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Replication Capacity in Relation to Immunologic and Virologic Outcomes in HIV-1 infected, Treatment-Naïve Subjects

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Abstract

Objectives—To evaluate the association between baseline (BL) replication capacity (RC) [RC_{BL}] and immunologic/virologic parameters (at BL and after 48 weeks on therapy) in HIV-1 infected subjects initiating antiretroviral therapy.

Methods—RC_{BL} was determined using a modified Monogram PhenoSense HIV drug susceptibility assay on plasma HIV-1 from 321 treatment-naïve subjects from ACTG384. Univariate and multivariable analyses were performed to determine the association of RC_{BL} with BL and on-therapy virologic and immunologic outcomes.

Results—Higher RC_{BL} was associated with lower baseline CD4 ($CD4_{BL}$) ($r=-0.23$, $p<0.0001$), higher baseline HIV-1 (RNA_{BL}) ($r=0.25$, $p<0.0001$), higher $CD4_{BL}$ activation percent ($r=0.23$, $p<0.0001$) and lower $CD4_{BL}$ memory count ($r=-0.21$, $p=0.0002$).

In a multivariable model, week 48 CD4 increase ($\Delta CD4_{48}$) was associated with lower $CD4_{BL}$ memory count and higher $CD4_{BL}$ naive percent ($p=0.004$, $p=0.015$, respectively). The interaction between $CD4_{BL}$ and RC_{BL} was significant ($p=0.018$), with a positive association between RC_{BL} and $\Delta CD4_{48}$ in subjects with higher $CD4_{BL}$, and a negative association at lower $absCD4_{BL}$.

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Conclusions—At baseline, higher RC was significantly associated with higher HIV-1 RNA, higher CD4 cell activation, lower CD4 cell count, and lower CD4 memory cell count. These factors may interact, directly or indirectly, to modify the extent to which CD4 recovery occurs in patients starting antiretroviral therapy at different baseline CD4 counts.

Keywords

HIV; replication capacity; viral fitness; pathogenesis; immune reconstitution; activation; memory

Introduction

Immunologic recovery following the initiation of a potent antiretroviral regimen remains a central goal of HIV-1 therapy. Greater CD4 cell recovery during antiretroviral therapy (ART) has been associated with younger age, female sex, higher BL viral load, and virologic suppression to fewer than 50 copies/ml (1–7). Virologic suppression, however, does not guarantee immunologic recovery. Other factors, such as lower BL CD4 naïve percent, higher BL CD4 and CD8 activation and persistent CD4 or CD8 activation on therapy, have been associated with lower CD4 cell recovery in some individuals despite achieving virologic suppression (1, 8–12).

Another factor which may influence immunologic recovery is viral fitness. Although viral fitness is dependent on many factors *in vivo*, including immunologic pressure and antiretroviral therapy, RC, as measured in vitro by recombinant virus assays, represents a component of viral fitness. RC is defined as the intrinsic ability of a virus to replicate in the absence of drugs under standardized laboratory conditions, in comparison to a reference wild-type strain (13). RC has been shown to be a predictor of clinical progression to AIDS that is independent of HIV RNA and CD4 cell count (14). Furthermore, higher RC values were significantly associated with a greater rate of decline of CD4 cell count during follow-up (14).

The association between RC and virologic or immunologic response to ART has been tested in several HIV-1 cohorts with varying results. An association between lower pre-therapy RC and greater CD4 cell recovery on-therapy has been suggested in two analyses of patients with acute or early HIV infection who initiated antiretroviral therapy (15, 16). A third analysis of chronically infected patients who achieved virologic suppression on their first ART regimen, however, did not show a relationship between RC_{BL} and CD4 cell recovery after 1 year of therapy (17). In treatment-experienced patients initiating a new regimen, higher RC_{BL} was associated with lower on-therapy CD4 cell gains in patients who achieved virologic suppression and higher on-therapy viral load in non-suppressed patients (18, 19). Thus, while several studies have shown an association between RC measurements and response to treatment, to date no adequately powered and controlled study has demonstrated a definitive role for RC measurements in the management of HIV-1 infection.

The ACTG 384 study provides a unique opportunity to evaluate RC in a large, prospective, randomized, controlled clinical trial, in which many predictors of virologic and immunologic outcomes have been defined (1, 20, 21). RC measurements at a pre-therapy BL were analyzed in the context of other BL covariates and as related to week 48 on-therapy immunologic and virologic response.

