# HIV viral load markers in clinical practice

**Plasma HIV RNA determinations are an important prognostic marker of disease progression and, when used appropriately, provide a valuable tool for the management of individual patients. But what constitutes appropriate use?**

M.S. Saag, M. Holodniy,
D.R. Kuritzkes, W.A. O'Brien,
R.W. Coombs, M.E. Poscher,
D.M. Jacobsen, G.M. Shaw,
D.D. Richman & P.A. Volberding

The development of new molecular techniques designed to detect circulating virion-associated HIV RNA in plasma has created an opportunity to study viral dynamics and HIV pathogenesis in substantial detail. Prior beliefs, based on the concept of a prolonged phase of relative virologic latency in the period before symptoms become evident have been replaced by a new paradigm of ongoing, high-level viral replication from the time of initial infection until death (Fig. 1). Indeed, as many as 10 billion new HIV virions are produced per day, with a half-life in plasma of 6 hours[1,2,3]. CD4+ lymphocytes, one of the principal cell targets responsible for viral replication *in vivo*, are also produced in high numbers and, once productively infected, have a half-life of about 1.6 days. The life-cycle of the virus, from infection of one cell to the production of new progeny, which infects the next cell, is 2.6 days[3]. This extraordinarily high level of viral replication, cell destruction and cell replacement has led to a dramatic shift in clinical management of HIV-infected patients, and, in particular, the use of antiretroviral therapy.

Before the development of these new molecular techniques, quantitative culture of peripheral blood mononuclear cells (PBMCs) or plasma was used to estimate the infectious titer of HIV in the blood[4,5]. Increasing plasma virus titers were associated with clinical progression while decreases in plasma virus accompanied treatment with active drugs. However, fewer than 50% of patients with CD4+ counts greater than 200 cells/µl had positive plasma cultures, and inherent biologic variability in virus quantitation required that a 25-fold (approximately 1.4 log) increase was seen before it was likely to be clinically meaningful. In contrast, HIV RNA detection techniques revealed measurable virus in the plasma of virtually all HIV-infected patients regardless of clinical stage[6]. Moreover, plasma RNA levels exhibited a wide dynamic range, correlated significantly with clinical stage, and fell rapidly following the initiation of effective antiretroviral therapy. Despite these promising attributes, it was not known whether plasma virus levels could be accurately and reproducibly measured in a clinical setting or whether they would be predictive of clinical outcome. Recent findings indicate that plasma HIV RNA determinations are an important prognostic marker of disease progression and provide a valuable tool for the management of individual patients.

## The assays
Three commercially available plasma HIV RNA assays — branched DNA (bDNA), RT-PCR, and Nucleic Acid Sequence-Based Amplification (NASBA) — have not yet been approved by the US Food and Drug Administration for use in routine patient management. The bDNA technique amplifies the signal from a captured viral RNA target by sequential olignonucleotide hybridization steps while RT-PCR and NASBA use enzymatic methods to amplify target HIV RNA into measurable amounts of nucleic acid product[7,8,9]. Target RNA sequences in plasma are quantified by comparison with internal or external reference standards, depending on the assay used. Despite the differences in methodologic approach, plasma HIV RNA measurements obtained with the three assays are strongly correlated ($R \sim 0.90$)[10,11] and each has low intra-assay sample variability (approximately 0.12 to 0.2 log on repeated testing of single samples)[10–12] (Table 1). Plasma HIV RNA levels are relatively stable on a week-to-week or month-to-month basis in clinically stable patients, as long as antiretroviral therapy is not instituted or changed (biologic variability ~0.3 log)[13]. Therefore, sustained changes in the plasma HIV RNA levels of >0.5 log (that is, greater than threefold) generally reflect biologically relevant changes in the level of viral replication.

