Expert Opinion

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ACAM2000: a newly licensed cell culture-based live vaccinia smallpox vaccine

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Background: Due to concern over i) expiration of currently available calf-lymph vaccine (Dryvax®); ii) calf lymph as a vaccine (bovine spongiform encephalopathy [BSE], other possible contaminations and animal welfare); and iii) use of variola as a weapon for bioterrorism, a new and safer vaccinia-based smallpox vaccine derived from new cell culture-based technology was proposed. Federally funded work by Acambis, Inc. resulted in FDA approval for ACAM2000 in August 2007. *Objectives*: This paper describes the development from conception to FDA approval of the new vaccinia cell cultured-based smallpox vaccine ACAM2000. *Methods*: Data were compiled from available public reports. *Results/conclusions*: The studies with ACAM2000 indicate that it closely matches the safety of Dryvax in both non-clinical and clinical trials. ACAM2000 met two of the four primary surrogate efficacy end point criteria established for the Phase III clinical trials. Concern over the incidence of myopericarditis with ACAM2000 and Dryvax exists. So far the cardiac events seem to be self-limited. There are no pediatric safety data for ACAM2000. Overall, clinical trial results were sufficient to convince the FDA that ACAM2000 is a suitable replacement for Dryvax in the event of bioterrorism involving variola (smallpox).

Keywords: ACAM2000, bioterrorism, myopericarditis, smallpox, vaccine, vaccinia

1. Introduction

Smallpox was a serious, highly contagious, and sometimes fatal infectious disease (20% to > 50% mortality) as a consequence of infection with the orthopox virus variola major. There are no specific treatments for smallpox disease, although some evidence exists for use of anti-vaccinia immune globulin in the hemorrhagic variant of the disease. Public health measures focus on the highly effective preventive vaccination with vaccinia virus, a closely related orthopox virus.

In 1965, WHO (World Health Organization) established criteria and standards for smallpox vaccines, reducing the virus derivatives that could be used for vaccine production to three strains: Elstree (Lister Institute, UK), EM63 (Moscow Research Institute of Viral Preparation, Russia) and the New York City Board of Health (NYCBH) strain VV (Dryvax®, Wyeth Pharmaceutical, Inc., Philadelphia, USA and a smallpox vaccine made by Aventis Pasteur, Swiftwater, PA, USA) [1,2]. In order to pass WHO standards, a vaccine against smallpox was required to produce a major skin reaction in 95% of primary vaccines, and in 90% of those vaccinated 10 or more years previously, using an inoculum titer of 10^8 plaque-forming units (PFU) per ml [2].

Vaccines were a key element to the success of the WHO smallpox eradication efforts with the last naturally occurring case of smallpox in the world diagnosed in Somalia in 1977. A coordinated and effective global vaccine campaign made

this possible and included Dryvax in the Western Hemisphere and African eradication campaigns. Without an animal reservoir for variola, WHO considered the disease fully eradicated in May 1980. At the same time eradication was succeeding, it was realized that smallpox vaccination could cause a significant number of adverse events with a morbidity of approximately one to four per million people vaccinated depending on whether they were vaccinia-experienced (i.e., previously vaccinated) or naive, respectively. Smallpox vaccination ceased in the US in the mid-1970s and worldwide about 10 years later. In the United States, calf-skin vaccine (i.e., Dryvax) production ceased in 1982 [3]. Vaccination of the military ceased in 1989.

Dryvax vaccination of selected groups including military, first responders, and those involved in orthopox virus research was restarted in 2002 due to the concern over bioterrorism. In addition, a report to the 60th World Health Assembly on May 18, 2007 ('Smallpox eradication: destruction of variola stocks') recalls resolution WHA55.16, which defines the accidental or deliberate use of biological agents a global public-health concern. Simply said, the release of variola in a developing rural nation could result in generations of transmissions and fatalities, exhausting vaccinia vaccine stockpiles worldwide. The current supply of Dryvax is sufficient for only a few million people [2,4].

Dryvax was prepared before 1982 under standards not likely to be approved today by the FDA. Dryvax was prepared from purulent lymph material scraped from the torso of bovine calves infected with NYCBH strain VV [1,5]. The material was clarified and lyophilized in the presence of antibiotics. Reconstituted with a diluent containing 0.25% phenol, it is administered by bifurcated needle (Kravitz technique) onto the skin surface. Within 7 – 10 days of inoculation, a vesicular or pustular lesion called a pock forms and is referred to as a 'take'; a take is a surrogate of protective immunity [6-8]. After 1 – 2 weeks the pock ulcerates and scabs over, healing with a scar in 3 – 5 weeks. Side effects include mild to severe local reactions as well as unusual and sometimes serious adverse events. In addition to the pock reaction (lesion at the vesicle stage and beyond), Dryvax is associated (vaccinia naive, vaccinia-experienced, respectively) with local swelling (72%, 28%), lymphangitis (24%, 4%), regional adenopathy (78%, 18%) and urticaria and benign exanthemas (4%, rare) [9,10]. More serious adverse events per million doses included 529 inadvertent inoculation, 242 generalized vaccinia, 39 eczema vaccinatum, 12 encephalitis, 1.5 vaccinia necrosum and ~ 1 death (even higher for infants < 1 year, 5/million doses) [11-13]. Recent studies have identified myopericarditis as an adverse event that appears more frequently than initially reported (~ 124 cases/million doses) or was seen historically (< 1 per 10,000) [7,14-18].

