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LETTER OF AMENDMENT

DATE: June 6, 2024

TO: ACTG CTU Principal Investigators, CRS Leaders, and CTU/CRS Coordinators

FROM: A5355 Protocol Team

SUBJECT: Letter of Amendment #2 for Protocol A5355 Version 2.0

The following information affects the A5355 study and must be forwarded to your institutional review board (IRB)/ethics committee (EC) as soon as possible for their information and review. This Letter of Amendment (LOA) must be approved by your IRB/EC before implementation.

The following information may also affect the Sample Informed Consent. Your IRB/EC is responsible for determining the process of informing participants of the contents of this LOA.

Upon receiving final IRB/EC and any other applicable regulatory entity approvals for this LOA, sites should implement the LOA immediately. Sites are still required to submit an LOA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center. Sites will receive a registration notification for the LOA once the DAIDS PRO verifies that all required LOA registration documents have been received and are complete. An LOA registration notification from the DAIDS PRO is not required prior to implementing the LOA. A copy of the LOA registration notification, along with this letter and any IRB/EC correspondence, should be retained in the site's regulatory file.

The following are changes (noted in bold or strikethrough) to A5355, Version 2.0, dated 03Feb2023, titled "Phase II, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Modified Vaccinia Ankara (MVA)-based anti-Cytomegalovirus (CMV) Vaccine (Triplex), in Adults with Both Human Immunodeficiency Virus (HIV)-1 and CMV Who Are on Potent Combination ART with Conserved Immune Function". These changes will be included in the next version of the A5355 protocol if it is amended at a future date.

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1. SECTION 1.0, HYPOTHESIS AND STUDY OBJECTIVES

1.1 Primary Hypotheses

1.1.1 Safety Two injections of MVA Vaccine Encoding CMV Antigens (Triplex) administered according to a 4-week, two-injection schedule will be safe over 48 weeks.

~~1.1.2 Cellular immunogenicity Anti-CMV CD8+ T cell responses (measured as pp65-specific CD137+ CD8+ T cells) between Day 0 and study week 12 will increase in participants receiving the active vaccine, compared to placebo.~~

1.1.2 ~~1.1.3~~ Inflammation Blood plasma levels of soluble receptors for tumor necrosis factor type II (sTNFRII) will decrease over the first 48 weeks in participants receiving the active vaccine, compared to placebo.

1.2 Secondary Hypotheses

1.2.1 Inflammation The blood plasma levels of selected soluble inflammatory biomarkers (including but not limited to IL-6, sCD163, IP-10, sTNFRII, D-Dimers) at study weeks 12, 24, 48, and 72 will decrease in participants receiving the active vaccine, compared to placebo. Inflammation might temporarily increase early after vaccine administration.

~~1.2.2 Cellular immunogenicity (IE1 and IE2) Anti-CMV CD8+ T cell responses (measured as IE1 and IE 2 -specific CD137+ CD8+ T cells) between Day 0 and study week 12 will increase in participants receiving the active vaccine, compared to placebo.~~

~~1.2.3 Prolonged cellular immunogenicity The increase in anti-CMV CD8+ T cell responses (pp65, IE1 and IE2) will be higher in participants receiving the active vaccine, compared to placebo, over the first 48 weeks (area under the curve).~~

1.2.2 ~~1.2.4~~ CMV DNA shedding Vaccination will reduce the frequency and levels of CMV DNA in peripheral blood mononuclear cells (PBMC), urine, genital secretion, and oral secretion at study week 12, 48, and 72 in participants receiving the active vaccine compared to participants receiving placebo.

1.2.3 ~~1.2.5~~ Prolonged safety Two injections of MVA Vaccine Encoding CMV Antigens (Triplex) administered according to a 4-week, two-injection schedule will be safe over the 96-week study period.

~~**1.2.4** 1.2.6 Detection of persistence of MVA DNA after immunizations Viral DNA derived from the recombinant MVA vaccine will decay in blood and will be undetectable by study week 12.~~

1.3 Exploratory Hypotheses

1.3.1 Cellular immunogenicity Anti-CMV CD8+ T cell responses (measured as pp65-specific CD137+ CD8+ T cells) between Day 0 and study week 12 will

increase in participants receiving the active vaccine, compared to placebo.

