

This is the Statistical Analysis Plan Version 3.0 for A5315 with names of authors, names of publication writing team members and analysis timeline redacted.

A5315

A Phase I/II Study of Single Dose Romidepsin in HIV-Infected Adults with Suppressed Viremia on Antiretroviral Therapy to Assess Safety, Tolerability, and Activation of HIV-1 Expression

ClinicalTrials.gov Identifier: NCT01933594

Statistical Analysis Plan

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1 INTRODUCTION

This document describes the content proposed for the primary statistical analysis of ACTG 5315. The focus is on analyses that address the key safety, tolerability and efficacy outcome measures, including those needed to address the study's primary and major secondary objectives. A subset of these analyses (as described herein) will form the basis of reports provided to the Study Monitoring Committee (SMC) while the study is ongoing. This analysis plan therefore includes the key analyses which might lead to modification or termination of the study, and hence also form the core of any presentation or publication used to disseminate the primary conclusions of the study. It is, however, recognized that this analysis plan may be modified by the study team as new information becomes available outside of the study, or to reflect recommendations made by the SMC or changes in the study design. In addition, some analyses, tables, or figures may be omitted at interim analyses if there are insufficient data to warrant analysis or at the request of the SMC.

2 CORE TEAM ROSTER

Information redacted

3 STUDY SCHEMA

Note: The following material is extracted from the study protocol version 2.0

DESIGN

A5315 is a phase I/II, double-blinded, randomized, placebo-controlled, dose-escalation study to evaluate the safety and efficacy of single dose and multiple dose administration of romidepsin (RMD). Four cohorts (1-4) of 15 participants each will be sequentially enrolled into the study (depending on safety outcomes, which will determine whether to dose escalate or not). Toxicity related to the administration of RMD will be evaluated systematically in all cohorts. The effect of RMD on HIV expression will be evaluated in all cohorts. The effect of RMD on total virus recovery (TVR) will be assessed in most cohorts. Samples will also be collected before and after most infusions for pharmacokinetic (PK) evaluation of antiretroviral drugs (ARVs) and RMD.

DURATION

For participants in Cohorts 1 – 3, study duration is 4 weeks. For participants in Cohort 4, study duration is a minimum of 24 weeks; study duration will be increased if any of the infusions after the first one must be delayed. The maximum study duration for Cohort 4 participants will be 48 weeks.

SAMPLE SIZE

60 evaluable participants (approximately 15 evaluable participants in each cohort)

POPULATION

HIV- infected adults at least 18 years of age with CD4+ counts >300 cells/mm³ who have suppressed viremia on a raltegravir (RAL), dolutegravir (DTG), or efavirenz (EFV)-based regimen (plasma HIV-1 RNA levels <50 copies/mL and no blips >50 copies/mL on standard commercial assays) and, for Cohorts 1-3 only, who have ≥0.4 HIV-1 RNA copies/mL by single-copy assay (SCA) at screening. Plasma HIV-1 RNA ≥0.4 HIV-1 RNA copies/mL by SCA is not required as part of eligibility for Cohort 4.

REGIMEN

Participants will be sequentially enrolled to cohorts and randomized 4:1 to receive RMD or placebo as shown below.

Cohort 1: 12 participants will receive 0.5 mg/m² RMD in 0.9% saline
3 participants will receive placebo in 0.9% saline

Cohort 2: 12 participants will receive 2 mg/m² RMD in 0.9% saline
3 participants will receive placebo in 0.9% saline

Cohort 3: 12 participants will receive 5 mg/m² RMD in 0.9% saline
3 participants will receive placebo in 0.9% saline

Cohort 4: 12 participants will receive a total of 20 mg/m² RMD in 0.9% saline
(5 mg/m² RMD at each of four dosing time points)
3 participants will receive placebo in 0.9% saline (at each of four dosing time points)

4 HYPOTHESES AND OBJECTIVES

4.1 Hypothesis

We hypothesize that adjunctive therapy with the potent histone deacetylase inhibitor (HDACi) romidepsin (RMD) administered at doses well below the maximum tolerated dose (MTD), will be well tolerated and lead to activation of proviral HIV-1 expression in latently-infected cells in HIV-infected participants receiving suppressive antiretroviral therapy (ART).

4.2 Primary Objectives

- To determine the safety and tolerability of the intravenous administration of a single dose of RMD to HIV-infected participants with HIV-1 RNA levels <50 copies/mL on a stable ART regimen in Cohorts 1-3, and of multiple doses of RMD in Cohort 4.
- To assess the induction of HIV-1 expression in HIV-infected participants with suppressed viremia by measuring plasma viremia using a single copy HIV-1 RNA assay prior to and following a single dose of RMD in Cohorts 1-3.
- To assess the induction of HIV-1 expression in HIV-infected participants with suppressed viremia by measuring plasma viremia using a single copy HIV-1 RNA assay prior to and following each of multiple doses of RMD in Cohort 4.
- To assess changes in cell-associated HIV-1 RNA levels in resting CD4+ T-cells obtained prior to and following a single dose of RMD in Cohorts 1-3 and prior to and following each of multiple RMD doses in Cohort 4.
- To examine the efficacy of multiple doses of RMD for reducing the latent, inducible HIV-1 reservoir, as measured by the total virus recovery (TVR) assay in Cohort 4.

