To determine whether markers of T cell activation and maturation are independently predictive of the response to potent antiretroviral therapy, the Immunophenotypic Markers and Antiretroviral Therapy study applied a novel data-sharing strategy across 5 Adult AIDS Clinical Trial Group trials that counted naive and activated CD4+ and CD8+ T cells in 324 subjects. Regression models—adjustment for baseline CD4 cell count, human immunodeficiency virus (HIV) RNA, and study—revealed that high pretreatment CD8+ T cell activation predicted virologic failure (P = .046). Additional models showed the greatest increase in CD4+ T cell counts in subjects with highest pretreatment naive CD4+ T cell counts (P < .0001), which was enhanced by high CD4+ and low CD8+ T cell activation. Total lymphocyte count also predicted a subsequent CD4+ T cell change. These results document the utility of T cell markers in predicting treatment outcome and their potential value for the study and management of HIV-1 infection.

Accelerated development of new drugs for the treatment of HIV-1 infection has been made possible by the validation of 2 essential biomarkers that serve as surrogates for clinical efficacy in antiretroviral trials—CD4+ T lymphocyte counts and plasma HIV-1 RNA concentrations [1–4]. Together, these markers do not fully explain the benefit of potent antiretroviral therapy (ART) on clinical outcomes, such as AIDS progression and death, as would the ideal biomarker [1, 5–9]. Still, their relevance to the pathogenesis of HIV infection and disease has been well established in natural history studies [10]. Their consistent performance across trials as indicators of ART activity, together with the demonstrated relationship between such activity and a reduction in subsequent clinical events, has led to the uniform (including regulatory) acceptance of HIV RNA and CD4+ T cell count as measurements signifying antiretroviral and immunorestorative efficacy, respectively [11, 12].

The goal of the present study was to examine whether naive CD4+ T lymphocytes and activated CD4+ and CD8+ T lymphocytes, as measured by flow cytometry, contribute independent information to HIV RNA load and CD4 cell count in predicting the response to ART. The Immunophenotypic Markers and ART (IMART) study is a cross-protocol analysis of 5 current multicenter Adult AIDS Clinical Trials Group (AACTG) trials of potent ART that assayed these T cell immunophenotypic markers by consensus methods, in subjects who were previously untreated or treated only with nucleosides. Because the timely evaluation of the markers would require access to data from ongoing trials, novel data-sharing conditions were developed to limit the possible negative effect on contributing parent studies.
SUBJECTS AND METHODS

Patient population and laboratory evaluations. The IMART data set was created with access to 5 ongoing or recently completed AACTG trials that evaluated potent ART in therapy-naive subjects or in those with exposure either to zidovudine or to zidovudine and lamivudine [13–17]. Institutional review boards approved all protocols; all patients provided written, informed consent. The data set included demographic information, study number, and baseline through week-40 values of T lymphocyte subsets and HIV RNA, measured by quantitative HIV-1 RNA polymerase chain reaction assays (Roche Molecular Systems, lower limit of quantitation 400 or 50 copies/mL or Nuclisens; Organon Teknika, lower limit of quantitation 100 copies/mL). T lymphocyte subpopulations were enumerated in fresh whole blood, according to AACTG consensus immunophenotyping methods [18] and quality assured across laboratories by the Immunology Quality Assurance program [18], using 3-color flow cytometry for the quantitation of CD3+, CD4+, and CD8+ T cells. Naive CD4+ T cells were defined as CD45RA+CD62L+, and activated CD4+ and CD8+ T cells were defined as CD38+/HLA-DR+. Values for total lymphocyte count (TLC) and hemoglobin (Hgb) concentrations were derived from automated complete blood counts.

Data-sharing methods and statistical analysis. The following data-sharing conditions were implemented as safeguards to preserve the integrity of ongoing contributing trials: (1) no treatment codes were included in the data set; (2) a week-24 end point was selected for analysis, to prevent compromise of longer-term end points of contributing trials; (3) only associations with predictive factors were examined—no summary data of actual CD4+ T cell or HIV RNA responses are included in the results; (4) subjects’ data were randomly excluded, to limit the maximum contribution from any single trial to ≤50% of the analysis population; (5) no study-specific results are reported.

