



Published in final edited form as:

Expert Rev Vaccines. 2016 September ; 15(9): 1197–1211. doi:10.1080/14760584.2016.1175305.

Defending Against Smallpox: a Focus on Vaccines

Emily A. Voigt¹, Richard B. Kennedy¹, and Gregory A. Poland¹

¹Mayo Vaccine Research Group, Mayo Clinic, Rochester, MN 55905 USA

Abstract

Smallpox has shaped human history, from the earliest human civilizations well into the 20th century. With high mortality rates, rapid transmission, and serious long-term effects on survivors, smallpox was a much-feared disease. The eradication of smallpox represents an unprecedented medical victory for the lasting benefit of human health and prosperity. Concerns remain, however, about the development and use of the smallpox virus as a biological weapon, which necessitates the need for continued vaccine development. Smallpox vaccine development is thus a much-reviewed topic of high interest. This review focuses on the current state of smallpox vaccines and their context in biodefense efforts.

Keywords

smallpox; smallpox vaccine; bioterrorism; biological warfare; vaccinia virus

1. Introduction

Smallpox disease, the history of smallpox vaccination and eradication, and current concerns about smallpox are briefly reviewed and presented here. The authors refer readers to comprehensive works outside the scope of this review for more information on the history and eradication of this disease [1-8][9-13].

1.1 Smallpox disease

1.1.1 History—The origin of smallpox is lost to history, with signs of smallpox present for as long as human records are available. Phylogenetic and historical studies suggest that smallpox may have first appeared before 10,000 B.C., or as recently as the 16th century B.C., in regions with large human populations such as Mesopotamia, east Africa, or the Indus valley [14-16]. As smallpox was an acute disease with no non-human reservoir, it was passed around the world in a chain of human infection following human movement until human populations became large enough to support the disease endemically. Descriptions of a disease resembling smallpox have been found in texts from ancient China and India, and

Declaration of Interests: R Kennedy and G Poland have provided consultative advice on smallpox vaccine safety to the World Health Organization. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

mentions of smallpox-like epidemics are found throughout Asia; for example, references are made to the Hittites (1350 B.C.), Egyptians (1157 B.C.), Carthaginians and Athenians (5th and 4th centuries B.C.) and Chinese (250 B.C.) [9,13].

Smallpox eventually spread into Europe; the first reliable description of smallpox in western Europe was recorded by Gregory of Tours in 581 A.D. [9,13]. The movement of Europeans between Europe and West Asia during the Crusades spread smallpox throughout Europe, where it became endemic during the medieval period. European explorers and colonists then spread the disease more widely to areas of the world where the disease was not yet endemic, resulting in devastating epidemics throughout the Americas in the 16th century onwards, and throughout Africa, Australia, and southeast Asia by the 19th century [9].

1.1.2 Pathophysiology—Smallpox is caused by the variola virus (VARV), a large, enveloped double-stranded DNA virus in the Orthopoxvirus genus of the Poxviridae family [17]. Natural smallpox infection typically spreads through close personal contact with ill persons through inhalation of droplets containing infectious virus or, less frequently, by direct contact [18]. There are indications that contaminated clothing or bedding can also transmit infection, though the details of this mode of transmission are less clear [9]. The virus can also enter through the skin, although this is uncommon outside of deliberate inoculation. The mode of transmission appears to affect the severity of the disease [18].

After initial infection with VARV, smallpox multiplies in the respiratory tract, then migrates to regional lymph nodes. Primary viremia is asymptomatic, and occurs 3-4 days after infection, further disseminating the virus to spleen, bone marrow, and distal lymph nodes. Secondary viremia occurs 7-11 days after infection, followed by the onset of fever, headache, backache, and extreme malaise. Maculopapular lesions develop on the mouth, face, and arms, spreading quickly to the trunk and legs. These lesions quickly ulcerate, become vesicular, and then pustular – typically with all lesions simultaneously in the same stage of development. Patients are most infectious during the first week of the skin rash. After 8-9 days, pustules become crusted, and scarring is typical [9].

1.1.3 Mortality—Two major forms of smallpox exist: variola major, a severe illness; and variola minor, a much less frequently fatal disease (mortality < 1%) that exhibits similar initial symptoms to variola major, but less severe and extensive. About 5-10% of people with the variola major strain develop either a hemorrhagic or a malignant (flat) variant of the rash. The hemorrhagic form is almost uniformly fatal. The malignant form classically exhibits confluent, flat, nonpustular skin lesions, and is also largely fatal [9]. Overall mortality is typically 30%, but can vary widely between outbreaks. Death typically occurs during the second week of illness, and likely results from a massive inflammatory response, shock, and major organ failure [9].

1.2 A brief history of smallpox vaccination

1.2.1 Variolation—Variolation is the deliberate introduction of infectious smallpox virus from the pustule of an infected person into a healthy, nonimmune person to induce a typically milder form of disease than normal. The practice of variolation appears to have arisen independently in several areas of the world in response to smallpox breakouts. In

India, variolation became a part of certain Hindu rituals and is described in early Sanskrit texts written as early as 1500 B.C. [13]. In the 10th century, Chinese physicians were known to inoculate smallpox from powdered scabs intradermally or intranasally to bolster immunity [9].

The practice of variolation was brought to Europe from Asia in the 18th century and quickly spread throughout European societies and from there to the American continent and worldwide. Variolation was a great medical advance, but had many shortcomings. While improving on the 30% mortality rate associated with natural smallpox infection, variolation still carried a risk of developing severe smallpox disease and had a resulting 1-2% mortality rate. The live virus could also spread to contacts of the variolated individual, causing smallpox cases that further contributed to outbreaks.

1.2.2 Jenner and early vaccines—In 1796, Edward Jenner, an English country doctor, examined a dairymaid with a cowpox infection. Interested in folklore suggesting that people infected with cowpox could not later be infected with smallpox, Jenner inoculated his gardener's eight-year-old son subcutaneously in the arm with cowpox, resulting in mild cowpox symptoms, and then variolated the boy a month and a half later. The boy did not develop any signs of smallpox and proved resistant to smallpox in the future. In 1798, Jenner published his research, including follow-up experiments, confirming that cowpox protected against smallpox infection, without the substantial dangers of variolation [19-21].

Jenner was not the first to have deliberately inoculated people with cowpox to produce protection against smallpox. However, Jenner's publication of his work and championship of cowpox inoculation as a safer replacement for variolation succeeded in sparking a major change in the accepted medical practice [21]. Despite early problems, some 100,000 people were vaccinated worldwide by 1801 [21].

Early smallpox vaccines were largely propagated by inoculation of cowpox material from one human to another with a wide variety of transfer techniques; however, concurrent transfer of bloodborne diseases became evident and, by the latter half of the 19th century, this process was converted in most places to propagation of cowpox virus in animals [9]. Typically, this involved calf lymph collected after infection of calf skin, although sheep, water buffalo, or other available animals were also used [9]. Contamination of vaccines was common and not well documented. By 1900, the poxvirus strains used for vaccination were no longer of cowpox derivation, having been contaminated by vaccinia virus (VACV), an orthopoxvirus closely related to, and possibly derived from, horsepox [9,22,23].

VACV was the basis of remarkably effective smallpox vaccines and remains the virus of choice for smallpox vaccines today. The widespread use of smallpox vaccine caused smallpox infections and outbreaks to start disappearing from large portions of the human population. The last known case of smallpox in the U.S. was in 1949.

1.2.3 Eradication—In 1959, the World Health Organization (WHO) launched a massive campaign to globally eradicate smallpox. At the time, an estimated 15 million annual cases were still occurring worldwide. As part of this campaign, teams of health workers traveled

throughout the world vaccinating large populations. The development of a freeze-drying technique rendered the vaccine heat-stable and transportable to remote regions [24], and the bifurcated needle that allowed simple, reliable, and efficient intradermal delivery of vaccine and resulted in formation of an easily-monitored pock indicating vaccine take [25]. These were advances that allowed effective vaccination of large populations by minimally trained health workers. Additionally, the fact that no natural reservoir for VARV exists outside of human populations greatly aided eradication efforts. The vaccination strategy for the final stages of eradication was termed “ring vaccination,” where disease surveillance teams traced smallpox patients and vaccinated their immediate contacts to prevent further disease spread. This strategy was highly effective. The last natural case of smallpox was reported in 1977. In 1980, the WHO declared smallpox to be eradicated worldwide, the first eradication of a human pathogen.

1.2.4 Current concerns—Currently, the only known remaining stocks of VARV are in secure frozen storage in two locations – the U.S. Centers for Disease Control and Prevention in Atlanta, GA, and the State Research Centre of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk region, Russia. Destruction of these final stocks is a topic of much discussion in scientific and political circles [26].

With the rise in terrorism, concerns about the use of smallpox as a bioweapon have arisen. While the only registered stocks are in secure facilities, other sources of infectious VARV are possible, as was demonstrated by the recent discovery of misplaced VARV samples at NIH [27]. It is known that the Soviet government adapted smallpox for use as a bioweapon in the 1980s [28]. Little information is known about the results of this research, and what became of any viral stocks resulting from that effort.

Natural or synthetic sources of infectious smallpox virus are also possible. VARV has been shown to remain infectious for more than a decade in a temperate climate without special storage [29], raising the possibility that infectious virus could be found in old medical supplies or human remains from the pre-eradication era [30,31]. With appropriate advances in biotechnology, VARV may also be deliberately synthesized in a laboratory setting; the sequence of VARV has been publicly available since the early 1990s [32,33].

Outbreaks of related emerging and zoonotic poxviruses in human populations are also a public health concern [22]. Standard smallpox vaccines have been shown to induce strong cross-protection against other poxviruses, and outbreaks of zoonotic and emerging poxviruses represent an increasing threat, as immunity in human populations decreases due to the cessation of smallpox vaccination [34]. Human monkeypox outbreaks have occurred in Africa throughout the last 25 years, with case-fatality rates of 1.5-10% [34-40]. Monkeypox was transferred to the U.S. through importation of small animals, causing a multi-state outbreak in 2003 [34,35,41]. In the U.S. outbreak, prior vaccination was not found to be protective against monkeypox disease [42]. Some studies examining the protective efficacy of smallpox vaccination against human monkeypox have not found any evidence of a protective effect, while others have determined that smallpox vaccination does confer protection [42-45]. These reports examined individuals immunized at least 25 years before monkeypox exposure. Whether or not more recent vaccination, or even post-exposure

vaccination, is protective against MPXV is still an unanswered question. Similarly, cowpox, buffalopox, camelpox, and other orthopoxviruses have been known to overcome the species barrier and infect humans [35,46,47]. Vaccinia virus outbreaks in South American cattle and humans appear to be a result of emerging disease rather than escaped vaccine strains [35,48]. Recent reports of humans infected with a novel poxvirus in the U.S. underscore the potential of poxviruses as emerging human pathogens [49]. Safe and effective vaccines without significant safety contraindications designed to protect against smallpox would be highly useful in containing such outbreaks.

1.3 First-generation live vaccines used for eradication

During the WHO eradication program, vaccinia-based smallpox vaccines were manufactured worldwide by 71 independent manufacturers that used different production methods and viral strains [9]. Vaccines in the U.S. largely used the New York City Board of Health (NYCBH) strain, which was developed from seed virus obtained from England in the 1850s. These vaccines have proven protective efficacy, but are no longer in production and are being replaced by second- and third-generation products. First-generation vaccines are not likely to play a major role in future biodefense activities, but are the gold standard for smallpox vaccines and are briefly discussed here for comparison purposes. For additional information, readers are referred to more comprehensive sources [9,10,17,50].