4.3 Secondary Objectives

- To assess changes in cell-associated HIV-1 RNA levels in total CD4+ T-cells obtained prior to and following a single dose of RMD in Cohorts 1-3 and prior to and following each of multiple RMD doses in Cohort 4.
- To assess changes in histone acetylation levels and P-TEFb expression in total CD4+ T-cells prior to and following a single dose of RMD in Cohorts 1-3 and prior to and following each of multiple RMD doses in Cohort 4.
- To assess changes in total HIV-1 DNA and 2-LTR circles in resting and total CD4+ T-cells prior to and following a single dose of RMD in Cohorts 1-3 and prior to and following each of multiple RMD doses in Cohort 4.

- To assess the induction of HIV-1 expression in HIV-infected participants with suppressed viremia by measuring plasma viremia using single copy HIV-1 RNA assay prior to and following each of multiple RMD doses in Cohort 4.
- To obtain pharmacokinetic (PK) data for RMD and coadministered antiretroviral drugs prior to and following a single dose of RMD in Cohorts 1-3 and at and following the third and fourth doses in Cohort 4 to assess potential drug-drug interactions.

5 ANALYSIS REPORTS

This section describes the various analysis reports that will be created for this study.

5.1 SMC (interim) review

5.1.1 SMC report for annual reviews

As outlined in the A5315 monitoring plan, an analysis report will be prepared and distributed to the SMC members for each SMC review. The report will include analyses from the following subsections of **Section 7** broken down by treatment arm and dose cohort:

- Accrual
- Baseline characteristics
- Study status
- Treatment status
- HIV-1 RNA level at day 7
- Primary safety endpoint
- List of events

and a pooled (over treatment arm) summary of:

- Data and stored sample completeness

Note: Only a closed report will be generated.

5.1.2 SMC report for dose escalation

For the SMC review after completion of each cohort, to assess whether dose escalation can occur, an abbreviated analysis report will be prepared in order to expedite the dose escalation review. This report will include the following subsections of Section 7 broken down by treatment arm and dose cohort:

- Accrual
- Baseline characteristics
- Study status
- Treatment status
- HIV-1 RNA level at day 7
- List of events

Note: Only a closed report will be generated.

5.2 Final Analysis report

Unless otherwise noted, the final analysis report will include all subsections of **Section 7**.

6 GENERAL ANALYSIS CONSIDERATIONS

6.1 Lists of Data in Reports

Listings of data by individual study participants are described below to facilitate the interpretation of the study results. To help protect confidentiality of data, the content of these lists will be limited and will not include dates, participants' ACTG identifier numbers or other combinations of information that might identify an individual participant (except that these may be included in the confidential closed reports prepared for SMC review).

6.2 Study Treatment

The study treatment is Romidepsin (RMD) or Placebo for RMD (placebo).

6.3 Analysis Populations

Safety: All participants who have been exposed to study treatment/placebo will be included in the safety analyses.

Efficacy: For Cohorts 1-3, all participants who completed the study treatment infusion at Day 0 and with available outcome data will be included in the as-treated efficacy analyses.

For Cohort 4, all participants who completed all study treatment infusions and with available outcome data will be included in the as-treated efficacy analyses.

6.4 Definition of "Day 0" and "baseline"

"Day 0" is defined as the day of study registration (variable **ONSTDT** from **ADM0020**). In the study protocol, this is often referred to as entry date. "Baseline" is defined as the last evaluation on or before Day 0.

6.5 Period of follow-up

For Cohorts 1-3:

Per protocol Section 6.2.3, Week 4/Day 28 evaluations will serve as the study completion evaluations for participants who have maintained an HIV-1 RNA level of < 200 copies/mL by standard ultrasensitive assay at the Day 7 visit. Also, these evaluations will be the study completion evaluations for participants who did not experience a Grade \geq 3 AE or clinical event (per protocol Section 7.1).

Participants who experience a Grade \geq 3 AE or clinical event must return for a Week 8/Day 56 visit (per protocol Section 7.1.2).

Per protocol Section 6.2.4, participants with an HIV-1 RNA level of \geq 200 copies/ml at the Day 7 visit will be contacted immediately and asked to return as soon as possible (preferably within 72 hours) for a repeat HIV-1 RNA PCR. If the repeat HIV-1 RNA PCR level after Day 7 is \geq 200 copies/ml, the participant will complete both Week 4 /Day 28 and Week 8 /Day 56 visits.

For Cohort 4:

Per protocol Section 6.1, participants will be followed for 18 weeks after the 4th infusion. Note: infusions can be delayed per protocol.

6.6 Visit schedule and definition of “Day” and “Week” for analysis purposes

Days and Weeks on study will be based on the study treatment administration dates (i.e., will be “anchored” on the infusion timing) and will use the protocol-specified windows. The protocol schedule of events for Cohort 4 is provided in **Appendix 1**.