## Correlation of HIV RNA levels to stage of disease
Early studies demonstrated a clear association between the titer of culturable virus in the plasma and the clinical stage of disease[4,5,14]. In patients with the acute seroconversion syndrome and those with advanced HIV disease (<100 CD4+ lymphocytes/µl), plasma viral titers were generally in the range of 100 to 10,000 tissue culture infectious doses (TCID)/ml while in asymptomatic patients with higher CD4+ lymphocyte counts (>300 cells/µl) plasma titers were low or non-detectable. The use of quantitative HIV RNA techniques has further defined the association between plasma virus levels and clinical disease sta-
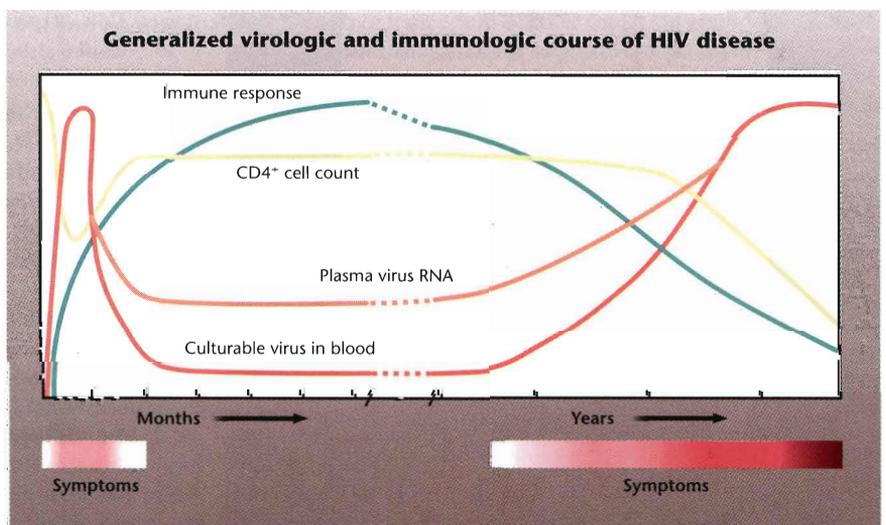


**Generalized virologic and immunologic course of HIV disease**

Immune response
CD4+ cell count
Plasma virus RNA
Culturable virus in blood
Months
Years
Symptoms
Symptoms

Fig. 1   Schematic representation of natural history of HIV disease.

tus. Most importantly, in virtually all un-treated patients, viral RNA is detectable in plasma regardless of clinical stage[6,10,15]. Plasma HIV RNA levels are highest in acute (primary) infection (former CDC stage I) and late-stage disease (CDC IV) and are intermediate in titer in earlier clinical stages (CDC II and III). The relative correlation between plasma virus titer (by culture methods) and quantitative PCR values is shown in Fig. 2.

## Ability of plasma viral RNA to predict clinical outcome

CD4+ lymphocyte counts have been viewed as the best predictor of the risk of develop-ing AIDS-related complications. The risk of developing HIV disease or dying over the next 24 months is <5% among individuals with CD4+ counts above 500 cells/µl and >70% among those having fewer than 50 cells/µl (ref. 16). Despite its value as a gen-eral marker of disease stage, the CD4+ count alone is inadequate as a means of measuring prognosis and response to antiretroviral therapy. CD4+ counts are subject to substan-tial biologic variability and exhibit a limited dynamic range (approximately 2 log)[17,18]. Most importantly, decreases in CD4+ cell counts occur as a result of viral replication and, in that sense, represent a clinical endpoint rather than a "surrogate marker" of disease activity. It is this very process — HIV-me-diated lymphocyte destruction — that physicians attempt to prevent rather than observe.

Higher HIV RNA levels correlate with lower baseline CD4+ counts, more-rapid declines in CD4+ counts, and more rapid disease progression[19–23]. Patients with >100,000 HIV RNA copies/ml of plasma within six months of seroconversion were tenfold more likely to progress to AIDS over five years than