Stocks of smallpox remain in secured locations within the US and Russia but there is concern that other stocks remain but are not declared and could be used for bioterrorism [19].

In 1998, a program was begun to develop a strategic national stockpile (SNS) of vaccines, particularly noting that only 15 million doses of smallpox vaccine, manufactured in 1978, remained in the US [20]. In July 1999, the Center for Disease Control and Prevention (CDC) awarded to Acambis, Inc. (Acambis, Inc. of Cambridge, UK and Cambridge, MA, USA) the initial contract that was twice amended after 11 September 2001 to secure 209 million doses of a new cell culture-derived vaccine. In order to deliver such a large quantity of vaccine, Acambis partnered with Baxter BioScience (Baxter International, Inc., Deerfield, IL, USA) with its large-scale bioreactor capacity at its facilities near Vienna Austria. The balance of doses between Acambis and the US population was to be drawn from the original Dryvax stock, which could be diluted 1:5 for administration in emergencies, and recently discovered stocks of Aventis Pasteur smallpox vaccine [21,22]. Acambis received FDA approval of its cell-cultured vaccinia vaccine ACAM2000 in August 2007.

2. ACAM1000, the seed strain for ACAM2000

2.1 Plaque selection, clone characteristics and preclinical animal testing

Vaccine strain (seed) candidates were purified from a pool of 30 vials from three different production lots of Dryvax, which is a vaccine comprised of a heterologous mixture of vaccinia variants. Sequential plaque selection was used to isolate particular clones to be tested in animal models of neurovirulence. Six clones were isolated by three sequential rounds of plaque purification in MRC-5 cells (diploid human embryonic lung fibroblasts). Candidates were tested for pock formation in rabbit skin. Lesion size was greater than Dryvax for clones 1, 3 and 5. Clones 2, 4 and 6 produced either no central lesions or lesions that were similar in diameter to Dryvax. Candidates were compared by intracerebral injection of suckling mice. Clones 1, 3 and 5 were more neurovirulent than Dryvax by survival analysis and clones 2, 4 and 6 were similar to Dryvax or less virulent. Clones 2, 3, 4 and 6 were tested for replication in suckling mouse brain tissue; clones 2 and 4 grew to lower titers than Dryvax. Based on its attenuated phenotype, clone 2 was selected as ACAM1000. In repeated testing ACAM1000 appeared stable after 18 passages in MRC-5 cells. A pilot GMP (good manufacturing practice) lot of ACAM1000 manufactured in MRC-5 cells grown in cell factories, harvested from disrupted cells and purified by ultrafiltration and diafiltration, was used in human clinical trials.

To confirm the attenuated neurovirulence phenotype, 12 young adult rhesus monkeys were inoculated with either ACAM1000 or with Dryvax by the intrathalamic route with 7 $\log_{10}$ PFU. Three of six monkeys injected with Dryvax died of neurologic illness, while all six monkeys survived injection with ACAM1000 with minimal effects [20].

ACAM1000 was similar to Dryvax in plaque morphology and genetic sequence homology [3]. Efficacy was determined in mice challenged with cowpox (strain Brighton) and vaccinia Western Reserve (WR) viruses by intranasal (IN) route and by small particle aerosol respiratory challenge of mice with ectromelia virus. Survival after vaccination with ACAM1000 at 7 or 8 $\log_{10}$ PFU/ml was similar to Dryvax, although lower doses showed a reduced survival compared with similar doses of Dryvax. ACAM1000 was similar to Dryvax in inducing skin pock lesions, neutralizing antibodies, and T-cell responses in monkeys and mice [3,20].

2.2 ACAM1000 clinical trials

Data from two Phase I clinical trials have been reported, one in 60 healthy 18 – 29- year-old vaccinia-naive adults (H-300-001) and another as an open-labeled study in 70 vaccinia-naive subjects (H-300-003). The first clinical trial was a randomized, double-blinded, non-inferiority study involving two groups inoculated with ACAM1000 or Dryvax and followed for 6 months. Exclusion criteria eliminated those with immunodeficiency and eczema [23]. The vaccines contained 1.0×10^8 PFU/ml of virus which elicited dermal pock reactions in 30 of 30 ACAM1000 recipients by day 10, compared with 29 of the 30 Dryvax recipients. Similar results of non-inferiority were observed for vaccinia-specific neutralizing antibodies at 45 days postvaccination with geometric mean titer (GMT) in the ACAM1000 and Dryvax groups of 142 and 248, respectively. T-cell responses were seen to both vaccines in the vast majority of subjects. No serious adverse events (SAEs) occurred and the rates of mild and moderate side effects in the two groups did not differ statistically. Immune responses to ACAM1000 were not inferior to that seen with Dryvax [3].