6.7 Validation of programs

All programs creating treatment codes, derived datasets, formats, and primary analysis programs will be validated in accordance with SOP PROG.10066 and PROG.10067 (or PROG.10083, as applicable). All user options files will be validated in accordance with SOP PROG.10071.

The primary analyses (safety and efficacy) will be additionally validated through independent coding.

7 ANALYSES

Analysis summaries will be presented for each dose cohort active arm, for the pooled dose cohort arms (if applicable), the pooled placebo arms (if applicable), and overall. Separate analyses may be performed by cohort in which summaries will be by active dose cohort, placebo arm and overall.

For SMC reports all summaries will be presented by randomized treatment, and overall, separately by dose cohort.

Lists will be indexed by the alternate participant identifier (**PUBLICID** variable), and visit week/date as needed.

7.1 Accrual

a. Table: number (%) of participants enrolled by month/year

b. Table: number (%) of participants enrolled by site

[**STATUS** dataset]

7.2 Eligibility Violations

a. Table: violations of eligibility criteria by site (if any)

b. List: details of exclusions from analyses (if any)

7.3 Participant Replacement

Table: reasons for replaced participants (if any), as described in protocol Section 9.4.

7.4 Baseline Characteristics

a. Table (demographic information):

- Age at randomization date (years): N, median, 25th and 75th percentiles, min, max; number (%) by category (18-34, 35-49, ≥50)
- Sex: number (%)
- Self-reported race/ethnicity: number (%)
- IV drug use: number (%) by category (never used, previously used, currently used)
- BMI (kg/m²): N, median, 25th and 75th percentiles, min, max
- Background ART regimen: number (%) by category (raltegravir, dolutegravir, efavirenz)

[STATUS dataset, PKW0348 form, ANSTAB table (for background regimen)]

b. Table (health status information):

- Time since first HIV-1 RNA below the detection limit: N, median, 25th and 75th percentiles, min, max and number (%) by category (1-3, 3-5, 5-10, 10-15, 15-20, 20+)
- History of VF on an ART regimen: number (%)
- Nadir CD4 count: N, median, 25th and 75th percentiles, min, max
- Screening CD4 count (cells/mm³): N, median, 25th and 75th percentiles, min, max
- Screening HIV-1 RNA level by SCA (copies/mL): N, median, 25th and 75th percentiles, min, max
- Screening negative HCV antibody: number (%) by category (Yes, No)
- Pre-entry ECG result: QTC interval (msec): N, median, 25th and 75th percentiles, min, max

[HXW182 form, HXW183 form, F1015 form, ANSTAB table, F0875 form]

7.5 Study Status

a. Table:

- Number (%) of participants who completed the protocol
- Number (%) of participants who did not complete the protocol
 - Number (%) of participants off study due to death
 - Number (%) of participants off study prior to completion of protocol for reasons other than death

b. List of lost-to follow-up participants (if any) with follow-up duration and reason off study

c. List of deaths (if any) including study week of death and cause of death information

[STATUS dataset]

7.6 Treatment Status

For Cohorts 1-3:

a. Table:

- Number (%) of participants who completed RMD/placebo infusion
- Number (%) of participants who discontinued prematurely RMD/placebo infusion

c. List: details (including reasons and % infused) for participants with incomplete treatment, if any

For Cohort 4:

a. Table:

- Number (%) of participants who completed all 4 RMD/placebo infusions
- Number (%) of participants who did not complete all 4 RMD/placebo infusions
 - Reasons why not all 4 RMD/placebo infusions were not completed

b. List: details (including reasons and % infused) for participants with incomplete treatment, if any

NOTE: For final analysis the duration of infusion (hours) will also be summarized (N, median, 25th and 75th percentiles, min, and max)

[TXW0279 form, STATUS dataset]

7.7 HIV-1 RNA level at day 7 after each infusion

a. Table: HIV-1 RNA level (copies/mL) at day 7 after each infusion: number (%) by category (<40 (G), <40 (J), 40-199, ≥200)

NOTE: The table will have a footnote listing all the HIV-1 RNA levels at day 7 that are ≥40 copies/mL

[RNALDMS dataset]

7.8 Data and stored sample completeness (interim analysis only)

Tables summarizing completeness of selected data and stored sample (using the CBAR macro %DAR):

- BLD/DPE/CEL/DMS or BLD/EDT/CEL/DMS PBMC (for virology/immunology studies)
- BLD/EDT/PLH or BLD/DPE/PLH aliquots (for SCA testing)
- LPK/SCI/CEL/DMS or LPK/ACD/CEL/DMS (Leukopak)
- LPK/SCI/LPK/RPM or LPK/ACD/LPK/RPM (Monogram)
- BLD/SCI/PL2 (D-dimer)
- BLD/DPE/PL1 (PK for raltegravir, dolutegravir, efavirenz)

NOTE: The completeness of the stored samples will be based on the LPC-required number of aliquots and a lower number (depending on the sample).

