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Residual Plasma Viremia and Infectious HIV-1 Recovery from Resting Memory CD4 Cells in Patients on Antiretroviral Therapy: Results from ACTG A5173

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Abstract

Background—In HIV-1-infected patients receiving antiretroviral therapy (ART), the relationship between residual viremia and ex vivo recovery of infectious virus from latently-infected CD4 cells is uncertain.

Methods—We measured residual viremia (HIV-1 RNA copies/mL) by single-copy assay (SCA) and the latent reservoir by infectious virus recovery from resting memory CD4 cells (infectious units per million cells [IUPM]) in patients who initiated ART. We assessed immune activation by measuring CD38 expression on T cells.

Results—Ten patients who initiated ART and maintained a plasma HIV-1 RNA level <200 copies/mL had residual viremia and IUPM measured every 24 weeks. Five of 10 patients had longitudinal IUPM measured at weeks 24–96; the remainder had IUPM measured 1–3 times over 24–72 weeks. Analyses of 29 paired measurements revealed a positive association between level of residual viremia and IUPM (0.56 higher $\log_{10}$ HIV-1 RNA copies/mL per 1 $\log_{10}$ higher IUPM, p=0.005). Residual viremia level was positively associated with CD38 density and percentage on CD8+ T-cells in concurrent samples and with pre-ART HIV-1 RNA levels.

Conclusions—In patients with HIV-1 RNA levels <200 copies/mL 24–96 weeks after initiating ART, the level of viremia is positively associated with infectious virus recovery from resting memory CD4 cells. Whether this association persists after longer-term suppressive ART needs to be determined. If additional studies show that residual viremia measured by SCA reflects the size of the latent reservoir in patients who have had virologic suppression for longer periods of time, this could facilitate testing of potentially curative strategies to reduce this important reservoir.

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Conflicts of Interest
For the remaining authors, none were declared.

Keywords

HIV-1; reservoir; residual viremia; single-copy assay

Introduction

HIV-1-infected patients on combination antiretroviral therapy (ART) continue to harbor a latent viral reservoir in resting memory CD4 cells that can be activated to produce infectious HIV-1[1]. The long half-life of these latently-infected cells is thought to be one of the main barriers to eradication of HIV-1 infection. In addition to HIV-1 persistence in resting memory CD4 cells and other reservoirs, the majority of patients on ART with HIV-1 RNA levels below the detection limit of commercial assays have low-level residual viremia measurable with more sensitive methods, such as a HIV-1 single copy assay (SCA)[2]. The origin of residual viremia is uncertain but may arise, at least in part, from induction of latently-infected cells to produce HIV-1[3]. However, the relation between residual viremia and the size of the latent reservoir is not known. If the level of residual viremia reflects the size of the latent reservoir, this could facilitate testing of therapeutic strategies to deplete or eliminate HIV-1 reservoirs.

We therefore compared the size of the latent reservoir (measured in infectious units per million [IUPM] resting memory CD4 cells) to the level of residual viremia (HIV-1 RNA copies/mL measured by SCA) in patients enrolled in AIDS Clinical Trials Group (ACTG) study A5173. ACTG A5173 was a single-arm pilot study designed to measure the decay rate of the latent reservoir in treatment-naive patients who initiated therapy with a multi-target antiretroviral regimen of enfuvirtide, a ritonavir-boosted protease inhibitor and two nucleoside reverse transcriptase inhibitors. The main result of this study, as previously reported[4], was that there was no measurable decay of the latent reservoir in patients who received this intensive regimen. In the current study, we also examined the relations among residual viremia, latent reservoir size, T cell activation (as measured by CD38 density and percentage), pre-therapy CD4 cell count and pre-ART plasma HIV-1 RNA level.

