DMID 01-650 PROTOCOL

TITLE: A Multicenter, Randomized Dose Response Study of the Safety, Clinical and Immune Responses of Dryvax® Administered to Children 2 to 5 Years of Age

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27 Sept 2002
Version 2.0

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ABSTRACT OF PROTOCOL

Purpose: This study is to evaluate the potency, dose and safety of vaccinia virus vaccine (Dryvax®) administered to children in the event there is a smallpox terrorist event. The objective of this study is to evaluate the safety and the rate of clinical and immune responses with stockpiled Dryvax vaccine when administered to children 2-5 years of age. It will be evaluated undiluted and at a 1:5 dilution, with 5 skin punctures; additionally, the safety of semi-occlusive dressings to limit self-inoculation and secondary transmission will be evaluated. Revaccination of non-responders will help define the value of a second dose. The vaccine take and immune response rate will be compared between pediatric subjects in the two study groups and by comparisons with results from similar trials conducted in adults.

Research Environment: Two NIH Vaccine and Treatment Evaluation Units (VTEU): 1) UCLA Center for Vaccine Research, and 2) Cincinnati Children's Hospital.

Volunteers: A total of 40 volunteers will participate in the study.

Volunteer Participation: The volunteers will be immunized by scarification with Dryvax on day 0. All volunteers will be observed by their parents/legal guardians at their homes and will be excluded from daycare and school for at least 30 days from the date of immunization and until a scab is well formed. At visit days 6-8, if there is no "take", the subject will be re-vaccinated with the same dose and method of administration of Dryvax as for the first vaccination. Each subject's participation will last for at least 6 months. During this time volunteers will return periodically for dressing changes, assessment of any adverse safety events, and for blood draws to check immune responses. A subset of subjects will have immune responses followed for 3 years.

Variables to be Investigated: Adverse events to and side effects of the vaccine, and clinical and immunogenicity responses, including vaccine " take", antibody responses, intracellular cytokine production and IFN-γ responses to the vaccine.

Risk/Benefits: The risks of participating in this study are those known to exist for vaccination with vaccinia vaccine (Dryvax) and those of blood draw. The benefit to the volunteer is potential protection against smallpox disease if the volunteer has a "take". The benefit to society is to determine whether smallpox vaccine can be diluted to provide an increased number of doses in the event of a release of smallpox into the environment, at the same time providing adequate protection in children.

Confidentiality: Volunteers will have unique study identification numbers and will not be identified by name.

1.0 STUDY OBJECTIVES

1.1 Overall objectives

The overall objective of this study is to evaluate Dryvax in children and assess the clinical and immune responses, as well as the safety of Dryvax administered undiluted and at a 1:5 dilution, with 5 skin punctures and with the use of a semi-occlusive dressing.

1.2 Specific objectives

1.2.1 Primary Objective

To evaluate the cutaneous responses (take rates) 6-8 days after vaccination in children given undiluted and diluted vaccine (1:5 dilution).

1.2.2 Secondary Objectives

1. To evaluate immunologic responses in children given undiluted and 1:5 diluted vaccine

2. To ascertain the clinical and immunologic responses and safety of 5 intradermal punctures with a bifurcated needle.

3. To assess the safety profile of vaccine in the vaccinated individual and assess the risk to contacts.

4. To qualitatively assess the cutaneous and serologic response rates in children relative to those observed in adults (comparison to a separate but parallel study).

5. To assess the revaccination immunologic response rates of non-responders.

2.0 Background/study rationale

2.1 Background

In light of recent terrorist attacks, particularly those involving the transmission of anthrax from contaminated mail, there has been heightened concern over the use of infectious agents as population weapons. The two agents thought by experts to be potentially most dangerous are smallpox (variola) and anthrax (1, 2). Both are characterized by features which make them ideal weapons of bioterrorism, including: 1) high mortality rate (up to 30% in unvaccinated persons) 2) stability, 3) transmission by aerosol, 4) capability of large-scale production and storage, 5) potential to cause widespread panic, 6) delayed disease recognition, and 7) a requirement for intense utilization of health-care resources (3).

At the present time, the only two World Health Organization-approved repositories of smallpox virus are located at the Center for Disease Control and Prevention (CDC) and the Russian State Research Center on Virology and Biotechnology, Koltsovo, Novosibirsk Region (2, 4). The number of potential covert stockpiles at other sites is unknown. Following the last known naturally occurring human case of smallpox in 1977, it was resolved by the World Health Assembly that all stocks of smallpox virus be destroyed in June 1999 (2,4). Subsequently, concerns over the potential use of smallpox for biologic warfare were felt to mandate a need for further study of variola virus, and plans for its destruction have been postponed.

Variola virus infects only humans and is transmitted by aerosol. Prior to 1979, smallpox had worldwide distribution (4,5) and required an intensive public health vaccination effort to eradicate. The incubation period lasts 7-17 days, after which fever, aching pains and prostration develop (4,6). Two to three days later a papular rash develops over the face, hands and forearms, extending to the trunk and lower extremities over the next week. The lesions quickly evolve into pustular vesicles, and finally scab and heal, leaving scars. Unlike varicella, the development and resolution of lesions are synchronous. A more malignant rapidly fatal form of the disease, hemorrhagic smallpox, develops in 5%-10%. Smallpox is transmissible from the time of the appearance of rash until all the scabs separate. The secondary attack rate among unvaccinated household contacts is 25-40% (4).

Vaccination against smallpox is protective if administered before exposure or within 2-3 days thereafter (7). Following eradication of smallpox, Dryvax production for general use in the US was discontinued in 1982 and general distribution of smallpox vaccine was discontinued in 1983 (8). Few physicians in the United States have actually seen a case of smallpox, so in the event of an outbreak, smallpox may not be diagnosed until late in the course, after secondary transmission has already occurred. A single case of smallpox would be expected to result in 10-20 more cases through aerosol transmission (2). Although it is estimated that approximately 20% of the adult population has some residual immunity from prior vaccinations, it is not known how protective this immunity would be in an outbreak (5). In the event of a bioterrorist threat, to prevent further transmission, infected patients would need to be isolated in negative pressure facilities (7). Although the probability of such an event is low, the effect in a largely unimmunized population would be significant and could result in millions of deaths in the U.S. alone (2).

Currently, there is no FDA approved therapy for smallpox and only a limited stockpile of vaccine prepared twenty years ago is available for use (7). Dryvax (Wyeth Laboratories) is a purified and lyophilized product prepared from lymph obtained from the skin of vaccinia-infected calves. Wyeth was the sole producer of smallpox vaccine, terminating production of the vaccine in 1983 (8,9). Although there are an estimated 15 million doses of vaccine currently in storage at the CDC, its use for prophylaxis of the general population is not recommended, nor is it generally available.

Essentially the entire U.S. population is now susceptible to smallpox and

the standards for vaccine safety are now much more stringent than they were 30 years ago. Studies of Dryvax in adults conducted at St. Louis University and other VTEUs are utilizing Dryvax in ways for which it was not licensed or recommended. In these studies a double, semi-occlusive dressing with gauze and a double layer of a semi-permeable membrane (Opsite) was used to reduce contact with the vaccination site and secondary transmission to others. This dressing allows moisture to escape, but virus does not penetrate. Two layers of Opsite are required to obtain negative cultures from the top of the dressing. Virus can be cultured from the lesions for 30-60 days. Although the use of occlusive dressings is contraindicated by American Academy of Pediatrics (AAP) Committee on Infectious Diseases and Advisory Committee on Immunization Practices (ACIP) recommendations, this recommendation is based on historical data from 30-40 years ago before the semi-permeable dressings were available and before there was such a risk of secondary transmission. Concerns regarding the use of semi-occlusive dressings include an increase in the risk of adverse viral reactions and increased risk of bacterial superinfection. None of these adverse events has been found in about 740 adult subjects studied in multi-centered Dryvax studies sponsored by the NIH.

Aventis Pasteur recently revealed that they were in possession of a liquid form of live vaccinia virus vaccine prepared from calf lymph. The bulk product was released to the National stockpile. However, limited data are available on its use; the vaccine has been in storage and has not been tested in humans in over 40 years.

In addition, the Department of Defense is promoting the development of an uncontaminated vaccinia virus vaccine produced in tissue culture. Pending availability and the ability to test a new cell culture-derived vaccine, it is necessary to maximally utilize the currently existing stockpile in the event of a biological emergency. One way to increase the available number of doses would be to dilute the vaccine; however, the number of dilutions that could be performed without compromising vaccine immunogenicity and effectiveness is unknown for children.

2.2 Vaccinia vaccine trials

Two studies have been conducted in vaccinia naïve adults with diluted and undiluted Dryvax vaccine. A pilot study has been completed with undiluted vaccine, 1:10 and 1:100 vaccine dilutions and a second, larger study was conducted to determine if a 1:5 or 1:10 dilution of Dryvax would provide an adequate “take” (and by implication, adequate protection), should widespread vaccination be necessary (10,11). The studies and results are summarized below.

2.2.1 Studies of Primary Vaccination of Adults with Dryvax Dilutions

2.2.1.1 Pilot Study of Dilutions of Dryvax in Vaccinia-naïve Adults

Design

The NIAID conducted a single-center, double blind, randomized trial of three dilutions of Dryvax vaccine in 2000 in an effort to see if the limited remaining stocks of Dryvax could be diluted and still induce successful viral replication at the inoculation site, and promote development of serologic and cell-mediated immune responses (10).

Methods

Sixty healthy young adults who had no history of previous smallpox vaccination were randomized to one of three dose groups for inoculation with Dryvax, administered by 15 insertions of a bifurcated needle: 20 each to receive undiluted, a 1:10 dilution, or a 1:100 dilution. Back titrations revealed that the mean potencies of the undiluted and diluted vaccines were $10^{7.8}$ (range, $10^{7.4}$ to $10^{8.3}$), $10^{6.5}$ (range, $10^{6.1}$ to $10^{7.0}$), and $10^{5.0}$ (range, $10^{4.2}$ to $10^{5.9}$) pfu/mL, respectively. The vaccination sites were covered with a folded gauze pad and two layers of a semi-permeable adhesive membrane until the lesion formed an eschar.

Results – Clinical and Serologic Takes

Of the 20 volunteers who were vaccinated with undiluted Dryvax ($10^{7.8}$ pfu/mL), 19 (95%) developed Jennerian vesicles or "take" seven to nine days after vaccination. Seventeen of the 19 with a take had four fold or higher increases in their neutralizing antibody titers at one month, and 15 of those 17 had detectable neutralizing antibodies one year after vaccination. Fourteen of the 19 with a take had four fold or higher increases in their vaccinia binding titers by ELISA at one month and all 17 tested had detectable vaccinia binding antibodies one year after vaccination.

Of those vaccinated with the $10^{6.5}$ pfu/mL vaccine, 14 (70%) developed a clinical take. All 14 had four fold or higher increases in their neutralizing antibody titers at one month and 9 of the 13 tested had detectable neutralizing antibodies one year after vaccination. Eleven of the 14 with a take had four fold or higher increases in their vaccinia binding titers by ELISA at one month and 10 of 13 tested had detectable vaccinia binding antibodies one year after vaccination.

