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Cartesian coordinate analysis of viral burden and CD4+ T-cell count in human immunodeficiency virus type-1 infection[1]

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Abstract

We have used a Cartesian coordinate plot to analyze the inverse relationship between viral burden (x-axis) and peripheral blood CD4+ cell count (y-axis) to extend our understanding of the mechanisms of antiviral drugs and differences in outcome resulting from variability in virus and host responses. Each of 186 subjects studied were assigned to one of four response quadrants. Quadrants **A** ($x-$, $y+$) and **D** ($x+$, $y-$) defined the effect of a change in virus load on the inverse change in CD4+ cell count expected from the natural history of HIV infection or antiretroviral therapy. Quadrants **B** ($x+$, $y+$) and **C** ($x-$, $y-$) defined the dissociation of the inverse relationship between the relative changes in CD4+ cell count and viral load that resulted from the hypothesized effect of putative virologic or immunologic response modifiers. Of the response modifiers studied, only the syncytium-inducing phenotype resulted in a complete dissociation of this inverse relationship. The analysis provided an integrated virological and immunological approach to better understand therapeutic responses and potential dissociation between changes in viral RNA and CD4+ cell count. This type of analysis may be helpful for individualizing patient management as well as designing and analyzing studies of HIV-1 antiretroviral drugs and disease pathogenesis.

Keywords: Cartesian coordinate analysis; CD4+ cell; HIV-1 RNA; Viral burden; Virulence; Virologic response modifiers; Pathogenesis; Clinical trials

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1. Introduction

Current evidence strongly supports the supposition that the progression of Human Immunodeficiency Virus type-1 (HIV-1) infection is primarily

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driven by viral burden and host immunologic factors (Fauci, 1993; Henrard et al., 1995; Fauci, 1996). Although the viral etiology of the acquired immunodeficiency syndrome is accepted, the precise roles of viral and immune pathogenesis remain to be more fully defined (Fauci, 1996). Despite this lack of a full understanding of the viral and immune pathogenesis of HIV-1 infection, both the CD4+ cell count in peripheral blood and the level of HIV-1 RNA in plasma are generally accepted as the standards with which antiretroviral response is measured and disease progression is assessed (Carpenter et al., 1997; Mellors et al., 1997; O'Brien et al., 1997). Specific limitations of the CD4+ cell count entail a partial explanation for the clinical benefit of antiretroviral therapy (Choi et al., 1993; DeGruttola et al., 1997) and a weak correlation with the level of cell-associated or cell-free HIV-1 (Corey and Coombs, 1993; Piatak et al., 1993; Fiscus et al., 1995; Mellors et al., 1995; Katzenstein et al., 1996; Mellors et al., 1996; Wain-Hobson et al., 1996; Welles et al., 1996). HIV-1 RNA is independent of other markers, including CD4+ cell count, as a measure of disease progression (Coombs et al., 1996; O'Brien et al., 1996; Welles et al., 1996). However, markers of viral phenotype such as syncytium-induction (Koot et al., 1993; Richman and Bozzette, 1994) and zidovudine resistance (D'Aquila et al., 1995; Japour et al., 1995) also are independent markers of diseases progression. Important but not unexpected interactions between viral RNA and CD4 cell count (Mofenson et al., 1997; Marschner et al., 1998), and the recovery of infectious virus from patients with undetectable viral RNA in plasma despite potent antiretroviral therapy (Finzi et al., 1997; Wong et al., 1997), argue against a single measure of either a viral or host parameter being sufficient to manage patients or fully understand pathogenesis (DeGruttola et al., 1997). We believe, therefore, that an approach based on measures of virus load, CD4 cell count and other markers of disease progression will be required to more fully explain the clinical benefit of therapy for the individual patient (DeGruttola et al., 1996, 1997).

Previous studies have demonstrated an inverse association between increasing viral burden and decreasing CD4+ cell count (Holodniy et al., 1991; Semple et al., 1991; Aoki-Sei et al., 1992; Katzenstein et al., 1992; Piatak et al., 1993) but only recently has the dynamic interaction between viral replication and CD4+ cell decline been described (Ho et al., 1995; Wei et al., 1995; Perelson et al., 1996). To date, multivariate analyses have utilized the inverse relationship between viral replication, CD4+ cell count and other factors to define the risk of disease progression for groups of clinical trial subjects (Coombs et al., 1996; Katzenstein et al., 1996; O'Brien et al., 1996; Welles et al., 1996; Mellors et al., 1997). For the clinician, the translation of this information into a simple graphical format would help with individual patient management by showing the important interaction between viral burden and CD4 cell count (McLean and Frost, 1995; Merigan, 1995; Wain-Hobson et al., 1996; Marschner et al., 1998). For the investigator, simple graphical approaches can be used to explore relationships among variables in categorical data analysis. To address both of these needs, we used an approach based on a Cartesian coordinate plot analysis (Reichelderfer and Coombs, 1996) to characterize the inverse linear relationship between CD4+ cell count and viral burden for individual patient data from several published clinical studies.

