Immune activation, CD4+ T cell counts, and viremia exhibit oscillatory patterns over time in patients with highly resistant HIV infection.

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Abstract

The rates of immunologic and clinical progression are lower in patients with drug-resistant HIV compared to wild-type HIV. This difference is not fully explained by viral load. It has been argued that reductions in T cell activation and/or viral fitness might result in preserved target cells and an altered relationship between the level of viremia and the rate of CD4+ T cell loss. We tested this hypothesis over time in a cohort of patients with highly resistant HIV. Fifty-four antiretroviral-treated patients with multi-drug resistant HIV and detectable plasma HIV RNA were followed longitudinally. CD4+ T cell counts and HIV RNA levels were measured every 4 weeks and T cell activation (CD38/HLA-DR) was measured every 16 weeks. We found that the levels of CD4+ T cell activation over time were a strong independent predictor of CD4+ T cell counts while CD8+ T cell activation was more strongly associated with viremia. Using spectral analysis, we found strong evidence for oscillatory (or cyclic) behavior in CD4+ T cell counts, HIV RNA levels, and T cell activation. Each of the cell populations exhibited an oscillatory behavior with similar frequencies. Collectively, these data suggest that there may be a mechanistic link between T cell activation, CD4+ T cell counts, and viremia and lends support for the hypothesis of altered predator-prey dynamics as a possible explanation of the stability of CD4+ T cell counts in the presence of sustained multi-drug resistant viremia.

Introduction

Current therapeutic strategies for HIV-infected persons include the use of antiretroviral therapy to fully inhibit viral replication, as defined by achieving and maintaining undetectable plasma HIV RNA levels. The vast majority of patients who are treatment naive and able to adhere to a recommended regimen are able to achieve durable and perhaps indefinite viral suppression. A poorly described but significant subset, however, are not able to achieve this outcome, either due to pre-existing resistance and/or the inability to fully adhere to therapy. Most, but not all, of these patients eventually develop drug resistance mutations and, hence, have limited long-term options for complete viral suppression.

The natural history of incomplete or partial viral suppression with combination therapy is complex. As compared to untreated disease, those who remain on a stable regimen despite the presence of drug-resistance mutations have slower rates of CD4+ T cell decline and a lower risk of progressing to AIDS and/or death [1,2,3]. This effect appears to be more strongly associated with failure of protease inhibitor-based regimens than with failure of non-nucleoside reverse transcriptase inhibitor based regimens [1,4]. Although partial reduction in viral load clearly contributes to the residual benefit of therapy [5], the delayed risk of disease progression in treated versus untreated disease remains significant, even after controlling for viral load [1,2].

Among untreated individuals, the level of viremia is only partially predictive of the rate of disease progression, as defined by the rate of CD4+ T cell loss and/or by the risk of progressing to AIDS and death [6,7]. T cell activation (as defined by expression of CD38 and HLA-DR) is an independent predictor of CD4+ T cell loss and disease progression among untreated patients [8,9]. Theoretically, activated T cells may contribute to a poor prognosis by supporting higher levels of viral replication and/or by causing inflammation-associated damage to the immune system and other organ systems. Given the central role of T cell activation in untreated diseases our previous work explored the impact of drug-resistance on the complex relationship between T cell activation and viral load. We have found that, after controlling for the levels...
of viremia, CD8+ T cell activation was lower in those with drug resistance than those with wild-type HIV. This effect appeared to be more strongly associated with the presence of protease inhibitor resistance rather than direct exposure to the immunomodulatory effects of protease inhibitors [10].

To understand the role of the T cell activation, progressive immunodeficiency, and drug resistant HIV, we performed detailed immunologic and virologic measurements among a cohort of treated patients with detectable viremia who were maintained on a stable regimen pending more effective therapeutic options. The overall objectives of this prospective cohort were to determine the impact of replicative capacity, T cell activation and HIV-specific T cell response on both viremia and peripheral CD4+ T cell counts over time. In the current analysis, we describe how many of these factors evolve over time. We found that CD4+ T cell activation was most strongly associated with the CD4+ T cell counts while CD8+ T cell activation was more strongly associated with viremia. Spectral analysis [11] in a subset of subjects revealed evidence of oscillatory behavior in CD4+ T cell counts, CD4+ T cell activation, CD8+ T cell activation, and plasma HIV RNA levels. Taken together, the data suggest a mechanistic link between T cell activation, viremia and CD4+ T cell depletion and support the hypothesis of altered predator-prey dynamics as a possible explanation for the stability of CD4+ T cell counts even in the present of sustained multi-drug-resistant viremia.

Materials and Methods

Subjects

All subjects were enrolled in the Partial Controllers on Antiretroviral Therapy cohort (PCAT) [12]. All subjects provided written informed consent. The study was approved by the University of California, San Francisco Human Research Protection Program Committee on Human Research, Laurel Heights Panel. Eligibility criteria included having a detectable viral load between 200 and 10,000 copies/mL while on a stable optimized combination antiretroviral regimen. Subjects were enrolled and followed in the period prior to the widespread availability of integrase inhibitors, CCR5 inhibitors, and second generation non-nucleoside reverse transcriptase inhibitors, and had hence had limited options for complete viral suppression. Because of concerns that high level viremia (>10,000 copies RNA/mL) would pose substantial risk to the study participant, subjects were encouraged to consider treatment modification once viral loads increased above this level. We chose a threshold of 10,000 copies of HIV RNA/mL (using the bDNA method) for this study because previous data suggested that viral loads above this threshold might be associated with rapid CD4+ T cell loss. Given this data, we felt that subjects and their health care providers should have no perceived barriers to modifying therapy. For the current analysis, subjects were censored if their HIV RNA levels exceeded this threshold on two subsequent visits or if the optimized antiretroviral therapy was modified or discontinued.