1.3.1. Dryvax—Dryvax, a vaccine produced by Wyeth Laboratories, is based on the NYCBH VACV strain and was first licensed in 1931 [9]. Produced by infection of the skin of calves, Dryvax was successfully used in the U.S. during the eradication era. However, rare but serious adverse events (AEs) were linked to large-sale immunization with this vaccine, resulting in 1-2 deaths per million vaccinees. Dryvax was used in the early 2000s to vaccinate large numbers of military personnel and select civilians with a high level of screening for contraindications to avoid inoculating those at highest risk of adverse reactions. During the U.S. Department of Defense (DoD) vaccination campaigns, there were fewer serious AEs than anticipated based on historical data, likely due to more rigorous screening and exclusion criteria; however, new findings of cardiac complications became a cause for concern [51-53]. Due to these findings and the development of more modern cell culture-based production methods, the FDA license for Dryvax was revoked as of February 29, 2008.

1.3.2. Elstree (Lister)—The Elstree vaccine, based upon the Lister strain of VACV, was used extensively throughout Europe, Africa, and Asia during the WHO eradication campaign. It stimulated excellent protective immune responses, including long-term neutralizing antibodies to poxviruses [54,55]. Large-scale retrospective studies showed the Lister/Elstree vaccine strain induced a higher rate of serious adverse reactions than NYCBH-based vaccines (approximately 8.4 deaths per million vaccinees relative to 1.4), but this number was significantly lower than various other VACV strains also in use at the time [9]. Lister vaccines, which have been stockpiled in many countries worldwide as a biodefense countermeasure, have been demonstrated to retain immunogenicity even if diluted 1:10 from the stored stock concentrations [55].

1.3.4 Aventis Pasteur (APSV)—APSV, produced by Aventis Pasteur from 1956 to 1957, was a frozen vaccine preparation based on the NYCBH VACV strain, and some 85 million doses were stockpiled by the DoD. The APSV vaccine effectively induces neutralizing antibody levels and produces high take rates similar to Dryvax, even at a 1:10 dilution of frozen stock preparations. Later trials in the 2000s suggested that APSV has a higher reactogenicity (larger lesion size, higher incidence of fever) than Dryvax, and thus stockpiles APSV is unlikely to be used in future non-emergency vaccination efforts [8,56].

1.3.5 EM-63—The EM-63 vaccine was used widely in the USSR in the 1960s and 1970s, and appears to have originated from the NYCBH VACV strain. Large-scale use of the EM-63 vaccine resulted in low rates of serious AEs (17 per million doses) similar to Dryvax and similarly effective protection against smallpox disease [9]. Depending on remaining stockpiles, this vaccine may be utilized in biodefense preparations or in response to a smallpox outbreak.

1.3.6 Lancy-Vaxina—The Lancy-Vaxina Berna vaccine was based upon the Lister VACV strain and was manufactured on the skin of sheep. Lyophilized vaccine has been stockpiled by several European countries against the threat of bioterrorism. Efficacy, safety, and immunogenicity were tested in the early 2000s and found to be similar to the original Lister/Elstree vaccine. Vaccine stored since the 1970s has been found to be still viable and retains immunogenicity at dilutions up to 1:10 [57].

1.3.7 Temple of Heaven/Tiantan—The Tiantan vaccine, from the Temple of Heaven strain, was developed and used heavily in China during the eradication era. It was originally derived from a smallpox patient, passaged several times in animals, and during this process was contaminated with the VACV it contains today [9]. This vaccine induces antibody responses similar to the Lister or NYCBH strains, but has a very high rate of AEs relative to Lister and NYCBH-derived vaccines, and is more pathogenic in animals than these other vaccines.

1.4 Immune Responses to First-Generation Vaccines

1.4.1 Cutaneous Responses—After successful primary vaccination, a pock forms at the vaccine site 3-4 days after vaccination; this pock forms a vesicle two to three days later, then pustulates. The pock reaches maximum size 8-12 days after vaccination, scabs over, and separates 14-21 days post-vaccination [9]. A lack of viremia after vaccination has been reported, suggesting infection remains localized to the vaccination site [58]. Vaccination also elicits strong immune responses that peak several weeks after immunization. A high-level overview of these responses are provide here. For more detailed descriptions of humoral and cellular immunity following smallpox vaccination, the readers are referred to the following references: [6,59-65].

1.4.2 Cytokine responses—Cytokine responses to smallpox vaccines begin shortly after primary vaccination. A strong inflammatory response occurs, with elevated serum levels of IFN γ , TNF α , IL-1, and IL-6 [66-68]. IFN γ serum levels clearly peak 8-9 days after vaccination [66]. Other cytokines relevant to induction of cellular responses are also present

at elevated levels, including IFN-inducible protein 10 (IP-10), monokine induced by IFN γ (MIG), granulocyte colony-stimulating factor (G-CSF) and granulocytemacrophage colony-stimulating factor (GM-CSF) [66,67]. Levels of cytokine production have been shown to be affected by sex [69] and race [70].

1.4.3 Cellular responses—T cell responses are responsible for containing VACV infection in a naïve host after vaccination [71]. VACV-specific T cells are detected in humans by day 7 after primary vaccination, and peak at 14 days post-vaccination [72]. Strong CD4+ and CD8+ T cell responses peak and then contract to form a stable memory population [73]. Conberg *et al.* have demonstrated that memory CD8+ T cells alone play a major role in immunity to VACV [74], but CD4+ T cell memory populations have been shown to be better maintained over time [75] and have been shown in mice to be more critical than CD8+ T cell populations in fighting VACV infection [76]. The important role of T cell memory in containing initial vaccinia infection is also suggested by the fact that defects in subjects' cellular immunity allow for uncontrolled VACV infection [77]. VACV-specific T-cell responses slowly wane over a period of decades, but appear to retain the ability to respond for up to 50 years [63,78].

1.4.4 Humoral responses—IgM neutralizing antibodies appear as early as 4 days after primary vaccination [79]. IgG responses appear later, typically after 10 days post-vaccination [73,80]. For smallpox vaccination, IgG antibodies to poxvirus antigens are the immune measure most correlated with protection against smallpox disease. Four weeks after vaccination, VACV-specific IgG B cells expand to approximately 1.5% of the circulating IgG memory B cell compartment [64]. B cell responses are likely necessary for long-term protection regardless of the robustness of T cell responses, as demonstrated in mice [81]. Antibody responses initially decline in the first years after vaccination, followed by maintenance of long-term stable levels for 50 years or longer [63,64]. Full protection requires antibody responses to both of the major virions formed during infection: the intracellular mature virion (IMV) and extracellular virion (EEV) [82,83].

The rapid induction of cellular and humoral antibody by smallpox vaccines, and relatively long time required for disease development (secondary viremia and onset of full symptoms begin 7-11 days after infection), allow for successful post-exposure vaccination [9]. Primary vaccination within four to five days of exposure is typically at least partially protective; vaccination reduces the severity of disease if it develops at all. Revaccination in this timeframe typically prevents illness entirely, and attenuates disease to some degree even into the second week of the incubation period [84-87].

1.5 Adverse Events

VACV-based smallpox vaccines are associated with significant rates of AEs, resulting in a minimum of 1-2 deaths per million primary vaccines [88] and an overall higher chance of hospitalization across vaccinated populations in the year postvaccination [89]. Routine civilian smallpox vaccination has ceased due to these risks, risks that must be considered in any biodefense-related vaccination campaigns.

Common non-serious reactions to smallpox vaccines include the development of inflammation and/or satellite lesions around the site of vaccination, localized edema, headache, fever, myalgia, lymphadenopathy, and erythema [9,90,91]. Autoinoculation, or the inadvertent transfer of VACV from the vaccination site to secondary locations on the vaccinee or close contacts, is also a common complication of smallpox vaccination [90].

More serious AEs resulting from smallpox vaccines (see Table 1) include generalized vaccinia (GV), eczema vaccinatum (EV), progressive vaccinia (PV), postvaccinial central nervous system disease, and fetal vaccinia. Treatments for adverse reactions to smallpox include vaccinia immune globulin (VIG) as the first-line therapy, and/or the potential use of cidofovir, an antiviral with broad-spectrum effects against DNA viruses. These drugs are available through Investigational New Drug protocols from the U.S. Centers for Disease Control and Prevention and the Department of Defense [90].

1.5.1 Generalized vaccinia—Generalized vaccinia is a generalized eruption of skin lesions that typically appear approximately a week after vaccination. This condition is usually benign and self-limiting, except in some immunosuppressed individuals who may require VIG. The underlying causes of generalized vaccinia are not fully understood. In some cases, it appears to be the result of VACV viremia after vaccination, often associated with an immunodeficiency [92]. In others, generalized erythematous rashes may be a result of hypersensitivity reactions [51].

1.5.2 Vaccinia keratitis—Ocular vaccinia infections are typically a result of autoinoculation of the eye area after touching the area of vaccination. Ocular vaccinia poses a risk to eyesight, particularly with infection of the cornea (keratitis). Prevention is the focus; topical antivirals may be used to treat the cornea during VACV infections to reduce scarring [90,93,94].

1.5.3 Fetal vaccinia—Fetal vaccinia is a rare complication of pregnancy after smallpox vaccination. Transmission of VACV from the mother to the fetus has been reported in a small number of cases (<50), usually resulting in stillbirth or death of the infant [90]. Pregnancy is a standard counter indication for smallpox vaccine. During recent smallpox vaccination campaigns, women inadvertently vaccinated during early pregnancy were tracked and no associations were found between vaccination and preterm delivery or birth defects [95].

1.5.4 Myopericarditis—DoD vaccine campaigns during the early 2000s identified myopericarditis as a possible AE resulting from smallpox vaccination [51,53]. Further investigation of possible cardiac AEs resulted in the identification of 59 carditis cases found in 492,671 predominantly male vaccinees in a 2003 cohort, an estimated carditis incidence rate approximately 7.4 times higher than in an unvaccinated control cohort [96], a rate estimated to be closer to 214-fold in a recent prospective study [52]. The etiology of these side effects remains unclear. Cardiac pre-screening has been shown not to significantly reduce cardiac events [97].

1.5.5 Eczema vaccinatum—Eczema vaccinatum (EV) is a rare, but serious, systemic complication of VACV inoculation in individuals with eczema/atopic dermatitis, regardless of the severity of the skin condition or whether the condition is active at the time of vaccination. It is characterized by a rash and severe systemic illness. Due to the heightened risk of EV, eczema/atopic dermatitis is a contraindication for smallpox vaccine. Many EV cases are, however, caused by contact of susceptible individuals with primary vaccines [98,99]. EV historically has a high mortality rate between 30-40% of those affected; however, VIG given in a timely manner has been shown to significantly reduce the mortality rate to less than 10% [100].

1.5.6 Progressive vaccinia—Progressive vaccinia (PV), also known as vaccinia necrosum, is a severe and often lethal complication of smallpox vaccination, generally in individuals with serious defects in cell-mediated immunity and underlying immune-related diseases such as leukemia and HIV/AIDs, conditions that are contraindications for the vaccine. Initially, the vaccination lesion necroses in a painless and progressive manner, eventually resulting in massive destruction of tissue. PV was universally fatal before VIG, though survivability has increased since VIG treatment became available [77,99].

