Date: August 1, 2024
To: FirstNameA LastNameA, FirstNameB LastNameB, FirstNameC LastNameC, FirstNameD LastNameD, FirstNameE LastNameE, FirstNameF LastNameF
From: FirstNameA LastNameA, FirstNameB LastNameB
RE: Title of Your Submission
cc: FirstNameA LastNameA, FirstNameB LastNameB, FirstNameC LastNameC, FirstNameD LastNameD, FirstNameE LastNameE
Contact: FirstNameA LastNameA, foo@bar.com

Contents

Overview

A short summary about your report.

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1 Summary of Main Results

Summarize the main highlights from the Results section. This can be in bullet format. Any significant results mentioned should include p-values and references to appropriate figures and tables. There should be no information in the Summary section that is not contained in the Results section (see 13).

2 Background

[CAVD PI and number] is a [insert study title].

Describe trial - product, primary hypothesis.

Describe participant enrollment - number per group planned and what those ppts received. If study was amended to reduce planned group sample sizes or study allowed for over/under enrollment such that number enrolled per group would not be the same as what is in the schema, mention it here and cite source.

Table 1: TEST000ADCCtemp study schema.

Group	Sample Size	Week 10	Week 20
Group A	10	Dose A	Dose A
Group B	10	Dose B	Dose B

Describe this report - "This report presents [blinded/unblinded] [list assay] data in [list groups, including descriptions] at [list timepoints and corresponding relationship to vaccination, e.g., week 0 (1st vaccination), week 10 (2 weeks post-2nd vaccination), and week 26 (2 weeks post-3rd (final) vaccination)] as of [ldo_processing_date]." Note that LDO processing date comes from qdata and pdata. Anything special about the report could also be mentioned here (e.g., This PT report presents neutralization data to Tier 1A and Tier 2 versions of the vaccine strain, assessed by the TZM-bl assay. Additionally this report summarizes epitope mapping isolate pairs.")

Reference test (Huang and Gottardo (2013)).

2.1 Report Amendments

If previous reports were provided, note if this report supplements or supersedes the previous reports. For example, "the previous PT report (distributed on DDMonthYYYY) presented peak data. This report summarizes additional durability data."

If this is an updated report, also briefly describe additional data included and/or analysis done since the previous report (e.g., additional visits, participants (include pubIDs), antigens, comparisons, new/changed tables, figures).

3 Objectives

List primary and secondary (if applicable) objectives. Objectives can be found on ATLAS, in the study protocol, or in the SAP.

4 Biological Endpoints

5 Biological Endpoints GTL

ADCC-mediated antibody responses were measured using Luciferase GranToxiLux (GTL) assays from specimens obtained at *[describe visits; include visit number, timepoint in weeks or months, and relation to SPA. E.g. week 26 (2 weeks post-4th vaccination, visit 10)]*. The GTL ADCC assay measures percent Granzyme B activity, defined as the percentage of antigen-coated target cells positive for proteolytically active Granzyme B out of the total viable target cell population. Endpoints are the response rate and magnitude of ADCC-mediated antibody responses against a panel of *[number of antigens]* HIV-1 antigens representing *[include description of viruses: those included in the vaccine product (vaccine-matched), Env matched in clade to vaccine products, and other Env to identify the breadth of the responses against HIV-1 subtypes]*.

6 Biological endpoints Luciferase

ADCC-mediated antibody responses were measured using Luciferase ADCC assays from specimens obtained at *[describe visits; include visit number, timepoint in weeks or months, and relation to SPA. E.g. week 26 (2 weeks post-4th vaccination, visit 10)]*. The Luciferase ADCC assay tests reactivity against Infectious Molecular Clone (IMC)-infected target cells by measuring percent reduction in Relative Luminescence Units (RLUs), reported as percentage specific killing. Endpoints are the response rate and magnitude of ADCC-mediated antibody responses against a panel of *[number of IMCs]* HIV-1 IMC expressing Env representing *[include description of IMCs: those included in the vaccine product (vaccine-matched), Env matched in clade to vaccine products, and other Env to identify the breadth of the responses against HIV-1 subtypes]*.

