

ACAM2000
Smallpox Vaccine

Vaccines and Related Biological Products Advisory Committee (VRBPAC) Briefing Document

Sponsor: Acambis Inc.
38 Sidney Street
Cambridge, MA 02139
Telephone: (617) 761-4200
Fax: (617) 494-1741

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LIST OF ABBREVIATIONS

| Abbreviation | Definition |
|------------------|--|
| ACAM2000 | ACAM2000 Smallpox Vaccine (Vero Cells), lyophilized Smallpox Vaccine |
| ACIP | Advisory Committee on Immunization Practices |
| AE | Adverse Event |
| AnE | Antibody evaluable |
| ANOVA | Analysis of variance |
| AST | Average Survival Time |
| AV | Atrio-ventricular |
| CAP | Cardiology Advisory Panel |
| CDC | Centers for Disease Control and Prevention |
| CI | Confidence interval |
| CK | Creatine kinase |
| CK-MB | Creatine kinase-myocardial band |
| CNS | Central nervous system |
| CPK | Creatine Phosphokinase |
| CTL | Cytotoxic T lymphocyte |
| DoD | Department of Defense |
| DHHS | Department of Health and Human Services |
| DSMB | Data Safety Monitoring Board |
| ECG | Electrocardiogram |
| ED ₉₀ | 90% Effective Dose |
| EV | Eczema vaccinatum |
| FDA | Food and Drug Administration |
| GMP | Good Manufacturing Practice |
| GMT | Geometric mean neutralizing antibody titer |
| GV | Generalized vaccinia |
| HBsAb | Hepatitis B Surface Antibody |
| HBsAg | Hepatitis B Surface Antigen |
| HCV | Hepatitis C Virus |
| HIV | Human immunodeficiency virus |
| IRC | Independent Review Committee |
| ITT | Intent-to-treat |
| LD ₅₀ | Lethal Dose, 50% |
| LPA | Lymphoproliferation Assay |
| MedDRA | Medical Dictionary for Regulatory Activities |

| Abbreviation | Definition |
|---------------------|--|
| NA | Not applicable |
| NYCBH | New York City Board of Health |
| P | Passage |
| PFU | Plaque-forming units |
| PRNT ₅₀ | 50% Plaque reduction neutralization test |
| PV | Progressive vaccinia |
| PVE | Post-vaccinial encephalopathy |
| PVG | Pharmacovigilance |
| RFP | Request for Proposal |
| SAE | Serious adverse event |
| SD | Standard deviation |
| SNS | Strategic National Stockpile |
| US | United States |
| USP | United States Pharmacopeia |
| VAERS | Vaccine Adverse Event Reporting System |
| VIG | Vaccinia immune globulin |
| VRBPAC | Vaccine and Related Biological Products Advisory Committee |
| WHO | World Health Organization |
| WR | Western Reserve |

1. HISTORY OF SMALLPOX AND CURRENT NEED FOR VACCINE

1.1. Smallpox (Variola Virus) Infection and Smallpox (Vaccinia Virus) Vaccination

Smallpox was one of the most important causes of morbidity and mortality worldwide through the first half of the 20th century. Smallpox in humans is caused by the variola virus, which, along with vaccinia (cowpox) and monkeypox, is a member of the Orthopoxvirus genus. The disease is defined by an acute onset of fever >101°F (38.3°C) followed by a rash characterized by firm, deep-seated vesicles or pustules in the same stage of development (i.e., on any one part of the body, all lesions are vesicles or all are pustules) without other apparent cause. Infection is spread person-to-person via the respiratory route by contact (droplets), and possibly by aerosol [1]. However, since the virus can survive on objects for approximately 1 week, infection can be spread by contact with contaminated objects such as bed linens. Smallpox is fatal in approximately 30% of cases.

Between 1967 and 1977, a global program of smallpox eradication resulted in the elimination of the natural disease. The systematic use of vaccine (live, attenuated vaccinia virus) contributed significantly to this effort [1]. The vaccines used in the eradication campaign were derived from several strains of vaccinia, including the New York City Board of Health (NYCBH) strain. Dryvax[®], the only smallpox vaccine currently approved in the United States (US), was derived from the NYCBH strain of vaccinia. In turn, ACAM2000 (Acambis' live, second generation smallpox vaccine), the subject of this briefing document, was derived from Dryvax[®].

The last cases of smallpox in the US occurred in 1949 in Texas [1]. Because of the absence of smallpox and the risk of vaccine-associated adverse events, routine vaccination of children ceased by 1972 and of hospital personnel by 1976. Vaccinations of military populations ceased in 1989, but were subsequently renewed in 2002 in the context of the imminent war in Iraq. By the mid 1980s, there were only 2 known repositories of variola virus: the Institute of Virus Preparations in Moscow, Russia, and the United States Centers for Disease Control and Prevention (US CDC) in Atlanta, GA.

1.2. Threat of Smallpox as a Biological Weapon

In the mid- to late 1990s, the US government became concerned about the threat of smallpox as an agent of biological terrorism [2]. In the US, approximately half of the population was born after the cessation of vaccination, and therefore has no immunity to smallpox. The remaining population has variable residual immunity. The eradication of smallpox and the cessation of vaccination, therefore, have created vulnerability to covert attack or biowarfare using variola virus.

The threat of a deliberate release of smallpox is a high-consequence event that is considered credible and possible. As a result, numerous governments around the world are building stockpiles of smallpox vaccine as a deterrent and management measure should such an attack occur. The terrorist attacks in New York and Washington in September 2001 and the anthrax

attacks of October 2001 in the US heightened the urgency of developing stockpiles of vaccines and treatments for potential bioweapon agents, and in November of 2001, Acambis was awarded a contract from the CDC for the development and manufacture of a second-generation smallpox vaccine using modern methodology and quality controls (see Section 2.3).

1.3. Measurements of the Protective Efficacy of Vaccination against Smallpox

Smallpox vaccination was started by Edward Jenner at the end of the 18th century before there was any understanding of measurable immunity. His classic studies on the protection of vaccination against smallpox were based on a dermal response to percutaneous vaccination manifested by pustule formation followed by a scar. This dermal response remained the standard for measurement of protection for over a hundred years. In the 20th century, standardized smallpox vaccines were developed and the dermal reaction that was observed to correspond to the development or the presence of humoral antibodies was defined by the World Health Organization (WHO) as major dermal reaction (6-8 days after vaccination the presence of a pustular lesion or an area of definite induration surrounding a central ulcer or scab) [3].

Although the measurement of dermal response is well established as an indicator of immunity for primary vaccination, it does have its drawbacks since the absence of a major cutaneous reaction can result either from poor vaccination technique or from an existing degree of immunity [4]. Dermal response rates, particularly in the previously vaccinated, are highly dependent upon the attributes of the vaccine and the technique of administration. Subjects who have been previously vaccinated and are revaccinated may manifest a reduced cutaneous response compared to vaccinia-naïve subjects, yet also have an immune response to the vaccine. Therefore, the measurement of neutralizing antibody is considered by many to be a better correlate to immunity against smallpox in the previously vaccinated [5]. Nevertheless, the measure of immunity has historically been based on the appearance of a major cutaneous reaction, confirmed by the presence of a vaccination scar.

Using this standard of dermal response, there were several retrospective studies that established that absolute protection against smallpox lasted 3 to 5 years after vaccination, then waned with protection appearing to persist for up to 50 years [6, 7, 8].

It is not entirely clear what the separate role of T cell response and circulating antibodies play in protection against smallpox. According to Fenner's extensive review of orthopox virus infections in animal models, circulating antibodies do have an effect on viral replication and spread of pox virus infection but cell mediated immunity may be more important [9]. In all probability both are very important. There is evidence to support the protective value of neutralizing antibodies against smallpox infection in humans [10, 5]. The observations of the value of neutralizing antibodies also are supported in the findings of another study where the administration of VIG (human gamma globulin serum with high titer neutralizing antibodies from vaccinated individuals) after exposure to smallpox during the incubation period seems to have modified the disease [11].

While the level of neutralizing antibody as determined by plaque reduction assays in cell culture may potentially provide a better correlate of immunity than dermal response, there is not an extensive database to precisely establish the neutralizing antibody titer required for protection

based on qualified assays. However, historical data and limited prospective studies suggest a putative titer of greater than 32 [1:32 dilution of plasma giving a 50% reduction in plaque-forming units (PFUs)] as protective [5].

1.4. Expected Adverse Events with Smallpox Vaccination

Frequent and non-serious adverse events (AEs) historically associated with smallpox vaccination include erythema, pruritus, and swelling at the vaccination site with or without regional lymphadenopathy or lymphangitis, as well as constitutional symptoms, including fatigue, headache, and myalgia [12,13].

Other possible, more severe and potentially serious adverse reactions to smallpox vaccination include inadvertent inoculation (nonocular), superinfection of the vaccination site or regional lymph nodes, ocular vaccinia [14], generalized vaccinia (GV), eczema vaccinatum (EV), progressive vaccinia (PV), postvaccinial central nervous system (CNS) diseases including encephalitis, post-vaccinial encephalopathy (PVE) and encephalomyelitis (PVEM), myocarditis and/or pericarditis, and fetal vaccinia [15,16,17,18,19]. Such complications may result in severe disability, permanent neurological sequelae, and/or death [20, 21, 22].

Estimates of the risks of occurrence of serious complications after primary vaccination and revaccination based on safety surveillance studies conducted when smallpox vaccine was routinely administered are presented in Table 1 [15]. ACAM2000 is a live vaccinia virus that can be transmitted to persons who have close contact with the vaccine or vaccinees. The risks for close contacts who are exposed to a vaccinee are the same as those stated for vaccinees.

Table 1: Rates of Reported Complications Associated with Vaccinia Vaccinations (Cases/Million Vaccinations) [a]

| Age (yrs) | <1 | | 1-4 | | 5-19 | | ≥20 | | Overall rates [g] | |
|------------------------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|-------------------|-----------------------|
| Vaccination status | Vaccinia-naïve | Previously vaccinated | Vaccinia-naïve | Previously vaccinated | Vaccinia-naïve | Previously vaccinated | Vaccinia-naïve | Previously vaccinated | Vaccinia-naïve | Previously vaccinated |
| Inadvertent Inoculation [b] | 507.0 | -- [f] | 577.3 | 109.1 | 371.2 | 47.7 | 606.1 | 25.0 | 529.2 | 42.1 |
| Generalized vaccinia | 394.4 | -- [f] | 233.4 | -- [f] | 139.7 | 9.9 | 212.1 | 9.1 | 241.5 | 9.0 |
| Eczema vaccinatum | 14.1 | -- [f] | 44.2 | -- [f] | 34.9 | 2.0 | 30.3 | 4.5 | 38.5 | 3.0 |
| Progressive Vaccinia [c] | -- [f] | -- [f] | 3.2 | -- [f] | -- [f] | -- [f] | -- [f] | 6.8 | 1.5 | 3.0 |
| Post-vaccinial encephalitis | 42.3 | -- [f] | 9.5 | -- [f] | 8.7 | -- [f] | -- [f] | 4.5 | 12.3 | 2.0 |
| Death [d] | 5 | -- | 0.5 | -- | 0.5 | -- | unknown | -- | -- | -- |
| Total [e] | 1549.3 | -- [f] | 1261.8 | 200.0 | 855.9 | 85.5 | 1515.2 | 113.6 | 1253.8 | 108.2 |

- Adapted from Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. J Infect Dis. 1970; 122:303-309. [17]
- Referenced as accidental implantation.
- Referenced as vaccinia necrosum.
- Death from all complications.
- Rates of overall complications by age group include complications not provided in this table, including severe local reactions, bacterial superinfection of the vaccination site, and erythema multiforme.
- No instances of this complication were identified during the 1968 10-state survey.
- Overall rates for each complication include persons of unknown age.

More recent data on the incidence of AEs among adult military personnel and civilian first responders vaccinated with Dryvax® during pre-event programs starting in December 2002/January 2003 are presented in Table 2. These data show significant decreases in the incidence of preventable AEs (eczema vaccinatum, contact transmission, and auto-inoculation) as compared to historical data, probably reflecting better screening procedures and routine use of protective bandages over the inoculation site. Myocarditis and/or pericarditis was not a common AE reported in the surveys of the 1960s [23], but emerged as the most frequent serious adverse event (SAE) in the US Department of Defense (DoD) and Department of Health and Human Services (DHHS) programs with incidence rates of 117.71 [24] and 519.52 [25] cases per million vaccinations, respectively.

Table 2: Anticipated and Unanticipated SAEs Associated With Large-scale Immunization Programs by the DoD and DHHS

| Adverse event | DoD program (n=730,5801 [a]) as of Jan05 | | DHHS program (n=40, 422) [b] as of Jan04 | |
|---------------------------------|---|-----------------------|---|-----------------------|
| | N | Incidence /million | N | Incidence /million |
| Myo/pericarditis | 89 | 117.71 | 21 | 519.52 |
| Post-vaccinal encephalitis | 1 | 1.37 | 1 | 24.74 |
| Eczema vaccinatum | 0 | 0 | 0 | 0 |
| Generalized vaccinia | 43 | 58.86 | 3 | 74.22 |
| Progressive vaccinia | 0 | 0 | 0 | 0 |
| Fetal vaccinia | 0 | 0 | 0 | 0 |
| Contact transmission | 52 | 71.18 | 0 | 0 |
| Auto-inoculation (nonocular) | 62 | 84.86 | 20 | 494.78 |
| Ocular vaccinia | 16 | 21.9 | 3 | 74.22 |

a. 71% primary vaccination; 89% male; median age 28.5 yr.

b. 36% primary vaccination; 36% male; median age 47.1 yr.

2. PRODUCT OVERVIEW

2.1. ACAM2000 Product Description

Acambis Inc. has developed a live Smallpox Vaccine, ACAM2000 [Proper name: Smallpox Vaccine (Vero Cells), Lyophilized], containing live vaccinia virus derived by plaque purification cloning from the licensed calf-lymph produced vaccine, Dryvax[®] Dried Smallpox Vaccine (Dryvax[®], Wyeth Laboratories, Marietta, PA). The goal in developing ACAM2000 was to produce a clonally pure virus derived from Dryvax[®] that would provide effective protection against smallpox disease, possess an acceptable safety profile, and which could be manufactured efficiently in serum-free tissue culture (Vero cells) to produce a second generation, purified, and lyophilized smallpox vaccine. The advantages with cell culture based manufacturing over production in calf skin include better control for adventitious agents and a more consistent product quality with higher purity.

ACAM2000 is manufactured in compliance with current Good Manufacturing Practice (cGMP) regulations with cell harvest production performed by Baxter International, Inc. and bulk processing/purification and formulation being carried out by Acambis. Consistent cGMP manufacturing based on a well controlled process has been demonstrated with approximately 75 lots, representing over 190 million doses, being supplied to the UUS Strategic National Stockpile (SNS).

ACAM2000 is a lyophilized preparation of purified live vaccinia virus containing the following non-active excipients:

- 6-8 mM HEPES (pH 6.5-7.5)
- 2% human serum albumin United States Pharmacopeia (USP)
- 0.5 -0.7% sodium chloride USP
- 5% mannitol USP

Trace levels of residual neomycin and polymyxin B from the manufacturing process may be present in the vaccine. The diluent for ACAM2000 contains 50% (v/v) Glycerin USP, 0.25% (v/v) Phenol USP in Water for Injection USP, and is supplied in 3 mL clear glass vials containing 0.6 mL of diluent.

Each vial of ACAM2000 is reconstituted using 0.3 mL of the supplied diluent. After reconstitution, each vial of ACAM2000 vaccine contains 100 nominal doses (0.0025 mL/dose) and is stable at 2-8 °C for up to 30 days. The concentration of vaccinia virus in the reconstituted solution is 1.0-5.0×10⁸ PFU/mL determined by plaque assay in Vero cells. One vial of diluent, 100 bifurcated needles and a 1-mL syringe are necessary for use of each vial of vaccine.

2.2. Indication

ACAM2000 is indicated for active immunization against smallpox disease for persons determined to be at risk for smallpox infection. The determinations of those at risk are developed and managed in accordance with the policies governing the use of a SNS product and DoD regulations.

2.3. Strategic National Stockpile (SNS)

ACAM2000 was produced under a contract awarded by the CDC in November 2001 for the development and manufacture of a second generation smallpox vaccine. Under this contract more than 192.5 million doses have been manufactured and supplied to the US Government SNS. This vaccine may be provided for military vaccination and to others at risk of smallpox infection post licensure. Acambis is also awaiting a contract grant (pending licensure) to maintain an ongoing manufacturing capacity of ACAM2000

ACAM2000 will not be marketed for general commercial distribution. The product will be manufactured solely for the following groups:

1. The CDC of the US Government for stockpiling at the SNS and for limited use in accordance with CDC and DoD regulations,
2. The World Health Organization (WHO) for stockpiling, and
3. Foreign governments for stockpiling and potential use outside the US.

3. DEVELOPMENT OF ACAM2000 (SMALLPOX VACCINE)

3.1. Required Vaccine Specifications

As part of the Request for Proposal (RFP) issued by the CDC on November 1, 2001, specifications for a new smallpox vaccine were established. Acambis modeled the development of a second generation smallpox vaccines based on these specifications. These specifications included, but were not limited to:

- Vaccine administration via the percutaneous route by bifurcated needle.
- Packaged as wet-frozen or freeze-dried preparation in units of 100 doses per container.
- Minimum potency of 1×10^8 PFU/mL.
- Produced in cell-culture from fully qualified, current cGMP compliant cell banks.
- Derived from the NYCBH Laboratory strain of vaccinia virus, or comparable strains of known efficacy against smallpox virus.
- Scalable manufacture capable of production of up to 250 million doses in a 12 month period.