Methods

We determined RC_{BL} on plasma HIV-1 from 321 treatment-naïve subjects who participated in the immunology substudy of ACTG 384 (980 subjects randomized to start d4T/ddI or

AZT/3TC with NFV, EFV or both NFV/EFV (20, 21). For this analysis, subjects were required to have BL and week 48 CD4 cell counts, flow cytometry, plasma HIV-1 RNA by PCR, and stored plasma samples. In addition, subjects had to have remained on their initial ART regimen assignment through week 48.

Subjects were grouped into pre-defined BL CD4 cell count strata 1 through 5 (<= 50, 51–200, 201–350, 351–500, and > 500 cells/mm^3)(1). For subjects with fewer than 350 CD4 cells/mm^3 at BL, we randomly identified 48 subjects with virologic suppression (HIV-1 RNA < 50 copies/ml at week 48) from each of 3 strata. In order to enrich the lower CD4 sample for the evaluation of immunologic endpoints, up to 7 additional subjects per stratum were identified who had virologic suppression and immunologic failure (HIV-1 RNA <50 c/mL and <100 CD4 cell/mm^3 rise in CD4 cell count) at week 48. Stored samples were available on a total of 148 subjects in the lower 3 strata. All subjects with greater than 350 CD4 cells/mm^3 who had stored samples available were assayed for RC, regardless of virologic or immunologic response; this was a total of 173 subjects. A total of 321 subjects were included in the RC substudy (Figure 1).

The institutional review board of participating sites approved the ACTG 384 trial, all subjects signed informed consent forms which included the use of stored samples in future ACTG-approved research, and subsequent analyses were approved by the ACTG. All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

RC was assessed using a modification of the PhenoSense HIV drug susceptibility assay, as previously described (13, 22, 23). Briefly, plasma-derived reverse transcriptase (RT) and protease (PR) sequences [constructs containing gag (3' end from p7), all of PR (aa 1–99), and RT (aa 1–305)] were inserted into a modified retroviral vector (RTV) containing a luciferase indicator gene in place of the HIV-1 envelope gene. Plasmids containing the RTV and a plasmid containing amphotropic murine leukemia virus (aMLV) were co-transfected in HEK-293 cells to generate pseudotyped virus particles containing the patient-derived PR/RT sequences with the aMLV envelope. Fresh HEK-293 cells were infected with the pseudotyped viruses in the absence of drugs, then RC was determined by comparing luciferase activity in infected cells with that of a wild-type reference virus (NL4-3) following a single round of replication. RC was expressed as a percentage relative to the reference strain (NL4-3). An RC of 100% indicates that the RC of the pseudotyped virus population closely approximates the median value of a wild-type (i.e., drug-sensitive) virus population.

Plasma HIV-1 RNA, CD4 and CD8 cell counts and advanced immunology flow cytometry were performed as previously reported (1). Plasma HIV-1 RNA levels were measured using the Roche Ultrasensitive Amplicor v1.0 PCR assay, with a lower limit of detection of 50 copies/mL at a central laboratory. Three-color flow cytometry was performed on fresh cells according to the ACTG advanced flow protocol at BL and week 48. Naïve T cells were defined as triple positive for CD45RA, CD62L and either CD4 or CD8; memory T cells were defined as positive for CD45RO, negative for CD45RA and positive for CD4 or CD8. Activated T cells were defined as triple positive for CD38, HLA-DR and either CD4 or CD8 (1).

BL covariates that have been shown to significantly affect disease progression, CD4 cell decline and/or on-therapy virologic or immunologic outcome were analyzed by univariate and multivariable analysis for association with RC_{BL}. Spearman correlations, logistic regressions and Cox Proportional Hazard models, Wilcoxon Rank Sum and Fisher's Exact tests, as appropriate, were performed to determine the effect of RC_{BL} on virologic and

immunologic outcome measures. Multiple linear regression was performed to determine the effect of RC_{BL} on $\Delta CD4_{48}$ restricted to subjects with HIV-1 RNA < 50 copies/mL at week 48, controlling for $CD4_{BL}$, RNA_{BL}, BL CD8 count, BL CD4 memory count and BL CD4 naïve percent (all covariates were continuous). All other analyses were based on all 321 subjects. Statistical tests were two-sided exploratory analyses and 0.05 was used for the nominal level of significance (without adjustments for multiple testing).

