STATISTICAL ANALYSIS PLAN

Predictive value of the CD4/CD8 ratio after two years of successful ART in HIV-infected patients

September 18, 2018

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1 Study Rationale [from DACS322]

Although the reasons remain to be elucidated, subjects on successful antiretroviral therapy (ART) still present increased morbidity and mortality with respect to uninfected individuals [1–3]. This shortening of the expected life span has been recently associated with increased risk of the so-called “non-AIDS” complications, which include cardiovascular disease, renal impairment, liver disease, neurocognitive disorders, non-AIDS defining cancers, osteoporosis, and frailty [4–6]. Most of these non-infectious conditions have been related to the on-going immune activation and low-level systemic inflammatory status that occurs in chronic HIV infection despite effective ART [7]. While several studies have described immunovirological factors that predict age-associated disease risk, such as inflammatory markers of immune activation, most are not clinically available [8,9].

New data suggest that the CD4/CD8 ratio, a surrogate marker of immunosenescence and a predictor of all-cause mortality in the elderly [10–14], might identify a subset of ART-suppressed HIV-infected individuals at increased risk of clinical progression. Early in the HIV epidemic, the CD4/CD8 ratio was recognized as a predictor of AIDS, providing similar prognostic information to that of CD4 count as a predictor of opportunistic infections [15,16], a finding that was corroborated in subsequent studies [17,18]. More recently, it has been shown in treated HIV-infected individuals that a low CD4/CD8 ratio is reflective of higher levels of T cell activation and T cell senescence [19,20]. Interestingly, in a study using multiparametric bioinformatics to analyze predictors of immune changes secondary to HIV infection, the CD4/CD8 ratio was more strongly associated than other laboratory parameters, including viral load, CD4 counts and CD8 counts [21]. In a large Canadian cohort the CD4/CD8 ratio showed no additional predictive value to the CD4 counts for the risk of AIDS-defining events and mortality; however, the CD4/CD8 ratio has demonstrated to predict non-AIDS morbidity and mortality after adjustment for proximal CD4 counts in two European studies [22,23]. In a recent study analyzing several cohorts and focusing on ART-treated individuals achieving more than 500 CD4+ T cells/mm³, a low CD4/CD8 ratio was associated with increased inflammation, immune activation, and increased risk of serious non-AIDS events [24]. Data from this study suggest that while the CD4/CD8 ratio has prognostic significance in treated adults across all-ranges of CD4 counts, much of the risk associated with a low ratio in those with low CD4 counts is driven by the CD4 count (and presumably immunodeficiency), while the risk associated with low ratio in those with higher CD4 counts is driven by CD8 counts (and presumably inflammation).

Collectively, these observations suggest that monitoring the CD4/CD8 ratio might be useful to guide decisions during ART-mediated HIV RNA suppression. If the prognostic value of the CD4/CD8 ratio is further confirmed, the CD4/CD8 ratio may become an end-point for clinical trials evaluating the efficacy of ART regimens, and patients failing to normalize the CD4/CD8 ratio during ART might be candidates for treatments targeting immune dysfunction in chronically treated HIV-infected individuals. In addition, this subset of patients may benefit from screening programs or aggressive management of concomitant risk factors for aging-associated disease.
2 Study objectives

- To investigate whether the CD4/CD8 ratio and CD8 counts after 2 years of suppressive ART predicts the probability of clinical progression through year 7, independently of the CD4 count reached at the same time-point.
- To determine the best CD4/CD8 ratio and CD8 counts cut-offs for the prediction of clinical progression.
- To assess the previous objectives in the subset of individuals with >500 CD4+ T cells after two years of suppressive ART.

3 Study population

All participants were included if they

- Were from ART-naïve studies (384,388, A5095, A5142, A5202 and A5257)
- And had CD4/CD8 ratio data at year two (average of weeks 80-96)
- And were virologically suppressed at year two (<200 cp/ml at week 96) and on ART at year two.

4 Statistical Considerations

4.1 Outcomes

Our primary outcome will be any (first event after year two) of the following events from year 2 through year 7

- AIDS-defining event
- Non-accidental death
- Liver EOD (including ascites, esophageal/gastric varices, hepatic encephalopathy)
- Cardiovascular EOD (including myocardial infarction, stroke, coronary artery intervention, pulmonary embolism, thrombosis)
- Renal disease: confirmed, at least 3 months apart, eGFR<60ml/min/1.73m^2 using the MDRD equation for eGFR
- Non-AIDS cancers (all cancers except those already included as AIDS-defining events)
- Fractures: hip, spin, and wrist (often non-traumatic)
- Diabetes
- Serious bacterial infection: pneumonia (non AIDS-related), meningitis, sepsis, bacteremia.

Regarding non-AIDS-defining events, we will follow the definitions used by the DACS 280 and DACS 286 team, so all ALLRT analyses use the same definition, and we can save resources. Regarding AIDS-defining events, we will follow the definition of the ACTG.
### 4.2 Covariates

Covariates considered included in these analyses and their detailed definitions are listed in the table below. Baseline refers to parent study entry.

