Summary report on first, second and third generation smallpox vaccines

Content

The first report was prepared for WHO in August 2013 by H. Meyer, Paul-Ehrlich-Institut and updated in October 2013 following comments received after the expert meeting on smallpox vaccines

Abbreviations

AE: adverse event

CAM: chorioallantois membrane

CMI: cell-mediated immunity

ECG: electrocardiography

ECTV: Ectromelia virus

EEV: extracellular enveloped virus

GMP: Good manufacturing practice

GMT: geometric mean titer

IMV: intracellular mature virus

MPXV: monkeypox virus

NYCBH: New York City Board of Health

PFU: pock forming units

PRNT: plaque reduction neutralization test

pvE: post-vaccinial encephalitis

RPXV: rabbitpox virus

SCR: seroconversion rate

SAE: Serious adverse event

WHO: World Health Organisation

Background

Smallpox has been successfully eradicated by a global effort in the 1960s and 1970s using a panel of live vaccinia virus vaccines. The last naturally occurring case was reported in 1977 in Somalia and in May 1980 the World Health Organisation (WHO) declared that smallpox had been eradicated. In the last decades fears of deliberate release of Variola virus or genetically modified orthopox viruses by bioterrorist attacks led to an intensified research on the molecular and immunogenic characteristics of orthopox viruses as well as to the conduct of vaccination campaigns involving first generation vaccines produced during the eradication era and the development of new smallpox vaccines.

Smallpox is a devastating disease caused by the Variola virus. Variola virus is transmitted by aerosols and droplets from person-to-person or through contact with contaminated clothing and bedding. After infection and a prolonged incubation period of usually 10-14 days (range 7-17 days) the first disease symptoms develop. Typically smallpox disease is characterized by the sudden onset of influenza-like symptoms including high fever, malaise, headache, prostration, severe backache and nausea that last 2-4 days. During the prolonged incubation period there is no evidence of viral shedding and infected persons do not infect others. After this incubation period, lesions erupt in the oropharynx and mouth and one day later the characteristic rash appears on the face, hands and forearms, which eventually spreads over the entire body. Soon after formation of first mucosal lesions large amounts of smallpox are released in the mouth and throat and infected individuals can transmit smallpox to others. The skin lesions start as small reddish macules, which enlarge and become pustule. Over the next 2-3 weeks crusts are formed, which eventually fall off and leave extensive scarring behind. All lesions in a given area progress together through these stages. Other long-term sequelae can include blindness, encephalitis, secondary bacterial infections and arthritis. Two principal forms of smallpox were clinically differentiated during natural outbreaks, Variola major and Variola minor. Variola major epidemics resulted in high case-fatality rates of about 30%, whereas Variola minor caused a much milder form with case fatality rates of approximately 1%. Rarely two fatal disease forms occurred, haemorrhagic and malignant smallpox.

Smallpox vaccines produced and successfully used during the intensified eradication program are called first generation vaccines in contrast to smallpox vaccines developed at the end of the eradication phase or thereafter and produced by modern cell culture techniques. Second generation smallpox vaccines use the same smallpox vaccine strains employed for manufacture of first generation vaccines or clonal virus variants plaque purified from traditional vaccine stocks, whereas third generation smallpox vaccines represent more attenuated vaccine strains specifically developed as safer vaccines at the end of the eradication phase by further passage in cell culture or animals. Second and third generation vaccines are produced using modern cell culture techniques and current standards of Good Manufacturing Practices (GMP). Other groups of vaccines include inactivated vaccines used to prime vaccinia-naïve subjects prior to administration of traditional smallpox vaccines or live smallpox vaccines administered orally as tablets.

This review summarizes published information on different smallpox vaccines stockpiled and/or licensed today primarily for emergency use.

First generation smallpox vaccines

Smallpox has been successfully eradicated by a global effort using a panel of live vaccinia virus vaccines. Manufacture, use and clinical experience with these first generation smallpox vaccines in the pre-eradication phase were described in detail by Fenner, Henderson, Arita, Jezek, and Ladnyi in 1988. In the following sections main characteristics of first generation smallpox vaccines are summarized as described by Fenner et al 1988.

During the intensified eradication program coordinated by the (WHO) smallpox vaccines produced by 71 manufacturers worldwide were used. Manufacture of first generation vaccines was based on different vaccinia virus strains and production systems (Table 1). The most widely used vaccine strains were Lister, New York City Board of Health (NYCBH), Tiantan (Temple of Heaven), and EM63. These vaccine strains were propagated and harvested primarily from skin of live animals (e.g. calf, sheep, water buffalo), calf lymph or chorioallantois membrane (CAM) of embryonated hens' eggs. From 1967 onwards more standardized vaccine production methods were implemented to meet the WHO requirements for potency, stability and safety of vaccine batches (WHO TRS 1965, No 5). Lyophilisation was recommended and all freeze-dried vaccine batches released had to contain at least $1x10^8$ pock-forming units (PFU) per milliliter (mL). Absence of pathogenic microorganism had to be ensured. Standardisation of manufacture and control of vaccine production was one important factor for the success to eradicate smallpox (Fenner et al 1988).

Table 1: Pathogenicity of various vaccinia virus strains used during the eradication campaign in endemic settings

Vaccinia strain and derivatives	Area used	Pathogenicity in animals and humans
NYCBH • EM63 • Ecuador	USA, Central and South America Africa USSR Asia	low
Lister • L-IVP • Merieux 37 • Nigeria strains	Europe USSR Africa Asia	moderate
Patwadanger	India	moderate
B-51	USSR	moderate
TianTan	China	high
Copenhagen	Europe	high
Bern	Europe	high
Ikeda	Japan	high
Tashkent	USSR	high

During the intensified eradication program the vaccines were commonly administered by scarification using a bifurcated needle. In general a minute drop of 2-3 microliter reconstituted vaccine corresponding to a human dose of ~$2x10^5$ PFU was delivered by 10-15 punctures to the skin over the deltoid muscle.

Experience with first generation vaccines in the pre-eradication phase

Field effectiveness, post-exposure protection and clinical correlates

Although controlled clinical trials were never performed with first generation smallpox vaccines, field effectiveness was demonstrated by their success in the global eradication campaign.

Information on protective post exposure efficacy derives from several sets of data on secondary attack rates among vaccinated and unvaccinated family contacts of smallpox cases. Vaccine efficacy has been estimated to be between 90.7% and 97.1%. Moreover the data indicate that the longer the time

between exposure and vaccination the higher the risk that post-exposure vaccination is ineffective to prevent infection or to mitigate severity of smallpox disease (Fenner et al 1988). Based on historical data from the UK it was found that primary vaccination within 3-4 days after smallpox exposure and revaccination within one week after exposure may protect against disease and/or death (Mortimer 2003).

A vaccine-related major cutaneous reaction (vaccine "take") was recognized as clinical correlate of protection against smallpox. No common serological correlate of protection has been established. Although neutralising antibodies are the most reliably parameter correlated with immunity and protection against smallpox infection and disease, it is known from individuals with certain T-cell immune deficiencies that cellular immune responses contribute to protection. After successful vaccination, the duration of protection was thought to be at least three years, with at least some degree of protection likely persisting for 10 years or more (Fenner et al 1988).

Safety

Expected vaccine-related common local and systemic reactions with usually mild to moderate severity are local reactions including pain, intense erythema and inflammation at the vaccination site as well as systemic reactions such as fever, malaise, myalgia, headache, chills, nausea, fatique, and lymphadenopathy. These reactions usually resolve within 2-3 weeks. Other complications of smallpox vaccination are autoinoculation and inadvertent infections of close contacts, which are generally self-limited. Vaccinia keratitis was reported after inadvertent autoinoculation and results in lesions of the cornea, which eventually may lead to ocular impairment.

During the eradication phase rare but serious postvaccinial complications were documented. Reports describe generalized vaccinia, eczema vaccinatum, progressive vaccinia (vaccinia necrosum), postvaccinial encephalitis and death.

- Generalized vaccinia is marked by swelling and tenderness of the draining lymphnodes and a viremia detectable between 3 to 10 days, e.g. in pharyngeal swabs of individuals with postvaccinial tonsillitis. Viremia is followed by a disseminated rash. The rash develops 6 to 9 days after vaccination and is composed of lesions that follow a similar progression as the lesion at the vaccination site. Primary vaccinees are at greater risk of developing generalized vaccinia, however in most cases it is self-limited.

- Eczema vaccinatum emerges with constitutional symptoms and vaccinial rash mostly at current or previous eczematous locations. Vaccinated and unvaccinated contacts with eczema or atopic dermatitis are at increased risk even if the eczema/atopic dermatitis is not active at the time of vaccination or contact. Severe cases and fatalities have been observed.

- Progressive vaccinia is a severe complication associated with T cell deficiency. It is characterized by the development of a progressive often necrotic lesion at the vaccination site followed by spreading of lesions to other parts of the body. Early after vaccination patients can lack symptoms of inflammation but later on the disease progresses and is frequently fatal.

- Postvaccinial encephalitis (pvE) is the most serious complication in otherwise healthy persons and is associated with high mortality and morbidity. Approximately 25-35% of vaccine recipients with pvE died within a week, and about 25% had permanent neurological sequelae.

Another serious complication is foetal vaccinia following primary vaccination of women in early stages of pregnancies. It results in stillbirth or death of the newborn.

The frequency of postvaccinial complications was found to be dependent on the vaccine strain employed and was generally higher in primary vaccinees then following revaccination.
Occurrence of serious adverse events during the intensified eradication campaign was intensively reviewed in recent years (Aragón et al. 2003, Kretzschmar et al. 2006).

Kretzschmar et al analysed historical vaccination data on the frequency of post-vaccinial encephalitis (pvE) and death after primary and revaccination with smallpox vaccine with respect to age and vaccinia strain used. The data sets analysed derived from Germany, Austria, Sweden, France, the UK, the Netherlands, the former Soviet Union and from the USA. For primary vaccination the frequency of pvE differed hugely between various vaccine strains. The highest rate of pvE was found with the Bern strain with 44.9 expected cases per million vaccines, followed by the Copenhagen strain (33.3 expected cases per million), the Lister strain (26.2 expected cases per million) and the NYCBH strain with the lowest rate of 2.9 expected cases per million vaccinations. With respect to age-related effects the frequency of pvE was highest among infants in their first year of life and increased constantly with age. For the NYCBH strain the highest frequency was reported in children aged 1 to 3 years. A similar pattern was observed for vaccination related mortality. After primary vaccination highest mortality rates were expected with the Bern strain (55 deaths per million), followed by the Copenhagen strain (31.2 deaths per million) and the Lister strain (8.4 deaths per million). The lowest death rate is expected for the NYCBH strain with only 1.4 deaths per million primary vaccinations. For the Bern, Lister and NYCBH strains mortality is expected to be highest in children younger than 1 year of age and lowest in children approximately 2 years of age. After revaccination the mortality rates are generally much lower than after primary vaccination and the differences between strains after revaccination are similar to those after primary vaccination. In revaccinees older than 20 years of age the rates were 4 to 16-fold lower than in primary vaccinees with the lowest rate expected with the NYCBH strain.

