



# Utility of *pol*/Replication Capacity in Predicting Immunologic Course Among HIV-Infected Patients with Drug-Resistant Viremia

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## Background

- There are a substantial number of HIV-infected patients with drug-resistant viremia for whom there does not exist salvage therapy which has high probability of suppressing viral replication with low risk of toxicity.
- The ability to predict immunologic course among these patients with drug-resistant viremia would greatly aid in decisions as to whether to maintain or change their antiretroviral therapy.
- Currently, the only available laboratory values by which to gauge the need to change antiretroviral therapy are the immediate CD4+ T cell count and plasma HIV RNA level.
- We hypothesized that, among patients with drug-resistant viremia, assessment of *pol* replication capacity (RC) – one of few functional assays of HIV available in high throughput – could predict subsequent CD4+ T cell count and hence be useful in clinical management.

## Objective

Among HIV-infected patients with drug-resistant viremia, assess whether the rate of change of CD4+ T cell count over time (CD4 slope) differs according to a single baseline RC value or a short-term change in RC.

Hypothesis: Lower baseline RC values will subsequently result in less CD4 count loss over time or even in gains in CD4 counts.

## Participants

HIV-infected subjects participating in the UCSF SCOPE cohort (Study of the Consequences of the Protease Inhibitor Era) who met the following criteria:

- On a stable antiretroviral medication regimen (of any kind) for at least 120 days
- Plasma HIV RNA level > 500 copies/ml at time 0 (to ensure ability to amplify viral nucleic acid to evaluate RC) and at least one other plasma HIV RNA level > 100 copies/ml at least 90 days prior to time 0

- Presence at time 0 of any genotypic mutation, including the minor mutations, except L63P, listed on the October 2003 IAS-USA “Drug Resistance Mutations in HIV-1” chart

- Stable antiretroviral medications and the concomitant presence of at least one CD4+ T cell count determination taken 2 weeks or more after time 0 (to ensure that all subjects have at least some observation time)

- For subjects with more than one eligible time period, the three longest time periods were chosen.

## Measurements

- Clinical**
- Antiretroviral medication use was assessed by prospective interviewer-administered questionnaire
- Laboratory**
- RC assessed by modified Phenoseq HIV phenotypic drug susceptibility assay (Monogram Biosciences).
    - HIV RNA is extracted from plasma, followed by reverse transcription PCR amplification of the C-terminal end of gag (the last 83 amino acids), all of protease, and the N-terminal 305 amino acids of reverse transcriptase.
    - Amplified gene segments are inserted into a viral vector containing a luciferase gene.
    - Following a single round of viral replication in the absence of drug, luciferase activity is measured and compared to that for a reference virus (NL4-3).
    - RC values are expressed as a percentage of the NL4-3 reference and adjusted so that the median value of wild-type viruses approximates 100%. Values <100% imply reduced replicative capacity.

- Plasma HIV RNA levels assessed by Quantiplex® HIV RNA, version 3.0 (Chiron Corporation, Emeryville, CA).
- Genotypic resistance tested by GeneSeq HIV (Monogram).
- Phenotypic resistance measured by Phenoseq HIV (Monogram). Phenotypic susceptibility score (PSS) generated by summing susceptibility (0=resistant; 0.5=intermediate; 1=susceptible) to all current drugs (“current regimen PSS”) or to each of 16 available drugs (“global PSS”).

## Analysis

- Analysis subsets**
- Effect of baseline RC on subsequent CD4 counts:
    1. All episodes of drug-resistant viremia in all subjects
    2. PI users only: All episodes of drug-resistant viremia in all subjects using protease inhibitors at time 0
  - Effect of within-subject change in RC on subsequent CD4 counts:
    3. All episodes of drug-resistant viremia for which there is a follow-up RC value taken within 45 to 180 days after the first RC value (median of 160 days). The change in RC in these subjects (“delta RC”) is the primary variable of interest and the time of the follow-up RC is the time 0 for this analysis.

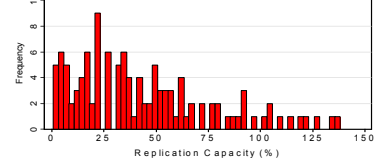
- Regression modeling**
- Prediction of subsequent CD4+ T cell counts by baseline (time 0) RC (or change in RC) assessed by mixed effects linear models implemented by proc MIXED in SAS (Cary, NC).
  - Outcome variable: square root CD4 chosen to improve model fit
  - Coefficient of interest is interaction term of RC and time (RC\*time). Coefficient is interpreted as mean change in square root CD4 per month per 1% increment of baseline RC
  - Models accommodate random slopes and intercepts
  - Observation was censored upon any change in antiretroviral therapy or at latest available CD4 count up to 14 months from time 0
- Sensitivity analyses**
- RC (or delta RC) specified both as continuous variable and dichotomized at a variety of cutpoints
  - Plasma HIV RNA specified as continuous and categorical variables
  - Global PSS and current regimen PSS assessed