Of those vaccinated with the $10^{5.0}$ pfu/mL vaccine, only three (15%) developed a take. All 3 had four fold or higher increases in their neutralizing antibody titers at one month and detectable neutralizing antibodies one year after vaccination. Two of the three with a take had four fold or higher increases in their vaccinia binding titers by ELISA at one month and all three had detectable vaccinia binding antibodies one year after vaccination.

Cell-mediated Immunity Assays

Cytotoxic T-lymphocyte (CTL) responses were detected at six months in all 19 who developed a vesicle after vaccination with undiluted Dryvax ($10^{7.8}$ pfu/mL). Sixteen of them had positive results of enzyme-linked immunospot (ELISPOT) assays for detection of vaccinia virus-specific interferon-ã in peripheral blood mononuclear cells at six months post-vaccination. All 19 demonstrated positive lymphocyte proliferation in response to vaccinia virus. The individual who did not develop a vesicle had negative results for these assays.

Ten of eleven of those who developed a vesicle after vaccination with the $10^{6.5}$ pfu/mL vaccine and were tested for CTL responses tested positive. Ten of twelve who developed a vesicle and were tested with the ELISPOT assay tested positive. Eleven of twelve who developed a vesicle and were tested for lymphocyte proliferation tested positive. None of the six who did not develop a vesicle tested positive on any of these assays.

All three of those who developed a vesicle after vaccination with the $10^{5.0}$ pfu/mL vaccine tested positive for CTL responses as well as with the ELISPOT and lymphocyte proliferation assays. One volunteer who did not develop a vesicle tested positive for a CTL response. None of the others tested positive.

In comparing the three dose groups, the CTL responses were greatest in the undiluted vaccine group. The difference was statistically significant ($P<0.001$) between the undiluted and the $10^{5.0}$ pfu/mL vaccine groups; however, the difference between the undiluted and the $10^{6.5}$ pfu/mL vaccine groups did not quite reach statistical significance ($P=0.052$). With respect to the ELISPOT results, the magnitude of the interferon-ã responses was significantly lower among those given the $10^{6.5}$ pfu/mL and $10^{5.0}$ pfu/mL vaccines ($P=0.038$ and $P<0.001$, respectively.) No significant differences were observed among the groups with respect to the lymphocyte proliferation in those who developed a vesicle.

Local Reactions

If a vesicle developed, the resulting lesion was approximately 10 mm in diameter, regardless of the dose of the vaccine. Vaccinia virus was isolated from swab samples of the skin lesions in 35 of 36 volunteers with vesicles. There was no evidence of bacterial infection in this study.

Complications

No serious adverse events were reported.

2.2.1.2 Multi-center Study of Dilutions of Dryvax Vaccine in Vaccinia-naïve Adults

Design

Building on the results of the pilot study described above, the NIAID conducted a multi-center (four centers, five sites) study in late 2001 to obtain more precise estimates of the success rates and incidence of adverse events with dilutions of Dryvax against smallpox. For this expanded study, the doses examined were undiluted vaccine and dilutions at 1:5 and 1:10. The results of the pilot study suggested that the success rates with the 1:10 dilution (and, perhaps, the 1:5 dilution) would not be satisfactory if a single vaccination was relied upon; therefore, this protocol incorporated a revaccination strategy. Volunteers were to be given a second vaccination with the same dose of vaccine if a clinical take was not present at the day 7 to 9 visit after the first attempt at vaccination (11).

Methods

Across the four centers, 680 healthy young adults who had no history of previous smallpox vaccination were randomized to one of three dose groups for inoculation with Dryvax administered by 15 insertions of a bifurcated needle: 106 to receive undiluted, 234 to receive a 1:5 dilution, and 340 to receive a 1:10 dilution. Back titrations revealed that the mean potencies of the undiluted and diluted vaccines were $10^{8.1}$ (range, $10^{7.8}$ to $10^{8.4}$), $10^{7.2}$ (range, $10^{6.9}$ to $10^{7.5}$), and $10^{7.0}$ (range, $10^{6.0}$ to $10^{7.2}$) pfu/mL, respectively. The vaccination sites were covered with a folded gauze pad and two layers of a semi-permeable adhesive membrane until the lesion formed an eschar.

Results – Clinical Takes (Immune assay results are not currently available)

The initial vaccination was successful in 665 of the 680 subjects (97.8%) and no significant difference was observed over the range of titers tested. Three of the four participating sites had successful initial vaccination in all of their subjects. Revaccination of 14 of the 15 without takes after the initial vaccination resulted in seven additional successes for an overall take rate of 98.8 percent.

Of the 106 volunteers who were vaccinated with undiluted Dryvax ($10^{8.1}$ pfu/ML), 103 (97.2%) developed a Jennerian vesicle or "take" seven to nine days after the initial vaccination. Neither of the two who failed to react to the primary vaccination was successfully revaccinated. Of the 234 vaccinated with the $10^{7.2}$ pfu/mL vaccine, 232 (99.1%) developed a clinical take after the initial vaccination. Both of the failures were successfully revaccinated. Of the 340 vaccinated with $10^{7.0}$ vaccine, 330 (97.1%) developed a clinical take after the initial vaccination. One of the ten failures could not be revaccinated due to logistic constraints; five of the other nine were successfully revaccinated for a final take rate for the $10^{7.0}$ vaccine of 98.5%.

Local Reactions

If a vesicle/pustule developed, the resulting lesion reached its maximum size (mean diameter 12.4 mm) by days 13 to 14 post-vaccination. The maximum size of surrounding erythema (mean diameter 51.4 mm) and induration (mean diameter 48.1 mm) were observed on days 10 to 12. Some successfully vaccinated subjects had very large areas of redness and swelling; 10% of the 665 successfully vaccinated with the primary vaccination had areas of redness exceeding 100 mm in diameter. Satellite lesions near the inoculation site occurred in 5.8% on days 13 to 14. Regional lymphadenopathy occurred in 30.5% on days 7 to 9. Pain at the vaccination site was graded as moderate or severe in 33.8% of the subjects with a take on days 7 to 9, and 30.4% on days 10 to 12.

The mean pustule sizes on days 13 to 14 post-vaccination were the same for the three dose groups. The mean diameters of erythema and induration were significantly larger and the frequency and severity of regional lymphadenopathy were significantly greater in those who received the undiluted vaccine compared with those who received either dilution of vaccine. Curiously, the occurrence of satellite lesions was most frequent in those receiving the $10^{7.2}$ vaccine; whereas, no satellite lesions were observed in those receiving the undiluted vaccine.

Systemic Reactions

Systemic signs and symptoms included fever, headache, muscle aches, chills, nausea, fatigue and rashes at remote sites. Headaches, muscle aches, fatigue were graded as severe by more than two percent of the 665 subjects with vesicle formation at varying periods after vaccination. There were no significant differences among the dose groups with respect to systemic signs and symptoms, with the exception of a higher incidence of muscle aches during the first week post-vaccination among those who received the $10^{7.2}$ vaccine.

Complications

Twelve subjects had serious adverse events (SAE). Seven of these events involved visits to emergency rooms or hospitalizations that were considered unrelated to the vaccination. Two other SAEs were classified as probably not related: a case of bronchitis twelve days after vaccination and an episode of syncope four days after vaccination. One subject had a high fever (40.1^{o}C) on day 11 that was classified as possibly related to the vaccine. Two subjects had an SAE that was classified as definitely related to the vaccine: a case of severe headache with nausea lasting between days 5 to 14 post-vaccination and a case involving a very large area of erythema (290 mm x 65 mm) around the vaccination site that developed on day 8 and resolved by day 15.

2.2.2 Prior Studies of Primary Vaccination of Children with Dilutions of Dryvax

2.2.2.1 NIAID Studies by Galasso, Cherry, and others, 1970 to 1973

Design

In the early 1970s, the NIAID conducted a series of studies to define the best vaccine and/or vaccination procedure for use in areas of the world that were then free of smallpox with the goal of minimizing the morbidity associated with vaccination (12). One of these investigations focused on primary percutaneous vaccination of 786 children between the ages of one and nine years (13). A large proportion (58.4%) of these children were between the ages of one and two years; 3.9% were six years or older.

Methods

Three concentrations (10^6, 10^7, and 10^8 pock-forming units (pfu) per mL) of four different vaccine strains were administered by

scarification with five insertions of a bifurcated needle: 1) New York City calf lymph (NYC-CL), a licensed vaccine prepared by Wyeth Laboratories (Dryvax); 2) New York City chorioallantoic membrane (NYC-CAM), a licensed vaccine prepared by Lederle Laboratories; 3) the attenuated vaccinia strain CVI-78 (which was mistakenly labeled "CV-1" in this series) produced by Wyeth Laboratories (14); and 4) the Elstree strain of vaccinia prepared by the Lister Institute in England. Only the results of the primary vaccinations with Dryvax will be discussed below, as they are most relevant to the present study.

Results – Clinical and Serologic Takes

Of the 77 children who received undiluted Dryvax (10^8 pfu/mL), 75 (97%) had a major cutaneous reaction defined as the presence of a Jennerian vesicle one week post-vaccination and a serologic take defined as detectable neutralizing and/or hemagglutination-inhibition antibodies. (1:10) at 28 days post-vaccination. Of the 78 children who received Dryvax diluted at 1:10 (10^7 pfu/mL), 58 (74 %) had major reactions and serologic takes and an additional child had a serologic take without developing a major reaction. Of the 72 children who received Dryvax diluted at 1:100 (10^6 pfu/mL), 31 (43%) had major reactions and serologic takes, one had a major reaction without a serologic take, and five had serologic takes without major reactions.

Local Reactions

The mean central lesion diameter at 10 days post-vaccination was approximately 9 mm in the children vaccinated with either the undiluted Dryvax or 1:10 dilution versus approximately 7 mm in those vaccinated with the 1:100 dilution. The mean diameter of erythema was approximately 27 mm in the children vaccinated with the undiluted Dryvax versus approximately 21 mm in those vaccinated with the 1:10 or 1:100 dilutions.

Complications

Eight children out of 148 who had been vaccinated with Dryvax (and for whom adequate follow-up information was available) developed satellite lesions. Three developed erythema multiforme. One child developed a mild illness with generalized vesicular lesions that was classified as generalized vaccinia but this diagnosis was not confirmed. Information about the concentrations of Dryvax received by the children experiencing these complications was not provided.

2.2.2.2 Dryvax Dilutions Administered to Children by Jet Injection

Design

Millar and others at the Centers for Disease Control conducted a series of studies on the safety and efficacy of intradermal jet injection of Dryvax vaccine in the 1960s. In one of the studies, they compared the responses in 625 children in Jamaica to primary vaccination with dilutions of Dryvax administered by jet injection with multiple pressure inoculation of undiluted vaccine (15).

