# Residual HIV-RNA levels persist for up to 2.5 years in peripheral blood mononuclear cells of patients on potent antiretroviral therapy

Fischer, M; Günthard, H F; Opravil, M; Joos, B; Huber, W; Bisset, L R; Ott, P; Böni, J; Weber, R; Cone, R W

Abstract: The long-term response of 10 asymptomatic, antiretroviral therapy-naive HIV-1-infected patients to potent combination antiretroviral therapy was characterized by monitoring levels of HIV-1 RNA in plasma, peripheral blood mononuclear cells (PBMC), and lymphoid tissue using highly sensitive HIV-1 RNA assays. Although plasma viral loads were continuously suppressed to levels below 50 HIV-1 RNA copies/ml for up to 2.5 years (60-128 weeks), HIV-1 RNA was still detectable at very low levels (1 to 49 HIV-1 RNA copies/ml) in 25% of the samples. In corresponding PBMC specimens, residual HIV-RNA was detectable in as much as 91% of samples tested (1 to 420 HIV-1 RNA copies/microg total RNA). Similarly, HIV-1 RNA levels in lymphoid tissue also remained detectable at a high frequency (86%). A highly significant correlation was demonstrated between therapy-induced change in PBMC HIV-1 RNA levels and change in plasma HIV-1 RNA levels ($r2 = 0.69$; $p = 0.003$). These findings support the concept that measurement of HIV-1 RNA in the easily accessible PBMC compartment is relevant for evaluating the potency of current and future antiretroviral therapies.

DOI: https://doi.org/10.1089/088922200414974

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 16, Number 12, 2000, pp. 1135–1140
Mary Ann Liebert, Inc.

## Short Communication

# Residual HIV-RNA Levels Persist for Up to 2.5 Years in Peripheral Blood Mononuclear Cells of Patients on Potent Antiretroviral Therapy

MAREK FISCHER,[1] HULDRYCH F. GÜNTHARD,[1] MILOS OPRAVIL,[1] BEDA JOOS,[1] WERNER HUBER,[1,5] LESLIE R. BISSET,[2] PETER OTT,[3] JÜRG BÖNI,[4] RAINER WEBER,[1] and RICHARD W. CONE[1]

### ABSTRACT

**The long-term response of 10 asymptomatic, antiretroviral therapy-naive HIV-1-infected patients to potent combination antiretroviral therapy was characterized by monitoring levels of HIV-1 RNA in plasma, peripheral blood mononuclear cells (PBMC), and lymphoid tissue using highly sensitive HIV-1 RNA assays. Although plasma viral loads were continuously suppressed to levels below 50 HIV-1 RNA copies/ml for up to 2.5 years (60–128 weeks), HIV-1 RNA was still detectable at very low levels (1 to 49 HIV-1 RNA copies/ml) in 25% of the samples. In corresponding PBMC specimens, residual HIV-RNA was detectable in as much as 91% of samples tested (1 to 420 HIV-1 RNA copies/$\mu$g total RNA). Similarly, HIV-1 RNA levels in lymphoid tissue also remained detectable at a high frequency (86%). A highly significant correlation was demonstrated between therapy-induced change in PBMC HIV-1 RNA levels and change in plasma HIV-1 RNA levels ($r^2 = 0.69$; $p = 0.003$). These findings support the concept that measurement of HIV-1 RNA in the easily accessible PBMC compartment is relevant for evaluating the potency of current and future antiretroviral therapies.**

COMBINATION ANTIRETROVIRAL DRUG THERAPY can suppress HIV-1 replication,[1] resulting in marked clinical benefit.[2–4] Plasma HIV-1 RNA quantitation is most widely used to assess an individual's virological status both for prediction of the subsequent clinical course[5] and for monitoring the treatment response.[6] Similar to plasma viral load, levels of cell-associated HIV-1 RNA are predictive of clinical progression.[7,8] In addition, levels of HIV-1 RNA in lymphoid tissue, the presumed major reservoir of HIV-1,[9] may provide information about residual viral activity in this reservoir for patients with undetectable plasma RNA levels.[10,11] However, the limited accessibility of lymphoid tissue precludes the use of routine sampling for monitoring HIV therapy.