Results

Patients

BL characteristics and virologic and immunologic outcomes at week 48 are shown in Table 1. The subgroup of individuals with 350 or fewer CD4 cells/mm^3 at BL ($CD4_{BL}$) had significantly higher BL HIV-1 RNA (RNA_{BL}) levels than the subgroup with >350 cells/mm^3 ($p<0.0001$).

Replication Capacity

The distribution of RC_{BL} for each of the five BL CD4 cell strata is illustrated by a box-and-whiskers plot in Figure 2. Median RC_{BL} values for strata 1 through 5 were 148, 99, 85, 90, and 85%, respectively. RC_{BL} was significantly higher in stratum 1 ($CD4_{BL}$ <= 50) than in the other 4 CD4 strata ($p<0.0001$).

Baseline Associations Between RC and Demographic and Immunologic/Virologic Factors

Higher RC_{BL} was associated with lower $CD4_{BL}$ ($r = -0.23$, $p<0.0001$; adjusting for RNA_{BL} $r = -0.12$, $p=0.033$), higher RNA_{BL} ($r = 0.25$, $p<0.0001$; adjusting for $CD4_{BL}$ $r = 0.16$, $p=0.005$), higher BL CD4 activation percent ($r = 0.23$, $p<0.0001$) and lower BL CD4 memory count ($r = -0.21$, $p = 0.0002$). RC_{BL} was not significantly associated with BL naïve/memory CD4 ratio, BL CD8 activation percent, gender, intravenous drug use (ever versus never), or race/ethnicity.

Association Between RC_{BL} and Immunologic/Virologic Outcomes at Week 48

In unadjusted analyses, RC_{BL} was not significantly associated with $\Delta CD4_{48}$, immunologic success (defined as a greater than 100 cells/mm^3 rise in CD4 cell count from BL to week 48), time-to-first virologic failure or time to the primary endpoint of the parent ACTG 384 study (defined as time from first treatment date to second regimen failure for three-drug arms, or time to first regimen failure for four-drug arms)(20,21).

In order to assess the contribution of baseline RC to CD4 recovery on treatment, a multiple regression model was constructed with $\Delta CD4_{48}$ as the outcome variable. Univariate analyses (Spearman correlation) in the entire RC substudy cohort (N = 321) demonstrated that $\Delta CD4_{48}$ was significantly associated with BL CD4 naïve percent ($r = 0.21$, $p = 0.0002$), BL naïve:memory CD4 ratio ($r = 0.20$, $p = 0.0003$), RNA_{BL} ($r = 0.18$, $p = 0.001$), BL memory CD4 count ($r = -0.15$, $p = 0.008$), BL CD8 cell count ($r = -0.13$, $p = 0.02$) and age ($r = -0.13$, $p = 0.02$). Additional significant baseline variables were considered from the multivariable model of Gandhi, et al, from the parent ACTG 384 Immunology Study (1). The final model included RC_{BL}, $CD4_{BL}$, $CD8_{BL}$, RNA_{BL}, BL CD4 memory count and BL CD4 naïve percent. CD4 naive/memory ratio was not included because of the inclusion of CD4 memory and CD4 naïve variables, and age and gender were not included because they did not remain significant in our multivariable model. This analysis was restricted to subjects with HIV-1 RNA suppression to fewer than 50 copies/ml at week 48 (N = 288).

The multivariable model demonstrated that BL memory CD4 cell count was negatively associated with $\Delta CD4_{48}$ (coefficient −0.35, $p = 0.004$) and BL naïve CD4 percent was

positively associated with $\Delta CD4_{48}$ (coefficient 1.47, p = 0.015). $CD8_{BL}$ and RNA_{BL} failed to show a significant association with $\Delta CD4_{48}$ after adjusting for other covariates. In order to put these results in the context of other studies limited to subjects with early HIV infection who, in general, had higher $CD4_{BL}$ counts, a subgroup analysis was performed for subjects with $CD4_{BL}$ <= 350 or > 350 cells/mm^3. An interaction between $CD4_{BL}$ and RC_{BL} was suggested, which was then formally tested by including the interaction term ($CD4_{BL}$ * RC_{BL}) in the multiple regression model (Table 2). This interaction was significant (coefficient 0.002, p = 0.018), precluding interpretation of a direct association between RC_{BL} and $\Delta CD4_{48}$.

