Application Type	BLA Supplement
STN	125158/297
CBER Received Date	October 27, 2023
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Division / Office	DVP/OVRR
Committee Chair	Edward Wolfgang
Clinical Reviewer(s)	Sixun Yang
Project Managers	Maria Bagh Lynsay Ehui
Priority Review	No
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Review Completion Date / Stamped Date	
Concurrence	Lei Huang, Ph.D. Concurring Reviewer, VEB, DB, OBPV
Concurrence	Tsai-Lien Lin, Ph.D. Branch Chief, VEB, DB, OBPV
Applicant	Emergent Product Development Gaithersburg, Inc.
Established Name	Smallpox (Vaccinia) Vaccine, Live
(Proposed) Trade Name	ACAM2000
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc	Reconstituted Lyophilized Antigen Component, Live, with 0.3 mL of diluent. A single dose of reconstituted vaccine is approximately 0.0025 mL (droplet).
Dosage Form(s) and Route(s) of Administration	Administered via percutaneous route (scarification)
Dosing Regimen	One dose
Purpose of Supplement	To expand the indication to include for active immunization against mpox disease for persons determined to be at high risk of mpox infection.

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1. Executive Summary

ACAM2000 is currently indicated for the active immunization against smallpox disease for persons determined to be at high risk for smallpox infection. The applicant, Emergent Product Development, submitted an efficacy supplement to Biologics License Application (BLA), STN 125158/297, to expand the indication of ACAM2000 to include active immunization against mpox disease for persons determined to be at high risk for mpox infection. The expansion of the indication is primarily supported by the sponsor's nonclinical mpox virus challenge study in non-human primates (NHPs), which was included in the original BLA (study report T-400-001). No new clinical data are provided in this efficacy supplement. This statistical review memo focuses on the immunogenicity and protective efficacy data in nonclinical Study T-400-001.

In nonclinical study T-400-001, 24 NHPs were allocated equally to three arms: ACAM2000, Dryvax, and a negative control. Antibody production was determined by 50% vaccinia plaque reduction neutralization assay. Geometric mean titers (GMTs) and 95% confidence intervals (CIs) 60 days after vaccination (prior to challenge) were 174 (95% CI: 91 to 335) in the ACAM200 group and 190 (95% CI: 126 to 287) in the Dryvax group. All monkeys in the negative control group remained seronegative after 60 days. Monkeys were challenged with virulent mpox virus administered intravenously 61 days after vaccination. All monkeys in the ACAM2000 and Dryvax groups survived the challenge, and no monkey in the negative control group survived. No monkey in the ACAM2000 group had viral shedding, viremia, or lesions after mpox challenge. There were no major statistical issues with the analyses conducted in the study.

The nonclinical descriptive NHP study results support that ACAM2000 is effective in protecting monkeys against mpox infection. I defer to the other reviewers on the suitability of the animal model, the extrapolation of these results to humans, and the overall adequacy of evidence to support the expansion of the indication.

2. Clinical and Regulatory Background

ACAM2000 was originally approved for the prevention of smallpox infection in 2007. On October 26, 2023, Emergent submitted an efficacy supplement application to expand indication of ACAM2000 to the prevention of mpox disease in the high-risk population.

3. Submission Quality and Good Clinical Practices

3.1 Submission Quality and Completeness

The quality of the submission was sufficient for the statistical review of T-400-001. Verification of the relevant analyses was based on the provided data tables in the final report (including Appendices).

3.2 Compliance With Good Clinical Practices And Data Integrity

No data integrity issues were identified.

4. Significant Efficacy/Safety Issues Related to Other Review Disciplines

I defer to reviewers from other disciplines.

5. Sources of Clinical Data and Other Information Considered in the Review

5.1 Review Strategy

This memo focuses on the statistical review of relevant immunogenicity and protective efficacy data in nonclinical study T-400-001, the primary source of evidence in support of the expanded indication to the prevention of mpox disease.