7 Lab Methods

8 Lab Methods GTL

The qualified GranToxiLux Antibody-Dependent Cell-Mediated Cytotoxicity (GTL-ADCC) assay was performed as previously described (Pollara et al. 2014). Target cells were a clonal isolate of the CEM.NKRCCR5 CD4+ T-cell line (NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: from Dr. Alexandra Trkola (Trkola et al. 1999). These cells were coated with recombinant gp120s representing the HIV-1 envelopes of the subtype [specify subtype and antigens, e.g. C (TV1 and 1086c)]. Effector cells were PBMCs obtained from a HIV-seronegative donor with heterozygous for FcγR3A at position 158 (158F/V). PBMCs were obtained by leukapheresis to collect enough cells for completion of the study with a single donation, minimizing potential effector cell population variability effects on the study outcome. PBMCs were used at an effector cell to target cell ratio of 30:1. Serum samples were tested after five-fold serial dilutions starting at 1:50 (1:50, 1:250, 1:1250, 1:6250, 1:31250, and 1:156250). Each plate has one standardized positive control in duplicate and one standardized negative control in duplicate.

ADCC is quantified as net percent granzyme B activity, which is the percent of target cells positive for GTL (an indicator of granzyme B uptake) minus the percent of target cells positive for GTL when incubated with effector cells in the absence of a source of antibodies. Flow cytometry is used to quantify

the frequency of granzyme B positive cells.

9 Lab Methods Luciferase

We utilized a modified version of a previously published ADCC luciferase procedure Fisher et al. (2019) . Briefly, CEM.NKRCCR5 cells (Trkola et al. 1999) were used as targets for ADCC luciferase assays after infection by one of the following HIV-1 [vaccine-matched, if all IMCs are vaccine-matched; if not, indicate match in table below] IMCs: Complete IMC name Accession Number Abbreviated name Vaccine Match [Full name, as uploaded by lab] [provided by lab] [SRA-derived abbreviated name shown in tables and figures] [if a mix of matched and unmatched IMCs were tested, indicate here which were matched]

Peripheral blood mononuclear cells (PBMCs) were obtained from a HIV-seronegative donor by leukapheresis and cryopreserved until the day of the assay. After thawing and overnight resting in RPMI 1640 supplemented with antibiotics, 10% fetal bovine serum (R10), and 10 ng/mL of IL-15, the PBMCs were used as effector cells at an effector-to-target ratio of 30:1.

Target and effector cells were plated in white 96-well half-area plates and co-cultured with 4-fold serial dilutions of trial participant serum starting at the 1:50 dilution. For each sample, percent specific killing was measured in duplicate at dilutions of 1:50, 1:200, and 1:800, 1:3200, 1:12800, and 1:51200. Co-cultures were incubated for 6 hours at 37°C in 5% CO2. The final readout was the reduction of luminescence intensity generated by the presence of residual intact target cells that had not been lysed by the effector population in the presence of ADCC-mediating serum antibodies. The percentage of killing was calculated using the formula: percent specific killing $= 100 * \frac{\text{RLU of target and effector well– RLU of test well)}}{\text{RLU of target and effector well}}$.

In this analysis, the Relative Luminescence Units (RLU) of the target plus effector wells represents spontaneous lysis in the absence of any source of antibody and is used to calculate background activity. The monoclonal antibody [insert antibody name from lab study plan, e.g. Synagis] and a cocktail of HIV-1 monoclonal antibodies [insert antibody names from lab study plan, e.g. (A32, 2G12, CH44, and 7B2)] were used as negative and positive controls, respectively.

10 Statistical Methods

10.1 Statistical Endpoints GTL

10.1.1 Peak activity

Peak net percent granzyme B activity defined as the maximum activity across the six dilution levels ("peak activity"). Peak activity less than 0% is set to 0%.

10.1.2 AUC

Area under the net percent granzyme B activity vs log10 (dilution) curve is ("AUC") calculated using the trapezoidal rule, setting any net percent granzyme B activity below 0% to 0%.

10.1.3 Response call

A positive response is defined as peak activity greater than or equal to 8%. Tables show positive response rates and corresponding 95% confidence intervals calculated by the Wilson score method (Agresti

and Coull 1998), as well as summary statistics among positive responders and both responders and non-responders [update as needed; must have a table of summary statistics for the same population as comparisons described below].

10.2 Statistical Endpoints Luciferase

10.2.1 Percent loss

Percent specific killing was averaged over wells within participant, timepoint, and dilution. Baseline-subtracted percent loss activity was calculated for each dilution as baseline activity subtracted from post-baseline activity. Negative values were truncated at zero.

