

VRBPAC Background Document

ACAM2000 (Live Vaccinia Virus Smallpox Vaccine)
May 17, 2007 VRBPAC Meeting

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1.0 General Information

Applicant: Acambis, Inc.

Proper Name: Smallpox Vaccine, Vero Cells

Proposed Trade Name: *ACAM2000*

Product Formulation Including Preservatives:

ACAM2000 vaccine contains vaccinia virus derived by plaque purification from Dryvax® vaccine (Smallpox Vaccine Dried, Calf Lymph Type, Wyeth Laboratories), which is derived from the New York City Board of Health (NYCBH) vaccinia strain and grown in African green monkey kidney (Vero) cells.

ACAM2000 is a lyophilized preparation of purified live virus in HEPES buffer, pH 7.4 containing 1-4% (w/w) human serum albumin (USP) (HSA), 0.6% NaCl (USP), 5% Mannitol, and traces of antibiotics (70 ug/mL neomycin and 70 U/mL polymyxin B).

Potency $1.0-5.0 \times 10^8$ PFU/mL

It is reconstituted by addition of 0.3 mL glycerol-phenol diluent (50% [v/v] Glycerin, USP; 0.25 [v/v] Phenol, USP, in water for Injection, USP)

Applicant: Acambis, Inc.

Proposed Indication: Prevention of smallpox

Proposed Population: Persons determined to be at high risk for smallpox. Not for routine vaccination of the general population, due to risk of serious adverse events.

Dosage Form and Route of Administration: Approximately 0.0025 mL of vaccine (1 drop), containing approximately 250,000 PFU, administered via percutaneous scarification using 15 punctures with a bifurcated needle (supplied).

Executive Summary

This document contains the summary of the safety and immunogenicity data provided by Acambis, Inc. to support approval of *ACAM2000*, a live vaccinia virus smallpox vaccine. Clinical effectiveness was based on the demonstration that *ACAM2000* is non-inferior to the licensed smallpox vaccine, Dryvax®. Efficacy was demonstrated in 2 pivotal clinical trials (Trials H-400-009 and H-400-012) using surrogate endpoints; major cutaneous reaction, or *take rates*, and serum neutralizing antibody respectively.

Clinical trial H-400-009 was a Phase 3 randomized, placebo-controlled, double-blinded study in subjects who were naïve to previous smallpox vaccination. Subjects were 18 to 30 years of

age (mean age 23 years; 65% male; 74% Caucasian; 12% African-American; 10% Hispanic; 1% Asian). Enrollment totaled 1,162 persons; 873 received *ACAM2000* and 289 received Dryvax®. Ninety six percent of subjects randomized to receive *ACAM2000* had a “take” (major cutaneous response) (95% CI: 0.95, 0.97). Ninety nine percent of subjects randomized to receive Dryvax® had a “take” (major cutaneous response). The lower bound of the 97.5% 1-sided CI on the difference in rates of successful vaccination between *ACAM2000* and Dryvax® was -4.67%; *ACAM2000* was non-inferior to Dryvax® based on this outcome. By Day 30 after vaccination neutralizing antibody Geometric Mean Titers (GMT) were 166 in the *ACAM2000* group and 255 in the Dryvax® group. The lower bound of the 97.5% 1-sided CI on the difference in the mean log₁₀ GMT between *ACAM2000* and Dryvax® was -0.307; a lower bound of ≥ -0.301 was required to establish non-inferiority.

Clinical trial H-400-012 was a Phase 3 randomized, placebo-controlled, double-blinded study in persons previously vaccinated with smallpox vaccine. Subjects aged 31 to 84 years (mean age 49 years; 49% male; 81% Caucasian; 7% African-American; 12% Hispanic and Asian) were enrolled. Enrollment totaled 1,647 subjects; 1,242 received *ACAM2000* and 405 received Dryvax®. Eighty four percent of subjects receiving *ACAM2000* had a “take” (major cutaneous reaction) (95% CI: 0.82, 0.86). Ninety eight percent of subjects receiving Dryvax® had a “take” (major cutaneous reaction). The lower bound of the 97.5% 1-sided CI on the difference in rates of successful vaccination between *ACAM2000* and Dryvax® was -17.00%; *ACAM2000* was not non-inferior to Dryvax® based on this outcome (needed to exceed -10%). By Day 30 after vaccination, serum neutralizing antibody GMTs were 286 in the *ACAM2000* group and 445 in the Dryvax® group. The lower bound of the 97.5% 1-sided CI on the difference in the mean log₁₀ GMT between *ACAM2000* and Dryvax® was -0.275; *ACAM2000* was non-inferior to Dryvax® (a lower bound of ≥ -0.301 was required to establish non-inferiority).

ACAM2000 met two of the four primary endpoint criteria established for the phase 3 clinical trials. The primary determinant for an effective immune response in those naïve to vaccine is a major cutaneous reaction. *ACAM2000* was non-inferior to Dryvax® in clinical trial H-400-009 with regard to eliciting a major cutaneous reaction. The measure of the strength of the generated antibody response was similar but did not meet the predefined criterion for non-inferiority. The percentage of vaccinees developing a major cutaneous response after revaccination with vaccinia-based smallpox vaccines may not provide an accurate measure of the strength of the immune response since the pre-existing immunity modifies the scope of the cutaneous response. A more informative measure of the immune response in persons previously vaccinated may be the strength of the neutralizing immune response, as measured by the plaque reduction neutralization test (PRNT). In clinical trial H-400-012 *ACAM2000* was non-inferior to Dryvax® with regard to the strength of the immune response (GMT of the PRNT). Therefore, *ACAM2000* was non-inferior to Dryvax® in what may be the two most important measurements of efficacy: the rate of the major cutaneous reaction in those naïve to the vaccine, and the strength of the immune response in those previously exposed to vaccinia-based smallpox vaccines.

There are serious safety concerns associated with the administration of live vaccinia virus smallpox vaccines such as *ACAM2000* and Dryvax® in vaccinated persons and their close

contacts; these include the traditionally recognized serious, albeit rare, adverse effects such as generalized vaccinia, eczema vaccinatum, postvaccinial encephalitis, fetal vaccinia, and death. It was demonstrated in clinical trial H-400-009 that in naïve subjects, smallpox vaccination is associated with myopericarditis at a rate much higher than previously suspected (1 case per 145 vaccinees including suspected/probable). However, the potential benefits of administration of *ACAM2000* during a smallpox outbreak to persons who are determined to be at high risk of exposure or who have been recently exposed outweigh potential risks. If *ACAM2000* is approved, the availability of additional smallpox vaccine to the National Strategic Stockpile may provide meaningful benefit for national emergency preparedness. Acambis, Inc. has stated in its April 2006 submission to the BLA that they do not intend to make this vaccine commercially available in the U.S.

Because *ACAM2000* will be used routinely for forward deployed troops in the Department of Defense DoD, a Risk Minimization Action Plan (RiskMAP) will be an essential component of the license application package to ensure vaccinees are fully informed of risks and benefits and steps are taken to minimize risks.

2.0 Introduction and Background

Epidemiology of Smallpox Infection

Smallpox (*variola major*) is a particularly dangerous biological weapon threat because of its clinical and epidemiological properties. *Variola major* can be manufactured in large quantities, stored for an extended period of time, and delivered as an infectious aerosol. Reported evidence implicating the possession of smallpox virus by potential enemies of the United States has led to concerns regarding the susceptibility of U.S. troops and civilians to smallpox virus and the need to develop defense strategies in case of attack. With the success by 1980 of the World Health Organization's (WHO) campaign to eradicate naturally occurring smallpox, and the subsequent discontinuation of vaccination, it is estimated that a large proportion of the population has no immunity. Case fatality rates could be higher than 25 percent if smallpox was ever released as a bioterrorist weapon.

The strain of vaccinia virus commercially prepared in the United States during the campaign to eradicate smallpox was the New York City Board of Health (NYCBH) strain. It was used in the Western Hemisphere and Africa and shown to be effective in preventing smallpox infection. The new smallpox vaccine, *ACAM2000*, is a clonal isolate prepared from the licensed NYCBH commercial smallpox vaccine originally manufactured by Wyeth, Dryvax®. The selected clonal isolate was amplified in tissue culture (Vero cells) to generate the Master Viral Seed and Production Viral Seed. Although the specific correlates for protection against smallpox are not known, the efficacy of *ACAM2000* may be deduced by comparing its elicited immune response to the one generated by Dryvax®.