**Table 1  Characteristics of Plasma HIV RNA Assays**

| Assay (manufacturer) | Linear Dynamic range (copies/ml) | Observed intra-assay standard deviation range (log10)[a] | Preferred anticoagulant |
|---|---|---|---|
| RT-PCR (Roche Molecular Systems) | $10^{2.6}$–$10^{5.9}$ | <0.15–0.33 | ACD/EDTA |
| bDNA (Chiron) | $1 \times 10^4$–$1.6 \times 10^6$ | 0.12–0.2 | EDTA |
| NASBA (Organon Teknika) | $4 \times 10^2$–$4 \times 10^7$ | 0.13–0.23 | ACD/EDTA/HEP |

Higher values can be measured with dilution of the specimen into the linear dynamic range for each assay.  [a]Ranges are representative of an ongoing HIV RNA certification program sponsored by the National Institutes of Health, Division of AIDS, Virology Quality Assurance Program[12]. ACD = acid citrate dextran (citrate; yellow-top tube) EDTA = ethylenediaminetetraacetic acid (purple-top tube). HEP = heparin (green-top tube).

were those with <100,000 copies/ml (ref. 22). Patients who con-sistently maintained an HIV RNA copy number of less than 10,000/ml did not progress to AIDS during the next five years; HIV RNA levels tended to increase among progressors. Thus, maintenance of plasma HIV RNA levels below 10,000/ml in early HIV disease appears to be associated with decreased risk of progression to AIDS. However, in patients with more advanced disease (median CD4+ cell count, 89/µl), disease progression oc-curred in up to 30% of patients with fewer than 10,000 HIV RNA copies/ml (ref. 20, 21).

In a recent study, a single determination of plasma viral RNA in 181 seropositive individuals provided important prognostic information concerning time to AIDS and death[23]. Subjects were stratified by plasma HIV RNA level (quartiles of <4,530; 4,531–13,020; 13,021–36,270; and >36,271 HIV-1 RNA copies/ml plasma) and followed for as long as 11.2 years. Substantial increases in disease progression rates were associ-ated with higher baseline viral levels. For patients with CD4+ cell counts above 500/µl (median, 780/µl), >70% progressed to AIDS and died within 10 years if their baseline RNA level was >10,200; in contrast, <30% of those with <10,200 copies/ml died within 10 years. A threefold higher baseline HIV RNA level was predictive of a 60% increased hazard of death. A single plasma virus RNA determination predicted clinical events oc-
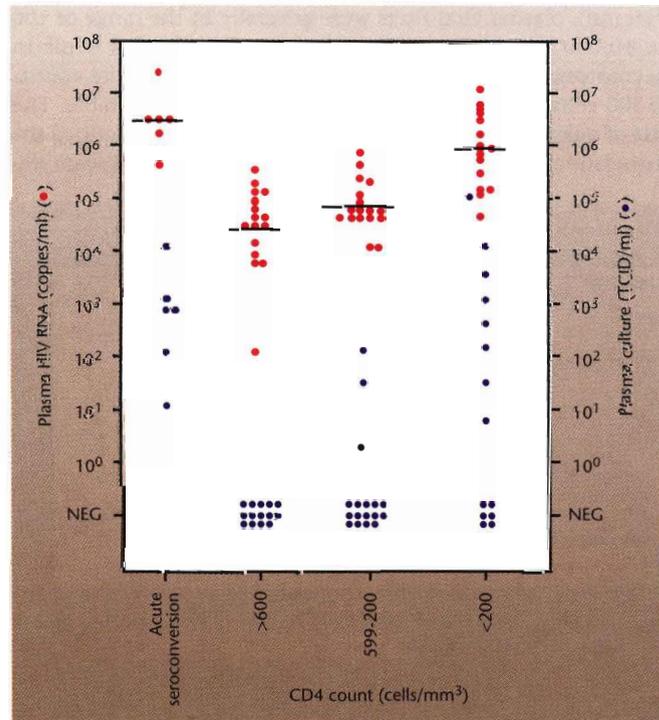


Fig. 2   Comparison of plasma HIV levels as measured by infectious titer in tissue culture (tissue culture infective dose/ml (TCID/ml); blue circles) versus amplified genomic RNA via QC-PCR (copies/ml; red circles) in patients at different stages of HIV disease. Mean val-ues of HIV RNA levels are indicated by horizontal bars. All values obtained for each assay were determined from paired specimens, as reported in Piatak et al.[6] The observed discrepancy between total virus levels determined by direct RNA measurements and those determined by culture (generally 100–10,000 to 1) is typical of retroviruses, which are known to exhibit high frequencies of ge-netic and phenotypic defectiveness. The fact that direct bDNA, RT-PCR and NASBA methods detect primarily virus that is non-culturable is not relevant to their clinical utility since plasma virus, infectious or not, is a direct measure of virus production and the processes sustaining HIV infection and pathogenesis.