The interaction between $CD4_{BL}$ and RC_{BL} in relation to $\Delta CD4_{48}$ is illustrated in Figure 3A. Each dot represents one subject. A pattern of $\Delta CD4_{48}$ according to RC_{BL} and CD4 stratum is suggested by the univariate least squares fitted lines. It was noted that the vast majority of subjects with very high RC_{BL} (above 200% of reference) were in stratum 1 ($CD4_{BL}$ <= 50) and that these high RC subjects had a $\Delta CD4_{48}$ at or below the median for the entire group. In contrast, the highest CD4 stratum ($CD4_{BL}$ > 500) included a group of subjects with low RC_{BL} and a net loss in CD4 cell count from BL to week 48.

The value for $\Delta CD4_{48}$ was associated not only with RC_{BL} and $CD4_{BL}$ as depicted in Figure 3A, but also other BL factors, such as absolute memory CD4 count and the percentage of naïve CD4 cells. In order to focus on the relationship between $CD4_{BL}$, RC_{BL} and $\Delta CD4_{48}$, the multiple regression model described above was used to generate fitted regression lines by controlling for (at their median values) BL CD8 count (779 cells/mm^3), RNA_{BL} (4.85 log_{10} copies/ml), BL memory CD4 count (165 cell/mm^3) and BL naïve CD4 percent (33%). $CD4_{BL}$ was placed in the model at values corresponding to the median values for the five BL CD4 strata. Figure 3B illustrates the fitted regression lines generated and the median RC_{BL} for each of the five strata. Although there is some variability in these estimates, these lines illustrate the significant interaction term ($CD4_{BL}$ * RC_{BL}) discussed above, with a positive association between RC_{BL} and $\Delta CD4_{48}$ in subjects with higher $CD4_{BL}$ and a negative association in subjects with lower $CD4_{BL}$.

Discussion

Variation in CD4 recovery has been noted in previous analyses of treatment-naïve, HIV-1 infected individuals who achieve virologic suppression after initiating ART (1, 2, 8–10, 13, 24, 25). Although overall gains in CD4 cell count do not differ by BL CD4 count strata ((7) and G Robbins, submitted), a small but significant number of subjects do not achieve at least a 100 cell rise in CD4 count. In the analysis of the larger ACTG 384 Immunology substudy, of 608 subjects with virologic suppression to fewer than 50 copies/ml at week 24, only 255 (42%) achieved this definition of “immunologic success”(1). Although 87% of the 148 subjects who maintained virologic suppression through week 144 achieved immunologic success at this time point, 13% did not. These immunologic substudies not only describe changes in lymphocyte subsets on therapy, but attempt to understand why not all patients are able to achieve immunologic success.

The current study was broadly designed to evaluate the relationship between RC and other BL factors, and to evaluate RC_{BL} in relation to immunologic and virologic response to ART. As described above, RC_{BL} was significantly associated with $CD4_{BL}$, RNA_{BL}, BL CD4 activation percent, and BL memory CD4 cell count.

Three previous studies evaluated similar BL associations with RC_{BL} in individuals who were naive or minimally exposed to antiretroviral therapy (14–15,17). The significant negative association between RC_{BL} and $CD4_{BL}$ in our study is in agreement with these

previous analyses. In the recently infected cohort of Barbour, et. al., a significant inverse association between RC_{BL} and $CD4_{BL}$ cell count was seen (r = −0.29, P = <0.0001)(15). In contrast, in study populations with more advanced disease, weaker inverse associations between RC_{BL} and $CD4_{BL}$ were seen (r = −0.197, P = 0.03(14) and r = 0.065, P = 0.0031(17)). The current analysis demonstrated a highly significant association (r = −0.23, p < 0.0001), which remained significant after controlling for HIV-1 RNA_{BL} (r = −.012, p = 0.033) in a large and uniformly antiretroviral treatment-naïve population across a broad spectrum of $CD4_{BL}$.