10.2.2 Peak percent loss

Baseline-subtracted peak percent (%) specific killing was defined as the maximum baseline-subtracted activity across the six dilution levels.

10.2.3 AUC

Nonparametric partial Area under the baseline-subtracted curves ("pAUC"), calculated using the trapezoidal rule on three dilutions of the baseline-subtracted curves, setting baseline-subtracted percent specific killing less than 0% to zero.

10.2.4 Response call

A response is defined as positive if the peak baseline-subtracted % specific killing activity greater than or equal to 10% for either the 1:50 or 1:200 dilution.

11 Statistical Methods GTL and Luciferase

11.1 Graphical analysis

Plots of peak activity and AUC show both response rates and the distribution of magnitude. Positive responses are indicated by dots color-coded by treatment group, and negative responses by gray triangles. A boxplot is superimposed on the distribution, including only positive responses. The mid-line of the box denotes the median and the ends of the box denote the 25^{th} and 75^{th} percentiles. The whiskers that extend from the top and bottom of the box extend to the most extreme data points that are no more than 1.5 times the interquartile range (i.e., height of the box) or if no value meets this criterion, to the data extremes.

[If working with durability data and calculating fold change from peak, be sure to specify direction of difference, e.g. log10(durability visit) − log10(peak visit).]

11.2 Statistical tests

[If statistical tests are used to compare responses, describe here. Follow protocol and assay specific SAP, converting the language to past tense. Generally, response rates are compared between groups using

Barnard's test, and response are compared using Wilcoxon rank sum tests. Typically, two sets of magnitude comparisons are done: restricted to positive responders only, and all data i.e. positive and nonresponders.]

12 Participant Cohort

The study enrolled [describe the total number enrolled to date and, if unblinded, the number in each treatment arm]. Include a table with data availability by key variables and red highlights for counts that are less than expected. Refer to the table and comment on reasons for missing data if known.

13 Results

The results section addresses how each endpoint supports the main objectives. Include summary statistics and significant results as applicable, including p-values and table and figure references. The results section should provide supporting evidence for all statements made in the summary section.

13.1 Section 1

Consider breaking up the results section by objective or by statistical endpoint.

13.2 Section 2

Make sure to include p-values and references to relevant tables and figures. See Figure 1 and Table 2.