Fig. 3 Composite of plasma HIV RNA and CD4⁺ cell count responses among a group of patients receiving three different antiretroviral regimens: zidovudine monotherapy (ZDV, 600 mg/day; solid lines), zidovudine plus lamivudine (ZDV/3TC, 600 mg/day, 300 mg/day, respectively; long-dashed lines), and the protease inhibitor, indinavir (MK-639, 2400 mg/day; short-dashed lines). All patients were naive to their respective treatment regimens. For each treatment group, the relative HIV RNA and CD4⁺ count treatment responses appear inversely proportional, although individual exceptions to this association exist.
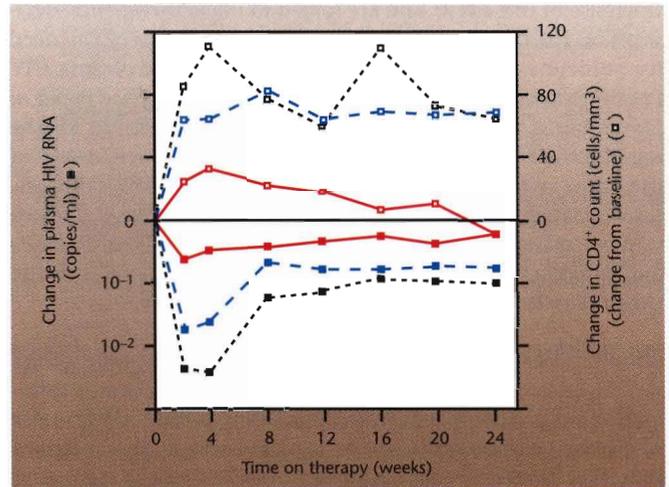


curring as long as 10 years later, similar to predictions determined through the staging of certain malignancies (for example, Hodgkin's disease). Placed in the context of clinical practice, plasma HIV RNA levels appear to be the best predictor of long-term clinical outcome while CD4⁺ lymphocyte counts remain the best predictor of immediate or short-term risk of developing a new opportunistic disease process.

### Response of plasma HIV RNA to antiretroviral therapy

Plasma HIV RNA measurements are useful for rapidly evaluating the relative antiretroviral effect of new or available drugs or regimens in clinical trials[1,19,24,25]. Effective antiretroviral therapy significantly decreases HIV RNA levels in plasma within one week of the start of treatment. No significant decrease in the plasma levels within this period suggests that the regimen has no antiretroviral activity. Zidovudine monotherapy results in a median 0.7 log decrease in the plasma HIV RNA level within two weeks, which returns toward baseline values by 24 weeks[19,20]. Lower HIV RNA troughs (~1.5 log decreases) and higher CD4⁺ lymphocyte peaks are observed with nucleoside combinations and the responses are generally more durable, often persisting for more than one year[26] (Fig. 3). Protease inhibitors in combination with nucleoside therapy results in dramatic and sustained reductions in plasma viral RNA (~2.0 log) and, in one trial, treatment-associated reductions were associated with a survival benefit in patients with advanced disease[1,2,27–29]. Decreases in plasma HIV RNA levels generally correlate with increases in the CD4⁺ lymphocyte count in patients in whom effective antiretroviral therapy is initiated. In patients in whom plasma HIV RNA levels initially decline but return to pretreatment values, the loss of antiviral effect has been associated with the emergence of drug-resistant strains of HIV (ref. 1, 24, 30, 31).