General Safety concerns with Live Vaccinia Virus Smallpox Vaccines

Serious complications may follow live vaccinia smallpox vaccination: myocarditis and/or pericarditis, encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia (*vaccinia necrosum*), generalized vaccinia, severe vaccinial skin infections, erythema multiforme major

(including Stevens-Johnson syndrome) and eczema vaccinatum. The risk of acute myocarditis and/or pericarditis, both symptomatic and asymptomatic, following live vaccine smallpox vaccination is highest among vaccinia naïve individuals and occurs at a rate of approximately 1 case per 150 primary vaccinations. Such complications may result in severe disability, permanent neurological sequelae, and/or death. Serious complications and death have also been reported in unvaccinated contacts of individuals who have been vaccinated.

Ischemic cardiac events, including fatalities, have been reported following smallpox vaccination; the relationship of these events, if any, to vaccination has not been established. In addition, cases of non-ischemic, dilated cardiomyopathy have been reported following smallpox vaccination; the relationship of these cases to smallpox vaccination is unknown.

Accidental infection of the eye (ocular vaccinia) may result in ocular complications including keratitis, corneal scarring and blindness.

Persons with eczema of any description such as, atopic dermatitis, neurodermatitis, and other eczematous conditions, regardless of severity of the condition, or persons who have a history of these conditions at any time in the past, are at higher risk of developing eczema vaccinatum. Vaccinees with close contacts who have eczematous conditions, may be at increased risk because live vaccinia virus can shed and be transmitted to these close contacts.

The risk of serious adverse events following vaccination with live vaccinia virus, e.g., encephalitis, is higher in infants.

Live vaccinia virus vaccines can cause fetal harm when administered to a pregnant woman. Congenital infection, principally occurring during the first trimester, was rarely associated after vaccination with live vaccinia smallpox vaccines. Generalized vaccinia of the fetus, early delivery of a stillborn infant, or a high risk of perinatal death have been reported.

Risk minimization activities such as vaccinee and provider education on vaccination site care, dressing changes, hand washing and other measures to prevent vaccinia transmission and autoinoculation are extremely important. Such activities, when incorporated into a smallpox vaccination program can significantly reduce the occurrence of serious adverse events.

Basis for Licensure

There is no generally accepted serological correlate of protection against smallpox. The most reliable indicator for protection against smallpox is the generation of the major cutaneous reaction, or “take,” after inoculation with vaccinia. The incidence of lesion formation, or “take rate,” was calculated for Dryvax® and *ACAM2000* in both vaccinia naïve and previously vaccinated individuals. The second measure of the immune response was the quantification of anti-viral activity, in tissue culture, using the Plaque Reduction Neutralization Test (PRNT). The Geometric Mean Titer (GMT) of the PRNT was calculated from both vaccinia naïve and previously vaccinated volunteers inoculated with *ACAM2000* or Dryvax®.

The efficacy of *ACAM2000* would be inferred by demonstration of non-inferiority to Dryvax® vaccinia-naïve and previously vaccinated adults. The pivotal clinical trials had two primary endpoints in each population:

- The percentage of patients that demonstrate the formation of the major cutaneous reaction or “take rate.”
- The GMT of the PRNT.

Regulatory Timeline

January 2006 initial component of “rolling” BLA submitted.

August 2006 BLA submission complete

January 2007 CBER Complete Response Letter issued informing sponsor 



February 2007 Response to Complete Response letter received.

3.0 Clinical Overview

A summary table of all *ACAM2000* clinical trials can be found in the Appendix (Table 1a). The Phase 3 clinical trials, H-400-009 and H-400-012, are summarized and discussed below.

Summary of Clinical Trials

Clinical Trial *ACAM2000* H-400-009 “The Safety, Tolerability, and Immunogenicity of *ACAM2000* Smallpox Vaccine in Adults without Previous Smallpox Vaccinations.”

Objective/Rationale H-400-009:

The safety of *ACAM2000* and Dryvax® vaccines was compared in healthy adults 18 to 30 years of age without a history of prior smallpox vaccination. Safety was evaluated by examination of the local cutaneous reaction, collection of adverse events, physical exams, and laboratory analysis.

The immune response of *ACAM2000* and Dryvax® vaccines was compared in healthy adults 18 to 30 years of age without a history of prior smallpox vaccination. Immune response was evaluated by comparing the proportion of subjects in each treatment group who developed a successful vaccination as determined by a major cutaneous reaction, i.e., *take rate*, and the proportion of subjects in each treatment group who developed neutralizing antibodies and the geometric mean vaccinia neutralizing antibody titer on Day 30.

Design Overview:

The clinical trial was a randomized, double-blind, controlled, and multi-center study in which subjects 18-30 years of age (inclusive) who were naïve to smallpox vaccine were to be randomized 3:1 to receive either *ACAM2000* or Dryvax®.

Inclusion/Exclusion Criteria

Healthy adults 18-30 years of age who had no history of smallpox vaccination (were not previously vaccinated) were included. In particular, subjects were excluded if they had 3 or more risk factors for coronary artery disease, had a history of palpitations or abnormalities of cardiac rhythm, or had an ECG pattern that would have complicated the recognition of new changes due to pericarditis or myocarditis.

Endpoints

Efficacy:

The co-primary efficacy end-points were: 1) The proportion of subjects in the Efficacy Evaluable (EE) population with a successful vaccination, based on a major cutaneous reaction [defined as a pustular vesicular, or ulcerative central lesion of measurable size assessed on Day 7 or Day 10 (i.e., during the interval Day 6-11)], and 2) the Geometric mean neutralizing antibody titer (GMT) on Day 30.

Safety:

The safety of *ACAM2000* was assessed by documentation of adverse events, physical examination findings, electrocardiogram findings, lymph node assessments, and measurements of vital signs and cardiac troponin I. Subjects with dermatologic, neurological, and potential cardiac adverse events were to have additional evaluations performed as specified in the applicable algorithms in the clinical study protocol. Subject diaries were maintained and structured interviews conducted to facilitate accurate collection of adverse events. Safety evaluations were to be performed for the safety population, defined as all subjects who received vaccination.

1. Vital signs were taken at the screening visit and on days 1 (30 minutes before and after vaccination) and 31. Subjects were given an oral thermometer and diary card for home monitoring. ECGs and troponin I were done at the screening visit.
2. Subjects were required to return to clinic on Days 7, 10, 21, 30 for assessments of vaccination site, limited exam and history, dressing changes and care instructions, temperature, blood and urine specimens, regional lymphadenopathy. ECG and troponin I tests were done on Day 10 and Day 21.
3. Telephone interviews were made after Day 15 for subjects who had not yet developed a dry scab.
4. Subjects were examined on Day 30 for: limited physical examination and history, vaccination site exam, regional lymph node exam, temperature, blood sample for neutralizing antibody and T cell assays. Urine pregnancy for females.
5. A telephone interview was scheduled at 6 months post vaccination.

Take Rates:

The site of vaccination was to be examined prior to vaccination (Day 0) and on Study Days 3, 7, 10, 15, 21, and 30. During the examination on Day 10 and 30, a digital photograph was taken of the vaccination site and a copy placed with the subject's source documents. The photograph included a millimeter scale within the field of the photograph so that the dimensions of the vaccination site could be measured.

Statistical Considerations:

A test of non-inferiority of *ACAM2000* to Dryvax® intended to rule out a greater than 5% margin of superiority of Dryvax® for successful primary vaccination was used. The significance level for detecting non-inferiority of *ACAM2000* to Dryvax® was to be one-sided at an alpha-level of 0.025. The rates of successful vaccination were to be analyzed for non-inferiority of *ACAM2000* to Dryvax® through the use of a lower bound 97.5%, one-sided confidence interval on the difference in rates of response between *ACAM2000* and Dryvax®.

Analysis of GMT was to be performed using a test of non-inferiority of neutralizing antibody titer between *ACAM2000* and Dryvax®, intending to ensure that the ratio of the GMTs of *ACAM2000*: Dryvax® was at least 0.5 (equivalent to the difference of the log₁₀ (GMT) being at least - 0.301). The one-sided, 97.5% lower bound of the confidence interval on the difference of the mean log₁₀ (GMT) between *ACAM2000* and Dryvax® was to be calculated using results from analysis of variance on the log-transformed neutralizing titer at Day 30 (±3

days). If this lower bound was at least - 0.301 (log base10 of 0.5), non-inferiority was to be established.