Immunologic and virologic measurements

Viral load and CD4+ T cell counts were measured every 4 weeks. T cell activation (CD38+/HLA-DR+) was measured every 16 weeks using cytokine flow cytometry, as previously described [12]. HIV replicative capacity was also measured longitudinally, using a modified version of an HIV phenotypic drug susceptibility assay (Monogram Biosciences). Briefly, HIV RNA was extracted from the subject’s plasma and the terminal 18 codons of the gag gene, the entire pro gene, and a portion of the RT gene were PCR amplified. The amplified gene segments were inserted into a viral vector containing a luciferase gene. Following a single round of viral replication in the absence of drug, luciferase activity was measured and compared to that for a reference virus (NL4-3).

Statistical analysis

The primary variables considered for modeling CD4+ T cell counts over time included CD4+ and CD8+ T cell activation, CD4 nadir, duration of infection, HIV-specific T cell response, viral burden, and viral replication capacity. Plotting revealed significant skews in the distributions of CD4+ T cell counts, CD4+ and CD8+ T cell activation, and plasma HIV RNA levels, and these values were log_{10} transformed to meet model assumptions. Given the less frequent measurements of immune activation than CD4+ T cell counts, CD8+ T cell counts, and viral load, we used multiple imputation to address the missingness in the T cell activation and replication capacity data. Missing data can lead to bias in estimates as well as a loss of power [13,14,15]. The extent of the bias depends on the cause of the missingness. Multiple imputation can be used when the missing data are “missing at random”, that is the probability of the observation being missing for a particular subject does not depend on the value of the marker, conditional on other observed variables. In this case the assumption was justified in that it was solely a cost issue related to the missingness and not an underlying disease process. Multiple imputation has been used successfully in a wide variety of fields of application from astrophysics [16] to chemistry [17] to clinical research [13,14,15,18]. Multiple imputation has not only been used in regression settings but also used in spectral analysis [16,17,19]. Instead of filling in a single value for each missing value, such as last observation carried forward (LOCF), multiple imputation replaces each value with a set of draws from a random sample of plausible values reflecting the uncertainty of the missing values [18]. In this way, one creates multiple datasets, each with different draws from the random sample of the missing values resulting in multiple complete case datasets. Standard statistical techniques can then be applied to each complete case imputed dataset and the results are then combined for the final inference. We used PROC MI in SAS version 9.2 for multiple imputation, using the Markov Chain Monte Carlo (MCMC method) with multiple chains. We discarded the first 8000 iterations as “burn in” and used a total of 10000 iterations for inference. We used the Jeffreys (noninformative) prior and took the results of the Expectation-Maximization (EM) algorithm as our starting point. For the multiple imputed estimates, time-series and autocorrelation plots showed that our MCMC sampler was mixing well and that the estimates reached convergence. We then used linear mixed models with random intercepts and slopes to assess the association of T cell activation and viremia in CD4+ T cell counts over time in each imputed dataset. An unstructured covariance matrix was used to assess the serial correlation. This approach allows one to model the association of continuous predictor variables measured longitudinally on a continuous longitudinal outcome variable while accounting for within-subject correla-

We also measured relative efficiency, which is a function of the number of imputations, m, and the percent missingness of the data. It is defined in units of variance as the differential utility of using m (in this case m = 30) imputations instead of an infinite number of imputations, where 100% is fully efficient. We conducted 30 imputations because results have shown that although the estimate is unbiased, more imputations are needed than previously suggested to attain good statistical power [20].
MIAnalyze was used for statistical inference of parameters from the 30 imputed complete datasets.

**Spectral analysis**

Individual viral and immune cell dynamics were analyzed using spectral analysis [11] in a subset of subjects. Subjects with at least 20 observation points for all compartments (i.e., CD4+ T cell counts, HIV viremia, and T cell activation) were included for analysis (N = 11). Spectral analysis can be used to detect cyclical patterns in data, with the purpose of decomposing complex time series with cyclical components into sinusoidal functions. In a sense, performing spectral analysis on time series data can be compared to putting these data through a prism that identifies the underlying cyclical (sinusoidal) components. As a result, data that might initially look like random noise can be meaningfully interpreted in terms of underlying recurring cycles. The decomposition is performed using the Fourier transform. The time series can then be presented as a mixture of functions expressed as \( y(t) = \text{amplitude} \cdot \sin(2 \pi \cdot \text{frequency} \cdot t + \text{phase}) \).

The phase is determined by the initial displacement of the wave at time \( t = 0 \). In this case the y would be the population of interest such as viremia. Amplitude is the maximum height of the wave (in absolute value). Frequency is the reciprocal of the period which is the length of time for the wave to repeat. This signal is thus presented in the frequency domain, where frequency is expressed in units of \([1/\text{time}]\) and significant peaks are recorded. Raw data are first de-trended (the mean and the linear fit are subtracted) and then the Lomb-Scargle periodogram is constructed. This algorithm generates a Fourier spectrum for data that are not equally spaced [21]. Signals with peaks below the 50% critical limit were discarded. For this analysis, spectral decomposition was used to determine signal composition for HIV RNA levels, CD4+ T cell activation, CD8+ T cell activation, and CD4+ T cell counts. From the analysis, we determined each signal from noise, identified the presence of oscillatory behavior, and drew conclusions about correlations between the populations. The results show the averages of the quantities of interest averaged across all imputations.

While spectral analysis can only examine one patient over time, non-linear mixed models can examine all subjects simultaneously. We therefore constructed a non-linear mixed effects model with a random intercept to model log viremia over time. To model the oscillatory trend, we used a mixture of two sine waves with frequencies at \( 2 \pi \) and \( 8 \pi \), as suggested by the results of the spectral analysis, to account for any cyclical patterns in the data. We fit a non-linear mixed effects model with random intercept model using the first-order method of Beal and Sheiner [22] that included an indicator for protease inhibitor use, CD8+ T cell activation, baseline viremia, the oscillatory component and protease inhibitor use. Estimates from the spectral analysis were used as the starting values.