1.5.7 Postvaccinial encephalitis—Postvaccinial encephalitis (PVE) is a rare adverse event that reflects cerebral damage. Symptoms include headache, drowsiness, coma, seizures, and other non-specific neurological symptoms occurring 6-10 days postvaccination. This AE has a case-fatality rate of approximately 15-30%, and 15-50% of survivors experience neurological damage [90,92,99]. It appears to have a higher incidence rate in infants <12 months, but may affect any age group. The pathology of PVE is not well understood, the low incidence rate makes causality difficult to investigate, and autoimmunity is suggested as a mechanism, though not definitively demonstrated. There are some indications that the incidence of PVE varies based on the VACV strain used for immunization [90]. Therapy is supportive.

1.5.8 Adverse Event Summary—Although proper screening for contraindications results in lower rates of significant AEs after smallpox vaccination, as demonstrated in the 2002-2003 DoD vaccination campaign [51], large population-based immunization programs would result in some level of serious AEs, including death. Looking forward, the incidence of AEs may increase as an increasing fraction of the population are now immunosuppressed or have autoimmune skin conditions such as eczema.

2. Contemporary vaccines

Modern smallpox vaccines have been developed since the eradication era in attempt to improve vaccine manufacturing processes and safety profiles. These include tissue-culture-based live vaccines, attenuated live-virus vaccines, and subunit vaccines. Currently marketed vaccines and promising vaccine candidates are discussed here and summarized in Table 2.

2.1 Tissue-culture-based live vaccines

2.1.1 ACAM2000—Acambis and Baxter labs created two new vaccines, ACAM1000 and ACAM2000, from a plaque-purified viral isolate of Dryvax (NYCBH), grown in Vero cell

culture. The ACAM1000 and ACAM2000 viral strains differed by only 7 passages in cell culture, were identical at the genomic level, induced similar responses and ACAM2000 was further developed [105]. ACAM2000 showed an improved safety profile in animal infection models [106]. ACAM2000 has been tested extensively in human clinical trials and demonstrated to have a similar take rate to Dryvax in primary vaccinees, and to induce equal levels of neutralizing antibodies and both T-cell and lymphocyte proliferation [105].

Revaccination with ACAM2000, however, was shown to be inferior to revaccination with Dryvax (84% vs. 98% take rates), possibly due to lower virulence of the viral vaccine strain [107]. Antibodies induced by ACAM2000 have epitope profiles distinguishable from antibodies induced by Dryvax, likely due to the monoclonal nature of the ACAM2000 VACV strain relative to the highly polyclonal Dryvax [108,109]. The safety profile in humans has been shown to be similar to that of Dryvax in terms of cardiac and other AEs, though careful prescreening avoided most serious AEs [107]. ACAM2000 was licensed in the U.S. in 2007 and millions of doses have been produced for the U.S. national stockpile [106]. It should be noted that, in contrast to Dryvax, ACAM2000 did not retain full immunogenicity when diluted. This vaccine is a major component of the US Strategic National Stockpile and is being stockpiled in other countries as well.

2.1.2 CCSV—CCSV is a new cell-cultured vaccine developed by DynPort Vaccine Company, LLC, from the NYCBH vaccinia strain; it is produced in MRC-5 cells. In phase I clinical trials, CCSV was demonstrated to induce similar levels of neutralizing antibodies and T-cell responses, and was immunogenic at doses 50-times lower than approved Dryvax doses [110]. This study was underpowered to properly assess adverse events. This vaccine is no longer in development.

2.1.3 Elstree-BN—Elstree-BN is a Bavarian Nordic vaccine based on the Lister/Elstree strain and adapted for production in cell culture. The Elstree-BN vaccine induces comparable immune responses to the traditional Elstree vaccine in preclinical studies in macaques [111]. In a small clinical study in 2004, Bavarian Nordic reported similar safety profiles and efficacy to the traditional Elstree Lister-based vaccine. This vaccine is not being pursued for further development

2.1.4 CJ-50300—CJ-50300 is a cell-culture adapted derivative of the NYCBH strain of VACV developed in South Korea and produced in MRC-5 cells, which induces similar responses in mice to the Lancy-Vaxina strain. Lyophilized virus, given at two different doses in >100 subjects, showed a 99% take rate and cellular immunogenicity similar to that of other second-generation (tissue-culture based live) smallpox vaccines with one possible case of generalized vaccinia [112]. Further phase III studies conducted in Korea showed similar immune responses, and no occurrence of severe AEs [113]. It is unclear if there are any advantages to this vaccine strain relative to other, more-heavily studied vaccine strains such as ACAM2000 and CCSV.

2.2 Attenuated vaccines

2.2.1 Modified Vaccinia Ankara—Modified vaccinia Ankara (MVA) is a highly attenuated strain of VACV formed by over 500 serial passages of a Turkish smallpox vaccine strain in primary chicken embryo fibroblast cells, during which 15% of its genome was lost [114]. MVA is largely replication incompetent in most mammalian cell lines and is considered safe for humans, including immunosuppressed individuals. It has been widely studied both as a potential smallpox vaccine and as a potential backbone vector for many recombinant non-smallpox vaccines. Over 120,000 people have been immunized by an MVA-based vaccine, with an excellent safety profile (i.e., no reported severe AEs). MVA is considered a strong candidate for a safer, well-tolerated modern smallpox vaccine. Several MVA-based vaccines are in development, most notably IMVAMUNE (Bavarian Nordic) and ACAM3000 (Acambis), which have each been through phase I and II clinical trials with similar results.

MVA-based vaccines administered in a prime-boost regimen have been shown to induce similar immune (neutralizing antibody and T-cell) responses to ACAM2000 in macaques [115], indicating strong immunogenicity. The safety profile of MVA-based vaccines has been well-characterized. Tests for myo-/pericarditis in several hundred people showed no such AEs, and that the vaccine was safe and well tolerated. Of particular note are studies of MVA vaccines in people with eczema/atopic dermatitis or under immunosuppressive conditions. IMVAMUNE has been tested in several hundred atopic dermatitis (AD) patients, including many with active disease. While the AD individuals showed somewhat higher levels of minor AEs, no major AEs were found and the levels of VACV-specific antibody titers were similar between AD and healthy groups [116,117]. Similarly, the vaccine was tested in HIV-infected patients and was demonstrated to be safe, immunogenic, and well tolerated, without cardiac complications [118]. Lyophilization of MVA-based vaccines for long-term stable storage has been shown not to affect immunogenicity, and intradermal injection of low doses has been demonstrated to have similar immunogenicity to the standard subcutaneous injection route typically used in smallpox vaccination [119]. As a result of such studies, IMVAMUNE was added to the U.S. National Strategic Stockpile in 2010 for individuals with HIV and atopic dermatitis, and since has been approved for the entire adult population in Europe and in Canada for adults with immune deficiencies or skin disorders.

There is some concern about the protection provided by the MVA vaccines relative to the traditional smallpox vaccines. A single dose of MVA-based vaccine failed to protect macaques against aerosolized monkeypox in a recent trial, though protective effects and neutralizing antibody titers similar to ACAM2000 were induced by a prime-booster sequence [120]. MVA-vaccinated monkeys challenged with monkeypox also demonstrated higher levels of viremia than Dryvax or MVA/Dryvax vaccinated monkeys, and some developed self-resolving minor monkeypox lesions while remaining otherwise healthy. It is unclear if this difference is due to the method of inoculation or differences in vaccine immunogenicity [115].

The necessity of such a prime-boost vaccination regimen suggests that MVA may be more suitable for long-term smallpox prevention strategies than in combating serious smallpox outbreaks or bioterror events. The delay in inducing full protective immunity with two doses of an MVA vaccine is a serious drawback relative to traditional smallpox vaccines in situations where time is of the essence.

2.2.2 LC16m8—The live, attenuated, cell-culture vaccine LC16m8 was created in Japan toward the end of the eradication effort in an attempt to create a safer vaccine with fewer AEs. This low-virulence, temperature-sensitive, but replication-competent, VACV strain was created by serial passage of the Lister VACV strain through rabbit kidney cells, with the loss of membrane protein B5R expression due to a frameshift mutation [121]. LC16m8 was used to vaccinate infants in Japan during the 1970s eradication era without serious AEs. In recent primate studies comparing the virulence of VACV vaccine strains, LC16m8 was shown to be less virulent than both NYCBH and Lister virus strains [122]. Despite lower virulence, LC16m8 successfully protected mice from fatal vaccinia challenge similarly to its parental Lister VACV strain, [123] and successfully protected monkeys from monkeypox [124]. Protection against fatal VACV infection has been demonstrated using LC16m8 even in immunodeficient mice lacking either CD4, MHC class I, MHC class II or MHC classes I and II [123].

Over 100,000 people have been vaccinated with LC16m8 in Japan, with no serious AEs, reported evidence of cardiac toxicity, or deaths, suggesting a possibly improved safety profile over Lister and NYCBH strains. A recent study has demonstrated that in macaques depleted of T or B cells prior to vaccination, LC16m8 vaccination did not result in AEs, while progressive vaccinia resulted from vaccination with Dryvax [71]. These results suggest that LC16m8 may be a safer and effective vaccine for immunocompromised individuals, or those with atopic dermatitis. Recent clinical studies demonstrated safety and efficacy in Japanese military cohorts (3221 and 268 subjects) [125,126] and U.S. volunteers (125 subjects); however, although LC16m8 was demonstrated to induce robust cellular immune responses, lower neutralizing antibody titers were found relative to volunteers immunized with Dryvax [127], possibly due to the lower virulence of the LC16m8 strain. The sample sizes of recent clinical studies in humans and rarity of AEs are small; hence, larger clinical studies should be done to better characterize the safety profile of LC16m8.

Observations have been made that the attenuated LC16m8 VACV strain could spontaneously revert to higher virulence [128], suggesting that the frameshift mutation nature of LC16m8 attenuation is not genetically stable, which is a major potential issue for vaccine stability. A genetically stable version of the LC16m8 vaccine strain has been created by completely deleting the B5R gene (m8), and has been demonstrated in mice to induce antibody responses and confer protective immunity at similar levels to the original LC16m8 strain and significantly higher than MVA-induced immunity at similar virus doses [128]. This is a potential genetically stable variant of the LC16m8 strain for further vaccine development.

LC16M8, currently licensed in Japan, is proposed as a candidate for bioterrorism prevention stockpiles. This smallpox vaccine is a promising candidate for future large-scale use, but

questions regarding its genetic stability and lower antibody responses than Dryvax must still be fully addressed.

2.2.3 NYVAC—NYVAC, a highly attenuated VACV strain, was derived from the Copenhagen vaccine strain by targeted deletion of 18 open reading frames from the viral genome that were suspected to affect pathogenicity and virulence [129]. NYVAC is highly immunogenic despite its attenuation, and is also being considered as a backbone vector for recombinant HIV vaccines [130]. Genetic comparisons of NYVAC and MVA have been conducted; they identified differences in the nature of replication attenuation between these virus strains [130]. NYVAC upregulates a largely different set of cytokines than MVA in *in vitro* studies on immature human dendritic cells, with MVA inducing a stronger overall cytokine response than the distinct NYVAC response [131]. NYVAC and MVA also trigger different cellular responses; NYVAC induces a predominantly CD4+ T-cell response, while MVA induces both CD4+ and CD8+ responses [132]. Immunization of humans with NYVAC induces significantly lower VACV-specific neutralizing antibody titers than both Dryvax and Lister strains, suggesting that NYVAC may not be an optimal smallpox vaccine strain, or may require multiple doses [133]. This vaccine has not been tested in clinical trials; however, NYVAC-vectored vaccines for other infectious agents have been tested in clinical trials and typically exhibit fewer local/systemic reactions than NYCBH.