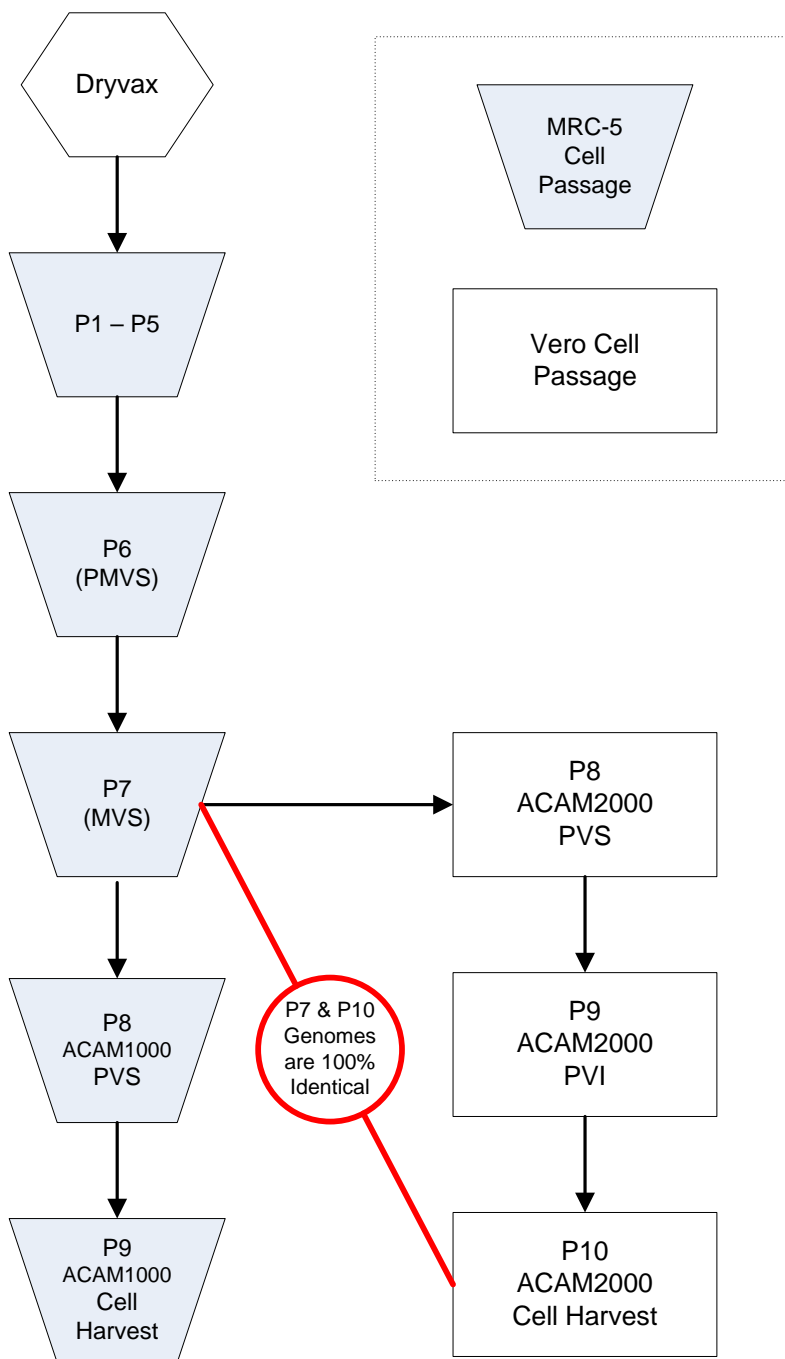
3.2. Development of ACAM2000

In order to develop a modern smallpox vaccine, Acambis first characterized six cloned vaccine candidates, isolated by plaque-purification, and a polyclonal virus; all were derived from the Dryvax® NYCBH vaccinia virus strain and grown in cell cultures of diploid human embryonic lung (MRC-5) cells [26]. Based on its reduced neurovirulence in suckling mice and similarity to Dryvax® in other characteristics, clone number 2 was selected as the best candidate for further development and was renamed ACAM1000.

The ACAM1000 master virus seed (Passage 7) was used to prepare the second candidate vaccine, ACAM2000, by growth in a continuous line of African green monkey kidney (Vero) cells under serum-free conditions (cell line established and provided by Baxter International, Inc.). The ACAM2000 production virus seed and smallpox vaccine are 8 and 10 passages, respectively, from Dryvax® (Figure 1). The ACAM2000 virus is treated with endonuclease enzyme (Benzonase) to digest cellular nucleic acids, and is purified by ultrafiltration and diafiltration to produce the vaccine product.

The vaccine product was shown to be negative for a variety of adventitious agents including mycoplasma and viruses. The genomes of ACAM1000 master virus seed (Passage 7) and ACAM2000 vaccine (Passage 10) were sequenced at St. Louis University, St. Louis, MO, and CDC, Atlanta, GA, respectively, and found to be identical [27]. Therefore, ACAM1000 (Passage 9) and ACAM2000 (Passage 10) may be considered equivalent. ACAM1000 and ACAM2000 were shown to have similar safety and immunogenicity profiles in nonclinical studies and in Phase 1 and 2 clinical studies. Based on these findings and the recommendation by a Joint Down-Selection Working Group of the National Vaccine Advisory Committee (NVAC) and the

Figure 1: Passage History of ACAM1000 and ACAM2000



MVS = Master Virus Seed; P = Passage; PMVS = Pre-master Virus Seed; PVI = Production Virus Inoculum;
PVS = Production Virus Seed

Defense Science Board, the CDC recommended in February 2003 that Acambis stop the co-development of ACAM1000 and focus its manufacturing and clinical study resources solely on the production and development program of ACAM2000. Data obtained in nonclinical and clinical studies of ACAM1000 prior to selection of ACAM2000 are considered to be supportive of data obtained with ACAM2000.

4. NON-CLINICAL STUDIES OF ACAM2000

4.1. Non-Clinical Evaluations

ACAM2000 was tested in nine non-clinical studies in order to evaluate its safety and efficacy. In designing the non-clinical testing program, Dryvax[®] treated groups were included in accordance with the CDC requirement that the vaccine candidate be developed to molecular and non-clinical biological criteria similar to DryVax[®]. Dryvax[®] is the only US Food and Drug Administration (FDA)-licensed smallpox vaccine in the US and provided a reliable standard against which to measure immunogenicity and virulence potential in animal systems. Table 3 presents a summary of the non-clinical studies conducted with ACAM2000 and with the ACAM2000 Master Seed Virus (ACAM1000).

4.1.1. Non-Clinical Efficacy

With the worldwide eradication of smallpox, protective efficacy studies are no longer possible for smallpox vaccine candidates. Therefore, the animal challenge studies, as summarized in Table 3, provide very important supportive data for the efficacy of ACAM2000.

The primate challenge study performed at the Battelle Memorial Institute is of particular note relative to vaccine efficacy. In this study twenty four (24) cynomolgus monkeys, were randomized into three groups (8 monkeys per group). Each group received inoculations of either ACAM2000 (potency= 4.4×10^8 PFU/mL), Dryvax[®] (potency= 1.5×10^8 PFU/mL), or a negative control material (an equivalent volume of ACAM2000 diluent). All were vaccinated on Day 0 and were challenged with a virulent dose (3.8×10^7 PFU) of monkeypox virus at Day 61. Monkeys were observed for morbidity and mortality for 30 days post challenge. The following study observations were made:

- Seroconversion (≥ 4 -fold rise in neutralizing antibodies between Days 0 and 30) rates were identical for ACAM2000 and Dryvax[®] (100% each) whereas placebo treatment resulted in 0% seroconversion. The geometric mean titer (GMT) for ACAM2000 was 160 (range 40-320) and Dryvax[®] was 174 (range 40-640).
- Those vaccinated with either ACAM2000 or Dryvax[®] developed cutaneous lesions characteristic of a "take" or positive dermal response as observed in human subjects.
- Post-challenge (day 91) antibody results for both ACAM2000 and Dryvax[®] showed greater than 200-fold increases in GMT. The control group also developed a detectable antibody titer post-challenge (Day 67 or 69) with a GMT of 227 (range 70 to 946); however, this was not protective as all 8 controls succumbed to the infection.
- All monkeys (8) vaccinated with the ACAM2000 vaccine survived the lethal challenge with little to no apparent clinical signs of infection, as did the 8 monkeys in the Dryvax[®] group.
- No detectable viremia was found in animals vaccinated with either the ACAM2000 or the Dryvax[®] vaccine at any point during the post-challenge period.
- No relevant clinical symptoms or pathological changes were observed in either the ACAM2000 or the Dryvax[®] vaccinated animals.

Based upon the results of this study, ACAM2000 was determined to be immunogenic and fully efficacious in protecting cynomolgus monkeys from a fatal monkeypox challenge. No post-challenge viral replication was observed in any biological sample tested from the ACAM2000 treatment group and no significant clinical symptoms were observed in any of the ACAM2000 vaccinated animals. All control monkeys receiving placebo (ACAM2000 diluent) succumbed to the virulent monkeypox challenge with viremia tissue virus replication, and numerous monkeypox-specific clinical symptoms.

In summary, the non-clinical studies summarized in Table 3 demonstrate that ACAM2000 elicits neutralizing antibody and T cell responses in mice and monkeys that are sufficient to provide protection against a lethal challenge dose of orthopox virus. The non-clinical studies further support the conclusion that the immunogenicity of ACAM2000 is comparable to Dryvax® based on dose response, and that ACAM2000 has less neurovirulence and cutaneous virulence potential than Dryvax® at similar dose levels.

Table 3: Non-Clinical Studies Conducted with ACAM2000 Vaccine, and Master and Production Virus Banks

| Study Type/Test Article | Study Results |
|---|--|
| Immunogenicity in mice following percutaneous administration / ACAM2000 Vaccine | ACAM2000 (1×10^6 or 1×10^8 PFU/mL) was associated with an increase in neutralizing antibody titer and a T cell response comparable to that observed for Dryvax [®] at similar doses. |
| Protection against Vaccinia Western Reserve (WR) Virus challenge in mice following percutaneous administration / ACAM2000 Vaccine | ACAM2000 provided equivalent protection to Dryvax [®] based on the survival of all vaccinia WR virus-challenged mice at ACAM2000 doses of 1×10^6 and 1×10^7 PFU/mL |
| Immunogenicity and protection against Monkeypox Virus challenge in monkeys following percutaneous administration / ACAM2000 Vaccine | ACAM2000 (4.4×10^8 PFU/mL) was associated with an increase in neutralizing antibody titer comparable to that observed for Dryvax [®] (1.5×10^8 PFU/mL) and provided equal protection of all monkeypox-challenged monkeys. |
| Cutaneous virulence in rabbits following percutaneous administration / ACAM2000 Vaccine | Average erythema and lesion diameters observed in the ACAM2000 groups were less than or equivalent to those in the Dryvax [®] groups at the same dose levels. |
| Neurovirulence in mice following intracerebral administration / Unpurified ACAM2000 Vaccine | Average survival times (AST) in ACAM2000-treated mice were greater than AST in Dryvax [®] -treated mice, except at the highest dose administered (2,000 PFU/mouse). 50% lethal dose (LD ₅₀) value was higher for ACAM2000 than for Dryvax [®] . |
| Neurovirulence in mice following intracerebral administration / ACAM2000 Vaccine | AST were greater in the ACAM2000 treated mice at all doses compared to the Dryvax [®] treated mice. LD ₅₀ value was higher for ACAM2000 than for Dryvax [®] . |
| Neurovirulence in mice following intracerebral administration / ACAM2000 Vero Production Virus Bank P8 | Mortality was significantly lower ($p=0.0003$) in mice treated with the ACAM2000 Production Virus Bank compared to mice treated with Dryvax [®] . |
| Neurovirulence in mice following intracerebral administration / ACAM2000 Vero-Vaccinia TFF Retentate (ACAM2000 Drug Substance) | Mortality was similar and not significantly lower in mice treated with the ACAM2000 Drug Substance compared to mice treated with Dryvax [®] . |
| Neurovirulence in monkeys following intrathalamic administration / ACAM1000 Master Seed Virus | Three of six monkeys treated with Dryvax [®] (4.9×10^7 PFU) died during the study. There were no deaths among six monkeys treated with ACAM1000 Master Seed Virus (1.25×10^7 PFU). Clinical illness scores were lower and clinical signs of vaccine-related meningitis were less severe in the ACAM1000 Master Seed Virus treated animals than the Dryvax [®] treated animals. |

5. CLINICAL DEVELOPMENT OF ACAM2000

5.1. Overview

ACAM2000 was evaluated in six clinical studies sponsored by Acambis Inc., summarized in Table 4. A total of 3881 subjects received either ACAM2000 (2983), ACAM1000 (30, all in Study H-400-002), or Dryvax[®] (868) in these studies. Of these subjects, 2981 were enrolled in two Phase 3 studies.

Table 4: Clinical Studies Conducted in the ACAM2000 Clinical Program

| | Study Number | Subject Population | Total Subjects |
|----------------|--------------|-----------------------|----------------|
| Phase 1 | H-400-002 | Vaccinia-Naïve | 90 |
| | H-400-008 | Vaccinia-Naïve | 100 |
| Phase 2 | H-400-003 | Previously Vaccinated | 357 |
| | H-400-005 | Vaccinia-Naïve | 353 |
| Phase 3 | H-400-009 | Vaccinia-Naïve | 1162 |
| | H-400-012 | Previously Vaccinated | 1819 |
| | | Overall Total | 3881 |

The Phase 1 studies were designed to evaluate the safety, tolerability, and immunogenicity of the ACAM1000 and ACAM2000 vaccines in vaccinia-naïve subjects (note that H-400-002 compared ACAM1000 to ACAM2000 to Dryvax[®]). The Phase 2 studies further assessed the safety and efficacy of ACAM2000 compared to Dryvax[®] in both vaccinia-naïve and previously vaccinated subjects and evaluated the dose response to the vaccine. The Phase 3 studies were designed to assess the safety and efficacy of ACAM2000 based on non-inferiority to Dryvax[®] in the vaccinia-naïve and the previously vaccinated populations, as well as the evaluation of clinical lot consistency for the ACAM2000 vaccine.

5.2. Standard Procedures in Clinical Studies of ACAM2000

The following research procedures and criteria were used in clinical studies conducted with ACAM2000.

5.2.1. Study Design

With the exception of Study H-400-008, all clinical studies in this series were parallel group, double-blind studies with Dryvax[®] as an active control. Following a screening period, all subjects were vaccinated on Day 0 and safety and efficacy assessments were obtained through Day 30 (Day 45 for study H-400-002) as described below.

5.2.2. Administration of Vaccine

Smallpox vaccine was administered percutaneously via fifteen vigorous strokes with a bifurcated needle in the skin of the upper arm over the deltoid muscle, with a small amount of blood subsequently appearing at the vaccination site. In all studies in the ACAM2000 clinical

program, subjects received a single administration of smallpox vaccine via 15 pokes with a bifurcated needle.

5.2.3. Subject Populations for Efficacy Studies

ACAM 2000 has been studied in two subject populations: 1. those who have not been previously vaccinated for smallpox (vaccinia-naïve), and 2. those previously vaccinated with vaccinia.

Subjects enrolled into studies designed to evaluate responses in vaccinia-naïve populations were aged 18 to 30 years and did not have a smallpox vaccination scar at Baseline.

Those enrolled into studies designed to evaluate responses in previously vaccinated populations ranged in age from 29 to 84 years. The majority ($\geq 97\%$) had a confirmed vaccination scar at Baseline. All subjects in this population met the protocol criterion of >10 years since previous vaccination. In studies with previously vaccinated subjects, the mean time between study vaccination and their last previous vaccination was similar in the ACAM2000 (42 years) and Dryvax[®] (41 years) groups.

Specific analysis populations were defined in each of the studies conducted with ACAM2000 to assist in interpretation of the study data. These populations are listed and defined in Table 5.

Table 5: Analysis Populations in Clinical Studies of ACAM2000

| Analysis Population | Definition | |
|--------------------------|---|--|
| | Vaccinia-Naïve subjects | Previously vaccinated subjects |
| Intent to Treat (ITT) | All subjects who received vaccination regardless of any post-vaccination assessments. These subjects may or may not have had cutaneous reaction assessments. | |
| Safety | All subjects who received vaccination (same as ITT population) and had follow-up for safety | |
| Dermal Evaluable | All subjects who received vaccination, had no major protocol violations, and were assessed for local cutaneous reaction between Days 6 and 11. | All subjects who received study vaccination, had no major protocol violations, were assessed for local cutaneous reaction between Days 6 and 8, inclusive, and were evaluated for vaccination response by the Independent Review Committee, based on photographic evidence. |
| Antibody Evaluable (AnE) | All subjects who were randomly assigned to have serum samples analyzed for neutralizing antibody response, received vaccination, were seronegative for vaccinia at Baseline [i.e., had a neutralizing antibody titer <10 (set to 5 for analysis purposes)], had no major protocol violations, and had sera collected for vaccinia antibody assessments at Baseline (Day 0) and on Day 30 (± 3 days). | All subjects who were randomly assigned to have serum samples analyzed for neutralizing antibody response, received vaccination, had no major protocol violations, and had sera collected for vaccinia antibody assessments at Baseline (Day 0) and on Day 30 (± 3 days). |

5.2.4. Primary Efficacy Endpoints

Based on FDA guidance pertaining to the development of second generation smallpox vaccines [28] and communications with the Agency, Acambis' clinical trials have been designed to evaluate the ACAM2000 vaccine based on two surrogate endpoints for efficacy, specifically, the rate of vaccination success based on the proportion of subjects displaying a major cutaneous response, and the vaccinia-specific neutralizing antibody GMT.

The two surrogate endpoints for efficacy were established based upon the following observations:

- a. In historical practice, vaccinated individuals were considered fully protected against smallpox after a major cutaneous reaction was observed [29].
- b. Two prospective studies have correlated higher titers of neutralizing antibodies with lower susceptibility to smallpox upon contact with a smallpox victim [10, 5]. Although the methods used to measure neutralizing antibody titers differ between these published studies and Acambis' clinical trials, the data indicate that neutralizing antibody titer serves as a qualitative, if not a quantitative, correlate of protection. The limited data from these studies suggested that neutralizing antibody titers greater than 32 may correlate with protection [10, 5] although this value cannot be validated due to eradication of the disease.