**Results**

We examined 54 subjects with partially controlled antiretrovi-
ral-resistant viremia on a stable antiretroviral regimen. There were 50 men (93%) and 4 women. The median baseline CD4+ T cell count was 303 cells/mm\(^3\) (IQR: 210–437) and the median log\(_{10}\) plasma HIV RNA level was 3.3 copies/ml (IQR: 2.5–3.7) (Table 1). The baseline percent of activated CD38+HLA-DR+CD4+ T cells was 6.4% (IQR: 5.0–8.9) and the baseline percent of activated CD38+HLA-DR+CD8+ T-cells was 21.1% (IQR: 15.1–30.0). The median self-reported CD4 nadir was 82 cells/mm\(^3\) (IQR: 20–164). Forty (74%) of the subjects were failing on a protease-inhibitor based regimen. Of those, 30 (75%) were failing a boosted protease inhibitor regimen. The median duration of follow-up was 44 weeks (IQR 18.1 to 71.0). Across all subjects, the average rate of CD4+ T cell change was \(-1\) CD4+ T cells/mm\(^3\)/month. Of interest, CD4+ T cell counts increased, on average, by 20 cells/mm\(^3\) from baseline in subjects taking boosted protease inhibitors, and decreased by 33 cells/mm\(^3\) in subjects not taking these drugs, although this difference did not reach statistical significance.

As expected, at baseline there was a negative correlation between log\(_{10}\) viral load and CD4+ T cell count (\( \rho = -0.28, p = 0.038 \)). There was also a negative correlation between T cell activation and CD4+ T cell counts with subjects with higher levels of T cell activation having lower CD4+ T cell counts at baseline (\( \rho = -0.38, p = 0.006 \) for CD4+ T cell activation and \( \rho = -0.30, p = 0.03 \) for CD8+ T cell activation). Log\(_{10}\) viremia and both CD4+ and CD8+ T cell activation were strongly correlated (\( \rho = 0.35, p = 0.012 \) and \( \rho = 0.52, p = 0.0001 \) respectively). The strong association between log\(_{10}\) viremia and CD8 activation persisted across time.

**Longitudinal predictors of CD4+ T cell counts and viremia**

We analyzed longitudinal CD4+ T cell counts using a mixed effects model with a random intercept and slope. Correlation over time was modeled using an unstructured covariance matrix. Not surprisingly, CD4+ T cell counts declined over time (adjusted p-value 0.0028). Across all subjects, higher levels of CD4+ T cell counts were temporally associated with lower CD4+ T cell activation, after adjusting for the log plasma HIV RNA levels. For every percentage increase in CD4+ T cell activation, CD4+ T cell counts declined by 2 cells/mm\(^3\) (adjusted p-value of 0.025; Table 2). Similarly, higher replication capacity was associated with lower CD4+ T cell counts (adjusted p-value 0.041). This effect of

| Table 1. Baseline characteristics of the study group. |
|---------------------------------------------------|
| **Baseline variable** | **Median** | **Interquartile range** |
| Age in years | 46 | 42–52 |
| CD4 T-cell, cells/mm3 | 303 | 210–437 |
| Plasma HIV RNA log 10 copies/ml | 3.3 | 2.5–3.7 |
| CD4 activation (%) | 6.4 | 5–8.9 |
| CD8 activation (%) | 21.1 | 15.1–30.0 |
| CD4 nadir, cells/mm3 | 82 | 20–164 |
| Follow up (weeks) | 44 | 18.1–71.0 |

Gender | N | Percent |
|------|----|--------|
| Male | 50 | 93 |
| Female | 4 | 7 |

Protease Inhibitor (PI) | N | Percent |
|----------------------|----|--------|
| Boosted | 30 | 55 |
| RTV/LPV | 23 | 43 |
| RTV-other PI | 7 | 13 |
| Non-boosted | 10 | 19 |
| None | 14 | 26 |
| Non-nucleoside RTI | 7 | 13 |
| Nucleoside RTI | 53 | 98 |

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Oscillatory Patterns in Chronic HIV Infection
oscillatory pattern

Immunologic and virologic measures follow an

Longitudinal predictors of viremia

CD4+ T cell activation on CD4+ T cell counts was significant in the complete case analysis (data not shown) and remained significant across all multiply imputed datasets. Table 2 gives the estimates from the final mixed effects model as well as the relative efficiencies of the multiple imputation estimates.

### Table 2. Parameters associated with CD4 T cell count loss among patients with partially-controlled drug resistant viremia using a mixed effects model.

| Effect                  | Estimate | Standard Error | 95% CI       | P       | Relative efficiency |
|-------------------------|----------|----------------|--------------|---------|---------------------|
| Intercept               | 437      | 31.8           | (375, 500)   | <0.0001 | 0.996               |
| Time (weeks)            | −0.356   | 0.119          | (−0.59, −0.123) | 0.0028  | 0.998               |
| Replication capacity    | −0.365   | 0.174          | (−0.715, −0.015) | 0.0412  | 0.975               |
| CD4 activation          | −1.695   | 0.742          | (−3.168, −0.222) | 0.0246  | 0.981               |
| Log viral load          | −19.95   | 6.131          | (−32.05, −7.85) | 0.0014  | 0.987               |

The model shows that CD4 T cell count decreased over time. Across all subjects higher levels of CD4 T cell activation were associated with lower levels of CD4 T cell counts controlling for log plasma HIV-1 viremia. Higher replication capacity was also associated with lower CD4 counts.