Aragón et al reviewed data on the experience of adverse events after smallpox vaccination in the USA with the NYCBH strain that were published by Neff, Lane, Melin and Ratner (Aragón et al 2003). The data were gathered in the years 1963 to 1968 and represent approximately 13 million primary vaccinations and 18 million re-vaccinations.
Following primary vaccination the risk of post-vaccinial encephalitis (pvE) and death from pvE was highest among infants under 1 year of age with estimated 6.8 cases of pvE and 3.0 deaths per million vaccinations, respectively. The risk in primary vaccinees 1 year of age and older was much lower and ranged between 1.8 and 3.3 cases of pvE per million vaccinations and 0-1.2 deaths per million vaccinations. The risk of developing progressive vaccinia (vaccinia necrosum) was 1.0 case per million vaccinations in all age groups and was highest in individuals 20 years of age and older (5.3 cases per million). Two deaths were reported in subjects experiencing progressive vaccinia. The risk of eczema vaccinatum did not differ across age groups and was reported to be 12.8 cases per 1 million vaccinations and no deaths were reported in subjects with eczema vaccinatum. However, among non-vaccinees that developed eczema vaccinatum after contact transmission from a recent vaccinee, 3 of 132 cases died. The risk for generalized vaccinia was highest in infants in their first year of life and in this age group 103.3 cases per million vaccinations were reported. In subjects 1 year and older the risk was determined to range from 22.4-49.9 cases per million vaccinations. Across all age groups no death due to generalized vaccinia occurred. Accidental infection (inadvertent autoinoculation) was reported for 64.9 cases per million vaccinees with no deaths associated and the risk was comparable across the different age categories.

In revaccinees the risks of developing specific complications and risks of dying from specific complications following smallpox administration were significantly lower than for primary vaccination. After revaccination the risk of pvE, progressive vaccinia (vaccinia necrosum), eczema vaccinatum and generalized vaccinia was 26 times, 1.5 times, 12 times and 29 times lower, respectively, compared to primary vaccinations. Only 2 subjects with progressive vaccinia died in the total group of revaccinees resulting in a risk of 0.1 deaths per million re-vaccinations.

Experience with first generation smallpox vaccine in the post eradication era

Based on the knowledge of the safety profile of smallpox vaccines of the pre-eradication era the vaccine use in the post eradication era was restricted to laboratory personnel, military or first responders. The vaccine was in principle contraindicated in pregnant women, persons with known immune deficiencies, patients under immune suppressive therapies, and persons with a history of eczema. More recently due to the experience in large-scale vaccination campaigns myopericarditis was recognised as a serious postvaccinial complication in healthy subjects and this led to further restrictions in the use of traditional smallpox vaccines.

Lister strain vaccines

In the last decades several Lister strain vaccines were evaluated in clinical trials or vaccination campaigns.

- *Lister vaccine produced on CAM (Israel)*

During the winter of 2002–2003, the Israeli health authorities launched a campaign to vaccinate first responders against smallpox (Orr et al 2004). In an open study, 159 healthy, previously vaccinated adults aged 24–52 years were immunised by scarification. The vaccine available in Israel is prepared from the Lister strain in chorioallantois membranes of chicken embryos and the virus titer of the vaccine suspension was given to be approximately 10^7 PFU per mL. Vaccine take and seroconversion rates of 61% and 56%, respectively, were observed. It was found that the level of pre-existing antibodies against vaccinia inversely correlated with the rates of clinical take and seroconversion. Most of the subjects enrolled had been vaccinated 2-3 times before the start of the campaign. The safety and reactogenicity were actively monitored at 3 time points within 18 days after revaccination. The most commonly adverse events among the vaccinees were pruritus, local and axillary pain, lymphadenopathy, fatique, and headache. Only 1 subject reported fever over 38.0°C. No serious adverse events were noted.

Occurrence of adverse reactions smallpox vaccine was defined in a vaccination campaign performed by the Israel Defense Force (IDF) among vaccinees aged 18 years and older between 1991 and 1996 and compared with previous surveys (Haim et al 2000). The majority of recruits were previously vaccinated and approx. 20% were primary vaccinees. Overall a postvaccinial complication rate of 0.4 per 10,000 vaccines was determined. The most frequent complications were eczema vaccinatum (0.15 per 10,000) and generalized vaccinia (0.09 per 10,000) followed by inadvertent inoculation (0.06 per 10,000), secondary infection (0.06 per 10,000) and erythema multiforme (0.03 per 10,000). No cases of postvaccinial encephalitis, progressive vaccinia or mortality were reported. The rates of eczema vaccinatum, generalized vaccinia and inadvertent inoculation were higher in the IDF recruits than in similar surveys conducted in the 1960s. Among 2,480 vaccinees included in a survey of new recruits 1.5% reported minor adverse events including local pain, local swelling, fever and lymphadenopathy. None required hospitalization.

- *Lancy-Vaxina Berna (SSI) manufactured on animal skin*

The first generation smallpox vaccine Lancy-Vaxina Berna was manufactured according to WHO requirements on the skin of sheep using the Lister strain derived from the Lister Institute. In the last decade efficacy, immunogenicity and safety of the vaccine Lancy-Vaxina were evaluated in clinical trials in Taiwan, South Korea and the UK (Hsieh et al 2006, Kim et al 2005, Auckland et al 2005, Pütz et al 2006).

Hsieh et al evaluated the clinical and immunological responses to diluted and undiluted Lancy-Vaxina vaccine in vaccinia naïve and previously vaccinated subjects in Taiwan. A total of 219 healthy adults aged 24 to 65 years were randomised to receive either undiluted or diluted vaccine. All vaccinia-naïve subjects (N=97), who received either undiluted ($10^{9.0}$ PFU/mL) or diluted (1:5 or 1:10) vaccine showed a major skin reaction (vaccine take). In the group of the previously vaccinated subjects (N=122) all subjects were successfully revaccinated by undiluted or diluted (1:10 and 1:30) vaccine except two subjects having received 1:30 diluted vaccine. Evaluation of the neutralising antibody response demonstrated that all vaccinia-naïve and vaccinia-experienced subjects elicited a robust vaccinia-specific immune response regardless of the vaccine preparation (diluted or undiluted) used or the occurrence of a vaccine take. As regards safety the data indicate that dilution of the vaccine was not associated with decreased local reactions and incidences of systemic reactions when compared with undiluted vaccines. However, in vaccinia-naïve subjects receiving undiluted vaccine the lesion size was significantly smaller and most systemic reactions, including fever, headache, muscle ache, fatigue and lymphadenopathy, were more frequently observed than in previously vaccinated subjects receiving undiluted vaccine. No serious or life-threatening events were observed in this study.

Similar results were reported from a study conducted in South Korea also using undiluted and diluted Lancy-Vaxina vaccine (Kim et al. 2005). Two vaccine dilutions (1:1 and 1:10) of Lancy-Vaxina vaccine (vaccine titer undiluted: $10^{7.7}$ PFU/mL; Berna Biotech, formerly SSI) were administered to vaccinia-naive persons (n=36) and persons previously vaccinated >25 years ago (n=76). All vaccinees responded successfully to vaccination as judged by vaccine ”take”. There were no significant differences in the size of the skin lesions, the number of adverse events, the amount of viral shedding, or the level of antibody responses between the undiluted and diluted vaccine groups. Compared with vaccinia-naive persons, previously vaccinated persons exhibited significantly smaller and more rapidly evolving skin lesions and fewer adverse events. No serious adverse events were documented.

The study conducted in health care workers in England and Wales assessed vaccine take rates and occurrence of adverse events following vaccination with the Lister vaccine manufactured by SSI in vaccinia-naïve and previously vaccinated individuals (Auckland et al 2005). The participants were actively followed-up for at least 21 days by diaries. Out of 232 subjects vaccinated 200 completed their diaries and were included in the efficacy and safety analyses. By day 7, 99% of the vaccinees had a vaccine ‘take’. The most commonly reported adverse events were redness (87%) and pain at the vaccination site (71%). Fever recorded as moderate or severe occurred in 25% of subjects and this peaked 4–9 days post-vaccination and lasted usually 3 days. There was no significant difference in the incidence of pain between vaccinia-naïve and experienced subjects. There was a general trend towards increased rates of common adverse events such as axillary lymphadenopathy, itch, erythema, general malaise or flu-like symptoms and headache in naïve vaccinees compared with those with a prior vaccination history. Clinically important but less common symptoms included local skin/soft tissue infection requiring oral antibiotics in 2.5% of patients. In addition, two serious adverse events occurred. One previously vaccinia vaccinated subject was hospitalised 7 days after vaccination with headache, fever and altered conscious level. A clinical diagnosis of encephalitis was made, classified as ‘possibly’ related to vaccination. The patient made a complete recovery. Another SAE occurred in a vaccinia-naïve individual, which was

admitted to hospital 12 days after vaccination with a fever and cellulitis around the vaccination site requiring treatment with intravenous antibiotics. No case of myopericarditis was reported.

An independent group quantified the antibody responses against the intracellular mature virus (IMV) and the extracellular enveloped (EEV) form using a panel of test systems including virus neutralisation assays (Pütz et al 2006). Sera from 92 health care workers enrolled in the vaccination campaign in England and Wales were analysed. The sera derived from vaccinia naïve subjects (N=18), subjects having previously received one (N=58) or two (N=6) smallpox vaccinations and 10 subjects with unknown vaccination status. The results show that both primary vaccinees and revaccinees elicit strong responses against the EEV surface proteins B5, A33 and A56, against the IMV surface proteins A27 and H3, but only low levels of L1-specific antibodies. Comparative analysis showed that the levels of antibodies to B5 induced in humans correlated closely with the magnitude of EEV-specific neutralization in vitro and that B5 is the only target of EEV-neutralizing antibodies after smallpox vaccination. In addition two IMV surface proteins (A27 and H3) were identified as targets for IMV-neutralizing antibodies present in human sera of primary vaccinees and revaccinees.

- *Pourquier vaccine (Lister strain produced from calf lymph, France)*

In 2003, a group of first responders were revaccinated in France using stockpiled smallpox vaccine (Bossi et al 2001). The lyophiilized vaccine was prepared using the Lister strain from calf lymph by the Pourquier laboratories. The virus titer was determined to be $10^{7.7}$ PFU/ml. A total of 226 healthy volunteers aged 27-63 years were vaccinated and actively followed up for vaccine take and adverse events for 28 days post vaccination. Most of the participants were previously vaccinated more than once (80%). Successful vaccination was observed in 95.6% of revaccinees. Side effects were experienced by 27% of participants, which resolved within two weeks. The most frequently reported local and systemic adverse reactions were fever (12%), fatique (6%), local pruritus (8%), axillary lymphadenopathy (3%), headache (3%) and myalgia (3%). No serious adverse events were notified and no inadvertent inoculation of close contacts was reported.

Regulatory status:

First generation Lister strain vaccines are stockpiled in many countries worldwide. Some are licensed and some have no active marketing authorisation. However, regulatory mechanisms are in place for approval of these vaccines allowing their immediate use in an emergency.