The second Phase I open-label study H-300-003 was designed to evaluate the safety, tolerability, and immunogenicity of ACAM1000 in 70 vaccinia-naive adults aged 18 – 29 years. In this study, all had takes by day 10. A fourfold increase in neutralizing antibody titer by day 30 was seen in 66 (94%) of the subjects and the GMT was 154 but the titers varied considerably from 20 to 20,480. T-cell responses were not studied. There were no SAEs [20].

3. ACAM2000

3.1 Manufacturing

ACAM2000 was the second prototype smallpox vaccine to be generated and was created because of the availability of validated large-scale manufacturing using Vero cells (Baxter International, Inc). ACAM1000 master seed virus (passage 7 in MRC-5 cells) was used to prepare ACAM2000, by growth under serum-free conditions in a continuous line of African green monkey (Vero) cells; thus the eighth passage of ACAM1000 was the first passage ACAM2000 in Vero cells. Two subsequent passages over Vero cells for 3 days in a 1200L bioreactor yielded the inoculum used

(passage 10) for growth of the ACAM 2000 vaccine. Virus is harvested by cellular disruption and cellular debris removed by large-pore depth filtration. Host cell DNA is digested with endonuclease (Benzonase) and virus particles are purified and concentrated by tangential-flow filtration and diafiltration. For safety purposes the seed viruses and vaccine loads were tested for: adventitious agents including bacteria, fungi, mycoplasma and viruses; neurovirulence in suckling mice; and residual Vero cell DNA [20]. In addition, ACAM1000-P7 and ACAM2000-P10 (3 passages in Vero cells) were found to have identical sequences [7,20,23]. The concentrated virus is formulated by dilution with a buffer to a potency of 5×10^8 PFU/ml with 0.3 ml dispensed into vials (100 doses) and lyophilized.

ACAM2000 is supplied as a lyophilized purified live vaccinia virus containing the following: 6 – 8 mM HEPES (pH 6.5 – 7.5), 2% human serum albumin USP (United States Pharmacopeia), 0.5 – 0.7% sodium chloride USP and 5% mannitol USP. There may be traces of residual polymixin B and neomycin. The diluent 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 containing 0.6 ml of diluent. The vaccine can be stored for 72 months from the date of manufacturing at -15 to -25°C. The diluent can be stored at 15 – 30°C for 60 months. Each vial is reconstituted with 0.3 ml of the supplied diluent. After reconstitution, each vial contains 100 doses (0.0025 ml/dose) and is stable at 2 – 8°C for up to 30 days. The concentration of the virus is $1.0 – 5.0 \times 10^8$ PFU/ml. Vaccine is administered using 15 punctures by a sterile bifurcated needle which delivers approximately 0.0025 ml of vaccine (1 drop) to the skin surface, containing approximately 250,000 PFU of virus [7].

Based the results of non-clinical studies and early clinical Phase I and II data (summarized in sections 3.2 and 3.3) and recommendations by a Joint Down-Selection Working Group of the National Vaccine Advisery Committee (NVAC) and the Defense Science Board, the CDC requested in February 2003 that Acambis stop further testing of ACAM1000 and focus on the development of ACAM2000. Data obtained with ACAM1000, prior to the down selection of ACAM2000, were considered supportive of data obtained with ACAM2000.

3.2 Preclinical studies

The results of nine non-clinical studies that evaluated the safety and efficacy of ACAM2000 are summarized [7,20,24]:

- Immunogenicity in mice following percutaneous administration of ACAM2000 (1×10^6 or 1×10^8 PFU/ml) was associated with an increase in neutralizing antibody 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 of

ACAM2000 was tested. 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 of ACAM2000 was tested. 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 equivalent protection for all monkeypox-challenged monkeys. No viremia was found in vaccinated groups and no clinical symptoms or pathological changes were observed in the vaccinated animals. Controls (diluent only) showed numerous monkeypox clinical symptoms and died.

- Cutaneous virulence following percutaneous administration of ACAM2000 to rabbits was evaluated. 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 of unpurified ACAM2000 found that the average survival time in ACAM2000-treated mice was greater than average survival time in Dryvax-treated mice, except at the highest dose administered (2000 PFU/mouse).

- The average survival time in mice following intracerebral administration of ACM2000 was greater than Dryvax-inoculated mice.

- Mortality in mice following intracerebral administration of ACAM2000 Vero Production virus Bank passage 8 was significantly lower in mice inoculated with the ACAM2000 Production Virus Bank compared with mice inoculated with Dryvax.

- Mortality in mice following intracerebral administration of ACM2000 Vero-Vaccine TFF Retentate (ACAM Drug substance) was similar to mice inoculated with Dryvax.