1.3.2 **Cellular immunogenicity (IE1 and IE2) Anti-CMV CD8+ T cell responses (measured as IE1- and IE 2 -specific CD137+ CD8+ T cells) between Day 0 and study week 12 will increase in participants receiving the active vaccine, compared to placebo.**

1.3.3 **Prolonged cellular immunogenicity The increase in anti-CMV CD8+ T cell responses (pp65, IE1, and IE2) will be higher in participants receiving the active vaccine, compared to placebo, over the first 48 weeks (area under the curve).**

1.3.4 **Detection of persistence of MVA DNA after immunizations Viral DNA derived from the recombinant MVA vaccine will decay in blood and will be undetectable by study week 12.**

1.3.5 ~~1.3.1~~ Inflammation The blood plasma levels of soluble inflammatory biomarkers other than those listed as secondary hypothesis (including but not limited to IL-18, IL-7, sCD14) at study weeks 12, 24, 48, and 72 will decrease in participants receiving the active vaccine, compared to placebo. Inflammation might temporarily increase early after vaccine administration.

1.3.6 ~~1.3.2~~ T cell dysfunction Participants completing the vaccination series will have less evidence for T cell dysfunction (including but not limited to, less activation, proliferation, and exhaustion of T cells) in their blood at study weeks 12 and 48, and 72 when compared to those receiving placebo.

1.3.7 ~~1.3.3~~ Quality of cellular responses (pending external funding) Broad and maintained anti-CMV T cell receptor (TCR) diversity in response to the immunogen will be observed post-vaccination at 12 and 48 weeks, as opposed to lower quality responses resulting from narrowing of the TCR repertoire due to expansion of limited anti-CMV TCRs present at Day 0.

1.3.8 ~~1.3.4~~ HIV Reservoir (pending external funding) Vaccination will reduce the size and transcriptional activity of the HIV DNA reservoir at study weeks 12, 24, 48, and 72 in participants receiving the active vaccine compared to participants receiving placebo.

1.3.9 ~~1.3.5~~ Microbiome (pending external funding) The rectal microbiome composition will predict the magnitude of the vaccine immune response.

1.3.10 ~~1.3.6~~ Substance use Use of non-prescribed stimulatory drugs (e.g., cocaine, methamphetamine) will be associated with increased immune activation in both arms.

1.4 Primary Objectives

1.4.1 Safety To determine whether a 2-injection regimen of MVA Vaccine Encoding CMV antigens is safe (over 48 weeks).

~~1.4.2 Cellular immunogenicity To determine the anti-CMV CD8+ T cell responses (pp65) in participants receiving the active vaccine versus placebo (week 12).~~

1.4.2 ~~1.4.3~~ Inflammation To determine whether the active vaccine decreases inflammation, as measured by sTNFRII, compared to placebo (week 48).

1.5 Secondary Objectives

1.5.1 Inflammation To determine if anti-CMV vaccination reduces plasma levels of selected soluble inflammatory biomarkers (e.g., IL-6, sCD163, IP-10, TNFRII, D-Dimers).

~~1.5.2 Cellular immunogenicity (IE1 and IE2) To determine the anti-CMV CD8+ T cell responses (IE1 and IE2) in participants receiving the active vaccine versus placebo (week 12).~~

~~1.5.3 Prolonged cellular immunogenicity To determine the anti-CMV CD4+/CD8+ T cell immune responses (pp65, IE1 and IE2) to vaccine in participants over 48 weeks of follow-up.~~

1.5.2 ~~1.5.4~~ CMV DNA shedding To determine if vaccination influences the shedding of CMV DNA in PBMC, oral secretion, genital secretion, and urine.

1.5.3 ~~1.5.5~~ Prolonged safety To determine whether a 2-injection regimen of MVA Vaccine encoding CMV antigens is safe over 96 weeks of follow-up.

1.5.4 ~~1.5.6 Detection of persistence of MVA DNA after immunizations To determine the persistence of viral DNA derived from MVA vaccine in blood specimens.~~

1.6 Exploratory Objectives

1.6.1 Cellular immunogenicity To determine the anti-CMV CD8+ T cell responses (pp65) in participants receiving the active vaccine versus placebo (week 12).

1.6.2 Cellular immunogenicity (IE1 and IE2) To determine the anti-CMV CD8+ T cell responses (IE1 and IE2) in participants receiving the active vaccine versus placebo (week 12).

1.6.3 Prolonged cellular immunogenicity To determine the anti-CMV CD4+/CD8+ T cell immune responses (pp65, IE1 and IE2) to vaccine in participants over 48 weeks of follow-up.

1.6.4 Detection of persistence of MVA DNA after immunizations To determine the persistence of viral DNA derived from MVA vaccine in blood specimens.

1.6.5 ~~1.6.1~~ Inflammation To determine if anti-CMV vaccination reduces plasma levels of soluble inflammatory biomarkers (other than those listed in section 1.2.1).

