaccine was determined, and it was demonstrated, that it was still in the specified range.

Vaccine lots included in this EUL submission were manufactured several years ago (2003, 2004, 2005, 2009). In this regard, all lots available today for outbreak response are labelled as per the Japanese

standard and in Japanese language. However, the EUL applicant has developed a package insert in English, as well as, instructional materials for health care providers and for those receiving the vaccine and their families.

This Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" vaccine is produced in cultured primary rabbit kidney (PRK) cells using the attenuated strain LC16m8.

The outcome of the WHO review on programmatic aspects and suitability for LMICs highlighted the absence of VVM and preservative, as the vaccine is only available in a multidose presentation. This is a life viral vaccine - toxicity is extremely weakened - for which the storage and handling conditions are specific and very different to vaccines used in national immunization programmes (e.g., LC16m8 is administered using a bifurcated needle, special care of the skin, preparation before and after administration).

Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" vaccine is administered by puncturing the skin approximately 15 times with a special bifurcated needle. Puncture is performed to the extent that blood oozes (scarification). Because live vaccine virus is present at the vaccination site, special care should be paid not to touch other sites of the body or to touch other people with the hand that touched the vaccination site.

The vaccination with Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" vaccine induces antibodies against smallpox virus and mpox virus, making people less likely to contract these viruses.

The virus of this product is vulnerable to sunlight and is rapidly inactivated. Avoid exposure to light both before and after reconstitution.

WHO strongly recommends the observation and compliance with Multidose Vial Policy (MDVP) and policies on open vials. In this regard any printed material (e.g., package insert, instructions for use, etc.) should include the WHO MDVP.

The EUL applicant did not provide the validation of the procedure that will be followed for international shipping of the vaccine. This will depend on the negotiations and discussions on logistics with the WHO/UN deployment agency.

This vaccine was initially developed by Chiba Serum Institute (1975) in a frozen formulation, and a lyophilized formulation was developed in 1980. The routine vaccination against smallpox in Japan halted in 1976; therefore, Chiba Serum Institute ended the production of the vaccine accordingly, without distributing the product in the market. The license and all product-related rights of LC16m8 vaccine were transferred to the Chemo-Sero Therapeutic Research Institute (hereafter, "Kaketsuken") in September 2002 upon the discontinuation of Chiba Serum Institute's business, and thereafter to KM Biologics in July 2018 due to the transfer of pharmaceutical business from Kaketsuken.

The review of the dossier submitted for the EUL application produced several rounds of questions and answers. Possibly originating from the fact that this vaccine was produced some time ago for the national stockpile. This, coupled with the existence of special requirements of the Japanese regulatory framework (e.g., masking of what they considered sensitive information).

Consequently, PEG agreed that if an EUL recommendation will be issued for this vaccine based on a positive benefit – risk, the EUL applicant should commit to provide the missing information as post EUL commitments. In any case, the applicant should provide a time frame for the submission of the information that needs to be reported or completed, as per the content of sections 6.2 and 10.1 of this report.

4.2 Clinical

This clinical assessment raised a limited number of queries and comments from the reviewers on clinical submitted evidence and issues related to the RMP. These have either been considered addressed by KM Biologics have been incorporated into the recommendations listed below as well as in the conclusion section of this report. The available data may not be generalizable to populations in low and middle-income countries (LMIC) who have profiles that can impact on the efficacy of this vaccine (for example, ethnicity, concomitant infections and malnutrition).

The PEG recommends that an EUL may be granted by WHO to "LC16 (KMB)" (LC16m8 vaccine) (freeze-dried prepared in Cell Culture Smallpox vaccine) provided that KM Biologics commits to meet the following conditions post-EUL:

- Provide a revised Package Insert specifying the low age indication for the vaccine.
- Once available any relevant data coming from post EUL effectiveness studies should be shared with WHO, as this might change the benefit/risk profile of the vaccine.
- The RMP should also include/address the following:

    i. Safety specifications detailed description

        - Identified risk: *Convulsion, febrile convulsion.*
        - Potential risks: *Serious skin symptoms, anaphylaxis, Autoinoculation (ectopic inoculation), horizontal transmission, Vaccinia virus infection, Incorrect route of drug administration.*
        - Missing information: add *long term effectiveness and safety, clinically immunocompromised individuals, elderly, infants, safety experience in mass vaccination due to smallpox outbreak, interactions with other vaccines and concomitantly administered immunoglobulins, concomitant infection pregnancy.*

    ii. Pharmacovigilance plan

        - *Use in pediatric population based on the updated variant, morbidity and mortality in this vulnerable population in LMIC.*
        - *The applicant is urged to conduct additional pharmacovigilance activities (non-interventional and interventional that include effectiveness, the timeline is relevant to monitor the advances of this activity. It is necessary to explain how this information will be collected in LMIC.*