- Three of six monkeys given an intrathalamic administration of Dryvax (4.9×10^7 PFU) died. No deaths occurred among six monkeys inoculated with ACAM2000 Master Seed Virus (1.25×10^7 PFU).

These preclinical animal studies found ACAM2000 comparable to Dryvax with less neurovirulence and cutaneous virulence and similar immunogenicity to Dryvax at similar dose levels.

3.3 Clinical trials

Acambis conducted six clinical trials (Table 1) with ACAM2000 including two Phase I (H-400-008-naive and H-400-002-naive), two Phase II (H-400-005-naive and H-400-003-experienced), and two Phase III trials (H-400-009-naive and H-400-012-experienced).

Efficacy for both Phase III trials used the surrogate end points of take rate and serum neutralizing antibody against vaccinia. Both studies used lots with potencies from 1.3 to 2.2×10^8 PFU/ml for ACAM2000 and 1.5×10^8 PFU/ml for Dryvax. All studies with the exception of H-400-008 were parallel group, double-blind trials with Dryvax as the control. Subjects were followed for 30 days after vaccination except for H-400-002 which had a 45-day follow-up. Vaccination was performed via 15 strokes with a bifurcated needle in the skin of the upper arm. Vaccinia-experienced subjects were aged 29 – 84 years and it was at least 10 years since their last vaccination while all vaccine-naive subjects were less than 30 years old. Vaccination takes were assessed by the site investigator between day 6 and 11 for vaccinia-naive subjects and day 6 – 8 for vaccinia-experienced. In H-400-012 takes were assessed by both the site investigator between day 6 and 8 and by an independent review committee based on photographic evidence. Serum collected on days 0 and 30 after vaccination were analyzed for the neutralizing antibodies in a validated $PRNT_{50}$ (50% plaque reduction neutralization test) against the vaccine vaccinia strain plated on Vero cells. The serum titer was reported as the reciprocal of the dilution resulting in 50% plaque reduction. To compare population responses, GMTs were calculated. In order to more easily compare immunogenicity of ACAM2000 to controls, Table 2 summarizes and compares data for vaccinia-naive subjects across all Phase III vaccinia-naive trials.

After March 28, 2003, ongoing Phase II trials and the entire Phase III program monitored subjects for chest pain, SOB, palpitations and reduced tolerance to exercise. The Phase III trials added scheduled ECGs and cardiac injury lab tests post-vaccination. With the exception of H-400-008, a Data Safety Monitoring Board reviewed the trials and for the Phase III trials could request a blinded Cardiology Advisory Panel to review serious cardiac events, abnormal ECGs and troponin I levels [7,18].

H-400-002 was a Phase I, single-center, randomized, double-blinded study of ACAM1000, ACAM2000 and Dryvax smallpox vaccine. All 90 subjects, three groups of 30 subjects 18 – 29 years old, received a dose of 1×10^8 PFU/ml dose of vaccine and all had a take. The GMT on day 45 was 124, 103 and 172 in the ACAM1000, ACAM2000 and Dryvax groups with no significant differences between groups. All subjects were evaluated for T-cell responses: cytotoxic T lymphocytes (CTL assay), cytokine-producing cells (γ-IFN ELISPOT assay), and vaccinia-specific lymphoproliferation assay (LPA assay). All 30 subjects in the ACAM2000 group and 28 of 30 (93%) in the Dryvax group demonstrated positive T-cell immune responses to smallpox vaccine in at least one of the three T-cell assays (ACAM2000, Dryvax): CTL (87%, 73%); ELISPOT (100%, 90%); LPA (97%, 87%) [7,18]. H-400-002 was supplemented by measurements of virus shedding from the vaccination site. The median duration of virus shedding at the vaccination site was 16 – 20 days with ACAM2000 and Dryvax. Only one (3%) ACAM2000 recipient in H-400-002 had evidence of virus on the outside

Table 1. Clinical trials of ACAM2000 licensed vaccine.

Study	Phase	Number of subjects enrolled (VN or VE)	Title
H400-002	I	90-VN	The effect of ACAMM1000, ACAM2000 and Dryvax on safety, tolerability and immunogenic response in adults without previous smallpox vaccination
H400-008	I	100-VN	A Phase I, open-label, single-arm, fixed-dose study designed to evaluate the safety, tolerability and immunogenicity of ACAM2000
H400-005	II	353-VN	The effect of dose on safety, tolerability, and immunogenicity of ACAM2000 smallpox vaccine in adults without previous smallpox vaccination
H400-003	II	357-VE	The effect of dose on safety, tolerability and immunogenicity of ACAM2000 smallpox vaccine in adults with previous smallpox vaccination
H400-009	III	1162-VN	The safety, tolerability and immunogenicity of ACAM2000 smallpox vaccine in adults without previous smallpox vaccination: a randomized, double-blind, fixed-dose, Phase III comparison between ACAM2000 and Dryvax smallpox vaccines
H400-012	III	1819-VE	The safety, tolerability and immunogenicity of ACAM2000 smallpox vaccine in adults with previous smallpox vaccination: a randomized, double-blind, fixed-dose, Phase III comparison between ACAM2000 and Dryvax smallpox vaccines

VE: Vaccinia-experienced; VN: Vaccinia-naive.