[ALIQ dataset, TXW0279 form]

7.9 Safety data

7.9.1 List of events

List showing all the grade > 0 safety events for each study participant. The list will include the following:

- public ID
- treatment dispensation days
- core team assigned code (regarding the relatedness to study treatment)
- visit week
- onset day
- resolution day
- event description
- value and units (for laboratory results)
- grade
- site assigned code (regarding the relatedness to study treatment)

NOTE: This information corresponds to the ToxSum report prepared by the DMC, with the addition of the site assigned code. The site assigned code is derived from the TRAC table for events, except for those who originate from the EVW0206; the site assigned code for these events is derived from the EVW0206_UPDATE dataset

[TRAC table, EVW0206_UPDATE dataset]

7.9.2 Dose escalation criteria (protocol Section 9.4.1, for dose escalation review only)

Criteria to be used to guide the SMC when determining dose resumption or dose escalation for each cohort are defined as:

- a) No more than three subjects have experienced a Grade 3 AE that is probably or possibly related to study treatment (as judged by the core team, blinded to treatment arm) prior to or on Day 14 after the treatment administration;

and
- b) None of the subjects has experienced a Grade \geq 3 AE that is definitely related to study treatment or that is Grade \geq 4 and probably or possibly related to study treatment (as judged by the core team, blinded to treatment arm) prior to or on Day 14 after the treatment administration.

NOTE: The occurrence of a Grade 3 infusion site pain or tenderness that is sustained for less than 48 hours will be excluded from the dose escalation evaluation

7.9.3 Primary safety outcome (protocol Section 9.2.1.1)

List: Details of the following:

Occurrence of Grade ≥ 3 AE including signs/symptoms, lab toxicities, and /or clinical events that is probably, possibly or definitely related to study treatment (as judged by the core team, blinded to treatment arm) any time from the time of study treatment administration until 28 days after the administration.

7.10 Cohorts 1-3 primary efficacy outcomes (protocol Section 9.2.1.2)

These analyses will be 'as-treated', such that only participants who completed study treatment/placebo administration will be included.

7.10.1 Change in plasma HIV-1 RNA levels from baseline (average of pre-entry and entry values) as detected by single copy assay (SCA) at 24 and 48 hours (average) after the administration of RMD or placebo.

The analysis of 7.10.1 will be combined with 7.11.1.

SCA values below the limit of detection (LOD) will be imputed as equal to half the limit of detection (i.e., if LOD=0.4 copies/mL, results < 0.4 copies/mL will be imputed as 0.2 copies/mL).

a. Tables:

- Completeness of data for all measurement time points
- N (%) of SCA value < LOD at all measurement time points
- Baseline SCA: N, median, 25th and 75th percentiles, min, max
- 24/48 hour SCA: N, median, 25th and 75th percentiles, min, max
- Change (24/48 hour – baseline) SCA: N, median, 25th and 75th percentiles, min, max
- SCA at 6 and 12 hours, and 7, 14, and 28 days: N, median, 25th and 75th percentiles, min, max
- Change (at each above time point – baseline) SCA: N, median, 25th and 75th percentiles, min, max

Note: Baseline and 24/48 hour averages will be calculated using log₁₀-transformed SCA values.

A Wilcoxon rank sum test will be used for the comparison between groups.

b. Figure:

- Longitudinal, participant-specific, (spaghetti) plots for all measurement time points with open symbol for values below the LOD and different symbols/colors for RMD and placebo.

7.10.2 Change in cell-associated HIV-1 RNA levels in resting CD4+ T-cells from baseline to 24 hours after the administration of RMD or placebo.

The analysis of 7.10.2 will be combined with 7.11.2.

a. Tables:

- Completeness of data for all measurement time points
- Baseline CA-RNA in resting CD4+ T-cells: N, median, 25th and 75th percentiles, min, max
- 24 hour CA-RNA in resting CD4+ T-cells: N, median, 25th and 75th percentiles, min, max
- Day 14 CA-RNA in resting CD4+ T-cells: N, median, 25th and 75th percentiles, min, max
- Change (24 hour – baseline) of CA-RNA in resting CD4+ T-cells: N, median, 25th and 75th percentiles, min, max
- Change (Day 14 – baseline) of CA-RNA in resting CD4+ T-cells: N, median, 25th and 75th percentiles, min, max

A Wilcoxon rank sum test will be used for the comparison between groups.

b. Figure:

Longitudinal, participant-specific, (spaghetti) plots for all measurement time points using different symbols/colors for RMD and placebo.

7.11 Cohorts 1-3 secondary outcomes

Unless otherwise specified, these analyses will follow the approach for the primary efficacy outcomes.

7.11.1 Changes in plasma HIV-1 RNA levels as detected by single copy assay from baseline to 6 and 12 hours, and 7, 14, and 28 days after the administration of RMD or placebo.

Included in the analysis of 7.10.1

7.11.2 Change in cell-associated HIV-1 RNA levels in resting CD4+ T-cells from baseline to 14 days after the administration of RMD or placebo.