- Risk minimization activities

- *Include the material of the educational intervention as part of the risk minimization activities.*

5 Technical considerations

5.1 Pharmaceutical particulars

Qualitative and Quantitative composition

"LC16 (KMB)" vaccine after reconstitution with 0.5 mL of the co-packed diluent solution (water for injection containing 20 vol% glycerin) includes not less than 5.0 x 10^7 PFU/0.5mL (plaque forming unit) of live attenuated vaccinia virus LC16m8 strain.

When using the designated bifurcated needle (manufactured by NIPRO) that can hold 1±0.5 µL (nominal/specification value) of LC16m8 vaccine solution, the volume per dose after reconstitution with 0.5 mL is 1.5 µL (0.0015mL) to 1.8 µL (0.0018mL). According to documents submitted to WHO, "the antigen content per dose of vaccination picked up using the designated bifurcated needle does not change, whether 50 doses or 250 doses were picked from one vial.

Each dose contains an estimated potency of not less than 1.5~1.8 x 10^5 PFU.

List of excipients

- D-Sorbitol
- Peptone
- Phenol red
- Gelatin
- 199 Medium
- Sodium bicarbonate
- Concentrated glycerin
- Streptomycin sulphate (traces)
- Erythromycin lactobionate (traces)

Special precautions for storage and handling proposed by the applicant

Storage of Vaccine

The finished product is stable at –20°C or below for up to 143 months from the manufacturing date. Based on real time data, the recommended storage temperature is at –20°C or below this temperature but not at −35°C or below because the rubber stopper could deteriorate or be damaged. When stored at –20°C, the shelf-life of the vaccine is 10 years, from the date it is released by national control laboratory.

LC16 (KMB) vaccine can be stored at 2°C to 8°C for 2 years, without reconstitution.

The virus of this product is vulnerable to sunlight and is rapidly inactivated. Avoid exposure to light both before and after reconstitution.

Once the vaccine is moved to refrigerated storage, it should be used without being returned to frozen storage, and within the expiration period or within 2 years after being moved to refrigerated at 2°C to 8°C.

After reconstitution with the provided vaccine diluent, the vaccine should be used during the immunization session, discarded after 6 hours or whatever becomes first. However, because of the nature of this product and the handling conditions, it is preferable its use immediately after reconstitution.

Nature and contents of container

For the final product vials and rubber stoppers, acceptance testing is conducted in accordance with the Test for Glass Containers for injections and Test for Rubber Closure for Aqueous Infusions in the Japanese Pharmacopoeia.