Antiretroviral induced changes in plasma HIV RNA level and CD4+ lymphocyte count are both independent predictors of disease progression. In one study, each twofold (0.3 log) decrease in the HIV RNA level during treatment was correlated with a 27% reduction in the relative hazard of progression[20]. In another study, each three-fold (0.5 log) decrease in HIV RNA level was associated

with a 63% reduction in relative hazard of progression ($P = 0.02$)[19]. In a third study, a 1.0 log treatment-induced reduction in HIV RNA was associated with an 80% reduction in relative risk of disease progression and was a more powerful predictor of clinical outcome than CD4⁺ cell counts or other virologic measures[25]. In general, a decrease in plasma HIV RNA level along with an increase in the CD4⁺ lymphocyte count explain a significant part of the treatment effect observed in these studies.

### The effect of emerging data on clinical practice

As a result of the overlapping nature of clinical research and patient care and the rapid translation of clinical research findings (based on plasma HIV RNA endpoints) to patient management, many clinicians are using HIV RNA assays in their practices. As an example, among 915 HIV clinicians attending one of five International AIDS Society–USA-sponsored, advanced courses on the management of HIV disease, in spring 1995, 20% (172) used HIV RNA measurements in their practice. Yet despite increasing evidence demonstrating the value of viral load determinations, many practitioners are uncertain about the op-

**Table 2   Summary of interim recommendations**

| Parameter | Recommendation |
|---|---|
| • Plasma HIV RNA level that suggests initiation of treatment | • More than 5,000–10,000 copies/ml and a CD4⁺ count/clinical status suggestive of progression; >30,000–50,000 regardless of laboratory/clinical status |
| • Target level of HIV RNA after initiation of treatment | • Undetectable; <5,000 copies/ml is an acceptable target |
| • Minimal decrease in HIV RNA indicative of antiviral activity | • >0.5 log decrease |
| • Change in HIV RNA that suggests drug treatment failure | • Return to (or within 0.3 to 0.5 log of) pretreatment value |
| • Suggested frequency of HIV RNA measurement | • At baseline: 2 measurements, 2–4 weeks apart<br>• Every 3 to 4 months or in conjunction with CD4⁺ counts<br>• Shorter intervals as critical decision points are neared<br>• 3–4 weeks after initiating/changing therapy |

timal use of these tests and are forced to make therapeutic decisions on the basis of anecdotal experience. Because of the need for guidelines to assist practitioners in the use of plasma HIV RNA measurements, the IAS–USA convened an *ad hoc* panel of investigators and clinicians to make recommendations for the use of these assays in clinical practice. The recommendations of the panel were developed in conjunction with a series of questions related to the natural history of HIV disease, current understanding of correlations between plasma HIV RNA levels and prognosis or response to antiretroviral therapy, and the performance characteristics of the assays.

## Should plasma HIV RNA levels be used routinely in practice?

Yes. Monitoring plasma HIV RNA levels adds important information for patient management, including information on risk of disease progression, when to initiate therapy, the degree of initial antiretroviral effect achieved and when a drug regimen is failing. Although there are no published data from controlled clinical trials as yet, new data from controlled treatment trials and natural history studies strongly support their use in routine practice. The CD4+ lymphocyte count remains an essential index for making decisions regarding prophylaxis for opportunistic infections and for evaluating the immunologic effects of antiretroviral therapy. Since plasma HIV RNA levels and CD4+ lymphocyte count determinations are independent predictors of clinical outcome, their combined use provides a more complete picture of an individual patient's status and response to therapy.

## When should antiretroviral therapy be initiated?

The goals of antiretroviral therapy are to limit or delay disease progression and increase survival. Given the dynamic interaction between viral replication and CD4+ lymphocyte destruction and the results of clinical studies showing increased survival in association with significant reduction in plasma RNA levels, the best way to achieve these goals is by minimizing viral replication. Steady-state plasma viral RNA levels are directly related to rates of virus production in lymphoreticular tissues and changes in plasma viral load can be used to assess antiretroviral drug effects in otherwise inaccessible tissue compartments. Ideally, the goals of therapy are to reduce the plasma HIV RNA level as much as possible and for as long as possible.