Included in the analysis of 7.10.2

7.11.3 Changes in cell-associated HIV-1 RNA levels in total CD4+ T-cells from baseline to 6, 12, 24 and 48 hours, and 7, 14, and 28 days after completion of RMD or placebo.**7.11.4 Changes in histone acetylation in total CD4+ T-cells by flow cytometry from baseline to 12, 24, 48 hours and 7 and 14 days after the administration of RMD or placebo.**

NOTE: Mean and median FI records with sample acquisition counts < 10,000 will be excluded from the analysis.

Outcomes of interests: FICD3MED; FICD3MN, FICELMED, FICELMN

a. Tables:

- Completeness of data for all measurement time points
- Summaries for each outcome at each measurement time point: N, median, 25th and 75th percentiles, min, max
- Summaries for each outcome's absolute change from baseline to each measurement time point: N, median, 25th and 75th percentiles, min, max

Wilcoxon rank sum tests will be used for the comparisons of change (from pre-dose) between groups.

b. Figure:

Longitudinal, participant-specific, (spaghetti) plots for all measurement time points using different panels/symbols/colors for RMD and placebo, separately for each cohort.

Median (change from pre-dose) plots for combined cohorts and placebo groups.

7.11.5 Changes in total HIV-1 DNA and 2-LTR circles in resting or total CD4+ T-cells from baseline to 6, 12, 24, and 48 hours and 7, 14, and 28 days after the administration of RMD or placebo.

7.11.6 Pharmacokinetic parameters (AUC, Cmax, Cmin) for RMD and co-administered antiretroviral drugs (EFV, DTG, or RAL).

Table summarizing of the pharmacologist-estimated PK parameters.

7.11.7 Number/percent of subjects with HIV-1 RNA levels > 200 copies at the Day 7 visit

See 7.7

7.11.8 All reported Grade 2-4 AEs.

List showing all the events with grade 2-4 for each study participant. The list will include the following:

- public ID
- treatment dispensation date
- visit week
- onset date
- resolution date
- event description
- value and units (for laboratory results)
- grade

7.12 Cohorts 1-3 exploratory outcomes

Unless otherwise specified, these analyses will follow the same approach for the primary efficacy outcomes.

- 7.12.1 Change in CD4+ and CD8+ T cell percent from baseline to 48 hours, 7, and 28 days after the administration of RMD or placebo.
- 7.12.2 Changes in the percentage of CD3+ CD4+ and CD3+ CD8+ lymphocytes and cellular markers of immune activation (CD38/HLA-DR or CD69/CD25 expression on CD4+ and CD8+ T-cells) from baseline to 48 hours, and 7 and 28 days after the administration of RMD or placebo.
- 7.12.3 Changes in proportion of cycling CD4+ and CD8+ T-cells (Ki67 expression) from baseline to 48 hours, 7, and 28 days after the administration of RMD or placebo.
- 7.12.4 Changes in the levels of soluble markers IL-6, C-reactive protein (CRP), and D-dimer from baseline to 7 and 28 days after the administration of RMD or placebo.
- 7.12.5 Percentage of total lymphocytes expressing annexin V and 7 amino-actinomycin D (7-AAD) at baseline, 48 hours, 7 and 28 days after the administration of RMD or placebo.
- 7.12.6 Changes in ex vivo PMA/Ionomycin and RMD inducible HIV-1 RNA expression in resting CD4+ T-cells from baseline to 14 days after RMD dosing.

NOTE: Undetected values will be imputed with a value of 20 copies/mL; these are represented in the database as "< .".

Outcomes of interests: untreated (Y_{UNT}), PMA-treated (Y_{PMA}), and RMD-treated (Y_{RMD}). These Y variables will be average of the log transformed replicates, as long as the number of replicates is ≥ 3 . Note that data with replicate number < 3 will be excluded from the analyses.

a. Tables:

- Completeness of data for all measurement time points (no data, < 3 replicates and ≥ 3 replicates)
- Summaries for each outcome at each measurement time point: N, median, 25th and 75th percentiles, min, max
- Summaries for ratio of RMD-treated/untreated and PMA-treated/untreated at each measurement time point: N, median, 25th and 75th percentiles, min, max.
 - $\text{Ratio}_{\text{PMA}} = \exp(Y_{\text{PMA}})/\exp(Y_{\text{UNT}})$, $\text{Ratio}_{\text{RMD}} = \exp(Y_{\text{RMD}})/\exp(Y_{\text{UNT}})$, repeat for baseline and day 14.
- Summaries for each outcome fold change from baseline to week2 (week 2/pre-dose): N, median, 25th and 75th percentiles, min, max
 - $\text{FC}(Y_{\text{UNT}}) = \exp(Y_{\text{UNT at day 14}})/\exp(Y_{\text{UNT at baseline}})$, repeat for PMA and RMD
 - $\text{FC}(\text{PMA}) = (\text{Ratio}_{\text{PMA at day 14}})/(\text{Ratio}_{\text{PMA at baseline}})$, repeat for RMD

Wilcoxon rank sum tests will be used for the comparisons between groups.

b. Figure:

Longitudinal, participant-specific, (spaghetti) plots for all measurement time points using different symbols/colors for RMD and placebo.