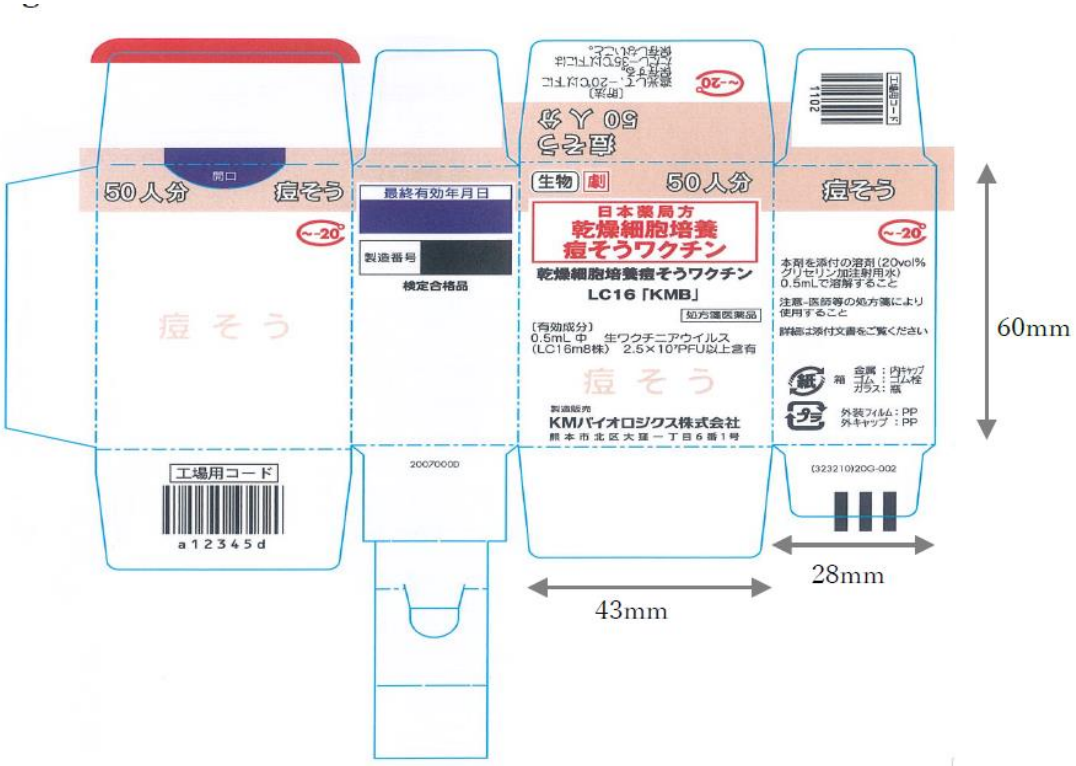
The vaccine vial is made from borosilicate glass Type I, with a nominal capacity of 2 mL (2R), with butyl rubber stopper and closed with a cap of aluminum and type of flip-off of polypropylene.

Packaging:

Carton box:

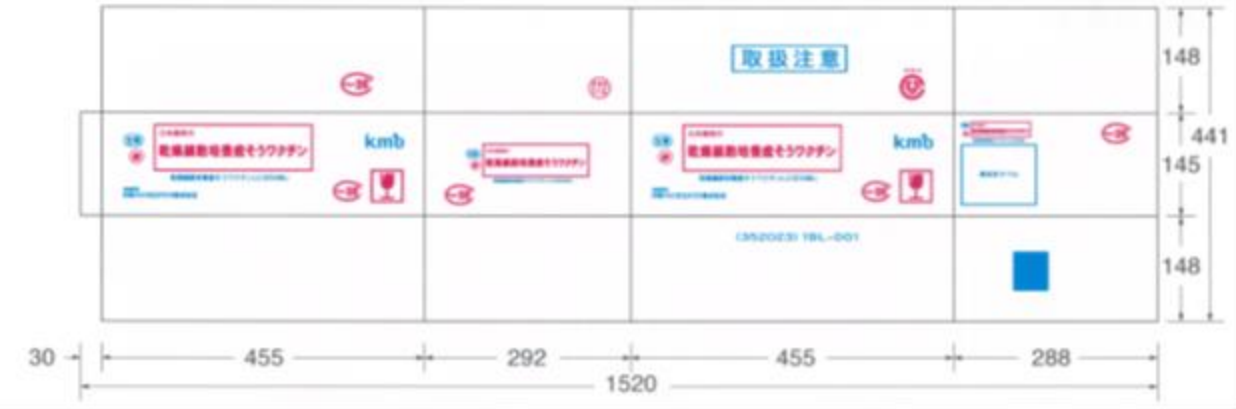
A carton box contains two vials: one vaccine vial and one co-packed diluent.

- Dimensions (length × width × height): 43 mm × 28 mm ×60 mm
- Weight: 19.5 g/box
- Dose 250 dose/box



Cardboard box

A cardboard box holds up to 200 carton boxes.
Dimensions (length × width × height): 455 mm × 292 mm × 145 mm
Weight: 4.4 kg/cardboard box (when fully loaded with 200 carton boxes)
Dose: 50,000 doses/cardboard box (when fully loaded, 250 doses × 200 carton boxes)



Tertiary Packaging (1)

One shipping box contains one cardboard box (=50,000 doses when fully loaded), surrounded by thermal storage material.
Transport temperature range: -30°C to -20°C
The shipping box is planned to be va-Q-proof 110P3 -26G

Shipping box dimensions:

- Outer dimensions [mm3] (length x width x height): 910mm x 700mm x 700mm
- Inner dimensions [mm3] (length x width x height): 670mm x 470mm x 395mm
- Total weight: shipping box: 28.0 kg / thermal storage material: 37.2 kg / cardboard box (fully loaded): 4.4 kg = 69.6 kg