2.2.4 dVV-L—dVV-L is a replication-incompetent VACV strain derived from the Lister vaccine strain. This virus was created by targeted deletion of the gene for the uracil-DNA-glycosylase (UDG) enzyme essential for viral replication; the dVV-L virus retains, however, the ability to infect human cells and express early viral genes [134]. It is grown on a rabbit kidney cell line (RK-13) engineered to provide the missing UDG enzyme. Prime-boost immunizations induced robust cellular and long-term immunity in mice similar to MVA-induced responses, and dVV-L was shown to be well tolerated, even by immunodeficient mice for which the normal Lister VACV strain is quickly fatal [134,135]. Data on human vaccinees is not available and this product is not likely to be utilized in biodefense efforts in the near future.

2.3 Subunit or other vaccines

Contemporary and future biodefense preparations have an increased emphasis on vaccine safety. In the following section, we briefly outline subunit vaccine approaches that avoid use of live, potentially pathogenic VACV currently under investigation.

2.3.1 Gene-based vectors—Various gene-based subunit vaccines are currently in development, with the goal of inducing lasting immunity without the risks of live virus. These vaccines consist of combinations of plasmids carrying individual VACV genes; and proteins from both the IMV and EEV virion forms were demonstrated to be necessary for complete protection in early efforts [136-138].

The 4pox-VRP (virus-like replicon particle) smallpox vaccine expresses VACV A33R, B5R, A27L, and L1R genes that successfully induce protective immunity in mice and nonhuman primates [139]. Studies of this vaccine, when combined with molecular adjuvants, show

protection in monkeys against severe monkeypox disease at levels at least as high as MVA, though vaccinated monkeys in both MVA and 4pox-VRP immunized groups developed some form of disease [140].

An obstacle in the development of gene-based subunit vaccines is the fact that vaccinia and variola viruses are different, and the cross-reactivity of VACV subunit-induced antibodies with smallpox (VARV) antigens may be diminished relative to whole virus vaccines. For example, the VACV B5 gene has 23 amino acid differences from the B6 VARV homologue, resulting in many polyclonal antibodies against B5 that do not cross-react against B6 [141]. Heterogeneity in VACV A33 protein also affects the efficacy of the vaccine [142], and as such careful design of subunit vaccines is necessary. As live virus is not used in subunit vaccines, use of VARV rather than VACV genes should be safe and may avoid these problems. Some efforts in this area have been made. For example, a DNA-based vaccine expressing three VARV antigens and their recombinant protein counterparts has been shown to induce high-titer, cross-reactive antibody responses in mice that protect against VACV infection [143]. Due to lack of replicating virus, adjuvants may be needed to boost immune responses to levels necessary to provide protective immunity; efforts to identify appropriate adjuvants are currently underway [144].

2.3.2 Protein-based subunit vaccines—Subunit vaccines based on viral proteins are also in development. Mice vaccinated with adjuvanted VACV proteins from both the intracellular mature virus and extracellular virus forms were protected from lethal VACV challenge [145]. A similar three-protein combination vaccine also protected mice against live VACV challenge when adjuvanted and given in three doses [146]. A four VACV protein vaccine (A33, B5, L1, A27, adjuvanted with alum) was shown to partially protect nonhuman primates from a lethal dose of monkeypox virus, and to protect fully when further adjuvanted with CpG [147]. Other similar protein subunit vaccines have been proposed and studied [148,149]. As with gene-based vector vaccines, cross-reactivity between poxviruses is a concern with protein-based subunit vaccines. Finally, a vaccine has been proposed that combines DNA and protein vaccine technologies with a DNA-prime, peptide-boost methodology that induces protective immunity in mice against VACV using T-cell epitopes alone [150].

The need for prime-boost regimens limits the use of gene- and protein-based vaccines in the early stages of reacting to a bioterror event or poxvirus outbreak where time is critical; however, such vaccines may be of great use in long-term prevention strategies.

3. Issues facing new vaccine development, testing, and regulation

3.1 Lack of variola virus infection models to prove efficacy

New smallpox vaccines face major challenges in their development, testing, and licensure. The absence of VARV and the seriousness of smallpox disease make human trials with VARV challenge ethically impossible. While safety can be demonstrated, the effectiveness of protection against smallpox disease cannot be directly tested. Correlates of protection may be used, such as observation of vaccine take and neutralizing antibody titers, and levels of poxvirus-specific immune cells. These may or may not be well related to protection

against smallpox challenge. The traditional observation of vaccine take, for example, cannot be used as an endpoint for highly attenuated or subunit vaccines that do not form classic vaccinia lesions.

Animal models of smallpox vaccination and infection are frequently used in vaccine development. Monkeypox virus challenge of nonhuman primates, such as macaques, is currently considered the gold-standard animal model for smallpox. Prior to nonhuman primate testing, smallpox vaccine candidates are often tested in rabbits by challenge with rabbitpox, and mice by challenge with ectromelia. Cross-reactivity between smallpox vaccines and diverse poxviruses allows for some confidence that smallpox vaccines tested in the monkeypox system will induce human protection against VARV.

3.2 Regulatory testing

In 2002, the U.S. FDA introduced a new “Animal Rule” (21 CFR 601.90) to guide testing and regulation of products, such as smallpox vaccines, whose efficacy cannot be field-tested in humans due to ethical or feasibility concerns. This rule allows for approval of products that have been established as safe in human trials and have a well-understood pathophysiological mechanism as “reasonably likely to provide clinical benefit in humans” based on appropriate animal studies conducted in more than one well-characterized animal model species. Licensure of smallpox vaccines for human use is currently based on an acceptable safety profile in humans, efficacy based on monkeypox virus studies in nonhuman primates, efficacy demonstrated in a second animal model such as mice or rabbits, and the “non-inferiority” of human immune responses relative to currently licensed vaccines. The appropriateness of the non-inferiority requirement is often debated, with the fear that this may incrementally decrease the efficacy and protection thresholds each time that it is applied to a new vaccine. One potential example is that, despite the relative similarities between ACAM2000 and Dryvax, only Dryvax retains full immunogenicity upon dilution. In this case non-inferiority does not equate with identical efficacy in some situations.

3.3 Uncertain markets

As smallpox is currently eradicated, no clear markets currently exist for smallpox vaccines outside of biodefense efforts. The high cost of developing a new vaccine, and the large size of clinical trials needed to demonstrate incidence of rare AEs, prove a significant challenge to current smallpox vaccine development efforts.

4. Promising developments in the field

New development of safer and more effective smallpox vaccines is progressing rapidly due to a better understanding of poxvirus virology allowing for new vaccine formulations, the introduction of new paradigms for both developing novel targeted vaccine candidates designed to maximize immunogenicity while minimizing adverse side effects (vaccinomics [151]), and identifying genetic markers of individuals predisposed for AEs (adversomics [152]) for vaccine personalization.

4.1 Vaccine delivery and formulation advances

Better understanding of poxvirus virology and immunology may lead to improvements in vaccine formulations. Concurrent smallpox vaccination and antiviral drug administration, such as with the anti-poxvirus Tecovirimat (ST-246), may help reduce vaccine reactogenicity without affecting humoral or cell-mediated immune responses [153]. In the case of an epidemic or bioterrorist attack involving poxviruses, administration of both the vaccine and antiviral may be an appropriate dual treatment/prevention strategy for potentially exposed populations. Application of a povidone iodine ointment to smallpox vaccination sites has been demonstrated to reduce viral shedding without altering the immune response, thereby reducing the risk of contact transmission from the vaccination site [154]. Additionally, studies indicate that altering the smallpox vaccination schedule to include an initial vaccination with MVA, followed by a subsequent dose of a highly immunogenic smallpox vaccine such as Dryvax, may induce an ideal combination of low reactogenicity and high immunity [115].

4.2 Systems Vaccinology

Systems vaccinology – the application of systems biology tools to the study of vaccines – shows great potential for elucidating mechanisms of human responses to vaccines, which may result in an improved understanding of vaccine design factors that lead to greater vaccine efficacy [155]. Such approaches have been used to great effect in the study of other human vaccines. For example, a systems vaccinology approach to studying yellow fever vaccine discovered gene signatures that correlated with T and B cell responses with 90% and nearly 100% accuracy [156]. These signatures identified specific genes and proteins involved in yellow fever vaccine responses crucial to the development of protective immunity. Similarly, use of systems vaccinology approaches to the study of seasonal influenza vaccine identified novel genes that correlated with vaccine response, and identified baseline predictors of post-vaccination responses [157-160]. Application of systems vaccinology to smallpox vaccines may result in similar elucidation of the mechanisms of vaccine responses, and lead to more directed design of safe and effective vaccines.

4.3 Vaccinomics

The emerging field of vaccinomics – the holistic application of immunogenetics, immunogenomics, and systems biology to understanding vaccine-induced immune responses – allows for engineering of new viral vaccine candidates that optimize immunogenicity at the population or individual level [161,162]. For example, identification of SNPs associated with poor protective responses in smallpox-immunized individuals identifies genes important to the development of full cellular and humoral memory responses [163]. This information can be used to elucidate immune mechanisms, and predict individuals' responses to the smallpox vaccine.

Vaccinomics and systems vaccinology can also guide the design of safe and effective vaccines. Specific VACV proteins associated with virulence and pathogenicity can now be identified, and selective deletion of the corresponding genes from the VACV genome can occur; this leaves highly immunogenic proteins intact, which can create attenuated VACV strains with improved safety profiles retaining high levels of immunogenicity [164].

Transcriptomic studies are similarly elucidating the key biological pathways involved in the generation and maintenance of poxvirus immunity [165]. Mass spectrometry has also been used to identify naturally processed VACV-derived peptides with high immunogenicity. Selection of a subset of these peptides conserved between VACV and VARV show great promise as components of new, safe, and highly effective subunit vaccines against smallpox [166].

4.4 Adversomics

With contraindications to the current smallpox vaccines existing in up to 30% or more of the population, safer smallpox vaccines are increasingly needed. Efforts to identify genetic markers associated with vaccine AE phenotypes are being made at the genetic level in such an attempt. For example, genetic factors associated with AEs are being identified [167], and the mechanisms underlying these AEs may then be studied. This emerging field of *adversomics*—using genomic and other information to predict the complex interactions that result in non-random AEs—may be used to establish and refine genomic-level counter indications for smallpox vaccines and reduce the future incidence of AEs, and/or design vaccines that bypass the mechanisms that trigger serious AEs [152].

5. Expert Commentary

By now, second-generation vaccines have largely replaced their first-generation counterparts. The new manufacturing processes offer greater control over the contents of the vaccines, lower levels of adventitious agents, and have allowed the removal of potentially dangerous strains and quasi-species. Development of third-generation vaccines provides potentially safer alternatives that may be more appropriate for today's populations with higher rates of immune deficiencies, skin disorders, and cardiovascular issues, and a lower risk of wild virus exposure/infection. Although immune reactivity seems to be lower with these attenuated vaccines, prime-boost strategies may be able to balance safety and immune protection. Work continues on protein and plasmid DNA vaccines and provides not only opportunities to reduce side effects, but also to explore the utility of poxvirus-based vaccines for other diseases.

6. Five-year View

We believe that the next 5 years will see significant advances in our understanding of poxvirus biology and host response. The following are just some of the developments that can be expected to occur:

1. The ability to manipulate poxvirus genomes will allow us to expand the use of poxvirus vectors as vaccine “backbones” for vaccines against other pathogens of interest.
2. Poxvirus immunomodulatory proteins possess remarkable capabilities that are beginning to be examined and tested for clinical utility.