Methods

Four dilutions of Dryvax were tested with the jet injector: $10^{6.1}$, $10^{5.4}$, $10^{5.1}$, and $10^{4.1}$ pfu/mL. The volume of vaccine injected was 0.1 mL per dose. This is 40 times the volume of vaccine that the bifurcated needle is supposed to deliver (0.0025 mL). Therefore, comparable doses for delivery by multiple insertions of a bifurcated needle would be $10^{7.7}$, $10^{7.0}$, $10^{6.7}$, and $10^{5.7}$ pfu/mL or roughly concentrations corresponding to undiluted, 1:5, 1:10, and 1:100 dilutions, respectively. The undiluted Dryvax administered by multiple pressure technique had a titer of $10^{7.6}$ pfu/mL.

Results – Clinical and Serologic Takes

The two most concentrated dilutions (equivalent to undiluted and 1:5 dilution) produced clinical takes in more than 97% of the children, $10^{5.1}$ pfu/mL (equivalent to 1:10 dilution) produced takes in 90%, and $10^{4.1}$ pfu/mL (equivalent to 1:100 dilution) produced takes in 62%. All but two of the children who developed Jennerian vesicles seroconverted and none without a vesicle seroconverted.

Local Reactions

The vesicles and scars were generally smaller in those who received the vaccine by jet injection compared to those who were vaccinated with the bifurcated needle.

Complications

No vaccinial complications occurred in the children in this study.

2.3 Rationale for the current study

Given the limited amount of smallpox vaccine currently available, there is a public health need to specify an optimal minimal dose and optimal vaccination strategy to protect the maximum number of people in the event of a terrorist release of the smallpox virus. The current adult trials summarized above have focused on the immunogenicity and take rate of Dryvax vaccine in varying dilutions. However, in a pediatric population, the safety assessment is particularly important. Even though Dryvax was a licensed vaccine with prior widespread use in millions of children, the Food and Drug Administration (FDA) currently regards it as an investigational new drug (IND). The US population is no longer immune to smallpox and there are other reasons for concern about the use of this stockpiled vaccine.

This is a non-sterile bovine product proposed for use at a time when standards of vaccine safety are much more stringent than they were 30 years ago. Our population must now be considered essentially non-immune and there is greater potential for secondary transmission of virus to susceptible children and adults, and to those with immunologic deficiency states who have greater potential for serious complications. There is little information available concerning the risk of secondary transmission of vaccinia in a largely non-immune population. Control of possible autoinoculation and transmission from vaccinees to contacts is more problematic for infants or young children than adults. The effectiveness of placing a semi-occlusive dressing over the vaccination site, to minimize risk of transmission has never been adequately studied. Finally, fewer intra-dermal punctures (3-5 punctures) have been used to administer the vaccine to children than in adults (10-15 punctures).

3.0 STUDY DESIGN

3.1 Overview

There are multiple considerations concerning safety and a sense of national urgency that influence the study design. First this is a difficult and time-consuming study that must be done with some urgency; thus, limiting the sample size and number of study groups is imperative. Second, there is significant concern about safety and secondary transmission to research personnel, family members and other contacts over a prolonged period, especially for our non-immune populations (the vaccine has not been used since 1971). To limit the risk of transmission, vaccination sites will be covered with a semi-occlusive dressing, a procedure not recommended in prior guidelines. Because virus can be cultured from the vaccination site for 30-60 days after inoculation, it seems prudent to additionally exclude child subjects from daycare or school for at least 30 days after immunization. This effectively precludes from the study the enrollment of children in grade school, middle school or high school. It is unlikely that the safety profile and immune responses in adolescents (10-17 years) would be different from those found in adults. Third, to avoid confusion about potential systemic vaccine reactions, it is wise to avoid giving vaccine in proximity to

receipt of measles, mumps, rubella and varicella (MMR,V) vaccine or any other childhood vaccines. Therefore, it is preferable to avoid subjects in the first and second years of life. For these reasons, it seems appropriate to limit the study to children ages 2-5 years of age. Observations in children of this age should be relevant to children of any age. This study employs phase I type objectives and methods and only healthy children will be recruited. Options are presented for a secondary supplementary phase of study, which will require a protocol amendment, if needed.

3.2 Dryvax vaccine

Dryvax is a lyophilized vaccinia vaccine prepared from calf lymph. It has previously been licensed for immunization against smallpox. The diluent for Dryvax contains 50% glycerin, 0.25% phenol in Sterile Water for Injection. Each reconstituted vaccine vial contains approximately 100 million live vaccinia virus particles per mL. The vaccine has low levels of bacterial contamination (<200 organisms/mL).

Each vial must be reconstituted with 0.25 mL of standard diluent. The dosages to be studied are: undiluted vaccine, and a 1:5 dilution of reconstituted vaccine. A copy of the package insert is available in Appendix A and specific reconstitution and dilution instructions are available in the Manual of Procedures.

3.3 Study population

Healthy children, 2 to 5 years of age, will be enrolled equally at two NIAID Vaccine Treatment and Evaluation Units (VTEUs): 1) UCLA Center for Vaccine Research and 2) Children's Hospital, Medical Center of Cincinnati.

The UCLA Center for Vaccine Research will enroll children from two sources: 1) children receiving healthcare through the Kaiser Permanente Health Plan and 2) children enrolled from the community surrounding the Harbor-UCLA Medical Center. The study population should be accessible for multiple evaluations and be racially and ethnically representative of the Southern California population shown below:

	American Indian/ Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female	0.5%	3.5%	6%	22%	18%	0	50%
Male	0.5%	3.5%	6%	22%	18%	0	50%
TOTAL	1%	7%	12%	44%	36%	0	100%

The Children's Hospital Medical Center of Cincinnati will enroll children from pediatric practices in the greater Cincinnati area. The gender and ethnic breakdowns of the study population from the greater Cincinnati area are shown in the table below:

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female	0%	0%	11.5%	0%	37.75%	0.75%	50%
Male	0%	0%	11.5%	0%	37.75%	0.75%	50%
TOTAL	0%	0%	23%	0%	75.5%	1.5%	100%

3.4 Inclusion and exclusion criteria

Inclusion criteria

- Age: 2-5 years
- Good health as ascertained by medical history, screening evaluation form and clinical assessment
- Negative serology for HIV (negative Western blot for those with positive HIV serology)
- Availability for follow-up for planned duration of the study (6 months; 3 years for a subset in each group)
- Parental signed informed consent
- Complete immunization status for age (No live attenuated vaccines within 30 days of enrollment; no inactivated vaccine within 14 days of enrollment)
- Normal serum creatinine for age

Exclusion criteria

- Current or history of atopy, eczema or other exfoliative skin disorders/conditions
- Any acute skin conditions e.g., burns, impetigo, lacerations not likely to resolve by day of vaccination
- Primary or acquired immunodeficiency disease (including HIV), cancer or use of immunosuppressive medications
- Any child with recurrent pyogenic infections suggestive of an immune deficiency
- Known or suspected impairment of immunologic function including, but not limited to clinically significant liver disease; diabetes mellitus; moderate to severe kidney impairment; failure to thrive; malignancy; autoimmune disease (lupus, rheumatoid arthritis, etc.); use of immunosuppressive medications, including topical or systemic steroids (steroid nasal sprays are permitted)
- Acute febrile illness (temperature greater than 38°C or 100.4°F, axillary measurement) on the day of vaccination
- Daycare or school attendance, unless the child can remain out of daycare/school for a minimum of 30 days after immunization, and until the lesion is well scabbed

- Receipt of a live attenuated vaccine within 30 days or a non-living vaccine within 14 days of enrollment.
- Any child expected to receive a vaccine within 30 days following Dryvax administration.
- Receipt of blood products or immunoglobulin in the 3 months prior to enrollment
- Use of any investigational agents within 30 days prior to or during study entry
- History of vaccinia vaccination or receipt of other pox vectored vaccine
- Allergies to the following:
  - any component of the vaccine (e.g., polymyxin B sulfate, dihydrostreptomycin sulfate, chlorotetracycline hydrochloride, neomycin sulfate)
  - any known component of the diluent
  - any known component of VIG, i.e., thimerosal or previous allergic reaction to immunoglobulins
  - cidofovir or probenecid
- Medical or psychiatric condition or occupational responsibilities of the parent which preclude subject compliance with the protocol
- Behavioral, developmental, or psychiatric conditions in the child which preclude subject compliance with the protocol
- Household members or contacts with any of the following:
  - pregnancy
  - <12 months of age
  - Immunodeficiency disease (including HIV), cancer, or use of immunosuppressive medications
  - Current or history of atopy, eczema, other exfoliative skin disorders/conditions, or any acute skin conditions such as those listed above
- Any condition which, in the opinion of the investigator might interfere with the study objectives.

3.5 Study groups

This study will assess whether vaccine in a 1:5 dilution is equivalent to undiluted vaccine, both administered in 5 skin punctures. This requires 2 study groups.

3.5.1 Initial stage of study

Study Group	Purpose	Sample Size (N)	Vaccine Dilution	Skin Punctures with Bifurcated Needle	Semi-Occlusive Dressings
1	Positive control with dressing	20	Undiluted	5	Yes
2	Asses dilution	20	1:5	5	Yes

Group 1: This is a control group that uses the previous WHO recommended standard vaccine dose and 5 skin punctures but with a semi-occlusive inoculation site dressing as used in the adult Dryvax vaccine trials. It is designed to assess the take rate, immunologic response and reaction rates with undiluted vaccine. Results from this group will be useful to compare to prior experience in children (12), data from adults (10,11) as well as in comparison with the 1:5 dilution group.

Group 2: This group is designed to evaluate the take rate and safety in children when given a 1:5 dilution of the vaccine using the same 5 skin punctures as the undiluted vaccine (Group 1). Results will also be compared to adults and to results from previous pediatric studies (12).

Subjects in either group without a take after the first vaccination will be revaccinated.