Published data suggest that in France two Lister vaccines including the Pourquier vaccine are licensed. In Russia the vaccine FCP, a freeze-dried vaccine derived from the L-IVP strain and prepared on calf skin, holds a marketing authorisation for emergency use (Onishchenko et al 2006).

NYCBH strain vaccines

Most information on NYCBH strain vaccines derive from Dryvax vaccine, which was intensively used in vaccination campaigns in the USA.

Dryvax

Dryvax was produced by infection of skin of calves using the NYCBH strain as seed virus. Recently, studies were performed evaluating clinical and immunologic responses to diluted vaccine in volunteers who had not previously been immunized. The lyophilized undiluted vaccine, when reconstituted, had a virus titer content of $10^{8.1}$ PFU/mL. A total of 680 healthy adults aged 18-32 years were randomized to either

receive undiluted or a 1:5 dilution or a 1:10 dilution of the vaccine. It was shown that at dilutions of 1:5 or 1:10, the vaccine was able to elicit adequate immune responses with 99.1 % and 97.1% developing a vaccine take, respectively. Undiluted vaccine resulted in a success rate of 97.2 % (Frey et al 2002). Determination of the neutralising antibody responses revealed that all participants were seropositive by day 28 and all but one subject were seropositive at day 56 (Belshe et al 2004). In the group that had received undiluted Dryvax 103 out of 106 subjects had a major cutaneous reaction and neutralising antibodies increased from <20 prior to vaccination to a GMT 1262 on D28 but had fallen to 796 on D56. In addition it was found that higher neutralising antibody titers at day 28 and 56 were significantly associated with larger skin lesions, erythema and fever.

In response to fears of bioterrorist attacks two large-scale vaccination campaigns were initiated in 2002 in the USA. The results of the safety assessment of subjects vaccinated as of 30 June 2004 were reviewed by Neff et al 2008. In the program conducted by the Department of Defense (DoD) in military personnel 628,414 individuals were vaccinated with Dryvax. Of the subjects included 71% received primary immunisation and 29% were revaccinated. The majority of vaccinees were males (88%) and the median age of all vaccinees was 26 years. A second program initiated by the US Department of Health and Human Services (DHHS) was designed to immunize health care workers and first responders and 39,566 individuals received Dryvax by scarification. In the DHHS program the rate of vaccinia-naïve subjects and the number of male subjects enrolled was lower than in the DoD program. Primary vaccinations were given to 24% of the participants and 37% of the total cohort were males. The median age was 48 years.

In both programs no cases of progressive vaccinia, eczema vaccinatum, or occupational transmission occurred. Two of 48 cases reported met the case definition for superinfection of the vaccination site, but no pathogenic organism could be isolated. One individual experienced erythema multiforme major (Stevens-Johnson syndrome) and recovered within one week. Out of 42 cases reported as suspect or probable generalized vaccinia only one case could be confirmed. All suspected generalized vaccinia cases were mild and self-limited. None of these case patients had atopic dermatitis or underlying conditions that might be related to an impaired immune system. Other adverse events recorded in the two programs were 97 cases of autoinoculation including inadvertent infection of the eye (17 cases). Of note, inadvertent infection of close contacts of vaccinees (family, friends or intimate contacts) occurred in 47 cases. All contact vaccinations resolved without sequelae.
Among the 628,414 vaccinees in the DoD program one case of encephalitis was reported, the patient recovered fully. In the civilian population one case of post-vaccinial encephalitis was recorded. This individual has some persistent memory loss.
Despite screening inadvertent vaccination was recorded in 236 pregnant women. No unexpected findings were found with respect to spontaneous abortion, preterm birth or congenital abnormalities. No case of foetal vaccinia occurred. Overall 10 subjects with undiagnosed HIV infection (3 vaccinia naïve, 5 revaccinees and 2 with unknown vaccination status; mean CD4 cell count: 483 cells/mm^3 post vaccination) were included. All had major reactions to smallpox vaccination, with normal healing and no adverse reactions.
Unexpectedly cardiac complications (i.e. myopericarditis, ischemic cardiac disease and dilated cardiomyopathy) became a cause for concern. Overall 79 cases of myopericarditis have occurred among the 628,414 vaccinees in the DoD program, specifically and disproportionally in male subjects. In the 66 subjects who have been intensively followed, all have recovered normal heart function. In the DHHS program 21 cases of myopericarditis were recorded, 86% occurred in revaccinees and 67% were females. In 14% some persistent mild symptoms continued approx. 40 weeks after diagnosis. A causal relationship of myopericarditis and smallpox vaccination is supported by the close temporal clustering. Onset of symptoms was coincident with peak viral replication at the vaccination site (at 7-14 days) and likely with occurrence of inflammatory responses. In an other publication it was estimated that the observed

incidence within the 30 day observation window of 16.11 per 100,000 was nearly 7.5-fold higher following primary vaccination than the expected background rate among non-vaccinees (2.16 per 100,000) (Poland et al 2005). No increased incidence of myopericarditis was found for the group of revaccinees (2.07 per 100,000).
Ischemic cardiac events occurred in a total of 26 cases (5 fatal) in both programs, but were not clustered temporally after vaccination. An epidemiological analysis identified no difference in the frequency observed in unvaccinated individuals. Consequently the data did not support an excess of ischemic cardiac events among recent smallpox vaccinees.
Seven cases of dilated cardiomyopathy (DCM) were reported 5-40 weeks after vaccination in previously healthy individuals. Out of the 7 DCM cases 2 patients received disability discharges and successful heart transplants. Four DCM cases recovered sufficiently to return to work. Currently it is difficult to determine whether DCM is linked to vaccination because no epidemiological background data from unvaccinated persons are available. There is however a possible link to infection and autoimmune processes.

Regulatory status
Dryvax manufactured by Wyeth Laboratories until the early 1980s and manufactured by Aventis Pasteur are no longer licensed in the USA. The use of Dryvax in the USA was revoked as of Feb 29, 2008. (MMWR, weekly, Feb 29 2008 / 57(08); 2007-2008.)

Second generation vaccines

Advantages of the second generation vaccines produced in cell culture are improved manufacturing processes primarily as regards consistency between lots and minimizing the risk of contaminations by adventitious agents.

- **Lister vaccine produced in primary rabbit kidney cells (RIVM)**

The first cell-culture derived smallpox vaccine was produced in the late 1960s by growing the Lister strain on primary rabbit kidney cells (Hekker et al 1973). The seed virus for the production was prepared in calves and was the same as that used for the production of calf lymph vaccine. The virus was thus not more than one passage removed from animal skin. Satisfactory vaccine potency and stability was demonstrated for the freeze-dried vaccine and initial field studies conducted in the Netherlands with app. 1600 vaccinia-naïve and vaccinia experienced individuals showed that the cell-culture based Lister vaccine induced comparable take rates, immune responses as measured by neutralising antibody titers and had a similar safety profile than the calf lymph derived vaccine (Fenner et al. 1988). Because of these results the cell culture derived vaccine was used together with a calf lymph vaccine in a field trial in Lombok, Indonesia, in 1973 (Hekker et al 1976). Both vaccines tested had comparable virus titers of $3x10^8$ PFU/ml. A total of 61,808 children aged 0 to 14 years were vaccinated: 51,268 (82.9%) with tissue culture vaccine and 10,540 (17.1%) with calf lymph vaccine; 15,390 (30.0%) of those vaccinated with tissue culture vaccine and 2992 (28.4%) of those vaccinated with calf lymph vaccine were primary vaccinees. None of the children in either group experienced disseminated vaccinia, eczema vaccinatum, or vaccinia necrosum. A number of cases of severe malaria, gastroenteritis, and pneumonia occurred in both vaccinated and unvaccinated children. Among those in the study group, illnesses were randomly distributed according to the time of onset and their occurrence appeared to bear no relationship to vaccination. One case of encephalitis occurred that may have been vaccine-related. A 5-month-old girl had a major reaction at the vaccination site and died 2 weeks after vaccination after it had developed high fever, convulsions, head stiffness and felt unconscious. Similar results were obtained with both

vaccines in primary vaccinees and in revaccinees as regards the take rates (appr. 97% in primary vaccinees and 71-74% in revaccinees).

- **Elstree-BN (Bavarian Nordic)**

Elstree-BN is a smallpox vaccine developed by Bavarian Nordic. The Lister vaccine strain was derived from a vaccine sample manufactured by a local German manufacturer. The vaccine is produced in embryonic chick fibroblast cells under current GMP standards and contains not less than $1x10^8$ PFU per mL. Preclinical studies demonstrated that comparable immune responses were induced in macaques by Elstree-BN and the traditional smallpox vaccine Elstree/RIVM produced on calf skin as measured by ELISA and PRNT (Stittelaar et al., 2005). Bavarian Nordic reported similar safety and efficacy compared with the traditional vaccine in a clinical study conducted in 2004 in a small number of subjects (N= 32) (referenced in Wiser et al 2009).

- **VV Lister/CEP (Sanofi Pasteur)**

The second-generation smallpox vaccine Lister/CEP was developed from a batch of a first-generation Lister/Elstree vaccine originally produced by Sanofi Pasteur. The vaccine strain has not been cloned and has undergone only three passages in chicken embryo primary cells during manufacture. Preclinical studies confirmed that further passaging in CEP cells has not resulted in selection of specific viral populations. VV LISTER/CEP showed a comparable safety and immunogenicity profile and induced comparable results in a lethal cowpox virus mouse model as the parental first generation Lister vaccine (Ferrier-Rembert et al 2007).

Regulatory status:

Although cell-culture derived Lister vaccines are stockpiled in various countries for emergency use, their regulatory status is not clear. It is assumed that they are currently not licensed in all countries having a stockpile. However, regulatory mechanisms are in place for approval of these vaccines allowing their immediate use in an emergency.

- **ACAM2000 (Acambis/Sanofi Pasteur)**

ACAM2000 is a live attenuated smallpox vaccine produced from a single plaque purified vaccinia virus strain, which originates from the Dryvax vaccine (NYCBH strain). Interestingly it was found that the original Dryvax vaccine represents a pool of vaccinia virus subpopulations which show varying degrees of virulence. Six individual clones were isolated by plaque purification and these clones were evaluated in virulence tests in rabbits and suckling mice. Among the six individual strains isolated significant differences in the neurovirulence was observed with clone CL2 having a reduced neurovirulence in suckling mice but being comparable to Dyrvax vaccine in other characteristics such as lesion size (Weltzin et al 2003, Acambis briefing document 2007). Clone 2 was selected for further development of a new smallpox vaccine initially in human embryonic lung fibroblast cells (MRC-5, ACAM1000) and further on in Vero cells (ACAM2000) (Acambis briefing document, 2007, Nalca and Zumbrun, 2010). Initially safety, efficacy and immunogenicity of ACAM1000 was investigated in a limited number of healthy vaccinia naïve adults (N=60) in a Phase I clinical trial and compared with Dryvax (Weltzin et al 2003). All ACAM1000 recipients had a `vaccine take' and seroconverted, but the mean neutralizing antibody response following vaccination with ACAM1000 was twofold lower than after vaccination with Dryvax (GMT 142 vs 248). No serious adverse events were reported following vaccination with ACAM1000 or

Dryvax (Weltzin et al 2003). Additional human data from a second phase I study in 70 adult subjects confirmed the results of the first trial (Monath et al 2004).