3. As more and more VACV genomes are sequenced, it becomes possible to identify correlations between virus genetic heterogeneity and safety/efficacy of smallpox vaccines [168-170].
4. A large number of poxvirus genes remain uncharacterized. Further studies into poxvirus biology may yield important insights into viral pathogenesis and host responses.
5. Increasingly sophisticated immune monitoring and high dimensional technology, combined with integrated systems biology approaches, may identify more pertinent correlates of protection and predictive immune response biomarkers that can be applied toward the development of diagnostic tools and new vaccine development.
6. Advances in animal models of poxvirus infection will allow us to better characterize vaccine immunogenicity and protection, an area of particular importance given the absence of disease.
7. The discovery of genetic markers linked to AEs will improve contraindications for smallpox vaccines and guide the development of new vaccines that do not induce serious adverse reactions.
8. Continued research into third-generation (DNA, protein, or peptide-based) vaccines may allow for the development of products with greatly improved safety profiles.

Acknowledgments

The authors wish to thank Caroline L. Vitse for her editorial assistance with this manuscript.

This project was funded by federal funds from the National Institute of Allergies and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN266200400025C (N01AI40065). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. G Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. G Poland offers consultative advice on vaccine development to Merck & Co. Inc., CSL Biotherapies, Avianax, Dynavax, Novartis Vaccines and Therapeutics, Emergent Biosolutions, Adjuvance, and Microdermis. G Poland holds two patents related to vaccinia and measles peptide research. R Kennedy is funded by Merck Research Laboratories to study waning immunity to mumps.

Reference annotations

* Of interest

** Of considerable interest

1. Wiser I, Balicer RD, Cohen D. An update on smallpox vaccine candidates and their role in bioterrorism related vaccination strategies. *Vaccine*. 2007; 25(6):976–984. 2007. [PubMed: 17074424]
2. Paran N, Sutter G. Smallpox vaccines: New formulations and revised strategies for vaccination. *Hum Vaccin*. 2009; 5(12):824–831. [PubMed: 19946203]
3. Walsh SR, Dolin R. Vaccinia viruses: vaccines against smallpox and vectors against infectious diseases and tumors. *Exp Rev Vaccines*. 2011; 10(8):1221–1240. 2011.

4. Verardi PH, Titong A, Hagen CJ. A vaccinia virus renaissance: new vaccine and immunotherapeutic uses after smallpox eradication. *Hum Vaccin Immunother.* 2012; 8(7):961–970. [PubMed: 22777090]
5. Kennedy RB, Ovsyannikova I, Poland GA. Smallpox vaccines for biodefense. *Vaccine.* 2009; 27(Suppl 4):D73–79. [PubMed: 19837292]
6. Kennedy RB, Ovsyannikova IG, Jacobson RM, Poland GA. The immunology of smallpox vaccines. *Curr Opin Immunol.* 2009; 21(3):314–320. [PubMed: 19524427]
7. Cohen J. Bioterrorism. Smallpox vaccinations: how much protection remains? *Science.* 2001; 294(5544):985. [PubMed: 11691969]
8. Parrino J, Graham BS. Smallpox vaccines: Past, present, and future. *J Allergy Clin Immunol.* 2006; 118(6):1320–1326. [PubMed: 17157663]
- 9**. Fenner, F.; Henderson, DA.; Arita, I.; Jezek, Z.; Ladnyi, ID. Smallpox and its Eradication. World Health Organization; Switzerland: 1988. Definitive book on smallpox disease, epidemiology, and the eradication effort, written by key members of the smallpox eradication effort
10. Henderson DA. The eradication of smallpox--an overview of the past, present, and future. *Vaccine.* 2011; 29(Suppl 4):D7–9. [PubMed: 22188929]
11. Bazin, H.; Jenner, E. The eradication of smallpox : Edward Jenner and the first and only eradication of a human infectious disease. Academic Press; San Diego, Calif.: 2000.
12. Dixon MF. Smallpox vaccination. *Br Med J.* 1970; 2(5708):539. [PubMed: 5468134]
13. Hopkins, DR. The Greatest Killer: Smallpox in History. University of Chicago Press; Chicago: 2002.
14. Li Y, Carroll DS, Gardner SN, Walsh MC, Vitalis EA, Damon IK. On the origin of smallpox: correlating variola phylogenics with historical smallpox records. *Proc Natl Acad Sci U S A.* 2007; 104(40):15787–15792. [PubMed: 17901212]
15. Shchelkunov SN. Emergence and reemergence of smallpox: the need for development of a new generation smallpox vaccine. *Vaccine.* 2011; 29(Suppl 4):D49–53. [PubMed: 22185833]
16. Shchelkunov SN. How long ago did smallpox virus emerge? *Arch Virol.* 2009; 154(12):1865–1871. [PubMed: 19882103]
- 17**. Kennedy, RB.; Lane, JM.; Henderson, DA.; Poland, GA. Smallpox and Vaccinia. In: Plotkin, SA.; Orenstein, WA.; Offit, PA., editors. Vaccines. Elsevier; 2013. p. 718-745. Comprehensive book chapter dealing with smallpox disease history, historical aspects of smallpox vaccine, and vaccine-induced immunity
18. Milton DK. What was the primary mode of smallpox transmission? Implications for biodefense *Front Cell Infect Microbiol.* 2012; 2:150. [PubMed: 23226686]
- 19**. Miller, G. Letters of Edward Jenner and other Documents Concerning the Early History of Vaccination. Johns Hopkins University Press; 1983. These are historical documents related to Edward Jenner's pioneering work on smallpox vaccination
20. Jenner, E.; Miller, G. Letters of Edward Jenner, and other documents concerning the early history of vaccination. Johns Hopkins University Press; Baltimore: 1983.
21. Crookshank, EM. History and pathology of vaccination. P. Blackiston; Philadelphia: 1889.
22. Shchelkunov SN. An increasing danger of zoonotic orthopoxvirus infections. *Plos Path.* 2013; 9(12):e1003756.
23. Tulman ER, Delhon G, Afonso CL, et al. Genome of horsepox virus. *J Virol.* 2006; 80(18):9244–9258. [PubMed: 16940536]
24. Collier LH. The development of a stable smallpox vaccine. *J Hyg (Lond).* 1955; 53(1):76–101. [PubMed: 14367805]
25. Pattanayak S, Arora DD, Sehgal CL, Raghavan NG, Topa PK, Subrahmanyam YK. Comparative studies of smallpox vaccination by the bifurcated needle and rotary lancet techniques. *Bull World Health Org.* 1970; 42(2):305–310. [PubMed: 5310142]
26. Lane JM, Poland GA. Why not destroy the remaining smallpox virus stocks? *Vaccine.* 2011; 29:2823–2824. [PubMed: 21376120]
27. Arita I. Discovery of forgotten variola specimens at the National Institutes of Health in the USA. *Exp Rev Anti Infect Ther.* 2014; 12(12):1419–1421.

28. Alibek, K.; H, S. Biohazard. Random House Inc; New York, NY: 1999.
29. Wolff HL, Croon JJAB. The survival of smallpox virus (variola minor) in natural circumstances. *Bull World Health Org.* 1968; 8:492–493. [PubMed: 4299528]
30. Reardon S. Infectious diseases: Smallpox watch. *Nature.* 2014; 509(7498):22–24. 2014. [PubMed: 24784198]
31. Biagini P, Theves C, Balaesque P, et al. Variola virus in a 300-year-old Siberian mummy. *N Engl J Med.* 2012; 367(21):2057–2059. [PubMed: 23171117]
32. Shchelkunov SN, Resenchuk SM, Totmenin AV, Blinov VM, Marennikova SS, Sandakhchiev LS. Comparison of the genetic maps of variola and vaccinia viruses. *Febs Letters.* 1993; 327(3):321–324. [PubMed: 8394246]
33. Massung RF, Liu LI, Qi J, et al. Analysis of the complete genome of smallpox variola major virus strain Bangladesh-1975. *Virology.* 1994; 201(2):215–240. [PubMed: 8184534]
34. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. *Lancet Infect Dis.* 2004; 4(1):15–25. [PubMed: 14720564]
35. Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. *Vet Microbiol.* 2010; 140(3-4):229–236. [PubMed: 19828265]
36. Parker S, Nuara A, Buller RM, Schultz DA. Human monkeypox: an emerging zoonotic disease. *Future Microbiol.* 2007; 2(1):17–34. [PubMed: 17661673]
37. Hutin YJ, Williams RJ, Malfait P, et al. Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. *Emerg Infect Dis.* 2001; 7(3):434–438. [PubMed: 11384521]
38. McCollum AM, Nakazawa Y, Ndongala GM, et al. Human Monkeypox in the Kivus, a Conflict Region of the Democratic Republic of the Congo. *Am J Trop Med Hyg.* 2015; 93(4):718–721. [PubMed: 26283752]
39. Meyer H, Perrichot M, Stemmler M, et al. Outbreaks of disease suspected of being due to human monkeypox virus infection in the Democratic Republic of Congo in 2001. *J Clin Microbiol.* 2002; 40(8):2919–2921. [PubMed: 12149352]
40. Rimoin AW, Mulembakani PM, Johnston SC, et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. *Proc Natl Acad Sci U S A.* 2010; 107(37):16262–16267. [PubMed: 20805472]
41. Gross E. Update on emerging infections: news from the Centers for Disease Control and prevention. Update: Multistate outbreak of monkeypox--Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *Ann Emerg Med.* 2003; 42(5):660–662. discussion 662- 664. [PubMed: 14581919]
42. Croft DR, Sotir MJ, Williams CJ, et al. Occupational risks during a monkeypox outbreak, Wisconsin, 2003. *Emerg Infect Dis.* 2007; 13(8):1150–1157. [PubMed: 17953084]
43. Huhn GD, Bauer AM, Yorita K, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin Infect Dis.* 2005; 41(12):1742–1751. [PubMed: 16288398]
44. Hammarlund E, Lewis MW, Carter SV, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. *Nat Med.* 2005; 11(9):1005–1011. [PubMed: 16086024]
45. Karem KL, Reynolds M, Hughes C, et al. Monkeypox-induced immunity and failure of childhood smallpox vaccination to provide complete protection. *Clin Vaccine Immunol.* 2007; 14(10):1318–1327. [PubMed: 17715329]
46. Bhanuprakash V, Venkatesan G, Balamurugan V, et al. Zoonotic infections of buffalopox in India. *Zoonoses Public Health.* 2010; 57(7-8):e149–155. [PubMed: 20042058]
47. Bera BC, Shanmugasundaram K, Barua S, et al. Zoonotic cases of camelpox infection in India. *Vet Microbiol.* 2011; 152(1-2):29–38. [PubMed: 21571451]
48. Trindade GS, Emerson GL, Carroll DS, Kroon EG, Damon IK. Brazilian vaccinia viruses and their origins. *Emerg Infect Dis.* 2007; 13(7):965–972. [PubMed: 18214166]
49. Osadebe LU, Manthiram K, McCollum AM, et al. Novel poxvirus infection in 2 patients from the United States. *Clin Infect Dis.* 2015; 60(2):195–202. [PubMed: 25301210]

50. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part I. Background, vaccination technique, normal vaccination and revaccination, and expected normal reactions. *Clin Infect Dis*. 2003; 37(2):241–250. [PubMed: 12856217]
- 51*. Poland GA, Grabenstein JD, Neff JM. The US smallpox vaccination program: a review of a large modern era smallpox vaccination implementation program. *Vaccine*. 2005; 23:2078–2081. This paper offers a summary of adverse event rates in modern smallpox vaccination programs. This reference contains the largest collection of adverse event data on smallpox vaccine use since eradication. [PubMed: 15755574]
- 52*. Engler RJ, Nelson MR, Collins LC Jr, et al. A prospective study of the incidence of myocarditis/pericarditis and new onset cardiac symptoms following smallpox and influenza vaccination. *PLoS One*. 2015; 10(3):e0118283. This is a prospective study examining cardiac adverse events in smallpox vaccine recipients. The results indicate a much higher rate of myopericarditis among vaccine recipients than previously assumed. [PubMed: 25793705]
53. Halsell JS, Riddle JR, Atwood JE, et al. Myopericarditis following smallpox vaccination among vaccinia-naive US military personnel. *JAMA*. 2003; 289:3283–3289. [PubMed: 12824210]
54. Putz MM, Midgley CM, Law M, Smith GL. Quantification of antibody responses against multiple antigens of the two infectious forms of Vaccinia virus provides a benchmark for smallpox vaccination. *Nat Med*. 2006; 12(11):1310–1315. [PubMed: 17086190]
55. Hsieh SM, Chen SY, Sheu GC, et al. Clinical and immunological responses to undiluted and diluted smallpox vaccine with vaccinia virus of Lister strain. *Vaccine*. 2006; 24(4):510–515. [PubMed: 16139395]
56. Talbot TR, Stapleton JT, Brady RC, et al. Vaccination success rate and reaction profile with diluted and undiluted smallpox vaccine: a randomized controlled trial. *JAMA*. 2004; 292(10):1205–1212. [PubMed: 15353533]
57. Kim SH, Yeo SG, Jang HC, et al. Clinical responses to smallpox vaccine in vaccinia-naive and previously vaccinated populations: undiluted and diluted Lancy-Vaxina vaccine in a single-blind, randomized, prospective trial. *J Infect Dis*. 2005; 192(6):1066–1070. [PubMed: 16107961]
58. Cummings JF, Polhemus ME, Hawkes C, Klote M, Ludwig GV, Wortmann G. Lack of vaccinia viremia after smallpox vaccination. *Clin Infect Dis*. 2004; 38(3):456–458. [PubMed: 14727224]
59. Kennedy JS, Frey SE, Yan L, et al. Induction of human T cell-mediated immune responses after primary and secondary smallpox vaccination. *J Infect Dis*. 2004; 190(7):1286–1294. [PubMed: 15346340]
60. Kennedy, RB.; Lane, JM.; Henderson, DA.; Poland, G. Vaccines. Elsevier Inc.; 2012. Smallpox and Vaccinia; p. 718-745.
61. Amanna IJ, Slifka MK, Crotty S. Immunity and immunological memory following smallpox vaccination. *Immunol Rev*. 2006; 211:320–337. [PubMed: 16824139]
62. Amara RR, Nigam P, Sharma S, Liu J, Bostik V. Long-lived poxvirus immunity, robust CD4 help, and better persistence of CD4 than CD8 T cells. *J Virol*. 2004; 78(8):3811–3816. [PubMed: 15047796]
63. Hammarlund E, Lewis MW, Hansen SG, et al. Duration of antiviral immunity after smallpox vaccination. *Nat Med*. 2003; 9(9):1131–1137. [PubMed: 12925846]
64. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J Immunol*. 2003; 171(10):4969–4973. [PubMed: 14607890]
65. Chaudhri G, Panchanathan V, Bluethmann H, Karupiah G. Obligatory requirement for antibody in recovery from a primary poxvirus infection. *J Virol*. 2006; 80(13):6339–6344. [PubMed: 16775322]
66. Cohen JI, Hohman P, Fulton R, et al. Kinetics of serum cytokines after primary or repeat vaccination with the smallpox vaccine. *J Infect Dis*. 2010; 201(8):1183–1191. [PubMed: 20214479]
67. Simon WL, Salk HM, Ovsyannikova IG, Kennedy RB, Poland GA. Cytokine production associated with smallpox vaccine responses. *Immunotherapy*. 2014; 6(10):1097–1112. [PubMed: 25428648]

68. Ovsyannikova IG, Haralambieva IH, Kennedy RB, et al. Impact of cytokine and cytokine receptor gene polymorphisms on cellular immunity after smallpox vaccination. *Gene*. 2012; 510(1):59–65. [PubMed: 23009887]
69. Kennedy RB, Ovsyannikova IG, Pankratz VS, et al. Gender effects on humoral immune responses to smallpox vaccine. *Vaccine*. 2009; 27(25-26):3319–3323. [PubMed: 19200827]
70. Haralambieva IH, Ovsyannikova IG, Kennedy RB, Larrabee BR, Shane Pankratz V, Poland GA. Race and sex-based differences in cytokine immune responses to smallpox vaccine in healthy individuals. *Hum Immunol*. 2013; 74(10):1263–1266. [PubMed: 23806267]
71. Gordon SN, Cecchinato V, Andresen V, et al. Smallpox vaccine safety is dependent on T cells and not B cells. *J Infect Dis*. 2011; 203(8):1043–1053. [PubMed: 21450994]
72. Kennedy JS, Frey SE, Yan L, et al. Induction of human T cell-mediated immune responses after primary and secondary smallpox vaccination. *J Infect Dis*. 2004; 190(7):1286–1294. [PubMed: 15346340]
73. Amanna IJ, Slifka MK, Crotty S. Immunity and immunological memory following smallpox vaccination. *Immunol Rev*. 2006; 211:320–337. [PubMed: 16824139]
74. Cornberg M, Sheridan BS, Saccoccio FM, Brehm MA, Selin LK. Protection against vaccinia virus challenge by CD8 memory T cells resolved by molecular mimicry. *J Virol*. 2007; 81(2):934–944. [PubMed: 17079318]
75. Amara RR, Nigam P, Sharma S, Liu J, Bostik V. Long-lived poxvirus immunity, robust CD4 help, and better persistence of CD4 than CD8 T cells. *J Virol*. 2004; 78(8):3811–3816. [PubMed: 15047796]
76. Xu R, Johnson AJ, Liggitt D, Bevan MJ. Cellular and humoral immunity against vaccinia virus infection of mice. *J Immunol*. 2004; 172(10):6265–6271. [PubMed: 15128815]
77. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: Results of ten statewide surveys. *J Infect Dis*. 1970; 122(4):303–309. [PubMed: 4396189]
78. Demkowicz WE, Littau RA, Wang JM, Ennis FA. Human cytotoxic T-cell memory: Long-lived responses to vaccinia virus. *J Virol*. 1996; 70(4):2627–2631. [PubMed: 8642697]
79. Moyron-Quiroz JE, McCausland MM, Kageyama R, Sette A, Crotty S. The smallpox vaccine induces an early neutralizing IgM response. *Vaccine*. 2009; 28(1):140–147. [PubMed: 19822230]
80. McCarthy K, Downie AW, Bradley WH. The antibody response in man following infection with viruses of the pox group. II. Antibody response following vaccination. *J Hyg (Lond)*. 1958; 56(4):466–478. [PubMed: 13611243]
81. Chaudhri G, Panchanathan V, Bluethmann H, Karupiah G. Obligatory requirement for antibody in recovery from a primary poxvirus infection. *J Virol*. 2006; 80(13):6339–6344. [PubMed: 16775322]
82. Viner KM, Isaacs SN. Activity of vaccinia virus-neutralizing antibody in the sera of smallpox vaccinees. *Microbes Infect*. 2005; 7(4):579–583. [PubMed: 15848274]
83. Benhnia MR, Maybeno M, Blum D, et al. Unusual features of vaccinia virus extracellular virion form neutralization resistance revealed in human antibody responses to the smallpox vaccine. *J Virol*. 2013; 87(3):1569–1585. [PubMed: 23152530]
84. Dixon CW. Smallpox in Tripolitania, 1946; an epidemiological and clinical study of 500 cases, including trials of penicillin treatment. *J Hyg (Lond)*. 1948; 46(4):351–377. [PubMed: 18129313]
85. Mortimer PP. Can postexposure vaccination against smallpox succeed? *Clin Infect Dis*. 2003; 36(5):622–629. [PubMed: 12594644]
86. Bicknell WJ, James K. Smallpox vaccination after a bioterrorism-based exposure. *Clin Infect Dis*. 2003; 37(3):467. [PubMed: 12884184]
87. Massoudi MS, Barker L, Schwartz B. Effectiveness of postexposure vaccination for the prevention of smallpox: results of a delphi analysis. *J Infect Dis*. 2003; 188(7):973–976. [PubMed: 14513416]
88. Feery BJ. Adverse reactions after smallpox vaccination. *Med J Aust*. 1977; 2(6):180–183. [PubMed: 20557]
89. Jacobson IG, Smith TC, Smith B, Wells TS, Reed RJ, Ryan MA. US military service members vaccinated against smallpox in 2003 and 2004 experience a slightly higher risk of hospitalization postvaccination. *Vaccine*. 2008; 26(32):4048–4056. [PubMed: 18586364]

90. Cono J, Casey CG, Bell DM. Smallpox vaccination and adverse reactions. Guidance for clinicians. *MMWR*. 2003; 52(RR-4):1–28. [PubMed: 12617510]
91. Sejvar J, Boneva R, Lane JM, Iskander J. Severe headaches following smallpox vaccination. *Headache*. 2005; 45(1):87–88. [PubMed: 15663622]
- 92*. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. *Clin Infect Dis*. 2003; 37(2):251–271. This paper is an excellent discussion of the rare but serious adverse events that can occur after smallpox vaccination. [PubMed: 12856218]
93. Pepose JS, Margolis TP, LaRussa P, Pavan-Langston D. Ocular complications of smallpox vaccination. *Am J Ophthalmol*. 2003; 136(2):343–352. [PubMed: 12888060]
94. Fillmore GL, Ward TP, Bower KS, et al. Ocular complications in the Department of Defense Smallpox Vaccination Program. *Ophthalmology*. 2004; 111(11):2086–2093. [PubMed: 15522376]
95. Ryan MA, Gumbs GR, Conlin AM, et al. Evaluation of preterm births and birth defects in liveborn infants of US military women who received smallpox vaccine. *Birth Defects Res A Clin Mol Teratol*. 2008; 82(7):533–539. [PubMed: 18496830]
96. Arness MK, Eckart RE, Love SS, et al. Myopericarditis following smallpox vaccination. *Am J Epidemiol*. 2004; 160(7):642–651. [PubMed: 15383408]
97. Eckart RE, Shry EA, Atwood JE, et al. Smallpox vaccination and ischemic coronary events in healthy adults. *Vaccine*. 2007; 25(50):8359–8364. [PubMed: 17981378]
98. Vora S, Damon I, Fulginiti V, et al. Severe eczema vaccinatum in a household contact of a smallpox vaccinee. *Clin Infect Dis*. 2008; 46(10):1555–1561. [PubMed: 18419490]
99. Lane JM, Ruben FL, Abrutyn E, Millar JD. Deaths attributable to smallpox vaccination, 1959 to 1966, and 1968. *JAMA*. 1970; 212:441–444. [PubMed: 4392370]
100. Kempe CH, Berge TO, England B. Hyperimmune vaccinal gamma globulin. Source, evaluation, and use in prophylaxis and therapy. *Pediatrics*. 1956; 18:177–188. [PubMed: 13349330]
101. Neff JM, Levine RH, Lane JM, et al. Complications of smallpox vaccination United States 1963. II. Results obtained by four statewide surveys. *Pediatrics*. 1967; 39(6):916–923. [PubMed: 4381735]
102. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968. *N Engl J Med*. 1969; 281(22):1201–1208. [PubMed: 4186802]
103. Neff JM, Lane JM, Pert JH, Moore R, Millar JD, Henderson DA. Complications of smallpox vaccination. I. National survey in the United States, 1963. *N Engl J Med*. 1967; 276(3):125–132. [PubMed: 4381041]
104. Ratner LH, Lane JM, Vicens CN. Complications of smallpox vaccination: surveillance during an island-wide program in Puerto Rico, 1967–1968. *Am J Epidemiol*. 1970; 91(3):278–285. 1970. [PubMed: 4391875]
105. Frey SE, Newman FK, Kennedy JS, et al. Comparison of the safety and immunogenicity of ACAM1000, ACAM2000 and Dryvax in healthy vaccinia-naive adults. *Vaccine*. 2009; 27(10):1637–1644. [PubMed: 19071184]
106. Monath TP, Caldwell JR, Mundt W, et al. ACAM2000 clonal Vero cell culture vaccinia virus (New York City Board of Health strain)--a second-generation smallpox vaccine for biological defense. *Int J Infect Dis*. 2004; 8(Suppl 2):S31–S44. [PubMed: 15491873]
107. Nalca A, Zumbrun EE. ACAM2000: the new smallpox vaccine for United States Strategic National Stockpile. *Drug Des Devel Ther*. 2010; 4:71–79.
108. Pugh C, Keasey S, Korman L, Pittman PR, Ulrich RG. Human antibody responses to the polyclonal Dryvax vaccine for smallpox prevention can be distinguished from responses to the monoclonal replacement vaccine ACAM2000. *Clin Vaccine Immunol*. 2014; 21(6):877–885. [PubMed: 24759651]
109. Qin L, Upton C, Hazes B, Evans DH. Genomic analysis of the vaccinia virus strain variants found in Dryvax vaccine. *J Virol*. 2011; 85(24):13049–13060. [PubMed: 21976639]
110. Greenberg RN, Kennedy JS, Clanton DJ, et al. Safety and immunogenicity of new cell-cultured smallpox vaccine compared with calf-lymph derived vaccine: a blind, single-centre, randomised controlled trial. *Lancet*. 2005; 365(9457):398–409. [PubMed: 15680454]