Methods

The trial design and study population of ACTG A5173 have been previously described[4]. All patients provided written informed consent for the study (NCT00051831). Briefly, in this single-arm study, treatment-naïve HIV-1-infected patients initiated therapy with enfuvirtide, ritonavir-boosted saquinavir mesylate, tenofovir disoproxil fumarate with emtricitabine or lamivudine. Patients with virologic suppression (no confirmed plasma HIV-1 RNA ≥ 200 copies/mL) at or after week 24 who continued enfuvirtide-containing ART were tested at week 24 and then every 24 weeks for the frequency of latently-infected resting memory CD4 cells (IUPM), using methods described elsewhere[5]. Plasma HIV-1 RNA testing using a commercial assay (Roche Amplicor HIV Monitor assay, ultrasensitive version 1.5) was performed in a central laboratory. CD4 and CD8 cell activation on fresh cells was assessed at entry and every 24 weeks by measuring the percentage of cells that expressed CD38 and by estimating CD38 cell surface density from the mean fluorescence intensity (MFI) of this marker[4].

A total of 19 patients enrolled in the trial and initiated the multi-target regimen. Patients who achieved a HIV-1 RNA < 200 copies/mL and who had at least one IUPM measurement underwent testing to quantify residual viremia using a real-time PCR assay that can detect a single copy of HIV-1 RNA in a plasma sample[6]. Patients were excluded if the HIV-1 RNA amplification efficiency by SCA on a pre-ART sample was < 10% of the Roche HIV

Monitor assay, suggesting primer mismatch. Time points were excluded from analysis if plasma HIV-1 RNA was detectable (≥200 copies/mL by Roche assay or ≥50 copies/mL by SCA).

Repeated measures models (generalized estimating equations) were used to examine the relation between residual viremia (HIV-1 RNA copies/mL) and IUPM, CD38 expression on T cells, pre-therapy CD4 count and pre-therapy plasma HIV-1 RNA level. In addition, a rank-based correlation was estimated, based on a resampling approach appropriate for repeated measures data[7]. The limit of detection for SCA varied because of differences in plasma volume (range 2–5 mL) available for analysis. HIV-1 RNA values "<1" copy/mL and "<0.6" copies/mL were imputed a value of 0.5 copies/mL and 0.3, respectively. If two SCA results existed for one specimen date, their average was used for the analyses.

Results

Nineteen patients enrolled in ACTG A5173; the participants' characteristics have been previously reported[4]. Of the 19 patients, 6 were excluded from the current analysis because they did not have IUPM results (they had stopped enfuvirtide while continuing the other antiretroviral drugs); 1 patient was excluded because the plasma HIV-1 RNA value was >200 copies/mL at the time of IUPM testing. Twelve patients achieved and maintained a plasma HIV-1 RNA level <200 copies/mL and underwent IUPM and SCA testing every 24 weeks; 2 of these patients were excluded because of inefficient PCR amplification. Thus, we analyzed paired SCA/IUPM measurements from a total of 10 patients (Figure 1).

All 10 patients analyzed had plasma HIV-1 RNA by Amplicor assay of <200 copies/mL at the time of IUPM testing, although 4 had RNA levels between 51–200 copies/mL at week 24. Five of 10 patients had longitudinal IUPM measurements over 24–96 weeks; the remainder had IUPM testing 1–3 times over 24–72 weeks (Table 1). One patient had two SCA results (<0.6 and 2 copies/mL) on the same specimen date; the average of the two results was used for the analyses.

Analyses of 29 paired IUPM/SCA measurements from the 10 patients revealed a positive association between IUPM and SCA with a rank correlation of 0.54 (Figure 2). More specifically, each 1.0 $\log_{10}$ higher IUPM value was associated with a 0.56 $\log_{10}$ higher HIV-1 RNA value (copies/mL) by SCA (Table 2; p=0.005). This association between IUPM and HIV-1 RNA by SCA persisted after adjustment for pre-therapy HIV-1 RNA.

In addition to the relation between residual viremia and IUPM, we found a positive correlation between residual viremia on ART and pre-ART HIV-1 RNA level (0.35 $\log_{10}$ HIV-1 RNA copies/mL by SCA per 1.0 $\log_{10}$ higher pre-ART HIV-1 RNA copies/mL, p=0.009) (Table 2). No association was observed between SCA HIV-1 RNA values and pre-ART CD4 cell count.