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### Table 3. Parameters associated with log HIV viremia among patients with partially-controlled drug resistant viremia using a mixed-effects model.

| Effect                  | Estimate | Standard Error | 95% CI       | P       | Relative efficiency |
|-------------------------|----------|----------------|--------------|---------|---------------------|
| Intercept               | 2.81     | 0.122          | (2.57, 3.05) | <0.0001 | 0.99               |
| Time (weeks)            | −0.002   | 0.0017         | (−0.005, 0.001) | 0.232  | 0.997               |
| CD8 activation          | 0.018    | 0.0036         | (0.010, 0.025) | <0.0001 | 0.976               |

The model suggests that increases in CD8 T cell activation was associated with increases in log HIV viremia.
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Longitudinal predictors of viremia

We analyzed longitudinal plasma HIV RNA levels using a mixed effects model with random intercepts and slopes and an unstructured covariance matrix. Across all subjects, higher levels of plasma HIV RNA levels were temporally associated with lower CD8 T cell activation. For every percentage increase in CD8 T cell activation, log transformed plasma HIV RNA levels increased by 0.02 logs (adjusted P-value<0.0001; Table 3). There was no consistent relationship between replicative capacity and plasma HIV RNA levels. This effect of CD8 T cell activation on plasma HIV RNA levels was significant in the complete case analysis (data not shown) and remained significant across all multiply imputed datasets.

Immunologic and virologic measures follow an oscillatory pattern

Next, we examined the correlation structure between CD4+ T cells, T cell activation and viral burden over time in eleven subjects using spectral analysis. Spectral analysis allows for modeling the time series of each population as a mixture of sine waves. Each time series was decomposed into a collection of sine waves with different frequencies, amplitude, and phases.

Table 4 lists the demographic characteristics of the subjects included in this analysis. Subjects in this subset did not differ significantly from subjects not included in this analysis in terms of average level of T cell activation, viremia or CD4+ T cell count. In these eleven subjects we found evidence of oscillatory patterns in the CD4 compartment with subjects exhibiting 2-6 separate waves. Figure 1 shows, for a representative patient, the results from the spectral analysis in terms of T cell activation, log viremia and CD4 counts over time. Using mixed effects models with a compound symmetric correlation structure, we found that subjects with higher levels of CD4+ T cell counts over time had fewer CD4 waves and these waves had lower amplitudes (p = 0.0100 and p = 0.0144 respectively). Similarly, we found cyclical behavior in the T cell activation compartments. Subjects with higher levels of viremia had higher amplitudes of CD8 activation (p<0.0001) and lower phase shifts (p = 0.0001) controlling for CD4 nadir. We found a tight correlation between viremia amplitude and CD8+ T cell activation amplitude (rho = 0.61, p = 0.04). Take together this yields further evidence of the mechanistic linkage between viremia and CD8 T cell activation. Figure 2 plots, for four representative patients, log CD8 activation and log viremia over time.

Overall, we found strong evidence of complex dynamics, with subjects exhibiting 0 to 7 separate sine waves and with different frequencies for each component. One or greater sine waves is an indication of the presence of oscillatory behavior in activated CD4+ T cells, activated CD8+ T cells, plasma HIV RNA levels, and total CD4+ T cells. Tables 5-8 list, for each subject and each parameter the average of the wave parameters (frequency, amplitude and phase) as well as the total number of waves in the model for CD8 T cell activation, HIV viremia, CD4 activation and CD4 T cell counts respectively. Two subjects did not exhibit oscillatory behavior in viremia. These two subjects also had the lowest CD4 nadirs in the sample with nadirs of 2 and 24 cells/mm³. These subjects were also amongst those with the highest maximum viremia. However these subjects did not differ significantly from other subjects in terms of length of follow-up, average CD4+ count or average viremia.

To examine the determinants of oscillatory behavior in these subjects, we constructed a non-linear mixed effects model with random intercepts. We found evidence of an interaction with protease inhibitor use and oscillatory behavior. Subjects who were not on a protease-inhibitor containing regimen had a stronger
oscillatory signal than subjects on a protease-inhibitor containing regimen ($p = 0.04$). In this model CD8$^{+}$ T cell activation remained a significant predictor of HIV viremia over time ($p = 0.0007$). Table 9 lists the parameter estimates for this model.

**Discussion**

T cell activation is a central component of HIV and SIV infection. Among untreated HIV-infected persons, measures of T cell activation predict risk of subsequent disease progression [23,24]. Among treated subjects, T cell activation is associated with CD4$^{+}$ T cell count changes during therapy, at least cross-sectionally [10,25]. T cell activation rather than viral load appears to be the primary characteristic that defines pathogenic versus non-pathogenic SIV infection in non-human primate models [26]. Despite extensive investigation, the temporal associations between immune activation, viral load, and peripheral CD4$^{+}$ T cell counts have not been fully defined. Here, we examined a unique cohort of HIV-infected persons in which immune activation was thought to have a strong independent effect on disease outcomes, and measured changes in several relevant biologic outcomes over time. We found that, among treated subjects with low levels of detectable drug-resistant viremia (<10,000 copies/mL), higher CD4$^{+}$ T cell counts were predicted by lower levels of CD4$^{+}$ T cell

### Table 4. Demographic profile of patients included in the spectral analysis.

| Patient | Maximum CD4 T cell count (cells/mm$^3$) | Average CD4 T-cell count (cells/mm$^3$) | CD4 nadir (cells/mm$^3$) | Maximum HIV-1 RNA (copies/mL) | Average HIV-1 RNA (copies/mL) |
|---------|----------------------------------------|----------------------------------------|--------------------------|------------------------------|-------------------------------|
| 2004    | 310                                    | 228                                    | 2                        | 4.65                         | 2.92                          |
| 3025    | 412                                    | 273                                    | 39                       | 3.78                         | 3.1                           |
| 3035    | 319                                    | 225                                    | 60                       | 3.66                         | 3.24                          |
| 3037    | 364                                    | 258                                    | 220                      | 4.21                         | 3.79                          |
| 3040    | 481                                    | 365                                    | 90                       | 4.27                         | 3.97                          |
| 3042    | 232                                    | 145                                    | 69                       | 4.21                         | 2.97                          |
| 3077    | 445                                    | 293                                    | 285                      | 3.84                         | 3.35                          |
| 3089    | 567                                    | 376                                    | 150                      | 4.11                         | 2.542                         |
| 3102    | 420                                    | 254                                    | 24                       | 4.86                         | 3.95                          |
| 3135    | 644                                    | 433                                    | 250                      | 4.12                         | 2.97                          |
| 3153    | 462                                    | 356                                    | 274                      | 4.78                         | 4.2                           |