In this report we have determined HIV-1 RNA levels in plasma, PBMC, and lymphoid tissue in patients on potent long-term antiretroviral therapy to assess the sensitivity of these virological measurements for detection of residual HIV-RNA.

HIV-1-infected, asymptomatic, treatment-naive patients with >400 CD4 cells/$\mu$1 at enrollment were randomly assigned to receive triple therapy [AZT 300 mg bid, 3TC 150 mg bid, and ritonavir (RTV) 600 mg bid] in a prospective, open-label trial called the Swiss EARTH study.[12] A subset of 10 individuals treated initially with triple drug regimens [50% female, average age 32 ± 5 years (mean ± SD)] with follow-up data available at least up to Week 96 and plasma viral load suppressed permanently to levels below 50 copies/ml during the second and the third year of therapy was selected for the present study. Due to side effects, two patients (patients 9 and 10) discontinued RTV and in two patients RTV was replaced by nelfinavir (NFV) 750 mg tid (Table 1). From a subset of five patients bi-

Divisions of [1]Infectious Diseases and [2]Clinical Immunology, Department of Internal Medicine, and the [3]Clinic for Ear, Nose and Throat Surgery, University Hospital Zurich, [4]Swiss National Centre for Retroviruses, University of Zurich, Zurich, Switzerland.
[5]Current address: F. Hoffmann-La Roche, Basel, Switzerland.

TABLE 1. PATIENT CHARACTERISTICS

| Patient ID | Therapy start | Change (week[a]) | Plasma[b] | | PBMC[d] | | Lymphoid tissue[d] | | Follow-up weeks |
|---|---|---|---|---|---|---|---|---|---|
| | | | Baseline | Nadir[c] | Baseline | Nadir[c] | Baseline | Nadir[e] | |
| 1 | AZT/3TC/RTV | AZT/3TC/NFV (7) | 5028 | ≤5 | 99 | 5 | nd | nd | 96 |
| 2 | AZT/3TC/RTV | | 1406 | ≤12 | 214 | 16 | 54056 | 6 | 114 |
| 3 | AZT/3TC/RTV | | 9995 | ≤9 | 252 | ≤5 | 115812 | 343 | 124 |
| 4 | AZT/3TC/RTV | AZT/3TC/NFV (12) | 3725 | <6 | 288 | 13 | nd[f] | nd | 96 |
| 5 | AZT/3TC/RTV | | 32595 | <7 | 431 | 6 | 557124 | <12 | 132 |
| 6 | AZT/3TC/RTV | | 30202 | 17 | 978 | 24 | 21025 | 6 | 108 |
| 7 | AZT/3TC/RTV | | 36835 | ≤10 | 2789 | 49 | 36450 | 11 | 126 |
| 8 | AZT/3TC/RTV | | 9154 | ≤9 | 3155 | 111 | 192514 | nd | 126 |
| 9 | AZT/3TC/RTV | AZT/3TC (80) | 35574 | ≤14 | 2138 | 49 | nd | nd | 102 |
| 10 | AZT/3TC/RTV | AZT/3TC (13) | 8555 | ≤8 | 244 | ≤4 | 14109 | 477 | 132 |

[a]Study week in which therapy was changed.
[b]HIV-1 RNA copies/ml.
[c]Mean HIV RNA levels after study Week 47 (< signifies that undetectable values were obtained throughout the observation period and ≤ means that some HIV-RNA levels were detectable and some were not).
[d]HIV-1 RNA copies/$\mu$g total RNA.
[e]HIV-RNA levels at study Week 48 (< signifies the detection limit of an undetectable value).
[f]nd, no data available.

lateral tonsil biopsies were obtained at Weeks 0, 4, 24, and 48 and from 1 subject at Weeks 0, 4, 24, 48, and 96. In one patient fine-needle biopsies from lymph nodes were available at Weeks 0 and 24.

After initiation of treatment, plasma HIV-RNA in all study patients reached levels below 50 copies/ml in a median (min; max) time of 12 (4; 36) weeks and subsequently remained below 50 copies for a median duration of 105 (60; 128) weeks. Overall, in 25% of plasma specimens obtained during the second and third year of therapy HIV-1 RNA (time points after Week 47) was measured at very low but detectable levels. For individual patients detectable plasma viral loads were observed with a frequency of 9% to 50% at time points after Week 47. From one patient (patient 6), all specimens and from two patients (patients 4 and 5) none were found to have detectable plasma HIV-1 RNA.