5.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

The following documents served as the basis for this review memo:

- 125158/297.0 (27 October 2023)
  - Module 1.6.3: Clinical Information Amendment, Briefing Package
  - Module 2.4: Nonclinical Overview
  - Module 2.6: Nonclinical Written and Tabulated Summaries
    - Module 2.6.1: Introduction
    - Module 2.6.2: Pharmacology Written Summary
    - Module 2.6.3: Pharmacology Tabulated Summary
  - Module 4.2.1.1: T-400-001 Final Study Report
- 125158/297.2 (16 January 2024)
  - Module 1.2: Reviewer's Guide

6. Discussion of Individual Studies

6.1 Non-clinical Study T-400-001

Immunogenicity and Protective Activity of ACAM2000 and Dryvax Smallpox Vaccines in Cynomolgus Macaques Challenged with Monkeypox Virus by Intravenous Route

6.1.1 Objectives

The objective of this Good Laboratory Practice (GLP) study was to assess whether cynomolgus monkeys inoculated with either ACAM2000 or Dryvax via percutaneous (scarification) route would be protected against a lethal monkeypox virus challenge.

6.1.2 Design Overview

Twenty-four (24) cynomolgus monkeys were randomized (by gender and weight) to be immunized by either ACAM2000, Dryvax, or a negative control (ACAM2000 diluent) via percutaneous (scarification) route on Day 0. Clinical observations were performed twice daily, in addition to inspection of the vaccination site on the day of vaccination, as well as Days 3, 5, 7, 10 and 15. On Day 61 post-immunization, all monkeys were challenged with virulent mpox virus (target of 5×10^7 pfu, actual dose delivered 3.8×10^7

pfu) delivered by the intravenous route. Blood was drawn prior to vaccination on Day 0 and was drawn on Days 30, 60 and 91 (survivors only) or at the time of death (when possible) for immunogenicity determinations. Antibody production was determined by 50% plaque reduction neutralization assay (PRNT50) using the same vaccinia plaque reduction neutralization test as the human clinical studies evaluated in the original BLA submission. Blood was drawn on Days 63, 65, 69, 71, 75, 77 and 91 for viremia determination (plaque assay). Throat swabs were collected on Days 63, 65, 67, 69, 71, 73, 75, 77, 79 and 91 for virus production as well (by plaque assay). The study completed on Study Day 91 (30 days after challenge).

6.1.3 Population

The population consists of male and female cynomolgus macaques approximately 2 years of age on Study Day 0, free of clinical signs of disease or malformation, and testing negative for poxvirus antibodies and Herpes B virus.

6.1.4 Study Treatments or Agents Mandated by the Protocol

The three treatment arms, lot numbers, and test material concentrations are given as follows:

- ACAM2000 (Lot No. VV03-015A): 4.4 x 10^8 pfu/mL
- Dryvax (Lot No. WI-100955): 1.5 x 10^8 pfu/mL
- Negative control/ACAM2000 diluent (Lot No. DV03-011): N/A

All inoculations were given via the percutaneous (scarification) route, with a minimum of 15 jabs to the subscapular region using a bifurcated needle.

Challenge material: 0.5 mL containing a total of 3.8 x 10^7 pfus of Monkeypox strain Zaire 79 virus (CDC V79-I-005, Battelle Lot No. ACAM424-C) in HEPES buffer, infused into femoral vein in a single intravenous injection.

6.1.6 Sites and Centers

Battelle Memorial Institute, Medical Research and Evaluation Facility (MREF)

6.1.7 Surveillance/Monitoring

N/A.

6.1.8 Endpoints and Criteria for Study Success

The following endpoints (in no order) were studied in T-400-001:

- Reactogenicity
    - Area of erythmia and/or central lesion by day.
    - Lesion appearance scores.
- Neutralizing antibody levels, based on the 50% Vaccinia Plaque Reduction Neutralization test ($PRNT_{50}$), on days 0, 30, 60 (pre-challenge), 91, and on a terminal/final blood sample for any moribund or sacrificed animal.
- Survival following challenge.
- Temperature by day, starting from day of challenge (day 61).

- Clinical chemical parameter measurements on days 0, 60, 67, 73, and 79.
- Clinical hematology parameter measurements on days 0, 60, 67, 73, and 79.
- Lesion counts and lesion classification, following challenge.
- Viremia, as measured in:
    - Peripheral blood mononuclear cells (PMBCs).
    - Plasma/serum.
    - Saliva and/or throat swabs (viral shedding).