![Figure 1](https://doi.org/10.1371/journal.pone.0021190.g001)
activation. In contrast, higher plasma HIV RNA levels were more strongly predicted by higher levels of CD8 T cell activation. We also observed that replication capacity, measured by an *in vitro* assay, was associated with lower CD4+ T cell counts but not with the level of viremia. Using a sophisticated statistical method that seeks to define the temporal cause and effect association of these

![Figure 2. Log viremia and Log CD8 activation over time in 4 patients.](image)

**Table 5.** Spectral analysis estimated parameters for CD8 T cell activation.

| Patient | Number of waves | Mean Frequency | Std Dev | Mean Amplitude | Std Dev | Mean Phase | Std Dev |
|---------|-----------------|----------------|---------|----------------|---------|------------|---------|
| 2004    | 3               | 0.059          | 0.039   | 8.710          | 1.274   | 3.163      | 0.518   |
| 3025    | 3               | 0.105          | 0.011   | 5.838          | 1.500   | 2.744      | 1.999   |
| 3035    | 5               | 0.067          | 0.038   | 5.038          | 1.833   | 1.795      | 1.463   |
| 3037    | 2               | 0.086          | 0.077   | 5.213          | 3.135   | 3.738      | 1.236   |
| 3040    | 4               | 0.061          | 0.043   | 4.543          | 5.630   | 2.167      | 0.658   |
| 3042    | 4               | 0.018          | 0.009   | 42.990         | 45.323  | 3.320      | 2.573   |
| 3077    | 4               | 0.078          | 0.062   | 31.924         | 21.029  | 4.064      | 1.675   |
| 3089    | 3               | 0.092          | 0.028   | 5.354          | 1.560   | 2.833      | 2.128   |
| 3102    | 4               | 0.073          | 0.024   | 16.464         | 9.540   | 2.350      | 1.476   |
| 3135    | 4               | 0.044          | 0.024   | 34.648         | 32.257  | 2.643      | 2.493   |
| 3153    | 3               | 0.073          | 0.022   | 42.089         | 36.380  | 2.755      | 2.333   |
| **Mean** |                | 3.55           | 0.07    | 18.44          | 14.50   | 2.87       | 1.69    |
| **Std Dev** |            | 0.82           | 0.02    | 16.07          | 16.40   | 0.67       | 0.70    |

For each individual, the mean and standard deviations for wave components for CD8 T cell activation is presented.

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observations, we found evidence of complex oscillatory behavior in these populations. This behavior was more evident among individuals not receiving a protease-based regimen. This suggests that the various measurements are linked to each other in an as yet poorly defined mechanism, although the temporal changes might be consistent with a previously hypothesized “ predator-prey” association, as described below [27].

Our data regarding the relationship between CD4+ T cell activation and CD4+ T cell counts is generally in agreement with prior work in untreated HIV-infected persons. For example, Catalfamo and colleagues reported that the degree of CD4+ T cell activation (or proliferation) is driven by homeostatic responses and that CD8+ T cell activation is mainly a direct pro-inflammatory consequence of viral replication [28]. Of note, presently it is not possible in these observational studies to clearly define the causal pathway. Although some have argued that the higher levels of CD4+ T cell activation in subjects with lower CD4+ T cell counts reflects a homeostatic response to low CD4+ T cell counts [28], others have argued that higher CD4+ T cell activation is mechanistically involved in CD4+ T cell depletion. Support for this latter perspective is based on several consistent observations, including: (1) measures of T cell activation rather than viral load predict outcome in the natural host of SIV [29], (2) measures of T cell activation in humans predict subsequent disease outcome independent of viral load [8,9,30], and (3) generalized T cell activation in the absence of SIV/HIV infection can cause CD4+ T cell but not CD8+ T cell depletion [31,32]. The striking and consistent relationship between viral load and CD8+ T cell

Table 6. Spectral analysis estimated parameters for HIV viremia.

| Patient | Number of waves | Mean Frequency | Std Dev | Mean Amplitude | Std Dev | Mean Phase | Std Dev |
|---------|----------------|----------------|---------|----------------|---------|------------|---------|
| 2004    | No oscillations |                |         |                |         |            |         |
| 3025    | 4              | 0.086          | 0.059   | 1245.86        | 692.327 | 4.436      | 1.328   |
| 3035    | 4              | 0.013          | 0.018   | 505.27         | 438.186 | 1.785      | 1.175   |
| 3037    | 4              | 0.034          | 0.048   | 3086.03        | 1102.75 | 2.734      | 1.924   |
| 3040    | 4              | 0.052          | 0.032   | 2429.39        | 704.876 | 4.759      | 1.805   |
| 3042    | 2              | 0.092          | 0.039   | 2568.66        | 483.054 | 3.000      | 1.044   |
| 3077    | 5              | 0.099          | 0.0319  | 4333.23        | 5465.362 | 4.298  | 1.3959   |
| 3089    | 2              | 0.043          | 0.0521  | 5882.81        | 1411.156 | 5.943  | 0.1204   |
| 3102    | No oscillations |                |         |                |         |            |         |
| 3135    | 4              | 0.044          | 0.0366  | 8829.99        | 7456.89 | 2.727      | 0.8222  |
| 3153    | 4              | 0.086          | 0.0324  | 41015.96       | 26361   | 4.718      | 2.1039  |
| Mean    | 3.67           | 0.06           | 0.04    | 7766.36        | 4901.73 | 3.822     | 1.30    |
| Std Dev | 1.00           | 0.03           | 0.01    | 12721.27       | 8431.66 | 1.322     | 0.61    |