SCA HIV-1 RNA values were also positively correlated with concurrently measured CD38 density and CD38 percentage on CD8 cells (0.85 $\log_{10}$ HIV-1 RNA copies/mL by SCA per 1.0 $\log_{10}$ CD38 density; 0.1 $\log_{10}$ HIV-1 RNA copies/mL by SCA per 10% increase in CD38 expression; both p-values ≤0.001) (Table 2 and Figure 3). The association between SCA values and CD38 expression persisted after adjustment for pre-therapy HIV-1 RNA (p ≤0.002; Table 2).

Discussion

We assessed the relationship between the level of residual plasma viremia on ART, measured by HIV-1 RNA SCA, and the size of the HIV-1 latent reservoir, measured in

IUPM, in a small number of intensively studied patients who achieved and maintained a plasma HIV-1 RNA of <200 copies/mL on a multi-target treatment regimen. Our major finding is that the level of residual viremia is positively associated with the size of the latent reservoir in resting memory CD4 cells that can be activated to produce infectious HIV-1 (rank correlation = 0.54, p = 0.005). We also found that residual viremia is positively correlated with pre-ART plasma HIV-1 RNA level and with CD38 expression on CD8 cells.

A number of studies have evaluated the relationship between residual viremia and other measures of HIV-1 reservoirs in patients on ART. A recent study found an association between residual viremia and the frequency of CD4 cells carrying HIV-1 DNA[8]. Similarly, Yukl et al. found mean plasma HIV-1 RNA in patients on suppressive ART correlated with HIV-1 DNA levels in peripheral blood mononuclear cells[9]. However, HIV-1 DNA is likely to include a high proportion of proviruses that are not capable of producing replication-competent virus. One strength of the current study is that we quantified the latent HIV-1 reservoir by measuring induction of replication-competent HIV-1 from resting memory CD4 cells. Such replication-competent virus may be a source of HIV-1 that rebounds when antiretroviral therapy is stopped[10].

Our finding that residual viremia is correlated with CD38 expression on CD8 cells differs from that of other recent studies. In ACTG A5244[11] and the study by Chun et al[8], no association was found between level of residual viremia and T cell activation. In both of those studies, patients had been virologically suppressed for a much longer period of time (median 5–6.5 years) than patients in the current study. In addition, different methods to measure immune activation were used in the current study compared with ACTG A5244. We measured activation on fresh cells in the current study and on frozen cells in ACTG A5244. Moreover, the primary measure of immune activation in the current study was the expression of CD38 (percentage of cells positive for this marker and mean fluorescence intensity of expression) whereas in ACTG A5244, the primary measure of immune activation was the percentage of cells that expressed CD38 and HLA-DR. By contrast, in a smaller study of 8 patients on suppressive ART, also for an extended period (median 6.7 years), there was a correlation between residual viremia and the percentage of CD4 cells that expressed CD38[9]. Whether there was also an association between residual viremia and CD8 activation in that study was not reported. Additional studies of larger cohorts of patients on long-term suppressive ART are needed to better define the relationship between residual viremia and T-cell activation.

Our study has several limitations. We did not measure HLA-DR expression on T cells; HLA-DR and CD38 co-expression is generally thought to be the most reliable indicator of activated T cells because CD38 may be expressed on naive T cells in the absence of activation[12–14]. Furthermore, residual viremia and the size of the latent reservoir were assessed in patients who had recently initiated ART and had just achieved virologic suppression. In fact, 5 of 29 samples from 4 of 10 patients had plasma HIV-1 RNA levels between 51–200 copies/mL by commercial assay (Roche HIV Monitor), indicating that viremia had not declined to a suppressed steady-state. In a study of residual viremia in patients on ART, Palmer et al. found that a steady state is not achieved until patients have been suppressed for several years[2]. Patients with shorter duration of virologic suppression, such as those in our study, may have HIV-1 released into plasma from at least two different sources: one that decays slowly and another that may not decay at all. Our finding that plasma HIV-1 RNA by SCA and infectious virus recovery from resting CD4 cells are correlated in patients who recently achieved virologic suppression suggests that the source of residual viremia during this phase of therapy may, at least in part, be from the latent reservoir. However, the predominant source of residual viremia in patients on longer term suppressive ART may not be from resting CD4 cells because of changes over time as

infected cell populations decay. Thus, the relationship between residual viremia and latent reservoir size needs to be also assessed in patients who have had longer term (> 3 years) virologic suppression. Moreover, understanding the source of residual viremia in patients soon after virologic suppression as well as in those with long-term suppression may shed light on how HIV-1 reservoirs decay over time.