14 Figures and Tables

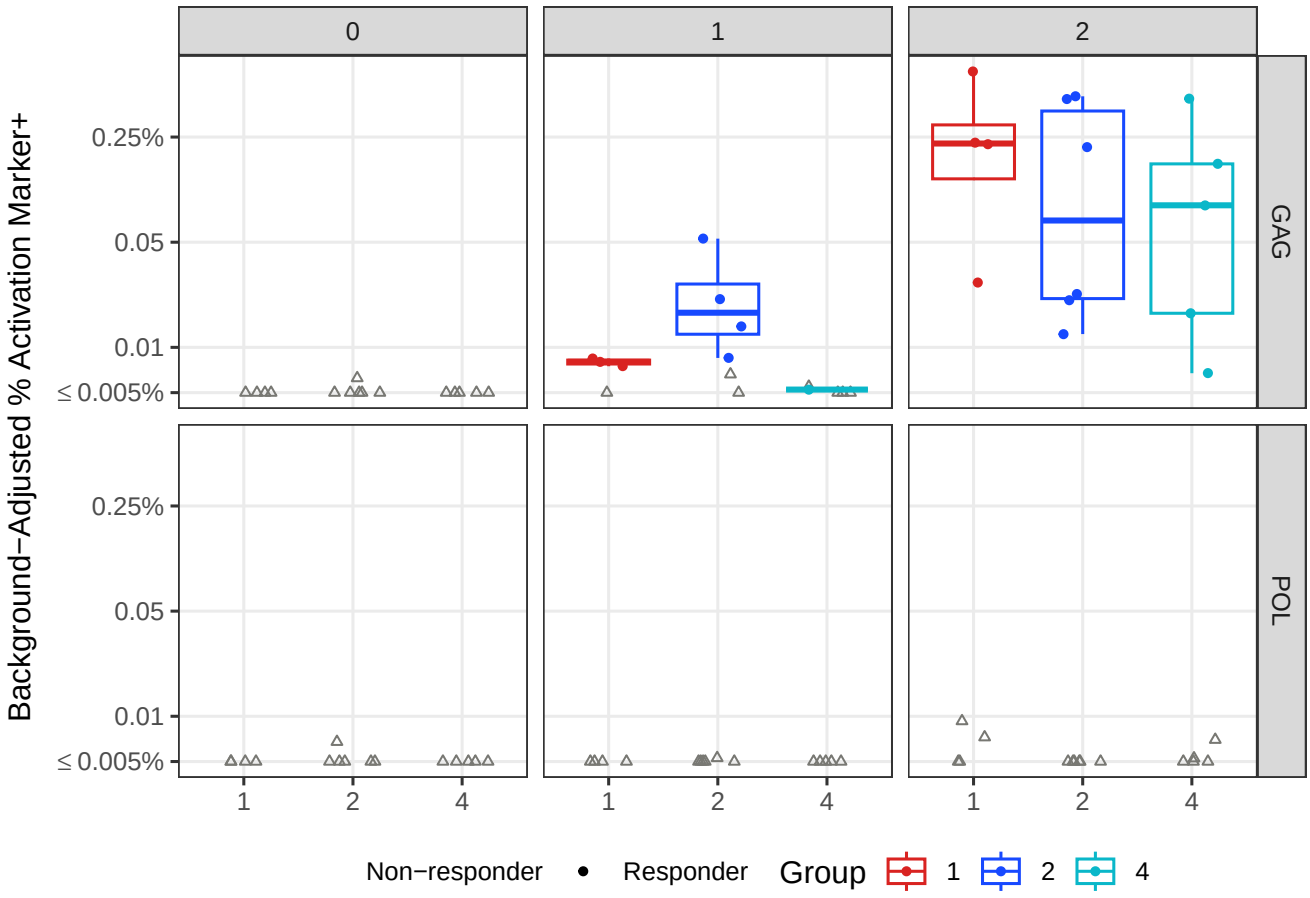

Figure 1: Longer caption that shows under the figure. Explain everything needed to understand the figure here.

Table 2: Long caption to show above table. Explain everything needed to understand the table here.

Stim	Visit	Comparison	SampleSizes	Median (Range)	Mean (SD)	MagnitudeTest
GAG	0	1 > 2	4 vs. 6	0.000 [0.000, 0.002] vs. 0.000 [0.000, 0.006]	0.001 (0.001) vs. 0.001 (0.003)	0.667
		1 > 4	4 vs. 5	0.000 [0.000, 0.002] vs. 0.000 [0.000, 0.000]	0.001 (0.001) vs. 0.000 (0.000)	0.444
		2 > 4	6 vs. 5	0.000 [0.000, 0.006] vs. 0.000 [0.000, 0.000]	0.001 (0.003) vs. 0.000 (0.000)	0.273
	1	1 > 2	4 vs. 6	0.008 [0.004, 0.008] vs. 0.011 [0.003, 0.053]	0.007 (0.002) vs. 0.018 (0.018)	0.871
		1 > 4	4 vs. 5	0.008 [0.004, 0.008] vs. 0.002 [-0.005, 0.005]	0.007 (0.002) vs. 0.002 (0.004)	0.032
		2 > 4	6 vs. 5	0.011 [0.003, 0.053] vs. 0.002 [-0.005, 0.005]	0.018 (0.018) vs. 0.002 (0.004)	0.009
	2	1 > 2	4 vs. 6	0.226 [0.027, 0.683] vs. 0.119 [0.012, 0.466]	0.291 (0.278) vs. 0.197 (0.215)	0.176
		1 > 4	4 vs. 5	0.226 [0.027, 0.683] vs. 0.088 [0.007, 0.450]	0.291 (0.278) vs. 0.145 (0.182)	0.143
		2 > 4	6 vs. 5	0.119 [0.012, 0.466] vs. 0.088 [0.007, 0.450]	0.197 (0.215) vs. 0.145 (0.182)	0.331
POL	0	1 > 2	4 vs. 6	0.000 [0.000, 0.003] vs. 0.000 [0.000, 0.007]	0.001 (0.002) vs. 0.001 (0.003)	0.667
		1 > 4	4 vs. 5	0.000 [0.000, 0.003] vs. 0.000 [0.000, 0.003]	0.001 (0.002) vs. 0.001 (0.002)	0.722
		2 > 4	6 vs. 5	0.000 [0.000, 0.007] vs. 0.000 [0.000, 0.003]	0.001 (0.003) vs. 0.001 (0.002)	0.697
	1	1 > 2	4 vs. 6	0.002 [0.000, 0.005] vs. 0.000 [0.000, 0.005]	0.002 (0.002) vs. 0.001 (0.002)	0.452
		1 > 4	4 vs. 5	0.002 [0.000, 0.005] vs. 0.000 [-0.005, 0.001]	0.002 (0.002) vs. -0.001 (0.002)	0.127
		2 > 4	6 vs. 5	0.000 [0.000, 0.005] vs. 0.000 [-0.005, 0.001]	0.001 (0.002) vs. -0.001 (0.002)	0.132
	2	1 > 2	4 vs. 6	0.004 [-0.001, 0.009] vs. 0.001 [-0.016, 0.004]	0.004 (0.005) vs. -0.001 (0.007)	0.381
		1 > 4	4 vs. 5	0.004 [-0.001, 0.009] vs. 0.001 [-0.002, 0.007]	0.004 (0.005) vs. 0.002 (0.004)	0.365
		2 > 4	6 vs. 5	0.001 [-0.016, 0.004] vs. 0.001 [-0.002, 0.007]	-0.001 (0.007) vs. 0.002 (0.004)	0.686