Results

Subject Demographics:

ACAM2000 and Dryvax® groups were well balanced with regard to demographic and baseline characteristics. Overall, the majority of subjects were male [65% (667 of 1037); 65% (508 of 780) of subjects in the *ACAM2000* group, and 62% (159 of 257) of subjects in the Dryvax® group]. Furthermore, the majority were Caucasian [74% (793 of 1037) of all subjects; 78% (606 of 780) of subjects in the *ACAM2000* group and 73% (187 of 257) of subjects in the Dryvax® group]. Of the remaining subjects, 12% were African-American, 10% were Hispanic, and 1% of subjects were Asian. Two percent (25 of 1162 subjects) of subjects were of an “other” race. The mean age was 23 years. No subject who was randomly selected for antibody testing was seropositive for vaccinia (i.e., neutralizing antibody titer ≥ 10) at Baseline. A table summarizing subject disposition can be found in the appendix (Table 2a)

Efficacy: H-400-009

Table 2 Vaccination Success (Take) Rates Trial H-400-009

Population / Vaccination success	Statistic	<i>ACAM2000</i>	Dryvax®
EE Population	N	776	257
Yes	n (%)	747 (96)	255 (99)
No	n (%)	29 (4)	2 (<1)
	Lower bound 97.5% CI on rate difference	-4.67%	

Non-inferiority criteria were intended to rule out a greater than 5% margin of superiority of Dryvax®. The significance level was one-sided at an α -level of 0.025. The rates of successful vaccination were analyzed using a 97.5%, one-sided confidence interval (CI) on the difference in rates of response between *ACAM2000* and Dryvax®. Non-inferiority was declared if the lower bound of the 1-sided 97.5% CI for the difference exceeded 0.5%.

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

As shown in Table 2, *ACAM2000* was shown to be non-inferior to Dryvax® with regard to vaccination success rates among subjects in the EE population, as indicated by the lower bound of the 1-sided 97.5% confidence interval on the difference between *ACAM2000* and Dryvax® of - 4.67%. Successful vaccination rates were 96% (747 of 776 subjects) and 99% (255 of 257 subjects) in the *ACAM2000* and Dryvax® groups, respectively. Findings were similar in the ITT population, in which *ACAM2000* was shown to be non-inferior to Dryvax® with regard to vaccination success rates as indicated by the lower bound of the 97.5% 1-sided confidence interval on the difference between *ACAM2000* and Dryvax® of - 4.52%.

There were no indications that *take rates* were different based on gender or race; however, the study was not powered to detect differences in the subpopulations. Data on *take rates* based on gender and race can be found in Tables 3a and 4a in the Appendix.

Table 3 Geometric Mean Antibody Titers at Baseline and on Day 30 per Vaccine Group: H-400-009

Parameter / Statistic	ACAM2000 (N=565)	Dryvax® (n=190)
PRNT Titer at Baseline (set for analysis)	5	5
PRNT Titer on Day 30	166	255
Log ₁₀ GMT	2.2	2.4
Lower bound 97.5% CI on difference in mean log ₁₀ (GMT)	-0.307	

Non-inferiority criteria were met if the ratio of the GMTs of ACAM2000:Dryvax® was at least 0.5. If the one-sided, 97.5% lower bound of the CI on the difference of the mean log₁₀ GMT between ACAM2000 and Dryvax® was at least -0.301 (log₁₀ of 0.5), non-inferiority was established.

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

As shown in Table 3, geometric mean neutralizing antibody titers of 166 and 255 were seen on Day 30 in the ACAM2000 and Dryvax® groups, respectively. Although the geometric mean neutralizing antibody titer was only ~1.5-fold higher in the Dryvax® group than in the ACAM2000 group, the geometric mean neutralizing antibody titer in the ACAM2000 group cannot be considered non-inferior to that in the Dryvax® group, as indicated by the lower bound of the 97.5% 1-sided confidence interval on the difference between ACAM2000 and Dryvax® of - 0.307. (A lower bound of ≥ -0.301 was required to establish non-inferiority; thus, ACAM2000 missed the requirement for non-inferiority to Dryvax® by a small margin.)

Safety: H-400-009

No significant difference between the ACAM2000 and Dryvax® groups was seen with regard to the overall incidence of treatment-emergent adverse events. The overall incidence of adverse events in the ACAM2000 and Dryvax® groups was 99% (864 of 873 subjects) and > 99% (288 of 289 subjects), respectively. Table 4 below summarizes the treatment-emergent adverse events reported by > 5% of subjects in each vaccine group.

Table 4 Treatment-emergent Adverse Events Reported by > 5% of subjects in Any Treatment Group

MedDRA SOC / Preferred Term	ACAM2000 (n=873) n (%)	Dryvax® (n=289) n (%)
At least 1 adverse event	864 (99)	288 (>99)
Blood and lymphatic system disorders	515 (59)	204 (74)
Lymph node pain	494 (57)	199 (69)
Lymphadenopathy	72 (8)	35 (12)
Gastrointestinal disorders	273 (31)	91 (31)
Nausea	170 (19)	65 (22)
Diarrhoea	144 (16)	34 (12)
Constipation	49 (6)	9 (3)
General disorders and admin. site conditions	850 (97)	288 (>99)
Injection site pruritus	804 (92)	277 (96)
Injection site erythema	649 (74)	229 (79)
Injection site pain	582 (67)	208 (72)
Fatigue	423 (48)	161 (56)
Injection site swelling	422 (48)	165 (57)
Malaise	327 (37)	122 (42)
Feeling hot	276 (32)	97 (34)
Rigors	185 (21)	66 (23)
Exercise tolerance decreased	98 (11)	35 (12)
Musculoskeletal and connective tissue disorders	418 (48)	153 (53)
Myalgia	404 (46)	147 (51)
Nervous system disorders	444 (51)	151 (52)
Headache	433 (50)	150 (52)
Respiratory, thoracic, and mediastinal disorders	134 (15)	40 (14)
Dyspnoea	39 (4)	16 (6)
Skin and subcutaneous tissue disorders	288 (33)	103 (36)
Erythema	190 (22)	69 (24)
Rash	94 (11)	30 (10)

Source: Table adapted from Acambis, Inc. BLA 125158 Original Submission Clinical Study Report H-400-009 Table 31 Page 124

Among the 873 subjects in the *ACAM2000* group, the most commonly reported adverse events included injection site pruritus (804 subjects; 92%), injection site erythema (649 subjects; 74%), injection site pain (582 subjects; 67%), lymph node pain (494 subjects; 57%), and headache (433 subjects; 50%). These events also were among the most commonly reported adverse events in the Dryvax® group.

Among the 289 subjects in the Dryvax® group, the most commonly reported adverse events were injection site pruritus (277 subjects; 96%), injection site erythema (229 subjects; 79%), injection site pain (208 subjects; 72%), lymph node pain (199 subjects; 69%), and injection site swelling (165 subjects; 57%). Of these events, there was a significant difference was seen between the *ACAM2000* and Dryvax® groups with regard to the incidence of injection site pruritus, lymph node pain, fatigue, and injection site swelling, with a significantly higher incidence of each event seen in the Dryvax® group than in the *ACAM2000* group.

Overall, a low incidence (1%) of other serious adverse events was reported, with 7 (1%) of 873 subjects in the *ACAM2000* group and 4 (1%) of 289 subjects in the Dryvax® group reporting at least 1 serious adverse event during the study. Overall, the most commonly reported serious adverse event was myocarditis / myopericarditis, which was identified in a total of 8 subjects, 5

(0.57%) of 873 subjects in the *ACAM2000* group and 3 (1.04%) of 289 subjects in the Dryvax® group.

Other serious events and adverse events reported in the *ACAM2000* group included pregnancy, appendicitis, and somatization disorder, each of which was reported for 1 subject. In the Dryvax® group, the only other serious adverse event reported was urticaria.

No other significant vaccination complications historically associated with smallpox vaccination, including ocular vaccinia, generalized vaccinia, postvaccinal encephalitis, progressive vaccinia, erythema multiforme, or eczema vaccinatum, were reported.

A total of 11 subjects [7 (<1%) of 873 subjects in the *ACAM2000* group and 4 (1%) of 289 subjects in the Dryvax® group] experienced a serious adverse event during the study, summarized in Table 5 below; the difference between groups was not significant in either subject population.