Definition of Successful Vaccination and Revaccination Based on Dermal Response

The definitions of successful vaccination and revaccination used in ACAM2000 clinical studies were consistent with the Advisory Committee on Immunization Practices (ACIP) [29] and WHO [3] definitions. Successful vaccination was defined as a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion that might be a crust or an ulcer. Since skin reactions may be less pronounced for revaccination, an independent review committee (IRC) was used in the evaluation of vaccination success for the previously vaccinated subjects in the Phase 3 study. The IRC reviewed digital photographs of the vaccination site taken by study center personnel and assessed subjects' responses to vaccination. The review was blinded to study treatment. This evaluation was conducted because of the acknowledged difficulty in determining successful revaccination based on the examination of the cutaneous response and the need to objectively assess vaccination success in a standardized fashion across all subjects in a multicenter study. The IRC consisted of 3 smallpox experts.

Determination of Neutralizing Antibody Response

Neutralizing antibody response to smallpox vaccination was determined using a 50% plaque reduction neutralization test (PRNT₅₀). The method determines the dilution of a test serum which, when pre-incubated with a fixed number of PFU of the vaccine virus strain, results in a 50% reduction in plaques when the virus-serum mixture is plated on Vero cells. The method was developed and validated by Acambis. The test serum titer is reported as the reciprocal of the dilution resulting in 50% plaque reduction. An arbitrary neutralizing antibody titer of 5 was assigned to a serum sample with no detectable neutralizing antibody at a dilution of 1:10. In order to determine the neutralizing antibody response in vaccinated populations, GMTs were calculated.

5.2.5. Evaluations of Vaccine Safety

The safety of study vaccine was assessed by structured interviews and subject diaries following vaccination, recording of concomitant medications, assessment of vital signs including body temperature, physical examination findings, and clinical laboratory tests. In a Phase 1 study (H-400-002) this was supplemented by measurements of virus shedding from the vaccination site, and in the Phase 3 studies and Study H-400-002, assessments were made post-vaccination of electrocardiogram (ECG) findings and cardiac troponin I measurements. Female subjects of child-bearing age were routinely tested for pregnancy using standard serum β -human chorionic gonadotropin (β -HCG) assays.

AEs were solicited via structured interviews at scheduled study center visits, with specific prompts for AEs historically associated with smallpox vaccination. A diary to be completed by the subject served as an aid to memory for the interview. In order to identify potential symptoms of myocarditis/pericarditis, subjects in the Phase 2 studies (after 28 March 2003) and Phase 3 studies were also questioned for symptoms of chest pain, shortness of breath, heart palpitations, and reduced tolerance to exercise.

Surveillance for selected cardiac, dermatologic and neurologic AEs was intensified due to reports of cardiac AEs in the civilian and US DoD vaccination programs utilizing Dryvax[®] [30,31]. In the Phase 3 studies (Studies H-400-009 and H-400-012) and one Phase 1 study (Study H-400-002), clinical algorithms were included to help the Investigator with the recognition of clinically apparent vaccinia-related myocarditis or pericarditis, specific dermatologic reactions, and neurologic complications associated with smallpox vaccination.

In clinical studies of ACAM2000, with the exception of Phase 1 Study H-400-008, serious and non-serious AE data were reviewed at scheduled intervals by a Data Safety Monitoring Board (DSMB) during the conduct of the studies, with stopping rules in place in the event any SAE was considered to be study-vaccine related in subjects or in contacts of subjects. Furthermore, a blinded Cardiology Advisory Panel (CAP) reviewed all serious cardiac events, abnormal ECG findings and troponin I levels in Studies H-400-009 and H-400-012 at the request of the DSMB, with the goal of providing a consensus diagnosis for each case of suspected/probable myocarditis or pericarditis and identifying additional cases by review of ECG and troponin I abnormalities.

6. CLINICAL EFFICACY RESULTS

This section provides a brief description of the major results and conclusions from each of the clinical studies which pertain to the clinical efficacy of the ACAM2000 smallpox vaccine. The Phase 3 efficacy results presented in this section are based on the analysis of clinical data which excluded four clinical sites (all treated subjects were considered for safety analyses). The FDA requested that these sites not be included due to possible Good Clinical Practice (GCP) violations. However, there were no differences in efficacy conclusions from the Phase 3 studies, with or without the inclusion of data from these four clinical study sites. Data from an efficacy analysis that includes all sites is provided as an appendix in Section 11.1 of this briefing document for reference. An integrated assessment of the efficacy of ACAM2000 based on all of the clinical studies, including comparison to the currently licensed vaccine, Dryvax[®], is provided in Section 6.4. The list of clinical studies conducted with ACAM2000 was provided in Section 5.1, Table 4.

6.1. Immunogenicity Results from Phase 1 Studies

Two Phase 1 studies, H-400-002 and H-400-008, were conducted in vaccinia-naïve subjects. Study H-400-008 was a Phase 1, open-label, single-arm, fixed-dose study designed to evaluate the safety, tolerability and immunogenicity of ACAM2000. The clinical lot used in this study had a potency of 7.7×10^7 PFU/mL, which was slightly below the nominal target of 1.0×10^8 PFU/mL. One hundred adults, aged 18 to 29 years, who were naïve to smallpox vaccine, were enrolled in the study. Ninety-nine percent of subjects (99 of 100 subjects) experienced a successful vaccination based on their cutaneous reactions. The GMT on Day 30 was 225.

Study H-400-002 was a randomized, double-blind study to compare the immunogenicity of ACAM1000 or ACAM2000 to Dryvax[®] in adults 18 to 29 years of age. A dose of 1.0×10^8 PFU/mL was delivered in all treatment groups. Thirty (30) subjects were vaccinated in each treatment group. All subjects in each treatment group (30 of 30 subjects, 100%) experienced a successful vaccination based on cutaneous reaction. The GMT on Day 45 was 124.1, 103.2 and 171.5 in the ACAM1000, ACAM2000 and Dryvax[®] groups, respectively; the differences between groups were not significant ($p \geq 0.2273$).

Protection against smallpox disease is mediated by neutralizing antibodies and cytotoxic T cells, and both B and T cells provide long-term memory [32]. In study H-400-002, all subjects were evaluated at Day 45 for cytotoxic T lymphocytes (CTL assay), cytokine producing cells (γ -interferon ELISPOT assay), and vaccinia-specific lymphoproliferation (LPA assay). The results are summarized in Table 6. All 30 subjects (100%) in the ACAM2000 group, and 28 (93%) of 30 subjects in the Dryvax[®] group demonstrated a positive cell-mediated immune response to smallpox vaccine in at least one of the three T-cell assays. These results are in agreement with studies performed with Dryvax[®] for development of cytotoxic T-cell responses and increases in virus specific γ -interferon-producing cells in vaccinia-naïve subjects [33, 34].

Table 6: Proportion of Subjects with Positive T Cell Responses in Study H-400-002, by Treatment Group

| Assay Method | Treatment Group | |
|-----------------------------------|----------------------|---------------------|
| | ACAM2000 (n = 30) | Dryvax® (n = 30) |
| CTL assay, n (%) | 26 (87) | 22 (73) |
| γ-interferon ELISPOT assay, n (%) | 30 (100) | 27 (90) |
| LPA assay, n (%) | 29 (97) | 26 (87) |
| At least one assay, n (%) | 30 (100) | 28 (93) |

6.2. Summary of Efficacy Results from Phase 2 Studies

The immune response to ACAM2000 as a function of administered dose was evaluated and compared to Dryvax® in two Phase 2 studies. Study H-400-005 assessed the dose response to ACAM2000 in vaccinia-naïve subjects, while study H-400-003 evaluated the dose response in previously vaccinated (Dryvax®) individuals. The results of these studies are summarized below.

6.2.1. Study H-400-005: Vaccinia-Naïve Subjects

Study H-400-005 was a Phase 2, double-blind, randomized, active controlled study evaluating the safety, tolerability and immunogenicity of 4 dose levels of ACAM2000 compared to Dryvax® in 353 healthy vaccinia-naïve adults, ages 18-30. The comparator vaccine, Dryvax®, was administered at a dose of 1.6×10^8 PFU/mL and doses of ACAM2000 ranged from a high of 6.8×10^7 PFU/mL to a low of 3.4×10^6 PFU/mL. No data were collected on the dose response to Dryvax® in this study.

Subjects were vaccinated on Day 0 and asked to return to the clinic on study Days 3, 7, 10, 15 and 30. The efficacy of each vaccine dose was evaluated based on the rates of major cutaneous reactions on Days 7 or 10 and the neutralizing antibody responses on Day 30. The results of the study are presented below, in Table 7.

Table 7: Vaccination Success and Neutralizing Antibody Response as a Function of ACAM2000 Dose for Study H-400-005: Vaccinia-Naïve Subjects

| Treatment group (PFU/Dose) | Dryvax® (1.6×10 ⁸) | ACAM2000 (6.8×10 ⁷) | ACAM2000 (1.4×10 ⁷) | ACAM2000 (6.8×10 ⁶) | ACAM2000 (3.4×10 ⁶) |
|--|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Vaccination Success based on Cutaneous Response | | | | | |
| Evaluable Subjects, n | 49 | 51 | 100 | 100 | 49 |
| Vaccination Success, n (%) | 49 (100) | 51 (100) | 86 (86) | 80 (80) | 29 (59) |
| Difference from Dryvax® [a] | -- | Undefined [b] | -22, -6 | -29, -11 | -57, -25 |
| Neutralizing Antibody Titer | | | | | |
| Evaluable Subjects, n | 49 | 51 | 100 | 100 | 50 |
| GMT | 158 | 154 | 101 | 61 | 31 |
| Ratio of GMT [c] | -- | 0.468, 1.995 | 0.339, 1.202 | 0.209, 0.741 | 0.095, 0.914 |
| Log ₁₀ GMT: Difference from Dryvax® [a] | -- | -0.33, 0.30 | -0.47, 0.08 | -0.68, -0.13 | -1.02, -0.39 |

- Lower and upper bound of 95% CI on ACAM2000 difference from Dryvax®, as derived by normal approximation
- Because the successful vaccination rates and the CIs for the ACAM2000 6.8×10⁷ dose group and Dryvax® were identical, the difference between ACAM2000 6.8×10⁷ versus Dryvax® could not be computed based on the normal approximation to the binomial.
- Lower and upper bound of 95% CI on the ratio of ACAM2000 GMT/Dryvax® GMT

With ACAM2000, rates of successful vaccination based on cutaneous response ranged from a low of 59% in subjects administered the lowest dose of vaccine to a high of 100% in subjects administered the highest dose, as presented in Table 7 and graphically in Figure 2. Based on a probit analysis, which models the probability of a successful reaction as a function of dose, the ACAM2000 dose necessary to achieve a successful vaccination in 90% of vaccinia-naïve subjects was 1.5×10⁷ PFU/mL, with an upper limit of the 95% confidence interval (CI) of 2.7×10⁷ PFU/mL. Vaccination success (as determined by cutaneous response) was also found to be dose dependent in a study performed with Dryvax® in vaccinia-naïve subjects [13]. Similarly, GMTs were sensitive to the administered dose of ACAM2000. In the highest dose ACAM2000 group, the GMT was considered equivalent to that in the Dryvax® group. The neutralizing antibody titer declined with decreasing doses of ACAM2000 (Table 7 and Figure 3).

Figure 2: ACAM2000 Dose Response and Comparison to Dryvax®: Vaccination Success based on Cutaneous Response in Vaccinia-Naïve Subjects (Study H-400-005)

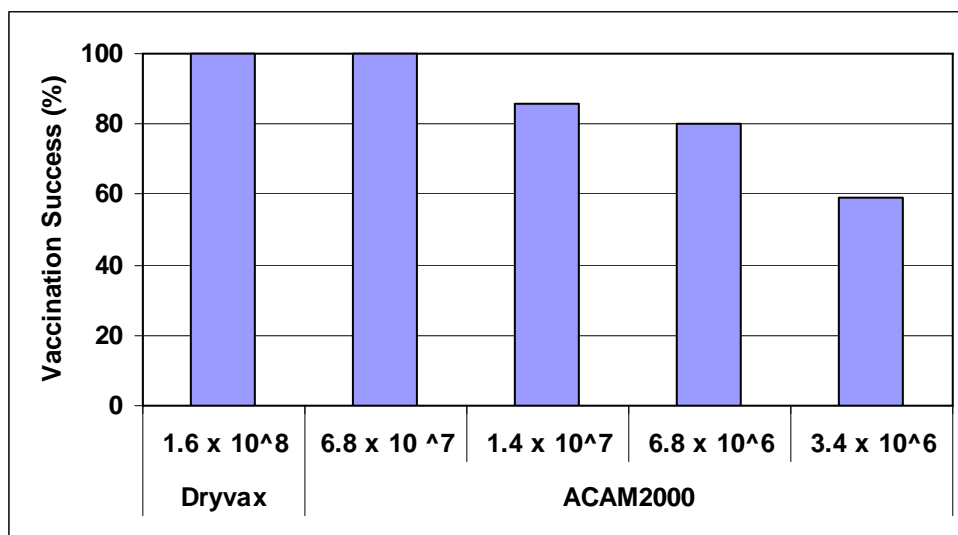
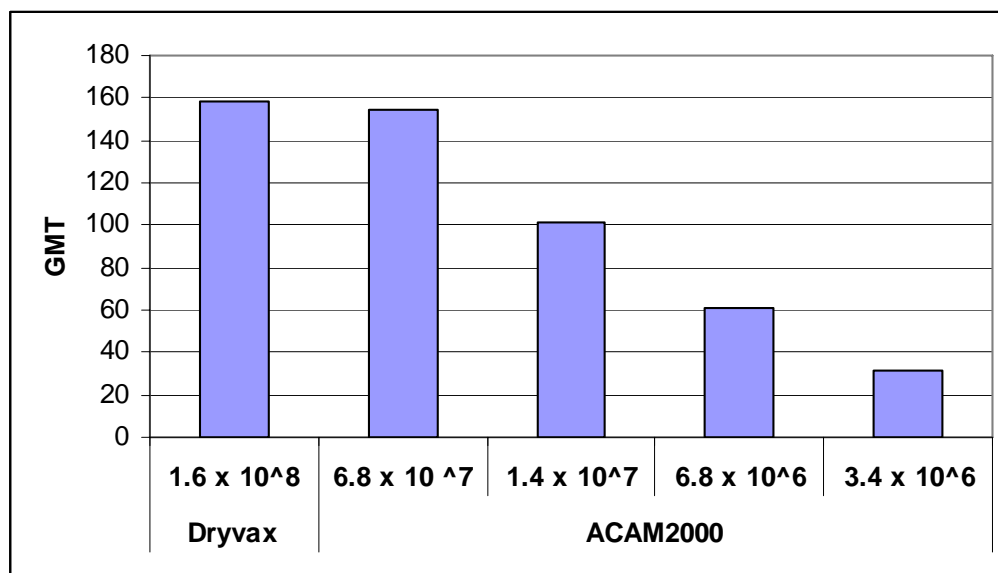


Figure 3: ACAM2000 Dose Response and Comparison to Dryvax®: Neutralizing Antibody GMT in Vaccinia-Naïve Subjects (Study H-400-005)



The highest dose ACAM2000 group (6.8×10^7 PFU/mL) was found to be substantially equivalent to Dryvax® for both measures of efficacy. The percentage of subjects with successful vaccination in the ACAM2000 6.8×10^7 PFU/mL dose group and the Dryvax® 1.6×10^8 group was 100% (Table 7). Each group had identical 2 sided exact 95% CIs of 93% and 100%. Similarly, the GMTs for the ACAM2000 6.8×10^7 dose group and the Dryvax® group (154 and 158, respectively) may be considered equivalent since the 2-sided 95% CI on the ratio of the geometric mean titers fell approximately between 0.5 and 2.0 (actual value 0.468 to 1.995). In contrast, all lower dose groups of ACAM2000 were found to be not statistically equivalent to Dryvax® for both primary efficacy measures.

6.2.2. Study H-400-003: Previously Vaccinated (Dryvax®) Subjects

Study H-400-003 was a Phase 2, double-blind, randomized study designed to evaluate the safety, tolerability and immunogenicity of four dose levels of ACAM2000 versus Dryvax® in subjects previously vaccinated with Dryvax® smallpox vaccine. Healthy adults, aged 28 years or older, who had received smallpox vaccine more than 10 years previously, were enrolled in the study at 3 centers in the US. There were no significant differences between subjects in each treatment with respect to demographic variables or baseline characteristics. The majority of subjects in all groups were seropositive for vaccinia antibodies at Baseline.

No data were collected on the dose response to Dryvax® in this study.

Subjects were inoculated on Day 0 and instructed to return to the clinic on Days 3, 7, 10, 15 and 30. At each visit, the size and appearance of the local cutaneous reaction was assessed and recorded. Blood samples were collected on Day 30 for serum neutralizing antibody assays.

Table 8 presents the rates of successful revaccination (based on cutaneous response) and the neutralizing antibody GMTs observed for each dose group at Day 30.