Tertiary Packaging for large shipping quantities (2)

Transport temperature range: -30°C to -20°C
The shipping container is planned to be va-Q-tainer USx -26G
Outer dimensions [mm3] (l × w × h): 1423mm x 1490mm x 1580mm
Inner dimensions [mm3] (l × w × h): 1200mm x 1250mm x 1170mm
Weight: shipping container: 420 kg / thermal storage material: 4.5 kg x 52 pcs = 234 kg

Special precautions for disposal and other handling

Once the stopper has been removed from the vial in a non-sterile environment, the solution remaining in the vial must always be disposed after 6 hours or at the end of the immunization session, which ever come first, without being stored again and used for the next vaccination session.

Pack the used bifurcated needle and vial and the items that might have come into contact with the drug solution in a sealable waste container that is easy to contain and is not easily damaged and dispose of them as infectious waste.

Freeze-dried Smallpox Vaccine Prepared in Cell Culture should be prepared and administered by a trained healthcare professional.

Indication, warnings and contraindications

Therapeutic indications

"LC16 (KMB)" vaccine is indicated for active immunization to prevent smallpox and mpox in individuals from 1 year of age and older.

The use of this vaccine should be in accordance with official recommendations.

Contraindications
Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.

Special warnings and precautions for use

- This drug should be used in compliance with the "Enforcement Regulations of Immunization" and "Guidelines for the Implementation of Routine Vaccinations".

- The health status of the recipient should be fully examined before vaccination through history taking, body temperature measurement, and medical examination (e.g., inspection and auscultation).

- This product contains stock solution-derived gelatin (not more than 0.15 w/v%). Cases of shock and anaphylaxis (e.g., urticaria, dyspnoea, lip oedema, laryngeal oedema) have been reported with administration of gelatin-containing products; the recipient should be interviewed carefully before vaccination and monitored carefully after vaccination.

- This product contains streptomycin as an excipient; it may cause hypersensitivity in individuals sensitive to this ingredient. After vaccination, the vaccine recipient should be monitored carefully, and if any symptom is observed, appropriate measures should be taken.

- The vaccine recipient or his/her guardian should be instructed in advance to avoid excessive exercise on the day of vaccination, keep the vaccination site clean, pay attention to health monitoring after vaccination, and seek immediate medical attention in the case of any abnormal local reaction, change in physical condition, or abnormal symptoms such as high fever, convulsion, and serious skin symptoms.

Persons to Be Vaccinated with Caution (Persons in Whom the Decision to Vaccinate Must Be Made with Caution)

If a recipient meets any of the following criteria, vaccination should be administered with care after carefully performing medical examination and making a judgment on the appropriateness of vaccination in consideration of his/her health status and constitution, giving sufficient explanation about the necessity, adverse reactions, and usefulness of vaccination, and successfully obtaining his/her consent.

- Person with a history of hypersensitivity such as shock or anaphylaxis (e.g., urticaria, dyspnoea, lip oedema, laryngeal oedema) to gelatin-containing pharmaceutical preparations or gelatin-containing foods.

- Persons with underlying diseases such as cardiovascular disease, renal disease, hepatic disease, hematological disease, and developmental disturbance.

- Persons who had pyrexia within 2 days after vaccination and those who had symptoms suggestive of allergy such as exanthema generalized.

- Persons with a history of convulsion Those who have been diagnosed with immunodeficiency and those who have a close relative with congenital immunodeficiency.

- Person who may be allergic to any of the ingredients of this product.

- Persons with Renal Impairment They are persons to be vaccinated with caution.

- Persons with Hepatic Impairment They are persons to be vaccinated with caution.

- Patients with Reproductive Potential. Women of childbearing potential should be vaccinated after approximately 1 month of contraception in advance and advised to avoid becoming pregnant for approximately 2 months after vaccination.

- Pregnant Women. This vaccine should not be administered to pregnant women.

- Brest-feeding Women. The benefit associated with vaccination and the benefit of breast milk nutrients should be considered before continuing or discontinuing breast-feeding.

Posology and method of administration

Posology

"LC16 (KMB)" vaccine is indicated for active immunization to prevent smallpox and mpox in individuals from 1 year of age and older.

The use of this vaccine should be in accordance with official recommendations.

