

Cover Page

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CARD8 Activation for Reservoir Reduction with Efavirenz (CARRE Trial): An open-label, single arm trial to assess the safety and efficacy of *in vivo* CARD8 activation with Efavirenz intensification on HIV-1 reservoir reduction in people living with HIV-1

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STATISTICAL ANALYSIS PLAN

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Statistical Analysis Plan (SAP)

TRIAL FULL TITLE	CARD8 Activation for Reservoir Reduction with Efavirenz (CARRE Trial): An open-label, single arm trial to assess the safety and efficacy of <i>in vivo</i> CARD8 activation with Efavirenz intensification on HIV-1 reservoir reduction in people living with HIV-1
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1. Introduction

This document describes the proposed presentation and analysis for the main paper(s) reporting results from the single center prospective single arm CARD8 Activation for Reservoir Reduction with Efavirenz (CARRE Trial): An open-label, single arm trial to assess the safety and efficacy of *in vivo* CARD8 activation with Efavirenz intensification on HIV-1 reservoir reduction in people living with HIV-1. The reader is referred to the trial protocol version 13 (5.31.23) for more detailed information [1].

Any deviations from the statistical analysis plan will be described and justified in the final study report.

Background

In people living with HIV (PLWH), the virus persists in a pool of dynamic CD4 T cells. After an individual is started on therapy, cART limits newly budded viral particles from causing productive infection; while cells already carrying integrated HIV remain unaffected. Over time, the landscape of latent viruses evolves. The reservoir expands in response to CD4 stimulation from cognate antigens or cytokines and is balanced by selective pressures such as cytotoxic T cells, NK cells and antibody dependent cellular cytotoxicity. Extensive work underscores that HIV is able to evade adaptive immune pressure through the viral diversity rapidly established in a host pre-initiation of cART. *HIV's evasion mechanisms highlight why non-canonical techniques for viral elimination are needed.* Recent work has identified an innate immune pathway, the CARD8 inflammasome, which can be harnessed for clearance of infected cells through the use of an FDA approved medication, Efavirenz. This strategy targets an essential viral protein function, making it impervious to classic resistance mechanisms. **We hypothesize that activating this mechanism will help reduce the active HIV reservoir in PLWH in a CARD8 dependent fashion.** We plan to assess if activating the CARD8 inflammasome in patients will help reduce the HIV reservoir as measured by cell associated DNA, cell associated RNA, inducible provirus, total intact provirus.

2. Definitions

ART: antiretroviral therapy

PLWH: people living with HIV

IPDA: intact proviral DNA assay

QVOA: quantitative viral outgrowth assay

EFV: Efavirenz

3. Scope

This document defines the statistical analysis for the analysis of the single arm prospective study of PLWH, comparing outcomes on the same patients before and after treatment intensification with EFV.

This document does not describe the analysis for any other study parts.

4. Objectives of the trial

4.1.1. Primary objective(s)

The primary objectives of the study were:

1. To determine the safety and tolerability of EFV intensification to PLWH on a stable antiretroviral therapy regimen with HIV-1 RNA levels <500 copies/mL
2. To determine the frequency of intact pro-viral DNA in different subsets of blood T cells before, during, and after EFV intensification.

Accordingly, the study has two co-primary endpoints.

4.1.2. Secondary objectives

Secondary objectives are to assess the effects of study treatments in terms of:

1. To assess changes in plasma viremia using single copy HIV-1 RNA assay before, during, and after EFV intensification.
2. To assess changes in cell-associated unspliced and spliced HIV-1 RNA in blood CD4⁺ T cells before, during, and after EFV intensification.
3. To determine frequency of replication competent viruses in blood CD4⁺ T cells by QVOA before, during, and after EFV intensification.
4. To describe the pharmacokinetics of EFV.

5.1.3 Exploratory Objectives

1. Assess changes in cellular immune activation by evaluating proportion of CD4⁺ and CD8⁺ T cells co-expressing activation markers CD38/HLA-DR and CD69/CD25 before, during, and after EFV intensification.
2. HIV specific T cell responses before, during, and after EFV intensification by ELISpot.
3. Determine if CYP2B6 metabolism characterization correlates with efficacy of Efavirenz in activating the CARD8 mechanism and likelihood of experiencing side effects

4.2. Trial

An open-label, single arm trial to assess the safety and efficacy of *in vivo* CARD8 activation with Efavirenz intensification on HIV-1 reservoir reduction in people living with HIV-1

4.3. Treatments

All eligible patients are evaluated during a period of at least 4 months:

- P1: Pre-treatment period (prior to date of first EFV intensification date [hereafter “time 0”]): Pre EFV intensification
- P2: Treatment period (4-6 months after date of first EFV intensification): EFV intensification.