2. Materials and methods

2.1. Analysis method

2.1.1. Cartesian coordinate plot analysis

In HIV-infected persons, it is assumed that the CD4+ cell count in the peripheral blood, at a given point in time is determined by the equilibrium between CD4+ cell proliferation and clearance of CD4+ cells; i.e. CD4+ influx less CD4+ efflux (Perelson et al., 1996). In HIV-1 infection, the efflux in CD4+ cell number is further influenced by the direct effect of virus replication (or load) and the additional effect of modifiers represented by viral pathogenicity and host response; e.g. viral resistance, viral phenotype, viral replicative efficiency, cytotoxic T-lymphocyte (CTL) activity, humoral immunity, co-receptor status, etc.

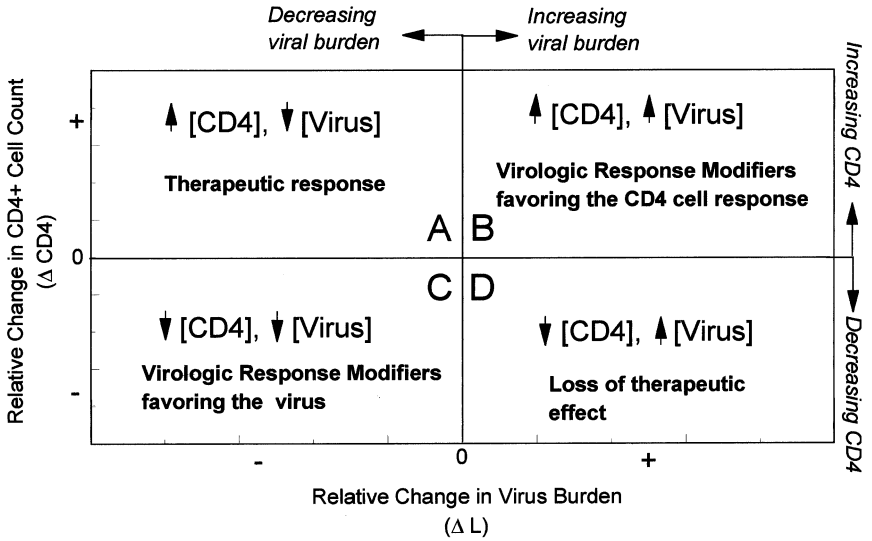

Fig. 1. Schematic of Eq. (1) showing the relationship between relative change in CD4+ cell count and HIV-1 burden. The x-axis represents the relative log change in measurement of virus burden between two time points [$\log(L_t/L_o)$; ΔL]; the y-axis represents the relative log change in measurement of CD4+ cell count between the same two time points [$\log(CD4_t/CD4_o)$; $\Delta CD4$]. Quadrants **A**, **B**, **C** and **D** catalogue the predicted outcomes between the change in virus burden and CD4+ cell count. In quadrants **A** and **D**, change in virus burden is the predominant factor driving the inverse change in CD4+ cell count; in quadrants **B** and **C**, virulence factors or CD4+ cell proliferation or both, rather than the change in virus burden, influence the change in CD4+ cell count. A positive response to antiretroviral therapy, as defined by both a decrease in the viral burden and an increase in the CD4+ cell count, is indicated by a shift from quadrant **D** to **A** while a negative response is indicated by a shift from quadrant **A** to **D** (modified after (Reichelderfer and Coombs, 1996)).

A Cartesian coordinate plot (Fig. 1) was obtained by plotting the proportional change in virus load (ΔL, plotted on the x-axis) versus the proportional change in CD4 cell count (ΔCD4, plotted on the y-axis) (Reichelderfer and Coombs, 1996). Generally, one compares a relative log change from the baseline value over a defined time interval (o, t): for example, for virus burden [$\log(L_t/L_o) = \Delta L$] versus CD4+ cell count [$\log(CD4_t/CD4_o) = \Delta CD4$]. As such, the proportional comparisons (or relative changes) are without units of measurement. However, other comparisons of virus and CD4 change can be assessed; for example, the rate of change over time (i.e. slope) for each parameter.