Method of administration (including precautions concerning administration of the vaccine)

At inoculation

- A sterilized inoculation needle (bifurcated needle) should be used. The inoculation needle must be replaced for each vaccine recipient.
- This drug should not be mixed with other vaccines.
- Before reconstituting the vaccine, the container stopper and its surroundings should be disinfected using alcohol. After that, the vaccine should be homogeneously reconstituted with 0.5 mL of provided diluent. After reconstitution, the rubber stopper should be removed by cutting the metal cap. The tip of the bifurcated needle should be soaked in the solution, and vaccine solution appropriate for one recipient should be sucked out.

- In cases such as mass vaccination where many persons need to be inoculated consecutively, approximately more than 250 recipients can be vaccinated if the 0.5 mL of vaccine solution is prepared by reconstituting this vaccine with 0.5 mL of provided diluent and bifurcated needles for smallpox vaccination with a single collection volume of 1 ± 0.5 µL (specified value) are used.

Inoculation site

- In principle, the inoculation site should be within a diameter of approximately 5 mm on the origin area of deltoid muscle on the lateral upper arm. The site should be disinfected with a tightly wrung alcohol cotton and dried well. At 1 to 3 minutes after inoculation, any excess vaccine should be wiped off with a tightly wrung alcohol cotton.

Inoculation method

- Multiple puncture technique: Hold a bifurcated needle perpendicular to the skin, rest the wrist of the hand holding the needle on the skin, and prick the skin by moving the wrist. Typically, a dedicated bifurcated needle to prick the skin 15 times (number of pricking as a guide). The skin should be pricked to the degree where blood will ooze from the skin. When using other bifurcated needles, consider their precautions for use before pricking the skin.

After inoculation

- Medical examination should be performed between 10 and 14 days after inoculation to confirm the “take” (successful vaccination)

Fertility, pregnancy and lactation

Patients with Reproductive Potential and *Pregnancy*

Women of childbearing potential should be vaccinated after approximately 1 month of contraception in advance and advised to avoid becoming pregnant for approximately 2 months after vaccination.

This vaccine should not be administered to pregnant women.

Breast-feeding

The benefit associated with vaccination and the benefit of breast milk nutrients should be considered before continuing or discontinuing breast-feeding.

Interaction with other medicinal products and other forms of interaction
Adverse Reactions

The following adverse reactions may occur. Patients should be carefully monitored, and if any abnormalities are observed, appropriate measures should be taken.

Clinically Significant Adverse Reactions
Shock, anaphylaxis (incidence of both unknown)
Urticaria, dyspnoea, lip oedema, and laryngeal oedema may occur. [See 8.3.]

Convulsion (<0.1%)
Febrile convulsion may occur

Other adverse reactions

	Incidence unknown
Hypersensitivity	Rash*, allergic dermatitis, erythema multiforme
Local symptoms (Inoculation site)	Inoculation site reactions
Others	Pyrexia*, enlarged axillary lymph nodes *

*It may occur around 10 days after vaccination

Clinical Studies

Clinical Studies for Efficacy and Safety
Japanese clinical studies (children): Approximately 50,000 children with primary vaccination, mainly 1 to 7 years old, were inoculated with this product. In 10,578 children with available detailed clinical symptom data, the “take” rate was 95.1%, the mean redness diameter (assessed on the 10th day) was 18.4 mm, the mean induration diameter was 6.1 mm, the incidence of axillary lymph nodes enlarged was 12% to 19%, and the incidence of pyrexia (between the 4th and 14th days after inoculation) was 7.7%.

Observed symptoms included febrile convulsion in 3 children, eczema vaccinatum in 1 child, autoinoculation in 9 children (infection with the vaccinia virus resulting from the virus inoculation by hands from the local inoculation site to other sites), vaccinola (blisters and pustules around an inoculation site) in 28 children, and vaccinal eruption in 8 children (allergic eczema in the form of urticaria, in the form of erythema, and in other forms occurring around the 7th to 10th days after inoculation).
Usually, the maximum temperature of pyrexia ranged from 38°C to 38.9°C, which accounted for 77.4% of the children. The duration of the fever was 1 day in 60% of the children and up to 2 days in 85% of the children. Regarding the indicators of the immune response, the HI antibody titer was 23.3 (n=513) and the NT antibody titer was 42.5 (n=97). Electroencephalography performed in 56 children on the 14th day after inoculation showed no abnormal findings.