111. Stittelaar KJ, van AG, Kondova I, et al. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J Virol.* 2005; 79(12):7845–7851. [PubMed: 15919938]
112. Jang HC, Kim CJ, Kim KH, et al. A randomized, double-blind, controlled clinical trial to evaluate the efficacy and safety of CJ-50300, a newly developed cell culture-derived smallpox vaccine, in healthy volunteers. *Vaccine.* 2010; 28(36):5845–5849. [PubMed: 20600480]
113. Kim NH, Kang YM, Kim G, et al. An open-label, single arm, phase III clinical study to evaluate the efficacy and safety of CJ smallpox vaccine in previously vaccinated healthy adults. *Vaccine.* 2013; 31(45):5239–5242. [PubMed: 24021303]
114. Antoine G, Scheiflinger F, Dorner F, Falkner FG. The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. *Virology.* 1998; 244(2): 365–396. [PubMed: 9601507]
115. Earl PL, Americo JL, Wyatt LS, et al. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature.* 2004; 428(6979):182–185. [PubMed: 15014500]
116. Greenberg RN, Hurley Y, Dinh DV, et al. A Multicenter, Open-Label, Controlled Phase II Study to Evaluate Safety and Immunogenicity of MVA Smallpox Vaccine (IMVAMUNE) in 18-40 Year Old Subjects with Diagnosed Atopic Dermatitis. *PLoS One.* 2015; 10(10):e0138348. [PubMed: 26439129]
117. von Sonnenburg F, Perona P, Darsow U, et al. Safety and immunogenicity of modified vaccinia Ankara as a smallpox vaccine in people with atopic dermatitis. *Vaccine.* 2014; 32(43):5696–5702. [PubMed: 25149431]
118. Overton ET, Stapleton J, Frank I, et al. Safety and Immunogenicity of Modified Vaccinia Ankara-Bavarian Nordic Smallpox Vaccine in Vaccinia-Naive and Experienced Human Immunodeficiency Virus-Infected Individuals: An Open-Label, Controlled Clinical Phase II Trial. *Open Forum Infect Dis.* 2015; 2(2):ofv040. [PubMed: 26380340]
119. Frey SE, Wald A, Edupuganti S, et al. Comparison of lyophilized versus liquid modified vaccinia Ankara (MVA) formulations and subcutaneous versus intradermal routes of administration in healthy vaccinia-naive subjects. *Vaccine.* 2015; 33(39):5225–5234. [PubMed: 26143613]
120. Hatch GJ, Graham VA, Bewley KR, et al. Assessment of the protective effect of Imvamune and Acam2000 vaccines against aerosolized monkeypox virus in cynomolgus macaques. *J Virol.* 2013; 87(14):7805–7815. [PubMed: 23658452]
121. Morikawa S, Sakiyama T, Hasegawa H, et al. An attenuated LC16m8 smallpox vaccine: analysis of full-genome sequence and induction of immune protection. *J Virol.* 2005; 79(18):11873–11891. [PubMed: 16140764]
122. Morita M, Aoyama Y, Arita M, et al. Comparative studies of several vaccinia virus strains by intrathalamic inoculation into cynomolgus monkeys. *Archi Virol.* 1977; 53(3):97–208.
123. Yokote H, Shinmura Y, Kanehara T, et al. Vaccinia virus strain LC16m8 defective in the B5R gene keeps strong protection comparable to its parental strain Lister in immunodeficient mice. *Vaccine.* 2015; 33(45):6112–6119. [PubMed: 26241947]
124. Saijo M, Ami Y, Suzaki Y, et al. LC16m8, a highly attenuated vaccinia virus vaccine lacking expression of the membrane protein B5R, protects monkeys from monkeypox. *J Virol.* 2006; 80(11):5179–5188. [PubMed: 16698998]
125. Nishiyama Y, Fujii T, Kanatani Y, Shinmura Y, Yokote H, Hashizume S. Freeze-dried live attenuated smallpox vaccine prepared in cell culture “LC16-KAKETSUKEN”: Post-marketing surveillance study on safety and efficacy compliant with Good Clinical Practice. *Vaccine.* 2015; 33(45):6120–6127. [PubMed: 26455406]
126. Saito T, Fujii T, Kanatani Y, et al. Clinical and immunological response to attenuated tissue-cultured smallpox vaccine LC16m8. *JAMA.* 2009; 301(10):1025–1033. [PubMed: 19278946]
127. Kennedy JS, Gurwith M, Dekker CL, et al. Safety and immunogenicity of LC16m8, an attenuated smallpox vaccine in vaccinia-naive adults. *J Infect Dis.* 2011; 204(9):1395–1402. [PubMed: 21921208]

128. Kidokoro M, Tashiro M, Shida H. Genetically stable and fully effective smallpox vaccine strain constructed from highly attenuated vaccinia LC16m8. *Proc Natl Acad Sci U S A*. 2005; 102(11): 4152–4157. [PubMed: 15753319]
129. Tartaglia J, Perkus ME, Taylor J, et al. NYVAC: a highly attenuated strain of vaccinia virus. *Virology*. 1992; 188(1):217–232. [PubMed: 1566575]
130. Gomez CE, Najera JL, Krupa M, Perdiguero B, Esteban M. MVA and NYVAC as vaccines against emergent infectious diseases and cancer. *Curr Gene Ther*. 2011; 11(3):189–217. [PubMed: 21453284]
131. Guerra S, Najera JL, Gonzalez JM, et al. Distinct gene expression profiling after infection of immature human monocyte-derived dendritic cells by the attenuated poxvirus vectors MVA and NYVAC. *J Virol*. 2007; 81(16):8707–8721. [PubMed: 17537851]
132. Mooij P, Balla-Jhagjhoorsingh SS, Koopman G, et al. Differential CD4+ versus CD8+ T-cell responses elicited by different poxvirus-based human immunodeficiency virus type 1 vaccine candidates provide comparable efficacies in primates. *J Virol*. 2008; 82(6):2975–2988. [PubMed: 18184713]
133. Midgley CM, Putz MM, Weber JN, Smith GL. Vaccinia virus strain NYVAC induces substantially lower and qualitatively different human antibody responses compared with strains Lister and Dryvax. *J Gen Virol*. 2008; 89(Pt 12):2992–2997. [PubMed: 19008384]
134. Coulibaly S, Bruhl P, Mayrhofer J, Schmid K, Gerencer M, Falkner FG. The nonreplicating smallpox candidate vaccines defective vaccinia Lister (dVVL) and modified vaccinia Ankara (MVA) elicit robust long-term protection. *Virology*. 2005; 341(1):91–101. [PubMed: 16061267]
135. Ober BT, Bruhl P, Schmidt M, et al. Immunogenicity and safety of defective vaccinia virus lister: comparison with modified vaccinia virus Ankara. *J Virol*. 2002; 76(15):7713–7723. [PubMed: 12097585]
136. Pulford DJ, Gates A, Bridge SH, Robinson JH, Ulaeto D. Differential efficacy of vaccinia virus envelope proteins administered by DNA immunisation in protection of BALB/c mice from a lethal intranasal poxvirus challenge. *Vaccine*. 2004; 22(25-26):3358–3366. [PubMed: 15308360]
137. Hooper JW, Custer DM, Schmaljohn CS, Schmaljohn AL. DNA vaccination with vaccinia virus L1R and A33R genes protects mice against a lethal poxvirus challenge. *Virology*. 2000; 266(2): 329–339. [PubMed: 10639319]
138. Sakhatskyy P, Wang S, Chou TH, Lu S. Immunogenicity and protection efficacy of monovalent and polyvalent poxvirus vaccines that include the D8 antigen. *Virology*. 2006; 355(2):164–174. [PubMed: 16919703]
139. Hooper JW, Ferro AM, Golden JW, et al. Molecular smallpox vaccine delivered by alphavirus replicons elicits protective immunity in mice and non-human primates. *Vaccine*. 2009; 28(2): 494–511. [PubMed: 19833247]
140. Golden JW, Josleyn M, Mucker EM, et al. Side-by-side comparison of gene-based smallpox vaccine with MVA in nonhuman primates. *PLoS One*. 2012; 7(7):e42353. [PubMed: 22860117]
141. Aldaz-Carroll L, Xiao Y, Whitbeck JC, et al. Major neutralizing sites on vaccinia virus glycoprotein B5 are exposed differently on variola virus ortholog B6. *J Virol*. 2007; 81(15): 8131–8139. [PubMed: 17522205]
142. Golden JW, Hooper JW. Heterogeneity in the A33 protein impacts the cross-protective efficacy of a candidate smallpox DNA vaccine. *Virology*. 2008; 377(1):19–29. [PubMed: 18482742]
143. Sakhatskyy P, Wang S, Zhang C, Chou TH, Kishko M, Lu S. Immunogenicity and protection efficacy of subunit-based smallpox vaccines using variola major antigens. *Virology*. 2008; 371(1):98–107. [PubMed: 17950773]
144. Martinez O, Miranda E, Ramirez M, et al. Immunomodulator-based enhancement of anti smallpox immune responses. *PLoS One*. 2015; 10(4):e0123113. [PubMed: 25875833]
145. Xiao Y, Aldaz-Carroll L, Ortiz AM, et al. A protein-based smallpox vaccine protects mice from vaccinia and ectromelia virus challenges when given as a prime and single boost. *Vaccine*. 2007; 25(7):1214–1224. [PubMed: 17098336]
146. Fogg C, Lustig S, Whitbeck JC, Eisenberg RJ, Cohen GH, Moss B. Protective immunity to vaccinia virus induced by vaccination with multiple recombinant outer membrane proteins of intracellular and extracellular virions. *J Virol*. 2004; 78(19):10230–10237. [PubMed: 15367588]