In conclusion, we find that the level of residual viremia and infectious virus recovery from resting memory CD4 cells are positively correlated 24–96 weeks after initiation of ART. Additional studies are needed to assess whether the association between residual viremia and latent reservoir size persists after longer-term suppression of HIV-1 replication. If future studies find that residual viremia measured by HIV-1 RNA SCA reflects the size of the latent reservoir in patients who have had virologic suppression for longer periods of time, this could facilitate testing of potentially curative strategies directed toward eliminating this reservoir. However, if the level of residual viremia correlates with the size of the latent reservoir in patients who have short-term virologic suppression (as seen in our study) but not in those with long-term suppression, this would imply that the predominant source of viremia changes during suppressive ART and that assays of infectious virus recovery will be needed to properly evaluate interventions designed to reduce HIV-1 reservoirs.

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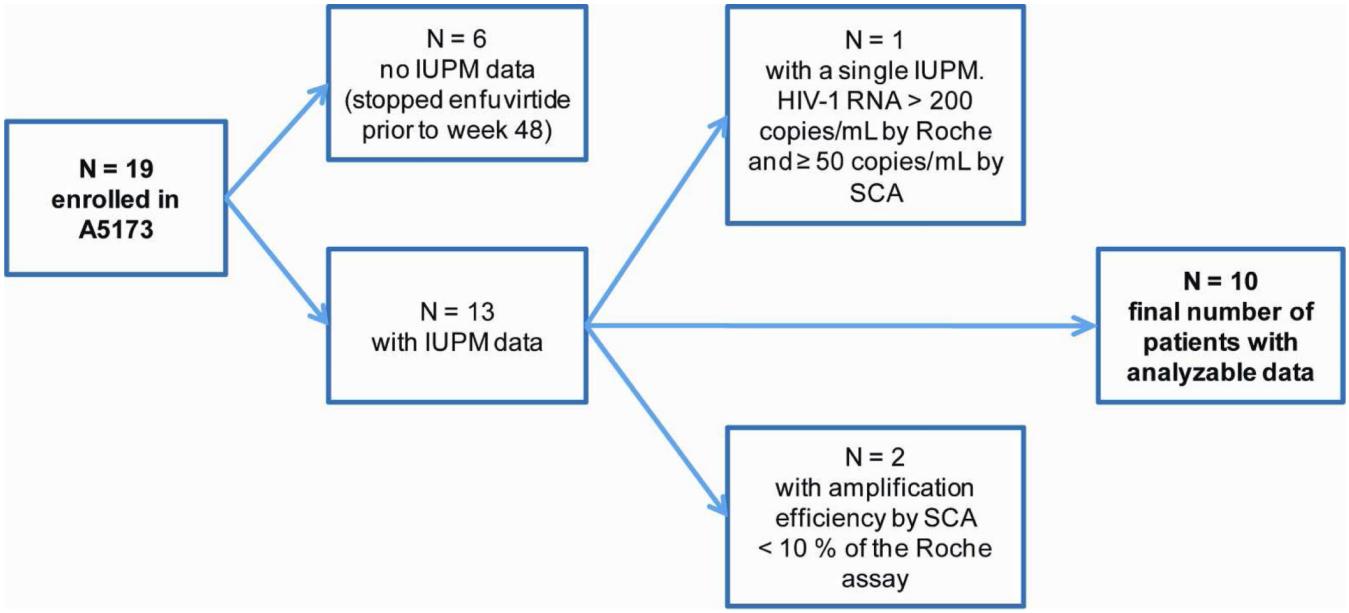

Figure 1.
Subject disposition. SCA: single-copy assay. IUPM: infectious units per million resting memory CD4 cells.