Post-marketing Surveillance, etc.
Drug use-results survey in Japan (adults):
This product was administered to 268 adults. The “take” rate was 91.0% (94.4% after the primary vaccination, 81.7% after the secondary vaccination), the mean redness diameter was 23.8 mm (n =98), and the mean blister diameter was 7.6 mm (n=87). Observed adverse reactions included swollen lymph nodes in 19.4% (52/268 subjects), injection site erythema in 5.2% (14/268 subjects), pyrexia in 1.5%

(4/268 subjects), malaise in 0.7% (2/268 subjects), postvaccination complication (satellite) in 0.7% (2/268 subjects), rash in 0.4% (1/268 subjects), injection site swelling in 0.4% (1/ 268 subjects), and post-vaccination autoinoculation (suspected ectopic inoculation) in 0.4% (1/268 subjects). Regarding the indicators of immune response, the NT antibody titer was 37 (n=68) before inoculation and 1400 (n=39) 1 month after inoculation in adults given the primary vaccination, and 206 (n=30) before inoculation and 782 (n=12) 1 month after inoculation in adults given the secondary vaccination. These results showed a significant increase in the antibody titer. There were no adverse reactions related to the priority survey items, which included cardiac disorder (chest radiograph and ECG), encephalitis, and vaccinola/vaccinal eruption.

Pharmacology

Mechanism of Action:

It is considered that the smallpox virus and the mpox virus are transmitted through the airway via droplets from patients or by contact with skin lesions or body fluids of patients and are proliferated in local lymph nodes. Then, by causing viremia, the virus is carried to the target organs in the whole body, leading to the onset of infection. When humoral and cellular immunity against the smallpox virus and mpox virus are acquired by inoculation of this product in advance, the proliferation of the infected virus is inhibited, thereby preventing onset.

Study to support Efficacy:

This vaccine (2.5×10^5 PFU/case) or phosphate-buffered saline (PBS) as a negative control was administered to 14 cynomolgus monkeys or 6 cynomolgus monkeys, respectively, for a single percutaneous vaccination using a bifurcated needle. Sixty days after vaccination, 79 strains of mpox virus Zaire (5×10^7 PFU/case) were intravenously administered to these cynomolgus monkeys. After intravenous administration of the mpox viruses, in the negative control group, multiple skin lesions were observed on the entire body, and all the animals either died or were euthanized by 12 days after the administration of the mpox viruses. In the Vaccine group, crusts were formed by 12 days after the administration of the mpox viruses without deaths.

6 Monitoring of performance of the vaccine in the field

6.1 Vaccine efficacy/effectiveness and safety Monitoring

Clinical research of safety and efficacy in children and adults is under consideration.

6.2 Programmatic aspects

Self-assessment against programmatic suitability criteria (PSPQ) has been provided by the EUL applicant. At least two PSPQ criterions are not met, one mandatory and one critical criterion. These are the following:

- **MANDATORY**: Though required, this is a multidose vaccine and is not preserved. To note that this is live viral vaccine. Nevertheless, the applicant did not discuss this fact against the feasibility or not of using a preservative.

- **CRITICAL**: Freeze-dried Smallpox Vaccine Prepared in Cell Culture LC16 "KMB" vaccine does not have VVM defined. The EUL applicant has not claim a VVM for this vaccine.

International shipping

The applicant has not yet validated the international shipping information based on the WHO shipping guidance. The vaccine has not been marketed outside of Japan; however DRC has given an emergency use authorization. Currently, (when this report is being prepared), there are discussions and coordination activities between the manufacturer, the Ministry of Health and Welfare of Japan and the WHO logistics department as well as Unicef for the deployment of this vaccine.

7 SAGE recommendations

Recommendations on the use of smallpox and mpox vaccines were issued by the WHO Strategic Advisory Group of Experts (SAGE) on Immunization at its meetings in September 2023 and March 2024 and endorsed by WHO thereafter. The emphasis of the mpox vaccine and immunization recommendations in this position paper is on outbreak response. Aside from the recommendation for laboratory personnel working with *Orthopoxviruses*, the development of a recommendation for broader preventive mpox vaccination in non-outbreak settings requires significant additional data, particularly in relation to a better epidemiological characterization of populations at risk. Additionally, further data are required on the duration of immunity provided by vaccines, and their effectiveness and safety profiles – particularly in children. However, future consideration may be given to preventive mpox vaccination if specific geographical areas and at-risk populations are more clearly identified and defined, and if vaccine performance becomes better characterized. The mpox vaccine and immunization recommendations are divided into 2 sections: one on preventive vaccination in laboratory personnel working with *Orthopoxviruses*, and another section on mpox outbreak response.[2]