This analysis defines four quadrants. Quadrants **A** $(x-, y+)$ and **D** $(x+, y-)$ define the effect of a relative change in virus load on the inverse relative change in CD4+ cell count. In quadrant **A**, a decrease in virus load is associated with an increase in the CD4+ cell count; in quadrant **D**, an increase in virus load is associated with a decrease in the CD4+ cell count. In both quadrants **A** and **D**, the inverse relationship between CD4+ cell count and virus load is maintained. The diagonal **A**–**D** defines the inverse relationship and is described by a negative slope.

Quadrants **B** $(x+, y+)$ and **C** $(x-, y-)$ define the dissociation of the inverse relationship between the relative changes in CD4+ cell count and viral load which we hypothesize results from specific virologic or immunologic response modifiers. For example, in quadrant **B**, either virologic response modifiers favoring the immune system (for example, a less replicatively fit virus or an increased CD4+ cell proliferation) may have a positive effect on CD4+ cell count despite an increase in virus load. In quadrant **C**, increased viral pathogenicity (that is, virulence) or an increase in CD4+ cell destruction or clearance have a negative effect on CD4+ cell count despite a decrease in virus burden. In both quad-

rants **B** and **C**, response modifiers or alterations in the CD4+ cell equilibrium (or both) and not virus burden per se, are the predominate factors driving the change in CD4+ cell count. The diagonal **B**–**C** defines the dissociation of the inverse relationship and is described by a positive slope.

2.1.2. Influence of other markers of disease progression (virologic response modifiers)

We defined the influence of other independent markers of disease progression (for example, virologic response modifiers such as antiretroviral susceptibility phenotype, syncytium-inducing phenotype or antiretroviral susceptibility genotype) on ΔCD4 and ΔL by the slope (V), whereby,

$$V = \Delta\text{CD4}/\Delta L. \quad (1)$$

The slope (V) defines not only the proportional decline in CD4+ cell count, but also the nature of the relationship between CD4+ cell count and viral load, when $\Delta L \neq 0$, and the influence of these virologic response modifiers on this relationship. Thus, when $V > 0$ (positive), subjects are located in either quadrant **B** or **C** and the inverse relationship between CD4 cell count and virus load is dissociated; in contrast, when $V < 0$ (negative), subjects are located in either quadrant **A** or **D** and this inverse relationship is sustained. Therefore, we hypothesize that deviations from this inverse relationship may arise from the influence of either virologic response modifiers, which in the extreme, will completely dissociate the inverse relationship between CD4 cell count and virus load, or the variability in the parameter measurement, or both.

2.2. Clinical studies used for analysis

Data from three published studies were available for this analysis. In the first study, a cohort of 41 subjects with a median CD4+ cell count of 577 cells/μl and who had received either no therapy ($n = 26$) or stable monotherapy ($n = 15$) with zidovudine, had quantitative peripheral blood mononuclear cell (PBMC) titers of HIV-1 (expressed as infectious units per million PBMC [IUPM]) assessed prospectively for a mean duration of 16 months (Paxton et al., 1997). We considered these patients to represent the short-term natural history of CD4 cell count and infectious PBMC-associated HIV-1 burden.

In the second study, 77 asymptomatic HIV-1 infected subjects with a median CD4+ cell count of 375 cells/μl who had received either didanosine or combination didanosine and zidovudine therapy as part of a multicenter clinical trial, AIDS Clinical Trials Group (ACTG) protocol 143, had uncensored quantitative PBMC titers of HIV-1 assessed at study entry and following 24 weeks of therapy (Fiscus et al., 1995).

In the third study, 68 HIV-1 infected subjects with a median CD4+ cell count of 85 cells/μl who had received either zidovudine or didanosine monotherapy as part of a multicenter clinical trial, ACTG 116B/117 (Kahn et al., 1992), had plasma HIV-1 RNA assessed at study entry and following 12 weeks of therapy (Coombs et al., 1996). In addition, phenotypic and genotypic zidovudine susceptibility, and syncytium-inducing phenotype data were available (D'Aquila et al., 1995; Japour et al., 1995).

2.3. Statistical analysis

The 95% confidence intervals for the within-subject variability in the estimate of parameter change were 0.10 log (1.25-fold or 25%) for CD4+ cell count, 0.41 log (3-fold) for plasma HIV-1 RNA, and 1.26 log (18-fold) for IUPM (Hughes et al., 1994; Lin et al., 1994; Fiscus et al., 1995; Raboud et al., 1996; Hughes et al., 1997; Paxton et al., 1997). Chi-square analysis was used for assessing the non-randomness of the distribution of subjects in each quadrant (categorical analysis) irrespective of the 95% CI. For each response modifier, median values for ΔCD4, ΔL, CD4_o and L_o were used to calculate V.