3.5.2 Potential second follow-up study

A second stage of the study permits a fine-tuning of needed comparisons or to expand the number of subjects evaluated. Utilizing results from the initial phase study, secondary objectives can be later evaluated with fewer subjects and in less time. The second phase could evaluate more subjects given 1:5 dilution with 5 punctures or, if necessary, may include groups given 1:10 or dilutions greater than 1:10. This second stage might require 1-3 additional study groups and the required sample size depends upon the observed safety and immune responses in the initial stage of study. The safety monitoring committee (SMC) will assist the investigators with need for and definition of the optimal second stage study groups. Dependent upon the initial results additional study groups might include one or more of the following study groups:

Study Group	Purpose	Sample Size (N)	Vaccine Dilution	Skin Punctures with Bifurcated Needle	Occlusive Dressings
3	To increase Group 2 sample size	20	1:5	5	Yes or No (depending on safety determination of initial stage)
AND / OR					
4	To evaluate more diluted vaccine, if Group 2 is adequate	20	1:10	5	Yes or No (depending on safety determination of initial stage)
AND / OR					
5	To evaluate a more diluted vaccine, if the response to Group 2 is adequate	20	> 1:10	5 or more	Yes or No (depending on safety determination of initial stage)

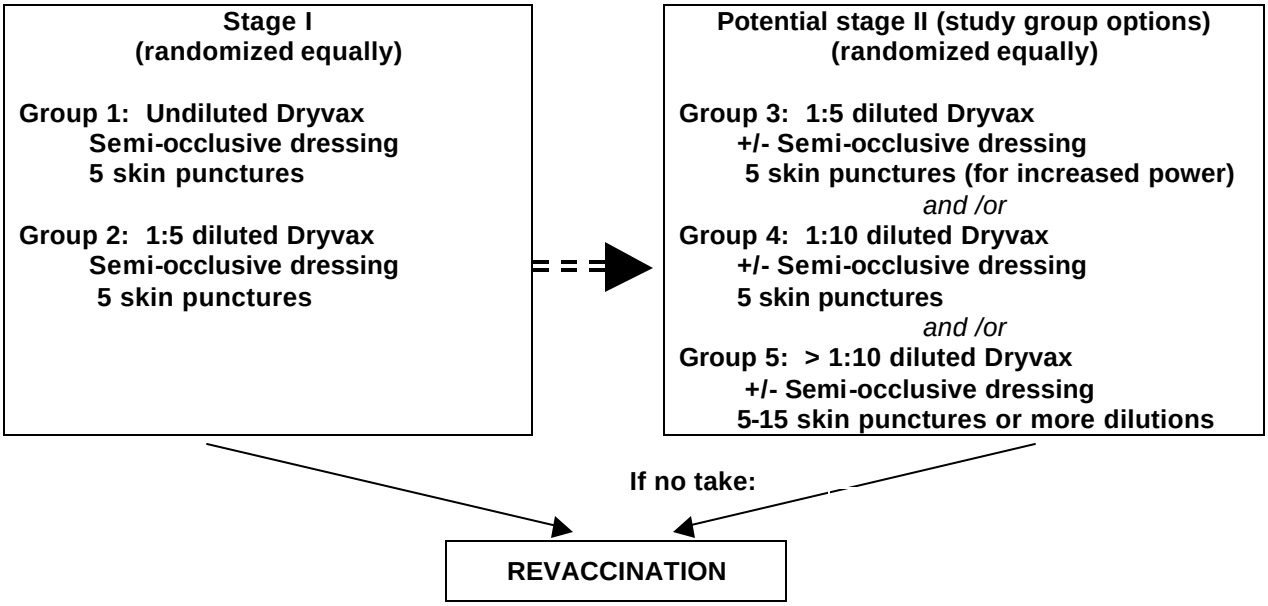
Group 3: If the 1:5 dilution has an acceptable response, then more subjects can be added to this group to increase the sample size.

Group 4: If the 1:5 dilution has a very good response, then a 1:10 dilution of vaccine could be evaluated in this group, presumably with 5 punctures.

Group 5: In addition, if the 1:10 dilution has a good response, a >1:10 dilution of vaccine might be considered and possibly 15 punctures can be evaluated, as in adults.

When the results of the first stage of the study are known, the investigators, NIH, and the SMC will decide the following: 1) which dilution requires further evaluation or increase in sample size for statistical precision 2) sample size, 3) safety and need for semi-occlusive dressing, and 4) optional study comparisons. Conceivably, a change in inclusion or exclusion criteria or in age may be needed. If a result in one of the first 2 groups is deemed to be acceptable, an expansion of the number of subjects in that group alone may be recommended. This second stage will involve an additional 15-45 subjects in 1-3 study groups. The joint recommendation will be submitted to the IRB as an amendment to the protocol and the safety and effectiveness data for justification will be provided.

3.5.3 Diagram of study groups and stages



3.6 Study procedures

3.6.1 Subject recruitment and education

Children will be recruited from the community, local pediatric practices, health maintenance organizations or other healthcare settings close to the study centers in Los Angeles and Cincinnati. Methods for distributing information about the study will vary between the study sites, but will generally include letters to pediatric healthcare providers, flyers or posters, local advertising (newspaper, radio, public service announcements, etc.), inservices to pediatric healthcare providers or community groups, and discussions held in small groups or individually with interested parents. All study advertising and promotional tools will be approved by the Institutional Review Board (IRB) according to local IRB policy. Attempts will be made to actively recruit and enroll all ethnic/racial groups and both genders.

Parents will be given written and verbal information about smallpox, the Dryvax vaccine and the study procedures to assist them in making a decision about their child's study participation. If requested by the parent, study educational materials will be provided for review prior to attending the screening visit. During the recruitment process, study personnel will ask the parents questions about the study procedures to ascertain their understanding of the requirements of participation and their willingness to attend the study visits.

If the parent wishes to enroll their child in the study, they will be asked to provide the name of the child's primary care physician

so that study personnel can notify the individual or group provider of the child's participation and verify the medical and immunization histories, when necessary.

3.6.2 Informed consent

Prior to the start of the study, the protocol and consent form will be approved by local IRBs as well as by the Office of Regulatory Affairs (ORA), DMID, National Institute of Allergy and Infectious Disease (NIAID)/National Institute of Health (NIH). The parents/legal guardians of possible study participants will be informed of the reason for the study, the study procedures, the risks and benefits associated with study participation, the alternatives to participation, and other elements as outlined in the Belmont Report. The parent will be given an opportunity to have all questions answered before signing the consent form. Informed consent will be obtained prior to any screening procedures. The parent will be given a copy of the consent form. As described below, additional consents will be used for HIV testing, use of VIG and use of cidofovir.

3.6.3 Randomization/Blinding

Subjects entered into the study will be randomized equally to one of the two study groups specified above. As each child enrolls in the study, he/she will be assigned a unique ID number. The EMMES Corporation is the data-coordinating center for this trial. Randomization will be implemented using an internet-based randomization system developed by the EMMES Corporation.

The total sample size for the trial will be 40 subjects (20 volunteers per group) randomized equally to group 1 (undiluted, with 5 punctures and a dressing) or to group 2 (diluted 1:5 with 5 punctures and a dressing). The number of subjects randomized to each study group will be monitored by the safety monitoring committee to assure that aggregate totals across the two participating sites achieve the target enrollment numbers.

This study will be conducted in a blinded fashion. Specified research personnel will inoculate and cover the vaccination site. Research staff will perform subsequent evaluations to document site reactions and take. Evaluators, investigators, and parents will have no knowledge of study group assignment.

3.6.4 Study visits and procedures

3.6.4.1 Overview (flowchart)

Shown in the table below is an overview of study visits and procedures.

Study Visits and Procedures from Initial Screening Period to Day 180

Study Procedure	Visit	1	2	3	4	5	6	7	8	9
	Day	-30 to –1	0	1 to 5	6 to 8	9 to 11	12 to 27	28 to 44	45 to 60	61 to 180
SCREENING										
Telephone screening		→								
Parent education		→	→	→	→	→	→	→	→	→
Informed consent		X								
Medical history		X								
Physical exam		X								
Inclusion/exclusion criteria		X	X							
Serology: HIV		X								
Serum creatinine		X								
PRIMARY VACCINATION										
Immune Assessment: Vaccinia[+]		X						X		
Randomization			X							
Dryvax vaccination			X							
Site evaluation and clinical assessment				X*	X*	X*	X*	X	X	
Dressing change				As needed	X	X	Every 2-4 days as needed until site is scabbed and dry			
Site photograph**					X	X				
Site viral culture**						X				
Solicited AEs (14 days)			→	→	→	→	→			
Unsolicited AEs (28 days)			→	→	→	→	→	→		
SAEs			→	→	→	→	→	→	→	→
Contacts log			→	→	→	→	→	→	→	→
REVACCINATION IF NO TAKE AFTER PRIMARY VACCINATION[#]										
Dryvax vaccination					X					
Dressing change					Every 2-4 days as needed until site is scabbed and dry					
Immune Assessment: Vaccinia								X		
Solicited AEs					→	→	→			
Unsolicited AEs					→	→	→	→		
SAEs					→	→	→	→	→	→

[+]A subset of subjects in each group will have repeat serum antibody titers and cell mediated immunity studies 1, 2 and 3 years after successful vaccination.
*Evaluation of the vaccination site will include measurements of erythema and induration and full description of the site. Additional measurement of vaccination site will be done between days 12-16.
**Additional photographs and viral cultures may be taken to evaluate satellite vesicular lesions, vaccinia rashes, lesions resulting from autoinoculation, or other reactions associated with Dryvax vaccination.
[#] All study procedures and safety follow-up for revaccination will be the same as those for primary vaccination.

Detailed description of study visits

Screening/Enrollment Visit	**Day -30 to –1**

- Parent is provided with written materials and verbal instructions on the requirements of the study. The level of parental interest, understanding and potential compliance is assessed by answering basic questions about the study.
- Parent/legal guardian provides written informed consent for their child's study participation prior to conducting any screening procedures.
- Parent/legal guardian provides written informed consent for HIV testing for their child.
- Parent is queried about the study inclusion and exclusion criteria.
- Research personnel obtain medical history and perform physical examination.
- A blood sample (approximately 5 to 10 mL) is obtained and processed for serum creatinine, HIV serology, and for the assessment of vaccinia humoral and cell-mediated immune responses.
- Prior to enrollment, parent is given the results of HIV test. (Counseling options are made available, if needed.)
- Assign unique study ID number.

Vaccination Visit	**Day 0**

- Reassess inclusion/exclusion criteria.
- Obtain axillary temperature.
- Record current medications and ascertain if there have been any changes in the child's health status since the screening visit.
- Confirm negative screening laboratory results.
- Using the internet based randomization system, determine the study group to which the child has been assigned.
- Administer the Dryvax vaccination in the deltoid area, according the study group assignment:
    - Group 1: Undiluted vaccine given with 5 skin punctures.
    - Group 2: Vaccine diluted 1:5 given with 5 skin punctures.
- Apply semi-occlusive dressing.

- Observe the child in the clinic for 30 minutes following vaccination to assess for any immediate adverse reactions. (Appropriate medical treatment and personnel should be available in the event of an emergency.)
- Provide parents with post-vaccination instructions.
    - Give parents digital thermometer and instructions on its use.
    - Give parents 14-day diary card and instructions on its completion. (The diary card includes an area for the parent to record answers to solicited adverse events and areas to record other complaints and medications given to the child.)
    - Set up a time for the next visit (clinic visit or home visit) to see the child, assess the vaccine site and change the dressing.
    - Give parent emergency dressing change kits and instructions on use (See Manual of Procedures for description of contents of the emergency dressing change kit).
    - Give parent study personnel contact information.
    - Give parent instructions on limiting and monitoring the child's contacts, and a log for recording contacts. (The parent will be given an information sheet that they can give to other parents, friends, and relatives to explain exposure to vaccinia virus.)