Table 8: Vaccination Success based on Cutaneous Response and Neutralizing Antibody Response as a Function of ACAM2000 Dose for Study H-400-003: Previously Vaccinated (Dryvax®) Subjects

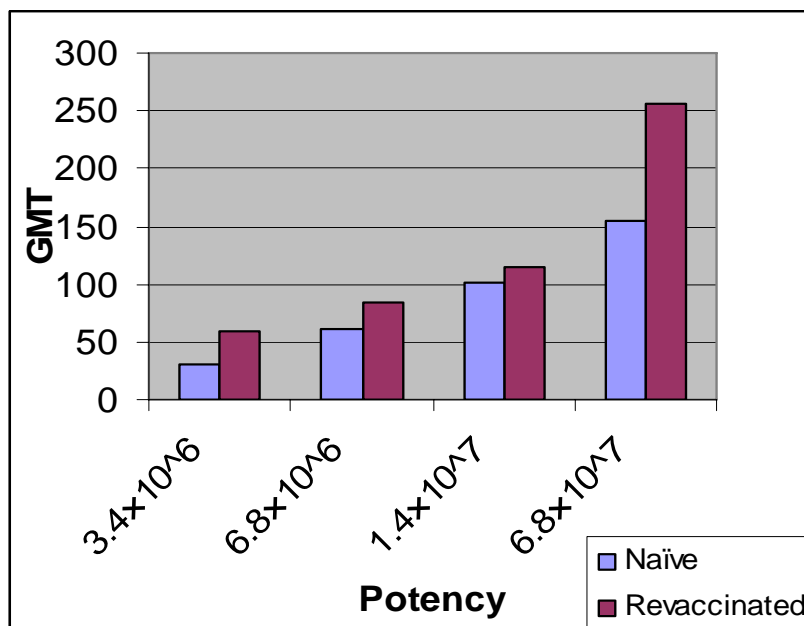
| Treatment group (PFU/Dose) | Dryvax® (1.6×10 ⁸) | ACAM2000 (6.8×10 ⁷) | ACAM2000 (1.4×10 ⁷) | ACAM2000 (6.8×10 ⁶) | ACAM2000 (3.4×10 ⁶) |
|--|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Revaccination Success based on Cutaneous Response | | | | | |
| Evaluable Subjects | 52 | 49 | 101 | 100 | 49 |
| Revaccination Success, n (%) | 52 (100) | 43 (88) | 52 (51) | 40 (40) | 13 (27) |
| Difference from Dryvax® [a] | -- | -23, -1 | -60, -37 | -71, -49 | -88, -59 |
| Neutralizing Antibody Titer | | | | | |
| Evaluable Subjects | 52 | 50 | 102 | 102 | 51 |
| GMT | 447 | 256 | 115 | 84 | 59 |
| Ratio of GMT [b] | -- | 0.309, 1.072 | 0.151, 0.447 | 0.110, 0.324 | 0.071, 0.245 |
| Log ₁₀ GMT: Difference from Dryvax® [a] | -- | -0.51, 0.03 | -0.82, -0.35 | -0.96, -0.49 | -1.15, -0.61 |

- a. Lower and upper bound of 95% CI on ACAM2000 difference from Dryvax®, as derived by normal approximation.
b. Lower and upper bound of 95% CI on the ratio of ACAM2000 GMT/Dryvax® GMT.

The rates of successful revaccination based on cutaneous response were 88%, 51%, 40%, and 27% in the ACAM2000 6.8×10⁷ PFU/dose, 1.4×10⁷, 6.8×10⁶ PFU/dose, and 3.4×10⁶ PFU/dose groups, respectively, and 100% in the Dryvax® group (1.6×10⁸ PFU/dose). As was observed for the vaccinia-naïve population, a dose response was seen for ACAM2000 with respect to revaccination success rate and GMT. This dose-dependency of the vaccination success rates is also consistent with the results of a study in previously vaccinated subjects when Dryvax® was diluted prior to administration [35]. In summary, this study demonstrated that in previously vaccinated individuals, the highest ACAM2000 dose group (6.8×10⁷) was not equivalent to Dryvax® with respect to revaccination success (as determined by cutaneous response) or GMT.

However, it should be noted that at all dose levels of ACAM2000 the GMTs were higher for the revaccinated subjects than the vaccinia-naïve subjects. (Figure 4).

Figure 4: ACAM2000 GMTs for Vaccine-Naïve vs. Previously Vaccinated Subjects (Studies H-400-003 and H-400-005)



6.3. Summary of Efficacy Results from Phase 3 Studies

The efficacy of ACAM2000 was evaluated and compared to Dryvax[®] in two Phase 3 studies. Study H-400-009 assessed the response to ACAM2000 and Dryvax[®] in a vaccinia-naïve population, while Study H-400-012 compared the response to both products in a previously vaccinated population. Both studies used ACAM2000 lots with potencies ranging from 1.3×10^8 to 2.2×10^8 PFU/mL and Dryvax[®] with a potency of 1.5×10^8 PFU/mL.

Study H-400-009 (vaccinia-naïve subjects) found that ACAM2000 was non-inferior to Dryvax[®] with respect to cutaneous response, but narrowly missed the endpoint for non-inferiority with respect to GMT (97.5% CI lower bound on the difference in \log_{10} GMT for non-inferiority was pre-defined at ≥ -0.301 and the study outcome was -0.307). On the other hand, study H-400-012 (previously vaccinated subjects) found that ACAM2000 was statistically non-inferior to Dryvax[®] with respect to GMT, but did not meet the endpoint for non-inferiority with respect to the cutaneous response.

The results of the final analysis of each study are summarized in further detail below, including a discussion of why ACAM2000 is considered efficacious despite the fact that the GMT of vaccinia-naïve subjects receiving ACAM2000 and Dryvax narrowly missed the statistical margin to demonstrate non-inferiority and that ACAM2000 does not elicit the same frequency of cutaneous response as Dryvax[®] in previously vaccinated subjects.

6.3.1. Phase 3 Study H-400-009: Vaccinia-Naïve Subjects

Study H-400-009 was a Phase 3, double-blind, randomized study designed to evaluate the safety and immunogenicity of ACAM2000 versus Dryvax® in adults 18 to 30 years of age, inclusive, who were naïve to smallpox vaccine. Subjects were randomized 3:1 to receive ACAM2000 and Dryvax®, respectively. In addition, consistency was confirmed by comparing 3 conformance lots of ACAM2000. The original design called for enrollment of 2720 healthy subjects. However, observed rates of occurrence of myocarditis/pericarditis were unexpectedly higher than those previously published for smallpox vaccine in both the ACAM2000 and Dryvax® treatment groups. Consequently, Acambis voluntarily suspended enrollment into this study. Following the DSMB review of the safety data, in conjunction with the analyses provided by a CAP, the DSMB concluded that if more data were needed to meet the primary efficacy end points, then the studies could continue as planned, with a revised informed consent describing the new findings on the risk of myocarditis/ pericarditis. However, Acambis calculated at the time that there was sufficient power to meet the objectives of the Phase 3 studies, and with the concurrence of the FDA, closed the study, resulting in a smaller sample size than originally planned.

Calculations performed after the studies were prematurely terminated and the 4 clinical sites removed (per FDA's request) indicate that the power for study H-400-009 (Vaccinia-Naïve) vaccination success (cutaneous response) was $\geq 80\%$ and the power for neutralizing antibody GMT was $>90\%$. For Study H-400-012 (Previously Vaccinated), the power for revaccination success (cutaneous response) and neutralizing antibody GMT was 72% and 85%, respectively.

A total of 1162 subjects were enrolled at 69 study centers in the US and its territories of which 1033 subjects at 65 sites qualified for the final analysis for dermal response. There were no significant differences between the Dryvax® and ACAM2000 groups with regard to demographic or baseline characteristics. A summary of demographic information for all patients enrolled in ACAM2000 studies, including Phase 3, is provided as an appendix in Section 11.2.

At Baseline (Day 0), subjects received a double-blind percutaneous vaccination in the deltoid region with ACAM2000 or Dryvax®. On Days 7, 10, 21, and 30 post-vaccination, study personnel examined the vaccination site and scored the local cutaneous reaction. Blood samples for assay of serum neutralizing antibodies were obtained at the final study center visit on Day 30.

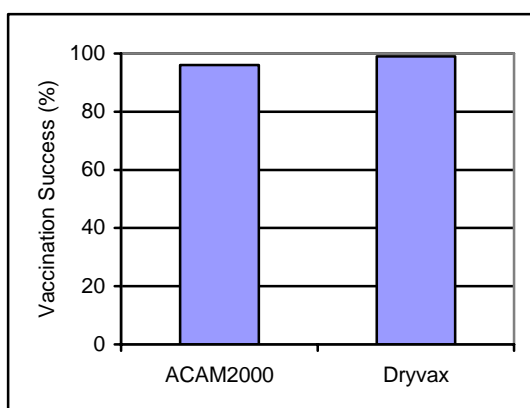
The effectiveness of study vaccine was determined by two co-primary endpoints: the proportion of subjects with a successful vaccination based on the investigators' assessments of cutaneous reaction on Day 7 and/or 10, and GMT of neutralizing antibodies on Day 30. The endpoints were evaluated based on statistical tests for the non-inferiority of ACAM2000 compared to Dryvax®. Table 9 summarizes the efficacy results of study H-400-009 for ACAM2000 versus Dryvax®. Vaccination success (as determined by cutaneous response) and GMT results are plotted in Figure 5.

Table 9: Phase 3 Efficacy Results for ACAM2000 versus Dryvax® in Vaccinia-Naïve Subjects (Study H-400-009)

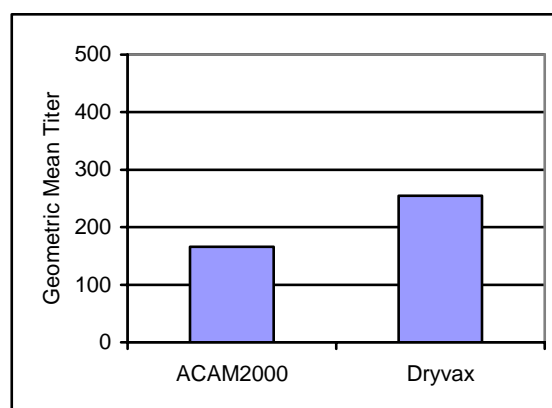
| Treatment group | ACAM2000 | Dryvax® |
|---|------------------|----------|
| Vaccination Success based on Cutaneous Response | | |
| Evaluable Subjects (Dermal Evaluable Population) | 776 | 257 |
| Vaccination Success, n (%) | 747 (96) | 255 (99) |
| 97.5% CI [a] (Criterion for Non-Inferiority) | -4.67 (>-5.00) | |
| Neutralizing Antibody Titer | | |
| Evaluable Subjects (Antibody Evaluable Population) | 565 | 190 |
| GMT | 166 | 255 |
| 97.5% CI [b] (Criterion for Non-Inferiority) | -0.307 (>-0.301) | |

- a. Lower bound of the 97.5% one-sided CI on the difference between ACAM2000 and Dryvax® derived by normal approximation to the binomial distribution.
- b. Lower bound of the 97.5% one-sided CI on the difference in the mean log₁₀ GMT between ACAM2000 and Dryvax® derived using analysis of variance (ANOVA).

Figure 5: Graphical Presentation of Phase 3 Efficacy Results in Vaccinia-Naïve Subjects (Study H-400-009)



Panel A: Vaccination Success



Panel B: Neutralizing Antibody Titer

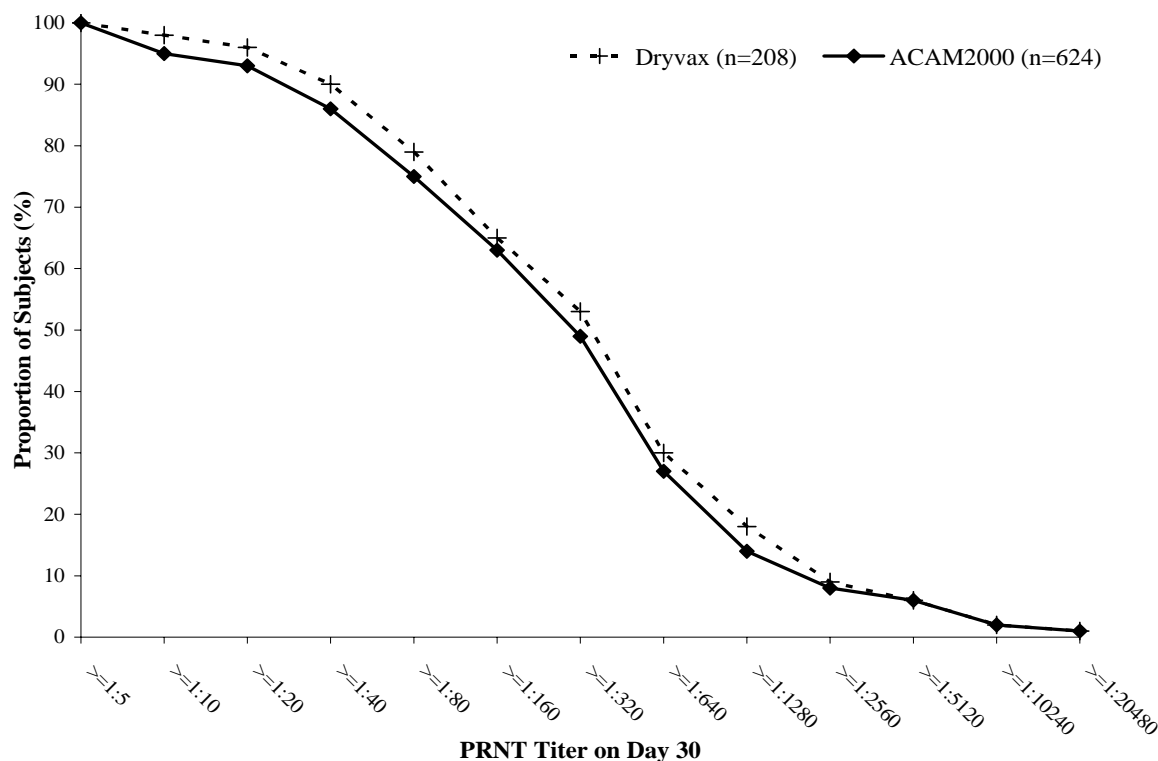
ACAM2000 was found to be non-inferior to Dryvax® with respect to vaccination success, as determined by cutaneous response rates, in the vaccinia-naïve population. Ninety-six percent (96%) of subjects in the ACAM2000 dose group and 99% of subjects in the Dryvax® group experienced a major cutaneous response after vaccination.

The GMT of the neutralizing antibody response was higher in the Dryvax® group (255) compared to the ACAM2000 group (166). Although only a 1.5-fold difference in GMT was observed between the two groups, ACAM2000 did not quite meet the statistical criterion for non-

inferiority relative to Dryvax[®]. The 97.5% CI (-0.307) was just below the lower bound required to establish non-inferiority (≥ -0.301).

A comparison of distribution of ACAM2000 and Dryvax[®] neutralizing antibody titers for the study is illustrated in Figure 6. The reverse cumulative frequency distribution curves for the two vaccines tracked well together with comparable profiles. It is interesting to note that about 93% of these subjects had neutralizing antibody titers above 20, a value that at least in one incomplete clinical study suggested as protective [10]. If one assumes that the positive cutaneous response observed with ACAM2000 (96%) correlates with protective immunity, then it is also worth noting that all subjects who did not have a positive dermal response and were evaluated for neutralizing antibody levels had titers ≤ 20 .

Figure 6: Reverse Cumulative Frequency Distribution of Neutralizing Antibody Titers on Day 30 (Study H-400-009)



6.3.2. Phase 3 Study H-400-012: Previously Vaccinated Subjects

Study H-400-012 was a Phase 3, double-blind, randomized study designed to evaluate the safety and immunogenicity of ACAM2000 versus Dryvax[®]. Subjects were randomized to receive either ACAM2000 or Dryvax[®] at a 3:1 ratio. The original design called for enrollment of 2720 healthy male and female subjects, at least 31 years of age, who were previously vaccinated against smallpox. However, due to the early termination of the study, as described in Section 6.3.1, a total of 1819 subjects was enrolled at 70 study centers in the US and its territories of which 1577 subjects at 66 sites were evaluable for efficacy.

Co-primary efficacy measures were the proportion of subjects with a successful cutaneous reaction on Day 7 (± 1), and neutralizing antibody GMT on Day 30. Vaccination success (based on cutaneous response) was determined by an IRC's assessment of digital photographs.

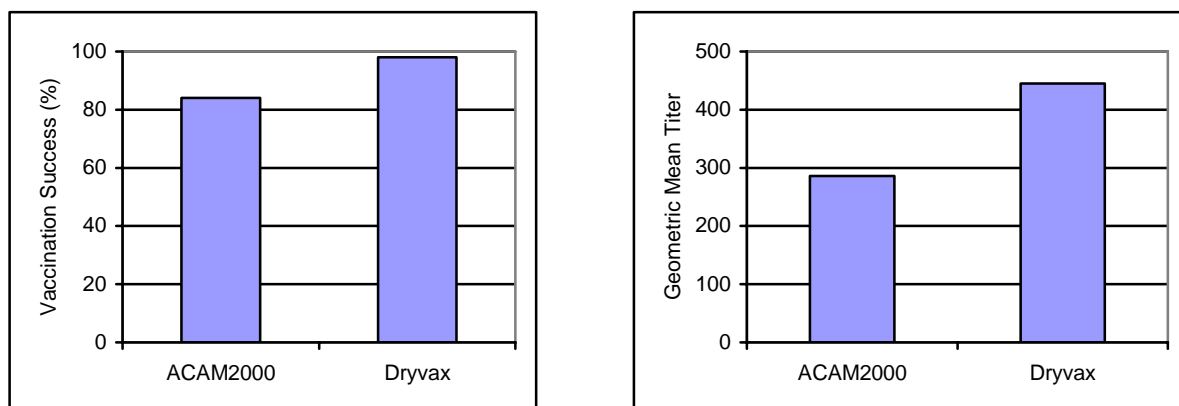
The treatment groups were well-balanced with respect to demographic variables and baseline characteristics, with the exception of baseline neutralizing titer. The baseline GMT was significantly higher in the ACAM2000 group than in the Dryvax[®] group (34 versus 28, respectively; $p = 0.036$). A summary of demographic information for all patients enrolled in ACAM2000 studies, including Phase 3, is provided as an appendix in Section 11.2. Table 10 summarizes the efficacy results in previously vaccinated subjects for ACAM2000 versus Dryvax[®]. These data are plotted in Figure 7.

Table 10: Phase 3 Efficacy Results for ACAM2000 versus Dryvax® in Previously Vaccinated Subjects (Study H-400-012)

| Treatment group | ACAM2000 | Dryvax® |
|---|------------------|----------|
| Revaccination Success based on Cutaneous Response | | |
| Evaluable Subjects (Dermal Evaluable Population) | 1189 | 388 |
| Revaccination Success, n (%) | 998 (84) | 381 (98) |
| 97.5% CI [a] (Criterion for Non-Inferiority) | -17.00 (>-10) | |
| Neutralizing Antibody Titer | | |
| Evaluable Subjects (Antibody Evaluable Population) | 734 | 376 |
| GMT | 286 | 445 |
| 97.5% CI [b] (Criterion for Non-Inferiority) | -0.275 (>-0.301) | |

- a. Lower bound of the 97.5% one-sided CI on the difference between ACAM2000 and Dryvax® derived by normal approximation to the binomial distribution.
b. Lower bound of the 97.5% one-sided CI on the difference in the mean log₁₀ GMT between ACAM2000 and Dryvax® derived using ANOVA.