2.3.1. Categorical analysis

The Cartesian coordinate plot provides for a categorical analysis based on the proportional distribution of subjects among quadrants. The distribution of subjects between quadrants **A** and **D** was used to assess antiviral effect. The number

of subjects within quadrants **A** and **C** versus **B** and **D** were used to assess the change in virus burden; similarly, quadrants **A** and **B**, and **C** and **D** were used to assess the change in CD4+ cell count, independently of therapeutic response. For our analyses, the distribution of subjects showing a change was considered to be statistically significant if the change was outside of the 95% CI estimate for the respective parameter change (Paxton et al., 1997). However, given the loss of statistical power by using a categorical analysis compared to one based on continuous data, the distribution or clustering of subjects within quadrants **A**–**D** and **B**–**C** were used to show the strength of the inverse relationship between CD4+ cell count and virus burden ($A+D/B+C>1$), irrespective of the 95% CI for each parameter estimate.

2.4. Laboratory methods

2.4.1. Sample preparation

Plasma and PBMC were collected following ficoll-hypaque density gradient centrifugation of heparinized anticoagulated whole blood according to standard methods of the ACTG Virology Group (Hollinger et al., 1992; National Institutes of Allergy and Infectious Diseases, 1994; Fiscus et al., 1995). Plasma aliquots were stored at −70°C. PBMC were either cultured for HIV-1 or cryopreserved using standard methods (National Institutes of Allergy and Infectious Diseases, 1994).

2.4.2. Assays of drug susceptibility, reverse transcriptase mutations at codon 215 and 41, and syncytium-inducing phenotype

The assay details are published elsewhere (D'Aquila et al., 1995; Japour et al., 1995). Briefly, virus stocks were prepared from either primary isolates or cryopreserved PBMC and infectivity was assessed by end-point dilution followed by zidovudine susceptibility determination using the ACTG/Department of Defense (DoD) drug susceptibility assay as previously described (Japour et al., 1993; D'Aquila et al., 1995). Viral isolates were categorized as susceptible if the IC_{50} was $<1.0\ \mu M$ and high-grade resistant if the IC_{50} was $\geqslant 1.0\ \mu M$. The mutation at codons 215 and 41 of the HIV-1 reverse transcriptase gene that are associated with zidovudine resistance were determined by a selective polymerase chain reaction analysis of $3-5\times10^6$ cells collected from HIV-1 stocks prepared in phytohemagglutinin-stimulated PBMC as described previously (Japour et al., 1995). Syncytium-inducing phenotype was assessed by cultivation of HIV-1 cell-free virus stocks with MT-2 cells in 96-well microtiter plates (Japour et al., 1994).

2.4.3. HIV-1 RNA analysis

Plasma HIV-1 RNA levels were determined from thawed heparinized plasma using quantitative reverse transcription PCR (RT-PCR) amplification (Roche Molecular Systems, Alameda, CA) (Lin et al., 1994; Mulder et al., 1994) or by an in-house assay (Paxton et al., 1997). HIV-1 RNA was purified from heparinized plasma by silica particle adsorption (Boom et al., 1990). The HIV-1 RNA copy number was assessed using either the manufacturer's or in-house reference standards.

3. Results

3.1. Natural history of infectious PBMC-associated virus burden

The inverse relationship between viral load and CD4+ cell count was first examined using unweighted slope data from a natural history study (Paxton et al., 1997). The Cartesian coordinate plot showed that 28 (68%) of 41 subjects who were either untreated or who received ineffective therapy were located in quadrant **D** where the natural decline in CD4+ count (log rate of cell change/month) was a function of increased viral load (log rate of viral RNA change/month) (Fig. 2). Two subjects were located in quadrant **A**. The virus burden increased over the study period in 80% of subjects ($B+D/A+B+C+D$: 33/41), remained unchanged in two (5%) and declined in six (15%). Some subjects were located in either quadrant **B** or **C** indicating that even in the natural history setting, the inverse **A**–**D** relationship was disassociated for some subjects.

3.2. Antiretroviral therapy

To show the effect of antiretroviral therapy on the inverse relationship between viral load and CD4+ cell count, we examined the relative changes in these two parameters for two study populations using the two different viral load assessments of PBMC infectivity (IUPM) and plasma viral RNA level (Fiscus et al., 1995; Coombs et al., 1996). First, the analysis indicated that subjects who received effective antiretroviral therapy and had a decline in viral burden concomitant with an increase in CD4+ cell count would be located in quadrant **A** (Fig. 3a). The Cartesian coordinate plot of ACTG protocol 143 data is shown in Fig. 3b. Irrespective of the type of therapy received by these subjects, most (48/77 or 61%) experienced a positive antiretroviral effect as assessed by the increase in CD4+ cell count and a decline in IUPM (quadrant **A**; χ^2 for quadrant distribution $P < 0.001$). Sixty subjects had changes in viral burden that were within the expected variability (95% CI) measurement for IUPM and of these, the majority were also clustered in the lower 95% CI bound of quadrant **A**. Thus, the diagonal **A**–**D** defined the inverse relationship associated with the antiretroviral response.