Each patient should receive daily EFV intensification over the 4-6-month period following enrollment, in accordance with the protocol.

4.4. Definitions of primary and secondary outcomes

4.4.1. Co-primary Endpoints

- 1.) Change in adverse events in period P1 compared to period P2.
- 2.) Change in reservoir size, as measured by IPDA, comparing periods P1 and P2

Primary outcome # 1 will be assessed at 3 months post completion of trial.

Primary outcome # 2 will be completed within two years of trial completion.

Analysis at other time points than those defined in this document will be considered posthoc analyses in reporting/publications.

We adopt the estimand framework in summarizing the components of the primary outcome.

Table 1: Definition of the primary outcome/estimand

Primary estimand	
Population	Adult (≥ 18) with HIV with VL <500
Treatment contrast	P1: Pre-treatment period (T=0): No EFV intensification P2: Treatment period (4-6 months): EFV intensification
Outcome definition	1. Safety 2. IPDA reservoir measurement
Population-level summary	1. Change in the number (%) of adverse events in period P1 compared to period P2. 2. Change in the mean IPDA from period P1 to period P2. Time 0 is defined as EFV intensification start.
Intercurrent event* and strategy	For the primary estimand, we follow a “treatment policy” strategy (akin to intention to treat) for ICEs from the time point at which treatment is initiated (i.e. at the start of period P2).

*intercurrent events (ICEs) are post-treatment initiation events which may preclude (or alter) the measurement of the primary estimand: For example (but not limited to) death, being lost to follow-up, not adhering to the treatment regime, not measured at the defined time, incomplete treatment regimen, non-compliant to obtain laboratory tests, initiation of alternative ART or medications with drug-drug interactions causing discontinuing trial

4.4.2. Secondary outcomes

S1: Average plasma viremia using single copy HIV-1 RNA assay over the 4-6 months following EFV intensification (period P2).

S2: Average changes in cell-associated unspliced and spliced HIV-1 RNA following EFV intensification.

1. P1: Pre-treatment
2. P2: Post-treatment initiation (up to 12 months)

S3: Frequency of replication competent viruses in blood CD4⁺ T cells by QVOA following EFV intensification.

1. P1: Pre-treatment
2. P2: Post-treatment initiation (up to 12 months)

S4: Plasma EFV concentrations (C_{max} and average, and median concentrations) during EFV intensification period P2.

4.5. Hypothesis framework

For each of the co-primary and secondary outcomes, the null hypothesis will be that there is no true difference in effect between measurements at time 0 and those following the EFV intensification period.

1.) Safety

- a. Null hypothesis: no difference in safety events
- b. Alternative hypothesis: more adverse events in post treatment phase than pre-intensification phase

2.) IPDA

- a. Null hypothesis: no difference in IPDA values pre and post EFV intensification
- b. Alternative hypothesis: lower IPDA values post EFV intensification phase (one sided)

4.6. Sample size

We did not perform a sample size calculation for the safety primary endpoint. A pre-enrollment power calculation was not possible as no prior clinical trial has been able to demonstrate a reduction in HIV reservoir size as quantified by QVOA or IPDA accelerated from baseline HIV reservoir decay established in the literature. We performed a posthoc power calculation at the time of the interim analysis (see later). This sample size calculation is for the IPDA primary endpoint.

At the time of the first reported adverse event, the trial was paused for internal IRB review, and an interim analysis was performed (see results below). Based on these results, a post-hoc power calculation was performed

1.) Safety

Pre EFV Intensification Safety Events	Post EFV Intensification Safety Events
0/3	1/3

Chi squared test of differences: no difference] Null hypothesis

2.) IPDA

Historic Half Life in Literature	Pre vs Post EFV Intensification Half-life
Mean 14.7 years (St. Dev. 5.83)	Mean 1.84 years (St. Dev. 1.57)

Based on the results from the IPDA interim analysis with 2 people's data, the estimated half-life of these patients 0.736 and 2.952 years, respectively. Based on an estimated mean half-life of 1.84 years, with a standard deviation of 1.57 yrs, recruiting an additional 5 people (total = 2 + 5 = 7) would enable detection of at least 2 standard deviations difference between periods P1 and P2 with 80% power and a two-sided

5% statistical significance level (Bonferroni corrected for the interim analysis, equivalent 2.5%). From prior literature, the IPDA half-life is estimated to be approximately 14.7 years with a ST Dev 5.83.