Previous evaluations of RC and plasma HIV-1 RNA levels have shown weak positive associations, at best. One study found no significant association (r = 0.08, p = 0.25), whereas another study found a weak association (r = 0.189, p = 0.03) (14, 15). These study populations differed from the current study in regard to stage of disease, presence of antiretroviral resistance, and/or previous antiretroviral experience. In contrast, our study of a larger number of uniformly treatment-naïve subjects found a highly significant positive association between RC_{BL} and RNA_{BL} (r = 0.25, p<0.0001) that remained significant after adjusting for $CD4_{BL}$ (r = 0.16, p = 0.005). Whether higher RC leads directly to an increase in circulating virus or whether indirect effects are exerted through another mechanism remains to be elucidated.

As a substudy of the larger ACTG 384 Immunology Study, this analysis was able to utilize advanced flow analysis results to investigate associations between RC_{BL} and subpopulations of CD4 cells. This analysis demonstrated, for the first time, the highly significant positive association between RC_{BL} and BL CD4 activation percent (r = 0.23, p <0.0001). The level of CD4 activation has been shown to be an independent predictor of CD4 decline and progression to AIDS (26–28). The relationship between immune system activation and lentivirus disease progression is complex and has been demonstrated to be independent of circulating viral load in humans (29–31) and non-human primates (32, 33). The association between RC and CD4 activation demonstrated in this study may suggest one mechanism by which RC contributes to CD4 cell decline and disease progression (14).

High RC_{BL} was also associated with low BL CD4 memory cell count (r = −0.21, p = 0.0002) in this population. Low levels of circulating CD4 memory cells may reflect loss by direct infection by R5 virus (34, 35), loss via activation-induced cell death (36), and/or sequestration into lymph nodes during viremia (37–39). High RC viruses may contribute to CD4 memory cell loss directly through one of these mechanisms, or indirectly by a positive association with high viral load or CD4 cell activation. Thus, viruses with higher RC may contribute to CD4 decline and disease progression through an association with higher viral load, through an increase in the level of CD4 cell activation, and/or through enhanced loss of CD4 memory cells.

This analysis also evaluated the association between RC_{BL} and immunologic/virologic outcomes on therapy. In initial univariate analyses, RC_{BL} was not significantly associated with $\Delta CD4_{48}$, immunologic success ($\Delta CD4_{48} \geq 100$ cells/mm^3), or time-to-first virologic failure. A subsequent model utilized $\Delta CD4_{48}$ as the outcome variable and independent variables that were highly associated with $\Delta CD4_{48}$ (RNA_{BL}, BL CD8 cell count, BL CD4 memory count and BL CD4 naïve CD4 percent) as well as $CD4_{BL}$.

BL memory CD4 cell count was a significant negative predictor and BL naïve CD4 percent a significant positive predictor of $\Delta CD4_{48}$. Accounting for these factors overwhelmed the effects of RNA_{BL} and BL CD8 cell count, such that $\Delta CD4_{48}$ was no longer significantly associated with either of these factors. This relationship between baseline naïve and memory

cell number, percent or ratio and CD4 recovery on ART is in agreement with other studies (1, 8, 9).

A significant interaction between RC_{BL} and $CD4_{BL}$ was demonstrated, as illustrated by the fitted regression lines in Figure 3B. The explanation for why a positive relationship between RC_{BL} and $\Delta CD4_{48}$ exists in subjects with higher BL $CD4_{BL}$ and a negative relationship exists in subjects with lower BL $CD4_{BL}$ is uncertain. However, as noted in Figure 3A, individual subject data points from the highest and lowest BL CD4 cell strata stand out and provide a basis for generating hypotheses as to the potential role of RC_{BL} in CD4 recovery.

From the model, in patients with higher $CD4_{BL}$, lower RC_{BL} was associated with a smaller $\Delta CD4_{48}$. Figure 2 illustrates that a cluster of subjects with $CD4_{BL} > 500$ had RC_{BL} values in the lower half of the entire group, and demonstrated a net fall in CD4 cell count from BL to week 48. This suggests that low RC viruses in this population are of low pathogenicity and that suppression of virus replication reverses what is at most a low level of CD4 cell destruction or redistribution. The occurrence of poor CD4 recovery in patients initiating ART at high CD4 counts has been noted by others (2, 40, 41), and the demonstrated association with low RC may in part explain this observation.