In order to expand the production capacity of the new smallpox vaccine it was decided to use an established large-scale production system based on Vero cells. Further passage of the MRC-5 derived vaccinia virus seed material in Vero cells demonstrated genetic stability of the DNA sequence of Clone 2. In addition, studies in mice and rabbits confirmed that the pathogenicity and immunogenicity of the Vero cell derived vaccine ACAM2000 was comparable to ACAM1000 (MRC-5 derived) and that both candidate vaccine strains were less virulent than Dryvax (Monath et al. 2004). The clinical development program of ACAM2000 encompassed six clinical studies with a total of 3881 adult subjects enrolled (ACAM2000 N=2983, ACAM1000 N=30 and Dryvax N=868). In two phase I clinical studies conducted in healthy vaccinia naïve adults aged 18-29 years it was confirmed that vaccination with ACAM2000 results in high take rates of nearly 100% and that the majority of subjects had antibody responses comparable with ACAM1000 and Dryvax. The lowest effective dose of ACAM2000 was determined in two phase II studies that enrolled either vaccinia naïve or previously vaccinated subjects. In these studies a control group was included that received Dryvax at a standard dose (1.6×10^8 PFU/ml). All naïve individuals vaccinated with Dryvax or a dose of 6.8×10^7 PFU/ml of ACAM2000 showed a take and 96% and 94% seroconverted, respectively, with comparable neutralizing antibody titers.
Additional groups of vaccinia naïve subjects received either 1:5, 1:10, or 1:20 dilutions of ACAM2000 or a 1:10 dilution of Dryvax vaccine. It was found that vaccinia naïve subjects receiving of diluted ACAM2000 had take-rates below the threshold set for efficacy (59% to 86%), whereas over 97% of vaccinees having received a 1:10 dilution of Dryvax had a vaccine take.
Data from vaccinia virus experienced subjects indicated that ACAM2000 was less effective than Dryvax. Only 88% of the ACAM2000 recipients had a take whereas all of the Dryvax vaccinees developed a major cutaneous reaction.

Since the dose range of the ACAM2000 and Dryvax vaccine evaluated in these phase I/II studies varied between the two study vaccines further clinical studies were initiated to investigate ACAM2000 and Dryvax at comparable dose ranges. Two clinical studies, one in vaccinia-naïve and one in revaccinees, compared a dose range of $1.3\text{-}2.2 \times 10^8$ PFU/ml of ACAM2000 vaccine with Dryvax given in a dose of 1.5×10^8 PFU/mL). In vaccinia-naïve subjects, the take-rates were 96% and 99% for ACAM2000 and Dryvax, respectively, indicating that both formulations were effective, although the geometric mean antibody titer elicited in the group receiving ACAM2000 were inferior to those receiving Dryvax. In the clinical study in vaccinia-experienced subjects, success rates of 84% and 98% were obtained in the ACAM2000 and Dryvax groups, respectively. Again, the geometric mean neutralizing antibody titers were higher in the Dryvax group than in the ACAM2000 group. In summary these data suggest that Dryvax is superior to ACAM2000 as regards robustness of the antibody response most likely due to the decreased level of virulence.

Serious and nonserious adverse events following vaccination of 2983 people with ACAM2000 (1307 naïve and 1676 experienced) were actively monitored and compared with that of Dryvax.
In the clinical studies 97% of vaccine-naïve subjects and 92% of revaccinees experienced one or more AEs after vaccination with ACAM2000. Common events included injection site reactions (erythema, pruritus, pain and swelling) and systemic symptoms (fatigue, headache, myalgia, malaise, feeling hot, and rigors). In general, occurrence of adverse events was similar in individuals vaccinated with ACAM2000 and Dryvax, but the frequency for some adverse events were lower for several specific adverse events like lymphnode and injection site pain in ACAM2000 vaccinees.

No cases of generalised vaccinia, ocular vaccinia, postvaccinial encephalitis, progressive vaccinia, erythema multiforme, or pvE were reported with ACAM2000. However, in the clinical development program of ACAM2000 10 cases of suspect or probable myocarditis, 7 (5.73 events per thousand vaccinations) in subjects treated with ACAM2000 and 3 (10.38 events per thousand vaccinations) in subjects treated with Dryvax, were identified. The rates of myopericarditis for ACAM2000 and Dryvax were not statistically different in these trials indicating no significant reduction in the risk of developing myopericarditis by ACAM2000 compared to Dryvax. All subjects who experienced myocarditis were previously naïve to vaccinia; no cases were detected in previously vaccinated subjects. Two of the 10 subjects were hospitalised while the others were sub-clinical and received no treatment.

Regulatory status:

ACAM2000 is licensed since August 2007 in the USA. The vaccine is manufactured by Sanofi Pasteur Biologics Co. of Cambridge, MA. ACAM2000 is indicated for active immunization against smallpox disease for persons determined to be at high risk for smallpox infection, however it is not currently recommended for or available to the general public in the U.S. The smallpox vaccine is only available under limited circumstances.

- **CJ-50300 (CJ CheilJedang Cooperation, South Korea)**

CJ-50300 is a freeze-dried smallpox vaccine prepared on MRC-5 cells under serum-free conditions employing the NYCBH strain (ATCC VR-118) without prior plaque purification. The vaccine contains a titer of vaccinia virus of $10^{8\pm 0\cdot5}$ plaque-forming units (pfu)/mL following reconstitution. Preclinical studies in mice and cynomolgus monkeys confirmed that CJ-50300 is comparable with the first generation Lancy Vaxina vaccine with respect to cutaneous reactogenicity, immunogenicity, protection and neurovirulence (Kim et al 2007).
In a phase I clinical trial all vaccinia naïve subjects enrolled (N=18, age range 18-27 years) exhibited cutaneous reactions after CJ-50300 vaccination, whereas no vaccine take was reported in the 6 subjects receiving only placebo (Kim et al 2007). Investigation of the kinetics of humoral and cell-mediated immune responses to CJ-50300 showed that all subjects achieved high neutralizing antibody titers 28 days after vaccination and cell-mediated immune responses 14 days after vaccination as measured by IFN--γ-producing T cell response by enzyme-linked immunospot (ELISPOT) assays. Antibody responses increased up to 28 days after vaccination and were maintained up to 56 days after vaccination. In contrast, cell-mediated immune responses increased up to 14 days after vaccination and steadily decreased thereafter. Frequently observed local and systemic adverse reactions were chill (11%), myalgia (11%), fatique (28%), headache (28%), axillary lymphadenopathy (28%), and pain at the vaccination site (22%). Another clinical study evaluated the efficacy and safety of two different doses of CJ-50300 (1.0×10^8 and 1.0×10^7 PFU/ml,) in healthy vaccinia-naïve adults aged 20-60 years (Jang et al 2010). A total of 123 volunteers were randomized to the two vaccine groups (82 received high dose and 41 received low dose vaccine) and were actively followed up for 28 days for vaccine take and safety. The success rates ("take") were 99% and 100% following administration of the high dose and low dose, respectively. The same rates were observed for seroconversion (4-fold increase in neutralising antibody titers (HD: 99%, LD 100%). Cellular immune response were somewhat lower with 89% in the high dose vaccine group and 95% in the low dose group Differences in the clinical, humoral and cellular responses were not significant between the two vaccine groups. No serious adverse events were observed, except of one case of a possibly vaccine related generalized vaccinia in the high dose group. The two vaccine groups did not differ in the occurrence of common adverse events. Fever, chill, myalgia, fatique, headache, lymphadenopathy and pain at the vaccination site were frequently reported. Satellite lesions were observed in both vaccine groups in 10% of vaccinees.

Regulatory status:

Further clinical evaluation of the vaccine CJ-50300 is ongoing in South Korea (NCT01056770). The current regulatory status in not known.

Third generation vaccines

At the end of the eradication campaign several attenuated smallpox vaccines were developed because of increasing concerns about serious adverse events following vaccination with first generation smallpox vaccines. These vaccines were evaluated in field studies and used for routine vaccination (e.g. LC16m8, MVA,), but they were never tested or used in endemic settings. Consequently they have no proven field effectiveness against smallpox virus infection or disease in humans. Otherwise they were found to have excellent safety profiles specifically as regards serious postvaccinal complications related to traditional smallpox vaccines.

Current knowledge of the immunological mechanisms of protection against smallpox suggests that both humoral and cellular immunity play an important role (reviewed by Amanna et al 2006, Panchanathan et al 2008, Moss 2011) although the underlying mechanisms are not fully understood. Circulating antibody and immune memory may be the more important factors for preventing or at least modifying the severity of clinically apparent smallpox, taking into account that there is enough time for clonal expansion of memory B-cells after exposure. Once clinical disease is established it seems that the ability to mount an adequate T-cell response is important for recovery.

LC16m8 – (replication competent)

Prof. Hashizume isolated a replication competent attenuated virus after serial passages of the Lister strain in primary rabbit kidney cells at 30°C. The selected vaccine strain LC16m8 formed small pocks on the chorioallantois membrane of embryonated eggs and was demonstrated to be less neurovirulent in mice, rabbits and monkeys after intracerebral inoculation than the parent Lister strain (Hashizume et al., 1985).

Pre-eradication era

Efficacy and safety of the LC16m8 vaccine was investigated between 1973 and 1974 in field studies and approximately 10.000 children 0-5 years of age were closely followed after administration of a single dose by scarification. The results were compared with data gathered with the parental Lister strain in field trials conducted between 1968 and 1971. The observed take rates for the LC16m8 and Lister vaccine were 95.1% (N=10,578) and 93.7% (N=3,662), respectively. One month after vaccination comparable neutralising and HI antibody responses were measured (Japan, Ministry of Health 1975).

Among 8,544 subjects receiving LC16m8 one case of eczema vaccinatum, nine cases of autoinoculation, 28 cases of statellite vesiculation, eight cases of postvaccinal exanthema and three cases of convulsions were reported, all of which were mild, but no case of encephalitis occurred. The causal relationship between the three cases of convulsion and vaccination was not established. The examination of a subset of vaccinated subjects by electroencephalography showed that the number of temporary anomalies was lower in LC16m8 (0/56) than in Lister recipients (6/37) (Fenner et al 1988). Fever rates (7.7% vs 26.6%) and local induration at the site of vaccination (6.1mm vs 15.3mm) were lower in LC16m8 recipients than after Lister vaccination (Japan, Ministry of Health 1975, Hashizume et al 1985, Fenner et al. 1988). Following licensure of the LC16m8 vaccine in 1975 in Japan 90,000 doses were distributed and no reports of serious adverse events were received.