4.7. Randomization and blinding

Due to the per-patient pre- and post-treatment measurement approach, there is no randomization. After enrollment, each subject has a pre EFV Intensification measurement and post EFV intensification period.

The study is open label.

4.8. Data collection and monitoring

Data collection and monitoring will be performed in line with the protocol.

5. Analysis populations

5.1. Primary analysis population

The target population includes all patients as defined by the trial's inclusion/exclusion criteria. The trial is based on the "intention to treat" (ITT) principle (equivalently, according to the ICH E9 (R1) addendum a "treatment policy" strategy for the treatment of post-treatment initiation events [4, 5]). The ITT population will also be used for analysis of efficacy and safety data, and is referred to as the Full Analysis Set (FAS).

6. Descriptive and comparative analyses

6.1. Comparison of patients in pre-treatment and post-treatment

Every patient in the FAS has all measurement(s) in both the pre-treatment and post-treatment phase. Characteristics will be described for all patients (see Table template T1 in the supplementary material to this document), and separately for patients in the pre- and post-treatment phases for those analyses in which not all patients are included in both phases (e.g. Table templates T2 and T3).

6.2. Follow-up

All reasonable efforts will be taken to minimize loss to follow-up. The overall number and percentage of participants with follow-up information at treatment initiation (time 0), 4-6 months after treatment initiation will be reported.

6.3. Treatment adherence

The number and proportion of patients who did not receive the treatment as defined in the protocol will be reported.

Any other treatment options received, instead of, or in addition to, the ECP treatment during the first 6 months after treatment initiations, will be reported. Details on non-standard treatment received will be reported.

6.4. Descriptive statistics

Baseline patient characteristics (i.e. those at the beginning of period P1) will be summarized as median (inter-quartile range) for continuous variables and number (percentage) as categorical variable. Inter-group differences will be investigated using the Wilcoxon non-parametric test for continuous variables and the chi-square test for categorical variables (see Table T1 in the supplementary material).

The mean, standard deviation, median, inter-quartile range, and minimum and maximum values for continuous variables will be summarized for all patients in the FAS, and stratified according to the pre- and post-treatment phase.

6.5. Primary analysis

For the safety endpoint, the number of adverse events in period P1 and P2 will be tabulated and reported.

The IPDA values pre and post EFV intensification will be tabulated for half life calculations. Mean half-lives. Non-parametric methods will be used for group comparisons for continuous variables (i.e. Mann-Whitney test).

6.5.1. Subgroup analyses

The following subgroup analyses will be performed with respect to the primary and secondary endpoints:

By early or late ART treatment phases

By low or high EFV plasma levels

By initial ART regimen

6.5.2. Missing data

We do not envisage there being a large (>5%) proportion of missing baseline covariate data. By definition, the FAS only includes complete case data.

6.5.3. Interim analyses

At time of the first reported adverse event in period P2, an interim analysis was performed to determine whether it was worthwhile to continue pursuing this trial in terms of safety.

6.5.4. Statistical software employed

Prism was used for statistical software.

6.5.5. Multiple testing and p-value considerations

Allowance for multiple treatment comparisons will be made, as appropriate. Unless stated otherwise, a p-value of less than 0.05 will be considered statistically significant throughout (unless stated otherwise).

References

- [1] "AMENDED STUDY PROTOCOL: Safety, Tolerability, and Efficacy of Efavirenz (EFV) Intensification on the HIV-1 reservoir" Version 13, 31st of March 2023

Supplementary material

Table T1. Baseline characteristics (prior to treatment intensification (beginning of period P1))

Patient characteristic	Mean (sd)	Median [IQR]	Range (min, max)
Age, years			
Gender			
Male			
Female			
IVDU			
Race			
Date of HIV Diagnosis			
ART initiation			
Prior ART regimens			
Adverse Events			

Table T2: Table of each participant's data values.

	Day since EFV intensification	CD4	VL	Ultra-sensitive VL	IPDA intact (per million)	IPDA 5'def (per million)	IPDA 3'def (per million)	IUPM	Ca-RNA
Time periods									
P1									
P2									

Table T3: Primary and secondary outcomes

Outcome	Time0 N, %	Time3 0 N, %	Time60	Time: d90	Time d120	Estimate of Difference (95% CI)	p-value
Primary outcome							
Adverse Events							
IPDA median [IQR] mean (SD)							
Secondary outcomes							
EFV plasma concentrations							
QVOA							
Ca-RNA							
Plasma cytokines							