Conversely, high RC viruses in this high $CD4_{BL}$ population are associated with a larger $\Delta CD4_{48}$. This suggests that high RC viruses are more pathogenic, contributing to greater CD4 cell destruction or redistribution, potentially by effects on the level of CD4 activation or CD4 memory cell numbers. It would follow, then, that abrogation of replication of these high RC viruses by effective antiretroviral therapy would lead to greater CD4 count gains in these subjects, when compared to those subjects with lower RC viruses.

In the lowest BL CD4 stratum ($CD4_{BL} <= 50$ cells/mm^3), higher RC_{BL} was associated with a smaller $\Delta CD4_{48}$. This suggests that high RC viruses may have exerted an effect on the body prior to the institution of ART, which prevented an optimal CD4 cell recovery. This would be the case if high RC viruses destroyed T cell precursors in the bone marrow or thymus, destroyed CD4 memory cells in lymphoid tissues, prevented their redistribution after viral suppression, or interfered with an effective immune response to the virus. The association of high RC viruses with high levels of CD4 activation and low CD4 memory cell counts at baseline may contribute to these mechanisms. In contrast to the situation in high $CD4_{BL}$/high RC_{BL} subjects, the poor CD4 recovery seen in low $CD4_{BL}$/high RC_{BL} subjects suggests that the damage from high RC viruses has already occurred and is relatively irreversible.

The potential role of RC as a contributing factor in HIV-1 disease progression and CD4 cell decline has been suggested by a previous study. In the Hemophilia Growth and Development study by Daar, et. al., RC_{BL} was shown to be a predictor of clinical progression to AIDS, independent of HIV RNA_{BL} and $CD4_{BL}$ ($p = 0.04$) (14). Furthermore, higher RC_{BL} values were significantly associated with a greater rate of decline of CD4 cell count during follow-up, controlling for time on study, antiretroviral use over time, $CD4_{BL}$ and RNA_{BL} ($P < 0.0001$) (14).

In summary, this analysis has demonstrated significant pre-therapy associations between higher RC viruses and either higher HIV-1 RNA level, higher levels of CD4 activation, lower CD4 count, or lower CD4 memory cell numbers. These factors may interact, directly or indirectly, to modify the extent to which CD4 recovery occurs in patients at different baseline CD4 counts. The contribution of other factors, such as viral coreceptor tropism (42), will be evaluated in ongoing analyses.

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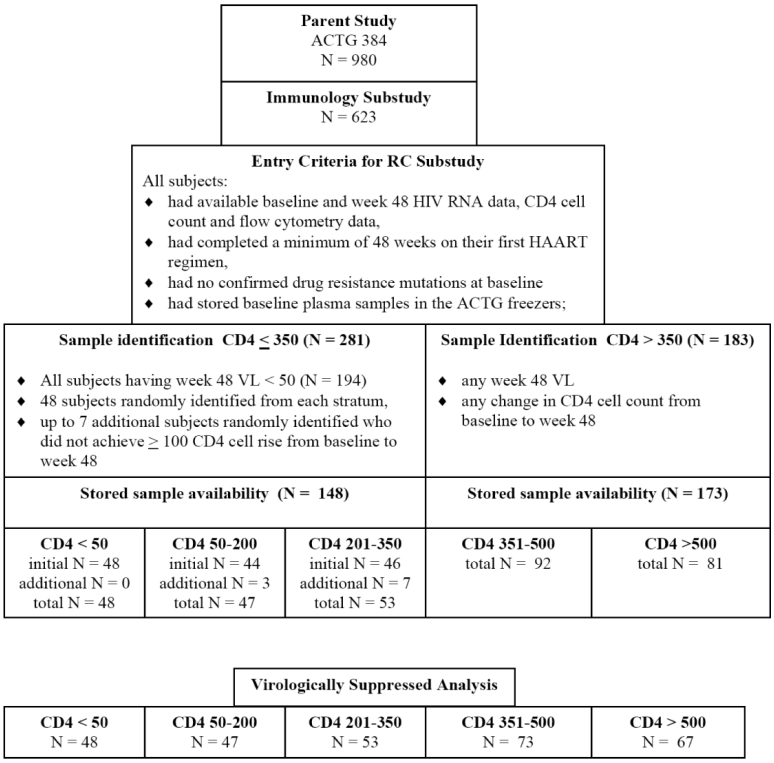

Figure 1. Subject Selection

Subject selection for the ACTG 384 RC substudy. See Methods.