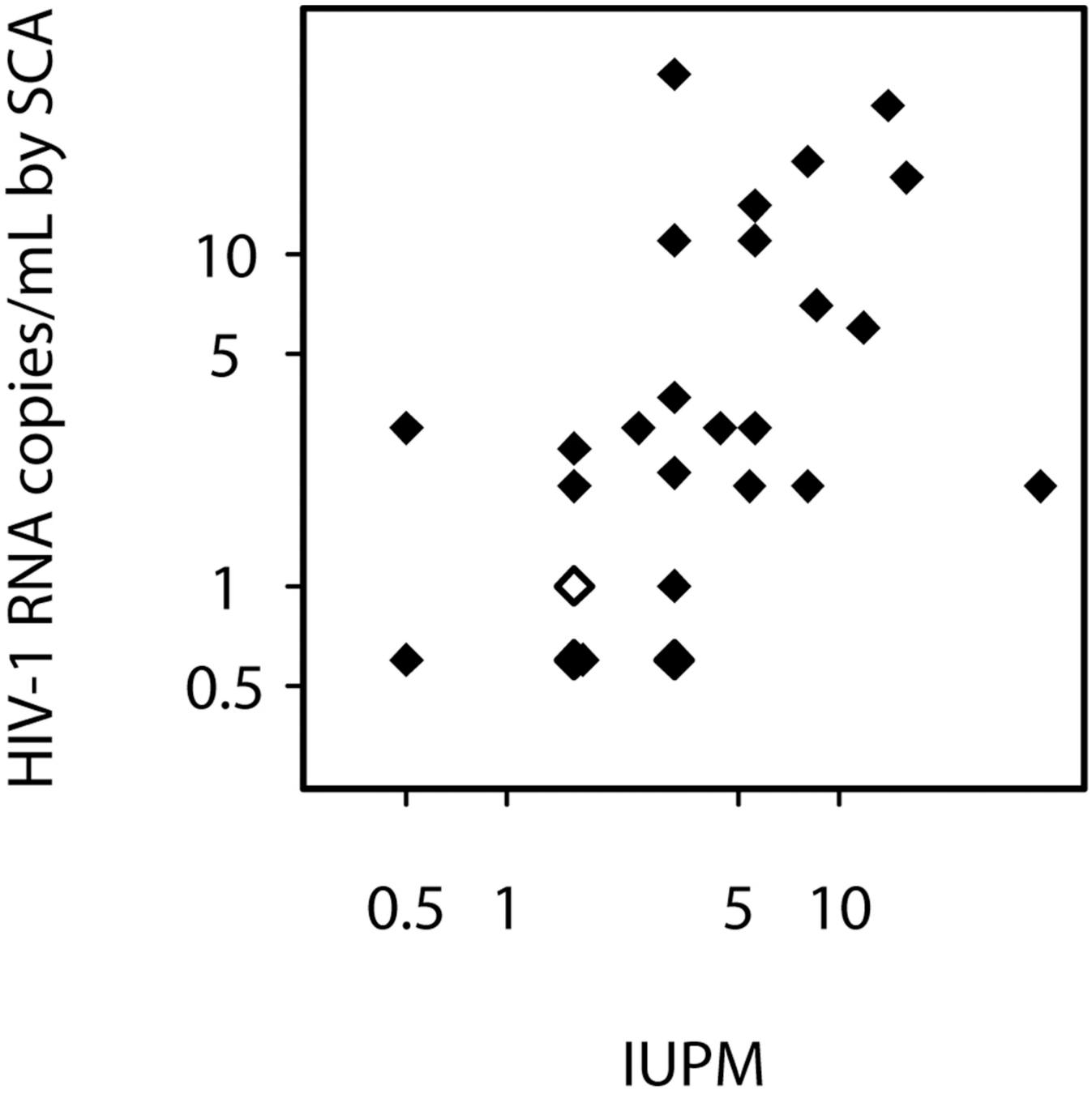

Figure 2.
Positive association between on-therapy plasma HIV-1 RNA level (by single-copy assay, SCA) and latent reservoir size (measured in infectious units per million cells, IUPM) (rank correlation =0.54; p = 0.005 from the repeated measures model). An open diamond represents a SCA value below limit of detection; for example, a SCA value of <1 copy/mL is plotted as an open symbol at 1 copy/mL. Several data points have the same value, so not all 30 observations are visible.