Recent disease progression cohort data and clinical trial results showed that there is a continuum of increased risk for AIDS and death as HIV RNA levels increase. Patients with very low HIV RNA levels (for example, <5,000–10,000 copies/ml) have a better clinical prognosis than those individuals with only modest elevations in RNA levels (for example, 10,000–25,000 copies/ml). Some investigators, including the authors, have concluded that plasma HIV RNA levels >30,000–50,000 copies/ml warrant the initiation therapy, regardless of CD4+ cell count or clinical status. In patients with plasma HIV RNA levels >5,000–10,000 copies/ml but less than 30,000, the decision to start therapy should be made in conjunction with CD4+ cell counts and clinical status. If antiretroviral treatment is initiated according to current clinical guidelines (based solely on CD4+ count values), plasma HIV RNA determinations may be helpful for patients who have CD4+ counts near the current threshold values (for example, 500 CD4+ cells/μl). A high viral load in such patients might provide additional impetus for initiation of treatment.

## What level of plasma HIV RNA should be sought?

Ideally, undetectable levels of plasma HIV RNA should be sought. However, maximal clinical benefit might be achieved by maintaining plasma HIV RNA levels below 5,000 copies/ml. It has not been shown whether plasma HIV RNA reduced to a particular level by antiretroviral therapy carries the same risk of clinical progression as that same HIV RNA level without antiretroviral therapy; prospective clinical trials are urgently needed to address this question. Because sustained suppression below 5,000 copies/ml may not be achievable for many patients using the currently available therapies, this recommendation represents a reasonable "target" level for some patients, but certainly less than "ideal" in others.

## For a patient, what suggests a drug is working?

A three-fold or greater sustained reduction (>0.5 log) of the plasma HIV RNA levels is the minimal response indicative of an antiviral effect, given within-assay variation (~0.15–0.2 log) and natural biologic variation of plasma HIV RNA *in vivo* (~0.3 log). Reductions of this magnitude have been associated with clinical benefit in treatment trials. It is not known whether a reduction in plasma viral RNA of any given magnitude has the same significance in terms of clinical benefit irrespective of the initial pretreatment RNA value. That is, it is not clear whether a 1.0 log (tenfold) reduction in virus load in a patient with a pretreatment level of 1,000,000 copies/ml has the same clinical significance as 1.0 log reduction in a patient with an initial pretreatment level of 10,000 or 100,000 copies/ml. It is likely that the clinical benefits of antiretroviral therapy are related to the duration as well as the magnitude of HIV suppression (that is, the area under the curve), although the precise duration of HIV suppression necessary to result in measurable clinical benefit still needs to be clearly defined.

With existing antiretroviral drug regimens, it is not realistic to expect that lowest plasma HIV RNA levels achieved can be maintained indefinitely. Thus, the return of HIV RNA levels to pretreatment values (or to within 0.3–0.5 log of the pretreatment value), confirmed by at least two measurements, is indicative of drug failure and should prompt consideration of alternative treatment regimens. Decisions to institute changes in therapy should be made using the plasma HIV RNA value in conjunction with CD4+ lymphocyte count and clinical status.

## How often should plasma HIV RNA levels be measured?

For the initial determinations of the HIV RNA plasma level, two measurements should be obtained 2–4 weeks apart. Subsequently, we suggest that measurements might be obtained along with the CD4+ lymphocyte count (every 3–4 months, according to current convention), since serial determination of both markers simultaneously provide useful information. Viral load assessments may be made at shorter intervals (for example, every 4 weeks) as critical clinical decision points — such as the return of the viral load level to baseline values — are approached. Ideally, plasma HIV RNA levels should be measured 3–4 weeks after initiating or changing antiretroviral treatment to determine the magnitude of the response. Because of the effects of immune activation on viral load, HIV RNA levels should not be measured within a month of acute illnesses or within a month after influenza and pneumococcus immunizations. Increases in HIV RNA levels in blood of as much as 300-fold have been observed within two weeks of routine immunizations against influenza, tetanus, or pneumo-

coccus[32–33]. These increases are transient, returning to preimmunization levels within four weeks of immunization. Increases are also associated with reactivated genital herpes and tuberculosis, and presumably occur with other acute illnesses.

