REVIEWS

# The current and future landscape of smallpox vaccines

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## Abstract

Smallpox is a potential weapon for bioterrorism. There is a need for better smallpox vaccines. The first generation vaccines such as Dryvax were made using crude methods that would not allow licensure today. Second generation vaccines, grown in modern tissue cultures but employing seed virus from first generation vaccines, have been developed. One, ACAM2000, has been licensed and added to the US National Stockpile. These second generation vaccines can produce the same complications as first generation vaccines. Myopericarditis has been well documented as caused by ACAM2000. This has created advocacy for third and fourth generation smallpox vaccines.

Third generation vaccines are viruses that have been attenuated by serial passage in non-human cells, or by careful laboratory deletions of selected genes. Two of these, Modified Vaccinia Ankara, and LC16m8, derived from Lister strain vaccinia, have been tested in human trials. These seem to be ready to apply for licensure if there proves to be a market.

Fourth generation vaccines, created in the laboratory as subunits of full-strength vaccinia, or fully engineered non-replicating molecules that express various epitopes of vaccinia and/or smallpox, have also been developed. Proving the efficacy of these vaccines may be difficult because smallpox no longer exists and there is no animal model that accurately reflects the human disease. These fourth generation vaccines include large DNA viruses into which immunogens from others agents such as HIV and malaria can be inserted. They thus may have a future as vector vaccines for a variety of other agents besides smallpox.

## Introduction

Smallpox is a potential weapon for biological warfare or bioterrorism. Therefore, after 9/11/2001 and the published claims made by the former head of the Soviet Biowarfare program (1-3), public health experts agreed that a safer yet fully effective vaccine against smallpox is needed. Shortly after 9/11/2001, the Department of Health and Human Services created the Biomedical Advanced Research and Development Authority (BARDA). BARDA's mission is to encourage the development of vaccines, antibiotics, and antivirals for agents which might become weapons of biowarfare. BARDA was also given funds to purchase such products and create a National Stockpile. Smallpox was high on the list of agents in BARDA's early interest, because only a few million doses of Wyeth Dryvax were available for use.

In last 17 years a great deal of modern virological research and development has been devoted to this work. Funds have been made available to stock newer vaccines in the US Government's National Strategic Stockpile. This stockpile now contains enough smallpox vaccine to immunize every man, woman and child in the United States. This paper reviews this effort. Readers needing a complete exposition of the virological and genetic information about third and fourth generation vaccines are directed to the chapter on Smallpox and Vaccinia in the 7th Edition of Plotkin, Orenstein, Offit, and Edwards's textbook ***Vaccines*** (4).

## First Generation Smallpox Vaccines

In the last half of the 20th Century, first generation smallpox vaccines in the United States were largely preparations of the New York City Board of Health (NYCBOH) strain of vaccinia. These vaccines were of proven effectiveness, although there has never been a controlled trial of any vaccine against smallpox. They were administered by scratch or multiple pressure and, after 1965, largely by using the bifurcated needle.

## Vaccination Technique

Most first generation vaccines used in the United States after 1965 were lyophilized for better shelf life. This enhanced their effectiveness when taken into tropical areas. A bifurcated needle is dipped into liquid vaccine (lyophilized vaccine after diluent has been added). Capillary action draws a droplet of vaccine into the crotch of the needle. About 15 firm but gentle downward strokes onto the skin of the arm near the insertion of the deltoid muscle are made. The first of these strokes dislodges the droplet of vaccine. The subsequent strokes through this droplet abrade the skin and allow entry of the vaccinia virus into the Malpighian layer. A tiny droplet of blood is often visible at the site, but frank bleeding indicates technique that is too vigorous. The site may be covered by a loose dressing, which helps avoid transfer of the virus to other sites (5, 6). Good technique with fully potent vaccine causes a major skin reaction to develop by about 7 days. This is a central lesion surrounded by

visible inflammation, the so-called "take". This is a viral infection, and thus there is frequently some mild fever and discomfort around 6 to 8 days after inoculation. (5, 6)

**Immunity**

Immunity following vaccination include both humoral antibodies and cellular immunity.