Table 2. Summary of comparative immunogenicity for Acambis experimental smallpox vaccines in vaccinia-naive subjects.

Immune response*	Number of studies[¶]	Dryvax	ACAM1000	ACAM2000
Number subjects		268	60	754
Skin takes (pock)	3	98.8%	98.3%	99.0%
nAb ($PRNT_{50} > 40$)[‡]	3	92.1%	100%	87.9%
Number subjects		60	60	30[#]
IFN-γ-ELISPOT assay (> 15 SFC)[§]	2	93.3%	98.3%	100%

*Three depicted responses represent quality control measures performed enabling comparison.
[‡]50% plaque reduction neutralization test ($PRNT_{50}$) positive titer is 1:40 or greater.
[§]Limit of detection: five spot forming cells (SFC)/million peripheral blood mononuclear cells (PBMC), and positive cut-off is SFC/million PBMC > 15.
[¶]Studies depicted are H-300-001, H-400-002, H-400-008 and H-400-009 with PRNT data available.
[#]ACAM2000 ELISPOT responses only conducted for one trial H-400-002.

the vaccination site dressing on day 7 and 516 environmental samples were negative, suggesting that ACAM2000 does not easily spread outside the dressing [25]. At least one subject may have had myopericarditis following an ACAM2000 vaccination [7,18].

H-400-008 was a Phase I open-label trial of ACAM2000 in 100 healthy vaccinia-naive adults 18 – 29 years old, with 56% males and 89% Caucasian. The lot used in this trial had a potency of 7.7×10^7 PFU/ml, which was slightly below the target of 1.0×10^8 PFU/ml. The results showed that the vaccine was well tolerated and elicited cutaneous and antibody responses in 99% (days 7 – 15 post-vaccination) and 96% of the subjects, respectively. The GMT neutralizing (50% plaque reduction) antibody titer on day 30 was 225. Four subjects with a take failed to achieve a fourfold rise in their GMT by day 30. One subject experienced a single new onset seizure on day 8 [7,18,20].

H-400-003 was a Phase II, multicenter, dose-finding study of ACAM2000 smallpox vaccine. A total of 357 enrolled with a planned distribution of 50 to receive ACAM2000 6.8×10^7 PFU, 100 each to receive ACAM2000 1.4×10^7 PFU and 6.8×10^6 PFU, 50 to ACAM2000 3.4×10^6 PFU and 50 to receive Dryvax. The rate of successful vaccination was 88, 51, 40 and 27% in the ACAM2000 groups and 100% in the Dryvax. A dose response was also seen in the GMT responses, with GMT of 256, 115, 84 and 59 for the ACAM2000 groups, respectively, and 447 for Dryvax. The study demonstrated that in previously vaccinated individuals, the highest ACAM2000 dose group was not equivalent to Dryvax with respect to revaccination or GMT. There was no mention of safety concerns [7,18,23].

In H-400-005 subjects were vaccinated with ACAM2000 6.8×10^8 PFU/ml and doses diluted 1:5, 1:10 and 1:20 (1.4×10^7, 6.8×10^6 and 3.4×10^6 PFU/ml, respectively)

in a randomized, double-blinded, controlled trial. Dryvax was the control at a 1.6×10^8 PFU/ml dose [23]. A total of 353 were enrolled and there were no significant differences in take rate between the Dryvax and the full-dose ACAM2000, both were 100%. None of the three lower doses met the 90% take rate. Similarly GMTs were sensitive to the administration dose of ACAM2000. There was a linear trend in seroconversion with increasing doses of ACAM2000 (i.e., 59%, 80%, 86% and 94%) and 96% for Dryvax. Overall, no significant differences were noted between the highest ACAM2000 group and Dryvax. These results suggest a difference between ACAM2000 and Dryvax as Dryvax can be diluted up to 1:10 without loss of efficacy in terms of take rate and neutralizing antibody titer [21,22]. The mean size of the maximum take cutaneous reaction among responders was not significantly different between the high-dose ACAM2000 and Dryvax groups. Maximum erythema was significantly greater in the Dryvax group. One subject in the high-dose ACAM2000 developed myopericarditis, diagnosed on day 11 (elevated CPK-MB and troponin I and ECG changes). Symptoms resolved after 36 h, labs by day 15 and ECG after 3 months. Overall, chest pain occurred in 3% of the ACAM2000 group and 6% in the Dryvax group; work-ups varied and not all had ECGs or labs, so etiologies were not clear. After 6 months, no change in health status was found in all those that could be contacted [7,18,23].