Second, the analysis described the differential response to two antiretroviral drugs (Fig. 4). A greater proportion of the subjects who received the more effective drug were located in quadrant **A**, while more of those who received the less effective drug were located in quadrant **D**; thus, the effectiveness of an antiretroviral drug or regi-

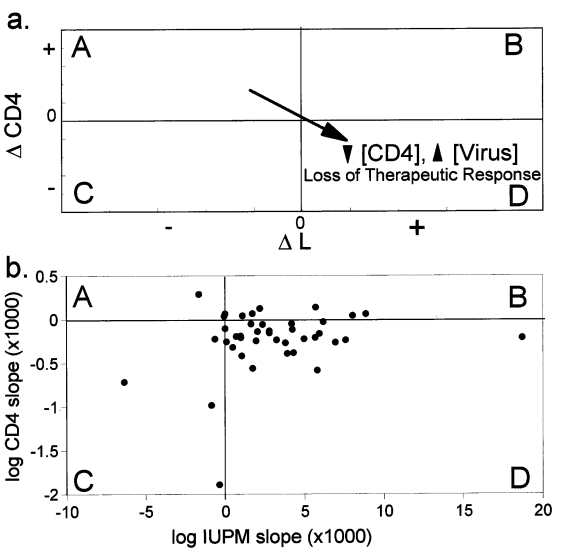

Fig. 2. (a) Expected relationship between the relative changes in viral burden (ΔL) and CD4+ cell count (ΔCD4) describing either the natural history of HIV-1 infection or loss of therapeutic effect. In quadrant **D**, increased virus burden is hypothesized to be the predominant factor driving the inverse decrease in CD4+ cell count. (b) The observed relationship between the unweighted change in viral burden ($\log_{10}$ slope of the infectious units per million peripheral blood mononuclear cells [IUPM] change per month) and CD4+ cell count ($\log_{10}$ slope of the CD4+ cell count change per month) for 41 subjects who received either ineffective zidovudine therapy or no antiretroviral therapy (Paxton et al., 1997). The median CD4+ cell count at the start of this study was 577 cells/μl; viral burden and CD4+ cell counts were assessed bimonthly for a mean of 16 months.

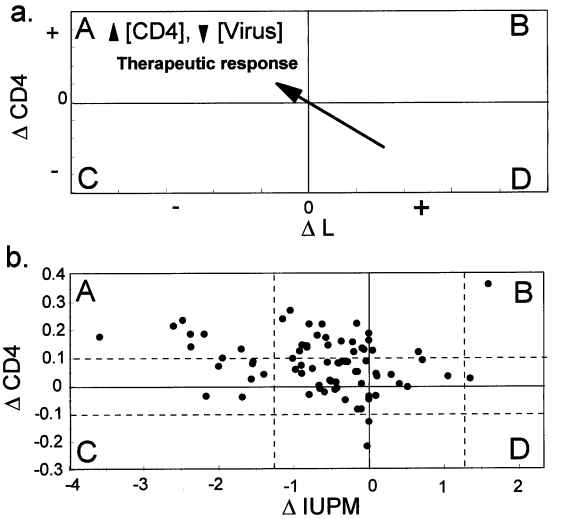

Fig. 3. (a) Expected relationship between the relative changes in viral burden (ΔL) and CD4+ cell count (ΔCD4) following effective antiretroviral therapy. In quadrant **A**, decreased virus burden was hypothesized to be the predominant factor driving the inverse increase in CD4+ cell count. (b) The observed relationship between the relative changes in viral burden (IUPM) and CD4+ cell count for 77 asymptomatic HIV-1 infected subjects (median CD4+ cell count of 375 cells/μl) after 24 weeks of either didanosine or combination didanosine and zidovudine therapy (Fiscus et al., 1995). The dashed lines indicate the estimated within-subject variability (95% CI) for relative change in IUPM ($\pm 1.26 \log_{10}$) and CD4+ cell count ($\pm 0.10 \log_{10}$ cells/μl).

men could be assessed by measuring the proportion of subjects between quadrants **A** or **D**. The data shown in Fig. 4b were derived from ACTG protocol 116B/117 in which subjects treated with didanosine received a greater clinical benefit than did those who received zidovudine when both agents were administered as monotherapy (Kahn et al., 1992). A greater proportion of the subjects who received didanosine (15 (38%) of 39) experienced a decline in HIV-1 RNA and rise in CD4 cell count (quadrant **A**), compared to five (17%) of 29 who received zidovudine alone (Fisher's exact test, $P = 0.07$). If one only considered those subjects who experienced a decline in HIV-1 RNA outside the 95% CI, then didanosine therapy was superior to zidovudine therapy.