For the outcome, the complete record closest to week 24 was chosen for inclusion in the data set. Because of the variability in scheduled evaluations and visits across trials, the 24-week follow-up window after the initiation of potent ART ranged from 12 to 40 weeks; 71% were between weeks 20 and 28. This follow-up time is referred to as “week 24” throughout. Values for HIV RNA obtained within 60 days before the initiation of potent ART were averaged on the log 10 scale, to obtain the baseline value; HIV RNA obtained within 60 days before the initiation of potent ART is referred to as “week 24” throughout. Values for scheduled evaluations and visits across trials, the 24-week follow-up time point is chosen for inclusion in the data set. Because of the variability in the 5 studies would supplement HIV RNA and CD4+ T cell counts in predicting week-24 responses to potent ART. Rank-based (Spearman’s) correlations were used to assess bivariate associations. The Wilcoxon signed rank test was used to assess changes from baseline for continuous variables, and McNemar’s test was used to test changes in the proportion of subjects with HIV RNA <1000 copies/mL. Regression models were constructed, using linear regression for changes in CD4+, CD8+, and naive CD4+ T cell counts. A censored-data normal-distribution regression for log10 RNA was used to model the week-24 HIV RNA, which accommodates results below the quantification limits of the assay; the baseline log10 HIV RNA was included as a covariate [19]. A forward stepwise procedure was used to select variables with statistical and biological importance; no corrections were made for multiple comparisons or for the stepwise selection. Interactions were also investigated (i.e., effect modifiers); when interactions were included in the model, each main effect was also included. A set of 4 indicator variables to represent the 5 contributing studies was included in each model; this essentially adjusted for nucleoside-naive versus nucleoside-experienced subjects, although ACTG 343/879 consisted of both zidovudine-experienced and zidovudine-naive subjects. The amount of variability explained by the covariates in the model was assessed by $R^2$; for the censored-data regression model, a generalization of $R^2$ was used [20]. Sensitivity analyses were also conducted, to assess whether results were consistent in subgroups of subjects. Because subsets of CD4+ and CD8+ T lymphocytes were not measured at early time points (e.g., week 12) in these studies, early changes in these markers could not be examined. With the data-sharing conditions, the available sample size of 324 provided 80% power to detect a correlation of 0.15.

RESULTS

Baseline characteristics and correlations. The analysis cohort consisted of 324 subjects from 5 AACTG studies. Of the 5 studies, 3 were completed at the outset (ACTG 315, 343/879, and 368/890), 1 has been recently completed (ACTG 384), and 1 is ongoing (ACTG 371; table 1) [13–17]. Subjects were predominantly male (82%) and white non-Hispanic (56%), with a median age of 37 years (25th–75th percentile, 32–45 years). As shown in table 2, the study population was immunodeficient at baseline, with a median baseline CD4+ T cell count of 260 cells/mm³, a median baseline CD8+ T cell count of 777 cells/mm³, and a median baseline naive CD4+ T cell count of 75 cells/mm³ (normal level, ≥300 cells/mm³) [21]. The proportion of T lymphocytes that expressed activation markers was increased; the median percentage of activated CD4+ and CD8+ T cell subpopulations was 16% and 46%, respectively (normal level, ≤5%) [21]. The median baseline log10 HIV RNA was 4.63 (43,000 copies/mL).