Figure 7: Graphical Presentation of Phase 3 Efficacy Results in Previously Vaccinated Subjects (Study H-400-012)



Panel A: Vaccination Success

Panel B: Neutralizing Antibody Titer

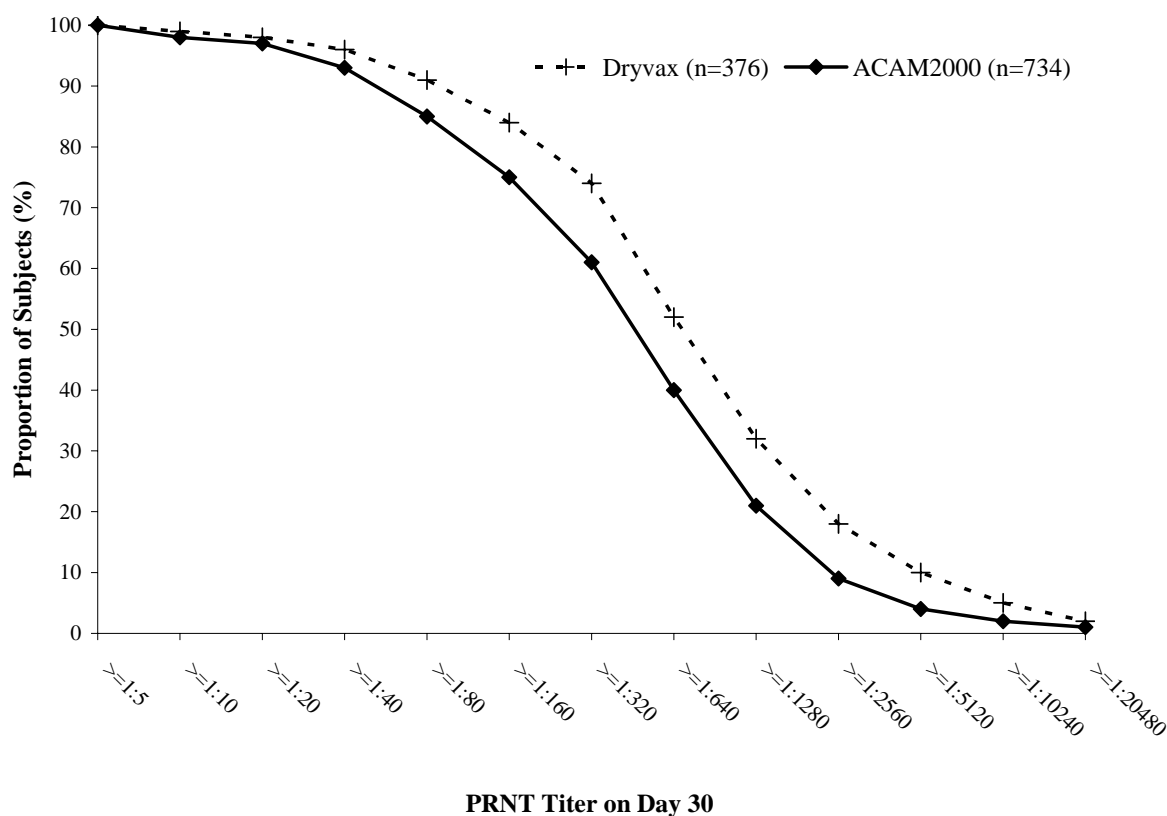
While the majority of subjects in the ACAM2000 evaluable population developed a cutaneous response after revaccination (84%), the response rate was lower than in the Dryvax® group (98%). Statistically, ACAM2000 failed to meet the criterion for non-inferiority to Dryvax® with regard to revaccination success rates based on cutaneous response, as indicated by a lower bound of the one-sided 97.5% CI on the ACAM2000 – Dryvax difference that did not exceed -10% (Table 10). The observation that the dermal response with ACAM2000 was somewhat suppressed in previously vaccinated subjects was not unexpected based on Phase 2 data and is consistent with experience with other smallpox vaccines where the cutaneous reaction is

affected by the level of neutralizing antibody in the vaccinee [29, 36] (see discussion of baseline immunity, below).

With regard to neutralizing antibody GMT on Day 30, ACAM2000 was statistically non-inferior to Dryvax[®] in this study, as indicated by a lower bound of the 97.5% one-sided CI that exceeded -0.301 (Table 10). Consistent with the findings in the vaccinia-naïve subjects, the neutralizing antibody response to ACAM2000 was approximately 1.5-fold lower than to Dryvax[®].

The reverse cumulative frequency distribution for ACAM2000 and Dryvax[®] neutralizing antibody titers demonstrate that these vaccines have similar response slopes, although there are generally higher titers in the Dryvax[®] treated group (Figure 8). However, it is important to emphasize that with both vaccines greater than 97% of the subjects had neutralizing antibody titers ≥ 20 .

Figure 8: Reverse Cumulative Frequency Distribution of Neutralizing Antibody Titers on Day 30 (Study H-400-012)



Influence of Baseline Immunity

It has been shown that pre-existing immunity can modulate the response to revaccination [36]. Therefore, a prospectively planned analysis of the relative influence of baseline immunity on the response to revaccination with ACAM2000 or Dryvax[®] was carried out using a covariate analysis of subjects who were in the evaluable populations. For ACAM2000, cutaneous response rates were found to be inversely proportional to baseline immunity, ranging from a high of 94% in subjects who were seronegative at baseline, to a low of 73% in subjects with

baseline titers of 160 or more (Table 11). Baseline immunity, however, had little effect on the cutaneous response to Dryvax[®]. It is possible that the restriction of cutaneous response by preexisting immunity is greater in the case of a slightly more attenuated virus (ACAM2000).

The magnitude of the antibody response to revaccination was also dependent on baseline immunity (Table 11). In both treatment groups, the mean fold-increase in neutralizing antibody titer was found to be inversely proportional to the titer of neutralizing antibodies at baseline. When mean fold-increases in neutralizing antibody titer were adjusted for baseline titer, a significant difference was found between the treatment groups ($p < 0.0001$), with a higher fold-increase in the Dryvax[®] group than in the ACAM2000 group in each baseline titer category. Thus, the influence of baseline immunity was greater on ACAM2000 than on Dryvax[®]. This could again be due to a slight attenuation of the ACAM2000 virus, which would result in a more modest immune response as compared to Dryvax[®].

Table 11: Phase 3 Efficacy Results for ACAM2000 and Dryvax[®], Adjusted for Baseline Neutralizing Antibody Titer (Study H-400-012)

| Baseline Titer | Revaccination Success n/N, (%) | | GMT Mean Fold-Increase from Baseline to Day 30 | |
|------------------|-----------------------------------|----------------------------------|---|----------------------------------|
| | ACAM2000 (n = 706) | Dryvax [®] (n = 364) | ACAM2000 (n = 734) | Dryvax [®] (n = 376) |
| < 10 | 151/161 (94) | 86/88 (98) | 29.6 | 36.4 |
| 10-20 | 158/186 (85) | 107/108 (99) | 13.3 | 23.8 |
| 40-80 | 168/199 (84) | 93/94 (99) | 5.9 | 10.9 |
| ≥160 | 116/160 (73) | 72/74 (97) | 2.2 | 4.8 |
| All subjects ≥10 | 442/545 (81) | 272/276 (99) | 5.8 | 12.0 |
| Overall total | 593/706 (84) | 358/364 (98) | 8.5 | 15.7 |
| p-value | <0.0001 [a] | | <0.0001 [b] | |

a. Overall test of difference between ACAM2000 and Dryvax[®].

b. p-value on difference between ACAM2000 and Dryvax[®] derived by Analysis of Covariance, adjusting for baseline neutralizing antibody titer.

6.4. Integrated Efficacy Analysis

This section presents a summary of the efficacy results across the entire clinical program with an interpretation of those results.

6.4.1. Summary of Efficacy Results

The clinical trial results for the principal efficacy endpoints (cutaneous response and neutralizing antibody GMTs) are summarized in Table 12 for vaccinia-naïve subjects and in Table 13 for previously vaccinated subjects. The data are presented by study phase, study number and treatment group.

From a clinical perspective, ACAM2000 elicited a strong immune response in all study populations. Across all studies, ACAM2000 induced positive cutaneous responses in >96% of vaccinia-naïve subjects and in >84% of previously vaccinated subjects. In addition, ACAM2000

induced a robust neutralizing antibody response across all studies, with GMTs >100 in vaccinia-naïve subjects and >250 in the previously vaccinated population. ACAM2000 also induced a positive cell-mediated immune response as determined by at least one assay method in 100% of subjects tested for this response (n=30, see Table 6).

There was no significant difference between the ACAM2000 and Dryvax[®] groups in respect to neutralizing antibody titer in the Phase I and Phase 2 clinical studies of vaccinia-naïve subjects. Indeed, the Phase 2 comparative study of these vaccines (Study H-400-005) showed nearly identical GMTs (154 and 158) for the two vaccine groups. In an earlier open label, Phase 1 study (H-400-008) with ACAM2000 the GMT was 225 (n=100), however, in the Phase 3 trial (Study H-400-009) the GMT was 166 (n=776). In part these differences reflect the inconsistency of vaccination techniques, the diversity of individual responses, the variability of serological assays, and explain why immune responses are often reported in log units.

Plots for the reverse cumulative frequency distribution of neutralizing antibody titers in the Phase 3 studies for vaccinia-naïve and previously vaccinated subjects are provided in Figure 6 and Figure 8, respectively. These graphs illustrate similar profiles for both vaccines, with Dryvax[®] having slightly higher antibody titers. Although the neutralizing antibody titers of the ACAM2000 group (GMT=166) in the vaccinia-naïve study (H-400-009) was within approximately 1.5 fold of the Dryvax[®] group (GMT=255), it narrowly missed the primary endpoint for non-inferiority (CI = -0.307 vs. >-0.301) in the Phase 3 study.

In regards to the previously vaccinated subjects, the Phase 2 study (H-400-003) indicates that the titers of the ACAM2000 and Dryvax[®] were not statistically equivalent, although, in the Phase 3 study (Study H-400-012) the primary endpoint for non-inferiority, based on GMT, was met (Table 13). The important point is that in all studies the previously vaccinated groups had higher GMTs than the primary vaccination (vaccinia-naïve) groups. Therefore, if protection is provided to the primary vaccination group it follows that the previously vaccinated group would also be protected (based on antibody titer).

With respect to the dermal response, no statistical difference was observed between ACAM2000 and Dryvax[®] groups in the Phase I and Phase 2 studies (H-400-002 & H-400-005) for primary vaccination, where there was a 100% positive dermal response in all groups. In the Phase 3 study of the vaccinia-naïve (Study H-400-009) there was a 96% positive dermal response with ACAM2000 and the primary endpoint for non-inferiority was met. This is a very significant outcome since it is recognized as the primary correlate for immunity with smallpox vaccines [29].

The greatest difference between the two vaccines was observed in the cutaneous response rates in previously vaccinated subjects. Eighty-four percent (84%) of subjects in the ACAM2000 group versus 98% of subjects in the Dryvax[®] group were scored for a positive cutaneous response. In this population, ACAM2000 did not meet the Phase 3 statistical endpoint of non-inferiority, relative to Dryvax[®], for dermal response. However, not meeting this endpoint is not considered negative from an efficacy standpoint since those with low neutralizing antibody titers (<10) prior to vaccination had 94% positive cutaneous responses and overall the neutralizing antibody levels were higher than in the primary vaccination group (see Section 6.4.2 and Section 6.5).

Table 12: Immunogenicity and Efficacy Results for Vaccinia-Naïve Subjects

| Study Phase | Phase 1 | | | Phase 2 | | Phase 3 | |
|---|--|--|----------------------------------|--|--|--|--|
| Study No. | H-400-002 | | H-400-008 | H-400-005 | | H-400-009 | |
| Treatment group (PFU/Dose) | ACAM 2000 (1.0x10 ⁸) | Dryvax [®] (1.6x10 ⁸) | ACAM 2000 (7.7x10 ⁷) | ACAM 2000 (6.8x10 ⁷) | Dryvax [®] (1.6x10 ⁸) | ACAM 2000 (1.3-2.2 x10 ⁸) | Dryvax [®] (1.6x10 ⁸) |
| Vaccination Success based on Cutaneous Response | | | | | | | |
| Evaluable Subjects (n) | 30 | 30 | 100 | 51 | 49 | 776 | 257 |
| Vaccination success, n (%) | 30 (100) | 30 (100) | 99 (99) | 51 (100) | 49 (100) | 747 (96) | 255 (99) |
| Statistical Interpretation | No difference between groups | | NA | No difference between groups | | ACAM2000 was non-inferior to Dryvax [®] | |
| Neutralizing Antibody Titer [a] | | | | | | | |
| Evaluable Subjects (n) | 30 | 30 | 100 | 51 | 49 | 565 | 190 |
| Geometric Mean Titer | 103 | 172 | 225 | 154 | 158 | 166 | 255 |
| Statistical Interpretation | No significant difference between groups | | NA | No significant difference between groups | | ACAM2000 did not meet the endpoint of non-inferiority to Dryvax [®] | |

Key: NA = Not applicable

a. PRNT₅₀ titer was determined on Day 45 in Study H-400-002 and on Day 30 in all other studies.

Table 13: Immunogenicity and Efficacy Results for Previously Vaccinated Subjects

| Study Phase | Phase 2 | | Phase 3 | |
|---|--|--|--|--|
| Study No. | H-400-003 | | H-400-012 | |
| Treatment group (PFU/Dose) | ACAM2000 (6.8 x 10 ⁷) | Dryvax [®] (1.6 x 10 ⁸) | ACAM2000 (1.3-2.2 x 10 ⁸) | Dryvax [®] (1.6 x 10 ⁸) |
| Vaccination Success based on Cutaneous Response | | | | |
| Evaluable Subjects | n = 49 | n = 52 | n = 1189 | n = 388 |
| Vaccination success, n (%) | 43 (88) | 52 (100) | 998 (84) | 381 (98) |
| Statistical Interpretation | ACAM2000 not equivalent to Dryvax [®] | | ACAM2000 did not meet the endpoint of non-inferiority to Dryvax [®] | |
| Neutralizing Antibody Titer | | | | |
| Evaluable Subjects | n = 50 | n = 52 | n = 734 | n = 376 |
| Geometric Mean Titer | 256 | 447 | 286 | 445 |
| Statistical Interpretation | Groups not equivalent | | ACAM2000 was non-inferior to Dryvax [®] | |

6.4.2. Effect of Baseline Immunity on Efficacy Outcome Measures

Clinical experience with other smallpox vaccines has shown that previously vaccinated subjects with baseline neutralizing antibody titers ≥ 10 are less likely to exhibit a primary-type cutaneous response upon revaccination than are subjects with undetectable levels of neutralizing antibody at the time of revaccination [29]. Consistent with these historical observations, cutaneous and neutralizing antibody responses to ACAM2000 in previously vaccinated subjects were strongly dependent on the level of pre-existing immunity to vaccinia in each subject. Both the cutaneous response rate and the mean fold change in neutralizing antibody titer from Baseline to Day 30 were inversely proportional to the titer at Baseline (Table 11). Revaccination success based on cutaneous response with Dryvax[®] was comparatively unaffected by baseline titer, which contributed to the overall differences observed between the two treatment groups. A recent study in Israel demonstrated that a lack of response to revaccination with Lister vaccine correlated with the interval since last vaccination [37]. In that study, 77.5% of subjects vaccinated <20 years previously responded to revaccination versus 97.2% for persons vaccinated >20 years before. Unlike ACAM2000 or the Israeli Lister vaccine, Dryvax[®] is able to elicit a more active cutaneous response that is independent of residual immunity [38]. Nevertheless, in previously vaccinated subjects where immunity has waned so that smallpox neutralizing antibody titers are below 10 or in vaccinia-naïve subjects, ACAM2000 elicited a positive cutaneous response in greater than 94% of the subjects, similar to Dryvax[®] (Table 11).

6.4.3. ACAM2000 Clinical Consistency and Dosing Recommendations

Three lots of ACAM2000, with potencies of 1.3×10^8 , 1.9×10^8 , and 2.2×10^8 PFU/mL, were released according to the proposed potency specification (1.0 - 5.0×10^8 PFU/mL) and utilized in the Phase 3 studies. Given that all three ACAM2000 lots utilized in the Phase 3 studies produced a cutaneous response in $\geq 94\%$ of vaccinia-naïve subjects and in previously vaccinated subjects who lacked baseline antibody, and given that data from Phase 2 study H-400-005 demonstrated that a lot with 4-fold lower potency (6.8×10^7 PFU/mL) than the lower limit of the release specification elicited responses comparable to Dryvax[®], the sponsor has concluded that ACAM2000 released according to the potency specification of 1.0 - 5.0×10^8 PFU/mL is effective in eliciting a protective response (cutaneous and serological) against smallpox infection in the majority of vaccinia-naïve and previously vaccinated individuals. This potency specification is also consistent with the requirements outlined in the original RFP issued by the CDC in 2001.

6.5. Efficacy Conclusions

Based on historical evidence, vaccinated individuals are considered protected against smallpox after a major cutaneous reaction is observed following primary vaccination [29]. Further, two prospective studies have correlated higher titers of neutralizing antibodies with lower susceptibility to smallpox upon contact with a smallpox victim [10, 5]. Although the methods used to measure neutralizing antibody titers differ between these published studies and Acambis' clinical trials, the limited data suggest that neutralizing antibody titer serves as a qualitative, if not a quantitative, correlate of protection with titers greater than 32 proposed as nominally protective [5].