Post eradication era

Since the initial development of LC16m8 vaccine, progress was made to further characterize the vaccine virus strain. LC16m8 retains the majority of the vaccinia genome and it was found that there is a 1-base deletion in the B5R gene, which introduces a stop codon within the open reading frame (Takahashi-Nishimaki et al. 1991, Morikawa et al. 2005). This mutation results in a truncated form of the B5R protein. B5R is related to the complement activation gene family and is a major target for neutralizing antibodies (Pütz et al 2006). It is involved in the efficient formation of extracellular enveloped virus (EEV), which is essential for cell-to-cell transmission of the virus and dissemination within the host (Smith et al. 2002). Recently it was found that virus revertants spontaneously emerged from plaque purified LC16m8 vaccine virus after passage in cell culture (Kidokoro et al 2005). These revertant virus clones were characterized by plaque size, dermal reaction in rabbits and pathogenicity in mice. Following i.p. injection in SCID mice the revertant virus clones induced severe rash and killed mice at low dose levels, while no overt symptoms were reported over a 4-week period in SCID mice receiving the LC16m8 vaccine. Sequence analyses showed that the full length open reading frame was reconstituted through a one base insertion that corrects the initial frameshift mutation. Synthesis of full-length B5 protein was demonstrated in western blot analysis (Kidokoro et al 2005).

Animal data

Since LC16m8 vaccine has never been tested against smallpox (e.g. variola virus) in the field, protective efficacy was evaluated in various animal studies using mouse, rabbit and monkeypox models (Morikawa 2005, Saijo et al 2006, Empig et al 2006, Meseda et al 2009, Gordon et al 2011).

Initial data from studies on the protective efficacy of LC16m8 and the parental Lister vaccine strain showed that mice were protected against lethal intranasal challenge with VACV strain WR (Morikawa et al. 2005). Another study compared the protective efficacy of LC16m8 with the parental Lister strain and placebo in the monkeypox model (Saijo et al 2006). Cynomolgus monkeys were immunized with LC16m8 or Lister vaccine by scarification and then 5 weeks later infected intranasally or subcutaneously with monkeypox virus (MPXV) strain Liberia or Zr-599, respectively. All animals in the vaccine and placebo groups survived the intranasal MPXV challenge with $1x10^6$ PFU of the Liberia strain. Whereas no clinical symptoms developed in and no virus could be isolated from animals vaccinated with LC16m8 or Lister, both animals in the placebo group showed clinical symptoms of MPXV infection such as body weight loss, rhinorrhea, papulovesicular lesions and decreased activity. Virus was isolated from peripheral blood starting 4 days after challenge. In a second experiment immunized monkeys received a subcutaneous MPXV challenge of $1x10^6$ PFU (strain Zr-599). Subcutaneous infection with MPXV Zr-599 was fatal to placebo -immunized monkeys and this was accompanied by a decrease in body weight, appearance of papulovesicular lesions and continuous detection of virus in peripheral blood. In contrast monkeys immunized with LC16m8 or Lister vaccine did not develop any MPXV related symptoms, except for local cutaneous lesions observed in the LC16m8 group at the inoculation site of MPXV. All animals maintained their body weight and none of the animals died. Histopathological examination 3 weeks after intranasal or subcutaneous challenge showed no MPXV related findings in the internal organs (lymphoid systems, lung, digestive organs, urogenital tract) of any of the monkeys in the LC16m8 and Lister group, but all organs were affected in animals in the placebo group.

Empig et al. presented data on efficacy employing rabbit and mouse challenge models. In both animal models LC16m8 was compared with Dryvax and placebo. Rabbits immunized with approx. $2x10^5$ PFU of LC16m8 or Dyrvax vaccine were protected against intradermal challenge with low (200 PFU) or high doses (1000 PFU) of lethal rabbitpox virus 28 days post infection. At the high dose challenge all animals in the placebo group died within 10 days whereas in the low dose group 9 of 10 rabbits died.

Determination of infectious RPV in lung tissue revealed no virus in the lungs of animals immunised with either LC16m8 or Dryvax. In contrast high titers of PRV were detected in the lung tissue of all animals of the placebo group. In the inhalation orthopoxvirus challenge model groups of mice were immunized either with $2x10^5$ PFU of LC16m8 or Dyrvax vaccine by scarification or received placebo. All mice were challenged 49 days later with aerosolized ECTV (approx. 1-2 PFU). Ninety percent of mice in the placebo group died within 15 days after ECTV challenge whereas all mice immunized with either LC16m8 or Dryvax vaccine were protected. Assessment of clinical symptoms revealed that most of the mice in each vaccine group had no signs of illness and continued to gain weight following challenge.

Meseda et al. presented data on comparative evaluation of the immune responses and protective efficacy engendered by LC16m8 and Dryvax smallpox vaccines in a mouse model. LC16m8 elicited a broad-spectrum antibody response that neutralized both EEV and the IMV form of vaccinia virus. Mice inoculated with LC16m8 had detectable but low levels of anti-B5 antibodies compared to Dryvax, but both Dryvax and LC16m8 sera neutralized vaccinia virus EEV in vitro. A truncated B5 protein (~8 kDa) was expressed abundantly in LC16m8-infected cells, and both murine immune sera and human vaccinia virus immunoglobulin recognized the truncated recombinant B5 protein in an antigen-specific ELISA. Immunization of mice with LC16m8 and Dryvax conferred similar levels of protection against a high-dose intranasal challenge (100 or 250 LD50) with vaccinia virus strain Western Reserve (WR). Among mice vaccinated with LC16m8, 80% survived either the 100 LD50 or the 250 LD50 challenge. All mice vaccinated with Dryvax survived the 100 LD50 and 60% survived the 250 LD50 challenge.

Studies conducted in immunocompromised animal models demonstrated that LC16m8 is well tolerated, with no serious adverse effects (Kidokoro et al 2005, Gordon et al 2011). Immunisation of SCID mice with high doses of LC16m8 did not induce mortality in contrast to Dryvax (Kidokoro et al 2005). Recently, Gordon et al investigated the immunological basis of the containment of vaccine in the skin by using macaques depleted systematically of T or B cells and vaccinated by scarification with either Dryvax or LC16m8 (2.5×10^5 PFU), . B cell depletion did not affect the size of skin lesions induced by either vaccine. Following simultaneous CD4+ and CD8+ T cell depletion LC16m8 was unable to spread and cause satellite or distal lesions whereas Dryvax led to progressive vaccinia with larger skin lesions, a longer resolution time of the lesions and in half of the animals spreading of lesions to secondary sites, indicating that LC16m8 might have a better safety profile in immunocompromised patients than traditional smallpox vaccines (Gordon et al 2011). All animals having received either LC16m8 or Dryvax survived lethal intravenous challenge ($5x10^7$ PFU MPXV strain Zaire 79)

<u>Human data</u>

Results of two clinical trials conducted in healthy subjects were recently 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 (Saito et al 2009). Of the 3468 subjects enrolled 247 were excluded from the study due to nonmedical or medical reasons such as active atopic dermatitis. In total 3221 subjects were vaccinated and followed for up to 30 days to assess successful vaccination ('vaccine take', neutralizing antibody response) and occurrence of adverse events including determination of troponin T levels in blood (Day 0 and Day 30) and electrocardiography (ECG) at day 30. Almost half of the subjects had never been vaccinated (47.5%), the vast majority of the vaccinees were men (98.4%) and all were of Asian origin.

Determination of successful vaccination indicates that the proportion of 'vaccine take' was significantly higher in primary vaccinees (94.4%) than in revaccinees (86.6%). In primary vaccinees take rates did not significantly differ across different age categories, although most participants belonged to the groups of the 20-29 (N=1122) and 30-39 years-old (N=367). Only 3 vaccinia naïve subjects were over 50 years of

age. Among revaccinees slightly higher take rates were observed in the 20-29-year-old group (N=84; 95.2%) but the number of subjects enrolled in this age category was low. In a subset of the study population neutralizing antibody responses were measured in serum samples collected prior and 30 days after vaccination by plaque reduction neutralization test (PRNT) using LC16m8 virus. Effective seroconversion or booster response was defined as a 4-fold increase in the $PRNT_{50}$ titer at day 30 days compared to the titer prior vaccination. The geometric mean titer (GMT) prior vaccination was significantly higher in revaccinees (21.0) than in vaccinia naïve subjects (6.1); among the revaccinees the GMT was significant higher in subjects born before 1964 (group D; GMT: 29.5) than in any other age group (group B and C: 14.4 and 19.4). The difference in baseline antibody titers might be explained by the number of doses and/or vaccine received through routine childhood vaccination in Japan. Post vaccination the GMTs were comparable in primary vaccinees and revaccinees. The percentage of primary vaccinees with 4-fold increases in neutralizing antibody titers (90.2%) was significantly higher than that of revaccinees (60.0%) and among the revaccinees it decreased with age. Further evaluations of the immune responses to specific VACV antigens and the neutralizing antibody response to EEV in a subset of sera from 42 vaccinia naïve vaccinees and 43 revaccinees revealed that the antibody responses to LC16m8 were qualitatively and quantitatively different from those seen for the Lister vaccine (Johnson et al 2011). In vaccinia-naïve subjects LC16m8 failed to elicit neutralizing antibody responses against the EEV form but induced a strong neutralizing antibody response to the IMV form of VACV. Antibody responses to specific proteins indicate that the vaccine induces antibodies against a variety of IMV proteins as well as against A56 protein, but not to B5. A56 and B5 are both components of EEV. Since non-neutralizing antibodies against EEV are capable to activate the complement system (Benhnia et al 2009) antibodies against A56 might induce such a response. In contrast, in revaccinees an anamnestic antibody response to B5 protein and neutralizing EEV response was found and these findings are most likely due to the presence of the truncated form of B5 expressed by LC16m8.

Throughout the study no serious adverse events were reported. No abnormal ECG findings or symptomatic heart disease occurred and the troponin T levels in all samples of a subset of the study population were below the detection limit. Due to the timing of the ECG and measurement of the troponin level on day 30 day instead of the more appropriate time from day 7 to14 post vaccination occurrence of asymptomatic myopericarditis can not be excluded. In general the frequency of adverse events was low (13.9%) with a significantly higher frequency of AEs in primary vaccinees than revaccinees. The most frequently reported AEs were swelling of axillary lymph nodes and low grade fever.

In a phase I/II clinical trial performed in the US the safety and immunogenicity of LC16m8 was compared with Dryvax in healthy vaccinia-naïve subjects 18 to 34 years of age (Kennedy et al 2011). The primary endpoints were the neutralizing antibody titers to intracellular mature virus (IMV) measured by PRNT 30 days after vaccination and the rates of AEs attributed to the vaccines. Exploratory endpoints included vaccine take, lesions at the vaccination site, neutralizing antibody titers against EEV and variola virus, cell-mediated immune (CMI) responses, viral persistence and viremia after vaccination. Of the 154 subjects randomized into one of the two vaccination groups 147 subjects (27 Dryvax, 120 LC16m8) had undetectable antibody titers prior immunization and completed the follow-up at day 30. All 125 participants vaccinated with LC16m8 developed a primary lesion at the vaccination site between 6 and 12 days after scarification. The take rate was significantly lower in the Dryvax group (24/28; 86%). The four subjects who did not develop a take most likely received subpotent vaccine due to handling issues. Lesions at the vaccination site as well as the extent of erythema and swelling were on average greater within the Dryvax group than in the LC16m8 group. Viral shedding was investigated in a subset of 27 vaccinees (4 Dryvax, 23 LC16m8). In 2 of the 4 Dryvax recipients viral shedding was observed on day 3 and by day 7 all subjects had detectable virus. In all LC16m8 participants evaluated virus was found

starting from day 7. PCR analyses of blood samples drawn at different time points (Days 0, 3, 7, 13, 22) confirmed absence of viremia at all time points and in all subjects.