Table 5 Subjects with Serious Adverse Events by Treatment Group

Subject Number	Serious Adverse Event Term		Day of Onset	Intensity	Relationship to Vaccine	Outcome
	Verbatim	Preferred				
ACAM2000, Naïve Subjects						
023-109 ¹	Subclinical myocarditis (suspected)	Myocarditis	9	Moderate	Probable	Resolved
032-106	Pregnancy	Pregnancy	9	Moderate	Definitely not	Resolved
035-109	Acute appendicitis	Appendicitis	4	Severe	Definitely not	Resolved
048-116 ¹	Acute myocarditis	Myocarditis	11	Moderate	Probable	Resolved w/ sequelae
048-125	Somatic transformation	Somatization disorder	PST ²	Severe	Definite	Resolved w/ sequelae
056-111 ¹	Subclinical myocarditis	Myocarditis	10	Mild	Definite	Resolved
080-112 ¹	Abnormal ECG changes	Electrocardiogram abnormal	9	Moderate	Possible	Resolved
094-114 ¹	Cardiac enzyme abnormalities	Cardiac enzymes increased	9	Mild	Probable	Resolved
Dryvax®, Naïve Subjects						
004-103 ¹	Suspected subclinical myocarditis	Myocarditis	20	Mild	Probable	Resolved
021-102	Urticarial rash	Urticaria	8	Mild	Possible	Resolved
054-106 ¹	Myopericarditis	Myopericarditis	11	Severe	Definite	Resolved
065-137 ¹	Probable subclinical myocarditis	Myocarditis	9	Mild	Probable	Resolved

Source: Table from Acambis, Inc. BLA 125158 Original Submission Clinical Study Report H-400-009 Table 46 Page 158

The most commonly reported serious adverse event was myocarditis / myopericarditis, which occurred at an incidence of <1% (5 of 873 subjects) and approximately 1% (3 of 289 subjects) in the *ACAM2000* and Dryvax® groups, respectively. All cases of myocarditis / myopericarditis were considered by the Investigator to be at least possibly related to study vaccine. Table 6 below summarizes the clinical information of the cases of myocarditis.

Table 6

Subjects with Treatment-emergent Myocarditis / Myopericarditis, by Treatment Group and Study

Subject No.	Sex / Race / Age (years)	Notable Medical History	Adverse Event Verbatim Term / MedDRA Preferred Term / Cardiac Expert Panel Classification	Clinical Signs	ECG Findings	Laboratory Test Results	Day of Onset	Intensity	Outcome
ACAM2000									
Study H-400-002									
2065	Male Caucasian 18 years	None	Non-specific T wave abnormality with subsequent non-specific ST and T wave changes Electrocardiogram abnormal Subclinical myocarditis ¹	None reported	Mild T wave flattening with very slight elevation of ST segment in leads I and aVL consistent with but not diagnostic of myocarditis.	Troponin and CPK normal	14	Mild	Resolved
Study H-400-005									
1238	Male Caucasian 18 years	None	Intermittent chest tightness Chest tightness Myocarditis	Chest tightness	T-wave inversion in Lead III and non-specific changes in Leads II and aVF	CPK elevated (400 IU/L; normal range 38 to 199 IU/L), troponin I elevated (3.8 ug/mL; normal range <0.5 ng/mL, CPK-MB fraction elevated (38%); and CPK-MB elevated (12%); normal range 0%)	10	Mild	Resolved
Study H-400-009									
023-190	Male Black 22 years	Current smoker	Subclinical myocarditis (suspected) Myocarditis Suspect subclinical myocarditis	None reported	Sinus arrhythmia, ST changes in anterior leads suggesting infarction	CPK elevated (344 U/L); troponin I and CK-MB normal	Day 9	Moderate	Resolved without sequelae
048-116	Male Caucasian 25 years	None	Acute myocarditis Myocarditis Probable myocarditis	Chest pain, exercise tolerance decreased	Sinus rhythm with sinus arrhythmia, minimal voltage criteria for LVH, ST elevation	Troponin I elevated (4.8 ng/mL), troponin T elevated (0.61 ng/mL), CK-MB elevated (36.6 ng/mL), CK-MB index elevated (8.9), CPK elevated (410 U/L)	Day 11	Moderate	Resolved with sequelae
056-111	Male Caucasian 24 years	None	Subclinical myocarditis Myocarditis Probable Subclinical Myocarditis	None reported	Elevated ST segments	Troponin I elevated (1.8 ng/mL)	Day 10	Mild	Resolved without sequelae
080-112	Male Hispanic 18 years	None	Abnormal ECG changes Electrocardiogram abnormal Probable myocarditis	Dyspnoea, palpitations	ST elevation suggesting lateral injury; inferior ST depression	Troponin I, CK-MB, and CPK normal	Day 8	Moderate	Resolved without sequelae
094-114	Male Caucasian 21 years	None	Cardiac enzyme abnormalities Cardiac enzymes increased Probable subclinical myocarditis	None reported	Sinus bradycardia with sinus arrhythmia and first degree AV block	Troponin I elevated (0.11 pg/mL)	Day 9	Mild	Resolved without sequelae
Dryvax®									
Study H-400-009									
004-103	Male Asian 20 years	None	Suspected subclinical myocarditis Myocarditis Suspect Myocarditis	None reported	ST and T wave changes	Troponin I normal; CPK and CK-MB not done	Day 20	Mild	Resolved without sequelae
054-106	Male Caucasian 23 years	None	Myopericarditis Myopericarditis Probable Myocarditis	Exercise tolerance decreased, palpitations, chest pain, dyspnoea	Abnormal ST-T wave elevation	Troponin I elevated (5.7 ug/mL); CK-MB normal	Day 11	Severe	Resolved without sequelae
065-137	Female Caucasian 21 years	Smoker, obesity; borderline high blood pressure; occasional sinus tachycardia	Probable subclinical myocarditis Myocarditis Probable myocarditis	None reported	No clinically significant findings	Troponin I elevated (3.2 ug/mL)	Day 9	Mild	Resolved without sequelae

Source: Table from Acambis, Inc. BLA 125158 Original Submission Clinical Study Report H-400-009 Table 43
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Nine of 10 subjects who experienced myocarditis / myopericarditis during the study were male. The mean age of subjects was 21 years, with a range of 18 to 24 years. Seven of these 10 subjects were Caucasian. Of the remaining 3 subjects, 1 each was Black, Asian, and Hispanic. No subject had a known history of cardiac disease. However, 2 subjects had at least 1 risk factor for ischemic coronary disease. Subject No. 023-190 (Study H-400-009, ACAM2000) was a current smoker, and Subject No. 065-137 (Study H-400-009, Dryvax®) was a current smoker and had borderline high blood pressure. In addition, this subject was obese (height 171 cm, weight 134 kg) and was receiving Depo Provera for birth control.

The mean time to onset of myocarditis / myopericarditis was 11 days, with a range of 9 to 20 days). 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, the exact time to onset of these events is unknown.

Two subjects, 1 subject who received *ACAM2000* (Subject No. 048-116, Study H-400-009) and 1 who received Dryvax® (Subject No. 054-106, Study H-400-009), who both experienced acute symptoms, were hospitalized and required concomitant treatment because of myocarditis.

Subject No. 048-116, a 23-year-old Caucasian male, presented to a local ER with signs and symptoms of acute myocardial infarction 11 days after vaccination, for which he was hospitalized. He had attended his scheduled study center visit earlier that day, during which the subject was asymptomatic but had ECG findings of inferior ST segment elevations. After presentation to the ER, the subject underwent emergency coronary angiography, and subsequently was diagnosed with acute myocarditis with decreased left ventricular systolic function without evidence of obstructive coronary disease. Treatment with Coreg and acetylsalicylic acid (ASA) were started. Three days later, the subject was considered stable, and he was discharged from the hospital.

Subject No. 054-106, a 23-year-old Caucasian male, experienced the acute onset of severe pressure and chest pain during the night, which woke him from sleep. The following day (11 days post-vaccination), he presented to the local ER with continued chest pain and diaphoresis. The subject initially received treatment with nitroglycerin, morphine, and ASA. The subject underwent cardiac catheterization, and a diagnosis of myopericarditis was made. Two days later, the subject was considered to be stable and was discharged from the hospital. Treatments for myopericarditis on discharge included Indocin and Vasotec.