H-400-009 was a Phase III randomized placebo-controlled, double-blind, multicenter study in which vaccinia-naive adults were randomized 3:1 to receive ACAM2000 or Dryvax. There were co-primary efficacy end points: take on day 7 or 10 and the GMT on day 30. ECG and troponin I tests were done on screening, day 10 and day 21. Subjects were excluded if they had three or more risk factors for coronary artery disease, had palpitations or abnormalities of cardiac rhythm, or had ECG pattern that would have complicated the recognition of new changes due to pericarditis or myocarditis. A total of 1162 subjects were enrolled with a mean age of 23 years, and were 65% male, 74% Caucasian, 12% African-American, 10% Hispanic and 1% Asian. Ninety-six per cent of subjects randomized to receive ACAM2000 had a take, 95% (CI 0.95 – 0.97) compared with 99% in the Dryvax group. ACAM2000 was non-inferior to Dryvax based on this outcome and 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. Day 30 mean GMTs were 166 in the ACAM group and 255 in the Dryvax group. The GMT results failed to meet the lower bound of the 97.5% one-sided CI indicating that ACAM2000 was inferior to Dryvax in GMT responses [7,18,23].

In H-400-009, no significant differences between the ACAM2000 and Dryvax groups were seen with regard to the overall incidence of treatment-emergent adverse events. The most common serious adverse event was myopericarditis, identified in eight subjects: five subjects in the ACAM2000 group and three subjects in the Dryvax group. Four of five in the ACAM2000 group resolved without sequelae, while one subject continued to have ECG changes and was prescribed carvedilol and aspirin. Two of the three subjects in the Dryvax group resolved without sequelae, while one 21-year-old female continued to have a cardiac ejection fraction of 27 – 32% with global hypokinesis 2.5 years after vaccination. The study planned to enroll 2720 healthy subjects but was suspended because of the observed frequency of myopericarditis [7,18,23].

H-400-012 had a 3:1 randomization to receive ACAM2000 or Dryvax. Subjects were excluded if they had three 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. Mean age was 49 years with 49% male, 81% Caucasian, 7% African-American, 12% Hispanic and Asian. Due to the same concerns as for H-400-009, only 1819 subjects of a planned 2720 were enrolled. ACAM2000 had an 84% (with 95% CI 0.82 – 0.86) take versus a 98% take for Dryvax demonstrating that the take rate for ACAM2000 was inferior to Dryvax. However, the GMT at day 30 was 286 for ACAM2000 and 445 for subjects in Dryvax group; the GMT results for ACAM2000 were found to be non-inferior to Dryvax. For both vaccines, 97% of the subjects had neutralizing antibodies. However, the neutralizing antibody achieved with ACAM2000 was ~ 1.5-fold lower than to Dryvax, consistent with the findings in H-400-009. Despite the difference in take rates, there was no indication that the difference was based on gender or race; however the study was not powered to detect differences in subpopulations [7,18]. All serious adverse events at least possibly due to ACAM2000 (chest pain in two subjects and one with atrial fibrillation) or Dryvax (one generalized vaccinia and one hypersensitivity reaction) resolved during the follow-up period [7,18].

3.4 Additional comments on clinical safety data

The most common SAEs during the clinical development program were pregnancy during the trials and myocarditis. No cases of congenital infection were documented. There was a total of 10 cases of myocarditis (seven with ACAM2000; 5.73 per 1000 compared with 10.38 events per 1000 with Dryvax), all in vaccinia-naive subjects. Of the 10 subjects, nine were male, seven were Caucasian, mean age was 22 years, and two had at least one risk factor for coronary heart disease. The mean time to onset was 11 days with a range of 9 to 20 days. Of the 10, four were symptomatic (three in the ACAM2000 group) and one from each group was hospitalized [7,18]. The incidence of myopericarditis for ACAM2000 is higher than that reported by the Department

of Defense and by the CDC for civilian programs (0.11 and 0.54 cases per 1000). Both programs vaccinated exclusively with Dryvax. Importantly, neither program had active surveillance protocols for myopericarditis. So far, no differences between the vaccines have been evident in the Acambis trials, suggesting that both vaccines retain a similar risk for this particular adverse event [14,26,27].

Non-specific positive serological responses to HIV, hepatitis B and C and syphilis were tested and ACAM2000 vaccination was found to elicit a false-positive test for syphilis (RPR, Rapid Plasma Reagin test). No notable differences in AE rates were seen by race, sex, age, body surface area or baseline neutralizing antibody titer. No SAEs historically associated with smallpox vaccine occurred in the ACAM2000 groups [7,18].

3.5 2007: Strategic National Stockpile and FDA approval and warnings

On January 17, 2007 Acambis delivered an additional 10 million doses increasing the Strategic National Stockpile (SNS) to 192 million doses. The vaccine continues to be manufactured for the US Government SNS, WHO stockpiling, and for foreign governments for stockpiling and potential use outside the US [7,18]. Acambis stated in its April 2006 BLA that they do not intend to commercialize ACAM2000. ACAM2000 will be used routinely for forward deployed troops.