Correlations between immunologic markers and HIV RNA at baseline are summarized in table 2 and figures 1 and 2 (percentages of CD4+ and CD8+ T cells are not shown). There were significant ($P<.001$ for all correlations $≥.20$ in absolute value) and strong positive correlations between CD4+ T cell...
count and naive CD4+ T cell count and percentage ($r = 0.89$ and $r = 0.48$, respectively). A stronger negative correlation was observed between CD4+ T cell count or percentage of CD4+ T cells and the percentage of activated CD4+ T cells ($r = -0.56$ and $r = -0.65$, respectively) than was found between CD4+ T cell count or percentage and HIV RNA ($r = -0.34$ and $r = -0.37$, respectively), whereas no correlation was noted between CD4+ T cell count and the percentage of activated CD8+ T cells. The baseline CD8+ T cell count was positively correlated with the CD4+ T cell count ($r = 0.38$), which reflects that calculations share the total lymphocyte count, whereas baseline percentages of CD4+ and CD8+ T cells were negatively correlated ($r = -0.52$). Percentages of activated CD4+ and CD8+ T cells were moderately associated with each other, and both were positively correlated with HIV RNA.

Additional correlations at baseline (data not shown) revealed that age was negatively associated with the CD4+ T cell count ($r = -0.21, P < .001$) and naive CD4+ T cell count ($r = -0.26, P < .001$) T cell count. For 317 subjects, a baseline Hgb measurement was available, and for 236 subjects, a baseline Hgb measurement was available. Hgb was strongly associated, and Hgb level was associated, with baseline CD4+ T cell count ($r = 0.63, P < .001$ and $r = 0.21, P = .001$, respectively). Hgb was negatively correlated with HIV RNA ($r = -0.18, P = .001$), whereas the relationship between Hgb level and HIV RNA was not significant ($r = -0.06, P = .39$).

**Changes in HIV RNA and immunologic measurements with treatment.** Significant increases were noted in the proportion of subjects with HIV RNA <1000 copies/mL by week 24 ($P < .001$). Significant reductions from baseline to week 24 were seen in the percentages of activated CD4+ and CD8+ T cells, and significant increases were observed in CD4+ T cell counts, naive CD4+ T cell counts, and the percentage of naive CD4+ T cells ($P < .001$). There was no evidence of a change in the number of CD8+ T cells ($P = .78$).

**Predicting HIV RNA response at week 24.** In the regression model shown in table 3, a lower HIV RNA at week 24 was predicted by a lower baseline HIV RNA ($P < .0001$), older age ($P = .008$), and, marginally, by a higher baseline CD8+ T cell count ($P = .06$). After adjustment for these, as well as for CD4+ T cell count and CD4+ and CD8+ T cell subpopulations, sex, and study, there was a significant independent prognostic effect of the baseline percentage of CD8+ T cell activation for the week-24 HIV RNA response, with an estimated 0.20 log_{10} higher HIV RNA per 10 percentage points of higher CD8+ T cell activation at baseline ($P = .046$). In addition, at week 24, a higher percentage of CD8+ T cell activation (above the median) defined a population with a higher week-24 HIV RNA (a greater proportion of subjects with HIV RNA >400 copies/mL; $P < .0001$), compared with those who had a week-24 percentage of CD8+ T cell activation below the median.

**Predicting change in CD4+ and naive CD4+ T cell counts.** In the regression model described in table 4, greater increases in CD4+ T cell counts through week 24 were seen with a longer time receiving potent ART ($P = .001$), a lower CD4+ T cell count ($P < .001$), and a higher HIV RNA ($P = .004$) at baseline. In addition, there was a strong independent prognostic effect of naive CD4+ T cell count. Of the CD4+ and CD8+ T lymphocyte subpopulations examined at baseline, naive CD4+ T cell count was the strongest predictor of subsequent change, with an estimated change in CD4+ T cell count of 6.35 cells/mm$^3$ per 10 naive CD4+ T cells ($P < .0001$) in this model (table 4), where $R^2$ was 22%. However, applying this model to subjects who had a baseline CD4+ T cell count <200 cells/mm$^3$, neither naive CD4+ T cell count nor the percentage of CD4+ or CD8+ T cell activation was predictive of a change in CD4+ T cell count, although the sample size in this subgroup was small. Analyzing all subjects, the baseline percentage of naive CD4+ T cells represented a strong predictor, similar to results seen for absolute naive CD4+ T cell count ($P = .001$, model not shown).