The remaining 8 subjects were neither hospitalized nor treated with medications because of myocarditis / myopericarditis. For 8 of 10 subjects, the myocarditis event was considered resolved without sequelae at last follow-up.

For Subject No. 048-116 (*ACAM2000*, Study H-400-009), myocarditis was considered resolved with sequelae at last follow-up. As described above, this subject was hospitalized because of acute myocarditis for 3 days. Although the subject was considered to be stable at the time of discharge, the event was considered resolved with sequelae because he still exhibited ECG changes and was continuing treatment with Coreg and acetylsalicylic acid (ASA) at the time of discharge. Approximately 3 months later, the consulting cardiologist considered that the subject's heart function would return to normal. At 6- and 12-month follow-up, the subject was reported to have no clinical signs or symptoms of myocarditis.

Clinical Trial H-400-012: “The Safety, Tolerability, and Immunogenicity of *ACAM2000* Smallpox Vaccine in Adults with Previous Smallpox Vaccination.”

Objective/Rationale H-400-012:

The safety of *ACAM2000* and Dryvax® vaccines was compared in healthy adults ≥ 31 years of age with a history of prior smallpox vaccination. Safety was evaluated by examination of the local cutaneous reaction, adverse events, physical exams, and laboratory analysis.

The immune response of *ACAM2000* and Dryvax® vaccines was compared in healthy adults ≥ 31 years of age with a history of prior smallpox vaccination was evaluated by comparing the proportion of subjects in each treatment group who develop a successful vaccination, i.e., *take rate* and the proportion of subjects in each treatment group who develop neutralizing antibodies and the geometric mean vaccinia neutralizing antibody titer on Day 30.

Design Overview:

The clinical trial was a randomized, double-blind, controlled, multi-center study in which subjects ≥ 31 years of age (inclusive) who were previously vaccinated with smallpox vaccine were to be randomized 3:1 to receive either *ACAM2000* or Dryvax®.

Inclusion/Exclusion Criteria

Healthy adults ≥ 31 years of age who were previously vaccinated were included. In particular, subjects were excluded if they had 3 or more risk factors for coronary artery disease, had a history of palpitations or abnormalities of cardiac rhythm, or had an ECG pattern that would have complicated the recognition of new changes due to pericarditis or myocarditis.

Endpoints

Efficacy:

The co-primary efficacy end-points were the proportion of subjects in the Efficacy Evaluable (EE) population with a successful vaccination, based on a major cutaneous reaction [defined as a pustular vesicular, or ulcerative central lesion of measurable size assessed on Day 7 or Day 10 (i.e., during the interval Day 6-11)] and the geometric mean neutralizing antibody titer (GMT) on Day 30.

Safety:

The safety of study vaccine was assessed by documentation of adverse events, physical examination findings, electrocardiogram findings, lymph node assessments, and measurements of vital signs and cardiac troponin I. Subjects with dermatologic, neurological, and potential cardiac adverse events were to have additional evaluations performed as specified in the applicable algorithms in the clinical study protocol. Subject diaries were maintained and structured interviews conducted to facilitate accurate collection of adverse events. Safety evaluations were to be performed for the safety population, defined as all subjects who received vaccination.

1. Vital signs were taken at the screening visit and on days 1 (30 minutes before and after vaccination) and 31. Subjects were given an oral thermometer and diary card for home monitoring. ECGs and troponin I were done at the screening visit.
2. Subjects were required to return to clinic on Days 7, 10, 21, 30 for assessments of vaccination site, limited exam and history, dressing changes and care instructions, temperature, blood and urine specimens, regional lymphadenopathy. ECG and troponin I tests were done on Day 10 and Day 21.
3. Telephone interviews were made after Day 15 for subjects who had not yet developed a dry scab.
4. Subjects were examined on Day 30 for: limited physical examination and history, vaccination site exam, regional lymph node exam, temperature, blood sample for neutralizing antibody and T cell assays. Urine pregnancy for females.
5. A telephone interview was scheduled at 6 months post vaccination.

Take Rates:

The site of vaccination was to be examined prior to vaccination (Day 0) and on Study Days 3, 7, 10, 15, 21, and 30. During the examination on Day 10 and 30, a digital photograph was taken of the vaccination site and a copy placed with the subject's source documents. The photograph included a millimeter scale within the field of the photograph so that the dimensions of the vaccination site could be measured.

Statistical Considerations:

A test of non-inferiority of *ACAM2000* to Dryvax® intended to rule out a greater than 10% margin of superiority of Dryvax® for successful primary vaccination was used. The significance level for detecting non-inferiority of *ACAM2000* to Dryvax® was to be one-sided at an alpha-level of 0.025. The rates of successful vaccination were to be analyzed for non-inferiority of *ACAM2000* to Dryvax® through the use of a lower bound 97.5%, one-sided confidence interval on the difference in rates of response between *ACAM2000* and Dryvax®.

A greater margin of 10% was used in Trial H-400-012, compared with 5% in Trial H-400-009 because a lower rate of response was expected in subjects previously vaccinated.

Analysis of GMT was to be performed using a test of non-inferiority of neutralizing antibody titer between *ACAM2000* and Dryvax®, intending to ensure that the ratio of the GMTs of *ACAM2000*: Dryvax® was at least 0.5 (equivalent to the difference of the log₁₀ (GMT) being at least -0.301). The one-sided, 97.5% lower bound of the confidence interval on the difference of the mean log₁₀ (GMT) between *ACAM2000* and Dryvax® was to be calculated using results from analysis of variance on the log-transformed neutralizing titer at Day 30 (±3 days). If this lower bound was at least -0.301 (log base10 of 0.5), non-inferiority was to be established.

Results

Subject Demographics

The *ACAM2000* and Dryvax® groups were well balanced with regard to demographic and baseline characteristics. In the *ACAM2000* group, 612 (49%) subjects were male and 630

(51%) were female. In the Dryvax® group, 192 (47%) subjects were male and 213 (53%) were female. The majority of subjects, 1008 (81%) of 1242 subjects in the *ACAM2000* group and 325 (80%) of 405 subjects in the Dryvax® group, were Caucasian. Of the remaining subjects, 7% (110 of 1647 subjects) were African-American 9% (158 of 1647 subjects) were Hispanic, and 1% (14 of 1647 subjects) were Asian. Two percent of subjects (32 of 1647) were of an “other” race. All subjects were at least 31 years of age, with a range of 31 to 84 years and mean age of 49 years in the *ACAM2000* group and 50 years in the Dryvax® group.

Efficacy: H-400-012

Table 7 Vaccination Success (*Take*) Rates Trial H-400-012

Population / Vaccination success	Statistic	<i>ACAM2000</i> 1189	Dryvax® 388
EE Population			
Yes	n (%)	998 (84)	381 (98)
No	n (%)	191 (16)	7 (2)
	97.5% CI	-17	

Non-inferiority criteria was intended to rule out a greater than 10% margin of superiority of Dryvax®. The significance level was one-sided at an α -level of 0.025. The rates of successful vaccination were analyzed using a 97.5%, one-sided confidence interval (CI) on the difference in rates of response between *ACAM2000* and Dryvax®. Non-inferiority was declared if the lower bound of the 1-sided 97.5% CI for the difference exceeded 10%.

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

As shown in Table 7 *ACAM2000* was shown to be inferior to Dryvax® with regard to revaccination success rates, as indicated by a lower bound of the 1-sided 97.5% confidence interval that did not exceed -10% (actual value - 17.00%). Successful revaccination rates in the EE population, based on the IRC’s assessment, were 84% (998 of 1242 subjects) and 98% (381 of 405 subjects) in the *ACAM2000* and Dryvax® groups, respectively. Findings were similar for previously vaccinated subjects in the ITT population, in which *ACAM2000* was not shown to be non-inferior to Dryvax® with regard to revaccination success rates as indicated by a lower bound of the 1-sided 97.5% confidence interval on the difference between *ACAM2000* and Dryvax® that did not exceed -10% (actual value: -17%).

There were no indications that *take rates* were different based on gender or race; however, the study was not powered to detect differences in the subpopulations. Data on *take rates* based on gender and race can be found in Tables 6a and 7a in the Appendix.