## Characteristics of Participants (N=92)

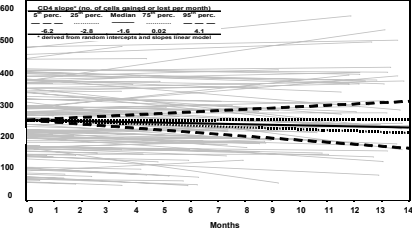
Characteristic	Median (IQR) or Percentage
Age, years	46 (40 to 51)
Male gender	85.9%
Ethnicity	
African-American	27.2%
White	53.3%
Latino	10.9%
Asian	2.2%
Mixed	6.5%
Plasma HIV RNA, log <sub>10</sub> copies/ml	3.8 (3.3 to 4.5)
CD4+ T cells, per mm <sup>3</sup>	232 (151 to 320)
No. of prior or current NRTIs	4 (4 to 6)
No. of prior or current NRTIs	1 (0 to 1)
No. of prior or current PIs	3 (2 to 4)
Abacavir phenotypic susceptibility <sup>a</sup>	5.2 (3.2 to 7.4)
Nevirapine phenotypic susceptibility	49 (0.8 to 400)
Lopinavir phenotypic susceptibility	7.0 (0.8 to 43)
Global PSS <sup>b</sup>	8 (4.5 to 11.5)
Current Regimen PSS <sup>c</sup>	1 (0.5 to 1.5)
No. of CD4 count determinations	5 (4 to 7)
Months of observation	8 (1.4 to 12.2)

<sup>a</sup> Not change observed (reference: none) or concentration of drug required to inhibit 50% replicable by 20%  
<sup>b</sup> PSS determined by aggregating susceptibility to 16 available antiretroviral agents  
<sup>c</sup> PSS determined by aggregating susceptibility to 5 current antiretroviral agents used by subject

## Distribution of Replication Capacity



## Overall Change in CD4+ T Cells



## No Strong Evidence for an Association Between Baseline RC and Subsequent CD4 Count Change

Factor	Unadjusted		Adjusted	
	Mean change in square root CD4+ T cell count (95% CI)	p value	Mean change in square root CD4+ T cell count (95% CI)	p value
Time, in months	-0.059 (-0.11 to 0.0043)	0.035	0.23 (-0.23 to 0.70)	0.33
RC, per 1% increase <sup>a</sup>	-0.036 (-0.057 to -0.015)	<0.001	-0.032 (-0.051 to -0.013)	<0.001
RC*time	-0.00061 (-0.0024 to 0.0012) <sup>b</sup>	0.51	-0.00065 (-0.0023 to 0.0010)	0.45
Plasma HIV RNA, per 1 log <sub>10</sub> increase <sup>c</sup>	-2.5 (-3.5 to -1.5)	<0.001	-2.4 (-3.4 to 1.5)	<0.001
Plasma HIV RNA*time	-0.058 (-0.14 to 0.020)	0.14	-0.064 (-0.16 to 0.028)	0.17
Global PSS, per 1 point increase <sup>d</sup>	0.012 (-0.17 to 0.20)	0.90	-0.0089 (-0.17 to 0.16)	0.92
Global PSS*time	0.00067 (-0.018 to 0.020)	0.94	-0.0021 (-0.022 to 0.018)	0.84

<sup>a</sup> Inferences unchanged when specifying RC dichotomized at 0% its median value; b) first quartile vs remainder; c) fourth quartile vs remainder; d) <10% vs >60%, or e) <10% vs >84%  
<sup>b</sup> Equals to the null change in CD4 count per month per 1% absolute increase in RC (95% CI: -0.069 to 0.043). For a 20% increase in RC, the change in CD4 count over 3 months is estimated to be -0.70 cells (95% CI: -4.1 to 2.6).  
<sup>c</sup> Inferences for RC unchanged when specifying plasma HIV RNA as a categorical variable  
<sup>d</sup> Inferences for RC unchanged when using the current regimen PSS