7.12.7 Changes in P-TEFb and HIV-1 expression; changes in PTEF-b phosphorylation in total CD4+ T-cells by flow cytometry from baseline to 12, 24, 48 hours and 7 and 14 days after the administration of RMD or placebo.

7.13 Cohort 4 primary efficacy outcomes

7.13.1 Change in plasma HIV-1 RNA levels from baseline (average of pre-entry and entry values) as detected by single copy assay before and 24 hours after each administration of RMD or placebo in Cohort 4.

The analysis of 7.13.1 will be combined with 7.14.1. The available timepoints are: pre-entry (-35 to -28 days), pre- & 24hr post-infusion for all 4 infusions, and 72hr post-2nd-infusion

SCA values below the limit of detection (LOD) will be imputed as equal to half the limit of detection (i.e., if LOD=0.4 copies/mL, results < 0.4 copies/mL will be imputed as 0.2 copies/mL).

a. Tables:

- Completeness of data for all measurement time points
- N (%) of SCA value < LOD at all measurement time points
- Baseline SCA: N, median, 25th and 75th percentiles, min, max
- Pre- and 24hr post-infusion SCA for each infusion and 72hr post-2nd-infusion: N, median, 25th and 75th percentiles, min, max
- Change (at each post-entry time point – baseline) SCA: N, median, 25th and 75th percentiles, min, max
- Change (post-infusion time point – pre-infusion time point for each infusion) SCA: N, median, 25th and 75th percentiles, min, max

NOTE: Baseline averages will be calculated using log10-transformed SCA values.

Wilcoxon rank sum test will be used for the comparison between groups. Wilcoxon signed rank test will be used to test within-group changes for RMD group. Two-sided 95% confidence intervals for the shift will be calculated based on Hodges-Lehmann estimator.

b. Figure:

- Longitudinal median (Q1, Q3) plots for all measurement time points by RMD and placebo.
- Longitudinal, participant-specific, (spaghetti) plots for all measurement time points with open symbol for values below the LOD and different symbols/colors for RMD and placebo.

7.13.2 Change in cell-associated HIV-1 RNA levels in PBMCs before and 24 hours after each administration of RMD or placebo in Cohort 4.

The analysis of 7.13.2 will be combined with 7.14.2. The available timepoints are the same as 7.13.1 and 7.14.1. The same analysis approach will be used as in 7.13.1 (except N (%) below LOD).

NOTE: Cell-associated RNA results below the detection limit are imputed with 1 copy/million PBMCs.

7.14 Cohort 4 secondary outcomes

7.14.1 Changes in plasma HIV-1 RNA levels as detected by single copy assay from baseline to after each administration of RMD or placebo in Cohort 4.

NOTE: Only 72hr post-2nd-infusion time point was tested for the primary analysis. Testing of remaining samples is pending on further team discussion.

Included in the analysis of 7.13.1.

7.14.2 Change in cell-associated HIV-1 RNA levels in PBMCs from baseline to after each administration of RMD or placebo in Cohort 4.

NOTE: Only 72hr post-2nd-infusion time point was tested for the primary analysis. Testing of remaining samples is pending on further team discussion.

Included in the analysis of 7.13.2.

7.14.3 Changes in histone acetylation in CD4+ and CD8+ T-cells from baseline to after each administration of RMD or placebo in Cohort 4.

The same analysis approach will be used as in 7.13.2.

7.14.4 Changes in total HIV-1 DNA in PBMCs from baseline to after each administration of RMD or placebo in Cohort 4.

The same analysis approach will be used as in 7.13.2.

Analysis of RNA/DNA ratio will be using the same approach.

7.14.5 Pharmacokinetic parameters for RMD and co-administered antiretroviral drugs (EFV, DTG, or RAL).

Table summarizing of the pharmacologist-estimated PK parameters.

7.14.6 Number/percent of participants in Cohort 4 with HIV-1 RNA levels ≥ 200 copies/mL at scheduled post-infusion 4 visits.

See 7.7

7.14.7 All reported Grade 2-4 AEs.

List showing all the events with grade 2-4 for each study participant. The list will include the following:

- public ID
- treatment dispensation date
- visit week
- onset date
- resolution date
- event description
- value and units (for laboratory results)
- grade

7.14.8 Change in CD4+ T cell percent from baseline to after each administration of RMD or placebo in Cohort 4.**7.14.9 Changes in the percentage of CD3+ CD4+ and CD3+ CD8+ lymphocytes and cellular markers of immune activation (CD38/HLA-DR or CD69/CD25 expression on CD4+ and CD8+ T-cells) from baseline to after each administration of RMD or placebo in Cohort 4.****7.14.10 Change in percentage of total lymphocytes expressing annexin V and/or 7 amino-actinomycin D (7-AAD) from baseline to after each administration of RMD or placebo in Cohort 4.****7.14.11 Changes in P-TEFb and HIV-1 expression; changes in PTEF-b phosphorylation in CD4+ and CD8+ T-cells from baseline to after each administration of RMD or placebo in Cohort 4.**

For 7.14.8-7.14.11:

Tables:

- Summaries for each outcome at each measurement time point: N, median, 25th and 75th percentiles, min, max
- Summaries for each outcome's absolute change from baseline to each measurement time point: N, median, 25th and 75th percentiles, min, max

Wilcoxon rank sum test will be used for the comparison between groups. Wilcoxon signed rank test will be used to test within-group changes for RMD group.