Table 8 GMT's at baseline and on Day 30 Trial H-400-012

Parameter/statistic	ACAM2000 (N=734)	Dryvax® (N=376)
PRNT Titer at Baseline GMT	33	28
PRNT Titer on Day 30 GMT	286	445
Log ₁₀ GMT	2.46	2.65
Lower bound 97.5% CI on difference in mean log ₁₀ (GMT)	- 0.275	

Non-inferiority criteria were met if the ratio of the GMTs of ACAM2000:Dryvax® was at least 0.5. If the one-sided, 97.5% lower bound of the CI on the difference of the mean log₁₀ GMT between ACAM2000 and Dryvax® was at least -0.301 (log₁₀ of 0.5), non-inferiority was established.

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

As shown in Table 8, *ACAM2000* was shown to be non-inferior to Dryvax® with regard to geometric mean neutralizing antibody titer on Day 30, with geometric mean neutralizing antibody titers of 286 and 445 in the *ACAM2000* and Dryvax® groups, respectively, as indicated by the lower bound of the 97.5% confidence interval for the difference in log₁₀ titers that exceeded -0.301 (actual value -0.275).

Safety: H-400-012

The overall incidence of adverse events was significantly higher in the Dryvax® group than in the *ACAM2000* group ($p = 0.012$, derived by Fisher's Exact test). The overall incidence of adverse events in the *ACAM2000* and Dryvax® groups was 97% (1325 of 1371 subjects) and 99% (443 of 448 subjects), respectively. Table 9 below summarizes the treatment-emergent adverse events reported by >5% of subjects in each vaccine group.

Table 9

Treatment-emergent Adverse Events Reported by ≥5% of Subjects in Any Treatment Group, by Subject Population and Treatment Group

MedDRA SOC / Preferred Term	ACAM2000 (n=1371) n (%)	Dryvax® (n=448) n (%)
At least 1 adverse event	1325 (97)	443 (99)
Blood and lymphatic system disorders	302 (22)	133 (30)
Lymph node pain	261 (19)	119 (27)
Lymphadenopathy	78 (6)	29 (6)
Gastrointestinal disorders	314 (23)	137 (31)
Nausea	142 (10)	63 (14)
Diarrhoea	158 (12)	77 (17)
Constipation	88 (6)	31 (7)
General disorders and admin. site conditions	1280 (93)	434 (97)
Injection site pruritus	1130 (82)	416 (93)
Injection site erythema	841 (61)	324 (72)
Injection site pain	505 (37)	209 (47)
Fatigue	468 (34)	184 (41)
Injection site swelling	384 (28)	188 (42)
Malaise	381 (28)	147 (33)
Feeling hot	271 (20)	114 (25)
Rigors	171 (12)	76 (17)
Exercise tolerance decreased	105 (8)	50 (11)
Musculoskeletal and connective tissue disorders	418 (30)	160 (36)
Myalgia	374 (27)	148 (33)
Nervous system disorders	453 (33)	174 (39)
Headache	437 (32)	166 (37)
Respiratory, thoracic, and mediastinal disorders	127 (9)	42 (9)
Dyspnoea	41 (3)	18 (4)
Skin and subcutaneous tissue disorders	425 (31)	139 (31)
Erythema	329 (24)	107 (24)
Rash	80 (6)	29 (6)

Source: Table adapted from Acambis, Inc. BLA 125158 Original Submission Clinical Study Report H-400-012 Table 36 Page 131

Among the 1371 subjects in the *ACAM2000* group, the most commonly reported adverse events included injection site pruritus (1130 subjects; 82%), injection site erythema (841 subjects; 61%), injection site pain (505 subjects; 37%), fatigue (468 subjects; 34%), and headache (437 subjects; 32%). These events also were among the most commonly reported adverse events in the Dryvax® group.

Among the 448 subjects in the Dryvax® group, the most commonly reported adverse events were injection site pruritus (416 subjects; 93%), injection site erythema (324 subjects; 72%), injection site pain (209 subjects; 47%), injection site swelling (188 subjects; 42%), and fatigue (184 subjects; 41%).

A total of 7 subjects [4 (<1%) of 1371 subjects in the *ACAM2000* group and 3 (<1%) of 448 subjects in the Dryvax® group] experienced a serious adverse event during the study; the difference between groups was not significant. Subjects experiencing serious adverse events during or after study participation are summarized in the table below.

Table 10**Subjects with Serious Adverse Events, by Treatment Group and Subject Population**

Subject Number	Serious Adverse Event Term		Day of Onset	Intensity	Relationship to Vaccine	Outcome
	Verbatim	Preferred				
ACAM2000, Previously Vaccinated Subjects						
033-516	Chest pain	Chest pain	35	Severe	Possibly	Resolved
045-511	Appendicitis	Appendicitis	8	Severe	Definitely not	Resolved
065-557	Atrial fibrillation	Atrial fibrillation	31	Mild	Possibly	Resolved w/ sequelae
097-544	Chest pressure	Chest discomfort	3	Mild	Possibly	Resolved
Dryvax®, Previously Vaccinated Subjects						
030-509	Coronary artery disease	Coronary artery disease	9	Severe	Definitely not	Resolved
041-503	Generalized vaccinia	Cow pox	9	Moderate	Definitely	Resolved
079-555	Allergic reaction	Hypersensitivity	0	Moderate	Possibly	Resolved

Source: Table from Acambis, Inc. BLA 125158 Original Submission Clinical Study Report H-400-012 Table 44 Page 151

Table 10 summarizes the serious adverse events that were reported in both vaccine groups. Appendicitis was reported as a serious adverse event for 1 subject in the *ACAM2000* group. Subject No. 045-511, a 42-year-old Caucasian female, presented with significant abdominal pain on Day 9 and was admitted to the hospital with a diagnosis of appendicitis. The following day, she underwent an appendectomy and was discharged later that evening. For both subjects, the event was considered by the Investigator to be unrelated to study vaccine.

Among the 448 subjects in the Dryvax® group, serious adverse events reported included coronary artery disease (CAD), cowpox (generalized vaccinia), and hypersensitivity. CAD was considered by the Investigator to be unrelated to study vaccine; the remaining events were considered to be related.

Subject No. 041-503, a 34-year-old Caucasian male, developed generalized vaccinia during the study that was considered by the Investigator to be definitely related to study vaccine. This subject, who developed a major cutaneous reaction by Day 7, reported at his scheduled study center visit on Day 10 that he had been experiencing pain, redness, itching, and swelling at the vaccination site for the past day. He also complained of lower back and left-sided chest pain. He reported malaise and a fever of 101°F the previous evening, but was afebrile at the time of the visit. It was noted that the central lesion at the vaccination site measured 110 mm by 90 mm. A satellite lesion measuring 50 mm by 30 mm, and 12 distinct “erythematous pustules,” all of which were at a similar stage of maturity, also were noted. The majority of the pustules were on the subject’s back, with several more on the proximal left upper extremity (LUE) and two on the anterior chest. The subject was admitted to a local hospital that day for observation, and a dermatological consultant determined that symptoms were consistent with generalized vaccinia (MedDRA preferred term “cow pox”). The subject was treated with diphenhydramine and was discharged from the hospital the following day. The event, which was assessed by the Investigator as moderate in intensity and study-vaccine related, resolved without sequelae on Day 13.

Subject No. 079-555 experienced fever and swelling, pain, and redness of the left upper extremity on the day of vaccination, for which he was seen in the ER. He was diagnosed with possible cellulitis and was hospitalized because of this event. A venous duplex scan of the upper arm revealed focal subacute non-occlusive thrombosis of the left basilic vein in the upper arm and of the cephalic vein in the forearm; no evidence of acute deep vein thrombosis was seen. The subject was discharged from the hospital 2 days later, and the event was considered resolved without sequelae 10 days thereafter. The hypersensitivity event was considered by the Investigator to be moderate in intensity and possibly related to study vaccine.

Serious Cardiac Events H-400-012

Subject No. 033-516 (*ACAM2000*), a 62-year-old Caucasian female with a history of hypercholesterolemia, experienced severe chest pain 35 days after study vaccine administration. On Day 5, this subject experienced an initial episode of chest pain, which resolved spontaneously. Routine ECG findings on Day 12 were clinically insignificant. The subject did not experience any subsequent episodes of chest pain within 30 days post-vaccination. On Day 35, the subject awakened with sharp, retrosternal chest pain. The pain continued for approximately one and a half hours and resolved after treatment with a nitroglycerin spray. The subject was brought to the ER and was admitted to the hospital for further evaluation to rule out myocardial infarction. ECG findings revealed trace aortic insufficiency and normal left ventricular systolic function. CPK was within normal range in 3 sequential tests, with values of 86, 80, and 75 U/L (normal range 26 to 174 U/L). Troponin I was slightly abnormal, with three sequential tests showing levels of <0.04, 0.10, and <0.04 ng/mL (normal range 0.04 to 0.80 ng/mL). Chest pain resolved the following day. The chest pain was considered by the Investigator to be severe in intensity and possibly related to study vaccine.