Note:
SD: standard deviation.

Table 3: Reproducibility software session information

name	value
version	R version 4.3.1 (2023-06-16 ucrt)
os	Windows 10 x64 (build 19045)
system	x86_64, mingw32
ui	RTerm
language	(EN)
collate	English_United States.utf8
ctype	English_United States.utf8
tz	America/Los_Angeles
date	2024-08-01
pandoc	3.1.1 @ C:/Program Files/RStudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
location	C:/Users/glemire/Documents/code/TEST000ADCCtemp/TEST_adcc
file name	TEST_adcc.Rmd
user	Gabrielle Lemire

Table 4: Reproducibility software package version information

package	version	date	source
conflicted	1.2.0	2023-02-01	CRAN (R 4.3.3)
dplyr	1.1.4	2023-11-17	CRAN (R 4.3.3)
forcats	1.0.0	2023-01-29	CRAN (R 4.3.1)
ggplot2	3.5.1	2024-04-23	CRAN (R 4.3.3)
kableExtra	1.3.4.9000	2023-10-18	Github (kupietz/kableExtra@3bf9b21a769c9e6c21c955689bf5f8175dc83350)
knitr	1.48	2024-07-07	CRAN (R 4.3.3)
lubridate	1.9.2	2023-02-10	CRAN (R 4.3.1)
purrr	1.0.2	2023-08-10	CRAN (R 4.3.1)
readr	2.1.5	2024-01-10	CRAN (R 4.3.3)
remotes	2.5.0	2024-03-17	CRAN (R 4.3.3)
rmarkdown	2.27	2024-05-17	CRAN (R 4.3.3)
rprojroot	2.0.4	2023-11-05	CRAN (R 4.3.3)
stringr	1.5.1	2023-11-14	CRAN (R 4.3.3)
tibble	3.2.1	2023-03-20	CRAN (R 4.3.1)
tidyr	1.3.1	2024-01-24	CRAN (R 4.3.3)
tidyverse	2.0.0	2023-02-22	CRAN (R 4.3.3)
VISCfunctions	1.2.2	2024-06-13	Github (FredHutch/VISCfunctions@28be2826df1c09cf2cac919ae2db82e05dde8dd9)
VISCtemplates	1.2.0.9000	2024-08-02	git2r (https://github.com/FredHutch/VISCtemplates.git@ea0cf015e9b0ea704f2447

15 Acknowledgements

[Contact the study PM to get an updated list of internal and external contributors, and then fill in the template below]

The authors thank the following individuals for their invaluable contributions to this report. From [insert group name or affiliation, for example: the CAVIMC/Duke team] we thank [insert individual names, and roles here, for example: Kelli Greene and Hongmei Gao (Experimental Design, Data Interpretation, Study Management); Nicole Yates (Scientific Research Laboratory Manager) and Sheetal Sawant (Biostatistician)]. From Fred Hutch Cancer Center, we also thank [insert names and roles here, for example: Ratana Som (SCHARP Lab Data Manager); Marie Vendettuoli and Valeria Duran (SCHARP Statistical Programmers); and Lindsey Mwoga and Drienna Holman (VISC Project Management)].

Note: names may be listed in bullet point format instead of paragraph format if that helps with readability, for example:

- Name 1 (Role 1)
- Name 2 (Role 2)

16 References

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