Although CD4+ and CD8+ T cell activation did not have direct effects on changes in CD4+ T cell counts, they were apparent modifiers of the effect of naive CD4+ T cells. As shown in table 4, the influence of greater numbers of naive CD4+ T cells at baseline was more pronounced in subjects with a higher percentage of CD4+ T cell activation (above the median) and in subjects with a lower percentage of CD8+ T cell activation (below
The greatest effect of naive CD4+ T cells at baseline on subsequent changes in CD4+ T cell count was observed in subjects who had both a high percentage of CD4+ T cell activation and a low percentage of CD8+ T cell activation (estimate, 13.41). Likewise, this advantage was consistently found when subjects with baseline CD4+ T cell counts >200 cells/mm3 were examined, as well as when the subgroups of subjects with virologic suppression at week 24 (HIV RNA, <400 copies/mL) and those without virologic suppression were investigated (models not shown).

A consistent high correlation was observed between changes in the CD4+ T cell count and changes in the naive CD4+ T cell count, overall and within each of the subgroups shown in table 4 (P < .0001 with correlations >0.79). The baseline naive CD4+ T cell count was a similarly strong predictor of a change in the naive CD4+ T cell count at week 24, and the percentage of CD4+ and CD8+ T cell activation similarly modified this association (table 4).

**Immunologic markers at week 24 among subjects with virologic suppression.** To characterize the immunophenotypic marker response in the setting of virologic suppression during ART, each of the CD4+ and CD8+ T cell subset percentages at week 24 (naive CD4+, activated CD4+, and activated CD8+) was added to the baseline predictive model described in the first row of table 4 (with naive CD4+ T cell count, without activation modifiers), restricted to subjects who had a virus load <400 copies/mL at week 24. This group represented the majority of study subjects. Greater increases in CD4+ T cell counts at week 24 were associated with a lower activated CD4+ T cell percentage at week 24 (P = .055), and the baseline naive CD4+ T cell count remained highly significant, but there was no evidence of an effect for the week-24 naive percentage of CD4+ T cells (P = .38) or the percentage of activated CD8+ T cells (P = .17). Alternatively, when change to week 24 in each of the CD4+ and CD8+ T cell subsets was added to the model, first among all subjects, greater increases in CD4+ T cell counts were seen, with greater declines in the percentages of activated CD4+ (P = .051) and CD8+ (P = .068) T cells. When the analysis was repeated in the restricted data set of subjects with virologic suppression at week 24, the effects were no longer statistically significant (P = .12 and P = .40, respectively), although the baseline naive CD4 T cell count remained highly significant (P = .0008 and P = .002, respectively).

**Effect of TLC and Hgb levels.** When TLC and Hgb levels were each added to the model described in table 3, neither was predictive of the week-24 HIV RNA, nor was either predictive when CD4+ T cell count, CD8+ T cell count, and their respective subsets were excluded from the model (P > .15). When examined in the models described in table 4, which included CD4+ and naive CD4+ T cell counts, neither was additionally predictive of a change in CD4+ T cell count. However, in a model that included only follow-up week, age, sex, and study, the baseline TLC was found to be predictive of a change in CD4+ T cell counts, with a greater increase in CD4+ T cell count predicted by a lower baseline TLC (P = .023). After adjusting for baseline HIV RNA, but not after adjustment for baseline CD4 cell count, the effect of TLC remained significant (P = .028 and P = .07, respectively). In similar assessments as for TLC, no apparent effect of Hgb was seen (P > .7).

**DISCUSSION**

Our results demonstrate that immunophenotypic markers of T cell activation and maturation, as measured by flow cytometry before the initiation of potent ART, independently distinguish subjects who will achieve the greatest and least favorable virologic and immunologic responses 24 weeks later. As a condition of the original data-sharing strategies described for the present cross-protocol analysis, treatment assignment was not examined, to protect the integrity of ongoing, contributing