| Variables                                      | Time points                                      |
|-----------------------------------------------|--------------------------------------------------|
| **Age**                                       | Continuous                                       |
| **Sex**                                       | 1=Male                                           |
|                                               | 2=Female                                         |
| **Race/Ethnicity**                            | 1=White, non-Hispanic                           |
|                                               | 2=Black, non-Hispanic                           |
|                                               | 3=Hispanic + other                               |
| **Injection drug use**                        | 0=Never                                          |
|                                               | 1=Currently or previously used                   |
| **Initial ARV regimen**                       | 1=Non-boosted PI+NRTIs                           |
|                                               | 2=Boosted PI + NRTIs                             |
|                                               | 3=NNRTI + NRTIs                                 |
|                                               | 4=INSTI + NRTIs                                 |
|                                               | 5=Other                                          |
| **CD4 cell count**                            | Continuous                                       |
|                                               | Categorical:                                     |
|                                               | 0=<=50                                          |
|                                               | 1=51-200                                        |
|                                               | 2=201-350                                       |
|                                               | 3=351-500                                       |
|                                               | 4=>500                                          |
|                                               | OR                                              |
|                                               | 0=<=500                                         |
|                                               | 1=>500                                          |
| **CD4 count change from ART initiation to year 2** | Continuous                                     |
| **CD8 cell count**                            | Continuous                                       |
| **CD8 count change from ART initiation to year 2** | Continuous                                     |
| **CD4:CD8 ratio**                             | Continuous                                       |
|                                               | Categorical:                                     |
|                                               | 1= <0.4                                         |
|                                               | 2= 0.4 - 1                                      |
|                                               | 3=>1                                            |
| **CD4:CD8 ratio change from ART initiation to year 2** | Continuous                                     |
| **HIV RNA viral load**                        | Continuous: in log_{10} scale                    |
|                                               | Categorical:                                    |
|                                               | Baseline                                        |
|                                               | Baseline, year 2 and other time-points at years 2-7 |
|                                               | Baseline, year 2 and other time-points at years 2-7 |
|                                               | Baseline and year 2                             |
|                                               | Baseline and year 2                             |
### 4.3 Statistical Analysis

#### 4.3.1 Primary Analysis

To investigate how the probability of experiencing a clinical event in years 2-7 depends on prognostic factors, had everyone remained on suppressive ART. The factors we will consider are CD4 count, CD8 counts and CD4/CD8 ratio at year two (the average of CD4, CD4/CD8 ratio at week 80,88 and 96 using +/- 4 week window). We will also evaluate whether findings are changed if we additionally adjust for baseline year 2 CD4 counts.

**IPCW approach:** follow-up time from year two (week 100 after ART initiation) through year 7 will be discretized into 16-week-intervals. Year 7 will be the interval (week 341 - 356). Participants in the analysis (N) will be those with a year two CD4/CD8 ratio, HIV RNA<200 at year two (last HIV RNA in 80-96 week window) and on ART at the end of 80-96 week window.

#### 4.3.1.1 The prognostic value of CD4:8 on clinical progression had everyone remained on suppressive ART

Had everyone remained on suppressive ART, our primary analysis will artificially censor participants at the first Q16 week window where they either a) stop ART for >21 days, b) viral failure (the first 2 consecutive HIV RNA >200 copies/ml) c) lost to follow up. This will provide inference for a population that remains on suppressive ART. To address informative censoring, we will use an Inverse probability of censoring weighting (IPCW) methods as in DACS286. Pooled logistic regression models for remaining in follow-up with suppressive ARTs at each Q16 week window, are fitted with sex, age, race/ethnicity, injection drug, time since year 2 and previous CD4 as covariates.,. The IPCW weight of each participant at a current week window, is the reciprocal of the cumulative estimated probability of remaining in follow-up with suppressive ARTs from the logistic regression models.

After applying the IPCW methods, we will obtain an analysis dataset containing N_1 participants with an observed clinical event between year 2 and year 7, and not artificially censored before events, and N_0 participants without a clinical event between year 2 and year 7 and without being artificially censored.

| History of AIDS or non-AIDS events | 0=No | 1=Yes | Year 2 |
|-----------------------------------|------|-------|--------|
| 0-1000                            |      |       |        |
| 1001-10000                        |      |       |        |
| 10001-100000                      |      |       |        |
| =>100000                         |      |       |        |

Note: * Cut-offs for CD4:8 ratios are based on:
- Ratnam I et al. *Incidence and risk factors for immune reconstitution inflammatory syndrome in an ethnically diverse HIV type-1-infected cohort.* Clin Infect Dis 42: 418-427, 2006
through year 7. Regarding accumulation of IPCW weights, the N_1 will accumulate IPCW weights up to the Q16 week window where a clinical event is observed. The N_0 will accumulate IPCW weights through the Q16 week window of year 7. The sum of weights for N_1 and N_0 should approximately equal to N. The N_0 participants (clinical event=0) + N_1 participants (clinical event=1) will be analyzed in a logistic regression model, each weighted by their accumulated IPCW weights, to evaluate the prognostic value of the CD4/CD8 ratio at year 2. We will further evaluate additional covariates: CD4 slope at year 2 (i.e., CD4 change from baseline to year 2), baseline log_{10} vRNA, AIDS diagnosis or serious non-AIDS-defining event before year 2, HCV co-infection before year 2, initial ART type, and demographics age, sex, race/ethnicity (white non-Hispanic/other), and injection drug use (ever/never).

This logistic regression model assumes each participant’s IPCW is true. However, these weights are estimated (by IPCW), therefore a bootstrap approach will also be used to calculate the confidence intervals. In the presented analysis, we will conservatively obtain the confidence intervals by assuming the weights are known.

4.3.2 Secondary Analysis

The secondary analysis will censor only using a) and c) in previous section. This will provide inference had everyone remained on ART regardless of VF.

In another secondary analysis, we will investigate the effect of the CD4/CD8 ratio and CD8 counts applying the same statistical method in previous section in the subset of individuals with CD4+ T cell >500 at year two of ART initiation.