ACAM2000 was approved by the FDA on August 31, 2007 for people at high risk of exposure to smallpox. FDA stated that the vaccine will be manufactured to provide vaccine for the SNS only. Based on clinical studies, myopericarditis occurs in 1 in 175 adults who are vaccinia-naive. The clinical trials did not include anyone < 18 years of age and the risk to this population is unknown. Interestingly, no cases were diagnosed in those previously vaccinated. Anyone severely immunocompromised for any reason should not receive the vaccine. There is a black box warning for ACAM2000 including not only the possibility of the known SAEs of live vaccinia virus vaccine but also acute myopericarditis. Warnings also include the risk of these events occurring in unvaccinated close contacts of the vaccine. The product will be labeled as a pregnancy category D risk, and will state that vaccinees living in the same household with or having close contact with a pregnant woman should be apprised of the potential hazard and information on how to report any transmission to the National Smallpox Vaccine Pregnancy Registry. Information about the availability of vaccinia immune globulin for the management of certain complications is found on the label.

ACAM2000 is the first licensed vaccine required to supply a medication guide to all potential vaccinees as required by CFR Part 208. The guide provides information about serious side effects that can occur with ACAM2000

vaccination and explains the proper care of the vaccination site. Severe immunosuppression is the sole contraindication to having the vaccine; eczema and atopic dermatitis are described as conditions with greater risk for a SAE. The FDA also implemented a risk minimization plan (RiskMAP) for ACAM2000. The evaluation program is to include an annual adverse event report with an analysis of data from the Vaccine Adverse Event Reporting System and other sources. In addition Acambis agreed to the following:

- A Phase IV prospective cohort study within the military population that includes 15,000 ACAM2000 vaccinees and an appropriately sized control group over a 2-year period. The study will evaluate the effectiveness of screening procedures and long-term follow-up of cases of myopericarditis.
- Acambis is to perform an enhanced surveillance program to include at least 75% of symptomatic cases of myopericarditis after ACM2000 vaccination. The enhanced surveillance will include up to 200,000 military members and use of all communication modalities.
- Acambis will implement a myocarditis registry to evaluate further the natural history of myopericarditis after ACAM2000 vaccination and potential risk factors in a minimum of 150 cases followed over at least 2 years.
- Acambis will conduct a study to examine how effectively the Department of Defense adheres to its own screening procedures to identify potential vaccinees who have risk factors for SAEs and should not be vaccinated.

4. Conclusions

The studies with ACAM2000 indicate that it closely matches the safety of Dryvax in both non-clinical and clinical trials. At best because it is a homogeneous product with less neurovirulence and cutaneous reactions in animal models, its pock response may be slightly attenuated in humans. Concern over the incidence of vaccination-related myopericarditis with ACAM2000 and Dryvax exists. So far the cardiac events seem to be self-limited [15]. There are no pediatric safety data for ACAM2000.

ACAM2000 met two of the four primary surrogate efficacy end-point criteria established for the Phase III clinical trials. ACAM2000 also induced a positive cell-mediated immune response as determined by at least one assay method in 100% of subjects (n = 30). Based on the lot consistency data, 1.0 to 5.0×10^8 PFU/ml of ACAM2000 will be required to achieve the results from the Phase III trials, suggesting that the vaccine would become ineffective if diluted. It will be recommended per CDC that those with a no take to ACAM2000 need to be revaccinated. Overall, these results were sufficient to convince the FDA that ACAM2000 is a suitable replacement for Dryvax in the event of bioterrorism involving variola (smallpox).

5. Expert opinion

5.1 Historical considerations

The successful completion of this clinical development program, from creation of a second-generation (cell culture-derived) smallpox vaccine to FDA approval, is an important achievement. It demonstrates that current vaccine manufacturing technology can be harnessed to develop a needed vaccine, in a manner consistent with GCP and FDA GMP specifications, in order to protect the population against a bioterrorism agent as significant as variola. Such a success would have been even more spectacular if countries worldwide had joined in the development of such a vaccine or similar ones (e.g., other vaccinia vaccines in development include LC16m8 and BN-MVA®), driving smallpox vaccine research not only by market forces but also by internationalism.

A major issue in developing vaccines against smallpox is the lack of endemic disease, and the lack of clear scientific understanding of the elements of protective immunity and immunological memory against smallpox. The dermal response to percutaneous vaccination has been the standard measurement protection. In 1964, the WHO defined as a major dermal reaction the presence of a pustular or indurated area surrounding a central ulcer or scab 6 – 8 days after vaccination and that this reaction to vaccination corresponded to the development of humeral antibodies and with protection against smallpox for up to 5 years.