Subject No. 065-557 (*ACAM2000*), a 56-year-old Caucasian male with a medical history notable for hypertension, experienced atrial fibrillation starting 31 days after study vaccine administration. At that time, the subject noticed an uncomfortable feeling in his chest and rapid heart action. He took his vital signs and noted a pulse of 126 bpm, for which he went to the hospital. On admission, the subject was determined to have atrial fibrillation with a rapid ventricular rate of 140 bpm. Treatment with diltiazem, Lovenox, warfarin, alprazolam, amiodarone, and simvastatin was started, and the event resolved 3 days later. Although the event was considered resolved, the subject remained hospitalized for additional diagnostic testing. Three days later, a chest computed tomography (CT) scan revealed pleural and diaphragmatic calcifications consistent with asbestosis. That same day, the subject was discharged from the hospital; treatment upon discharge included warfarin. Atrial fibrillation was assessed by the Investigator as mild in intensity and possibly related to study vaccine.

Subject No. 097-544 (*ACAM2000*), a 47-year-old Caucasian female, reported at her Day 7 study visit that she had experienced chest pressure beginning 3 days after study vaccine administration. She reported no associated symptoms or pertinent medical history. The subject was evaluated according to the cardiac algorithm in the study protocol. She was referred to a cardiologist and hospitalized for evaluation. No evidence of cardiac dysfunction or another cause of her chest pressure was found. Stress treadmill, chest x-ray, ECG,

echocardiogram, and cardiac enzymes all were normal. The Investigator considered the event to be possibly study-vaccine related. The event was considered to have resolved without sequelae 4 days after onset.

Subject No. 030-509 (Dryvax®), a 76-year-old Caucasian male, experienced chest pain while exercising 9 days after study vaccination. He was referred to a cardiologist and hospitalized 4 days later after another episode of chest pain with exercise. Cardiac catheterization revealed severe CAD and angioplasty and stenting of the right coronary artery was performed. The subject was released from the hospital after 2 days and the event was considered resolved at that time. The CAD was considered by the Investigator to be definitely not related to study vaccine.

4.0 Efficacy Summary/Conclusion

ACAM2000 met two of the four primary endpoint criteria established for the phase 3 clinical trials. The primary determinant for an effective immune response in those naïve to vaccine is a major cutaneous reaction. *ACAM2000* was non-inferior to Dryvax® in clinical trial H-400-009 with regard to eliciting a major cutaneous reaction. The measure of the strength of the generated antibody response was similar but did not meet the predefined criterion for non-inferiority. The percentage vaccines developing a major cutaneous response after revaccination with vaccinia-based smallpox vaccines may not provide an accurate measure of the strength of the immune response since the pre-existing immunity modifies the scope of the cutaneous response. A more informative measure of the immune response in persons previously vaccinated may be the strength of the neutralizing immune response, as measured by the plaque reduction neutralization test (PRNT). In clinical trial H-400-012 *ACAM2000* was non-inferior to Dryvax® with regard to the strength of the immune response (GMT of the PRNT). Therefore, *ACAM2000* was non-inferior to Dryvax® in what may be the two most important measurements of efficacy: the rate of the major cutaneous reaction in those naïve to the vaccine, and the strength of the immune response in those previously exposed to vaccinia-based smallpox vaccines.

5.0 Risk Management

Distribution of Product

Acambis, Inc. has stated to CBER that the company has no plan or intention to distribute *ACAM2000* in the United States outside of sales to the US Government for the Strategic National Stockpile.

Labeling

CBER recommends that a Black Box Warning be incorporated into the package insert as it is with another previously licensed live vaccinia virus smallpox vaccine. The Black Box Warning will describe the risk for acute myopericarditis as well as the other known serious adverse effects of live vaccinia virus vaccine.

Warnings and Precautions section will include information about potential serious adverse events with vaccinia virus, including the risk of these events occurring in unvaccinated close contacts of the vaccine. For women of childbearing potential, this product will have labeling that states it is Pregnancy Category D, and vaccinees that live in the same household with or

have close contact with a pregnant woman will be apprised of the potential hazard. Information on how to report to the National Smallpox Vaccine in Pregnancy Registry is provided in the label. Dose and Administration section will have steps recommended to avoid transmission/autoinoculation with proper care of the vaccination site, hand washing, and proper disposal of wound dressings and potentially contaminate materials.

Information on the availability of Vaccinia Immune Globulin (VIG) for the management of certain complication of smallpox vaccination is provided in the label.

Medication Guides and Provider Education

A Medication Guide should be provided to *ACAM2000* vaccinees to provide information on the care of the vaccination site, how to minimize autoinoculation and vaccinia virus transmission, and potential serious adverse events. The Medication Guide should be updated periodically to incorporate new information on the risk and nature of myocarditis and other serious adverse effects as learned in post-licensure surveillance program.

Provider education is needed to ensure health care workers administering the vaccine take steps to communicate warnings and precautions to vaccinees to ensure that compliance with instructions, such as, avoiding close contact with susceptible high risk non-vaccinees, and proper vaccination site care is maintained. Providers must ensure that vaccinees are fully informed of the risks and benefits and the steps needed to minimize risks.

Post-Licensure Pharmacovigilance Plan

Acambis, Inc. proposes the following post-licensure pharmacovigilance activities:

1. Phase 4 Study

A Phase 4 prospective cohort study is proposed to assess the risk and severity of myocarditis and to assess the risk of superinfection, contact transmission, autoinoculation and serious rash. The size of the cohort will be 10,000 participants who are naïve to previous smallpox. It is anticipated that it will detect 2.7 to 5.4 cases per 1000 vaccinations, or 27 to 54 cases myocarditis. Follow-up at 10 days after vaccination to solicit symptoms of possible cardiac or other adverse events through structured interview and to test serum cardiac troponin I levels. ECGs may be performed if there is clinical indication and will be required if there is an elevated troponin level. Participants will be instructed to report any cardiac-related symptoms or other serious adverse events within 21 days after vaccination. They will be provided with a toll free number and/or a website for such reporting.

2. Myocarditis Registry

A registry for cases of myocarditis following Dryvax® smallpox vaccination currently exists and is conducted by the DoD Vaccine Health Care Centers (VHC). Follow-up beyond 2 years has been incomplete and difficult in part because many patients have been discharged from the DoD and cannot access care. Acambis, Inc. proposes a supplement to the VHC registry. An estimated 27 to 54 cases of myocarditis will be identified in the Phase 4 cohort study and an additional 50 cases may be identified with enhanced routine surveillance for the registry. A follow-up visit with a cardiologist, preferably in a DoD military treatment facility (MTF), or at a VA clinic (if feasible), will be scheduled for between 3 and 6 months post diagnosis. A

cardiac evaluation will be scheduled for 6 months subsequent to the initial follow-up visit. A yearly ECG will be performed as a standardized assessment of functional capacity. Cases will be followed for at least 2 years after onset of disease unless continuing cardiac abnormalities are found. In this case, follow-up will be attempted for up to 5 years. Acambis, Inc. will facilitate follow-up of any myocarditis cases if they are discharged from DoD service and cannot access an MTF or the VA system. Acambis, Inc. will also cover follow-up expenses incurred by subjects with continuing abnormalities who are followed beyond 2 years. Annual registry status and patient status reports will be prepared using descriptive summary statistics and line listings and delivered to the *ACAM2000* Smallpox Vaccine Safety Board and PVG Steering Committees, as well as FDA.