Both dermal reactivity and neutralizing antibodies to smallpox infection were used as co-primary endpoints in two Phase 3 trials designed to show the non-inferiority of ACAM2000 to Dryvax[®]. In these Phase 3 studies the non-inferiority endpoint for cutaneous response was met and the neutralizing antibody titer was narrowly missed in the vaccinia-naïve population whereas in the previously vaccinated population the non-inferiority endpoint for neutralizing antibody titer was met and the cutaneous response was not met, consistent with ACAM2000 being a purified vaccine that is slightly more attenuated than Dryvax[®].

Although the Phase 3 clinical endpoints for non-inferiority were not all met, these results when taken together with historical information, earlier clinical and non-clinical studies, and further analysis of individual baseline immunity provide a preponderance of data supporting the efficacy of this vaccine. The following points further support this conclusion:

1. The ACIP guidance for protective efficacy against smallpox states: "Clinically, persons are considered fully protected after a successful response is demonstrated at the site of vaccination" [29]. Primary vaccination efficacy, based on this criterion, was demonstrated to be greater than 96% with ACAM2000 and was determined to be statistically non-inferior to Dryvax[®].
2. Neutralizing antibody titers greater than a putative value of 32 have been suggested as a correlate to immunity [5, 10]. Although this value cannot be validated due to the elimination of the disease, it is noted that $>93\%$ of subjects in the primary vaccination group had titers greater than 20. In the same group 96% were evaluated to be protected

based on a positive cutaneous response. Of those subjects who did not have a positive dermal response and were evaluated serologically (n=20), all had neutralizing antibody titers ≤ 20 .

3. The Phase 3 study resulted in neutralizing antibody GMTs of 166 and 286 for vaccinia-naïve subjects and previously vaccinated subjects, respectively. Therefore, if the primary vaccination group is considered protected (based on cutaneous response), so also should the previously vaccinated group with ever higher titers (GMT=286), assuming similar distribution curves for the antibody titer (Figure 6 and Figure 8). Even though ACAM2000 had a subdued dermal response with previously vaccinated, one may conclude that the neutralizing antibody titer for this group provides a reasonable indication of immunity.
4. In the previously vaccinated group, those at highest risk for infection (lowest neutralizing antibody titer) also exhibited a dermal response as defined by the ACIP. Of the 161 previously vaccinated subjects who had waning immunity, (titers <10), 151 (94%) of them had a positive dermal response with ACAM2000.
5. Plots of reverse cumulative frequency distribution of neutralizing antibody titers show similar profiles for both vaccines with Dryvax[®] having a slightly higher antibody level (Figure 6 and Figure 8). Since differences in immune responses are often measured in logs, these distribution curves are illustrative of product similarity. In the case of the previously vaccinated subjects, the vaccines directly overlapped in the frequency distribution curves for those with low neutralizing antibody titers (Figure 8).
6. Phase 1 clinical studies demonstrated that all 30 subjects (100%) in the ACAM2000 group, and 28 (93%) of 30 subjects in the Dryvax[®] group had a positive cell-mediated immune response to smallpox vaccine in at least one of the three T-cell conducted
7. Because of the serious nature of this disease and the inability to perform traditional efficacy studies it is important to consider supportive animal challenge studies as part of the efficacy assessment. The ability to provide complete protection of primates at lethal challenge doses of monkeypox virus (Orthopoxvirus genus) with no signs of disease, as described in Section 4, provides critical support for ACAM2000 effectiveness.

Since ACAM2000 is a purified vaccine derived from Dryvax[®] through 10 cell culture passages, it provides a slightly more attenuated vaccine and therefore a milder cutaneous reaction than does Dryvax[®]. Nevertheless, ACAM2000 elicited a robust immune response with neutralizing antibody GMTs >100 in vaccinia naïve subjects and >250 in previously vaccinated subjects across all clinical studies.

In conclusion, the immunogenicity of ACAM2000 and its efficacy compared to Dryvax[®] are adequate to support ACAM2000 vaccination against smallpox disease for those at risk of infection.

7. CLINICAL SAFETY

7.1. Safety Population

The Safety Population consists of all subjects who received vaccination during the clinical trials. The safety profile of ACAM2000 was evaluated in a total of 2983 subjects (1307 vaccinia-naïve and 1676 previously vaccinated) in doses ranging from 3.4×10^6 to 2.2×10^8 PFU/mL. A total of 868 subjects (368 vaccinia-naïve and 500 previously vaccinated) received single cutaneous doses of Dryvax® vaccine administered at doses ranging from 1.0×10^8 to 1.6×10^8 PFU/mL.

Comparison of the safety profile of ACAM2000 obtained from the Phase 3 studies with the safety profile of ACAM2000 when the safety data from all clinical studies were pooled indicated that the safety profile of ACAM2000 in the Phase 3 studies was similar to that seen in all studies overall. Therefore this summary will focus on Phase 3 results for the most common AEs, but the discussion regarding SAEs will include data from all clinical studies.

7.2. Serious Adverse Experiences (SAEs) and Deaths

There were no fatalities during any clinical study of ACAM2000. Other SAEs occurred rarely (<1%) with ACAM2000, with the most commonly reported SAEs being myocarditis and pregnancy (considered an SAE due to the potential risk of congenital infection, although no cases of congenital infection were documented). Not all reported events of myocarditis or pregnancy met the International Conference on Harmonization (ICH) definition of an SAE. However, Acambis treated all such events as SAEs as a conservative safety measure.

7.2.1. Myocarditis/pericarditis

Ten cases, seven in subjects treated with ACAM2000 (5.73 events per thousand vaccinations) and three in subjects treated with Dryvax® (10.38 events per thousand vaccinations), were reported in a total vaccinia-naïve population of 1675 subjects, for a combined calculated incidence of 5.97 cases per thousand vaccinations.

Recently, it was reported that myocarditis and/or pericarditis (myocarditis/pericarditis) is associated with administration of the Dryvax® vaccine with incidence rates of 0.11 cases per thousand vaccinations (cumulative for both vaccinia-naïve and previously vaccinated subjects) [24]. Reports of myocarditis/pericarditis in the US civilian and military smallpox vaccination programs using Dryvax® were issued in 2003 [30]. In addition, evidence of subclinical myocarditis/pericarditis following smallpox vaccination from the literature was highlighted at that time [30, 4]. Thus in 2003, when the ACAM2000 Phase 2 studies were being conducted, the structured interview for all ongoing and planned ACAM2000 clinical studies was revised to include symptoms of myocarditis/pericarditis [30]. Subjects with such symptoms were to have additional evaluations performed according to a cardiac algorithm to determine whether myocarditis/pericarditis had occurred. These studies excluded subjects with cardiac disease and specific risk factors for ischemic cardiac disease. (See 11.4, Appendix 4 – Cardiac Algorithm)

Scheduled serial ECGs and cardiac troponin I evaluations (capable of detecting subclinical myocarditis) were incorporated for all subjects in the Phase 3 studies and the H-400-002 Phase

1 study, to complement the structured interview. These studies also excluded subjects with cardiac disease and specific risk factors for ischemic cardiac disease.

The incidence rate for myocarditis in Acambis' Phase 3 clinical studies was higher than previously reported in Department of Defense (DoD) and civilian vaccination programs (0.11 [1] and 0.54 [25] cases per thousand vaccinations, respectively), which used only passive surveillance measures and did not prospectively test for sub-clinical cases.

Subjects with suspect or probable myocarditis as confirmed by the CAP or the Sponsor, are presented as an appendix in Section 11.3. Both local and central read ECGs and clinical laboratory findings obtained at the time of onset of the event (or at the first time-point after the event onset) are presented.

A total of 10 cases of suspect or probable myocarditis, 7 (5.73 events per thousand vaccinations) in subjects treated with ACAM2000 and 3 (10.38 events per thousand vaccinations) in subjects treated with Dryvax[®], were identified in the ACAM2000 clinical program (Phase 1 through 3). No ACAM2000 subjects were diagnosed with confirmed myocarditis or pericarditis (requiring histological evidence of myocardial or pericardial inflammation). All subjects who experienced myocarditis were previously naïve to vaccinia; no cases were detected in previously vaccinated subjects. Of these 10 subjects, 9 were male and 7 were Caucasian. The mean age of subjects was 22 years. No subject had a known history of cardiac disease. However, 2 subjects had at least 1 risk factor for ischemic coronary disease. The mean time to onset of myocarditis from vaccination was 11 days, with a range of 9 to 20 days. It is noted however that the exact time to onset of these events is unknown, because 8 of 10 cases were not characterized by acute clinical signs and were identified by routine study evaluation at the Day 10 study center visit, the first post-vaccination time-point at which cardiovascular evaluations were conducted.

Although the myocarditis incidence rate was higher in the Dryvax[®] group (10.38 events per thousand vaccinations) there was no statistically significant difference from the ACAM2000 group (5.73 events per thousand vaccinations). Of the ten subjects, four (three in the ACAM2000 group and one in the Dryvax[®] group) were symptomatic, of whom two (one each in the ACAM2000 and Dryvax[®] groups) were hospitalized with acute cardiac symptoms. In the 2 hospitalized subjects, these cardiovascular events were considered resolved with sequelae for the ACAM2000 subject and resolved without sequelae for the Dryvax[®] subject. At discharge the ECG for the ACAM2000 subject was still abnormal with nonspecific ST-T wave changes and he was put on Coreg 6.25 mg and aspirin 81 mg.

The remaining 8 subjects with suspect or probable myocarditis were neither hospitalized nor treated with medications. Suspect or probable myocarditis was considered resolved without sequelae at the last follow-up for all but one Dryvax[®] recipient, the sole female subject, who remains with persistent echocardiogram evidence of diminished ejection fraction (27-32%) and global hypokinesis. The subject has been followed by her cardiologist for 2.5 years after vaccination and remains asymptomatic with normal cardiac examination findings, and continuing abnormal echocardiogram findings (see Table 18).

7.2.2. Other Serious Cardiac Events

Serious cardiac events other than myocarditis were reported within 30 days after vaccination for 4 previously vaccinated subjects, and included single reports of atrial fibrillation, chest discomfort, chest pain, and coronary artery disease. All 3 events, except coronary artery disease, were considered to have a possible relationship to ACAM2000 as determined by the clinical investigator, and are described below. The event of coronary artery disease was considered by the clinical investigator to have no relationship to the study vaccine (Dryvax®).

The subject hospitalized with atrial fibrillation 31 days after vaccination converted to normal sinus rhythm while in the hospital and the event was considered resolved. The subject had a normal ejection fraction on echocardiogram, normal troponin and slightly elevated CK.

One subject was hospitalized with chest pressure beginning 3 days after vaccination. Follow-up testing was normal and the event was considered resolved 4 days after onset.

One subject was hospitalized with severe chest pain 35 days after vaccination. The subject's Day 12 ECG was abnormal; however the specific abnormality was not reported. Follow-up testing revealed normal ejection fraction on echocardiogram, normal chest x-ray and normal cardiac enzymes. The subject was discharged with nitroglycerin, beta blockers, aspirin and diet change.

7.2.3. Pregnancy

As reported in the literature [39], congenital infection, a very rare occurrence, principally occurring during the first trimester, may be associated with generalized vaccinia of the fetus (termed fetal vaccinia), resulting in early delivery of a stillborn infant or a high risk of perinatal death. Due to this potential risk, women of child-bearing potential who were pregnant or lactating were excluded from participation in any clinical study of ACAM2000. Reports of several pregnancies within 30 days of vaccination during the Phase 2 studies resulted in a two week interruption of enrollment while the protocols were amended to require more stringent birth control methods and pregnancy testing. In addition, a comprehensive plan to reduce the risk of inadvertent vaccination of pregnant women was developed and implemented, including more comprehensive screening procedures and enhanced subject education materials. These procedures also were incorporated in the Phase 3 studies.

In the ACAM2000 clinical program, a total of five subjects who were determined to be non-pregnant prior to vaccination subsequently were determined to be pregnant within 30 days after vaccination. The five pregnancies after vaccination resulted in two spontaneous abortions, one live birth, and one elective termination; the remaining subject was lost to follow-up. There were no reports of fetal vaccinia, congenital anomalies, or birth defects. Of the two spontaneous abortions, one was considered to be unrelated to study vaccine. In the other case, neither smallpox vaccination nor VIG administration could be ruled out as risk factors.

7.2.4. Other Serious Adverse Events

One vaccinia-naïve subject treated with ACAM2000 experienced a new-onset seizure 8 days after vaccination. Follow-up physical and neurological examination findings and imaging studies were normal, and the subject did not experience any subsequent seizures during the study period. Other predisposing factors (sleep deprivation, photic stimulation, paternal history of

seizures) were considered contributory to new-onset seizures in this subject, in addition to vaccination with ACAM2000. This event in this subject was not considered to be a symptom of a significant CNS disorder historically associated with smallpox vaccine (e.g., aseptic meningitis, post-vaccinal encephalitis, or myelitis). A neurologic algorithm was subsequently established in the Phase 3 studies in order to monitor closely for neurologic events associated with smallpox vaccination. No additional subject experienced convulsions of any etiology in any clinical study of ACAM2000 or any other significant CNS event historically associated with smallpox vaccine. This is consistent with the experience from the government smallpox vaccination programs [40].

Two cases of appendicitis were reported with ACAM2000, one each in a vaccinia-naïve and previously vaccinated subject. Both cases were considered unrelated to ACAM2000. One previously vaccinated subject treated with ACAM2000 was determined to be human immunodeficiency virus (HIV)-positive one day after vaccination. (Samples for screening virology tests had been collected ten days before vaccination; however, the test results were not available at the time of vaccination.) This subject received prophylactic treatment with VIG. No notable complications were reported through last follow-up approximately three months post-vaccination.

7.2.5. Other Relevant Safety Information

Urticaria was rarely (<1%) reported with ACAM2000; and no cases were considered to be serious. No cases of urticaria had an acute temporal relationship with ACAM2000 administration. No cases of anaphylaxis, erythema multiforme major/Stevens-Johnson syndrome, or other significant allergic responses were reported with ACAM2000. Urticarial rash and hypersensitivity were noted after Dryvax[®] in the Phase 3 studies.

7.3. Common Adverse Experiences

ACAM2000 was well tolerated by both vaccinia-naïve and previously vaccinated subjects. ACAM2000 and Dryvax[®] were associated with a high rate of AEs, with the majority (99%) of subjects, regardless of baseline vaccination status and vaccine dose, experiencing at least one treatment-emergent AE after vaccination.

As expected with a smallpox vaccine, the most commonly reported AEs among subjects generally were local signs or symptoms related to the cutaneous reaction or associated systemic symptoms. The incidence of these events was higher among subjects who experienced a successful vaccination based on dermal response than among those who did not, regardless of dose or baseline vaccination status. Solicited, as well as unsolicited events were collected, although the most common events came from a solicited checklist.

AEs commonly reported after vaccination with ACAM2000 generally fell into four distinct categories: reactions at the vaccination site, lymphadenitis, constitutional “flu-like” symptoms, and minor gastrointestinal symptoms. These commonly reported AEs generally occurred at a higher incidence in vaccinia-naïve subjects than in previously vaccinated subjects receiving revaccination. This finding is not unexpected, given that the clinical course of the response to revaccination is typically milder than that to primary vaccination. Furthermore, the incidence of these events generally was lower, in many cases statistically significantly lower, with ACAM2000 than with Dryvax[®], regardless of baseline vaccination status, suggesting that the

former is more attenuated. However, the clinical relevance of this difference is limited, considering the relatively benign nature of these events.

AEs reported by $\geq 10\%$ of subjects in either the ACAM2000 or Dryvax[®] group in the Phase 3 studies are summarized in Table 14 for the safety population by baseline vaccination status and treatment group.

Table 14: Commonly Reported ($\geq 10\%$ in the ACAM2000 or Dryvax[®] Groups) AEs by MedDRA Preferred Term, Baseline Vaccination Status and Treatment Group, Phase 3 Studies, Total Population

| MedDRA Preferred Term [a] | Vaccinia-Naïve Subjects | | | Previously Vaccinated Subjects | | |
|-------------------------------|-------------------------------|---|----------------|--------------------------------|---|----------------|
| | ACAM 2000 (n=873) n (%) | Dryvax [®] (n=289) n (%) | p-value [b] | ACAM 2000 (n=1371) n (%) | Dryvax [®] (n=448) n (%) | p-value [b] |
| At least 1 AE | 864 (99) | 288 (100) | 0.4661 | 1325 (97) | 443 (99) | 0.0124 |
| Injection site pruritus* | 804 (92) | 277 (96) | 0.0324 | 1130 (82) | 416 (93) | <.0001 |
| Injection site erythema* | 649 (74) | 229 (79) | 0.0976 | 841 (61) | 324 (72) | <.0001 |
| Injection site pain* | 582 (67) | 208 (72) | 0.0950 | 505 (37) | 209 (47) | 0.0003 |
| Lymph node pain* | 494 (57) | 199 (69) | 0.0002 | 261 (19) | 119 (27) | 0.0008 |
| Headache* | 433 (50) | 150 (52) | 0.4984 | 437 (32) | 166 (37) | 0.0493 |
| Fatigue* | 423 (48) | 161 (56) | 0.0354 | 468 (34) | 184 (41) | 0.0090 |
| Injection site swelling* | 422 (48) | 165 (57) | 0.0100 | 384 (28) | 188 (42) | <.0001 |
| Myalgia* | 404 (46) | 147 (51) | 0.1966 | 374 (27) | 148 (33) | 0.0222 |
| Malaise* | 327 (37) | 122 (42) | 0.1634 | 381 (28) | 147 (33) | 0.0478 |
| Feeling hot* | 276 (32) | 97 (34) | 0.5611 | 271 (20) | 114 (25) | 0.0114 |
| Erythema* | 190 (22) | 69 (24) | 0.4636 | 329 (24) | 107 (24) | 1.000 |
| Rigors* | 185 (21) | 66 (23) | 0.5643 | 171 (12) | 76 (17) | 0.0173 |
| Nausea* | 170 (19) | 65 (22) | 0.2728 | 142 (10) | 63 (14) | 0.0386 |
| Diarrhea* | 144 (16) | 34 (12) | 0.0593 | 158 (12) | 77 (17) | 0.0026 |
| Exercise tolerance decreased* | 98 (11) | 35 (12) | 0.6709 | 105 (8) | 50 (11) | 0.0248 |
| Rash* | 94 (11) | 30 (10) | 0.9128 | 80 (6) | 29 (6) | 0.6466 |
| Lymphadenopathy* | 72 (8) | 35 (12) | 0.0598 | 78 (6) | 29 (6) | 0.5632 |

* Event was listed on a checklist included in subject diaries and is therefore considered prompted.