For each individual, the mean and standard deviations for wave components for HIV viremia is presented.
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Table 7. Spectral analysis estimated parameters for CD4 T-cell activation.

| Patient | Number of waves | Mean Frequency | SD | Mean Amplitude | SD | Mean Phase | SD |
|---------|----------------|----------------|----|----------------|----|------------|----|
| 2004    | 6              | 0.066          | 0.051 | 2.438 | 1.061 | 2.98 | 1.37 |
| 3025    | 7              | 0.059          | 0.042 | 4.61  | 2.475 | 3.216 | 1.906 |
| 3035    | 5              | 0.043          | 0.034 | 5.62  | 2.011 | 3.191 | 1.702 |
| 3037    | 2              | 0.043          | 0.034 | 6.18  | 4.994 | 3.678 | 0.956 |
| 3040    | 4              | 0.069          | 0.044 | 8.04  | 4.561 | 1.846 | 1.383 |
| 3042    | 3              | 0.092          | 0.027 | 5.54  | 1.823 | 4.219 | 2.062 |
| 3077    | 5              | 0.101          | 0.046 | 5.42  | 3.978 | 3.282 | 1.932 |
| 3089    | 4              | 0.053          | 0.042 | 3.53  | 1.48  | 2.449 | 2.095 |
| 3102    | 4              | 0.075          | 0.04  | 3.10  | 3.524 | 2.339 | 1.929 |
| 3135    | 4              | 0.052          | 0.028 | 4.27  | 2.184 | 2.677 | 2.826 |
| 3153    | 3              | 0.090          | 0.008 | 3.94  | 1.434 | 3.550 | 0.414 |
| Mean    | 4.27           | 0.07           | 0.04  | 4.79  | 2.68  | 3.039 | 1.69  |
| Std Dev | 1.42           | 0.02           | 0.01  | 1.59  | 1.36  | 0.676 | 0.64  |

For each individual, the mean and standard deviations for wave components for CD4 T cell activation is presented.
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activation observed in our study is consistent with a robust literature suggesting that these two properties are highly associated in untreated and treated disease [7,28]. Viral replication is almost certainly causally associated with CD8+ T cell activation, as the latter parameter decreases consistently in response to combination therapy [33].

We observed no consistent association between replicative capacity and viremia. This lack of an association may be due to several factors, including the use of an assay that only incorporated parts of the patient-derived viral genome. It is also possible (and indeed we believe likely) that the true replicative capacity of the virus in vivo may not directly predict viremia. Bonhoeffer, Coffin and Nowak, for example, predicted that mutations which reduce the capacity of the virus to infect its target cells would lead to reduction in viremia, reduced CD4+ T cell activation, as the latter parameter decreases consistently in response to combination therapy [33].

The model found evidence of an oscillatory signal in patients whose regimens did not include a protease inhibitor. Although such a model is outside the scope of this paper, it is clear from our current data that, among reasonably stable subjects with drug-resistant HIV, there exists complex cell/virus interaction as indicated by the presence of sine waves at multiple frequencies in the spectral analysis. The presence of two or more frequencies of oscillation is a signature of more than one equilibrium state between the virus and the cells of the immune system. Furthermore, the existence of oscillations of high and low frequencies within the same system is indicative of processes taking place on different time scales. A variety of forces have been identified as drivers of periodicity in natural systems. Dynamic interactions between host and parasite populations cause oscillations in disease epidemics [41]. Climatic determinants have been described as the force behind epidemics of dengue fever and malaria [42,43,44]. Random noise is also capable of inducing oscillations in deterministic systems [45]. Such systems are commonly studied using spectral analysis, which is an important tool in describing oscillatory behavior as it provides additional

| Patient | Number of waves | Mean Frequency | SD | Mean Amplitude | SD | Mean Phase | SD |
|---------|----------------|----------------|----|----------------|----|------------|----|
| 2004    | 2              | 0.026          | 0.022 | 29.93         | 17.371 | 3.921      | 3.328 |
| 3025    | 5              | 0.088          | 0.064 | 173.08        | 100.792 | 4.024      | 2.17  |
| 3035    | 5              | 0.082          | 0.039 | 41.82         | 25.996  | 2.702      | 2.03  |
| 3037    | 5              | 0.048          | 0.036 | 49.70         | 45.099  | 2.772      | 1.071 |
| 3040    | 4              | 0.045          | 0.033 | 94.78         | 57.969  | 3.909      | 1.803 |
| 3042    | 6              | 0.069          | 0.04  | 252.48        | 220.891 | 3.281      | 2.016 |
| 3077    | 3              | 0.054          | 0.031 | 107.63        | 78.17   | 2.178      | 2.56  |
| 3089    | 4              | 0.093          | 0.013 | 70.36         | 25.979  | 3.189      | 1.071 |
| 3102    | 3              | 0.082          | 0.01  | 41.22         | 17.746  | 5.017      | 1.258 |
| 3135    | 4              | 0.033          | 0.037 | 6636.00       | 7656    | 2.063      | 1.755 |
| 3153    | 4              | 0.081          | 0.044 | 157.81        | 106.193 | 3.628      | 1.762 |
| Mean    | 4.09           | 0.06           | 0.03  | 695.89        | 759.29  | 3.335      | 1.89  |
| Std Dev | 1.14           | 0.02           | 0.01  | 1971.31       | 2288.15 | 0.881      | 0.66  |

For each individual, the mean and standard deviations for wave components for CD4 T cell counts is presented. doi:10.1371/journal.pone.0021190.t008