147. Buchman GW, Cohen ME, Xiao Y, et al. A protein-based smallpox vaccine protects non-human primates from a lethal monkeypox virus challenge. *Vaccine*. 2010; 28(40):6627–6636. [PubMed: 20659519]
148. Portocarrero C, Markley K, Koprowski H, Spitsin S, Golovkin M. Immunogenic properties of plant-derived recombinant smallpox vaccine candidate pB5. *Vaccine*. 2008; 26(43):5535–5540. [PubMed: 18706953]
149. Berhanu A, Wilson RL, Kirkwood-Watts DL, et al. Vaccination of BALB/c mice with Escherichia coli-expressed vaccinia virus proteins A27L, B5R, and D8L protects mice from lethal vaccinia virus challenge. *J Virol*. 2008; 82(7):3517–3529. [PubMed: 18199639]
150. Moise L, Buller RM, Schriewer J, et al. VennVax, a DNA-prime, peptide-boost multi-T-cell epitope poxvirus vaccine, induces protective immunity against vaccinia infection by T cell response alone. *Vaccine*. 2011; 29(3):501–511. [PubMed: 21055490]
151. Poland GA. Pharmacology, vaccinomics, and the second golden age of vaccinology. *Clin Pharmacol Ther*. 2007; 82(6):623–626. [PubMed: 17998905]
152. Poland GA, Ovsyannikova IG, Jacobson RM. Adversomics: the emerging field of vaccine adverse event immunogenetics. *Pediatr Infect Dis J*. 2009; 28(5):431–432. [PubMed: 19395950]
153. Berhanu A, King DS, Mosier S, et al. Impact of ST-246(R) on ACAM2000 smallpox vaccine reactogenicity, immunogenicity, and protective efficacy in immunodeficient mice. *Vaccine*. 2010; 29(2):289–303. [PubMed: 21036130]
154. Pittman PR, Garman PM, Kim SH, et al. Smallpox vaccine, ACAM2000: Sites and duration of viral shedding and effect of povidone iodine on scarification site shedding and immune response. *Vaccine*. 2015; 33(26):2990–2996. [PubMed: 25930115]
155. Hagan T, Nakaya HI, Subramaniam S, Pulendran B. Systems vaccinology: Enabling rational vaccine design with systems biological approaches. *Vaccine*. 2015; 33(40):5294–5301. [PubMed: 25858860]
156. Querec TD, Akondy RS, Lee EK, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol*. 2009; 10(1):116–125. [PubMed: 19029902]
157. Nakaya HI, Wrammert J, Lee EK, et al. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol*. 2011; 12(8):786–795. [PubMed: 21743478]
158. Tsang JS, Schwartzberg PL, Kotliarov Y, et al. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. *Cell*. 2014; 157(2):499–513. [PubMed: 24725414]
159. Bucasas KL, Franco LM, Shaw CA, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. *J Infect Dis*. 2011; 203(7):921–929. [PubMed: 21357945]
160. Nakaya HI, Clutterbuck E, Kazmin D, et al. Systems biology of immunity to MF59-adjuvanted versus nonadjuvanted trivalent seasonal influenza vaccines in early childhood. *Proc Natl Acad Sci U S A*. 2016; 113(7):1853–1858. [PubMed: 26755593]
161. Poland GA, Ovsyannikova IG, Jacobson RM. Personalized vaccines: the emerging field of vaccinomics. *Exp Opin Biol Ther*. 2008; 8(11):1659–1667.
162. Poland GA, Kennedy RB, McKinney BA, et al. Vaccinomics, adversomics, and the immune response network theory: individualized vaccinology in the 21st century. *Semin Immunol*. 2013; 25(2):89–103. [PubMed: 23755893]
163. Ovsyannikova IG, Kennedy RB, O'Byrne M, Jacobson RM, Pankratz VS, Poland GA. Genome-wide association study of antibody response to smallpox vaccine. *Vaccine*. 2012; 30(28):4182–4189. [PubMed: 22542470]
164. Kan S, Jia P, Sun L, et al. Generation of an attenuated Tiantan vaccinia virus by deletion of the ribonucleotide reductase large subunit. *Arch Virol*. 2014; 159(9):2223–2231. [PubMed: 24677065]
165. Kennedy RB, Oberg AL, Ovsyannikova IG, Haralambieva IH, Grill DE, Poland GA. Transcriptomic profiles of high and low antibody responders to smallpox vaccine. *Genes Immunity*. 2013; 14(5):277–285. [PubMed: 23594957]

166. Johnson KL, Ovsyannikova IG, Mason CJ, Bergen HR III, Poland GA. Discovery of naturally processed and HLA-presented class I peptides from vaccinia virus infection using mass spectrometry for vaccine development. *Vaccine*. 2009; 28(1):38–47. [PubMed: 19822231]
167. Reif DM, McKinney BA, Motsinger AA, et al. Genetic basis for adverse events after smallpox vaccination. *J Infect Dis*. 2008; 198(1):16–22. [PubMed: 18454680]
168. Li G, Chen N, Feng Z, et al. Genomic sequence and analysis of a vaccinia virus isolate from a patient with a smallpox vaccine-related complication. *Virology J*. 2006; 3:88. [PubMed: 17062162]
169. Osborne JD, Da Silva M, Frace AM, et al. Genomic differences of Vaccinia virus clones from Dryvax smallpox vaccine: the Dryvax-like ACAM2000 and the mouse neurovirulent Clone-3. *Vaccine*. 2007; 25(52):8807–8832. [PubMed: 18037545]
170. Garcel A, Crance JM, Drillien R, Garin D, Favier AL. Genomic sequence of a clonal isolate of the vaccinia virus Lister strain employed for smallpox vaccination in France and its comparison to other orthopoxviruses. *J Gen Virol*. 2007; 88(Pt 7):1906–1916. [PubMed: 17554021]

Key Issues

- Smallpox is a deadly debilitating disease with mortality rate of 30%
- Following a decade-long, worldwide effort involving vaccination and disease surveillance, smallpox was declared eradicated in 1980
- Despite its eradication, smallpox vaccine remains a public health issue due to concerns about bioterrorism and zoonotic orthopoxvirus outbreaks
- First-generation vaccines contained live vaccinia virus and elicited long-lasting, protective immunity against disease
- Current vaccines are second-generation vaccines (cell culture derivatives of 1st generation vaccines) and third-generation vaccines (containing attenuated vaccinia virus strains)
- Subunit-based (protein, peptide, and DNA) smallpox vaccines have shown promise in animal models
- Recent advances in adjuvants, “omics” technologies, and animal models, as well as vaccinomics and systems biology approaches, are yielding additional insights into poxvirus immunology and can be applied to developing novel vaccine candidates

Table 1
Serious adverse events resulting from smallpox vaccination (numbers reflect U.S. epidemiological data from NYCBH-based vaccines)
[9,52,77,90,99,101-104]

| Serious adverse event | Frequency after primary (P) or re-vaccination (R)(cases/million vaccinees) | Susceptible populations | Treatment | Mortality rate | Notes |
|--|--|--|------------------------------------|----------------|--|
| Generalized vaccinia | P: 23.4 – 241.5 R: 0.4-42.1 | Immuno-compromised | VIG in rare immunosuppressed cases | Low | |
| Eczema Vaccinatum | P: 8.0 – 80.5 R: 0.4 – 5.4 | Individuals with atopic dermatitis/eczema | Hospitalization and VIG | High | Can be caused by primary vaccinee with active lesions coming in contact with susceptible person. |
| Progressive Vaccinia (Vaccinia necrosum) | P: 0 – 2.7 R: 0.3 – 3.0 | Individuals with cell-mediated or humoral immune defects | VIG and antivirals | Very high | Those with cell-mediated defects have worst prognosis. |
| Post-Vaccinal Encephalitis | P: 1.9 – 3.4 R: 0 – 3.0 | Most common in infants <12 months. Autoimmunity or allergic reactions to vaccines? | Supportive care | High | Low rate means causality is difficult to prove. Not believed to be result of replicating VACV. |
| Myo-/pericarditis | P: 4,000-5,000 R: Unknown | Unknown | Unknown | Low | Effects first noted during the 2003 DOD vaccination campaign. |
| Death | P: ~2.9 R: ~0 | | | | Incidence rate is vaccine strain dependent. Most often due to encephalitis or progressive vaccinia |

Table 2
Modern smallpox vaccines [71,106,110,111,113,116,121,129,134,139,145,150]

Second-generation vaccines

| Vaccine | Original VACV strain | Production method | Usage | Efficacy and reactogenicity | Dose/route |
|-----------------|----------------------|---------------------------------|--|---|--|
| ACAM2000 | NYCBH | Vero cells | Part of US national stockpile, approved by FDA in 2008 | Immunogenicity equivalent to Dryvax. Safety profile similar to Dryvax. | 0.0025 mL of live VACV containing $2.5-12.5 \times 10^5$ pfu, 1 dose, percutaneous |
| CCSV (DynPort)* | NYCBH | MRC-5 cells | Clinical trials | Immunogenicity equivalent to Dryvax. Safety profile not well established. | 1 dose, delivered dose similar to Dryvax, percutaneous |
| Elstree-BN* | Lister | Chicken embryo fibroblast cells | Clinical trials | Similar efficacy and safety as Elstree/Lister. | 1 dose, percutaneous, 1×10^8 pfu/ml |
| CJ-50300 | NYCBH | MRC-5 cells | Clinical trials | Similar efficacy as Dryvax. | 1 dose, percutaneous, 1×10^8 pfu/ml |

* no longer in development

Third-generation vaccines

| Live, attenuated vaccinia virus strains | | | | | | |
|---|--|-------------------|---|---|---|--|
| Vaccine | Original VACV strain | Production method | Usage | Efficacy and reactogenicity | Dose/route | |
| MVA (IMVAMUNE, TBC-MVA, ACAM3000) | Ankara, serially passaged in chicken embryo fibroblast cells | Cell culture | Used in 120,000 people at end of eradication. Extensive clinical and animal trials. | Excellent safety profile. Immunogenicity may be lower than replication-competent vaccines. | 2 doses, subcutaneous, 5×10^7 TCID ₅₀ | |
| LC16m8 | Lister, serially passaged in rabbit kidney cells | Cell culture | Used in Japan at end of eradication. Stockpiled in Japan. | Excellent safety profile. Similar reactogenicity to Dryvax. | 1 dose, percutaneous, ~4 ul of 1×10^8 pfu/ml | |
| NYVAC | Copenhagen, with genomic deletions | Cell culture | Early clinical trials | 18 ORFs deleted, coding genes related to pathogenicity, virulence, and host range. Immunogenicity may be lower than live vaccines | Likely 2 doses, intramuscular | |
| dVV-L | Lister, with deleted UDG enzyme | Cell culture | Animal trials | Early animal studies show good immunogenicity and safety profiles in mice. | Likely 2 doses, intramuscular, 1×10^6 pfu in mouse | |
| Subunit vaccines | | | | | | |
| Protein-based, various | Various | | Non-human trials | Theoretically better safety profile. Sufficient immunogenicity in animals when adjuvanted and boosted. | 2-3 doses, intramuscular, likely adjuvanted, human dosage TBD | |
| DNA-based, various | Various | | Non-human trials | Theoretically better safety profile. Sufficient immunogenicity in animals when adjuvanted and boosted. | 2-3 doses, intramuscular, likely adjuvanted, human dosage TBD | |