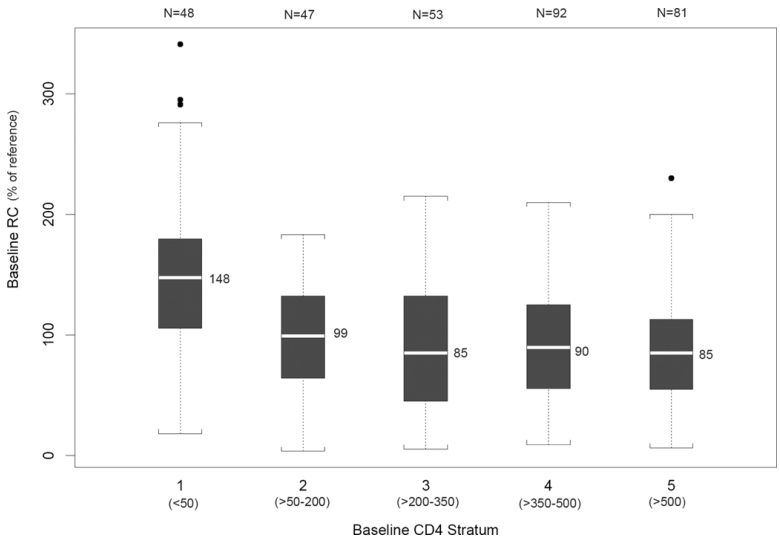

Figure 2. RC_{BL} versus $CD4_{BL}$ Cell Count

This box-and-whiskers plot illustrates the median RC value for each CD4 stratum (horizontal white line and adjacent value). The Q1 to Q3 (25^{th} to 75^{th} percentile) is depicted by the shaded box, the whiskers are drawn to the nearest value not beyond a standard span (1.5 × the interquartile range, defined as Q3 − Q1) from Q1 and Q3, and points beyond the whiskers (outliers) are drawn individually. RC_{BL} was significantly greater in stratum 1 (CD4 <= 50) compared with the other 4 CD4 strata ($p<0.0001$).

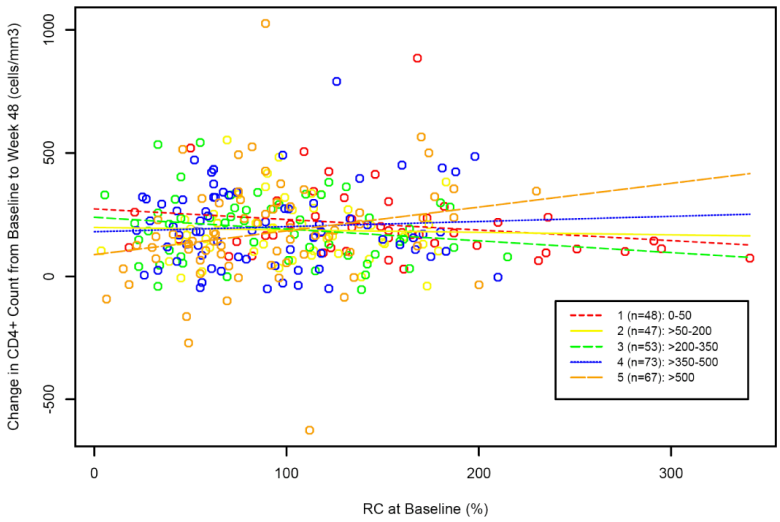

Figure 3A. $\Delta CD4_{48}$ and RC_{BL} by BL CD4 Stratum (restricted to subjects with virologic suppression at week 48)

Individual subjects are depicted by colored circles corresponding to the five BL CD4 strata. Each corresponding colored line represents the univariate least squares fit ($\Delta CD4_{48}$ on RC_{BL}) generated for each stratum. The lines are drawn beyond the individual points to illustrate the relationships among the lines.