8 Regulatory oversight

The Pharmaceuticals and Medical Devices Agency (PMDA) is the regulatory body that has granted marketing approval to "LC16 (KMB)" (LC16m8 vaccine) (freeze-dried prepared in Cell Culture Smallpox vaccine) on 2 August 2022. Therefore, PMDA is the regulatory agency of record for this vaccine as per the WHO EUL procedure. The WHO Vaccine Prequalification Team will continue to rely on the regulatory oversight of PMDA for this vaccine.

9 Benefit/Risk Assessment

LC16m8 vaccine is a third-generation vaccine, approved in 1975 in Japan, for preventing smallpox. LC16m8 vaccine has received emergency use authorization in DRC. The approval in Japan includes for use in children which may be important in the current clade 1b outbreak.

[2] WER9934-eng-fre.pdf (who.int)

The live attenuated, replicating, cell-cultured, third-generation smallpox vaccine LC16m8 was administered to vaccinia-naive infants in Japan during the 1970s without SAEs. Administration of the smallpox vaccine LC16m8 to healthy adults was associated with high levels of vaccine take and seroconversion in those who were vaccinia-naive and yielded an effective booster response in some previously vaccinated individuals. Therefore, the smallpox vaccine LC16m8 appears to be a viable alternative to first, second, and other third generation vaccines used for both smallpox and mpox preparedness programs, and the benefit risk profile is favorable in the indication for prevention of smallpox and mpox.

LC16m8 vaccine has been approved for children in Japan. The live attenuated, replicating, cell-cultured, third-generation smallpox vaccine LC16m8 was administered to vaccinia-naive infants in Japan during the 1970s without SAEs. No data from children outside Japan is available. Although the data in children did not raise safety concern, the generalizability of this data to children in low- and middle-income countries with malnutrition and other infections including malaria and HIV requires caution. Epidemiological data from DRC suggests that the morbidity and mortality of mpox are worse in younger age groups, and the reason for this is not clear. Thus, there is a need for vigorous monitoring of safety and effectiveness of LC16m8 when used in children.

No data is available in the use of the vaccine in immunocompromised individual. However, NHP data showed that animals depleted of B and Cell when vaccinated with LC16m8 were protected following lethal mpox viral challenge 12 months after, which suggests that the vaccine might be used in people with controlled HIV infection.

Administration of the smallpox vaccine LC16m8 to healthy adults was associated with high levels of vaccine take and seroconversion in those who were vaccinia-naive and yielded an effective booster response in some previously vaccinated individuals. Therefore, the smallpox vaccine LC16m8 appears to be a viable alternative to first-, second-, and other third generation vaccines used for both smallpox and mpox preparedness programs and/or outbreak response.

However, special precautions need to be observed before administering the vaccine in the following group of people: (1) Person with a history of hypersensitivity such as shock or anaphylaxis (e.g., urticaria, dyspnoea, lip oedema, laryngeal oedema) to gelatin-containing pharmaceutical preparations or gelatin-containing foods. (2) Persons with underlying diseases such as cardiovascular disease, renal disease, hepatic disease, haematological disease, and developmental disturbance. (3) Persons who had pyrexia within 2 days after vaccination and those who had symptoms suggestive of allergy such as exanthema generalized. (4) Persons with a history of convulsion. (5) Those who have been diagnosed with immunodeficiency and those who have a close relative with congenital immunodeficiency. (6) Person who may be allergic to any of the ingredients of this product. (7) Persons with Renal Impairment (8) Persons with Hepatic Impairment (9) Patients with Reproductive Potential. The vaccine is not recommended for (10) Pregnant Women and (11) Brest-feeding Women.

10 Conclusion

Considering the public health need to halt mpox morbidity and mortality and to continue immunizing the affected population to the largest extent possible, the introduction of vaccines that would protect the population from disease and, whenever possible, from mpox infection is needed.