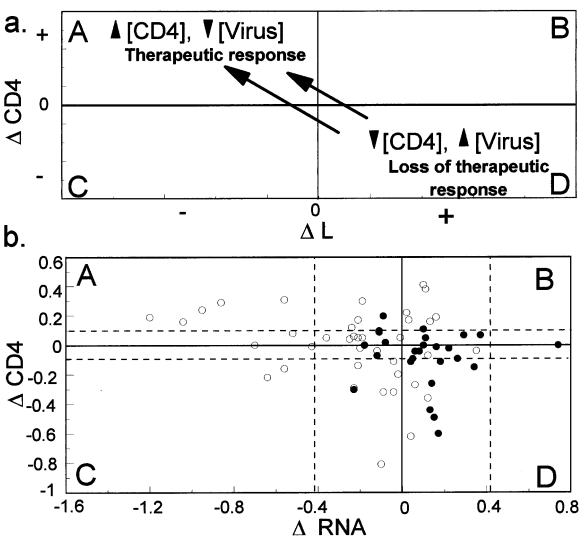

Fig. 4. (a) Differential effect of two antiretroviral drug regimens on the relative changes in viral burden (ΔL) and CD4+ cell count (ΔCD4). The more effective antiretroviral response was described by a greater proportional distribution between quadrant **A** and quadrant **D**. (b) The observed differential effect between the relative changes in viral burden (HIV-1 RNA) and CD4+ cell count for zidovudine (●) versus didanosine (○) monotherapy. 68 HIV-1 infected subjects (median CD4-cell count of 85 cells/μl) were assessed following 8 weeks of therapy (Kahn et al., 1992; Coombs et al., 1996). The dashed lines indicate the estimated within-subject variability (95% CI) for relative change in HIV-1 RNA ($\pm 0.41 \log_{10}$ copies/ml) and CD4+ cell count ($\pm 0.10 \log_{10}$ cells/μl).

3.3. Other markers of disease progression (virologic response modifiers)

Multivariate analyses of clinical trial data have shown that high-grade phenotypic drug resistance to zidovudine, genotypic mutations at codons 41 and 215, and the syncytium-inducing phenotype, all independently influence clinical progression of disease (Boucher et al., 1992; Veenstra et al., 1993; D'Aquila et al., 1995; Ho, 1995; Japour et al., 1995; Welles et al., 1996). For individual subjects, the slope, V (Eq. (1)), for each subject was used to assess how these virologic response modifiers influenced the inverse relationship between changes in CD4+ cell count and viral burden in response to antiretroviral therapy.

Only one of the possible response modifiers investigated, syncytium-inducing phenotype, resulted in a complete dissociation of the inverse relationship as defined by a positive slope ($V = 3.5$) (Table 1). As such, this virologic modifier effected a decline in CD4+ cell count ($-\Delta$CD4) despite an antiretroviral induced decline in virus burden ($-\Delta L$); i.e. a quadrant **C** assignment for these subjects. For the other seven putative virologic response modifiers investigated, the inverse relationship was maintained as defined by a negative slope and location in quadrant **A** or **D** (Table 1). None of the responses were located in quadrant **B**.

4. Discussion

The Cartesian coordinate plot has several potential implications for individualized patient management and the design and analysis of HIV-1 clinical trials. The current antiretroviral therapy paradigm is to lower virus burden (generally assessed by measuring viral RNA levels) and by this mechanism, raise the CD4+ cell count and delay disease progression (Coombs et al., 1996; Katzenstein et al., 1996; O'Brien et al., 1996; Welles et al., 1996; Carpenter et al., 1997). However, managing individual patients using this paradigm is complicated for the following reasons: first, viral RNA is only a partial surrogate for disease outcome (Sperling et al., 1996; DeGruttola et al.,

Table 1
Description of virologic response modifiers, therapy assignment and quadrant location by Cartesian coordinate quadrant analysis following 8 weeks of therapy with either zidovudine or didanosine for 61 subjects[a] enrolled in ACTG protocol 116B/117 (Kahn et al., 1992; D'Aquila et al., 1995; Japour et al., 1995; Coombs et al., 1996)