There are no prespecified study success criteria, and the nonclinical study is descriptive in nature.

6.1.9 Statistical Considerations & Statistical Analysis Plan

After obtaining the monkeys, a 6-week quarantine period was initiated. Randomization occurred during the quarantine period. Monkeys were stratified into two groups by sex (i.e., 12 male and 12 female) prior to randomization. Within each group, monkeys were then ranked according to their measured weights during quarantine. Monkeys were then assigned to the treatment groups in a randomized block design (3 blocks of 4 monkeys/sex/group).

In the protocol summary, the primary scientific hypothesis is that cynomolgus monkeys inoculated with either ACAM2000 or Dryvax will be protected against a lethal mpox challenge. Based on the proposed expanded indication to protection against mpox disease, the most relevant endpoints are survival after mpox challenge, immunogenicity after vaccination, viremia after challenge, and lesion counts/scores following challenge.

Statistical reviewer comment: *The protocol states that the "primary study variable is the evaluation and comparison of immunogenicity and protective activity of ACAM2000 compared with Dryvax and negative control material." Additionally, the protocol states, "[v]accine success will be defined as survival, reduction in clinical signs, lack of development of pox lesions (or a significant reduction in pock counts), or control of viremia as measured by plaque assay of PBMCs in the vaccinated groups compared to controls." This study does not formally list endpoints based on a primary, secondary, or exploratory classifications of objectives, nor are statistical hypotheses formally written; this approach is not atypical of preclinical animal studies. We consider this study to be descriptive in nature.*

A one-sided 0.05 type I error was used for comparative analyses of survival, viremia, and viral shedding proportions. Two-sided tests (with type I error equal to 0.05) were used for immunogenicity analyses. No multiplicity adjustment was made for the survival, immunogenicity, and viremia and virus shedding endpoints. For survival and viremia/viral shedding endpoints, the statistical methods were pre-specified in the protocol. For immunogenicity endpoints, the statistical methods were very broadly pre-specified.

Due to hair growth, there were some missing observations for lesion counts/classifications for the control group, which did not impact any conclusions. No missing data imputations were performed.

Statistical methods:

- Survival after challenge: The proportions of monkeys surviving the challenge were compared between groups using Fisher's exact test (at one-sided 0.05 level).
- Immunogenicity: GMTs were based on the mean of natural log-transformed titer values, which were then back-transforming to the original scale. The 95% CIs were estimated based on back-transforming (exponentiating) the lower and upper limits of the log-transformed 95% CIs based on the t distribution. The 95% CIs for geometric mean titer ratios (GMTRs) were based on calculating the confidence interval for the mean difference in the natural log-transformed titers between the ACAM2000 and Dryvax groups using a two-sample t-test assuming equal variance in the two groups, and then transforming back to the original scale. These methods assume that the natural log-transformed titers are normally distributed.

Statistical reviewer comment: *The 95% CIs for GMTs were calculated for Days 60 and 91 in ACAM2000 and Dryvax groups (Appendix J, statistical report). For Day 30 (Appendix H), no 95% CI was provided. The 95% CIs for GMTR between ACAM2000 and Dryvax was only provided for Day 91. The applicant stated that differences between ACAM2000 and Dryvax for Day 30 and Day 60 were based on rank-transforming titers (Appendix H); sufficient detail for the approach was not given. Nevertheless, this does not appear to impact the overall conclusion.*

- Viremia and viral shedding: Fisher's exact test (one-sided) was used to compare the proportions of monkeys with viremia or viral shedding on any day post-challenge between ACAM2000 and the negative control group. Additionally, the study results included the mean maximum, mean duration (in days), and mean Area Under the Curve (AUC) for virus.

Statistical reviewer comment: *Several analyses were conducted for viremia and viral shedding, including comparisons for each day. This review memo focuses on the proportion of monkeys in each group with viral shedding or viremia occurring at any point over the course of the study, as there was no viremia or viral shedding in the ACAM2000 group on any day after challenge. In general, the analyses of this study are descriptive in nature.*

- Lesion counts: The analysis of lesions was descriptive, as there were lesions in the negative control group only. Severity of lesions followed a World Health Organization (WHO) scale, classifying lesions by number of lesions in each location as None (<5), Mild (5-25), Moderate (26-100), Severe (101-250), and Grave (>250).