The orthopoxviruses are the largest viruses that infect humans. Their structure and functions are complex. The relationship between the various circulating antibodies against vaccinia virus antigens and the several cell-mediated immune responses evoked are complex. This is an area of active research, with many animal species utilized and hundreds of HLA class I and II epitopes identified (4, 7). No studies have definitively shown what level of antibodies, or what form of cellular immunity, is fully protective. We thus also don't know precisely how long immunity lasts. Currently the most accepted measure of neutralizing antibodies is PRNT (3, 4, 6, 7, 8). These antibodies become detectable by about the 6th day after vaccination and seem to last for several decades. Cellular immunity has been measured in several ways, with different researchers employing different tests for quantifying it. Such immunity lasts for several decades (4, 6).

Epidemiological evidence suggests that some residual immunity lasts for decades after a single primary vaccination. Patients several years after from vaccination sometime acquire smallpox, the disease is generally mild and death rates are low until about five decades post vaccination (7, 9, 10).

**Complications and Contraindications for Smallpox Vaccination**

Anyone who has had a documented, face-to-face contact with a smallpox patient should be vaccinated. There are no contraindications to vaccinating such patients because smallpox is always worse than any complications of vaccinia. There are several complications that can follow vaccination because vaccinia is a viral infection. Anyone who has not had direct contact with a smallpox case must be screened for contraindications (11, 12, 13).

Like many viral infections, vaccinia can occasionally cause post-vaccinial encephalitis, about one or two cases per million primary vaccinees. Encephalitis is more common in infants than in older patients, so infants should not be vaccinated unless they are in contact with smallpox patients.

Patients with eczema or a history of atopic dermatitis can develop eczema vaccinatum. This can be fatal, particularly for infants, in whom eczema vaccinatum can act like a serious burn with loss of protein and electrolytes. Thus, patients with atopic dermatitis should not be vaccinated, nor should family members who have close contact with them.

Patients with diseases or conditions that compromise their immune systems and those who are taking immunosuppressive medications are at risk for developing progressive vaccinia. This condition is often fatal, with vaccinia virus growing out of control and often spreading throughout the body.

Vaccinia does not increase fetal wastage or cause prematurity. However, since it is a viral infection, pregnant woman should not be vaccinated unless they have had direct contact with smallpox patients.

Vaccination leaves live virus on the skin and the developing Jennerian vesicle sheds copious amounts of virus. Infants often scratch the vaccination site and transfer vaccinia to areas such as the eye. The vaccination site can be covered with a loose dressing to reduce such spread.

Good photographs of patients with serious complications of smallpox vaccination can be found in Fenner (6), or at the smallpox section of the CDC website (14).

With no smallpox occurring in the US after 1949 and recognizing the frequency of complications of vaccination (11, 12, 13), the United States abandoned routine smallpox vaccination after 1971-72 (15).

**Production of First Generation Vaccines**

First generation vaccines were produced using technique that would prohibit licensure today (6). The skin of animals, such as cows or sheep, was shaved and then widely inoculated with vaccinia. The resulting inflammatory exudate was scraped off about seven days after inoculation. This exudate contained animal proteins, bacteria, and possibly unknown animal viruses. The FDA would not currently license such vaccines. Thus, there is a need for vaccines that can be produced using methods that meet modern standards of good practice.

**An Ideal New Vaccine**

An ideal new smallpox vaccine would be a live virus that could be lyophilized to prolong its shelf life. It should be amenable for administration via a bifurcated needle. It should produce a visible major reaction ("take") so that successful vaccination could be documented without requiring laboratory work. It should be made in standard cell cultures in large volumes. Clinical trials with such candidate vaccines should produce data showing fewer and less serious complications than first generation vaccines.

Proving the efficacy of new smallpox vaccines will be difficult. There are no simple markers for full effectiveness. Primary vaccination produces an array of circulating antibodies and a complex group of markers of cellular immunity (4, 6, 7). Eradication of smallpox has made field trials of efficacy impossible. Therefore, the FDA has developed the "Two Animal Rule" to substitute for direct data on efficacy. (16) This rule requires vaccine candidates for licensure to show efficacy in two animals in which infection with an orthopox closely related to smallpox has some similarity to humans' infection with Variola major. There is no animal model in which infection with live

Variola virus produces a disease quite similar to human smallpox. The orthopoxviruses that are virologically similar to Variola include monkeypox, ectromelia, buffalopox and vaccinia. These do not cause diseases in mammals (including non-human primates) that is similar to Variola in humans (17, 18, 19, 20). Jahrling has used large intravenous innocula of live Variola virus in monkeys. This often produces lesions on the skin similar to smallpox but does not include the widespread replication of the virus in reticuloendothelial tissues (18). These higher primates are expensive and difficult to work with, requiring special lab facilities. Such studies employing live Variola virus must be performed in the high security lab at CDC in Atlanta and require permission from the World Health Organization. Thus, meeting the two-animal rule will be difficult.