	Number subjects	Median log baseline value		Proportional change		Slope (V)[b]	Quadrant assignment
		CD4+ cell count ($CD4_o$)	HIV-1 RNA level (L_o)	CD4+ cell count ($\Delta CD4$)	HIV-1 RNA level (ΔL)		
Virologic response modifier							
ZDV resistance	10	1.58	5.09	−0.21	0.01	−21	D
ZDV sensitive	49	2.03	4.86	0.02	−0.08	−0.25	A
SI phenotype	37	1.71	4.87	−0.07	−0.02	3.5	C
NSI phenotype	21	2.27	4.83	0.05	−0.11	−0.45	A
Codon 215 wild type	11	2.25	4.66	0.04	−0.21	−0.19	A
Codon 215 mutant	31	1.89	5.10	−0.04	0.05	−0.80	D
Codon 215/41 mutant	16	1.92	4.82	0.05	−0.07	−0.71	A
Therapy							
Zidovudine therapy	23	2.1	4.81	−0.04	0.11	−0. 36	D
Didanosine therapy/500 mg/day	19	2.0	2.1	0.04	−0.21	−0. 19	A
Didanosine therapy/750 mg/day	19	1.91	5.44	0.05	−0.1	−0.5	A
Quadrant location							
A	19	2.19	5.07	0.10	−0.23	−0.43	
B	10	2.04	4.71	0.17	0.11	1.55	
C	14	1.8	5.22	−0.18	−0.16	1.13	
D	18	1.99	4.85	−0.11	0.14	−0.79	

[a] Seven additional subjects had a $\Delta CD4 = 0$ and were, therefore, not included in the analysis.
[b] $V = \Delta CD4/\Delta L$; $\Delta L \neq 0$.

1997); second, CD4+ cell count and function may not be fully reconstituted by antiretroviral therapy (Jacobson et al., 1997); third, there is an interaction between viral load and CD4+ cell count such that a dissociation between these two parameters may occur in some patients (O'Brien et al., 1996; Mofenson et al., 1997; Pitt et al., 1997; Marschner et al., 1998).

Moreover, we hypothesize that an antiretroviral regimen may not show an increase in CD4+ cell count if either the virus burden is below a critical level necessary for measuring a CD4+ cell response, or if pathogenic factors (e.g. syncytium-inducing phenotype) or inadequate CD4+ cell proliferation negate the ability of an antiretroviral drug regimen to effect an increase in CD4+ cell count through a decrease in virus burden (Drusano et al., 1992, 1994; Lane, 1995). Taken together these observations strongly suggest that viral RNA alone is insufficient to monitor disease progression (DeGruttola et al., 1996, 1997; Fessel, 1997). Thus, the Cartesian coordinate analysis may be used to assess the individual patient's response to therapy in the context of these considerations. As such, this graphical analysis may be helpful for categorizing an individual subject's response after either starting or changing therapy (compared to the group response) in clinical trials (Paxton et al., 1997; Marschner et al., 1998).

The Cartesian coordinate analysis presented in our study differs in important aspects from other relative change plots (Hughes et al., 1997; Marschner et al., 1998). These studies used a linear regression analysis to compare the relative change in viral RNA and CD4+ cell count for their study populations whereas we have analyzed each subject's response ($\Delta CD4/\Delta L$) independently of the study population response. As such, the additional information captured from each subject's response to therapy (i.e. either two assessments of response at two defined time points for relative change or multiple assessements described by the rate of change) is the basis for the individual's placement into one of the four response quadrants rather than to a position around a regression line as was done by Hughes, Marschner and co-workers.

The proportional response between quadrant **D** (no anti-viral effect) and quadrant **A** (antiretroviral effect) could be used to evaluate the relative antiretroviral activity for different regimens (diagonal **D**–**A**). For example, clinical trials designed to look at antiretroviral activity (quadrant **A**) should assess this activity at higher viral burden and CD4+ cell count, and independently of the effects of response modifiers that may dissociate the inverse relationship (i.e. a slope $V > 0$) between CD4+ cell count and viral load and place subjects into quadrant **C**. For an individual patient, a quadrant **B** assignment should probably be looked upon as a more favorable dissociation from the inverse relationship as this response may be seen with an effective immune-based therapy that raises the CD4+ cell count without effecting a decrease in virus load (Kovacs et al., 1996) or it could represent an increase in replicatively 'defective' cell-free virus as might be seen with protease inhibitor therapy (Meek et al., 1990; Craig et al., 1991). The quadrant **B** assignment also illustrates the interaction between viral RNA and CD4+ cell count whereby the rise in CD4+ cell count in response to therapy partially negates the adverse clinical effect of a rise in viral RNA (Marschner et al., 1998).