1.6.6 ~~1.6.2~~ T cell dysfunction To determine if anti-CMV vaccination reduces markers of T cell dysfunction.

1.6.7 ~~1.6.3~~ Quality of cellular responses (pending external funding) To determine the quality of the anti-CMV T cell response (i.e., the breadth and persistence of the anti-CMV TCR repertoire) induced by vaccination at weeks 12 and 48 as compared to Day 0.

1.6.8 ~~1.6.4~~ HIV Reservoir (pending external funding) To determine if vaccination influences the size and transcriptional activity of the HIV reservoir.

1.6.9 ~~1.6.5~~ Microbiome (pending external funding) To determine if the composition of the rectal microbiome predicts the magnitude of the vaccine immune response.

1.6.10 ~~1.6.6~~ Substance use To determine if stimulatory drugs influence immune activation in both arms.

2. SECTION 10.2, Outcome Measures

10.2.1 Primary Outcome Measures

10.2.1.1 Safety Occurrence of Grade ≥3 AEs over 48 weeks.

~~10.2.1.2 Cellular immunogenicity Change in pp65-specific CD137+ CD8+ T cells from Day 0 to Week 12.~~

10.2.1.2 ~~10.2.1.3~~ Inflammation Change in sTNFRII from Day 0 to Week 48.

10.2.2 Secondary Outcome Measures

10.2.2.1 Inflammation Change from Day 0 to Weeks 12, 24, 48, and 72 in levels of inflammatory biomarkers (including but not limited to IL-6, sCD163, IP-10, sTNFRII, D-Dimers).

~~10.2.2.2 Cellular immunogenicity Change in IE1 and IE2-specific CD137+ CD8+ T cells from Day 0 to Week 12.~~

~~10.2.2.3 Prolonged cellular immunogenicity Change in the percent of pp65-, IE1- and IE2-specific CD137+ CD8+ T cells over 48 weeks.~~

10.2.2.2 ~~10.2.2.4~~ CMV DNA shedding CMV DNA in PBMC, urine, and genital secretion, oral secretions at Weeks 12, 48, and 72.

~~**10.2.2.3** 10.2.2.5 Detection of persistence of MVA DNA after immunizations: Viral DNA from recombinant MVA vaccine at Week 12.~~

10.2.3 Other Outcome Measures

10.2.3.1 Cellular immunogenicity Change in pp65-specific CD137+ CD8+ T cells from Day 0 to Week 12.

10.2.3.2 **Cellular immunogenicity Change in IE1 and IE2-specific CD137+ CD8+ T cells from Day 0 to Week 12.**

10.2.3.3 **Prolonged cellular immunogenicity Change in the percent of pp65-, IE1- and IE2-specific CD137+ CD8+ T cells over 48 weeks.**

10.2.3.4 ~~10.2.2.5~~ **Detection of persistence of MVA DNA after immunizations: Viral DNA from recombinant MVA vaccine at Week 12.**

10.2.3.5 ~~10.2.3.1~~ Inflammation Change from Day 0 to Weeks 12, 24, 48, and 72 in levels of inflammatory biomarkers (including but not limited to IL-18, IL-7, sCD14).

10.2.3.6 ~~10.2.3.2~~ T cell dysfunction Change from Day 0 to Weeks 12, 48, and 72 in levels of T cell immune activation, proliferation, and exhaustion as well as CD4+/CD8+ T cell ratio.

10.2.3.7 ~~10.2.3.3~~ Prolonged safety Occurrence of Grade ≥3 AEs over 96 weeks.

10.2.3.8 ~~10.2.3.4~~ Quality of cellular responses (pending external funding): Frequency of unique anti-CMV T cell receptors at Day 0 and at Weeks 12 and 48.

10.2.3.9 ~~10.2.3.5~~ HIV Reservoir (pending external funding) Change from Day 0 to Weeks 12, 24, 48, and 72 in the size and transcriptional activity of the HIV DNA reservoir.

10.2.3.10 ~~10.2.3.6~~ Microbiome (pending external funding) Change from Day 0 to Weeks 12 and 48 in the composition of the microbiome and possible effect of the microbiome to the magnitude of the immune response.

3. SECTION 10.4, Sample Size and Accrual

10.4.1 **Inflammation** ~~Cellular Immunogenicity~~

The first co-primary objective is to determine if CMV-MVA Triplex influences inflammation. We hypothesize that it will significantly decrease sTNFRII when compared to placebo. Past ACTG trials have consistently shown an sTNFRII change SD between 0.15 and 0.20 $\log_{10}$ pg/mL over treatment and follow-up periods of varying duration.