All subjects with a vaccine take seroconverted as measured by PRNT assay using Dryvax vaccine strain. At day 30 post vaccination significantly higher geometric mean PRNT titers were elicited in Dryvax recipients than in LC16m8 vaccinees (919 vs 279). Additional immunogenicity analyses were performed on samples from a subset of LC16m8 and Dryvax vaccinees. Data on the kinetics of the neutralizing antibody response demonstrate a comparable timely course but significantly higher titers at day 30 and 60 in the Dryvax group. Comparison of different PRNT assays using various virus strains revealed that Dryvax vaccinees exhibited significantly higher GMT than LC16m8 recipients when Dryvax, Japan-NYCBH or monkeypox strains were used as test virus. On the other hand higher GMTs were observed in LC16m8 vaccinees than in Dryvax vaccinees when the virus used in the assay was LC16m8 or the parental Lister strain. Determination of anti-EEV PRNT titers were hampered by low antibody titers and it was only meaningful when the results were based on a 30% instead of a 50% reduction. Significantly higher anti-EEV GMTs were found in the Dryvax group compared to the LC16m8 group (24 vs 4). Finally, serum samples of 9 Dryvax and 11 LC16m8 recipients were assessed in an anti-variola PRNT conducted at CDC. Significantly higher GMTs were found in Dryvax group than in the LC16m8 group (274 vs 75). T cell responses were assessed by IFN-y ELISPOT and lymphoproliferation. LC16m8 produced somewhat higher lymphoproliferation but lower IFN y ELISPOT responses than Dryvax.

The safety assessment revealed no clinically significant differences in the reactogenicity and safety profile of Dryvax and LC16m8 with the exception that the vaccine site lesion was significantly smaller, less red, and less swollen in LC16m8 vaccinees compared to Dryvax recipients. No serious adverse events related to vaccination were reported. There were no cases of generalized vaccinia, progressive vaccinia, eczema vaccinatum, or myo-and pericarditis.

Regulatory Status of LC16m8

The Chiba Serum Institute was issued an unconditional license in 1975 in Japan. KAKETSUKEN was licensed to produce LC16m8 in 2003. LC16m8 has an approved 4-years shelf-life following storage at -20°C and a 2-years shelf-life after storage at 2-8°C. The vaccine is stockpiled in Japan.

Third generation vaccines based on the replication deficient MVA strain

Towards the end of the eradication campaign Anton Mayr and his group developed further attenuated vaccinia virus vaccines against smallpox by continually passaging the chorioallantois vaccinia Ankara (CVA) vaccine virus strain on chicken embryo fibroblast cells for more than 570 times. The resulting virus strain, called Modified Vaccinia Ankara (MVA), was found to have multiple mutations and six deletions accounting for a loss of approximately 30 kb of the CVA genome (Mayr et al 1978). MVA has been extensively tested and it was found that it has a limited ability to replicate and a low neuropathogenicity in humans and other mammals. It was also found that by a block in virus morphogenesis non-infectious immature virions and abnormal particles are produced but no infectious IMV and EEV particles. Despite these genomic modifications, MVA has retained stable immunogenic properties. MVA is a strong inducer of type I interferon (IFN) in human cells and it expresses a soluble interleukin-1 receptor, which has been implicated as an anti-virulence factor for certain poxviruses. In contrast to VV, MVA does not express soluble receptors for IFN-γ, IFN-α/β, tumour necrosis factor (TNF) and CC chemokines (Blanchard et al.1998). The virus is described as eliciting a protective immune response against any of the orthopox viruses.

At the end of the eradication campaign MVA was evaluated in clinical trials in 7098 subjects including 5691 children in a two-step vaccination regimen as priming dose prior to regular smallpox immunisation with Lister-Elstree vaccines (Stickl et al. 1974). MVA was administered mostly intradermally or subcutaneously. Each vial contained 2 x 10^6 freeze-dried infectious units (IU) and was used for two vaccinations after resolution in 0.5 ml saline. Most recipients were vaccinia-naïve children and adults considered to be at risk for adverse reactions to smallpox vaccines. A single dose of MVA elicited a weak haemagglutination inhibiting or virus neutralising antibody response. Following the subsequent vaccinia virus smallpox vaccine dose there was a marked immune response, which was interpreted as MVA priming of specific humoral and cellular immune responses. Mild local reactions including reddening and infiltration were observed at the site of injection (0.2 ml intradermal) in ~75% of 5308 individuals but there were no blisters, pustules or ulceration. Among 7098 subjects fever > 38°C occurred in 2.28% and non-specific general symptoms in 4.11%. There were no SAEs. Pre-vaccination with MVA resulted in a reduced number of side effects following a subsequent dose of vaccinia virus smallpox vaccine and the development of smaller pocks. The vaccine was licensed 1977 in Germany and was administered to over 120,000 subjects however at that time smallpox was no longer endemic in Germany. The MVA dose was well tolerated and the reactogenicity of the second traditional smallpox vaccine dose was reduced. In the majority of subjects the subsequent smallpox vaccination resulted in a revaccination reaction.

Based on these historical experiences several groups developed MVA vaccines or MVA based vectors for the development of new vaccines against other indications. In this review primarily data on Imvanex (Imvamune) manufactured by Bavarian Nordic (BN) is considered since this vaccine recently received a marketing authorization in Europe (ref to www.ema.europa.eu).

Other MVA based vaccines currently under development are ACAM3000 manufactured by Acambis/Sanofi Pasteur and TBC-MVA vaccine manufactured by Therion Biologics Cooperation.

Imvanex (Imvamune or MVA-BN, reference is made to the EPAR published by EMA)

BN has developed a third generation MVA vaccine, which is anticipated to represent a safer alternative to traditional first (manufactured on animal skin) and second generation vaccines (manufactured in cell culture) particularly in people with immune deficiencies and skin disorders. Imvanex (also designated Imvamune or MVA-BN) is derived from MVA-584 (i.e. 584th passage in CEF cells) and differs from all other MVA strains in that it has undergone six rounds of plaque purification resulting in a single clone (Suter et al 2009). The growth characteristics in different mammalian cell lines and the pathogenicity in immune-deficient mice demonstrated that the monoclonal MVA-BN strain is significantly different than other MVA strains. In contrast to polyclonal MVA strains, MVA-BN was incapable to replicate in different human cell lines tested. Moreover, experiments with immune deficient AGR129 mice demonstrate that after intraperitoneal inoculation of 1x10^7 TCID50 each of the various MVA strains evaluated, all animals having received MVA-BN survived the 100-day observation period while mice infected with other MVA strains died within 37 days (MVA-I721) or 94 days (MVA-572). Further experiments confirmed the safety profile of MVA-BN. Immune deficient AGR129 mice inoculated with MVA-BN appeared to be healthy over a 180 day time period and at no time point tested MVA could be isolated from the ovaries of these animals (Suter et al 2009).

MVA-BN has been developed as smallpox vaccines following a standalone regimen rather than in a sequential regimen of MVA followed by a replication competent smallpox vaccine. The vaccine was initially developed as freeze-dried formulation, but was reformulated and is now licensed as liquid formulation (refer to Imvanex EPAR published by EMA).

Animal data

Protective efficacy and immunogenicity of Imvanex were evaluated in animal studies using established animal models (ECTV/mouse, RPXV and MPXV models) and compared to first and second generation vaccines (EPAR 2013, Samuelsson et al 2008, Garza et al 2009, Stittelaar et al 2005, Hatch et al 2013)

Initial studies in mice with the challenge virus WR given by the intranasal route demonstrated a dose-response for morbidity and mortality (EPAR 2013). A dose equivalent to 50-fold the median lethal dose was taken further in testing the protective efficacy of Imvanex. Mice previously vaccinated with Imvanex survived this lethal intranasal dose after challenge. In characterising the dose-response for this protective effect a dose of $1x10^8$ TCID50 was found to be optimal. Lower doses protected mice from death but there was greater morbidity than with $1x10^8$ TCID50. In comparative testing of Imvanex with Dryvax and ACAM2000, 100% seroconversion rates were achieved by day 14 in mice given Imvanex but it took to days 26 to 40 for similar seroconversion rates with Dryvax and ACAM2000. Mice vaccinated with Imvanex were able to survive a lethal challenge with ECTV, whereas unvaccinated mice did not and only mice that seroconverted after Dryvax immunisation survived. All mice given Imvanex did seroconvert; however, only 79% of mice given Dryvax seroconverted. Lung titres of ECTV were reduced to 0 in mice vaccinated with Imvanex but 2 of 10 mice given Dryvax failed to completely clear the virus, although titres were much lower than in unvaccinated mice.

Samuelsson et al demonstrated that MVA-BN is capable to activate dendritic cells by TLR9-dependent and independent pathways and protect mice from lethal infection with ECTV. In wild type mice protection from death was achieved when $1x10^8$ TCID50 of MVA-BN was administered by subcutaneous injection at the same time as a lethal dose of ECTV ($1x10^5$ TCID50) given intranasally.

Garza et al evaluated the protective efficacy of Imvamune against aerosolized RPXV in a rabbit model. Rabbits were immunised with either PBS buffer, a single dose of Dryvax, a single low dose of Imvamune ($1x10^7$/dose), a single high dose of Imvamune ($1x10^8$/dose) or received two doses of a high dose of Imvamune at a 2 week interval. Four weeks after the last vaccination all animals were challenged with a lethal dose of aerosolized RPXV (~500 LD50). All animals in the control group succumbed to the disease, whereas all of the animals that received Dryvax or any of the different doses of Imvamune survived the challenge. The rabbits vaccinated with Dryvax, or a single low or high dose of Imvamune showed minimal to moderate clinical signs of the disease. The only clinical sign displayed by rabbits that had been vaccinated with two doses of Imvamune was mild transient anorexia in just two out of eight rabbits.