Time points tested for 7.14.8-7.14.10: pre-entry (-35 to -28 days), pre-entry (-14 to -3 days), 24hr post-1st-infusion, pre-4th infusion, 24hr post-4th-infusion, week 16

7.15 Cohort 4 exploratory outcomes

- 7.15.1 Change in total virus recovery (TVR) or qVOA from baseline to after administration of all doses of RMD or placebo in Cohort 4**
- 7.15.2 Changes in proportion of cycling CD4+ and CD8+ T-cells (Ki67 expression) from baseline to after each administration of RMD or placebo in Cohort 4.**
- 7.15.3 Changes in the levels of soluble markers IL-6, C-reactive protein (CRP), and D-dimer from baseline to after each administration of RMD or placebo in Cohort 4.**
- 7.15.4 Changes in HIV-specific immune responses from baseline to after the first and fourth administration of RMD or placebo in Cohort 4.**
- 7.15.5 Changes in host RNA expression profiles by RNAseq of PBMC from after the first and fourth administration of RMD or placebo in Cohort 4.**

Appendices

Appendix 1 Schedule of Events (protocol Section 6)

Cohort 4, Screening through Step 2

Evaluation (Cohort 4, Screening through Step 2; standard)	Screening	Pre-Entry	Pre-Entry	Step 1					Step 2					Prem. Tx and Study D/C	
	Day -60 to Day -36	Day -35 to Day -28	Day -14 to Day -3	Entry/ Hour 0 Pre-Infusion/Infusion	Week 1 / Day 1 (24 hr post-infusion)	Week 1/ Day 3 (72 hr post-infusion)	Week 1 / Day 7 (7 days post-infusion)	Weeks 2 to 8 (conditional) ¹	Step 2 Reg.						
	Week 2 / Day 14 (+/- 48hr)	Week 3 / Day 15 (24 hr post-infusion)	Week 3 / Day 17 (72 hr post-infusion)	Week 3 / Day 21 (7 days post-infusion)	Weeks 4 to 11 (conditional) ¹										
Documentation of HIV-1 Medical and Medication History	X			X											
Clinical Assessments	X	X	X	X	X	X	X	Once each week	X	X	X	X	Once each week	X	
Complete Physical Exam		X													
Targeted Physical Exam			X	X	X	X	X	Once each week	X	X	X	X	Once each week	X	
Hematology			X		X	X	X	Wk 2, 6		X	X	X	Wk 6, 10	X	
Potassium and Magnesium (see section 6.3.7 for timing at Pre-entry)			X				X	Wk 2, 6				X	Wk 6, 10		
LFTs/Blood Chemistries (see section 6.3.7)				X			X	Wk 2, 6				X	Wk 6, 10	X	

Evaluation (Cohort 4, Screening through Step 2; standard)	Screening	Pre-Entry	Pre-Entry	Step 1					Step 2					Prem. Tx and Study D/C
	Day -60 to Day -36	Day -35 to Day -28	Day -14 to Day -3	Entry/ Hour 0 Pre- Infusion/Infusion	Week 1 / Day 1 (24 hr post- infusion)	Week 1/ Day 3 (72 hr post- infusion)	Week 1 / Day 7 (7 days post- infusion)	Weeks 2 to 8 (conditional) ¹	Step 2 Reg.					
	Week 2 / Day 14 (+/- 48hr)	Week 3 / Day 15 (24 hr post- infusion)	Week 3 / Day 17 (72 hr post- infusion)	Week 3 / Day 21 (7 days post- infusion)	Weeks 4 to 11 (conditional) ¹									
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).														
Urinalysis	X													
Urine Pregnancy Testing	X	X	X	X					X					
HBV/HCV screening	X													
ECG		X		X Hr 4	As clinically indicated			X Hr 4	As clinically indicated					
CD4%			X	X	X	X			X	X	X			X
Stored Plasma and PBMC for immunology testing (see section 6.3.9)		X	X		X					X				X
Plasma HIV-1 RNA real time	X		X											X
Plasma HIV-1 RNA expedited							X	Once each week				X	Once each week	
Plasma HIV-1 RNA by SCA		X	X	X	X	X			X	X	X			X
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).														

Evaluation (Cohort 4, Screening through Step 2; standard)	Screening	Pre-Entry	Pre-Entry	Step 1					Step 2					Prem. Tx and Study D/C
	Day -60 to Day -36	Day -35 to Day -28	Day -14 to Day -3	Entry/ Hour 0 Pre-Infusion/Infusion	Week 1 / Day 1 (24 hr post-infusion)	Week 1/ Day 3 (72 hr post-infusion)	Week 1 / Day 7 (7 days post-infusion)	Weeks 2 to 8 (conditional) ¹	Step 2 Reg.					
	Week 2 / Day 14 (+/- 48hr)	Week 3 / Day 15 (24 hr post-infusion)	Week 3 / Day 17 (72 hr post-infusion)	Week 3 / Day 21 (7 days post-infusion)	Weeks 4 to 11 (conditional) ¹									
Resting CD4+ T cells (see section 6.3.10)		X	X	X	X	X			X	X	X			X
Stored PBMC for virology testing (see section 6.3.10)		X	X	X	X	X			X	X	X			X
Histone Acetylation				Hr 0	X	X			Hr 0	X	X			
Stored PBMC for TVR assay (see section 6.3.10)		X												
RMD/Placebo Infusion				X					X					
ART Adherence Assessment	X		X	X					X					X
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).														