Despite numerous recent publications attesting to long duration of detectable immune responses to vaccine, it is known from historical studies that smallpox disease symptoms become more pronounced with increased time since last vaccination. Although these historical studies lack many of the modern equivalent of immune response measures, it is probable that for such a virulent virus as variola, that a combination of intact cellular and humeral immunity together provide the most robust anti-viral immunity [5,28-30]. Two studies are often cited when comparing neutralization titers to full protection against smallpox: Sakar and associates showed protection against smallpox with a variola-specific neutralizing antibody titer > 1:20 (n = 57 subjects) and Mack and associates reported protection with vaccinia-specific neutralizing antibody titers > 1:32 (n = 142 subjects) [5,31-33]. Sakar *et al.* used variola virus in their neutralization assay and inoculated 12-day old chick eggs [34,35]. Six of 13 unvaccinated individuals with neutralization titers < 1:20 developed smallpox. All bloods were drawn 2 – 8 days after contact [32,35]. Mack *et al.* used a vaccinia strain identified as NIH reference smallpox vaccine lot no. 1b and inoculated tissue cultures [35]. They tested for neutralizing antibody in only 12 subjects that lacked a vaccination scar and were recent (i.e., less than 9 days) contacts of smallpox cases. Only 3 of 12 had titers < 1:32, of which 2 developed smallpox.

The number of those with vaccination scars studied was 130 and one of 12 with a titer < 1:32 developed smallpox. None in either group with a titer ≥ 1:32 developed clinical disease [31].

Similar data for T-cell responses is not available. Our work suggests that both LPA and γIFN-ELISPOT responses to vaccination correlate strongly with development of takes, the surrogate marker of protection. Nevertheless, it remains speculative as to how to apply immune biomarkers to determine whether vaccinated humans are protected against smallpox [31]. Moreover, it is not explained how the validated Acambis neutralization assay compares to the assays performed by Mack and Sakar and their associates. In fact, there is no fully accepted serological correlate of protection against smallpox. Recent studies have begun to compare long-term survivors of smallpox with vaccinated individuals or those infected with related poxviruses [36,37]. Such studies need to be expanded and conducted before the opportunity to do so with smallpox survivors becomes impossible.

5.2 What is clearly good with ACAM2000

ACAM2000 was not inferior to Dryvax in: safety and efficacy data in animals; take rates in vaccinia-naive subjects; and PRNT$_{50}$ titers in vaccinia-experienced subjects. In addition, the data suggested that vaccine-emergent reactions were slightly attenuated with ACAM2000. The assumption is that if ACAM2000 and Dryvax do not differ significantly in their cardiac risks (and there are no data to refute this), then ACAM2000 could be used as an emergency vaccine in place of Dryvax.

Along with the licensing of ACAM2000, there is a need to recognize that the eradication of smallpox is global and the unexpected release of variola could have global consequences for both the developed and resource limited world. Therefore, any new vaccine may be needed on a global scale. The WHO would be in the center of any global epidemic. It is time to review smallpox vaccines such as ACAM2000 on a global scale and decide how to organize manufacturing in case of need and to plan for better vaccines based on global concerns. With ACAM2000, the concept of worldwide public health security can move forward.

5.3 Are there concerns with ACAM2000?

There are concerns. The FDA's rigorous post-approval safety plan for ACAM2000 attests to the uncertainty of the incidence and sequelae of post-vaccination myopericarditis. The Acambis trials have established the incidence as 1:175 vaccinia-naive vaccinees. The trials did not find any myopericarditis in vaccinia-experienced subjects but the number of these enrollees may have been too small to detect events; prior exposure may have resulted in subclinical events, or may indeed indicate a group with less risk.

The vaccine-emergent reactions with ACAM2000 were slightly attenuated compared with those from

Dryvax vaccination. In addition, ACAM2000 could not be diluted and reliably remain effective by both take rate and $PRNT_{50}$ measurements. This is significant in light of a recent report that vaccine-associated morbidity may be diminished by dilution of Dryvax [38]. In fact, fewer take reactions were seen in ACAM2000 vaccinia-experienced subjects with residual $PRNT_{50}$ titers. Also, the method used to select the clone for ACAM1000 and ACAM2000, resulting in the selection of a less neurovirulent clone, raises concerns over the durability of ACAM2000 over time in storage, as the smallest drop off in titer may affect efficacy, and the potential for numerous no takes in vaccinia-experienced subjects. Durability, stability, inability to be diluted and decreased number of takes in vaccinia-experienced subjects are concerns that will require future study to assure that stockpiled vaccine maintains potency.

The ease of manufacture can overcome some of these potential problems through replenishment of vaccine but issues regarding durability of immune responses remain to be studied.

Declaration of interest

RN Greenberg is currently a principal investigator for clinical trials sponsored by the following companies: Massachusetts Biological Laboratory, BMS, VIRxSYS Corp., GSK, Dynport Vaccine Co., Emergent Biosolutions, Bavarian Nordic A/S, Wyeth Pharmaceuticals and Tibotec. In the past RN Greenberg was a principal investigator for Acambis on both Phase III ACAM-2000 trials as well as a principal investigator on four other Acambis vaccine trials (West Nile, Clostridium difficile and 2 ACAM-3000 trials).

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