- Medical Dictionary for Regulatory Activities (MedDRA) Preferred Terms are listed in descending order of occurrence in the ACAM2000 Naïve Subject Group.
- Fisher's Exact test.

AEs commonly reported with ACAM2000 generally were assessed by the Investigator as mild or moderate in intensity and study vaccine-related. Furthermore, the great majority of commonly reported AEs occurred within the first week (Days 0 to 6) after vaccination, with a general decline in the incidence of AEs thereafter.

A dose-response relationship was not seen with regard to the incidence of AEs overall across the range of doses evaluated in the Phase 3 program, 1.3×10^8 to 2.2×10^8 PFU/mL, in either vaccinia-naïve or previously vaccinated subjects.

7.3.1. Vaccination Site Reactions

After vaccination with ACAM2000, the majority (92%) of subjects experienced at least 1 vaccination site reaction, with the most common reactions being pruritus, erythema, pain, and swelling at the vaccination site; the incidence of all of these events was notably higher in vaccinia-naïve subjects than in previously vaccinated subjects. Less common vaccination site reactions with ACAM2000 included inflammation, burning, and vesicles at the vaccination site or another vaccination complication (2% each). All other vaccination site reactions reported with ACAM2000, including robust takes, satellite lesions, and autoinoculation/distal lesions, were uncommon (<1%).

A relatively small proportion (5%) of ACAM2000-treated subjects experienced a severe vaccination site reaction, with a higher incidence of such events in vaccinia-naïve subjects (9%) than in previously vaccinated subjects (2%). Approximately three-fourths (73%) of these subjects with a severe vaccination site reactions experienced severe injection site erythema, which was conservatively defined as an area of redness ≥ 1.5 inches (3.8 cm) in diameter. No significant vaccination site reactions or complications historically associated with smallpox vaccination, including GV, PV, erythema multiforme or EV, were reported with ACAM2000 in either vaccinia-naïve or previously vaccinated subjects. One case of GV was reported for a Dryvax[®]-treated subject.

7.3.2. Constitutional Symptoms

Commonly reported constitutional symptoms associated with the systemic response to smallpox vaccine included fatigue, headache, myalgia, malaise, feeling hot, and rigors. Constitutional “flu-like” symptoms were reported for approximately three-fourths (76%) of vaccinia-naïve subjects and 55% of previously vaccinated subjects treated with ACAM2000. All of these events occurred at a higher incidence ($p < 0.05$) in the Dryvax[®] group than in the ACAM2000 group in both vaccinia-naïve and previously vaccinated subjects (Table 14).

Fever is an expected reaction to primary vaccination and revaccination with smallpox vaccine, although it has been reported to occur less commonly in adults than in children after both primary vaccination and revaccination [12]. Of note, body temperature increase and pyrexia were uncommonly ($\leq 1\%$) reported as an AE with either the ACAM2000 or Dryvax[®] groups. Oral body temperature measurement $\geq 102^\circ\text{F}$ (38.9°C) at any time-point were uncommon (<1%) in both vaccinia-naïve and previously vaccinated subjects in either group.

7.3.3. Lymphadenitis

Lymph node pain, a prompted AE, was commonly reported with ACAM2000, occurring at an overall incidence of 57% and 19% in vaccinia-naïve and previously vaccinated subjects,

respectively. Lymphadenopathy was seen less commonly than lymph node pain (8% and 6% of vaccinia-naïve and previously vaccinated subjects, respectively). Specific reports of lymphadenitis were uncommon (1 subject; <1%) with ACAM2000.

As would be expected, lymph node enlargement and tenderness occurred more commonly ipsilateral rather than contralateral to the vaccination site.

7.3.4. Gastrointestinal Symptoms

Commonly reported gastrointestinal symptoms included nausea and diarrhea, which were reported at an incidence of 19% and 16%, respectively in vaccinia-naïve subjects and 10% and 12%, respectively, in previously vaccinated subjects treated with ACAM2000. In contrast with other commonly reported AEs, the incidence of diarrhea was higher with ACAM2000 (16%) than with Dryvax[®] (12%) in vaccinia-naïve subjects. However, in previously vaccinated subjects, the incidence of this event was lower with ACAM2000 than with Dryvax[®]. The incidence of nausea in both subject populations was lower with ACAM2000 than Dryvax[®]. Most cases of nausea were mild or moderate in intensity and were unaccompanied by vomiting.

7.4. Virus Shedding

Because virus shedding can lead to AEs for both the recipient and the recipient's contacts, this parameter was evaluated in Phase 1 study H-400-002 at the vaccination site and outside the vaccination site bandage.

ACAM2000 was compared to ACAM1000 and Dryvax[®]. The pattern of virus shedding over time was similar in each treatment group, with virus detectable at the vaccination site in all 3 treatment groups by Day 3, the first post-vaccination time-point assessed. The proportion of subjects with evidence of virus shedding was notably higher by Day 7 and remained high through Day 15. By Day 30, a relatively small proportion of subjects in each treatment group (3%, 10%, and 13% of subjects in the ACAM2000, ACAM1000, or Dryvax[®] groups) had evidence of virus shedding at the vaccination site. The median duration of virus shedding at the vaccination site, as determined by Kaplan-Meier estimates, was 16, 18, and 20 days with ACAM2000, ACAM1000, and Dryvax[®], respectively. Although the median duration of virus shedding was 4 days longer in the Dryvax[®] group compared to the ACAM2000 group, the p-value for the log rank comparison of the Kaplan-Meier curves for duration of virus shedding from the inoculation site missed the criterion for significance (p=0.0586).

Only 1 (3%) subject treated with ACAM2000 in Study H-400-002 had evidence of virus shedding outside the vaccination site dressing detected at any time-point.

7.5. Induction of Non-Specific Serologic Responses

In the Phase 1 Study H-400-002, serologic evaluations were performed before and after vaccination to determine whether subjects vaccinated with smallpox vaccine developed non-specific positive serological responses to unrelated viruses (HIV, hepatitis B and C viruses) and bacteria (syphilis). These tests were conducted at the request of the FDA based on historical data that indicated that other vaccines (e.g., influenza) occasionally cause non-specific false positive serological tests for HIV and that smallpox vaccine specifically caused a relatively high incidence of false positive tests for syphilis [41,45].

Serologic evaluations at Screening and on Days 15 and 45 included tests for HIV antibody (using an FDA approved HIV enzyme-linked immunoassay [EIA] test kit); hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb) (assessed only at Screening unless either was positive); hepatitis C virus (HCV); and syphilis antibody (using the Rapid Plasma Reagin test with reflex to titer). Samples that tested positive for HIV were confirmed by Western Blot, positive tests for HBsAg were confirmed by neutralization, positive tests for HBsAb were confirmed by endpoint, positive tests for HCV were confirmed by Recombinant Immunoblot Assay if there were no risk factors, and by polymerase chain reaction quantitation if there were risk factors, and positive tests for syphilis were retested using Florescent Treponemal Antibody. All subjects' retest results were negative. The results indicate that smallpox vaccination using calf lymph or cell culture derived vaccines, can elicit acute (i.e. transient) biologic false positive tests for syphilis (BFP) in a relatively high proportion of subjects.

7.6. Safety in Special Populations

Subgroup analyses of safety data were performed for the following factors: sex, race, body surface area, age, baseline neutralizing antibody titer (previously vaccinated subjects only), vaccination success (as determined by cutaneous response), and seroconversion (≥ 4 -fold rise in neutralizing antibodies between Days 0 and 30) status. As discussed in Section 7.3, the incidence of injection site reactions and constitutional symptoms was higher among subjects who experienced a successful vaccination and among subjects who seroconverted than among those who did not, regardless of dose or baseline vaccination status. No notable difference in AE rates was seen by race, sex, age, body surface area, or baseline neutralizing antibody titer.

7.7. Conclusions Regarding the Safety of ACAM2000

ACAM2000 was well tolerated and less reactogenicity than Dryvax[®]. These observations when taken in conjunction with the neurovirulence testing in animals (Table 3) suggest that ACAM2000 is more attenuated than Dryvax[®]. Therefore, the increased safety potential with ACAM2000 is balanced by a more modest cutaneous response observed in previously vaccinated subjects and slightly lower GMTs overall.

The AEs associated with ACAM2000 were, in general, predictable and manageable, with the most commonly reported events being mild to moderate vaccination site reactions, lymphadenitis, and constitutional and gastrointestinal symptoms. The incidence of these commonly reported AEs was higher in vaccinia-naïve subjects than in those who were previously vaccinated. Furthermore, a higher incidence of these events was generally seen in subjects who experienced a successful vaccination based on dermal response compared to those who did not, regardless of baseline vaccination status.

Although uncommon, myocarditis was associated with smallpox vaccination in vaccinia-naïve subjects, with an incidence of 5.73 cases per thousand vaccinations of ACAM2000 in the Phase 3 study. This event has only been identified in more recent years with Dryvax[®] vaccinated subjects [24] but has not been evaluated with the detail these studies have provided. No other SAEs historically associated with smallpox vaccine, including GV, ocular vaccinia, postvaccinial encephalitis, PV, erythema multiforme, or EV, were reported with ACAM2000.

8. POST-LICENSURE SURVEILLANCE

In accordance with established practice, Acambis will work with the various government agencies (DoD and CDC) to ensure complete reporting of safety information following incorporation of ACAM2000 post-licensure into their vaccination programs. Based upon the current reporting scheme for Dryvax[®] smallpox vaccine, most spontaneously reported ACAM2000 AEs will be captured by the CDC and/or DoD smallpox vaccine programs, and reported directly to the FDA using Vaccine Adverse Event Reporting System (VAERS) forms. Summaries of DoD and CDC spontaneous reports for ACAM2000 will be downloaded from the VAERS database by Acambis for safety review.

ACAM2000 AEs reported directly to Acambis will be entered, tracked and reported on FDA VAERS form via a validated pharmacovigilance (PVG) database. ACAM2000 spontaneous events reported directly by the CDC or DoD smallpox vaccination programs will not be entered into the Acambis PVG database to avoid duplication of reporting. During the early post-marketing period (first two years after licensure), all known 15 day reports provided directly to Acambis will be furnished to the FDA on a monthly basis.

As ACAM2000 is a new product, Acambis proposes additional safety measures in coordination with governmental agencies, to include the following three modules:

- A prospective post-marketing study with appropriate power estimates (minimum 10,000 subjects) for the specific primary endpoint of myocarditis and secondary endpoints of superinfection, contact transmission, autoinoculation, and rash.
- A myocarditis registry for extended follow-up of defined cases out to two years following onset of disease, to ascertain rates of long term serious outcomes (i.e. dilated cardiomyopathy (DCM), heart failure, and death associated smallpox vaccination-associated myocarditis/pericarditis).
- An enhanced surveillance program of between 1-2 years duration to facilitate signal detection following the introduction of ACAM2000.

9. OVERALL SUMMARY AND CONCLUSIONS: BENEFITS VERSUS RISKS

The eradication of naturally occurring smallpox was a major milestone in global public health, eliminating a disease with a case fatality rate of 30% [46]. However, the credible and possible risk of a deliberate release of smallpox necessitates a continued supply of vaccine against this virus. Dryvax[®], the only US-licensed vaccine for the prevention of smallpox, is effective and was instrumental in eliminating smallpox in the US, but is no longer manufactured. Consequently, an alternative smallpox vaccine is urgently needed to protect individuals at risk of exposure to smallpox and as an emergency safeguard stockpile for use in the general population in the event of a deliberate smallpox release.

Clinical and non-clinical studies conducted with ACAM2000 provide data supporting the safety and efficacy of this vaccine. While efficacy cannot be proven in the absence of naturally occurring smallpox, ACAM2000 provided protection from lethal challenges with other Orthopox viruses in two animal models and induced positive responses in the great majority of human subjects according to two markers of efficacy, cutaneous response and neutralizing antibody response. Among vaccinia-naïve subjects and previously vaccinated subjects in the Phase 3 studies, vaccination success (as determined by cutaneous response) rates of 96% and 84%, respectively, were observed. Notably, among previously vaccinated subjects where pre-existing immunity had waned (i.e., Baseline neutralizing antibody titers <10), successful rates were higher (94%) and more closely resembled the responses observed in vaccinia-naïve subjects. Robust neutralizing antibody responses to ACAM2000 were found in both vaccinia-naïve and previously vaccinated populations (GMTs of 166 and 286, respectively, in Phase 3 studies). In addition, vaccinia-specific T-cell responses were detected in all subjects assessed for this response (n=30) by at least one of three cell-mediated immunity assays (Table 6).

Overall, the most significant difference between ACAM2000 and Dryvax[®] is the lower rate of cutaneous response in previously vaccinated subjects: 84% vs. 98%, respectively. As noted, the lower response rate appears to be principally due to the confounding effect of residual immunity. A similar finding was reported in a recent study in Israel, where lack of response to revaccination with Lister vaccine correlated with the interval since last vaccination [37]. In that study, 77.5% of subjects vaccinated <20 years previously responded to revaccination versus 97.2% for persons vaccinated >20 years before. Unlike ACAM2000 or the Israeli Lister vaccine, Dryvax[®] is able to elicit a more active cutaneous response that is independent of residual immunity [38]. For ACAM2000, an inverse correlation was seen between response rate and level of residual immunity, i.e. response rates were higher in individuals with the lowest baseline neutralizing antibody titers.

As described in earlier sections of this package, not all statistical efficacy endpoints were met in the Phase 3 studies. ACAM2000 narrowly missed the criterion for non-inferiority to Dryvax[®] with regard to GMTs in the vaccinia-naïve population and did not meet the dermal response endpoint in the previously vaccinated population. Nevertheless, the entirety of the efficacy database supports the conclusion that ACAM2000 will be effective in providing protection against smallpox infection in these individuals. For example, if one accepts that the data are sufficient to support efficacy in the primary vaccination group (vaccinia naïve) based on the ACIP

definition for cutaneous reaction at the site of vaccination, then it follows that the previously vaccinated group should also be protected based on higher neutralizing antibody levels in that group. Furthermore, when one correlates the baseline antibody titer of the previously vaccinated group with the cutaneous reactivity, it is evident that the dermal reaction occurs in greater than 94% of the subjects who lack immunity (based on low neutralizing antibody titer).

ACAM2000 appeared to be somewhat more attenuated than Dryvax[®] in terms of immunogenicity and safety parameters. In both subject populations, cutaneous reaction rates and the magnitude of the antibody responses (GMT) were lower for ACAM2000 than Dryvax[®]. This phenomenon was also revealed by greater sensitivity to interference in cutaneous response in previously vaccinated persons with demonstrable residual immunity (neutralizing antibodies). Finally, ACAM2000 was less reactogenic than Dryvax[®] with respect to inoculation site, lymph node-related, and constitutional symptoms (see Risk Assessment discussion, below).

Revaccination

The CDC currently recommends revaccination of individuals who demonstrate no cutaneous response after Dryvax[®]. Acambis subscribes to the CDC's recommendation and has included it in the draft package insert for ACAM2000.

Risk Assessment

In the ACAM2000 program, risks of the vaccine were assessed in both the non-clinical and clinical studies. The non-clinical program demonstrated that ACAM2000 is associated with reduced neurovirulence relative to Dryvax[®]. It is unknown whether this non-clinical finding would translate into a lower risk of PVE. However, previous studies have demonstrated a relationship between mouse neurovirulence and the incidence of PVE associated with different vaccinia vaccine strains [48].

In the clinical program, common AEs were generally expected local signs or symptoms related to the cutaneous reaction to vaccination, or associated systemic symptoms indicative of vaccinia virus replication. These commonly reported AEs included pruritus, erythema, pain, and swelling at the vaccination site, constitutional symptoms (fatigue, headache, myalgia, malaise, feeling hot, and rigors), and lymphadenitis. These commonly reported AEs were generally predictable with regard to time of onset, mild or moderate in intensity, and tolerable. A higher incidence of these events was seen in vaccinia-naïve subjects than in previously vaccinated subjects. This finding is not unexpected, given that the clinical course of the response to revaccination is modulated by preexisting immunity and is typically milder than that to primary vaccination. In both subject populations, the incidence of these common AEs generally was lower, in many cases significantly lower, with ACAM2000 than with Dryvax[®].

Myocarditis and/or pericarditis is associated with smallpox vaccination (0.11 [24] and 0.54 [25] cases per thousand vaccinations in the DoD and civilian vaccination programs, respectively), which is a vaccinia virus-specific complication resulting from vaccination with a number of vaccine strains [18]. ACAM2000 was associated with myocarditis at an incidence of 5.73 cases per thousand vaccinations in vaccinia-naïve subjects in the Phase 3 study. The incidence was lower than that seen with Dryvax[®] (10.38 cases per thousand vaccinations), but the difference was not significant. Myocarditis/pericarditis was not seen in previously vaccinated subjects. The relatively high incidence of myocarditis in the Phase 3 program compared to the DoD and

civilian vaccination programs, where surveillance was largely passive, was attributed to prospective case finding and serial ECG and troponin I monitoring. These measures identified six asymptomatic cases (of the ten total cases reported) that would likely not have been identified by a passive surveillance program. Myocarditis resolved without permanent sequelae in all but one subject who received Dryvax®. It could not be determined whether that subject had preexisting heart disease.

No other SAEs historically associated with smallpox vaccine, including GV, fetal vaccinia, ocular vaccinia, PVE, PV, erythema multiforme, or EV, were reported with ACAM2000, although the studies were not powered to detect these SAEs historically associated with smallpox vaccine. Other adverse reactions noted after recent vaccination programs (including erythema multiforme major/ Stevens-Johnson syndrome, dilated cardiomyopathy, and contact transmission of vaccinia) have not been observed with ACAM2000 [23, 30, 31].