In contrast to plasma, HIV-1 RNA remained detectable in the vast majority (91%) of PBMC specimens. In seven patients (patients 1, 2, 4, 5, 7, 8, and 9) PBMC-associated HIV-1 RNA was detectable in 100% of the specimens. Lower frequencies (92, 89, and 42%, respectively) were observed in three patients (patients 3, 6, and 10, respectively). The mean PBMC-associated HIV-1 RNA levels measured after 1 year of therapy (> Week 47), henceforth referred to as nadir HIV-1 RNA levels, plateaued during the second and third year of therapy (Fig. 1B) as confirmed by the finding that no patient showed a significant trend for a decrease of PBMC-associated HIV-1 RNA during the second or third year of therapy (Spearman nonparametrical analysis, $p > 0.18$).

Similarly as in PBMC, very few specimens from lymphoid tissue had undetectable HIV-1 RNA: Only 3 of 27 samples were HIV-1 RNA negative: two from patient 5 and one from patient 8. The very low yield of total RNA extracted from each of these specimens may explain the negative results.

After initiation of therapy, changes in HIV-1 RNA levels in PBMC and lymphoid tissue paralleled changes in plasma HIV-1 RNA (Fig. 1). HIV-1 RNA levels in plasma and PBMC were significantly correlated at baseline ($r^2 = 0.41$; $p = 0.05$; $n = 10$) and throughout the observation period ($r^2 = 0.46$; $p < 0.0001$; $n = 62$) (Fig. 2). Significant correlation of HIV-RNA levels in lymphoid tissue compared to either PBMC or plasma HIV-1 RNA levels was demonstrated for the whole observation period ($r^2 = 0.40$; $p = 0.001$; $n = 23$ and $r^2 = 0.54$; $p = 0.002$; $n = 15$, respectively) but not for the data at baseline ($p > 0.25$; $n = 6$).

Virological response[13] to therapy was calculated as the ratio of the nadir HIV-1 RNA levels divided by the baseline level for each individual patient in plasma, PBMC, and lymphoid tissue. Virological responses in PBMC and plasma were highly correlated ($r^2 = 0.69$; $p = 0.003$; $n = 10$) (Fig. 3). This correlation remained significant, when the analysis was recalculated assuming that PCR-negative specimens contained zero copies ($r^2 = 0.54$; $p = 0.04$; $n = 8$). However, the comparison of virological response in lymphoid tissue to PBMC failed to show significant correlation ($p > 0.4$; $n = 6$).

Two main observations were made in this study:

1. In patients treated with potent antiretroviral therapy, PBMC-associated HIV-1 RNA persisted for up to 2 years or longer at levels as low as 5–10 copies/$\mu$g total RNA and did not show a tendency to decay further during the second year of therapy.
2. Virological response to therapy in plasma was mirrored by the response in PBMC-associated HIV-1 RNA with the measurement in the cellular compartment being much more sensitive.

Unlike plasma viremia, levels of HIV-1 RNA in PBMC and lymphoid tissue rarely became undetectable. PBMC-associated HIV-1 RNA was found to persist for up to 2.5 years of therapy. Furtado and colleagues[14] recently reported persistence of PBMC-associated HIV-RNA in five patients under potent an-