## How should samples be stored, handled and processed?

Optimal procedures for storage, handling, and processing of patient samples have yet to be fully defined. Practitioners should be familiar with the particular assay they have chosen for HIV RNA quantification and the specific sample-handling factors necessary for that assay. Each provider should adopt consistent procedures for handling specimens, including using the same collection tube and anticoagulant, processing techniques, transport and storage procedures, and the same assay for every sample from the same patient. To minimize signal degradation, all plasma specimens should be separated and frozen within 6 hours of collection. If this approach is not possible, the plasma should be removed and refrigerated. Less desirably, the whole blood could be refrigerated, but not for more than 24 hours before separation and freezing are completed.

Plasma HIV RNA quantitation has provided valuable insights into HIV pathogenesis and the activity of antiretroviral regimens. The optimal use of plasma HIV RNA assays will become better understood as more data become available. Of obvious importance is the continued demonstration of clinical benefit in association with treatment strategies that focus on suppressing the viral load as the primary objective of therapy, as is better characterization of the types of events that cause perturbations in plasma HIV RNA levels. Until these issues are further elucidated, these recommendations provide a reference point for the use of plasma HIV RNA as a marker today.

1. Wei, X. et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* **373**, 117 (1995).
2. Ho, D.D. et al. Rapid turnover of plasma virions and CD4+ lymphocytes in HIV-1 infection. *Nature* **373**, 123–126 (1995).
3. Perelson, A.S., Neumann, A.U., Markowitz, M., Leonard, J.M. & Ho, D.D. HIV-1 dynamics in vivo: Virion clearnace rate, infected cell life-span, and viral generation time. *Science* **271**, 1582–1586 (1996).
4. Ho, D.D., Moudgil, T. & Alam, M. Qunatitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* **321**, 1621–1625 (1989).
5. Coombs, R.W. et al. Plasma viremia in human immunodeficiency virus infection. *N. Engl. J. Med.* **321**, 1626–1631 (1989).
6. Piatak, M. Jr et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* **259**, 1749–1754 (1993).
7. Pachl, C. et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J. Acquir. Immune Defic. Syndr. Hum. Retrovir.* **8**, 446–454 (1995).
8. Mulder, J. et al. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. *J. Clin. Microbiol.* **32**, 292–300 (1994).
9. Kievits, T. et al. NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. *J. Virol. Methods* **35**, 273–286 (1991).
10. Cao, Y. et al. Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. *AIDS Res. Hum. Retrovir.* **11**, 353–361 (1995).
11. Lin, H.J. et al. Multicenter evaluation of quantification methods for plasma human immunodeficiency virus type 1 RNA. *J. Infect. Dis.* **170**, 553–562 (1994).
12. Brambilla, D. et al. Variation in estimates of HIV-1 plasma RNA concentration among laboratories and assay kits. *3rd Conference on Retroviruses and Opportunistic Infections*, Jan. 28–Feb. 1, 1996, 155, abst. 550 (Washington, DC).
13. Holodniy, M., Mole, L., Winters, M. & Merigan, T.C. Diurnal and short-term stability of HIV virus load as measured by gene amplification. *J. Acquir. Immune Defic. Syndr.* **7**, 363–368 (1994).
14. Clark, S.J. et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N. Engl. J. Med.* **324**, 954 (1991).
15. Holodniy, M. et al. Detection and quantification of human immunodeficiency virus RNA in patient serum by use of the polymerase chain reaction. *J. Infect. Dis.* **163**, 862–866 (1991).
16. Stein, D.S., Korvick, J.A. & Vermund, S.H. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. *J. Infect. Dis.* **165**, 352–363 (1992).