Protection against lethal challenges with MPXV given by the intravenous routes was investigated in macaques (EPAR, EMA). With the intravenous challenge, two subcutaneous doses of Imvanex given 28 days apart at $1x10^8$ TCID50 were shown to induce protection from death when monkeys were subsequently exposed to $5x10^7$ PFU/ml MPXV. A control groups having received ACAM2000 was also shown to be effectively protected. Results from two additional MPXV challenge studies investigating dose-response in a dose range of $1x10^2$ and $1x10^3$ to $1x10^7$ TCID50 were performed. One study used intravenous challenge and one used inhalational challenge methods. Immunogenicity was assessed using ELISA and PRNT. In the ELISA the MVA-derived antigen and in the PRNT assay vaccinia virus strain WR was used as test antigen. Statistically significant correlations were found between dose of Imvanex and probability of survival, and between dose of Imvanex and each of PRNT and ELISA titres. However, it was evident that vaccinated monkeys that developed PRNT and ELISA titres at levels comparable to those who survived could still succumb to the MPXV challenge. Despite the statistically significant correlation, survival of an individual monkey could not be accurately predicted based on its PRNT or ELISA response. In one study, there were two monkeys that did not survive despite seroconversion by day 42 and there were two monkeys who had not seroconverted by day 42 but survived. These results suggest that PRNT

and ELISA titres are not absolute correlates of protection and that other immune mechanisms, particularly cellular immunity, may contribute to protection.

Stittelaar et al investigated the protective efficacy of MVA-BN (Imvanex) in a lethal respiratory MPXV challenge model. MVA-BN was either administered subcutaneously in a 2-dose regimen (10^8 TCID50/dose) at an interval of 4 weeks or administered as single priming dose (10^6 TCID50) followed by a standard dose of a traditional smallpox vaccine given 10 days later intracutaneously. Two traditional smallpox vaccines prepared from the Lister Elstree strain in cell culture (Elstree-BN and Elstree-RIVM) were included in the study and six macaques each received a standard dose by intracutaneous vaccination. A control group of six animals received a sham vaccination. All animals were challenged intratracheally 15 weeks after the last vaccination with either 10^6 PFU (sublethal) or 10^7 PFU (lethal) of MPXV (strain MSF#6). All smallpox vaccination regimens used in this study evoked protective efficacy against sublethal and lethal MPXV challenge by the respiratory route. Differences were found in the viral loads of throat swabs. The strongest reduction in viral loads was found in animals vaccinated with the prime-boost regimen of MVA-BN/Elstree-RIVM followed by Elstree-RIVM alone.

In a recent study the protective effect against disease of either a single or two doses of Imvamune (Imvanex), and of a single dose of ACAM2000 was evaluated in cynomolgus macaques following an aerosolised severe/lethal dose (2.6×10^5 PFU) of the central African strain Zaire 79 of MPXV virus (Hatch et al 2013). It was found that all animals in the Imvamune 2-dose group and the ACAM2000 were protected from death, while 67% (4/6) in the Imvamune 1-dose group survived the lethal challenge. All animals in the control group having received TBS buffer succumbed to infection between day 7 and 11 post-challenge. Radiographs taken at the time of severe clinical signs at day 9 indicate that all animals in the ACAM2000 group were found normal, whereas all animals in the control group had severe clinical signs. A wide spectrum of conditions was observed in the Imvamune 1-dose and 2-dose group ranging from normal to severe oedema. One animal in the 2-dose group had moderate to severe pulmonary oedema but recovered fully.
Assessment of MPXV load in blood and throat swabs revealed that there was incomplete suppression of challenge virus replication in the blood and throat of monkeys vaccinated with one or two doses of Imvamune. In contrast, vaccination with ACAM2000 achieved complete viral suppression. The viral load detected in throat samples of animals in the Imvamune 1-dose group was comparable to the control group with 10^4-10^5 PFU live virus per ml. In the Imvamune 2-dose group live virus was detected in two out of 6 animals; one animal excreting MPXV at low levels (50 PFU/ml) and one animal at levels of 4.6×10^4 PFU/ml.
There was no significant difference ($p>0.05$) between the levels of neutralizing antibody in animals vaccinated with ACAM2000 (132 U/ml) and two doses of Imvamune (69 U/ml) 6 days prior to challenge with MPXV. However, significantly lower levels of neutralising antibody were detected in animals immunized with a single dose of Imvamune (13 U/ml).

Human data

Three clinical studies were performed, to assess the dose-response relation in healthy adults with or without prior history of smallpox vaccination, to evaluate the best route of administration and to define the optimal dosing of Imvanex with respect to immunogenicity and safety. In these studies an earlier freeze-dried formulation of Imvanex was employed. In summary 5 different dose levels ($1x10^6$, $1x10^7$, $2x10^7$, $5x10^7$, $1x10^8$ $TCID_{50}$) and two routes of administration (s.c. and i.m.) were investigated. The highest dose responses were found with the highest dose level of 10^8 $TCID_{50}$ regardless of the route of administration. A combined analysis of the GMT responses (ELISA and PRNT) and SCRs (ELISA and PRNT) 14 days post dose 2 in vaccinia-naïve subjects enrolled in the three dose finding studies indicate a linear dose response relationship based on serological data. Vaccinia-naive subjects having received the highest dose level subcutaneously achieved a SCR of 77% and a GMT of 29 based on the PRNT assay. Data from booster vaccinations of healthy vaccinia experienced subjects are only available from one study. Subjects with confirmed history of smallpox vaccination (last vaccination over 10 years ago) were given one dose of 10^8 $TCID_{50}$ subcutaneously. No other dose level or dosing regimens were evaluated. Based on the PRNT results 88.9% of these subjects seroconverted post booster and had a neutralising GMT of 41.3.

Of special interest is a study conducted in 2002, during a time when Dryvax was still licensed in the US and permitted for use in clinical trials (Frey et al 2007). This dose-finding study was sponsored by NIH and evaluated the immunogenicity of different vaccination regimens of Imvanex followed by administration of a single dose of traditional smallpox vaccine (Dryvax). In addition one group received Dryvax only. Post vaccination follow-up of clinical take rates revealed that clinical take rates were high in all vaccination groups (>90%) except for two groups having received either 2 doses of $5x10^7$ (53.8%) or 2 doses of $1x\ 10^8$ intramuscularly (66.7%). The clinical takes in subjects with prior Imvanex vaccinations were however attenuated as shown by the degree of the local lesions, the reduced healing times and viral titers in local lesions. In general the attenuated clinical takes appeared to be revaccination reactions as observed after revaccination with smallpox vaccines in vaccinia experienced subjects.

A subset of blood samples of this study was reanalysed by CDC in a PRNT using the variola strain strain Solaimen, a Bangladesh isolate (Damon et al., 2009, Hughes et al 2012). In total 106 sera from 53 of the 90 participants enrolled were retested. Blood samples were from subjects having received either the highest dose level of Imvanex of 10^8 TCID50 subcutaneously (26 subjects) or intramuscularly (15 subjects) or vaccinated with Dryvax (14 subjects). Paired sera collected prior to vaccine dose and at the times of 'peak' response, based on plaque PRNT data against either MVA or Dryvax, were evaluated by a variola (VAR) PRNT. Individuals' sera in the Dryvax arm were evaluated 28-30 days post-vaccination; individuals vaccinated with MVA were evaluated 14 days after the second s.c. or i.m. dose. When the individuals at peak times post-vaccination are scored for the ability to demonstrate a 60 or 90 % VAR PRNT titre at various serum dilutions, the ability to neutralize variola virus elicited by the MVA regimens is as robust as that elicited by Dryvax. A comparison of neutralisation assays using different test viruses (Dryvax, MVA or Variola) showed significantly different 90% neuralisation titers. It was found that using Dryvax as the neutralisation antigen results in significantly lower 90% PRNT titers than using variola. In contrast using MVA as test antigen resulted in significantly higher 90% PRNT titers than using variola as test antigen.

Different vaccination schedules as regards timing of the second dose were evaluated in another study (EPAR, EMA). The data clearly indicate that an accelerated vaccination scheme (2 doses at day 0 and 7) is unfavourable compared to the regular scheme of 2 doses given 4 weeks apart as the latter resulted in higher antibody titers and response rates.

Based on the results of the dose finding and regimen finding studies the standard regimen of 2 doses of Imvanex given 4 weeks apart was evaluated in vaccinia naïve healthy subjects, subjects with history of AD and HIV infected patients. In vaccinia experienced subjects one and two-dose vaccination regimens

were assessed. Moreover antibody persistence was studied over a two year period in a subset of initially vaccinia naïve subjects and in subjects with known history of smallpox vaccination. A subset of initially vaccinia naïve subjects received a booster vaccination given two years after primary vaccination and was followed for 4 weeks.

Immunogenicity and safety of Imvanex was evaluated in five main studies and several supportive studies. The studies were conducted in Europe, USA, Mexico and Puerto Rico. The study population was 18 years of age and older and male and female subjects were enrolled.

The neutralising antibody response was evaluated by plaque reduction neutralisation (PRNT) assays using the Vaccinia virus strain Western Reserve. An overview on the seroconversion rates (SCR) achieved in vaccinia-naïve and vaccinia experienced individuals, who received 2 doses of Imvanex 4 weeks apart is given below (ref to EPAR, EMA).

Seroconversion rates observed in vaccinia-naïve subjects as measured by PRNT

SCR - PRNT			**at Day 7 or 14**	**Day 28**	**Day 42**
Study	**Health status**	**n**	**SCR % (95%-CI)**	**SCR % (95%-CI)**	**SCR % (95%-CI)**
POX-MVA-005	Healthy	183	45.1 (37.7; 52.6)	56.7 49.1; 64.0)	89.2 (83.7; 93.4)
POX-MVA-008	Healthy	194	5.4 (2.6; 9.8)	24.5 (18.6; 31.2)	86.6 (81.0; 91.1)
	AD	257	5.6 (3.1; 9.3)	26.8 (21.4; 32.7)	90.3 (86.0; 93.6)
POX-MVA-009	Healthy	66	12.1 (5.4; 22.5)	10.6 (4.4; 20.6)	82.5 (70.9; 90.9)
POX-MVA-011	Healthy	88	11.1 (5.2; 20.0)	20.9 (12.9; 31.0)	77.2 (66.4; 85.9)
	HIV	351	15.7 (11.9; 20.1)	22.5 (18.1; 27.4)	60.3 (54.7; 65.8)

Seroconversion rates observed in vaccinia-experienced subjects as measured by PRNT

SCR - PRNT			**At Day 7 or 14**	**Day 28**	**Day 42**
Study	**Health status**	**n**	**SCR % (95%-CI)**	**SCR % (95%-CI)**	**SCR % (95%-CI)**
POX-MVA-005	Healthy	200	78.5 (72.2; 84.0)	69.8 (63.0; 76.1)	NA
POX-MVA-024	Healthy	61	73.8 (60.9; 84.2)	71.2 (57.9; 82.2)	NA
POX-MVA-011	Healthy	9	75.0 (34.9; 96.8)	62.5 (24.5; 91.5)	85.7 (42.1; 99.6)
	HIV	131	46.0 (37.0; 55.1)	59.7 (50.5; 68.4)	75.6 (67.0; 82.9)

The analyses of peak PRNT responses across the pivotal studies POX-MVA-005, POX-MVA-008 and POX-MVA-011 indicated that in vaccinia naïve healthy subjects SCRs of 89.2%, 86.6% and 77.2% were determined using the standard vaccine regimen of 2 doses given 4 weeks apart.