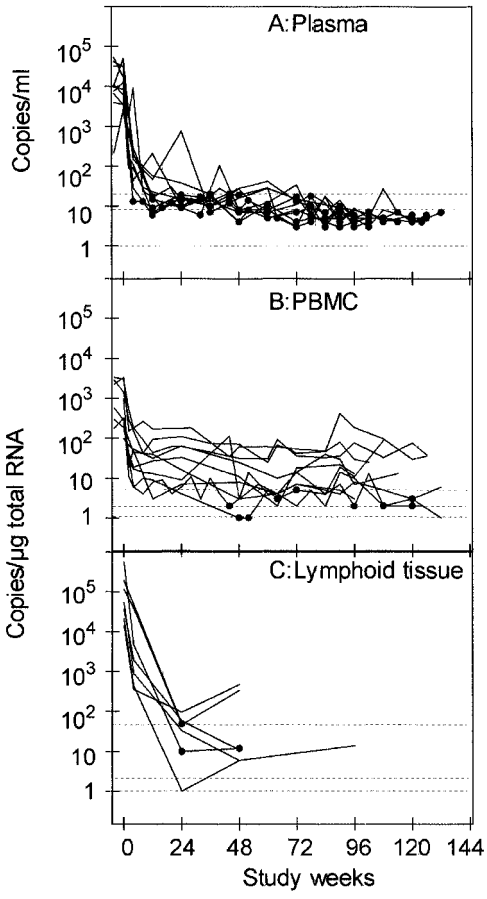


**FIG. 1.** HIV-1 RNA levels in plasma, PBMC, and lymphoid tissue during long-term antiretroviral therapy. Solid lines connect datapoints from individual patients. Filled circles indicate undetectable measurements and their individual limits of detection. Dotted lines parallel to *x*-axes show median, maximal, and minimal detection limits for RNA measurements in each compartment. (A) Plasma HIV-1 RNA was determined by the Amplicor HIV-1 Monitor Test and ultrasensitive modifications.[34,35] (B) PBMC HIV-1 RNA. (C) HIV-1 RNA in lymphoid tissue. Lymphoid tissue biopsies were processed as described previously.[17] Measurements of HIV-1 RNA in PBMC and lymphoid tissue were performed by a modification of the Amplicor Test.[12,35]

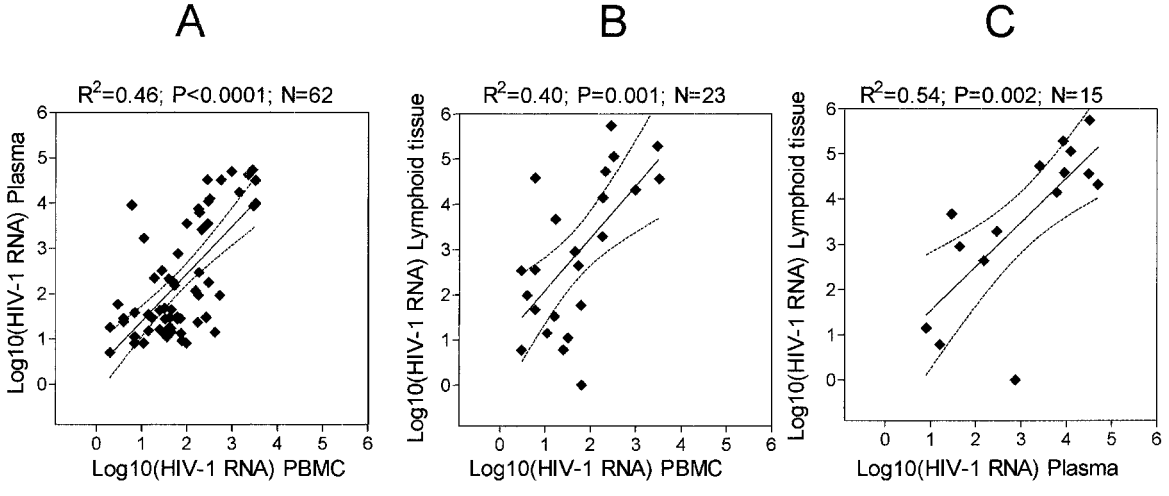


**FIG. 2.** Correlation between HIV-1 RNA levels in PBMC, plasma, and lymphoid tissue. Combined data from all patients during the whole observation period are shown. Detectable $\log_{10}$ transformed HIV-1 HIV RNA measurements were used for this analysis. *N*, number of datapoints; $R^2$, Pearson correlation coefficient; *P*, level of significance. Solid and dotted lines show calculated linear regression curves and their 95% confidence intervals, respectively, with the following underlying equations: (A) HIV-RNA in PBMC compared to plasma; $y = 1.05x + 0.34$. (B) HIV-RNA in PBMC compared to lymphoid tissue; $y = 1.15x + 0.93$. (C) HIV-RNA in plasma compared to lymphoid tissue; $y = 0.97x + 0.56$.