<table>
<thead>
<tr>
<th>Factor</th>
<th>Median (25th–75th quartile)</th>
<th>Correlation</th>
<th>Cell count</th>
<th>Percentage</th>
<th>Log10 HIV RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ cell count, cells/mm³</td>
<td>280 (129–434)</td>
<td></td>
<td>1</td>
<td>0.38</td>
<td>0.48</td>
</tr>
<tr>
<td>CD8+ cell count, cells/mm³</td>
<td>777 (565–1074)</td>
<td></td>
<td>1</td>
<td>0.24</td>
<td>–0.02</td>
</tr>
<tr>
<td>Naive CD4+ cell count, cells/mm³</td>
<td>75 (25–168)</td>
<td></td>
<td>1</td>
<td>0.80</td>
<td>–0.57</td>
</tr>
<tr>
<td>Percentage of naive CD4+ cells</td>
<td>31 (18–46)</td>
<td></td>
<td>1</td>
<td>–0.42</td>
<td>0.09</td>
</tr>
<tr>
<td>Percentage of activated CD4+ cells</td>
<td>16 (10–28)</td>
<td></td>
<td>1</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td>Percentage of activated CD8+ cells</td>
<td>46 (35–59)</td>
<td></td>
<td>1</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Log10 HIV RNA</td>
<td>4.62 (4.12–5.19)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NOTE. Not shown is the correlation between the CD4+ T cell count and the CD4+ T cell percentage (r = .85). P < .001 for all correlations with an absolute value ≥0.20.*
Figure 1. Scatterplot of baseline CD4+ T cell count vs. baseline log_{10} HIV RNA and CD4+ and CD8+ T cell subpopulation measurements. For plotting purposes, 2 subjects with baseline CD4+ T cell counts >1000 cells/mm^3 were excluded. Note the expected strong correlation between CD4+ T cells and naive CD4+ T cells, because naive CD4+ T cells generally represent a major component of the total CD4+ T cell count.

trials. These constraints notwithstanding, and despite the heterogeneity of regimens used in the 5 studies (table 1), we observed the characteristic patterns of response to potent ART—namely, decreases in virus load and increases in CD4+ and naive CD4+ T cell counts paralleled by decreases in CD4+ and CD8+ T cell activation markers, as was described in the contributing studies [13–16] and studies elsewhere [22–25].

A significant finding in the IMART study was the demonstration, in multivariate modeling adjusted for HIV RNA, CD4 cell count, and other baseline factors, including study as a proxy for treatment assignment, that a higher baseline level of CD8+ T cell activation predicted a poorer virologic response at week 24; 10 percentage points higher baseline level of CD8+ T cell activation resulted in an HIV RNA that was estimated to be 0.2 log_{10} higher 24 weeks later (P = .046). The model also identified a higher baseline HIV RNA, which is a validated prognostic marker, and younger age, which is a probable indicator of less-strict adherence to treatment [26–28], as independent predictors of an unfavorable HIV RNA response at week 24, along with a trend toward a lower baseline CD8+ T cell count, which is a possible reflection of the contribution of these cells to virus clearance, as was suggested by the viral dynamics substudy [29] of the contributing trial, ACTG 315 [13]. Consistent with these observations, subjects with a week-24 percentage of CD8+ T cell activation above the median were significantly more likely to have a detectable HIV RNA (≥400 copies/mL) at week 24 (P < .001).

The value of CD8+ T cell activation as a natural history marker predictive of disease progression [30, 31], independent of CD4+ T cell count and HIV RNA [32, 33], has been well established; so too has its relationship to decreases in CD4+ T cell counts and changes in virus load in untreated patients [34–36]. This marker is also a sensitive indicator of antiretroviral activity, with declining levels paralleling decreases in virus load [13, 15, 16, 23, 37, 38]. That CD8+ T cell activation might also predict the virologic response to treatment is consistent with results from small descriptive studies [37, 39] and 2 larger ART trials, 1 of which was an IMART parent study [16]. ACTG 890/368 identified a trend toward the correlation of higher baseline values of activated CD8+ T cell counts and virologic failure after adjustment for baseline HIV RNA and CD4 cell count [16]; the Quest study of primary HIV infection found, when controlling...
for baseline HIV RNA, that a higher baseline activated CD8⁺ T cell count was marginally predictive of a longer time to virus suppression [38]. To our knowledge, the present investigation is the only one to document that a higher percentage of CD8⁺ T cell activation is a significant predictor of virologic failure during potent ART, independent of virus load and CD4 cell count, whereas, of interest, a higher CD8⁺ T cell count trends in the opposing direction, toward predicting virologic success.