Vaccine Site Evaluation Visit(s)	**Day 1 to 5**

- Research personnel contact parent by telephone to check on the child's progress.
- Research personnel change the vaccination site dressing as needed (based on the need to maintain an intact dressing). (Study personnel may complete these visits through clinic or home visits. At least one visit should be performed during this interval.) During each dressing change, the vaccination site is assessed and measured, and the information is recorded.
- Parents are counseled about measures to minimize the spread of vaccinia to contacts.

Vaccine Take Assessment Visit	**Day 6 to 8**

- Parent brings the child to the clinic.
- Research personnel conduct a brief physical assessment.
- Research personnel assess and measure the vaccination site.
- A digital photograph of the vaccination site is taken.
- A new semi-occlusive dressing is applied.
- Research personnel review the diary card with the parent and answer questions about its completion.
- If there is no vaccine take, the child is revaccinated.
    - Administer Dryvax in the same dilution and number of skin punctures as the first dose. Administer the vaccine in the opposite deltoid region.

- Apply a semi-occlusive dressing.
- Have the parent stay with the child in the clinic for 30 minutes to assess for any immediate adverse reactions. (Appropriate medical treatment and personnel should be available in the event of an emergency.)
- Give the parent another 14-day diary card and instructions on its completion.
- Discuss the future clinic visit schedule with the parent.

- Parents are counseled about measures to minimize the spread of vaccinia to contacts.

Viral Culture Visit	**Day 9 to 11**

- Parent brings the child to the clinic.
- Research personnel assess and measure the vaccination site and apply a new semi-occlusive dressing.
- A digital photograph of the vaccination site is taken.
- A viral culture of the site is taken.
- Parents are counseled about measures to minimize the spread of vaccinia to contacts.

Vaccine Site Evaluation Visits	**Day 12 to 27**

- Research personnel contact parent by telephone to check on the child's progress.
- Research personnel assess the vaccination site and change the dressing every 2 to 4 days as needed (based on the need to maintain an intact dressing). (Study personnel may complete these visits through clinic or home visits.) The vaccination site is measured between days 12-16. Dressing changes will be continued until the site is scabbed and dry.
- After Day 14, the parent is asked to return the diary card to research personnel (card can be returned at the clinic or during a home visit).
- After Day 14, the parent is given another diary card and instructed on its completion. (The diary card includes an area for parents to record unsolicited adverse events and medications that are given to the child.) The parent is instructed to maintain this diary card until day 28.

Immune Assessment and Vaccine Site Evaluation Visit(s)	**Day 28 to 44**

- Parent brings the child to the clinic.
- Research personnel assess the vaccination site and apply a new semi-occlusive dressing, as needed. Dressing changes are continued as needed until the site is scabbed and dry.
- Collect diary card for days 15 to 28.
- Blood sample (5 to 10 mL) is obtained for vaccinia humoral and cell mediated immune responses 28 days (±2) after vaccination. [If the child

requires revaccination, this immune assessment sample will not be collected at this visit. An immune assessment sample, about 5 to 10 mL, will be collected 28 days (±2) following revaccination.]

Vaccine Site Evaluation Visit(s)	**Day 45 to 60**

- Parent brings the child to the clinic.
- Research personnel assess the vaccination site and apply a new semi-occlusive dressing, as needed. Dressing changes are continued until the site is scabbed and dry.

Site Evaluation Follow-up and Safety Assessment	**Day 61 to 180**

- Research personnel assess the vaccination site and apply a new semi-occlusive dressing, as needed. Dressing changes are continued until the site is scabbed and dry.
- Research personnel contact parents by telephone as needed to obtain an end date for any vaccine related adverse events.
- If the child was revaccinated, a blood sample, approximately 5 to 10 mL, will be obtained 45-60 days after revaccination.
- Log of contacts during the study is collected.
- At Day 180, research personnel contact parent by telephone to ascertain the occurrence of any serious adverse events.

Immune Assessment Visit	**Days 365, 730, 1095 ± 28 days**

- Parent brings the child to the clinic.
  Blood sample (5 to 10 mL) is obtained for vaccinia humoral and cell mediated immune responses.

3.6.5 Vaccine and study drug handling and administration

3.6.5.1 Dryvax

Dryvax vaccine may be requested through the DMID Office of Regulatory Affairs (ORA). Dryvax vaccine will not be shipped to the sites until the ORA has received all regulatory documents, including IRB approval and approved consent form(s). The Centers for Disease Control and Prevention will be responsible for the distribution of Dryvax. Dryvax is supplied as a combination package of one vial of lyophilized vaccinia virus vaccine, one container of diluent (0.25mL) and one tube of bifurcated needles (100 needles per tube). Each vial of Dryvax will be reconstituted with the 0.25 mL of the standard diluent for Dryvax according to

current ACIP recommendations (see Appendix B). The undiluted reconstituted Dryvax can be kept refrigerated at 2 to 8°C for up to 3 months (see Manual of Procedures).

Further dilutions of Dryvax will be made at each site. The diluent used will be the same as the original vaccine reconstitute. The 1:5 dilution of reconstituted vaccine is stable and can be kept refrigerated at 2 to 8°C for 56 days. (See Manual of Procedures). Dilutions will be back titrated to verify vaccinia plaque forming units in the 1:5 dilution. Disposal will be handled according to the instructions in the Dryvax package insert and ACIP recommendations (see Appendices A and B).

A CDC video on Dryvax administration will be employed at both study sites to standardize technique. The inoculation site will be cleaned with acetone. Then using a bifurcated needle, the vaccine will be given either above the insertion of the deltoid muscle or over the triceps muscle posteriorly. The standard pediatric recommendation for Dryvax has been to administer 2-3 punctures of the skin with a bifurcated needle stated in the package insert of Dryvax (see Appendix A). Earlier vaccine trials in children conducted by Dr. Cherry and others employed 5 punctures, which we will also use for both groups in this study. Dr. Cherry's studies noted differences in take rate at different study sites, which may relate to technique or subtle patient differences. Every effort will be made to employ the fewest number of vaccinators and to standardize technique. This could be an important issue with a small study being conducted at more than one study site.

3.6.5.2 Vaccinia Immune Globulin

Vaccinia immune globulin (VIG) is a sterile plasma derivative from individuals immunized with vaccinia virus vaccine. VIG is available in 5.0 mL vials and is supplied by the CDC on an as needed basis and will be supplied only upon request and receipt of a serious adverse event [SAE] report (facsimile acceptable) documenting emergency need. It will be stored between 2°C and 8° C (35°-46° F). Product will be disposed of as biohazardous waste in accordance with information contained in the IND. Instructions for request, storage and use of VIG are included in the Manual of Procedures.
Doses of VIG administered will also be recorded on an accountability log, provided in the Manual of Procedures.

3.6.5.3 Cidofovir

Cidofovir (VISTIDE®) will be supplied by NIAID only upon request and receipt of an SAE report (facsimile acceptable) documenting emergency need. Handling, storage and disposal of cidofovir will be according to the instructions of the manufacturer (Gilead Sciences, Inc). Instructions for request, storage and use of cidofovir are included in the Manual of Procedures. Cidofovir should be stored at controlled room temperatures between 20 and 25^{o} C. Cidofovir infusion admixtures may be stored refrigerated at 2 to 8^{o} C and must be administered within 24 hours of preparation. Doses of cidofovir administered will also be recorded on an accountability log, provided in the Manual of Procedures.

3.6.6 Limiting virus transmission to contacts

3.6.6.1 Semi-occlusive Dressing and Dressing Changes

The occlusive dressing is intended to prevent autoinoculation to other body sites of the subject (i.e. eyes) and prevent transmission to others. A nurse in the study clinic will assess the vaccination site and change the dressing as needed for days 1 to 11 following vaccination. From day 12 to 31, the dressing will be changed every 2-4 days or until the scab is well formed at which time, dressings will no longer be required. The dressing permits the child to shower, but care should be maintained to ensure that the child does not pick at the dressing or inadvertently remove it. Any concerns with the dressing or any signs of infection (redness, swelling and tenderness) observed by the parent/guardian should be immediately phoned to the on call study nurse. Most experts believe that the risk of secondary transmission is minimal once the scab is formed. It is important that even after the scab is formed, the child should be restrained from picking at it, as although the risk of transmission is lower, spread of the vaccine virus to other parts of the body or to others can occur.

3.6.6.2 Nurses / physicians

Nurses and physicians providing care to the child will have received the Dryvax vaccine according to the CDC recommendation and consent form that is directly supplied by the CDC. In addition, all study personnel will be educated regarding possible spread of vaccine virus.

3.6.6.3. Contacts

Although no absolute restrictions can be made regarding the subject's interactions with others, the following guidelines will be explained to the parents:

1. The child's bandage should be checked prior to contact with others
2. The child should wear a garment with sleeves when in contact with others
3. Visitors should be restricted, using the same guidelines as included in the exclusion criteria for family members i.e. no immune compromised individuals, no individuals with eczema, atopy, acute or chronic skin conditions, no pregnant women, and no children under 12 months of age. Information should be provided to visitors so that they can be informed of possible risks (a handout will be provided).
4. An educational handout with the guidelines will be provided to the parent/legal guardian to be shared with potential contacts who do not precisely qualify as close household contacts, but may have a significant temporal physical contact history with the vacinee.

3.6.7 Revaccination

If after 6-8 days there is no vesicle/pustule at the inoculation site, indicating a "take" of the vaccine, a second dose of the vaccine with the same concentration, method of administration, and local site care-instructions, will be administered. The same methods and procedure for evaluation and dressing changes at the vaccination site, follow-up visits, and safety evaluations will be pursued as for the initial dose of vaccine.

3.6.8 Study or Subject Termination

The NIH, FDA or IRB has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of adverse events indicates a potential health hazard
- Data recording is inaccurate or incomplete
- Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the study.

A subject may withdraw or be withdrawn from the study for the following reasons:

- Subject withdraws consent
- Development of serious adverse event warranting withdrawal (patient will roll over to treatment for adverse events protocol as warranted)
- Trial termination
- Any reason which, in the opinion of the investigator, precludes the subject's participation in the study.

If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms. The remaining follow-up safety evaluations will be conducted if the subject agrees. A subject who is withdrawn because of an adverse event (AE) or serious adverse event (SAE) must be followed until resolution of the event.

4.0 ASCERTAINMENT OF ADVERSE EVENTS

Evaluation of vaccine safety includes two major components: 1) assessment of the study subject for adverse reactions following vaccination, and 2) assessment of virus transmission to the subject's close contacts. The definitions and methods for assessment for each of these components are described below:

4.1 Adverse events

The investigator or designee is responsible for surveillance, detection and documentation of adverse events and serious adverse events in persons receiving Dryvax. As detailed in this protocol and the procedures manual, at each clinical evaluation and safety evaluation during the treatment and follow-up period, the investigator or site personnel should document any AEs or SAEs. AEs and SAEs should be reported to the Safety Monitoring Committee (SMC) and DMID as outlined in the following sections.