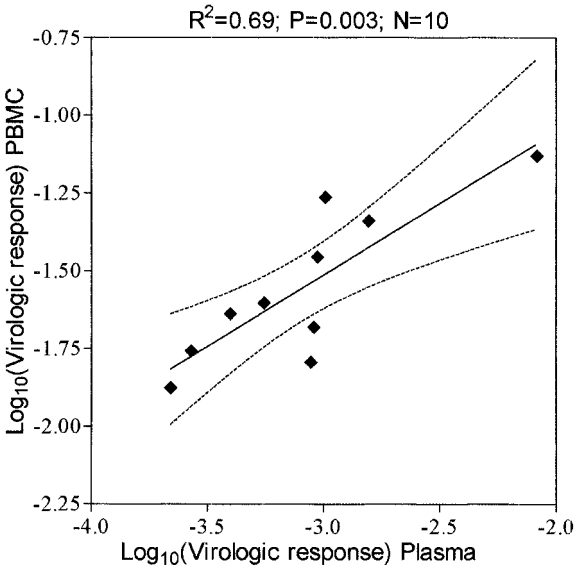


**FIG. 3.** Correlation between virologic response of HIV-1 RNA levels in PBMC and plasma to antiretroviral therapy. Virological responses[13] of HIV-1 RNA levels were plotted as $\log_{10}$ (nadir HIV-1 RNA levels/baseline HIV-1 RNA levels). *N*, number of data points; $R^2$, Pearson correlation coefficient; *P*, level of significance. The solid line shows the calculated linear regression curve and its 95% confidence intervals (dotted lines) with the following underlying equation: $y = 0.46x - 0.13$.

tiretroviral therapy. Our findings extend this observation showing that at least for the chronically infected asymptomatic patients studied here, such persistence can be stable in that no further decrease of PBMC-associated HIV-1 RNA was observed in the second year of treatment.

The question has been posed whether the residual cellular HIV-RNA persisting in PBMC reflects intracellular transcripts of infected cells or residual virions attached to the outside of uninfected cells[15]—in analogy to extracellular viral particles trapped in lymphoid tissue.[16–18] Consistent with the hypothesis that residual PBMC-associated HIV-1 RNA may be, in part or entirely, extracellular, it was observed that 25% of plasma specimens tested after Week 47 showed detectable HIV-1 RNA at low levels (all below 50 copies/ml). This observation, in agreement with a recent report,[19] shows that current antiretroviral therapies may not completely suppress virion production.

However, we did not observe elevated nadir levels of PBMC-associated HIV-1 RNA in the patients with actually detectable plasma viremia after 1 year of therapy (data not shown), which might be expected under the assumption that PBMC-associated HIV-1 RNA would merely reflect residual virions bound to the surface of uninfected cells.

If residual PBMC-associated HIV-1 RNA were located intracellularly, the question would arise whether such cellular transcripts originate from chronically or productively infected cells. Observations of other investigators indicate that both may apply. In particular, there is evidence that active viral replication may persist in the presence of treatment with potent antiretroviral therapy.[20–22] Conversely, the persistence of PBMC-associated HIV-1 RNA may be based on latency of long-lived infected cells[23–25] transcribing HIV-RNA at very low levels. Reminiscent of our observations, uninduced J1.1 cells—chronically infected T-lymphocytes—have been reported to express low levels of predominantly unspliced HIV-1 RNA in at least a fraction of cells.[26]

Comparison of HIV-1 RNA levels from the different compartments revealed that virological response in plasma mirrored response in PBMC with remarkable accuracy. The rational for having defined virological response in our patients by the ratio of baseline and nadir HIV-1 RNA levels[13] was based on observations that found "high baseline"[27–29] and "high nadir levels"[30,31] of plasma viremia to be predictors for virological failure of antiretroviral therapy. Reminiscent of the correlation of virological responses, PBMC HIV-1 RNA levels were also directly correlated with viral RNA levels in plasma at baseline and throughout the whole observation period. The finding that correlation was weaker ($r^2 = 0.46$) in the direct comparison than in the paired analysis of virological responses ($r^2 = 0.69$) may be due to the high patient-to-patient variation observed in PBMC-associated HIV-1 RNA levels (coefficients of variations at baseline and nadir of 111 and 120%, respectively). Correlation of PBMC-associated HIV-1 RNA levels with viral RNA load in lymphoid tissue was also demonstrated. However, baseline HIV-1 RNA levels as well as the virological response in lymphoid tissue failed to show significant correlation with either plasma or PBMC. Such discordance can be expected because the vast majority of HIV-1 RNA in lymphoid tissue of untreated patients represent extracellular virions bound to follicular dendritic cells,[16] which may be saturated at baseline.[16,32] It has also been claimed that HIV-1 RNA associated with mononuclear cells in lymph nodes reflects plasma viremia better than follicular dendritic cell-associated HIV-1 RNA.[32] Confirming the latter assumption, strong correlation between viral load in purified lymph node mononuclear cells and PBMC viral load has been reported.[33]