Based on the assessment of the information submitted, PEG is of the opinion that TAG has enough evidence to issue a recommendation on the emergency use of LC16m8 freeze-dried prepared in Cell Culture Smallpox vaccine "KMB" vaccine. This recommendation will be subject to the follow-up of several EUL post-listing actions and commitments, as indicated in the below sections.

Should new evidence become available that change the benefit-risk assessment the EUL recommendation could be reconsidered.

10.1 Quality (CMC) perspective

Based on the outcome of the review of the quality data provided by the applicant, the listing for emergency use of LC16m8 freeze-dried prepared in Cell Culture Smallpox vaccine "KMB" vaccine can be granted. However, for future manufacturing campaigns the manufacturer should gather and provide the following specific information as post-EUL commitments:

1. The vaccinia virus in the pooled harvest must be tested for identity by serological methods, **despite the identity test conducted on the finished product**. In addition, the specificity of the identity test by serological methods using viruses similar to the LC16m8 strain should be evaluated.
2. The vaccinia virus in the pooled harvest or the final bulk must be examined by tests that can determine that the **phenotypic and genotypic characteristics** of the vaccinia virus have not undergone changes during multiplication in production. This is more a characterization exercise.
3. Regarding the **working seed bank, genotypic characterization** should be provided. This is important as an additional criteria of production consistency.
4. As in-process control of the neutralized **single harvest**, the testing for adventitious agents must be conducted (rather than in the pooled harvest).
5. The assessment of the presence of **aggregation of viral particles** should be performed, at least as part of the vaccine characterization.
6. Regarding the **Reference** Smallpox Vaccine Prepared in Cell Cultures, which is an internal reference standard developed by the manufacturer, detailed data on the preparation, characterization, and establishment must be provided, including information on secondary reference standards. Data on the qualification tests of the reference supplied by the National Institute of Infectious Diseases (NIID) must also be submitted.
7. Tests for appearance, pH, bioburden and endotoxin for DP bulk must be part of **in-process** controls, considering their potential impact on product quality.
8. The evaluation of **extractables and leachables** must be conducted, and reports with sufficient detail on the studies performed must be provided. It should be noted that major metal leachables, such as iron, chromium, and nickel, are frequently associated with the use of container closure systems made of stainless steel for storage.

9. Given that vaccine stability data at **37°C for 4 weeks** show a decrease in potency, it is recommended that batch stability studies be performed with potency values at or near the lower limit of the specification.
10. The validation for the international shipping of the vaccine should be provided. The EUL applicant is recommended to follow the Guidelines for the international packaging and shipping of vaccines, 6th ed. (2020).
11. The EUL applicant is recommended to use WHO prequalified temperature monitoring devices for the international shipment of the vaccine (E006 Temperature Monitoring Devices at https://apps.who.int/immunization_standards/vaccine_quality/pqs_catalogue/).

10.2 Clinical perspective

The PEG recommends that an EUL may be granted by WHO to "LC16 (KMB)" (LC16m8 vaccine) (freeze-dried prepared in Cell Culture Smallpox vaccine) provided that KM Biologics commits to meet the following conditions post-EUL:

- Provide a revised Package Insert specifying the low age indication for the vaccine.
- Once available, any relevant data coming from post EUL effectiveness studies should be shared with WHO, as this might change the benefit/risk profile of the vaccine.
- The RMP should also include/address the following:

iii. Safety specifications detailed description

- Identified risk: *Convulsion, febrile convulsion.*
- Potential risks: *Serious skin symptoms, anaphylaxis, Autoinoculation (ectopic inoculation), horizontal transmission, Vaccinia virus infection, Incorrect route of drug administration.*
- Missing information: add *long term effectiveness and safety, clinically immunocompromised individuals, elderly, infants, safety experience in mass vaccination due to smallpox outbreak, Interactions with other vaccines and concomitantly administered immunoglobulins, concomitant infection, pregnancy.*

iv. Pharmacovigilance plan

- *Use in pediatric population based on the updated variant, morbidity and mortality in this vulnerable population in LMIC.*
- *The applicant agreed to conduct additional pharmacovigilance activities (non-interventional and interventional studies that include effectiveness, the timeline is relevant to monitor the advances of this activity. The details of these activities should be provided to WHO within twelve weeks of listing.*

- Risk minimization activities
  - *Include the material of the educational intervention as part of the risk minimization activities.*

KMB provided two documents as risk minimization efforts

a) Guide for healthcare professionals
b) Guide for vaccine recipients