Table 9. Parameters estimated from patients with partially controlled drug resistant HIV infection using a non-linear mixed-effects model.

| Effect                | Estimate | Standard Error | P     |
|-----------------------|----------|----------------|-------|
| Intercept             | 0.0714   | 0.4032         | 0.863 |
| Time (weeks)          | 0.00614  | 0.00947        | 0.1132|
| CD8 activation        | 0.8468   | 0.1739         | 0.0007|
| Baseline Viremia      | 0.6272   | 0.1171         | 0.0003|
| No protease inhibitor | 0.2237   | 0.1128         | 0.0755|
| Cyclical component    | -0.0183  | 0.009          | 0.07  |
| Cyclical component*No PI use | -0.02522 | 0.011 | 0.0453 |

The model found evidence of an oscillatory pattern in patients whose regimens did not include a protease inhibitor. doi:10.1371/journal.pone.0021190.t009

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Table 8. Spectral analysis estimated parameters for CD4 T cell count.

For each individual, the mean and standard deviations for wave components for CD4 T cell counts is presented. doi:10.1371/journal.pone.0021190.t008
insight into the complexity of system dynamics which could not be gained from analysis solely in the time-domain.

One caveat is that this study is based on a small population of patients who were selected because they had a steady-state viral load on a partially suppressive stable regimen. Further, for the specific analysis and nonlinear models patients were selected if they had at least 20 longitudinal observations. It was necessary to have this many time points to be able to identify the sometimes damped oscillatory behavior. Moreover, it is known that co-morbidities such as cardiovascular disease and diabetes can cause generalized immune activation. Although these patients did not have a coronary heart disease or diabetes diagnosis, we did not have data on markers that could indicate a preclinical condition. Further we did not have data on other potential confounders such as exposure to influenza, recent vaccinations or stress that could have caused increases in immune activation. Further detailed studies are needed. Although our study design does not allow for strong conclusions regarding the cause and effect relationship of activation and either viral load or CD4+ T cell counts, it does provide novel insights into this relationship and suggests that it may be altered by drug-resistant HIV. Along these lines, Bonhoeffer and colleagues predicted that a treatment-related decrease in viral fitness would result in a new dynamic between viral load and target cells [27]. Specifically, any decrease in fitness may act to preserve CD4+ T cell counts (due to reduction in pathogenicity); these preserved CD4+ T cells could increase as a consequence, although preservation of total body CD4+ T cells may or may not be reflected in the number of circulating cells. The critical role of target cell availability as a determinant of viral load in treated disease has also been postulated as cause of viral blips during treatment [46,47].

Author Contributions
Conceived and designed the experiments: CMRK LY SD RH ES JMM JNM. Performed the experiments: RH JMM ES. Analyzed the data: CMRK LY SD. Contributed reagents/materials/analysis tools: RH JMM ES SD. Wrote the paper: CMRK LY SD.