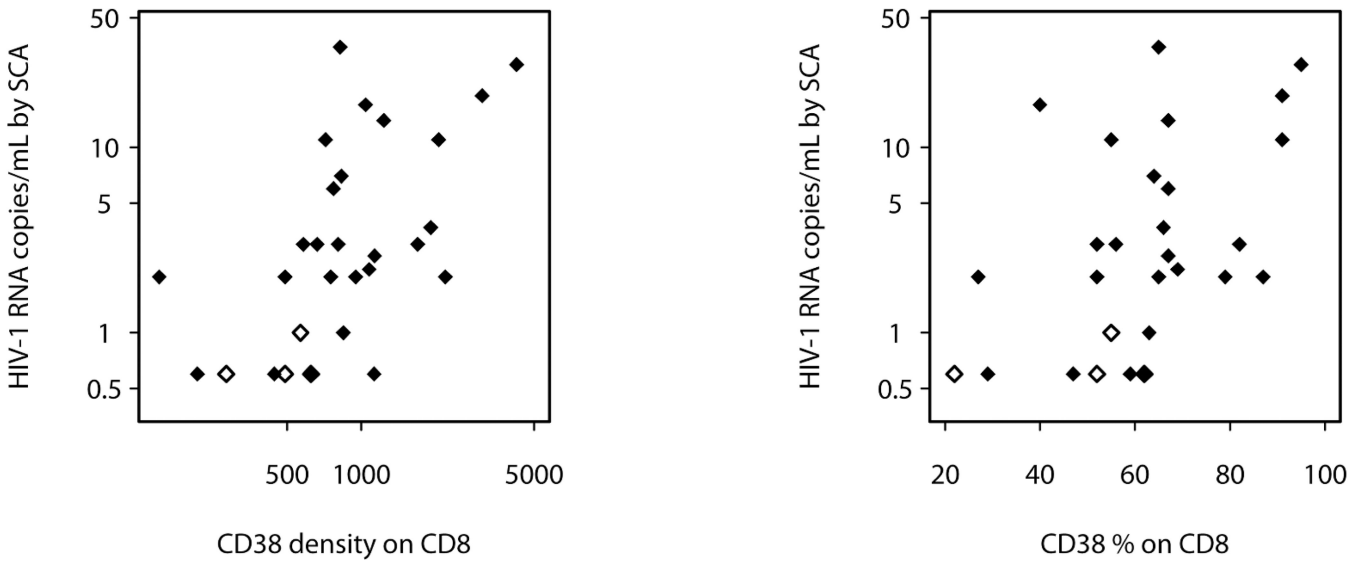

Figure 3.

Positive association between residual viremia (HIV-1 RNA by single-copy assay, SCA) and CD38 expression on CD8 cells (rank correlation for CD38 density by MFI = 0.52, $p < 0.001$ from the repeated measures model; rank correlation for CD38 percentage =0.35, $p = 0.001$ from the repeated measures model). An open diamond represents a SCA value below limit of detection; for example, a SCA value of <1 copy/mL is plotted as an open symbol at 1 copy/mL. Several data points have the same value, so not all 30 observations are visible.

Table 1

Latent reservoir (measured in infectious units per million (IUPM) resting, memory CD4 cells), residual viremia (measured by HIV-1 RNA single-copy assay, SCA) and CD38 expression on CD4 and CD8 cells in 10 patients who achieved and maintained a plasma HIV-1 RNA <200 copies/mL on antiretroviral therapy (ART).