Using a two-sample t-test, the change SD of 0.20 $\log_{10}$ pg/mL, and a two-sided 5% alpha, 72 participants (48 vaccine, 24 placebo), we will have 80% power to detect a 0.142 $\log_{10}$ pg/mL difference between the arms. A reduction of 0.142 $\log_{10}$ pg/mL corresponds to a 27% reduction in the geometric mean fold change compared to placebo. Per NWCS 329, each $\log_{10}$ increase in sTNFRII levels was associated with a 13.6-fold increased odds of a non-AIDS event

(using year 1 biomarker assessment), which corresponds to a 44% increased odds of a non-AIDS event for each 0.14 log_{10} increase in sTNFRII. Table 10.4.1-1 provides detectable effect size for various power and SD estimates.

~~The first co-primary objective is to determine if CMV-MVA Triplex influences CMV-specific immune responses. We hypothesize that CMV-MVA Triplex will significantly increase pp65-specific CD137$^+$ CD8$^+$ T cells compared to participants vaccinated with placebo. Published data from a Phase I vaccine trial [22] was used to estimate pp65-specific CD137$^+$ CD8$^+$ T cell distributions at Days 0 and 42. Using the minimum, median, and maximum values, and variance estimation methods [57], we assumed a standard deviation of **0.70** cells/μL at **Day 0** and **2.0** cells/μL at Day 42. For change in pp65-specific CD137$^+$ CD8$^+$ T cells we assumed a correlation between time points of 0.5 and thus a change standard deviation (SD) of 1.7 cells/μL. Because these SD estimates came from a small sample, our SD was further inflated [58] by 80% to 3.06 cells/μL.~~

~~Using a two-sample t-test, the change SD of **0.20 log_{10} pg/mL** 3.06 cells/μL and a would provide 80% power to detect a 2.2 cells/μL difference between the arms. This effect size corresponds to the observed change over 42 days in the previously cited vaccine trial [22]. Table 10.4.1-1 provides detectable effect size for various power and SD estimates.~~

~~Notably, the sample size calculation determination of using 2.2 cells/μL was based on the generation of a CMV-specific response in both CMV-immune and non-immune recipients, and thus was skewed toward a lower value than we expect to observe here. Since all our study participants will be CMV seropositive, we do expect a stronger immune response.~~

To account for 20% of participants not being included in the primary analysis due to missing samples, laboratory error, LTFU, not meeting per protocol definition, etc., the sample size was inflated to 90 participants (60 vaccine, 30 placebo).

Table 10.4.1-1: Effect Size Detectable with 72 Participants (48 CMV-MVA Triplex, 24 placebo) per Protocol Participants for Varying Power and SD

	Power		
	80%	**85%**	**90%**
SD=0.150	**0.107**	**0.114**	**0.123**
SD=0.175	**0.124**	**0.133**	**0.144**
SD=0.200	**0.142**	**0.152**	**0.164**
SD=0.225	**0.160**	**0.171**	**0.185**
SD=0.250	**0.178**	**0.190**	**0.205**

	~~Power~~		
	~~80%~~	~~85%~~	~~90%~~
~~SD=2.50~~	~~1.8~~	~~1.9~~	~~2.1~~
~~SD=2.75~~	~~2.0~~	~~2.1~~	~~2.3~~

	~~Power~~		
	~~80%~~	~~85%~~	~~90%~~
~~SD=3.06~~	~~2.2~~	~~2.3~~	~~2.5~~
~~SD=3.25~~	~~2.3~~	~~2.5~~	~~2.7~~
~~SD=3.50~~	~~2.5~~	~~2.7~~	~~2.9~~

While this study is not fully powered to detect treatment effects within cisgender women or differential treatment effects between men and women, those analyses are of interest. It is also important to understand treatment effects among transgender and gender non-binary participants. Similar to above, we will not have sufficient power to detect treatment effects for each group, so we will conduct exploratory analyses between groups (cisgender men, transmen, cisgender women, transwomen, and gender non-binary).