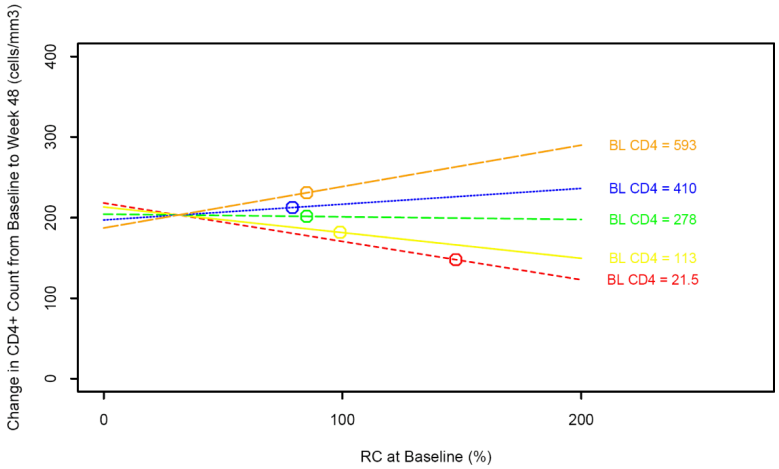

Figure 3B. Fitted Regression Lines for $\Delta CD4_{48}$ and RC_{BL} for Various $CD4_{BL}$ Values (restricted to subjects with virologic suppression at week 48)

Fitted regression lines are based on the model described in Table 2 (restricted to subjects with viral suppression at week 48), with controlled BL CD8 count, RNA_{BL}, BL CD4 memory count, BL CD4 naive percent at their median values (779 cells/mm3, 4.85 log10 copies/mL, 165 cells/mm3, and 33% respectively), and $CD4_{BL}$ at various values (medians for each of the 5 strata: 21.5, 113, 278, 410, and 593 cells/mm3). Circles represent the model-generated values of $\Delta CD4_{48}$ at their median BL RC_{BL} for the 5 strata. When $CD4_{BL}$ = 297, the slope of the fitted regression line is zero. The $CD4_{BL} \times RC_{BL}$ interaction was significant (p=0.018), with a positive association between RC_{BL} and $\Delta CD4_{48}$ in subjects with higher $CD4_{BL}$, and a negative association in subjects with lower $CD4_{BL}$. The lines are drawn beyond the data points used in the model in order to illustrate the relationships among the lines.

Table 1

BL Demographics and Immunologic and Virologic outcomes at Week 48

		CD4 <= 350	CD4 > 350	Total
Sample Sizes		148	173	321
Gender	Male	128 (86%)	143 (83%)	271 (84%)
Race/Ethnicity[a]	White	68 (46%)	91 (53%)	159 (50%)
	Black	50 (34%)	61 (35%)	111 (35%)
	Hispanic	28 (19%)	17 (10%)	45 (14%)
	Asian	2 (1%)	4 (2%)	6 (2%)
Intravenous Drug Use[b]		9 (6%)	10 (6%)	19 (6%)
Age (years)	Median (min-max)	37 (21–65)	35 (17–60)	36 (17–65)
CD4 count (cells/mm^3)	median (Q1 - Q3)	118.5 (37, 241)	479 (413, 593)	369 (135, 501)
Immunologic success ($\Delta CD4_{48} \geq 100$ cells/mm^3)	**N (%)**	**115 (78%)**	**118 (68%)**	**233/321 (73%)**
HIV-1 RNA(log_{10} copies/mL)	Median (Q1 - Q3)	5.26 (4.75, 5.66)	4.44 (4.02, 4.92)	4.83 (4.28, 5.40)
Proportion with RNA < 50 c/ml at week 48	**N (%)**	**148 (100%)**	**140 (81%)**	**288/321 (90%)**

[a] Intravenous drug use – ever (current or past)

[b] Self-reported

Table 2

Baseline factors associated with $\Delta CD4_{48}$ (restricted to subjects with virologic suppression at week 48)

BL Factor	Direction	Coefficient (95% C.I.)	P-value
Intercept	--	132.79 (−26.34, 291.92)	--
RC_{BL} (%)	neg	−0.51 (−1.01, −0.02)	0.043 +
$CD4_{BL}$ (cells/mm^3)	neg	−0.05 (−0.28, 0.18)	0.065
$CD8_{BL}$ (cells/mm^3)	neg	−0.03 (−0.07, 0.01)	0.144
RNA_{BL} (log_{10} copies/ml)	pos	24.38 (−1.91, 50.67)	0.070
BL CD4 Memory Count (cells/mm^3)	neg	−0.35 (−0.58, −0.12)	0.004 ++
BL CD4 Naïve %	pos	1.47 (0.30, 2.63)	0.015 +
Interaction: $CD4_{BL}$ *RC_{BL}	--	0.002 (0.0003, 0.003)	0.018 +

+ P < 0.05

++ P < 0.05