Statistical reviewer comment: *The protocol only specified that lesion counts were assumed to follow a Poisson distribution and that appropriate statistical methods would be used to compare counts between vaccine groups. In practice, there were no lesions in*

either ACAM2000 or Dryvax groups, and lesions were described according to the above classification in the control group.

6.1.10 Study Population and Disposition

6.1.10.1 Populations Enrolled/Analyzed

Table 1 summarizes baseline characteristics on Day 0 (just prior to vaccination, following quarantine period) for each of the 24 monkeys:

Table 1: Summary of baseline (Day 0) characteristics for monkeys in Study T-400-001

Animal ID	Treatment	Sex	Weight (kg)	Titer
CO9580	ACAM2000	M	2.5	< 10
CO9555	ACAM2000	M	2.6	< 10
19785	ACAM2000	M	2.6	< 10
19772	ACAM2000	M	2.8	< 10
CO9473	ACAM2000	F	2.5	< 10
CO8421	ACAM2000	F	2.8	< 10
CO8416	ACAM2000	F	2.7	< 10
19702	ACAM2000	F	2.5	< 10
19738	Dryvax	M	2.4	< 10
19749	Dryvax	M	2.5	< 10
CO9551	Dryvax	M	2.7	< 10
19765	Dryvax	M	2.5	< 10
19578	Dryvax	F	2.2	< 10
19690	Dryvax	F	2.4	< 10
19726	Dryvax	F	2.1	< 10
19717	Dryvax	F	2.6	< 10
CO9557	Control	M	2.8	< 10
CO9568	Control	M	2.6	< 10
19742	Control	M	2.3	< 10
19781	Control	M	2.9	< 10
CO9472	Control	F	2.5	< 10
CO8579	Control	F	3.2	< 10
19679	Control	F	2.3	< 10
19727	Control	F	2.7	< 10

Source: Adapted from T-400-001 Final Report, Table IX (Page A-13), Table XII (Page A-16).

All PRNT50 values were under the lower limit of quantification (LLOQ) (< 10 titers) prior to vaccination (seronegative) as expected.

Statistical reviewer comment: *The study report notes that weights were between 2.1 and 2.8 kg at time of randomization. On Day 0, two monkey weights were above 2.8 kg in the negative control group. Additionally, on Study Day 0, the mean weight in the Dryvax*

group (Mean = 2.425; SD = 0.2) was lower than the mean weight in the ACAM2000 group (Mean = 2.625; SD = 0.13), with a mean difference of -0.2 kg (95% CI: -0.38 to -0.02), using a two-sample t-test. This minor difference in weights was not likely to have impacted any substantive conclusions drawn, particularly in comparing ACAM2000 to the negative control for the endpoints relevant to the expanded indication.

6.1.10.1.3 Subject Disposition
All monkeys were challenged and assessed for safety, immunogenicity, and efficacy endpoints as per protocol.

6.1.11 Efficacy/Immunogenicity Analyses

Table 2 describes the main immunogenicity and protective efficacy results of ACAM2000.

Table 2: T-400-001 assessments of survival, immunogenicity, virus shedding, viremia, and lesions by vaccine group

Endpoint	ACAM2000	Dryvax	Control
Proportion surviving challenge n/N (%)	8/8 (100%)	8/8 (100%)	0/8[a]
Neutralizing antibody titers on day 30 (pre-challenge) GMT (95% CI)[b]	160 (86 to 297)	174 (85 to 359)	5 (N/A)[c]
Neutralizing antibody titers on day 60 (pre-challenge) GMT (95% CI)	174 (91 to 335)	190 (126 to 287)	5 (N/A)[c]
Neutralizing antibody titers on day 91 (post-challenge) GMT (95% CI)	43782 (24725 to 77529)	46072 (37470 to 56649)	N/A[d]
Proportion with detectable viral shedding on any day post-challenge (throat swab) n/N (%)	0/8 (0%)	3/8 (37.5%)	8/8 (100%)
Proportion with detectable viremia on any day post-challenge (PBMC) n/N (%)	0/8 (0%)	0/8 (0%)	6/8 (75%)
Proportion with detectable viremia on any day post-challenge (plasma/serum) n/N (%)	0/8 (0%)	0/8 (0%)	6/8 (75%)
Lesions, post-challenge n/N (%)	0/8 (0%)	0/8 (0%)	8/8 (100%)

Source: T-400-001 Final Report, pages 45-47, 54-56.
a: Five of the 8 monkeys were euthanized in the control group between 6 and 8 days after challenge.
b: 95% CIs based on reviewer's own calculations for Day 30.
c: All 8 neutralizing titers for control group remained below the LLOQ (10).
d: All 8 monkeys in the control group were dead or euthanized 6 to 8 days after challenge.