Although closely linked, CD8⁺ T cell activation and HIV RNA were only moderately correlated at baseline; in view of their independent effects on outcome, they likely represent distinct elements of pathogenesis. As shown here and elsewhere [21, 23, 24, 40], T cell activation can persist despite apparent virologic control during potent therapy, with attendant immunologic consequences [21, 41, 42]. Among individuals with virologic suppression, CD8⁺ T cell activation may reflect residual viral replication in plasma below the level of detection or in virus reservoirs [43–47]. Furthermore, considering that only a fraction of HIV virions are infectious [48, 49], markers of host response may be additionally predictive, because the level of CD8⁺ T cell activation could reflect that component of the virus load. CD8⁺ T cell activation could be measuring a functional supplement to HIV-1 RNA quantitation that corresponds to the component of the immune response elicited by viral replication but determined by the host—that is, the immunologic correlate of viral replication. Increased CD8⁺ T cell activation may also reflect ongoing immune dysregulation secondary to HIV infection, which, in turn, could significantly impair treatment-mediated virologic control as well as immunologic recovery, as our data also indicate.

Greater immune reconstitution at week 24, defined as a 

**Figure 2.** Scatterplot of baseline log₁₀ HIV RNA vs. CD4⁺ and CD8⁺ T cell subpopulation measures. For plotting purposes, 2 subjects with baseline CD4⁺ T cell counts >1000 cells/mm³ were excluded.

**Table 3.** Estimated log₁₀ HIV RNA (95% confidence intervals) at week 24 as influenced by baseline CD8⁺ T cell activation.

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Estimate (10 percentage-point higher baseline CD8⁺ cell activation)</th>
<th>Estimate (100-cell higher baseline CD8⁺ cell count)</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>0.20 (0.00–0.40)</td>
<td>−0.07 (−0.13–0.00)</td>
<td>.046</td>
<td>.061</td>
</tr>
</tbody>
</table>

**NOTE.** The model additionally was adjusted for follow-up week, CD4⁺ T cell count, CD8⁺ T cell count, HIV RNA, age, sex, study, and CD4⁺ T cell subpopulations. The significance and directionality of these factors are week (P = .35), CD4⁺ T cell count (P = .43), HIV RNA (P < .0001, with lower HIV RNA in those with lower baseline HIV RNA); age (P = .008, with lower HIV RNA in older subjects); sex (P = .14); study (P not reportable because of data-sharing conditions); naive CD4⁺ T cell count (P = .40); and percentage of activated CD4⁺ T cells (P = .74). A generalization of R² for this model is 21%.
greater increase in CD4+ and naive CD4+ T cell counts, was predicted in multivariate modeling by a lower baseline CD4+ T cell count, higher virus load, and longer time receiving therapy, which is consistent with results published elsewhere [24, 50]. Adjusting for these and other factors, including study, we identified a strong, independent, positive effect of baseline naive CD4+ T cell count (P<.001), the T cell subset that is disproportionately affected by HIV-1 infection [51, 52]. This demonstration persuasively supports the results of smaller studies [53–56] that showed the influence on ART-mediated CD4+ and CD8+ T cell counts, although not predictive individually, represent significant positive and negative modifiers, respectively, of the baseline naive CD4+ T cell count in predicting subsequent increases in CD4+ T cell counts—effects that were seen predominantly in individuals who had CD4+ T cell counts >200 cells/mm³.