References
1. Ledgergerber B, Lundgren JD, Walker AS, Sabin C, Justice A, et al. (2004) Predictions of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes. Lancet 364: 51–62.
2. Deeks SG, Barbour JD, Martin JN, Swanson MS, Grant RM (2000) Sustained CD4+ T cell response after virologic failure of protease inhibitor-based regimen in patients with human immunodeficiency virus infection. J Infect Dis 181: 946–953.
3. Vaidya N, Rong L, Marconi V, Kozinsky DR, Deeks SG, et al (2010) Treatment-mediated alterations in HIV fitness preserve CD4+ T-cell counts but have minimal effects on viral load. PLOS Computational Biology 6: e1000122.
4. Petersen ML, van der Laan MJ, Najavitsk S, Eron JJ, Moore RD, et al. (2008) Long-term consequences of the delay between virologic failure of highly active antiretroviral therapy and regimen modification. AIDS 22: 2097–2106.
5. Penn ML, Myers M, Eckstein DA, Leidger TJ, Hayden M, et al. (2001) Primary recombinant HIV-1 strains resistance to protease inhibitors are pathogenic in mature human lymphoid tissues. AIDS Research Human Retroviruses 17: 517–523.
6. Rodríguez B, Sethi AK, Cheruvu VK, Mackay W, Bosch RJ, et al. (2006) Predictive value of plasma HIV RNA level on rate of CD4+ T-cell decline in untreated HIV infection. JAMA 296: 1490–1496.
7. Mellois JW, Margolick JB, Phair JP, Rinaldo CR, Detels R, et al. (2007) Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 Cell count slope for progression to AIDS and death in untreated HIV-1 infection. JAMA 297: 2194–2201.
8. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, et al. (2004) Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 104: 942–947.
9. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, et al. (1999) Shorter survival time and advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 179: 859–870.
10. Hunt PW, Deeks SG, Bangser DR, Moso A, Sinclair E, et al. (2006) The independent effect of drug resistance on T-cell response to HIV infection in HIV infection. AIDS 20: 691–699.
11. Bloomfield P (2000) Fourier Analysis of Time Series: An Introduction; New York: Wiley & Sons, Inc.
12. Petersen ML, van der Laan MJ, Rinaldo CR, Detels R, et al. (2000) Sustained CD4+ T cell response after virologic failure of protease inhibitor-based regimen in patients with human immunodeficiency virus infection. J Infect Dis 181: 946–953.
13. Sterne J, White I, Carlin J, Spratn M, Kenward M, et al. (2009) Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. British Medical Journal 330: b2393.
14. White I, Carlin J (2010) Bias and efficiency of multiple imputation compared with complete-case analysis or using covariate values. Statistics in Medicine 29: 1920–2931.
15. Spratn M, Carpenter J, Sterne J, Carlin J, Heron J, et al. (2010) Strategies for multiple imputation in longitudinal studies. American Journal of Epidemiology 172: 478–487.
16. Lee H, Kashyap V, van Dyk D, Conners A, Drake J, et al. (2011) Accounting for calibration uncertainties in X-ray analysis: effective areas in spectral fitting. The Astrophysical Journal Letters 725: L37–L40.
17. Hoppe L, Liu C, Rubin DB (2001) Multiple imputation for multivariate data with missing and below-threshold measurements: time-series concentrations of pollutants in the arctic. Biometrics 57: 32–33.
18. Rubin DB (1996) Multiple imputation after 18+ years (with discussion). Journal of the American Statistical Association 91: 473–489.
19. Broersen P, de Waele S, Bos R (2004) Antiretroviral spectral analysis when observations are missing. Automatic 40: 1493–1509.
20. Graham JW, Orlowski AE, Gilreath TD (2007) How many imputations are really needed? Some practical clarifications of multiple imputation theory. Prevention Science 8: 206–213.
21. Press WH, Flannery BP, Teukolsky SA, Vetterling WT (2002) Numerical Recipes in C: the art of scientific computing; Cambridge University Press.
22. Seal S, Sheiner L (1980) Estimating population kinetics. Critical Reviews in Biomedical Engineering 8: 195–222.
23. Giorgi JV, Lyles RH, Matud JL, Yamashita TE, Mellors JW, et al. (2002) Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. J Acquir Immun Defic Syndr 29: 346–355.
24. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, et al. (1998) CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. J Acquir Immune Defic Syndr Hum Retrovirology 17: 322–340.
25. Hunt PW, Martin JN, Sinclair E, Breth B, Hages E, et al. (2003) T Cell Activation Is Associated with Lower CD4+ T Cell Gains in Human Immunodeficiency Virus-Infected Patients with Sustained Viral Suppression During Antiretroviral Therapy. J Infect Dis 187: 1534–1543.
26. Silvestri G, Paiardini M, Pandrea I, Lederman MM, Sodora DL (2007) Understanding the benign nature of SIV infection in natural hosts. J Clin Invest 117: 3404–3415.
27. Bonhoeffer S, Coffin JM, Novak MA (1997) Human immunodeficiency virus drug therapy and virus load. J Virol 71: 3275–3278.
28. Catalliano M, Di Mascio M, Hu Z, Sinivuvasa S, Thaker V, et al. (2008) HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T-cell transcripts. Proc Natl Acad Sci U S A 105: 19851–19856.
29. Silvestri G, Sodora D, Koup R, Paiardini M, O’Neil S, et al. (2003) Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunol 18: 441–452.
30. Hazenberg M, Otto S, van Benthen B, Roos M, Coutinho R, et al. (2003) Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 17: 1081–1088.
31. Tesalearu K, Arens R, van Schijndel G, Baas P, van der Valk M, et al. (2003) Lethal T cell immunodeficiency induced by chronic costimulation via CD27–CD70 interactions. Nature Immunology 4: 49–54.
32. Sumathilangam G, Perry M, Ward S, Brett S, Castleo-Cortes A, et al. (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. New England Journal of Medicine 355: 1018–1028.
33. Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, et al. (2009) Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 301. Clin Infect Dis 48: 350–361.
34. Bonhoeffer S, Coffin JM, Nowak MA (1997) Human immunodeficiency virus drug therapy and virus load. Journal of Virology 71: 3275–3278.
35. Wei X, Glish SK, Taylor ME, Johnson VA, Ennin EA, et al. (1995) Viral dynamics in HIV-1 infection. Nature 373: 117–122.
36. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, et al. (1995) Rapid turnover of plasma virions and CD4+ lymphocytes in HIV-1 infection. Nature 373: 129–136.
37. Spouge JL, Shragar Rl, Dimitrov DS (1996) HIV-1 infection kinetics in tissue cultures. Mathematical Biosciences 138: 1–22.
38. Nowak MA, Bangham CRM (1996) Population dynamics of immune responses to persistent viruses. Science 272: 74–79.
39. Phillips MN, McLean A, Johnson MA, Tyrer M, Emercy V, et al. (1997) HIV-1 dynamics after transient antiviral therapy: implications for pathogenesis and clinical management. Journal of Medical Virology 53: 261–265.
40. Perelson AS, Kirschner DE, De Boer R (1993) Dynamics of HIV infection in CD4+ T cells. Mathematical Biosciences 114: 81–125.
41. Anderson RM, May RM Infectious disease of humans: dynamics and control: Oxford Science Publications.
42. Hales S, Weinstein P, Souares Y, Woodward A (1999) El nino and the dynamics of vector borne disease transmission. Environmental Health Perspectives 107: 99–102.
43. Lindblade K, Walker E, Onapa A, Katungu J, Wilson M (1999) Highland malaria in Uganda: prospective analysis of an epidemic associated with El Nino. Transactions of the Royal Society of Tropical Medicine and Hygiene 93: 480–487.
44. Bouma MJ, Van der Kaay H (1996) The El Nino southern oscillation and the historic malaria epidemics on the Indian subcontinent and Sri Lanka: an early warning system for future epidemics? Tropical Medicine and International Health 1: 86–96.
45. Reuman DC, Deshamais RA, Constantino RF, Ahmad OS, Cohen JE (2006) Power spectra reveal the influence of stochasticity on nonlinear population dynamics. Proceedings of the National Academy of Sciences USA 103.
46. Havlir DV, Marschner IG, Hirsch MS, Collier AG, Tebas P, et al. (1998) Maintenance antiretroviral therapies in HIV infected patients with undetectable plasma HIV RNA after triple-drug therapy. AIDS Clinical Trials Group Study 343 Team. N Engl J Med 338: 1261–1268.
47. Grossman Z, Feinberg MB, Paul WE (1998) Multiple modes of cellular activation and virus transmission in HIV infection: a role for chronically and latently infected cells in sustaining viral replication. Proc Natl Acad Sci U S A 95: 6314–6319.