ID	Scheduled Study Visit (weeks)	CD4 cell count (cells/mm^3)	HIV-1 RNA by Amplicor assay (copies/mL)	Latent reservoir, IUPM	HIV-1 RNA by SCA (copies/mL)	CD38 density on CD4	CD38 density on CD8	CD38 % on CD4	CD38 % on CD8
1	0	317	39,281			.	.	.	.
	24	559	<50	3.20	1	1,325	845	59	63
	48	787	<50	5.40	2	1,111	152	54	27
	72	701	<50	1.60	<0.6	668	284	45	22
	96	768	<50	1.60	<0.6, 2	1,047	491	65	52
2	0	155	111,148			.	.	.	.
	24	192	<50	11.86	6	583	770	52	67
	48	219	<50	40.50	2	609	751	51	65
3	0	360	3,189,479			.	.	.	.
	24	776	68	8.07	19	2,420	3,078	83	91
	48	1067	<50	5.60	14	1,416	1,232	63	67
	72	1051	84	5.60	11	1,237	716	54	55
4	0	257	89,176			.	.	.	.
	24	511	64	16.00	17	2,270	1,040	57	40
	48	881	<50	8.56	7	1,922	829	70	64
5	0	158	217,338			.	.	.	.
	24	304	66	5.60	3	716	582	54	56
	48	503	<50	3.20	0.6	1,233	618	66	59
	72	607	<50	3.20	<0.6	1,216	625	64	62
	96	728	<50	1.60	0.6	1,196	217	57	29
6	0	262	39,278			.	.	.	.
	24	848	159	14.10	28	1,476	4,245	77	95
	48	1123	<50	1.60	2	672	2,188	57	87
	72	768	<50	0.50	3	487	1,689	41	82
	96	854	<50	3.20	2.2	424	1,075	34	69

ID	Scheduled Study Visit (weeks)	CD4 cell count (cells/mm^3)	HIV-1 RNA by Amplicor assay (copies/mL)	Latent reservoir, IUPM	HIV-1 RNA by SCA (copies/mL)	CD38 density on CD4	CD38 density on CD8	CD38 % on CD4	CD38 % on CD8
7	0	239	49,990			.	.	.	.
	24	391	<50	3.20	11	766	2,054	65	91
	48	398	<50	8.07	2	447	947	51	79
	72	363	<50	3.20	34.8	441	820	35	65
	96	475	<50	4.40	3	460	805	32	56
8	0	554	28,762			.	.	.	.
	24	948	<50	1.60	2.6	1,429	1,130	59	67
9	0	193	114,474			.	.	.	.
	24	313	<50	3.20	3.7	2,605	1,909	59	66
10	0	240	19,522			.	.	.	.
	24	468	<50	0.50	0.6	660	1,125	41	62
	48	.	<50	2.50	3	792	662	43	52
	72	549	<50	1.70	0.6	631	444	49	47
	96	594	<50	1.60	<1	900	567	57	55

Table 2

Associations between residual viremia and pre-therapy HIV-1 RNA level and CD4 cell count, on-therapy CD38 expression on T cells, and infectious virus recovery from resting CD4+ memory cells

		Association with Residual Viremia (HIV-1 RNA log_{10} copies/mL)					
		Unadjusted			Adjusted for pre-therapy HIV-1 RNA		
Covariate	**Number of observations**	**Estimate***	**95% Confidence limits**	**P-value**	**Estimate***	**95% Confidence limits**	**P-value**
Baseline HIV-1 RNA (per 1 log_{10} (copies/mL))	29	0.35	(0.09, 0.62)	0.009	-	-	-
Baseline CD4 cell count (per 50 cells/mm^3)	29	0.04	(−0.07, 0.15)	0.45	0.04	(−0.05, 0.13)	0.34
CD38 density on CD4 (per 1 log_{10})	29	0.91	(−0.04, 1.85)	0.059	0.80	(−0.16, 1.77)	0.10
CD38 density on CD8 (per 1 log_{10})	29	0.85	(0.58, 1.11)	<0.001	0.86	(0.49, 1.23)	<0.001
CD38 % on CD4 (per 10 percentage points)	29	0.09	(−0.05, 0.23)	0.21	0.07	(−0.07, 0.22)	0.34
CD38 % on CD8 (per 10 percentage points)	29	0.10	(0.04, 0.16)	0.001	0.10	(0.04, 0.16)	0.002
Latent reservoir, IUPM (per 1 log_{10})	29	0.56	(0.17, 0.96)	0.005	0.51	(0.10, 0.92)	0.015

* From repeated measures model for log_{10} SCA