Following 1 or 2 doses of Imvanex mean neutralising antibody titers of 6 and 45, respectively, were measured in healthy vaccinia naïve subjects, whereas a single dose of Imvanex elicited mean neutralising antibody responses of 192 in vaccinia experienced individuals 2 weeks after the booster immunisation. Analyses of antibody persistence revealed a fast decline in antibody titers in vaccinia naïve subjects following vaccination with one (MVA/Placebo group: GMT of 2) or two doses (MVA/MVA group; GMT of 7) of Imvanex. PRNT GMTs in both groups were lower two years after primary vaccination with Imvanex than baseline GMTs of vaccinia experienced subjects (GMT: 24), who had their last smallpox vaccination over 10 years ago. Moreover neutralising antibody titers observed 2 years post booster vaccination of vaccinia experienced subjects were significantly higher than in vaccinia naïve subjects having received either one or two doses of Imvanex. These findings suggest that the antibody response following traditional smallpox vaccination is more robust than that induced by Imvanex. Booster vaccination two years after primary vaccination of vaccinia naïve subjects with Imvanex however evoked a memory response in all subjects regardless whether they were primed with one or two doses. Based on the PRNT 98.7% of subjects seroconverted within 2-4 weeks following the booster vaccination. Following the booster neutralising GMTs of 166 and 117 were obtained for subjects primed with two or one dose of Imvanex, respectively. In vaccinia experienced healthy subjects 77.6% to 78.5% seroconverted after a single booster vaccination and 85.7% to 90.0% after two booster vaccinations.

In vaccinia naïve subjects with history of AD SCRs of 90.3% were observed after a standard 2 dose vaccination regimen suggesting sufficiently high titers especially as this group of patients are at high risk in developing serious adverse event following traditional smallpox vaccination.

Vaccinia naïve HIV infected subjects reached SCR of 60.3% post dose 2 and vaccinia experienced HIV infected subjects had SCRs of 75.6% post dose 2. In some subgroups even lower response rates were observed. No information is available whether these patients would benefit from additional vaccine doses and it remains unclear whether mitigation of smallpox disease is to be expected by vaccination with MVA alone.

The currently available data on the cell mediated immunity are variable and inconclusive.

Safety data from 14 clinical trials with a total 1547 vaccinia naïve and 534 subjects previously vaccinated either with smallpox vaccines or Imvanex were evaluated. The most frequently reported adverse reactions were injection site reactions and mild to moderate systemic symptoms such as headache, myalgia, nausea. These reactions typically resolved within one week. In general, occurrence and frequency of adverse events were comparable in primary vaccinees and revaccinees. In subjects with atopic dermatitis erythema and swelling at the injection site as well as headache, myalgia, nausea and fatique was more frequently observed than in healthy subjects. Worsening of atopic dermatitis was observed in 7% of subjects with atopic dermatitis.

Four serious adverse events possibly linked to vaccination were described. One healthy, vaccinia naïve subject experienced grade 3 extraocular muscle paresis after the second vaccination and one HIV patient with low CD4 counts experienced grade 3 pneumonia during the main study period. In the follow-up period 2 further SAEs were reported that were considered possibly related. One case of grade 2 sarcoidosis was observed in a vaccinia naïve healthy subject and one case of a cardiomyopathy was recorded in a HIV patient.

Inadvertant vaccination of pregnant women occurred in 13 cases, with no untoward findings.

No case of known postvaccinial complication was notified in human clinical trials. Moreover there was no case of myopericarditis, however, elevated troponin T levels were observed in some vaccinees and less than 25% of all subjects with abnormal troponin T levels or ECG findings were consistently investigated.

Regulatory Status of Imvanex (Imvamun)

Imvanex received a marketing authorization by the European Commission in July 2013. The European Public Assessment report is available and published by EMA. Results of some of the clinical studies performed to evaluate the immunogenicity and safety of Imvanex were published by Vollmar et al 2006, Frey et al 2007, von Krempelhuber et al 2010 and Greenberg et al 2013.

Data from studies with other MVA smallpox vaccines

Data from a recently published study evaluating MVA as an alternative to Dryvax in vaccinia-naïve and vaccinia-expierenced adult subjects show that intramuscular administration of two doses of 10^6 PFU of MVA three months prior to Dryvax challenge reduced the severity of lesion formation and a decreased magnitude and duration of viral shedding (Parrino et al 2007). Moreover an increased post-Dryvax vaccine specific CD8 T-cell response and augmented antibody response against EEV-specific A33R and B5R surface proteins were evoked in vaccinia naïve subjects. Pre-challenge however, lower neutralising antibody responses were elicited in the MVA vaccine groups compared to the Dryvax only vaccine group. Similar results were published by Seaman et al. 2010, who evaluated the effect of ACAM3000 given by different routes of administration and challenged with Dryvax. Interestingly the intradermal route was found to induce a immunological response comparable to those of a 10-fold-higher dose given subcutaneously.

Other smallpox vaccines

Inactivated vaccines

The inactivated vaccine Ospavir is based on the L-IVP strain (Lister derivative) and is produced on chorioallantois membrane of embryonated chicken eggs. Virus suspensions containing 1-5x10^9 PFU/mL are inactivated by gamma-irradiation and formulated with stabilisers prior to freeze-drying (Perekrest et al 2013). The vaccine was tested in a field trial in 1977 (Marennikova and Macevic 1978). A two-step procedure was evaluated with Ospavir administered intramuscularly as priming dose followed 1 to 7 days later by administration of a booster dose comprising of traditional live smallpox vaccine containing the L-IVP strain. One case of postvaccinal encephalitis occurred in a child with congenital macrocephaly among some 23,000 vaccinated subjects all of whom were over 3 years of age. It is unclear whether protective efficacy can be achieved by the two-step vaccination regimen, but the prime-boost vaccination regimen elicits a strong immune response (Prof George Ignatyev, personal communication). Further published data suggest that primary vaccination of children, adolescents and adults with the inactivated vaccine resulted in low to moderate reactogenicity and that both doses had comparable reactogenicity profiles (Gavrilova et al 2009).

Regulatory status

The inactivated vaccine Ospavir (priming dose) and the traditional freeze-dried smallpox vaccine (booster dose) are made from the strain LIVP on the skin of calves. Both are licensed in Russia by Microgen (personal communication Prof George Ignatyev, personal communication).

Oral smallpox vaccines

At the end of the eradication phase an oral form of live smallpox vaccine (TEOVac) has been developed in Russia. The vaccine contains vaccinia virus strain B-51 strain produced on the chorioallantois membranes of embryonated hens' eggs (Onishchenko et al. 2006). The B-51 strain was found to be of moderate pathogenicty in rabbits (Marennikova 1975) and the oral vaccine was used in a smallpox outbreak setting in Ethiopia in 1972-1973 (reviewed in Voroyev et al 2003). Of the 329 subjects vaccinated and with known contact to smallpox patients 70.2% (231 subjects) were primary vaccinees. Smallpox disease occurred in 1.7% of subjects vaccinated orally. Two children with confirmed vaccination status and 2 adults with unclear vaccination status developed smallpox disease within 1 to 9 days of vaccination. In a control group of 623 subjects with known contact to smallpox vaccine 133 were found to be vaccinia naïve. Smallpox disease was observed in 7.9%-9.1% of subjects having received smallpox vaccine by scarification 5 days after contact with smallpox patients. These historical data suggest that oral vaccination with TEOVac is protective against smallpox disease.

TEOVac was evaluated in a clinical trial in 6,000 adult subjects (Vorobyev et al 2003). The majority of subjects receiving TEOVac ($0.8\text{-}2x10^7$ PFU/tablet) were revaccinees having previously received oral smallpox vaccine (N=5047). Of the subjects enrolled 231 were vaccinia naïve subjects and received TEOVac for primary vaccination. Neutralising antibody titers of ≥1:25 were measured in 63% and 48% of revaccinees having received the vaccine 1 year or 12 years earlier by oral administration. All vaccinia-naïve subjects evaluated developed neutralizing antibodies titers of ≥1:25, respectively. No serious adverse events were reported in the group of revaccinees and no adverse events related to skin, neurological or allergic reactions were observed in revaccinees and primary vaccine recipients. Compared to traditional smallpox vaccine given by standard skin scarification (sc) TEOVac was found to be less reactogenic in revaccinees. Mild adverse events were notified in 5.2% of TEOVac participants and in 34.7% of subjects vaccinated by scarification. Moderate to severe general reactions were found in 0.2% of the orally vaccinated compared with 1.7% in the sc group. Local reactions were reported in 0.5% in the oral group and in 100% of the sc group (Vorobyev et al 2003, Onishchenko et al 2006).

Adverse events reported in the literature include fever, gingival edema, faucial hyperemia, enlargement of subaxillary lymph nodes, (ulceronecrotic) tonsillitis, lymphadenopathy asthenia and systemic postvaccinal reactions of medium severity. The main cause of local inflammatory reactions was found to be opportunistic superinfections (Melynikov et al 2005). Frequency of specific adverse events is unclear.

Regulatory status

TEOVac is licensed in Russia (Onishchenko et al 2006).

Ethical considerations

Recommendations for smallpox vaccination are based on benefit – risk assessments. Usually such assessments do not only consider the specific characteristics of the vaccine but include considerations on the vulnerability of the population as well as the probability of a smallpox attack and on the pathogenicity and virulence of the orthopox virus strain deliberately released.

In principle two scenarios must be assessed separately: the pre-event and the post-event scenario.

Due to the absence of smallpox disease and the known risks of severe adverse events with first and second generation vaccines a significant part of today's population has contraindications not allowing the general use of first and second generation smallpox vaccines in a pre-event scenario. Currently strict

restrictions on the use of smallpox vaccines are applied for vaccination of military personnel, first responders and laboratory workers with risk of exposure to orthopox viruses. With the availability of new and safer smallpox vaccines the decision to recommend these third generation vaccines will depend on the probability of a smallpox attack and the likelihood of their effectiveness to protect from exposure to smallpox or other orthopox viruses.

In the event of deliberate release recommendation of universal smallpox vaccination will depend on several factors, including attack rates, pathogenicity and virulence of the orthopox virus released, the effectiveness of universal immunization compared with ring vaccination, the expected harm of vaccination and the effectiveness of the vaccines used.
Recent publications estimated that use of first and most likely also second generation smallpox vaccines would result in high rate of complications and death, if a vaccine is used in a mass vaccination campaign today (Kretzschmar et al 2006). It was assumed that everybody over 30 years were vaccinated previously and that 20% of the population have contraindications. Under these assumptions a mass vaccination conducted in Germany with a population size of 82 million would lead to 46.2 deaths with the NYCBH strain and mass vaccination with the Lister strain would lead to 268.5 deaths. These death rates likely underestimate the actual numbers to be expected and make them most likely unacceptable for universal vaccination campaigns especially, if the virus strain deliberately released has low pathogenicity and/or low virulence. In contrast, due to missing information on the effectiveness of third generation vaccines their use in a post-exposure setting might not prevent smallpox disease or further virus spreading from person-to-person and therefore would make them most likely unacceptable for ring vaccinations except for high risk groups (e.g. AD and HIV patients).

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