~~10.4.3 Inflammation~~

~~The third co-primary objective is to determine if CMV-MVA Triplex influences inflammation. We hypothesize that it will significantly decrease sTNFRII when compared to placebo. Past ACTG trials have consistently shown an sTNFRII change SD between 0.15 and 0.20 $\log_{10}$ pg/mL over treatment and follow-up periods of varying duration. Assuming an SD of 0.20 $\log_{10}$ pg/mL, a Type I error of 5%, and an effective sample size of 72 participants (48 vaccine, 24 placebo), we will have 80% power to detect an effect size as small as 0.14 $\log_{10}$ pg/mL. A reduction of 0.14 $\log_{10}$ pg/mL corresponds to a 27% reduction in the geometric mean fold change compared to placebo. Per NWCS 329, each $\log_{10}$ increase in sTNFRII levels was associated with a 13.6-fold increased odds of a non-AIDS event (using year 1 biomarker assessment), which corresponds to a 44% increased odds of a non-AIDS event for each 0.14 $\log_{10}$ increase in sTNFRII.~~

~~These calculations are based on a two-sided two-sample t-test.~~

4. SECTION 10.6 Analyses

10.6.1 Primary Analyses

The primary efficacy analysis of the study will assess the effect of CMV-MVA Triplex on sTNFRII in participants with both HIV and CMV are well-controlled on ART. To address this, changes in sTNFRII from Day 0 to Week 48 will be compared between the CMV-MVA Triplex arm and the placebo arm by linear regression. For this model each participant will have a single outcome measure of sTNFRII change from Day 0 to Week 48. The predictor variables will be study arm, sex, and use of gender-affirming hormones (the stratification factor).

Three supplemental linear regression analyses will be performed. The first will additionally adjust for Day 0 sTNFRII values (continuous), while the second will assess differential CMV-MVA Triplex effects by Day 0 sTNFRII tertile by additionally adjusting for the sTNFRII tertile main effect and the study arm by sTNFRII tertile interaction. The third will perform the primary

analysis using the modified intent-to-treat (mITT) population.

For the primary safety analysis, Grade 3 or greater AEs will be summarized by treatment arm in the mITT population. The resulting difference in proportions and associated exact 95% CI will be provided.

~~The first primary efficacy analysis of the study will assess the effect of CMV-MVA Triplex on pp65-specific CD137+ CD8+ T cells in participants with both HIV and CMV are well-controlled on ART. To address this, changes in pp65-specific CD137+ CD8+ T cells from Day 0 to Week 12 will be compared between the CMV-MVA Triplex arm and the placebo arm by linear regression. For this model each participant will have a single outcome measure of pp65-specific CD137+ CD8+ T cell change from Day 0 to Week 12. The predictor variables will be study arm, sex and use of gender-affirming hormones (the stratification factor).~~

~~Three supplemental linear regression analyses will be performed. The first will additionally adjust for Day 0 pp65-specific CD137+ CD8+ T cell values (continuous), while the second will assess differential CMV-MVA Triplex effects by Day 0 pp65-specific CD137+ CD8+ T cell tertile by additionally adjusting for the pp65-specific CD137+ CD8+ T cell tertile main effect and the study arm by pp65-specific CD137+ CD8+ T cell tertile interaction. The third will perform the primary analysis using **the** modified intent-to-treat (mITT) population.~~

~~For the other primary efficacy analysis, which will assess the effect of CMV-MVA Triplex on sTNFRII change from Day 0 to Week 48, the same analysis approach will be used as with pp65-specific CD137+ CD8+ T cells above, including the three supplemental analyses.~~

10.6.2 Secondary Analyses

Similar to the initial primary analysis regression model, changes in other continuous outcome measures will be compared between the CMV-MVA Triplex and placebo arms by linear regression. For these models each participant will have a single outcome measure of change from Day 0 to Week 12. The predictor variables will be study arm, sex and gender-affirming hormones.

Additionally, for all continuous secondary outcomes and the primary outcome (**sTNFRII** ~~pp65-specific CD137+ CD8+ T cells~~), GEE models with an identity link will use change from Day 0 to all follow-up time points. These models will use appropriate correlation structures and splines if necessary.

For the CMV DNA shedding (yes/no) and Ki67 expression (<7%/≥7%) outcomes, GEE models with a logit link will examine Week 12, 48 and 72 data. Again, these models will use appropriate correlation structures and splines if necessary.

The secondary safety analysis of Grade 3 or greater AEs over 96 weeks will be summarized by treatment arm.

5. A Protocol Signature Page (PSP) is appended for submission to DAIDS Protocol Registration System (DPRS) as part of the LOA registration packet.

Phase II, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Modified Vaccinia Ankara (MVA)-based anti-Cytomegalovirus (CMV) Vaccine (Triplex), in Adults with Both Human Immunodeficiency Virus (HIV)-1 and CMV Who Are on Potent Combination ART with Conserved Immune Function

SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____
Print/Type

Signed: _____Date: _____
Name/Title