Given the central role of HIV-1–induced immune system activation in driving viral replication, CD4+ T cell loss, and disease pathogenesis [55, 59–66], it is not surprising that a higher level of pretreatment CD8+ T cell activation, as a modifier of the effect of naive CD4+ T cell counts, reduced immunologic recovery, a finding that is consistent with our parallel demonstration of the blunting effect by this marker on virologic response. In contrast, the enhancing effect of the baseline level of CD4+ T cell activation may be because the majority of HIV-1 replication occurs in activated CD4+ T lymphocytes [67–69]. This marker may reflect the cellular burden of infected, entrapped CD4+ T cells in lymphoid tissue or other compartments. Higher levels of CD4+ T cell activation would imply a greater potential for gain when these cells are redistributed during the initial phase of treatment [23–25, 52, 70]. Alternatively, CD4+ T cell activation may simply mirror lymphopenia, as indicated by the strong inverse relationship shown in univariate cross-sectional correlations between baseline CD4+ T cell count and activation, which is indeed stronger than that seen between CD4+ T cell count and baseline HIV RNA, as has been noted by others [71]. In advanced disease, with CD4+ T cell counts <200 cells/mm³, immune activation at the highest level, and the naïve cell reserve at its lowest levels, the independent influence of these immunophenotypic markers is less apparent, and the baseline CD4+ T cell count, adjusted for baseline HIV RNA, is sufficient to account for the subsequent increase in the CD4+ T cell count. Consistent with baseline predictive models, greater increases in CD4+ T cell counts at week 24 were seen in subjects who had greater decreases in CD4+ T cell activation or in those who had achieved virologic
suppression with concomitantly lower CD4+ T cell activation, similar to findings recently reported by Hunt et al. [72]. Taken together, these observations suggest that CD4+ and CD8+ T cell activation inform different aspects of treatment response, with CD4+ T cell activation more directly related to immune reconstitution and CD4+ T cell recovery and CD8+ T cell activation representing an expression of virologic activity and its secondary immunologic consequences, which is consistent with our findings in a smaller study [73].

IMART marker results, if validated like HIV RNA and CD4 cell count in analyses across trials that include treatment assignment and longer durations of therapy, have several important clinical implications. Together with CD4 cell count and HIV RNA, T cell activation and maturation markers can further accelerate the development of new drugs by serving as eligibility or stratification factors and end points in clinical trials of antiretroviral agents. They also have the potential to inform drug selection and the optimal timing for the start, intensification, or change in ART—decisions that are now almost exclusively driven by CD4 cell–count and virus-load criteria. For example, if a cutoff value was identified for nadir naive CD4+ T cell count that signals irreparable immune-system damage, this value could serve as a threshold for the initiation of ART. Similarly, the failure to normalize to predefined benchmark levels of CD4+ and CD8+ T cell activation could trigger adjustments to therapy, ideally before the occurrence of virologic breakthrough or the development of drug resistance. Our observation that the baseline total lymphocyte count predicts subsequent increases in CD4+ T cell count, adjusting for HIV RNA and other factors, corroborates and extends work published elsewhere [74–78] and has the potential for clinical application supporting the use of routine TLC measurements in settings where multiparameter flow cytometry is unavailable.

The present study has several limitations. The length of follow-up in IMART was only 24 weeks, to prevent the compromise of end points in parent trials. Our study was designed to evaluate markers that were common to all of the selected trials; additional key markers, such as naive CD8+ or memory CD4+ T cell counts, were not examined. Because improved treatment strategies may emerge from a better understanding of the mechanisms by which T cell activation and maturation, before and during treatment, influence outcome, it will be important to conduct additional intensive investigations to determine the proportion of the increase in CD4+ T cell count that is composed of activated or resting naive and memory subsets, using more-sensitive virus-load assays to relate these findings to the robustness of virologic suppression.

In conclusion, immunophenotypic markers of T cell activation and maturation are valuable prognostic and treatment-response indicators for ART, and they hold promise for application to the design of trials and guiding therapeutic choices to optimize outcome. The IMART study complements and adds new information to its contributing parent studies, thus confirming the usefulness of the developed methodology for cross-protocol marker analysis as a template for future investigations aimed at the timely evaluation of marker data from ongoing trials.

Acknowledgments

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