In conclusion, the data presented here suggest that measurements of PBMC-associated HIV-1 RNA may permit assessment of virological response even among patients with permanently undetectable plasma viral loads, when the distinction between "potent" and "very potent" antiretroviral therapy may otherwise be impossible.

## ACKNOWLEDGMENTS

We thank the participating patients for their commitment to the study, Ruedi Lüthy for helpful discussions, Joseph Wong for critical reading of the manuscript, Friederike Burgener, Alex Kallivroussis, and Baerbel Sauer for excellent laboratory assistance, and Christina Grube for her dedicated clinical assistance. This work was supported by grants from the Swiss National Science Foundation (3239-046016.95), the Swiss HIV Cohort Study (project 144), Glaxo Wellcome, and Abbott Laboratories.

## REFERENCES


1. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, et al.: Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 1997;387:188–191.
2. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al.: Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med 1997;337:734–739.
3. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al.: A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med 1997;337:725–733.
4. Ledergerber B, Egger M, Opravil M, Telenti A, Hirschel B, Battegay M, et al.: Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: A prospective cohort study. Swiss HIV Cohort Study. Lancet 1999;353:863–868.
5. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al.: Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946–954.
6. O'Brien WA, Hartigan PM, Daar ES, Simberkoff MS, and Hamilton JD: Changes in plasma HIV RNA levels and CD4+ lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure. VA Cooperative Study Group on AIDS. Ann Intern Med 1997;126:939–945.
7. Saksela K, Stevens C, Rubinstein P, and Baltimore D: Human immunodeficiency virus type 1 mRNA expression in peripheral blood cells predicts disease progression independently of the numbers of CD4+ lymphocytes. Proc Natl Acad Sci USA 1994;91:1104–1108.
8. Saksela K, Stevens CE, Rubinstein P, Taylor PE, and Baltimore D: HIV-1 messenger RNA in peripheral blood mononuclear cells as an early marker of risk for progression to AIDS. Ann Intern Med 1995;123:641–648.
9. Pantaleo G, Graziosi C, Butini L, Pizzo PA, Schnittman SM, Kotler DP, et al.: Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc Natl Acad Sci USA 1991;88:9838–9842.
10. Wong JK, Günthard HF, Havlir DV, Zhang ZQ, Haase AT, Ignacio CC, et al.: Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc Natl Acad Sci USA 1997;94:12574–12579.
11. Günthard HF, Wong JK, Ignacio CC, Guatelli JC, Riggs NL, Havlir DV, et al.: Human immunodeficiency virus replication and genotypic resistance in blood and lymph nodes after a year of potent antiretroviral therapy. J Virol 1998;72:2422–2428.
12. Opravil M, Cone RW, Fischer M, Vernazza P, Basseti S, Lorenzi P, et al.: Effects of early antiretroviral treatment on HIV-1 RNA in blood and lymphoid tissue: A randomized trial of double versus triple therapy. J Acquir Immune Defic Syndr Hum Retrovirol 2000;23:17–25.
13. Brown AJ, Günthard HF, Wong JK, D'Aquila RT, Johnson VA, Kuritzkes DR, et al.: Sequence clusters in human immunodeficiency virus type 1 reverse transcriptase are associated with subsequent virological response to antiretroviral therapy. J Infect Dis 1999;180:1043–1049.
14. Furtado MR, Callaway DS, Phair JP, Kunstman KJ, Stanton JL, Macken CA, et al.: Persistence of HIV-1 transcription of peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. N Engl J Med 1999;340:1614–1622.
15. Lewin SR, Vesanen M, Kostrikis L, Hurley A, Duran M, Zhang L, et al.: Use of real-time PCR and molecular beacons to detect virus replication in human immunodeficiency virus type 1-infected individuals on prolonged effective antiretroviral therapy. J Virol 1999;73:6099–6103.
16. Haase AT, Henry K, Zupancic M, Sedgewick G, Faust RA, Melroe H, et al.: Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science 1996;274:985–989.
17. Kuster H, Cone RC, Ott P, Schlaepfer E, Fischer M, Günthard HF, et al.: Treatment-induced decline of HIV-1 p24 and HIV-1 RNA in lymphoid tissue of patients with early HIV-1 infection. Am J Pathol 2000;156:1973–1986.
18. Schmitz J, van LJ, Tenner RK, Grosschupff G, Racz P, Schmitz H, et al.: Follicular dendritic cells retain HIV-1 particles on their plasma membrane, but are not productively infected in asymptomatic patients with follicular hyperplasia. J Immunol 1994;153:1352–1359.
19. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L, Ingerman MJ, et al.: Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. JAMA 1999;282:1627–1632.
20. Zhang L, Ramratnam B, Tenner RK, He Y, Vesanen M, Lewin S, et al.: Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. N Engl J Med 1999;340:1605–1613.
21. Günthard HF, Frost SD, Leigh BA, Ignacio CC, Kee K, Perelson AS, et al.: Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. J Virol 1999;73:9404–9412.
22. Sharkey ME, Teo I, Greenough T, Sharova N, Luzuriaga K, Sullivan JL, et al.: Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. Nat Med 2000;6:76–81.
23. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al.: Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA 1997;94:13193–13197.
24. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al.: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295–1300.
25. Wong JK, Hezareh M, Günthard HF, Havlir DV, Ignacio CC, Spina CA, et al.: Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291–1295.
26. Butera ST, Roberts BD, Lam L, Hodge T, and Folks TM: Human immunodeficiency virus type 1 RNA expression by four chronically infected cell lines indicates multiple mechanisms of latency. J Virol 1994;68:2726–2730.
27. Opravil M, Hill AM, DeMasi R, and Dawson D: Prediction of HIV-1 RNA suppression and its durability during treatment with zidovudine/lamivudine. Antivir Ther 1998;3:169–176.
28. Valdez H, Lederman MM, Woolley I, Walker CJ, Vernon LT, Hise A, et al.: Human immunodeficiency virus 1 protease inhibitors in clinical practice: Predictors of virological outcome. Arch Intern Med 1999;159:1771–1776.
29. Paris D, Ledergerber B, Weber R, Jost J, Flepp M, Opravil M, et al.: Incidence and predictors of virologic failure of antiretroviral triple-drug therapy in a community-based cohort. AIDS Res Hum Retroviruses 1999;15:1631–1638.
30. Raboud JM, Rae S, Hogg RS, Yip B, Sherlock CH, Harrigan PR, et al.: Suppression of plasma virus load below the detection limit of a human immunodeficiency virus kit is associated with longer virologic response than suppression below the limit of quantitation. J Infect Dis 1999;180:1347–1350.
31. Kempf DJ, Rode RA, Xu Y, Sun E, Heath-Chiozzi ME, Valdes J, et al.: The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. AIDS 1998;12:F9–14.