Survival

All monkeys in the ACAM2000 and Dryvax groups survived, and none of the monkeys in the negative control group survived. Fisher's exact test was performed with a reported p-value of < 0.0001 for the comparison of survival ratios between ACAM2000 and the negative control, and Dryvax and the negative control. There was no significant difference between the two vaccine groups in survival. However, the study was likely not powered for detecting smaller differences in survival between ACAM2000 and Dryvax.

Immunogenicity

GMTs for the negative control group remained under the LLOQ (< 10) on Days 30 and 60 after vaccination. The GMTs for ACAM2000 and Dryvax on Day 30 were 160 (95% CI: 86 to 297) and 174 (95% CI: 85 to 359), respectively. The GMTs on Day 60 (post-

vaccination, pre-challenge) were 174 (95% CI: 91 to 335) and 190 (95% CI: 126 to 287), respectively. The GMTR between ACAM2000 and Dryvax was 0.92 on Day 30 (95% CI: 0.39 to 2.17; p-value = 0.8326) and 0.92 on Day 60 (95% CI: 0.46 to 1.84; p-value = 0.7942). Seroconversion (defined as achieving ≥4-fold increase in titer from Day 0, i.e. titers of 20 or greater post vaccination since all monkeys were seronegative at Day 0) occurred in all (100%) ACAM2000 and Dryvax monkeys. On Day 91, the last day of the study, the GMTs for ACAM2000 and Dryvax were 43782 (95% CI: 24725 to 77529) and 46072 (95% CI: 37470 to 56649), respectively. The GMTR between ACAM2000 and Dryvax on Day 91 was 0.95 (95% CI: 0.55 to 1.65; p-value = 0.8456).

Statistical reviewer comment*: The report gives p-values of 0.8473 and 0.6505 (compared to 0.8326 and 0.7949 I reported above) for the geometric mean titer ratio between ACAM2000 and Dryvax on Days 30 and 60, respectively. The study report's p-values for Days 30 and 60 are based on the statistical analysis described in Appendix H. In Appendix H, rather than log-transforming GMTs and performing a two-sample t-test, antibody titers were rank-transformed. Given that the Day 30 immunogenicity is not of particular interest and the conclusions stay despite the difference in these two approaches, this difference in analysis method was not further pursued. In Appendix J, analyses of Day 60 and Day 91 data were based on log-transformed titers (GMTs and 95% CIs), as described above. Appendix J only reports the Day 91 GMTR between ACAM2000 and Dryvax.*

Viremia

In viremia analyses, no virus was detected in either vaccine group for peripheral blood mononuclear cells (PBMCs, for the cellular portion of cells) or plasma/serum samples (for non-cellular portion), compared to 6 of the 8 (i.e., 75%) control animals. The p-values from Fisher's exact test (one-sided) for comparisons between ACAM2000 and the negative control were 0.0035.

For oral virus shedding (throat swab samples), no monkey in the ACAM2000 group had any detectable measurement. Three (37.5%) in the Dryvax group had very low levels of virus shedding detected, compared to all 8 (100%) in the control group with virus shedding detected. The one-sided p-value was <0.0001 for the comparison of ACAM2000 to the negative control group based on Fisher's exact test.