The analysis was used to graphically show how modifiers of HIV-1 disease progression influence CD4+ cell decline and viral load. The influence of the response modifiers could be used to determine whether it was necessary to stratify in clinical trials by the response modifier for the purpose of analysis. For example, certain studies have suggested that the syncytium-inducing phenotype, high-grade phenotypic drug resistance to zidovudine and genotypic mutations at codons 41 and 215 which are each independently associated with disease progression, could have an effect on the association between the inverse relationship between viral load and CD4+ cell count (quadrant **D**–**A**) (Boucher et al., 1992; Veenstra et al., 1993; D'Aquila et al., 1995; Japour et al., 1995). In our analysis of the 116B/117 virology data set, neither high-level phenotypic nor genotypic resistance to zidovudine affected the inverse relationship between CD4+ cell decline and viral burden, whereas syncytium-inducing phenotype

was the only virologic response modifier that did. As such, the influence of syncytium-inducing phenotype could negate the expected CD4+ cell increase from successful antiretroviral therapy. Thus, for this modifier, in this study population, stratification might be necessary to assess the maximal antiretroviral response to therapy. It is not known if a similar response will be observed with more potent therapies.

The assumptions in our analysis do not preclude that when CD4+ cell proliferation and cell count are very low, the CD4+ cell count could remain essentially unchanged despite either rises or falls in virus load. This lack of CD4 response could reflect either a limitation of nucleoside therapy (Drusano et al., 1992, 1994) or exhaustion of the immune system, or both. As such, this suggests that for some patients with very low CD4+ cell counts, effective treatment may require immune augmentation in conjunction with more effective antiretroviral therapy (Lane, 1995).

There are limitations to this analysis based on the primary assumption of linearity. The cause-and-effect relationship between virus load and CD4+ cell decline may not hold true for all patients over the entire course of the disease; for example, primary infection and very late stage disease. Moreover, the relationship may not be valid for all measures of viral load (e.g. HIV-1 RNA versus IUPM) or for all antiretroviral drugs or drug combinations (e.g. combination nucleosides versus combination nucleosides and a protease inhibitor). The assumption of the linear relationship between virus burden and CD4+ cell count in the peripheral blood compartment ignored the contribution of other compartments such as lymphoid tissue, genital secretions and the central nervous system (Pantaleo et al., 1993; Lafeuillade et al., 1996; Liuzzi et al., 1996; Cavert et al., 1997). The effect of time on the linear relationship was only analyzed, as defined by rate of change, for the natural history study. Moreover, there may be a lag in the CD4 response which may be of particular importance for assessing protease inhibitor therapy. The analysis was not designed to assess the quadrant response by clinical outcome; however, it is reassuring that the interaction between viral RNA level and CD4 cell count shown elsewhere defined the poorest clinical outcomes for those with quadrant **C** or **D** responses (Marschner et al., 1998). Finally, immunologic response modifiers need to be investigated for their contribution to the quadrant **B** assignment as understanding this response may be particularly important for assessing immune-based therapies (Kovacs et al., 1996) and the dissociation between viral RNA and CD4+ cell count seen with protease inhibitors (Collier et al., 1996).

While we recognize the above limitations, there are several advantages to the Cartesian coordinate plot for analyzing both the CD4+ cell and viral load responses to antiretroviral therapy in clinical trials. First, it offers a simplified graphical approach to understand the proportional distribution and clustering of individual patient data among four different response quadrants for two distinct variables each of which has a different measurement variability: e.g. the relative change in CD4+ cell count and the relative change in virus load. Subjects were distinguished by their distribution into one of four possible outcomes despite small sample sizes and after accounting for within-subject variability in each parameter's measurement, thus allowing for a simple categorical analysis. Our estimates of the variability associated with the relative change estimates could be improved markedly by multiple sampling before and after the point of interest or by considering other assessments of change such as slope or area-under-the curve analysis (Paxton et al., 1997). Second, it identified those putative response modifiers that resulted in a dissociation of the inverse relationship and which should be considered for stratification. Third, it allows for data analysis of antiviral activity without a full understanding of the potentially complex interactions between the various virologic and immunologic factors that define the disease.

In summary, the Cartesian coordinate plot analysis utilizes the dynamic inverse relationship between virus load and CD4+ cell count to assess the effect of other markers of disease progression on this relationship; as such, this method may be helpful for designing trials based on individual patient management of antiretroviral ther-

apy by focusing individual patient response to therapy into one of four possible response quadrants. Similarly, this analysis also can be extended to evaluate HIV-1 antiretroviral therapies and studies of pathogenesis in prospective clinical trials that use smaller sample sizes (Loveday and Hill, 1995; Paxton et al., 1997).

Informed consent was obtained from each subject and human experimentation guidelines of the Human Subjects Review Committees of the participating institutions were followed.

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