3. Enhanced Surveillance Following *ACAM2000* Vaccination

Enhanced surveillance is proposed for at least the first year of use of *ACAM2000* in the DoD. It is estimated that there will be 100,000 to 200,000 recipients of *ACAM2000* vaccine in this time period. Enhanced surveillance will focus on all serious adverse events including serious cardiac and skin events. Additionally it will seek reports of pregnancy exposures, HIV-infected exposures, autoinoculation, superinfection, and contact transmission. All health care facilities administering the vaccine will receive mailings and materials in addition to the *ACAM2000* package insert. Acambis, Inc. will work with DoD clinicians to develop materials to supplement the existing materials distributed within the DoD. These educational materials will describe the need to immunize only appropriate patients and to be vigilant for adverse events occurring in the 2-4 week period following vaccination. These materials will emphasize skin reactions and signs and symptoms of cardiac adverse events. Materials aimed at vaccine recipients will also be distributed. In all the educational materials, the need to be sensitive to detection, potential attribution and reporting will be emphasized. Reporters will be asked to report via a customized reporting mechanism, such as the VOXIVA™ DoD Vaccine Monitoring System (<http://www.voxiva.net/safevax.asp>). This system has been used effectively in a pilot study involving 1400 DoD subjects after smallpox vaccination. Each vaccinee will report reaction data via a simple-to-use telephone or internet based monitoring system. If vaccinees don't report after smallpox vaccination, they will receive a reminder call (when feasible) from a call center operator who can help them submit their data.

Pregnancy Registry

CDC, in collaboration with the Department of Defense (DoD) and the Food and Drug Administration (FDA), has established the National Smallpox Vaccine in Pregnancy Registry. This registry will also be used to track pregnant women inadvertently exposed to *ACAM2000* Smallpox Vaccine.

Risk Minimization Action Plan (RiskMAP)

CBER has requested Acambis, Inc. to submit to FDA a formal RiskMap, with an obligation to provide FDA reports as described under 21 CFR 600.80 on an annual basis, or more frequently if agreed to. These reports will describe and evaluate how each element of the program has been implemented, provide implementation data, describe and evaluate the success of the risk management program in achieving program goals. In addition, the RiskMAP may include Phase 4 studies to further evaluate possible risk factors and potential minimization for serious adverse events (e.g., genetic biomarkers for myocarditis, etc.).

Appendix

Table 1a Summary ACAM2000 Clinical Studies

Study	Design	# Enrolled	Population
H-400-002, The Effect of ACAM1000, ACAM2000, and Dryvax® on Safety, Tolerability, and Immunogenic Response in Adults without Previous Smallpox Vaccination	Phase 1, single-center, randomized study of ACAM1000, ACAM2000, and Dryvax® smallpox vaccines	90 (30 each group)	18-29 years
H-400-008, The Safety, Tolerability, and Immunogenicity of ACAM2000 Smallpox Vaccine in Adults without Previous Smallpox Vaccination	Phase 1, single-center, open-label, single arm, fixed-dose study of ACAM2000 smallpox vaccine	100	18-29 years
H-400-003, The Effect of Dose on Safety, Tolerability, and Immunogenicity of ACAM2000 Smallpox Vaccine in Adults with Previous Smallpox Vaccination	Phase 2, multi-center, randomized, double-blind study of ACAM2000 and Dryvax® smallpox vaccines	357 (50 to ACAM2000 6.8-10 ⁷ , 102 each to ACAM2000 1.4-10 ⁷ and 6.8-10 ⁶ , 51 to ACAM2000 3.4-10 ⁶ , and 52 to Dryvax®)	Previously vaccinated > 28 years
H-400-005, The Effect of Dose on Safety, Tolerability, and Immunogenicity of ACAM2000 Smallpox Vaccine in Adults without Previous Smallpox Vaccination	Phase 2, multi-center, randomized, double-blind study of ACAM2000 and Dryvax® smallpox vaccines	353 (51 to ACAM2000 6.8-10 ⁷ , 101 to ACAM2000 1.4-10 ⁷ , 101 to ACAM2000 6.8-10 ⁶ , 51 to ACAM2000 3.4-10 ⁶ , and 49 to Dryvax®)	Naïve to smallpox vaccine, adults 18-29 yrs
H-400-009, The Safety, Tolerability, and Immunogenicity of ACAM2000 Smallpox Vaccine in Adults without Previous Smallpox Vaccine: A Randomized, Double-Blind, Fixed-Dose, Phase 3 Comparison Between ACAM2000 and Dryvax® Smallpox Vaccines	Phase 3, multi-center, double-blind, randomized study of ACAM2000 and Dryvax® smallpox vaccines	1162 (873 to ACAM2000 and 289 to Dryvax®)	Naïve to smallpox vaccine aged 18 to 30 years
H-400-012, The Safety, Tolerability, and Immunogenicity of ACAM2000 Smallpox Vaccine in Adults with Previous Smallpox Vaccine: A Randomized, Double-Blind, Fixed-Dose, Phase 3 Comparison Between ACAM2000 and Dryvax® Smallpox Vaccines	Phase 3, multi-center, double-blind, randomized study of ACAM2000 and Dryvax® smallpox vaccines	1819 (1371 ACAM2000 and 448 Dryvax®)	Previously vaccinated with smallpox vaccine aged >31 years

Source: Table adapted from Acambis, Inc. BLA 125158 Original Application 2.7.6 Synopses of Individual Studies

Table 2a Subject disposition Trial H-400-009

Subjects	ACAM2000	Dryvax®	Total
Screened			1744
Screen failures, n (%)			707 (41)
Enrolled	780	257	1037 (59)
Vaccinated	780 (100)	257 (100)	1037 (100)
In safety/ITT population, n (%)	780 (100)	257 (100)	1037 (100)
In EE population, n (%)	776 (99)	257 (100)	1033 (100)
In AnE population, n (%)	565 (72)	190 (74)	755 (73)

AnE=antibody evaluable; EE=efficacy evaluable; ITT=Intent-to-treat

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

Table 3a Vaccination Success (Take) Rates by Gender: H-400-009

Gender	ACAM2000 N Male = 505 N Female = 271	Dryvax N Male = 159 N Female = 98	95% CI on rate difference
Male	96%	100%	(-5.9%, -1.5%)
Female	96%	98%	(-5.2%, 3.5%)

Source: Data Acambis, Inc. BLA 125158 compiled by reviewer Efficacy Information Amendment February 20, 2007

Table 4a Vaccination Success (Take) Rates by Race: H-400-009

Race	ACAM2000 N Caucasian = 602 N Afr-American = 65 N Other = 109	Dryvax N Caucasian = 187 N Afr-American = 27 N Other = 43	95% CI on rate difference
Caucasian	97%	99%	(-4.9%, -0.5%)
Afr-American	94%	96%	(-12.3%, 12.9%)
Other	96%	100%	(-9.4%, 4.5%)

Source: Data Acambis, Inc. BLA 125158 compiled by reviewer Efficacy Information Amendment February 20, 2007

Table 5a Subject disposition Trial H-400-012:

Subjects	ACAM2000	Dryvax®	Total
Screened			2770
Screen failures, n (%)			1123 (41)
Enrolled	1242	405	1647 (59)
Vaccinated	1242 (100)	405 (100)	1647 (100)
In safety/ITT population, n (%)	1242 (100)	405 (100)	1674 (100)
In EE population, n (%)	1189 (96)	388 (96)	1577 (96)
In AnE population, n (%)	734 (59)	376 (93)	1110 (67)

AnE=antibody evaluable; EE=efficacy evaluable; ITT=Intent-to-treat

Source: Table adapted from Acambis, Inc. BLA 125158 Efficacy Information Amendment February 20, 2007

Table 6a Vaccination Success (*Take*) Rates by Gender Trial H-400-012

Gender	ACAM2000 N Male = 581 N Female = 608	Dryvax® N Male = 182 N Female = 206	95% CI on rate difference
Male	84%	98%	(- 18.3%, - 10.6%)
Female	84%	98%	(- 17.4%, - 9.8%)

Source: Data from Acambis, Inc. BLA 125158 compiled by reviewer Efficacy Information Amendment February 20, 2007

Table 7a Vaccination Success (*Take*) Rates by Race Trial H-400-012

Race	ACAM2000 N Caucasian = 966 N Afr-American = 73 N Other = 150	Dryvax® N Caucasian = 313 N Afr-American = 27 N Other = 48	95% CI on rate difference
Caucasian	83%	98%	(- 17.5% - 11.6%)
Afr-American	77%	96%	(- 31.8%, - 3.3%)
Other	91%	100%	(- 15.4%, - 1.9%)

Source: Data from Acambis, Inc. BLA 125158 compiled by reviewer Efficacy Information Amendment February 20, 2007