An adverse event is any event, side effect, or other untoward medical occurrence, including dosing errors, that may occur in relation to a pharmaceutical product or intervention and may be related to that product or intervention. An AE can, therefore, be any unfavorable and unintended sign (e.g., a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product or intervention, whether or not considered to be related to the medicinal product or intervention. An unexpected adverse event/experience is any adverse event that has not been previously described in available risk information (i.e., not included in the product labeling or the investigator's brochure).

Clinical adverse events are illnesses, signs, or symptoms that have appeared or worsened during the acute stage of the study or during the follow-up period. These may include the following: 1) the exacerbation of a pre-existing illness, 2) an increase in the frequency or intensity of a pre-existing episodic event or condition, 3) any condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study or 4) continuous persistent disease or symptoms present at baseline that worsen following the start of the study. Laboratory adverse events are abnormal values obtained on laboratory tests during the acute and follow-up period.

Any medical condition or clinically significant laboratory abnormality with an onset date before the date of vaccination is considered to be pre-existing, and should be documented on the Medical History case report form (CRF) page. Any AE (i.e., new event or exacerbation of a pre-existing condition) with an onset date equal to vaccination or within 56 days after primary vaccination, and if the subject was revaccinated, within 28 days after revaccination, should be recorded as an adverse event on the CRF. All AEs, whether believed to be drug-related or not, must be recorded on the Adverse Event page of the case report form (one adverse event per page).

Subjects must be monitored carefully for a vaccine "take" as well as for potential local or systemic adverse reactions. One expects to observe a take within the first week of inoculation, but local reactions may occur within the first 2-3 weeks, particularly if superinfection or dissemination becomes apparent. The influence of the double occlusive dressing is unknown. Lastly, we know that virus can be recovered from the local area of inoculation for 30-60 days.

There are a number of adverse events that have been described in association with Dryvax, are known to occur and are not unexpected. These include the following:

Local Reactions (expect crusting vaccinia lesion for up to 30 to 60 days)

a. Major:	Vaccinia necrosum (progressive vaccinia), eczema vaccinatum, bacterial superinfection, inadvertent inoculation of face, eyelid, nose, mouth, genitalia or rectum
b. Minor:	Excessive pain, tenderness, erythema, itching or induration at injection site, or limitation of limb movement

Systemic Reactions

a. Major:	Generalized vaccinia, post-vaccinial encephalitis, anaphylaxis and hypersensitivity, conjunctivitis, keratitis, uveitis, nausea, vomiting, diarrhea, erythematous or urticarial rash
b. Minor:	Myalgia, fatigue, headache, fever, and regional lymphadenopathy

For this protocol, vaccinia necrosum, eczema vaccinatum, bacterial superinfection, accidental/inadvertent inoculation, generalized vaccinia, ocular vaccinia, post vaccinial encephalitis and anaphylaxis will be reported via an SAE (serious adverse event) report within 24 hours of diagnosis. Other events listed above of severe intensity and requiring follow up should be reported to DMID within 24 hours (see flow chart on page 37).

The expected frequency of complications occurring in children 1 to 4 years of age following Dryvax vaccination from the 1968 US state and national surveys (16, 17) is shown in the table below:

Complications of smallpox primary vaccination, 1968: Ten State and National Surveys, 1-4 years of age (number of cases/1 million vaccinations)

Survey	Post-vaccinial encephalitis	Vaccinia necrosum	Eczema vaccinatum	Generalized vaccinia	Accidental inoculation	Other	Total
Ten State	9.5	3.2	44.2	233.4	577.3	236.6	1261.8
National	2.2	0.4	11.3	17.2	33.3	14.6	79.0

A more detailed description of some of the adverse events associated with Dryvax is provided below.

Progressive Vaccinia (Vaccinia Necrosum)

This entity involves progression of the vaccination lesion 15 days or more after vaccination. Secondary lesions sometimes appear. There may or may not be an associated erythema and lymphadenopathy. Management of progressive vaccinia requires hospitalization, an immunologic work-up for acquired or congenital immunodeficiency, surgical debridement and vaccinia immune globulin (VIG). In a 1963 national survey in the United States, no deaths were reported among 9 cases of vaccinia necrosum, while a 1968 US national survey reported 4 deaths among 11 patients with vaccinia necrosum (17, 18).

Post-Vaccinial Encephalitis

Patients can present with seizures, decreased level of consciousness, cranial nerve palsies or signs of increased intracranial pressure 10-35 days after vaccination. In one study, 17 out of 31 (54.2%) cases had virus isolated from the cerebrospinal fluid. VIG has not been shown to be effective in treating this complication so antiviral drugs should be employed. The mortality rate is 30%, and 20% can be left with permanent neurological sequelae.

Eczema Vaccinatum

This condition presents as the appearance of vaccinia lesions away from the vaccine inoculation site in a subject or contact with active or healed eczema. The patient should be hospitalized and VIG should be given immediately and repeated in 24 hours if new lesions occur. If an associated fever does not subside in 48 hours, or if new lesions are still forming after the second dose of VIG, use of antiviral agents should be considered to reduce the likelihood of viral dissemination.

Ocular Vaccinia and Vaccinial Keratitis

Vaccine immune globulin is recommended for cases of autoinoculation resulting in ocular vaccinia that are confined to the eyelids and/or conjunctiva. It is, however, contraindicated for the treatment of vaccinial keratitis (when the cornea is involved) because it may induce worsening of the corneal inflammation and scar formation (18). Subjects with ocular vaccinia will be evaluated by an ophthalmologist to determine the appropriate management.

Generalized Vaccinia

A generalized vaccinia-like rash can occur 6-9 days after primary vaccination in patients with otherwise normal skin. This rare complication is believed to occur from bloodborne viral dissemination in otherwise normal hosts. Hospitalization, viral and bacterial cultures of some of the lesions and VIG treatment if the patients appear toxic or have underlying predisposing high risk factors, should be carried out. Complete recovery is usual in most cases involving otherwise normal individuals.

Accidental Inoculation

The finding of vesicles around the vaccination site or discretely on the face, arms, trunk, buttocks, genitalia or eyelid are indicative of accidental inoculation. This occurs when a child scratches the vaccination site and transfers virus to another skin area. Rarely, they represent secondary impetigo and may require culture and antibacterial treatment. These secondary lesions usually resolve on their own without scarring. VIG is not generally indicated unless the child belongs to a high-risk group.

Erythematous or Urticarial Rash

These benign rashes commonly occur about ten days (range 4-17 days) after vaccination. They appear similar to roseola or erythema multiforme, and sometimes develop an associated vesicular component. The patient is usually afebrile. The rash usually subsides in 2-4 days

without treatment. If the patient appears ill and the rash is extensive, post vaccination Stevens-Johnson syndrome must be considered.

4.2 **Serious adverse events** (within 6 months of vaccination)

A serious adverse event (SAE) is any adverse event/experience occurring at any study drug dose that results in any of the following outcomes:

- Death
- Life-threatening (patient at immediate risk of death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect
- Results in a persistent or significant disability or incapacity
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events/experiences when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

In addition to the above, any medically attended visit subsequent to vaccination, such as a visit to an emergency room, during participation in this protocol will be considered an SAE.

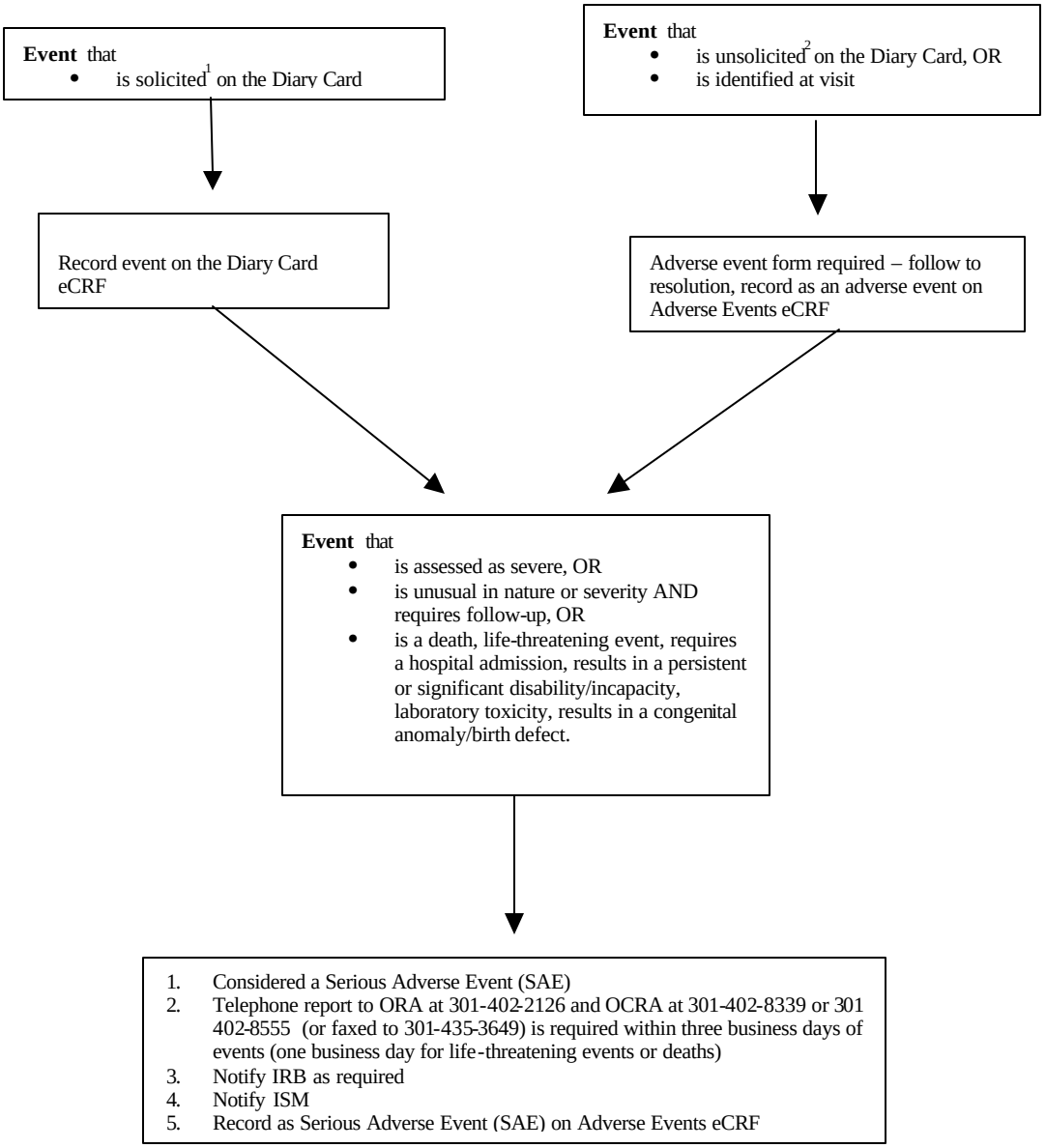
4.3 Assessment and reporting of adverse events

FDA regulations (21CFR 312.32, 312.33 and 312.64) require that an investigator notify the sponsor and the Institutional Review Board promptly of any serious adverse events, deaths, or life-threatening problems that may reasonably be regarded as caused by or associated with the administration of the investigational drug. Adverse events will be reported to the FDA in compliance with 21CFR 312.32.