**Second Generation Smallpox Vaccines**

Second generation vaccines are vaccinia strains that are clones of first generation strains of vaccinia of known effectiveness, but are grown on tissue culture and are thus free of bacteria and animal proteins. There are several such vaccines but only one, ACAM2000, has been subject to non-inferiority trials comparing it directly with the first generation vaccine Wyeth's Dryvax (a New York City Board of Health vaccinia vaccine that was used extensively in Africa and Asia during the Smallpox Eradication Program) (7). Dryvax is now known to be a mixture of closely related vaccinia strains, a single one of which was picked to yield ACAM2000.

Straightforward non-inferiority trials allowed licensure. Non-inferiority of ACAM2000 to DRYVAX was shown by measurement of neutralizing antibodies, rates of major reactions ("takes"), and measures of cellular immunity (7, 21). Such head to head comparison trials employing Dryvax or other first generation vaccines are no longer possible because of the documentation of myopericarditis following vaccination with both first generation vaccines and ACAM2000 (22, 23, 24, 25). ACAM2000 is now licensed in the United States and BARDA has added several hundred million doses to the United States Government's National Strategic Stockpile (26, 27).

There continue to be safety concerns about the use of first and second generation smallpox vaccines. There has been an increase in the prevalence of eczema since the studies of complications of vaccination performed in the 1960's (28). There has been a dramatic increase in the prevalence of immunocompromised patients, given HIV, oncological treatments, organ transplants and other conditions which jeopardize the immune system (28). Patients with severe immunological defects are at risk for developing progressive vaccinia, in which vaccinia virus continues to grow unchecked, frequently resulting in death. These concerns have led to vigorous efforts to develop third generation vaccines.

**Third Generation Smallpox Vaccines**

Several third generation vaccine candidates have been developed (4). During the 1960's, the Germans produced a vaccine called Modified Vaccinia Ankara (MVA). A first generation vaccinia strain derived from horses was passed 570+ times in chick embryo fibroblasts. The result is a live virus, but it does not replicate in human tissues. Thus, it acts somewhat like a killed virus vaccine. It does not produce a visible skin lesion. Modern genetic analysis shows that MVA has lost several genes from its parent vaccinia strain (29). Bavarian Nordic markets its MVA strain, IMVAMUNE, and has completed several trials in humans to demonstrate safety. IMVAMUNE has a potency of $10^8$ TCID after reconstitution. Optimal immunity requires two doses of 0.5 ml reconstituted vaccine delivered subcutaneously. Bavarian Nordic's many trials have included patients with HIV and patients with a history of eczema. They have employed several dosing schedules, although none have included young children (30-38).

MVA is not an optimal vaccine for controlling smallpox outbreaks. Optimal immunity requires two doses of MVA administered subcutaneously at about four weeks apart. IMVAMUNE is supplied in individual vials containing 0.5 ml of vaccine with $10^8$ TCID per dose. It must be refrigerated up to the time of use and must be injected with a needle and syringe. It does not produce a visible skin lesion. Meticulous records must be kept because health workers cannot tell at a glance whether a patient has or has not received the initial dose. Since optimal levels of immunity require two doses, contacts of cases may not be protected after initial processing and their first inoculation. IMVAMUNE may be a good vaccine in situations where there is no smallpox, but people with contraindications to vaccination with second generation vaccines require vaccination. These might include laboratory workers exposed to orthopoxviruses and medical workers who might form teams of caregivers during an actual smallpox outbreak.

The Japanese have developed a third generation vaccine named LC16m8, derived from first generation Lister strain vaccinia. While there are fewer published trials than with MVA, they have used LC16m8 extensively, and have experienced few serious complications (39, 40, 41, 42). This vaccine would be good for outbreak control. It is lyophilized and can be used without refrigeration and administered with a bifurcated needle. It produces a visible major skin reaction at the site of vaccination, so that a successful vaccination can be documented at a glance.