No fatalities were reported in the ACAM2000 clinical program. Historically, the rate of death as a result of complications from smallpox vaccination has been estimated to be 1/1,000,000 among primary vaccinees and 0.25/1,000,000 among revaccinees [15]. The ACIP has indicated that the risk associated with the receipt of smallpox vaccine should be weighed against the risk of exposure to Orthopoxviruses [49].

Conclusion

The license application for ACAM2000 Smallpox Vaccine is based on the safety and vaccination response (cutaneous and serological) demonstrated by the comprehensive clinical development program. There is strong evidence of efficacy in both vaccinia-naïve and previously vaccinated subject populations. The safety risks for ACAM2000 were expected based on historic experience with smallpox vaccines, with the observed incidence of myo/pericarditis with both Dryvax and ACAM2000 attributed to the increased surveillance that was performed in clinical trials for ACAM2000. On the basis of this clinical program, it is proposed that ACAM2000 vaccine be indicated for the prevention of smallpox disease in populations determined to be at risk for smallpox infection.

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11. APPENDICES

11.1. Appendix 1 - Analysis of Efficacy Data from ITT Population

This appendix presents a summary of the data and conclusions from the two Phase 3 studies comparing ACAM2000 to Dryvax[®], using data collected from all subjects vaccinated with either ACAM2000 or Dryvax (ITT population) at all sites, including the four sites excluded from the final analysis presented in Section 6 of this briefing document. ITT subjects with no evaluations on Day 7 or after were considered vaccination failures. The conclusions from the final analysis presented in Section 6 and the analysis presented below, based on the expanded data set, do not differ with respect to the assessment of non-inferiority of ACAM2000 relative to Dryvax[®] in both the vaccinia-naïve and previously vaccinated populations. The data for primary vaccination and for previously vaccinated subjects are provided in Tables 15 and 16, respectively.

Table 15: Phase 3 Efficacy Results for ACAM2000 versus Dryvax[®] in Vaccinia-Naïve Subjects from the ITT population (Study H-400-009)

| Treatment group | ACAM2000 | Dryvax® |
|---|------------------|----------|
| Vaccination Success based on Cutaneous Response | | |
| ITT Population, n | 873 | 289 |
| Vaccination Success, n (%) | 839 (96) | 287 (99) |
| 97.5% CI [a] (Criterion for Non-Inferiority) | -4.80 (>-5.00) | |
| Neutralizing Antibody Titer | | |
| AnE Population, n | 624 | 208 |
| GMT | 168 | 259 |
| 97.5% CI [b] (Criterion for Non-Inferiority) | -0.305 (>-0.301) | |

- Lower bound of the 97.5% one-sided CI on the difference between ACAM2000 and Dryvax[®] derived by normal approximation to the binomial distribution.
- Lower bound of the 97.5% one-sided CI on the difference in the mean log10 GMT between ACAM2000 and Dryvax[®] derived using analysis of variance (ANOVA).

Table 16: Phase 3 Efficacy Results for ACAM2000 versus Dryvax[®] in Previously Vaccinated Subjects from the ITT population (Study H-400-012)

| Treatment group | ACAM2000 | Dryvax® |
|---|------------------|----------|
| Revaccination Success based on Cutaneous Response | | |
| ITT Population, n | 1371 | 448 |
| Revaccination Success, n (%) | 1113 (81) | 427 (95) |
| 97.5% CI [a] (Criterion for Non-Inferiority) | -17.00 (>-10) | |
| Neutralizing Antibody Titer | | |
| AnE Population, n | 803 | 412 |
| GMT | 279 | 425 |
| 97.5% CI [b] (Criterion for Non-Inferiority) | -0.262 (>-0.301) | |

- Lower bound of the 97.5% one-sided CI on the difference between ACAM2000 and Dryvax[®] derived by normal approximation to the binomial distribution.
- Lower bound of the 97.5% one-sided CI on the difference in the mean log₁₀ GMT between ACAM2000 and Dryvax[®] derived using ANOVA.

11.2. Appendix 2 - Demographics

11.2.1. Summary of Demographics

Table 17 presents a summary of demographic and baseline characteristics by baseline vaccination status for subjects included in the safety population for the final analysis for each treatment regimen (ACAM2000 or Dryvax[®]) combined and by treatment groups. A total of 873 vaccinia-naïve subjects and 1371 previously vaccinated subjects were treated with ACAM2000 in the Phase 3 studies. A total of 289 vaccinia-naïve and 448 previously vaccinated subjects were treated with Dryvax[®] in these Phase 3 studies.

The racial distribution in the studies was generally similar to that of the overall US population (76.8% Caucasian, 10.1% African-American, and 9.3% Hispanic).

As anticipated, the vaccinia-naïve subjects were younger (mean age of 23.0 versus 49.0 years) than previously vaccinated subjects. The vaccinia-naïve subjects were slightly taller (174 cm versus 171 cm) and had a slightly lower mean body weight (81 kg versus 85 kg) than the previously vaccinated subjects. There was a higher percentage of male vaccinia-naïve subjects than male previously vaccinated subjects (65% versus 49%).

Table 17: Demographics and Baseline Characteristics, By Subject Population, Treatment Group, and ACAM2000 Lot, Final Analysis Safety Population

| Parameter / Statistic | Subject Population/Treatment Group | | | | | | | | | |
|-----------------------|---|---|---|-----------------------------------|---|--|---|---|------------------------------------|---|
| | Vaccinia-Naïve Subjects (Study H-400-009) | | | | | Previously Vaccinated Subjects (Study H-400-012) | | | | |
| | ACAM 2000 2.2×10 ⁸ (n=289) | ACAM 2000 1.9×10 ⁸ (n=298) | ACAM 2000 1.3×10 ⁸ (n=286) | ACAM 2000 All Doses (n=873) | Dryvax® 1.5×10 ⁸ (n=289) | ACAM 2000 2.2×10 ⁸ (n=455) | ACAM 2000 1.9×10 ⁸ (n=459) | ACAM 2000 1.3×10 ⁸ (n=457) | ACAM 2000 All Doses (n=1371) | Dryvax® 1.5×10 ⁸ (n=448) |
| Sex, n (%) | | | | | | | | | | |
| Male | 200 (69) | 187 (63) | 185 (65) | 572 (66) | 182 (63) | 218 (48) | 247 (54) | 220 (48) | 685 (50) | 214 (48) |
| Female | 89 (31) | 111 (37) | 101 (35) | 301 (34) | 107 (37) | 237 (52) | 212 (46) | 237 (52) | 686 (50) | 234 (52) |
| Race, n (%) | | | | | | | | | | |
| Caucasian | 220 (76) | 219 (73) | 221 (77) | 660 (76) | 205 (71) | 362 (80) | 365 (80) | 348 (76) | 1075 (78) | 348 (78) |
| Afr-Amer | 32 (11) | 43 (14) | 25 (9) | 100 (11) | 40 (14) | 38 (8) | 40 (9) | 58 (13) | 136 (10) | 51 (11) |
| Hispanic | 27 (9) | 28 (9) | 31 (11) | 86 (10) | 32 (11) | 39 (9) | 43 (9) | 39 (9) | 121 (9) | 38 (8) |
| Asian | 2 (1) | 4 (1) | 4 (1) | 10 (1) | 4 (1) | 5 (1) | 3 (1) | 3 (1) | 11 (1) | 4 (1) |
| Other | 8 (3) | 4 (1) | 5 (2) | 17 (2) | 8 (3) | 11 (2) | 8 (2) | 9 (2) | 28 (2) | 7 (2) |
| Age, (years) | | | | | | | | | | |
| N | 289 | 298 | 286 | 873 | 289 | 455 | 459 | 457 | 1371 | 448 |
| Mean (±SD) | 22.9 (3.5) | 23.2 (3.6) | 22.8 (3.5) | 23.0 (3.5) | 22.9 (3.5) | 44.8 (10.0) | 49.0 (10.4) | 48.9 (9.3) | 48.9 (10.0) | 49.2 (9.8) |
| Min, Max | 18, 30 | 18, 30 | 18, 30 | 18, 30 | 18, 30 | 31, 82 | 31, 78 | 31, 80 | 31, 82 | 31, 84 |
| Height, (cm) | | | | | | | | | | |
| N | 288 | 298 | 286 | 872 | 289 | 455 | 458 | 456 | 1369 | 448 |
| Mean (±SD) | 174 (9.7) | 174 (9.8) | 174 (10.4) | 174 (10.0) | 173 (9.8) | 171 (10.1) | 171 (9.5) | 170 (10.3) | 171 (10.0) | 171 (10.1) |
| Min, Max | 150, 201 | 147, 199 | 146, 203 | 146, 203 | 132, 203 | 149, 201 | 144, 196 | 142, 203 | 142, 203 | 142, 208 |

Table 17: Demographics and Baseline Characteristics, By Subject Population, Treatment Group, and ACAM2000 Lot, Final Analysis Safety Population (Continued)

| Parameter / Statistic | Subject Population/Treatment Group | | | | | | | | | |
|--------------------------|--|--|--|--------------------------------------|---|--|--|--|--------------------------------------|---|
| | Vaccinia-Naïve Subjects (Study H-400-009) | | | | | Previously Vaccinated Subjects (Study H-400-012) | | | | |
| | ACAM 2000 2.2×10 ⁸ (n=258) | ACAM 2000 1.9×10 ⁸ (n=264) | ACAM 2000 1.3×10 ⁸ (n=258) | ACAM 2000 All Doses (n=780) | Dryvax® 1.5×10 ⁸ (n=257) | ACAM 2000 2.2×10 ⁸ (n=258) | ACAM 2000 1.9×10 ⁸ (n=264) | ACAM 2000 1.3×10 ⁸ (n=258) | ACAM 2000 All Doses (n=780) | Dryvax® 1.5×10 ⁸ (n=257) |
| Weight, (kg) | | | | | | | | | | |
| N | 288 | 298 | 286 | 872 | 289 | 455 | 458 | 456 | 1369 | 448 |
| Mean (±SD) | 81 (20.7) | 82 (21.1) | 81 (19.4) | 81 (20.4) | 81 (19.1) | 85 (20.9) | 86 (21.1) | 83 (20.1) | 85 (20.7) | 85 (21.6) |
| Min, Max | 44, 158 | 43, 159 | 42, 159 | 42, 159 | 48, 158 | 45, 163 | 46, 173 | 47, 201 | 45, 201 | 46, 272 |

11.3. Appendix 3 – Subjects with Suspect or Probable Myocarditis

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG and ECHO Findings | | Laboratory Findings | PV Day of Onset and Outcome |
|---|-------------------------------|---|-------------------|--|---|---|---|
| | | | | Local | Core | | |
| ACAM2000 (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-002 | | | | | | | |
| 2063 (1.0x10 ⁸) Male Caucasian 19 years | None | Suspect subclinical myocarditis [a] | None reported | ECG: mild T wave flattening with very slight elevation of ST segment in Leads I and aVL consistent with but not diagnostic of myocarditis 3 month F/U ECHO LVEF = 64% | ECG Not done 3 month F/U ECHO LVEF = 55% | Troponin and creatinine phosphokinase (CPK) normal | 14 Resolved without sequelae |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG and ECHO Findings | | Laboratory Findings | PV Day of Onset and Outcome |
|---|-------------------------------|-----------------------------|-------------------|---|---|---|---|
| | | | | Local | Core | | |
| ACAM2000 (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-005 | | | | | | | |
| 1238 (6.8x10 ⁷) Male Caucasian 18 years | None | Probable myocarditis [a] | Chest tightness | ECG: T-wave inversion in Lead III and nonspecific changes in Leads II and aVF ECHO 16 month F/U LVEF = 60% | ECG Not done ECHO 16 month F/U LVEF = >55% | CPK elevated (400 IU/L; normal range 38 to 199 IU/L), troponin I evaluated (3.8 ng/mL; normal range <0.5 ng/mL, CPK-MM fraction elevated (88%); and CPK-MB elevated (12%; normal range 0%) | 10 Resolved without sequelae |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG and ECHO Findings | | Laboratory Findings | PV Day of Onset and Outcome |
|--|-------------------------------|---------------------------------------|---|--|---|---|---|
| | | | | Local | Core | | |
| ACAM2000 (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 023-190 (1.9x10 ⁸) Male African American 22 years | Current Smoker | Suspect subclinical myocarditis | None Reported | ECG: sinus arrhythmia, ST elevation, T- wave inversion Day 10 ECHO normal | Significant anterior T-wave inversion compared with baseline ECG Day 10 ECHO LVEF = 55 to 60% | Troponin I and CK-MB normal | 9 Resolved without sequelae |
| 048-116 (1.3x10 ⁸) Male Caucasian 23 years | None | Probable myocarditis | Chest pain, exercise tolerance decreased | ECG: sinus rhythm with sinus arrhythmia, minimal voltage criteria for LVH; ST-T elevations leads I, aVL, V5, V6; inverted T wave lead III Day 10 ECHO LVEF = <u>50%</u> | Significant inferolateral T-wave inversion on Day 21 as compared to Day 10 ECG Day 10 ECHO LVEF = ≥ 60% | Troponin I elevated (4.8 ng/mL; normal range 0.0 to 0.4 ng/mL), CK-MB 16.7 ng/mL (normal range 0.0 to 3.3 ng/mL) | 11 Resolved with sequelae (abnormal ECG on discharge with nonspecific ST-T wave changes) |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG and ECHO Findings | | Laboratory Findings | PV Day of Onset and Outcome |
|--|-------------------------------|--|--------------------------|---|--|--|---|
| | | | | Local | Core | | |
| ACAM2000 (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 056-111 (1.9X10 ⁸) Male Caucasian 23 years | None | Probable subclinical myocarditis | None reported | Elevated ST segments on ECG Day 56 ECHO LVEF = 60% | No significant ECG findings Day 56 ECHO LVEF = ≥ 60% | Troponin I elevated (1.8 ng/mL; normal range <0.5 ng/mL) | 10 Resolved without sequelae |
| 080-112 (1.9X10 ⁸) Male Hispanic 18 years | None | Probable myocarditis | Dyspnea; palpitations | ECG: 2 mm ST elevation in Leads I and aVL, suggesting lateral injury; T- wave inversion and 1 mm ST depression in Leads III and F Day 22 ECHO LVEF = 52% | Significant inferior T-wave inversion compared to Baseline ECG Day 22 ECHO LVEF = 60% 12 month Follow-up LVEF = ≥ 60% | Troponin I, CK- MB, and CPK and isoenzymes normal | 9 Resolved without sequelae |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG and ECHO Findings | | Laboratory Findings | PV Day of Onset and Outcome |
|--|-------------------------------|--|-------------------|--|---|--|---|
| | | | | Local | Core | | |
| ACAM2000 (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 094-114 (1.3X10 ⁸) Male Caucasian 21 years | None | Probable subclinical myocarditis | None reported | ECG: sinus bradycardia with sinus arrhythmia and first degree AV block Day 20 ECHO LVEF = 48% | No significant ECG findings Day 20 ECHO LVEF= ≥ 60% | Troponin I elevated (0.11; normal range <0.4) | 9 Resolved without sequelae |
| Dryvax® (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 004-103 (1.5X10 ⁸) Male Asian 20 years | None | Suspect myocarditis | None reported | Significant ST and T wave changes on ECG | Significant anterior T wave inversion on ECG 9 months post- vaccination LVEF= ≥ 60% | Troponin I normal; “all cardiac labs normal” | 20 Resolved without sequelae |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG Findings/ECHO | | Laboratory Findings | PV Day of Onset and Outcome |
|--|-------------------------------|-------------------------|---|--|---|---|---|
| | | | | Local | Core | | |
| Dryvax® (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 054-106 (1.5x10 ⁸) Male Caucasian 23 years | None | Probable myocarditis | Exercise tolerance decreased; palpitations; "crushing" chest pain; dyspnea | Abnormal ST-T wave elevations on ECG | Day 21 ECG showed minimal decrease in T- wave amplitude and ST flattening (possibly consistent with myopericarditis) compared with Day 10 ECG Day 42 MUGA LVEF = 70% | Troponin I elevated (6.7 ng/mL; normal range <0.2 ng/mL); CK-MB normal | 11 Resolved without sequelae |

Table 18: Subjects with Treatment-Emergent Suspect or Probable Myocarditis, by Treatment Group and Study
(Continued)

| Subject No. (Dose) Sex/Race/Age | Notable Medical History | CAP Classification | Clinical Signs | ECG Findings/ECHO | | Laboratory Findings | PV Day of Onset and Outcome |
|--|--|-------------------------|-------------------|--|--|---|-----------------------------------|
| | | | | Local | Core | | |
| Dryvax® (Vaccinia-Naïve Subjects) | | | | | | | |
| Study H-400-009 | | | | | | | |
| 065-137 (1.5x10 ⁸) Female Caucasian 21 years | Smoker, obesity; borderline high blood pressure; occasional sinus tachycardia | Probable myocarditis | None reported | No significant findings on ECG Day 15 ECHO LVEF = 52% Day 49 ECHO LVEF = 44% 1 year F/U LVEF = 35-42% 2 year F/U LVEF = 27-32% | No significant ECG findings Day 15 ECHO LVEF = 35% Day 49 ECHO LVEF = 30% 1 year F/U LVEF = 35% 2 year F/U LVEF = 25% | Troponin I elevated (3.2 ng/mL; normal range <0.3 ng/mL); CPK normal | 9 Ongoing |

AV=atrioventricular; CPK=creatine phosphokinase; CK-MB=creatine kinase-myocardial band; CPK-MM= creatine phosphokinase skeletal muscle;
PV=Post vaccination.

a. This case was not reviewed by the CAP, but was classified by the Sponsor's medical officer.

11.4. Appendix 4 – Cardiac Algorithm

The algorithm for diagnosis and management of subjects with suspect myocarditis/pericarditis, that was used in the ACAM2000 Phase 3 studies (H-400-009 and H-400-012) is presented in Figure 9 below.

Figure 9: Algorithm for Diagnosis and Management of Subjects with Suspect Myocarditis/Pericarditis