For those events meeting the previously described definition of Serious Adverse Events, the completion of an SAE form is required. Specific information on where to send this form is included in the Manual of Procedures for this study. Life-threatening SAEs or death must be reported via telephone to the Office of Regulatory Affairs (ORA), DMID (Wendy Fanaroff-Ravick at 301-402-2126) and the Office Clinical Research Affairs (OCRA), DMID (Holli Hamilton, MD, MPH at 301-402-8339 or Mamodikoe Makhene MD, MPH at 301-402-8544 or by fax to 301-435-3649) within one business day of site awareness of the event. All other SAEs must be reported via telephone to ORA and OCRA within 3 business days of site awareness of the event. All SAEs will be reported as required to all sites' participating Institutional Review Board (IRB.)

The investigator must also contact the Individual Safety Monitor (ISM) for evaluation any serious adverse events and adverse events that are unusual and severe and without alternative explanation within 24 hours of notification of the event. Contact with the ISM, along with the ISM's opinion, should be reported to NIAID, Office of Clinical Research Affairs (OCRA) and the Office of Regulatory Affairs (ORA) at 301-402-8339 or 301-402-8555.

See the flow chart below and the DMID Pediatric Toxicity Tables (Appendix D) for assessment and reporting of adverse events.



[1] Events recorded on the study-specific diary card are considered solicited. Examples are:

- oral temperature
- pain at injection site
- muscle aches
- chills
- headache
- nausea
- feeling tired
- underarm pain
- underarm swelling
- itchiness at vaccination site
- joint pain
- change in appetite

[2] Unsolicited events are events listed on page 3 of the diary cards or identified at or between study visits.

Any adverse event that is severe or unusual in nature AND requires follow-up is an SAE and requires expedited reporting.

4.4 Secondary Transmission: Surveillance and Isolation Procedures

Parents or guardians of the enrolled child will be questioned at each study visit about potential secondary infections among family members or other close contacts (i.e. playmates). They will be provided with an information sheet to distribute to close contacts of their child other than the immediate family. Children will be excluded from daycare or school during this study.

If enrolled children or close contacts develop skin lesions suggestive of secondary vaccinia, self-inoculations, or eczema, other than at the vaccine site, parents/legal guardians will be asked to have that individual examined by a study physician. A viral culture may be obtained for confirmation. Parents/legal guardians will be asked to call the study nurse immediately when such skin lesions are noted on their enrolled child or close contacts.

4.5 Safety Monitoring Oversight

Each site will have an on site independent safety monitor (ISM) who can review adverse events in a timely fashion. Each ISM will be approved by DMID/NIAID. The ISMs will have direct contact with the site PIs and will evaluate all adverse events on an ongoing basis. The study will have a Safety Monitoring Committee (see Manual of Procedures for DMID guidelines on safety monitoring). In the event of any safety concerns, the ISMs may be convened as part of the Safety Monitoring Committee in accordance with DMID guidelines.

4.6 Treatment of adverse events

Serious complications associated with smallpox vaccination are rare or uncommon. Their occurrence is influenced by age, vaccination status, and presence of eczema or underlying immune deficiency. Overall, it has been estimated that after the first vaccination, the risk of encephalitis or progressive vaccinia is less than 3 per 1,000,000 vaccinations while that of eczema vaccinatum is 38 per 1,000,000. The risk of other complications, including generalized vaccinia, or accidental infection of vaccinia on the eye, or bacterial infection of the vaccination site is less than 600 per 1,000,000. The risk of death is about 1 per 1,000,000 vaccinations. Following revaccination, the rates of adverse reactions decrease by approximately a factor of 10. Care of all adverse reactions will be managed by the PI and local physicians according to assessed medical need. This protocol does not purport to treat by protocol algorithm, but as deemed necessary by clinical need. Examples include:

4.6.1 Pruritis

Itching frequently occurs at the site of vaccination after 5-7 days. Treatment with cold compresses (e.g. ice water in a plastic Ziploc bag) or oral Benadryl elixir is usually helpful. DO NOT permit the dressing to be removed or opened.

4.6.2 Fever (axillary temperature ³ 38°C, 100.4°F) or pain

Mild fever or discomfort at the vaccination site can be controlled with acetaminophen or ibuprofen given as directed on the product label. Temperature over 101.5°F, severe or persistent pain, should be called to the attention of study personnel.

4.6.3 Vaccine Immune Globulin (VIG)

Subjects experiencing complications resulting from Dryvax vaccinations (except those with vaccinial keratitis) will be treated with vaccinia immune globulin (VIG). VIG is available for use under IND and its use is recommended by the Advisory Committee on Immunization Practices (ACIP) for the treatment of complications resulting from vaccinia vaccination. VIG will be administered intramuscularly (in the buttock or anterolateral aspect of the thigh) at a dose of 0.6 mL/kg. Doses greater than 10 mL will be divided and injected at two or more sites to reduce local pain and discomfort. VIG will be supplied by the CDC upon request, and packaged and labeled according to the IND specifications for VIG. The CDC will provide specific directions for use on a case-by-case basis. The ACIP Recommendations and the investigator's brochure for VIG are contained within the manual of operations for this study. Because this product is not FDA approved for treating complications of vaccinia vaccine, informed consent will be required for administration.

VIG is a sterile 16.5 % solution of the immunoglobulin fraction of plasma from individuals who were immunized with vaccinia virus vaccine. The solution is isotonic and contains 0.3 M glycine as a stabilizer and 0.01% thimerosal as a preservative. It is available in 5.0 mL vials. The recommended dose for treatment of post-vaccine complications is 0.6 mL of a 16.5% solution administered intramuscularly.

4.6.4 Cidofovir

At this time, there is no accepted alternative to treating severe complications resulting from vaccination with vaccinia virus. During the previous study, FDA requested that cidofovir be included as a back up to VIG treatment. Cidofovir (VISTIDE ®) is FDA

approved and marketed only for the treatment of CMV retinitis in HIV-infected patients. This anti-viral drug has in vitro efficacy against a variety of viruses including vaccinia orthopoxvirus. The drug must first be phosphorylated by cellular enzymes to its active diphosphate form that then acts to inhibit viral DNA synthesis. The drug is administered once a week due to its prolonged inhibition of viral replication and the long half-life of its active metabolites. Animal models of vaccinia infection in BALB/c mice (intranasal administration and pneumonia) and in SCID mice (tail lesion) showed marked reduction in mortality when cidofovir was administered as treatment after infection.

Specific product information about cidofovir for injection and its dosage and administration are provided in Appendix C. Pharmacokinetic studies of the intravenous preparations have been performed in adults but there not have been any phase I or II clinical trials of this drug in children. Several studies that have included some children have documented the clinical response of immunocompromised children with either HIV and CMV retinitis, or bone marrow transplant and disseminated CMV or adenovirus infections. It has been used in children for the treatment of viral warts without any adverse side effects.

Major side effects of the drug in adults include nephrotoxicity (reduced with concomitant probenecid administration), gastrointestinal upset, mild hepatitis and, on occasion, retinitis or bone marrow suppression. The nephrotoxicity can occur in subjects with previous normal renal function. It predominantly affects the renal tubules causing proteinuria, phosphaturia, and glycosuria.

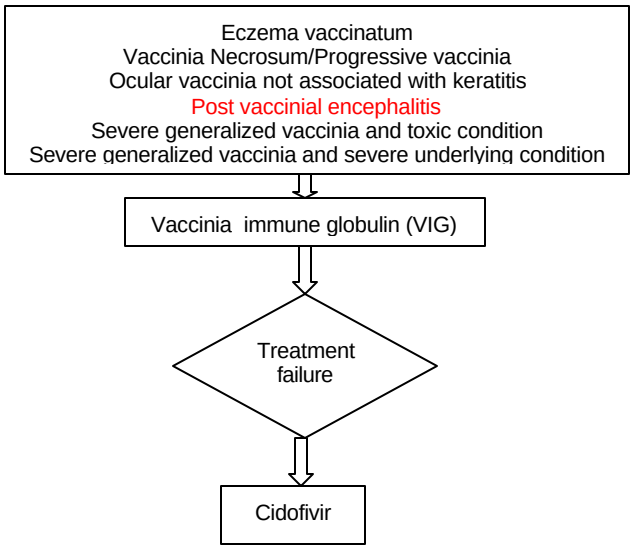
Cidofovir is a sterile, hypertonic aqueous solution for intravenous infusion only. The marketed dose for the treatment of CMV retinitis in HIV-infected patients is 5.0 mg/kg. The treating physician may administered a single infusion of 5.0 mg/kg in accordance with the procedures outlined in the package insert, which is provided in the manual of procedures for this study.

Cidofovir is to be administered only upon failure of VIG to control vaccinia infection. For this protocol, cidofovir may be used off-label as a secondary treatment, only if medically necessary. The decision to utilize cidofovir as a last resort will be made by the treating physician. *In vitro* and animal model data in several poxvirus infections models demonstrate the activity of cidofovir at clinically relevant doses. Thus, subjects who continue to experience disseminated vaccinia symptoms may be given a single dose of cidofovir. Treatment with cidofovir shall be secondary to clinical supportive care and treatment with VIG. If cidofovir treatment becomes medically necessary, cidofovir will be administered in accordance with the instructions in the package

insert. Briefly, patients will be given 25 mg/kg of probenecid three hours before administration of a single dose (5.0 mg/kg) of cidofovir. Each patient will receive 10-20 mL/kg of saline infused over a 1 to 2 hour period immediately before cidofovir infusion. Patients who can tolerate additional fluid load will be given a another 10-20 mL/kg of saline over a 1-3 hour period initiated at the start of the cidofovir infusion or immediately afterwards. Patients will receive an additional 10 mg/kg of probenecid 2 hours after the infusion and a final 10 mg/kg of probenecid 8 hours after infusion. All patients will be monitored for development of renal toxicity and hearing loss.

As this drug is not FDA approved for treating vaccinia infections and there are limited data available on its use in children, informed consent will be required. The NIH, in conjunction with the drug manufacturer, is developing a protocol for the use of this drug in children enrolled in this study. This protocol will be followed for any vaccinia complications that a study physician determines will require cidofovir therapy (see Manual of Procedures). Treatment with cidofovir will only be recommended after clinical failure following treatment with vaccinia immune globulin.

Management of Side Effects and Adverse Reactions



VIG is contraindicated in vaccinial keratitis and is not effective in post vaccinial encephalitis.