32. Tenner RK, Stellbrink HJ, van LJ, Schneider C, Jacobs JP, Raschdorff B, et al.: The unenlarged lymph nodes of HIV-1-infected, asymptomatic patients with high CD4 T cell counts are sites for virus replication and CD4 T cell proliferation. The impact of highly active antiretroviral therapy. J Exp Med 1998;187:949–959.
33. Yerly S, Rutschmann OT, Opravil M, Marchal F, Hirschel B, and Perrin L: Cell-associated HIV-1 RNA in blood as indicator of virus load in lymph nodes. J Infect Dis 1999;180:850–853.
34. Schockmel GA, Yerly S, and Perrin L: Detection of low HIV-1 RNA levels in plasma. J Acquir Immune Defic Syndr Hum Retroviral 1997;14:179–183.
35. Fischer M, Huber W, Kallivroussis A, Ott P, Opravil M, Lüthy R, et al.: Highly sensitive HIV-1 RNA quantitation from plasma, cells and tissues. J Clin Microbiol 1999;37:1260–1264.

Address reprint requests to:
*Marek Fischer*
*Division of Infectious Diseases*
*University Hospital*
*Rämistrasse 100*
*CH-8091 Zürich, Switzerland*

*E-mail:* marek.fischer@ dim.usz.ch