Cohort 4, Steps 3 and 4

Evaluation (Cohort 4, Steps 3 and 4; standard)	Step 3					Step 4								Prem. Tx and/or Study D/C	
	Step 3 Reg.					Step 4 Reg.					Post-Infusion 4 Follow Up				
	Week 4 / Day 28 (+/- 48hr)	Week 5 / Day 29 (24 hr post-infusion)	Week 5 / Day 31 (72 hr post-infusion)	Week 5 / Day 35 (7 days post-infusion)	Weeks 6 to 13 (conditional) ¹	Week 6 / Day 42 (+/- 48hr)	Week 7 / Day 43 (24 hr post-infusion)	Week 7 / Day 45 (72 hr post-infusion)	Week 7 / Day 49 (7 days post-infusion)	Week 8 / Day 56	Week 11 (±3 days)	Week 16 (± 7 days)	Week 24 (± 7 days)		
Clinical Assessments	X	X	X	X	Once each week	X	X	X	X	X	X	X	X	X	
Targeted Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Hematology		X	X	X	Wk 8, 12		X	X		X	X	X	X	X	
Potassium and Magnesium (see section 6.3.7 for timing at Pre-entry)				X	Wk 8, 12				X						
LFTs/Blood Chemistries (see section 6.3.7)				X	Wk 8, 12				X				X	X	
Urine Pregnancy Testing	X					X				As clinically indicated					
ECG	X	As clinically indicated				X Hr 4	As clinically indicated				X				
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).															
CD4%	X	X	X			X	X	X		X	X	X	X	X	

Evaluation (Cohort 4, Steps 3 and 4; standard)	Step 3					Step 4								Prem. Tx and/or Study D/C
	Step 3 Reg.					Step 4 Reg.					Post-Infusion 4 Follow Up			
	Week 4 / Day 28 (+/- 48hr)	Week 5 / Day 29 (24 hr post-infusion)	Week 5 / Day 31 (72 hr post-infusion)	Week 5 / Day 35 (7 days post-infusion)	Weeks 6 to 13 (conditional) ¹	Week 6 / Day 42 (+/- 48hr)	Week 7 / Day 43 (24 hr post-infusion)	Week 7 / Day 45 (72 hr post-infusion)	Week 7 / Day 49 (7 days post-infusion)	Week 8 / Day 56	Week 11 (±3 days)	Week 16 (± 7 days)	Week 24 (± 7 days)	
Stored Plasma and PBMC for immunology testing (see section 6.3.9)		X					X			X	X	X	X	X
Plasma HIV-1 RNA real time									X	X		X		X
Plasma HIV-1 RNA expedited				X	Once each week						X			
Plasma HIV-1 RNA by SCA	X	X	X			X	X	X		X	X	X	X	X
Resting CD4+ T cells (see section 6.3.10)	X	X	X			X	X	X		X	X	X	X	X
Stored PBMC for virology testing (see section 6.3.10)	X	X	X			X	X	X		X	X	X	X	X
Histone Acetylation	Hr 0	X	X			Hr 0	X	X						
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).														
Stored PBMC for TVR (see section 6.3.10)											X		X	
RMD/Placebo Infusion	X					X								

Evaluation (Cohort 4, Steps 3 and 4; standard)	Step 3					Step 4								Prem. Tx and/or Study D/C
	Step 3 Reg.					Step 4 Reg.					Post-Infusion 4 Follow Up			
	Week 4 / Day 28 (+/- 48hr)	Week 5 / Day 29 (24 hr post-infusion)	Week 5 / Day 31 (72 hr post-infusion)	Week 5 / Day 35 (7 days post-infusion)	Weeks 6 to 13 (conditional) ¹	Week 6 / Day 42 (+/- 48hr)	Week 7 / Day 43 (24 hr post-infusion)	Week 7 / Day 45 (72 hr post-infusion)	Week 7 / Day 49 (7 days post-infusion)	Week 8 / Day 56	Week 11 (±3 days)	Week 16 (± 7 days)	Week 24 (± 7 days)	
ART Adherence Assessment	X					X						X	X	X
PK RMD (Collection times are from the start of the infusion)	X Hr 0 & Hr 4					X Hr 0 & Hr 4								
PK ARVs (Collection time is from the start of the infusion)	X Hr 0	X				X Hr 0	X							
¹ These “conditional” evaluation weeks are only for participants whose infusions will be delayed for the next Step. (See section 6.2.3). If infusion will be delayed, go to the delayed infusion SOE (section 6.1.4).														