5.0 LABORATORY EVALUATIONS

5.1 Schedule of specimen collections

Shown in the table below is a summary of specimens and timing of collection:

	Time following vaccination					
Laboratory Test	**–30 to –1 days**	**9 to 11 days**	**28±2 days**	**365±28 days***	**730±28 days***	**1095±28 days***
HIV EIA	X					
Serum creatinine	X					
vaccinia antibody studies	X		X	X	X	X
vaccinia CMI studies	X		X	X	X	X
Viral Culture		X				

*serum antibody titers and cell mediated immunity studies will be repeated in a subset of children from each group

5.2 Materials for specimen collection

Blood samples

Phlebotomy supplies, red top serum separator vacutainer tubes, 2mL Sarstedt tubes, sterile pipettes, cardboard freezer boxes, bar coded labels. The assessment of immune response will include neutralization, ELISA and cell mediated immunity (CMI) studies.

5.3 Specimen labeling

Bar coded labels will be provided by the EMMES Corporation and will be sent to each site prior to the initiation of the study. Each label will contain a unique bar-coded number that will be associated with the site, DMID protocol number, volunteer ID, visit number, and date in the Internet Data Entry System. The date of specimen collection must be written on the label. In addition, for each specimen, an identical bar-coded label will be affixed to a source document upon which the same information will be recorded.

5.4 Specimen collection and processing procedures

Serum will be collected in 5-7 mL red top separator vacutainer tubes. Approximately 5 to 10 mL of blood will be collected at the screening visit and 28±2 days after vaccination. A subset of subjects will then have 5-10 ml of blood collected annually from the time of vaccination for 3 years

to measure serum antibody titers and cell mediated immune responses. At the screening visit, blood will be collected for serum creatinine and serum will be aliquoted for HIV EIA testing. Each study site will perform HIV EIA testing, and confirmatory Western blot as indicated. The residual serum will be aliquoted into Sarstedt tubes for vaccinia serology and CMI studies. The 28-day post-vaccination blood draw will have all the serum aliquoted into Sarstedt tubes for vaccinia serology and CMI studies. The subject's serum will be maintained in cardboard freezer boxes at -20^0 F or lower in the freezer, until shipment to the Repository. All specimens will be shipped to the NIAID designated repository.

5.5 Serologic responses

Subjects' sera will be collected for vaccinia neutralizing antibody titers by plaque reduction assay, and for vaccinia binding antibodies by ELISA (see Manual of Procedures for complete procedures). A subset of subjects from each group will be monitored with serum antibody titers for at least 3 years to gather data on long-term immunity.

5.6 Cellular immune responses

Blood will be collected at the screening visit before vaccination and 28±2 days after vaccination to measure IFNγ pre- and post-vaccination by ELISPOT assay and intracellular cytokine production (see Manual of Procedures for complete procedures). A subset of subjects from each group will be monitored for at least 3 years to gather data on long-term immunity.

5.7 Viral cultures and viral titers

Viral Cultures will require sterile Dacron tipped swabs, viral transport media and cryopreservative vials. Viral titers will be assessed quantitatively.

Each vial of diluted and undiluted vaccine will be back titrated by St. Louis University Virology Laboratory to determine the titer of virus used during subject inoculation. Vaccine site lesions will be swabbed on day 9-11 during the clinic visit. The viral swab of the vaccine site will be placed in viral transport media, the swab shaft broken off at the tip, and the tip and viral transport media frozen at -70^0F for later viral titer determination. Additional swabs of children with lesions suspicious for secondary transmission or self-inoculation will be processed in the same manner for qualitative vaccinia culture. Virus titration will not be performed on these skin lesion specimens.

The viral transport media with retained Dacron swab tip will be transported frozen on dry ice to St. Louis University Virology Laboratory

for viral culture and viral titer (see Manual of Procedures for complete procedures).

5.8 Laboratory safety precautions

All laboratory staff processing the serum and viral specimens will observe "Universal Precautions" as defined by the Centers for Disease Control and Prevention. Gloves, safety glasses and a lab coat will be worn at all times when aliquoting sera. The vacutainer blood tubes will be disposed of in biohazard waste containers after use. Closed sterile disposable plastic aspirators, not syringe and needles, will be used for aliquoting the sera. Personnel who perform viral cultures (St. Louis University) have been vaccinated to provide protection against smallpox.

6.0 DATA MANAGEMENT / CASE REPORT FORMS

Case report forms (CRFs) will be supplied by the EMMES Corporation under contract to NIAID. Data will be entered electronically. Copies of the screens, kept as workbooks, will be maintained at the site and will be considered the source documents. CRFs should be handled in accordance with instructions from NIAID and in the Manual of Procedures. All CRFs should be filled out completely by examining personnel or the study coordinator. The CRFs are reviewed, signed and dated by the investigator.

All CRFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced CRF copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

7.0 SAFETY MONITORING COMMITTEE

This protocol includes stage I type evaluations of safety and dose response, and it would be prudent to have a small group assist the investigators in the assessment of vaccine safety and to advise about the need and design of further studies. Additional information on safety monitoring committees may be obtained from DMID's guidelines on Safety Monitoring. In addition to monitoring safety and interim immune response data, this group will advise about protocol amendments for a second phase of studies.

8.0 SAMPLE SIZE, STATISTICAL METHODS AND ANALYSES

8.1 Sample Size Justifications

Given the nature of this trial as a preliminary evaluation of the effect of the vaccine in this patient population, as well as the assessment of the dilution, the sample size is based primarily on the ability to provide a

reasonable estimate of the take rate for each study group. As such, a 95% confidence interval for this rate for each group will be computed using the exact binomial approach (Clopper-Pearson interval). For instance, with a take rate of about 90%, the margin of error of this estimate is on the order of 10% with a sample size of 20 subjects. If the rate is as high as 95%, the margin of error drops to about 5%. On the other hand, if the take rate were as low as 70%, the margin of error would expand to about 20%. The following table gives the approximate 95% confidence intervals for a range of values (see table below). This stage of the study dictates a minimal sample size per group as indicated.

95% Confidence Intervals for True Take Rates Given Various Possible Study Outcomes

Possible Sample Take Rate (n=20)	95% Confidence Interval (Clopper-Pearson)
50%	27%, 73%
55%	32%, 77%
60%	36%, 81%
65%	41%, 85%
70%	46%, 88%
75%	51%, 91%
80%	56%, 94%
85%	62%, 97%
90%	68%, 99%
95%	75%, 100%
100%	83%, 100%

8.2 Safety and efficacy analyses

8.2.1 Effectiveness

8.2.1.1 Primary Endpoint

The primary study endpoint is the proportion of vaccinees who “take” (form a vesicle/pustule) approximately seven days after the **first** vaccination in each of the study groups: undiluted with 5 punctures or 1:5 dilution with 5 skin punctures. Inferences about this endpoint will be made by means of point and confidence interval estimation in each study group. No modeling assumptions beyond that of binomial response will be used in this analysis. The anticipated margin of error of these intervals will be as indicated in Section 8.1.

While the power is relatively low for this trial to examine any group differences, the study arms can be compared with the usual chi-square test for homogeneity, where the p-value is determined by exact (randomization) methods.

8.2.1.2 Secondary Endpoints

The following secondary endpoints will be evaluated during the trial:

- Immunologic responses in children to undiluted and diluted vaccine (1:5 dilution)
- Clinical and immunologic responses and safety of 5 intradermal punctures
- Safety profile of vaccine in the vaccinated individual and risk to contacts
- Cutaneous and serologic response rates in children compared to adults (comparison to a separate but parallel study).
- Immunologic response rates of non-responders to revaccination.

The analyses of the various secondary endpoints will be exploratory in nature. However, inferences can be generated for continuous variables using either the Kruskal-Wallis nonparametric analysis of variance or the Mann-Whitney test (Wilcoxon rank-sum test). Multichotomous data will be evaluated using the chi-square test for homogeneity employing a p-value calculation based on exact (randomization) methods.

8.2.2 Safety

Safety analyses will consist of an examination of reactogenicity, adverse events and serious adverse events. Since the size of this trial is small and intended for hypothesis-generating purposes, only descriptive analyses are planned for these safety data.

9.0 ADMINISTRATIVE AND REGULATORY ISSUES

9.1 Compliance with the protocol

Each investigator must adhere to the protocol as detailed in this document. Each investigator will be responsible for enrolling only those volunteers who have met protocol eligibility criteria. An investigator may implement a deviation from, or a change in, the protocol to eliminate immediate hazard(s) to trial subjects without prior NIAID and/or IRB/IEC approval. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate, the proposed protocol amendment(s) must be submitted:

- To the IRB/IEC for review and approval/favorable decision
- To NIAID for agreement; and if required

- To the regulatory authority(ies).

Any protocol violations must be reported to NIAID in a timely manner. Protocol violations that result in an adverse event must be reported on an AE/SAE form and on the protocol violation form. NIAID must be notified by fax (301-435-3649) within 24 hours of a protocol violation. A written report should be faxed to the NIAID as soon as possible. The report should include:

- Nature of the error
- Standard reporting information (subject's clinical status before and after the event)
- Steps taken to review the error
- Steps taken to assure the error will not recur.

Protocol violations must also be reported to the central IRB. Protocol deviations, which are departures from the protocol for which the staff has no control (e.g. subject misses a study visit for a given day), should be documented in the study records.

9.2 Informed consent

A sample informed consent document is provided in Appendix E. The informed consent must be signed by the subject's parent/guardian before participation in the study. A copy of the informed consent must be provided to the subject's parent/guardian. Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

9.3 Subject Confidentiality

Subject identifiers (name, social security number, medical record number) will not be included on data collection forms, specimens, reports or publications. A unique study ID number will be used for the data collection forms and specimens. Records will remain confidential and kept in a locked area, with access limited to personnel who have direct contact with the study. Study participant records will be available to the National Institutes of Health (NIH) or their designee, the Food and Drug Administration (FDA), and institutional regulatory groups who routinely audit research records.

9.4 IRB approval

This protocol and informed consent document and all types of subject recruitment or advertisement information will be submitted to the IRB/IEC for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB/IEC prior to implementing any changes in the study. The investigator is responsible for keeping the IRB/IEC apprised of the progress of the study and of any changes made to the protocol as

deemed appropriate, but in any case at least once a year. The investigator must also keep the IRB/IEC informed of any significant adverse events.

9.5 Study monitoring

Site visits will be conducted by an authorized representative of NIAID to inspect study data, subjects' medical records, and CRFs in accordance with ICH guidelines, GCPs and the respective local and national government regulations and guidelines.

The investigator will permit authorized representatives of NIAID and the respective local and national health authorities to inspect facilities and records relevant to this study, if needed.

9.6 Retention of records

Records and documents pertaining to the conduct of this study, including CRFs, source documents, consent forms, laboratory test results and medication inventory records, must be retained by the investigator for at least 15 years. No study records shall be destroyed without prior authorization from NIAID.

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11.0 APPENDICES

Appendix A
Dryvax® (smallpox vaccine) Package Insert

Appendix B
Vaccinia Vaccine Recommendations of the Advisory Committee on Immunization Practices, 2001

Appendix C
Cidofovir (VISTIDE®) Package Insert

Appendix D
DMID Pediatric Toxicity Tables

Appendix E
Sample Informed Consent