**Fourth Generation Smallpox vaccines**

Many new vaccinia-derived strains have been developed by genetic engineering. Several third and fourth generation vaccines have been created by deletion of genes from vaccinia or from creating

strains that have epitopes common to variola or vaccinia (4, 43, 44, 45). These are under development in laboratories, with a few that have progressed to animal experiments. Mostly, these have employed small mammals and challenged them after immunization with viruses such as vaccinia or ectromelia.

While these candidate vaccines may not result in a licensed immunologically excellent vaccine (see below), the interest in them may continue as we increase our understanding of the immune response, including which epitopes seem to be protective against other orthopoxviruses (45).

## Barriers to Developing Newer Smallpox Vaccines

While it would be good to have a safe and effective new vaccine to supplement or replace ACAM2000, development of such a vaccine is doubtful despite excellent viral generic and immunological work. In the absence of actual smallpox or credible threats of bioterrorist attacks, there probably is no market for such a vaccine, and indeed funding for research in this area is limited. Large scale production facilities capable of producing large lots do not exist and would be costly to create and operate. DA Henderson has estimated that the lab research, human field trials, large scale production, storage and marketing of a new vaccine would cost between $750 million and $1.75 billion US dollars (46). More recently, Koblentz pointed out that the US Government is the only possible purchaser of a new vaccine and BARDA has not shown interest in adding to the current National Strategic Stockpile (47). Indeed, BARDA is more interested in ensuring that the large stocks of ACAM2000 and IMVAMUNE will maintain their potency for a very long shelf life.

Given the need for vaccines against HIV, Ebola, SARS, Zika, and other viruses with serious potential as public health problems, justification of diverting the funds and expertise to create and actually produce an improved smallpox vaccine seems highly unlikely.

While first generation Dryvax costed less than a penny a dose during the Smallpox Eradication Program, new vaccines would be much more costly. The newer second and third generation vaccines that have been purchased by BARDA for the National Strategic Stockpile would be free to the public and only used after a documented need for vaccination. ACAM2000 and IMVAMUNE prices are not available, but from the amounts bought by BARDA for the National Strategic Stockpile, we can estimate that their cost is between $4 and $17 per dose.

Research on development of third and fourth generation vaccines will probably progress. Vaccinia and its many artificial variants such as MVA and NYVAC are large stable DNA viruses, relatively safe and easy to work with (4, 41, 42). Given their safety in humans, they may be excellent vectors for other vaccine antigens. In animal models, MVA vaccines are immunogenic and protective against various infectious agents, including HIV, simian immunodeficiency virus, influenza, parainfluenza, measles, malaria, tuberculosis and several cancers (48). An NYVAC based vaccine against HIV shows promise (49).

MVA and other engineered fourth generation viruses such as NYVAC probably have more of a future as engineered vectors than as smallpox vaccines (48). There have recently been infections with other zoonotic poxviruses documented, and the newer smallpox vaccines may be needed if one or more of these new viruses become pandemic or epizootic (51).

## Summary and Conclusions

Given the problems of serious side effects and outmoded production methods, the first generation of smallpox vaccines, despite their proven effectiveness, are not now acceptable. Second generation vaccines whose effectiveness can be assumed because they are made with the same vaccinia strains as first generation vaccines have been created. One, ACAM2000, has shown non-inferiority to first generation vaccine and has been added to the National Strategic Stockpile.

Third generation vaccines, which are derived from first generation vaccinia strains by serial passage in non-human tissues or by genetic modification of such strains in modern viral genetic laboratories, show promise as practical vaccines. MVA (Modified Vaccinia Ankara) has undergone several trials for safety in humans, including those who are HIV positive or have atopic dermatitis. It may be a good vaccine for use in persons who have contraindication to vaccination with first or second generation vaccines, but who require vaccination. MVA has been added to the National Strategic Stockpile. The Japanese vaccine LC16m8 seems good for outbreak control because it can be lyophilized, administered with a bifurcated needle, and produces a visible major reaction on the skin that proves its “take”. LC16m8 has not yet been submitted for licensure in the United States.

There are many fourth generation vaccine candidates, produced by modern immunologic and virologic techniques. These are subunits of vaccinia with several genes removed, or vaccines created *de novo* by adding various epitopes or other immunogens from vaccinia to artificially created molecules. The cost and difficulty in proving that such vaccines are effective against smallpox may inhibit their full development as smallpox vaccines. They may prove very good as “vector vaccines” for other infectious agents because immunogenic parts of such agents can be added to their genetic structure.

## About the Author

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**Competing Interests**

The author has no competing interests to declare.

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