## Inferences Unchanged When Limiting to Subjects Using Protease Inhibitors<sup>a</sup>

Factor	Unadjusted		Adjusted	
	Mean change in square root CD4+ T cell count (95% CI)	p value	Mean change in square root CD4+ T cell count (95% CI)	p value
Time, in months	-0.011 (-0.083 to 0.060)	0.75	-0.41 (-0.78 to -0.028)	0.036
RC, per 1% increase <sup>b</sup>	-0.042 (-0.071 to -0.013)	0.005	-0.042 (-0.067 to -0.017)	0.01
RC*time	0.0012 (-0.0094 to 0.0033)	0.28	0.00029 (-0.0018 to 0.0024)	0.79
Plasma HIV RNA, per 1 log <sub>10</sub> increase <sup>c</sup>	-2.4 (-3.8 to -0.99)	<0.001	-2.5 (-3.7 to -1.3)	<0.001
Plasma HIV RNA*time	0.0058 (-0.096 to 0.11)	0.91	0.051 (-0.037 to 0.14)	0.26
Global PSS, per 1 point increase <sup>d</sup>	-0.0031 (-0.24 to 0.24)	0.98	-0.041 (-0.27 to 0.19)	0.72
Global PSS*time	0.024 (0.0050 to 0.044)	0.014	0.025 (0.0065 to 0.044)	0.008

<sup>a</sup> 69 unique participants contributing 89 discrete episodes of drug-resistant viremia and 454 CD4+ T cell counts  
<sup>b</sup> Inferences unchanged when specifying RC dichotomized at 0% its median value; c) first quartile vs remainder; d) fourth quartile vs remainder;  
<sup>c</sup> <10% vs >60%, or e) <10% vs >84%  
<sup>d</sup> Inferences for RC unchanged when specifying plasma HIV RNA as a categorical variable  
<sup>e</sup> Inferences for RC unchanged when using the current regimen PSS

## No Strong Evidence for an Association Between Short-Term Change in RC<sup>a</sup> and Subsequent CD4 Count Change

Factor	Unadjusted		Adjusted	
	Mean change in square root CD4+ T cell count (95% CI)	p value	Mean change in square root CD4+ T cell count (95% CI)	p value
Time, in months	-0.048 (-0.12 to 0.023)	0.18	-0.22 (-0.78 to 0.34)	0.44
Delta RC, per 1% increase <sup>b</sup>	-0.033 (-0.071 to 0.0040)	0.080	-0.0091 (-0.039 to 0.020)	0.54
Delta RC*time	-0.00094 (-0.0045 to 0.0026)	0.61	-0.00045 (-0.0040 to 0.0030)	0.80
Plasma HIV RNA, per 1 log <sub>10</sub> increase <sup>c</sup>	-2.7 (-3.8 to -1.7)	<0.001	-3.0 (-4.3 to -1.8)	<0.001
Plasma HIV RNA*time	-0.050 (-0.18 to 0.080)	0.45	0.0046 (-0.12 to 0.13)	0.94
Global PSS, per 1 point increase <sup>d</sup>	0.050 (-0.15 to 0.25)	0.63	-0.17 (-0.34 to 0.0013)	0.052
Global PSS*time	0.019 (0.0080 to 0.038)	0.041	0.019 (0.0081 to 0.037)	0.041

<sup>a</sup> 69 participants contributing 89 episodes of drug-resistant viremia and 437 CD4+ T cell counts. Median delta RC was 0.2% (IQR: -11 to +10)  
<sup>b</sup> Inferences unchanged when specifying delta RC dichotomized at 0% its median value; c) first quartile vs remainder; d) fourth quartile vs remainder;  
<sup>c</sup> <12 vs >10, or e) <18 vs >19.5  
<sup>d</sup> Inferences for delta RC unchanged when specifying plasma HIV RNA as a categorical variable  
<sup>e</sup> Inferences for delta RC unchanged when using the current regimen PSS

## Conclusion

Among HIV-infected patients with drug-resistant viremia who maintained a stable antiretroviral regimen, neither a single RC value nor a short-term within-person measurement of the change in RC was strongly associated with subsequent CD4+ T cell count change.

## Limitations and Implications

- There is substantial variability in the RC of HIV obtained from treatment-naïve individuals. Therefore, any given RC value measured in a patient with drug-resistant viremia may represent a change from a variety of different pre-therapy RC starting values. The within-subject change in RC from wild-type to drug-resistant virus, which we did not have in this analysis, may be more predictive of subsequent CD4 count course than a single RC value obtained during period of resistance.

- Similarly, within-person changes in RC in patients with drug-resistant viremia taken over longer periods of time than occurred in our study could also be more predictive.

- RC measured by the current Monogram assay is just one method to ascertain the concept. Other approaches, particularly those that incorporate larger portions of the virus, require evaluation as to their ability to predict immunologic course in this setting.

- Larger sample sizes will be needed to assess for smaller effects of RC than could be observed with our available sample size.

## Acknowledgements

This research was supported by grants from the NIH (UCSF-Gladstone Institute of Virology & Immunology Center for AIDS Research [P30 A27763]; the UCSF-SFGH General Clinical Research Center [P41 RR000083]; and R01 A052745); the University of California, University of California AIDS Research Program, AIDS Research Center (CC99-SF-001); and the UCSF AIDS Research Institute. Phenotype and RC testing was supported by a NIH SBIR grant (R44 A050321) to Monogram.