Statistical reviewer comments*:*

- *Table XIII of the study report, as well as the results section of the study report, notes that 6 of 8 control monkeys had detectable virus in PBMC samples, for a one-sided p-value of approximately 0.0035 comparing ACAM2000 to the negative control group. The statistical report (Appendix J, Study No. 424-G004985, Tables 3-4) presents a proportion of 88% overall, or 7 of 8 monkeys, with detectable virus in the negative control group in PBMC samples post-challenge, for a one-sided p-value of approximately 0.0007. Regardless, the conclusion that ACAM2000 appears to protect against monkeypox virus replication remains valid based on either the main results or the statistical report, as 0 of 8 in the ACAM2000 had detectable virus in PBMC samples.*

- *For the proportion viremic based on plasma/serum, the main study results show 6 of 8 in the negative control group with detectable virus, which implies a one-sided p-value of 0.0035 for ACAM2000 as compared to the negative control group using Fisher's exact test. The statistical report in Appendix J suggests 5 of 8 in the negative control group with detectable virus, with a p-value of 0.0128. Similarly, the conclusion that ACAM2000 appears to protect against monkeypox virus replication remains valid based on either the main results or the statistical report, as 0 of 8 in the ACAM2000 had detectable virus in PBMC samples.*
- *Both the tables in the original BLA's pharmacology written summary (Table 2.6.2.2.3-3) and nonclinical overview (Table 2.4.2.4-1) documents suggest 5 of the 8 control monkeys had detectable virus in plasma or PBMC samples. The descriptions in these documents, however, indicate 6 of the 8 control monkeys had detectable virus in plasma or PBMC samples. The clinical information amendment (Module 1.6.2, Table 1) of the supplement suggests 6 of the 8 control monkeys had detectable virus in either PBMC or plasma, as it references results in the main study report. As discussed above, these discrepancies do not impact the conclusions drawn about viremia in ACAM2000.*
- *For the proportion with viral shedding (throat swab samples), the main study results show 8/8 in the negative control group, 3/8 in the Dryvax group, and 0/8 in the ACAM2000 group, while the statistical report suggests 1/8 in the Dryvax group had viral shedding. There is no disagreement for the ACAM2000 or negative control groups between the main study results and the statistical appendix, thus no conclusions relevant to the evaluation of ACAM2000 as compared to the negative control are impacted. The p-value reported in the statistical report for ACAM2000 vs. negative control is 0.0002 (page 335, Table 8), which appears to correspond to a two-sided p-value rather than a one-sided p-value from Fisher's exact test. The correct one-sided p-value is 7.77×10^{-5}.*

Lesions

No animal in the two vaccinated groups developed lesions. One animal in the ACAM2000 group and two animals in the Dryvax group had small, rash-like bumps on the legs on Day 66 that resolved by Day 68 and did not develop into pox-like lesions. Another animal in the Dryvax group had small, rash-like bumps on the back of the leg on Day 68 which resolved by Day 70 and did not develop into pox-like lesions. The development of lesions was substantial (varying by area on body) in the control group following challenge, starting on Day 66. On Day 66, all 8 negative control group monkeys had a moderate (26-100 count, 5 of 8) or severe (101-250 count, 3 of 8) classification of lesions on the face. Lesions on the legs, arms, chest, and back were also common (ranging from 5-25 to 101-250 counts). Of the 6 negative control group monkeys who survived until Day 68, several monkeys had a count of greater than 250 lesions for several areas of the body, including 4 of 6 for the face, legs and back, 3 of 6 for the arms and chest, and 2 of 6 for oral lesions (only counted on Day 68).

10. CONCLUSIONS

10.1 Statistical Issues and Collective Evidence

The small, descriptive nonclinical study T-400-001 indicates that ACAM2000 was effective in preventing mpox in NHPs as compared to the negative control group in response to an MPXV challenge. The geometric mean titers for the ACAM2000 and Dryvax groups were broadly similar both prior to and after challenge. All ACAM2000 and Dryvax monkeys survived the challenge, while none of the negative control group monkeys survived. The statistical analyses were generally appropriate. There were some discrepancies related to the proportions of monkeys in the control group or Dryvax group who were viremic or had viral shedding after challenge between the study report and the statistical report, but the conclusion that ACAM2000 appears to protect against monkeypox virus replication remains the same based on either the main results or the statistical report, i.e. there is no impact on the study conclusion.

10.2 Conclusions and Recommendations

There were no major statistical issues with the design or analysis of study T-400-001 for the endpoints relevant to the expanded indication. I defer to the clinical reviewer on the rationale and acceptability of extrapolating from NHPs to humans for evaluating the expanded indication to the prevention of mpox.