17. Raboud, R.M. et al. Quantification of the variation due to laboratory and physiologic sources in CD4 lymphocyte counts of clinically stable HIV-infected individuals. *J. Acquir. Immune Defic. Syndr. Hum. Retrovir.* **10** (suppl. 2), S67–S73 (1995).
18. Hoover, D.R. et al. Effect of CD4+ cell count measurement variability on staging HIV-1 infection. *J. AIDS* **5**, 794–802 (1992).
19. O'Brien, W.A. et al. Changes in plasma HIV-1 RNA and CD4+ Lymphocyte counts and the risk of progression to AIDS. *N. Engl. J. Med.* **334**, 425–431 (1996).
20. Coombs, R.W. et al. Association of plasma human immunodeficiency virus type-1 RNA level with risk of clinical progression in patients with advanced infection. *J. Infect. Dis.* (in the press).
21. Welles, S.L. et al. Prognostic value of plasma HIV-1 RNA levels in patients with advanced HIV-1 disease and with little or no zidovudine therapy. *J. Infect. Dis.* (in the press).
22. Mellors, J.W. et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann. Intern. Med.* **122**, 573–579 (1995).
23. Mellors, J.W. et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* (in the press).
24. Kappes, J.C. et al. Assessment of antiretroviral therapy by plasma viral load testing: standard and ICD HIV-1 p24 antigen and viral RNA j(QC-PCR) assays compared. *J. Acquir. Immune Defic. Syndr. Hum. Retrovir.* **10**, 139–149 (1995).
25. Hammer, S.M., Katzenstein, D.A., Hughes, M.D., Hirsch, M.S., Merigan, T.C. Virologic markers and outcome in ACTG 175. *3rd Conference on Retroviruses and Opportunistic Infections*, Jan. 28–Feb. 1, 1996, 175, abst. S24 (Washington, DC).
26. Eron, J. et al. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4 cells per cubic millimeter. *N. Engl. J. Med.* **333**, 1662–1669 (1995).
27. Danner, S.A. et al. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. *N. Engl. J. Med.* **333**, 1528–1533 (1995).
28. Markowitz, M. et al. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. *N. Engl. J. Med.* **333**, 1534–1539 (1995).
29. Heath-Chiozzi, M. et al. Anti-HIV activity and lymphocyte surrogate marker response dynamics to ritonavir therapy in advanced HIV immunodeficiency. *3rd Conference on Retroviruses and Opportunistic Infections*, Jan. 28–Feb. 1, 1996, 162, abst. LB6b (Washington, DC).
30. Havlir, D., McLaughlin, M.M. & Richman, D.D. A pilot study to evaluate the development of resistance to nevirapine in asymptomatic human asymptomatic human immunodeficiency virus-infected patients with CD4+ cell counts of >500/mm³: AIDS Clinical Trials Group Protocol 208. *J. Infect. Dis.* **172**, 1379–1383 (1995).
31. Holodniy, M. et al. Determination of human immunodeficiency virus RNA in plasma and cellular viral DNA genotypic zidovudine resistance and viral load during zidovudine-didanosine combination therapy. *J. Virol.* **69**, 3510–3516 (1995).
32. Staprans, S.I. et al. Activation of virus replication after vaccination of HIV-1 infected individuals. *J. Exp. Med.* **182**, 1727–1737 (1995).
33. O'Brien, W.A. et al. Human immunodeficiency virus- type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. *Blood* **86**, 1082–1089 (1995).

*From the University of Alabama at Birmingham (M.S.S. & G.M.S.); Stanford University and the Palo Alto Veterans Affairs Health Care System (M.H.); the University of Colorado Health Sciences Center (D.R.K.); the University of California Los Angeles School of Medicine and West Los Angeles Veterans Affairs Medical Center (W.A.O.); the University of Washington (R.W.C.); the University of California San Francisco School of Medicine (M.E.P. & P.A.V.); the International AIDS Society–USA (D.M.J.); and the University of California San Diego and San Diego Veterans Affairs Medical Center (D.D.R.).*

*Correspondence should be addressed to M.S.S. at the University of Alabama at Birmingham, 908 20th Street South Birmingham, Alabama 35294-2050, USA*