

IN VIVO DYNAMICS OF HIV INFECTION

The dynamics of HIV in vivo was discussed at the Atlanta meeting by Michael S Saag, MD, from the University of Alabama at Birmingham.

As related by Dr Saag, the novel techniques for quantitating viral burden in plasma, including most prominently the quantitative PCR and branched-DNA (b-DNA) assays for plasma HIV RNA, have altered the frameworks both for understanding HIV dynamics in vivo and for developing and implementing treatment strategies. The relative inability to culture virus from the peripheral circulation and the finding of relatively low numbers of infectious virus in cultures taken after resolution of the viremia following acute primary infection encouraged the belief that viral infection was quiescent during the prolonged asymptomatic stage of disease. However, pathology studies showing high levels of ongoing viral replication in lymphoid tissue, and PCR and b-DNA assay findings showing that plasma HIV RNA remains high throughout the course of infection, have combined to change the view of HIV as an infection with a quiescent stage. The plasma RNA assays have shown that the viral load in plasma at the time of initial infection is extraordinarily high (10^6 to 10^7 virions/mL) and that there is a dramatic decrease in viral level with the onset of HIV-directed immune response. Viral load nevertheless remains at significant levels (10^3 to 10^4 /mL) throughout the so called clinically latent period. In addition to demonstrating that viral replication is ongoing throughout infection, the assay findings indicate the potency of the initial immune response to infection.

As stated by Dr Saag, the plasma RNA PCR and b-DNA assays have been shown to have highly correlated results (Figure 6). The high degree of correlation indicates that the two assays are indeed providing an accurate measure of the same quantity,

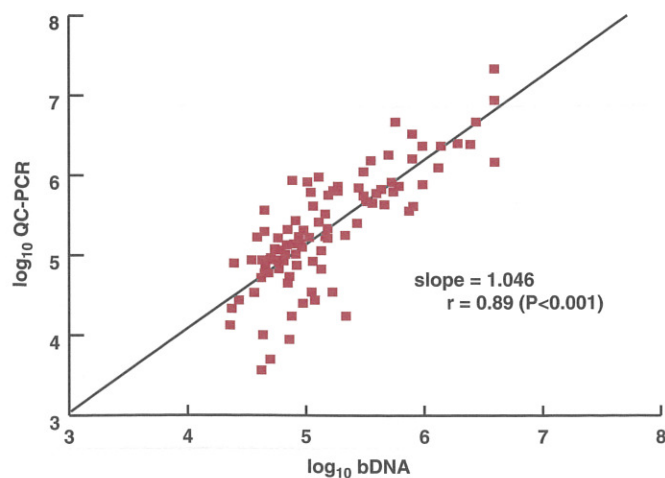


Figure 6. Correlation between quantitative competitive PCR (QC-PCR) and branched-DNA (b-DNA) measurements of plasma viral RNA. Adapted from Cao Y, et al. *AIDS Res Hum Retroviruses*. 1995.

namely the HIV RNA present in plasma samples. Early blinded findings with one of the RNA PCR (QC-PCR method) assays showed that all HIV-negative controls were negative for HIV RNA in plasma and that different stages of infection, corresponding to acute seroconversion, 'asymptomatic' disease, AIDS-related complex, and AIDS, were characterized by different RNA levels. In this study, patients with acute infection had 1 million to 20 million RNA copies/mL, those with asymptomatic disease had levels approximately three logs (a factor of 1000) lower, and those with later stages had successively higher viral loads. As

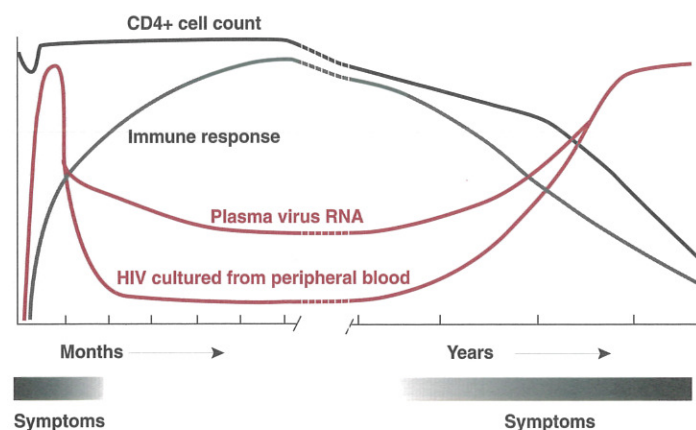


Figure 7. Generalized virologic and immunologic course of HIV disease. Plasma virus RNA curve reflects level of ongoing viral replication, presumably in lymphoid tissues and other areas.

noted by Dr Saag, such findings have resulted in a reformulation of the proposed general immunologic and virologic course of HIV disease (Figure 7), with the revised scheme representing the potency of host immune response in curtailing viral replication and, at the same time, the persistently high level of replication as indicated by the high level of plasma viral RNA.

Rapidity of Viral Turnover Demonstrated with Antiretroviral Treatment

Recent studies have investigated the dynamics of HIV replication in vivo, including the effects of antiretroviral therapy. A study initiated in 1992 by Dr Saag and colleagues included assessment of virologic responses to zidovudine treatment using a variety of assays, including RNA PCR and b-DNA assays. Figure 8 shows the virologic response in 12 patients, many of whom had been receiving zidovudine previously and had undergone a drug-washout phase. The data show that there was a 75% reduction in plasma viral burden that persisted throughout the 6 weeks of treatment and that the viral burden returned to pretreatment level within 1 week of stopping treatment. According to Dr Saag, an implication of these findings, although it was not fully appreciated at the time of the study, is that the viral turnover in vivo is

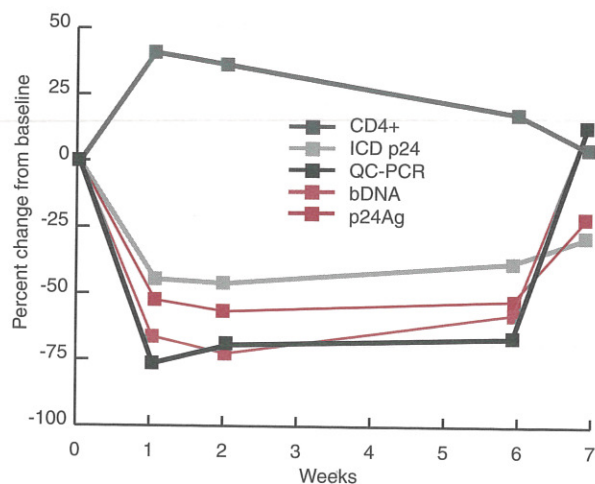


Figure 8. Virologic and CD4+ cell count response to initiation and withdrawal of zidovudine treatment. Zidovudine was administered to 12 patients for 6 weeks. Virologic response was assessed by standard p24 antigen assay, immune-complex dissociated (ICD) p24 assay, quantitative-competitive PCR plasma RNA assay, and b-DNA plasma RNA assay. Adapted from Cao Y, et al. *AIDS Res Hum Retroviruses*. 1995.

extraordinarily rapid, as indicated by the rapid decrease in viral level with initiation of treatment and the rapid increase in viral level with stopping of treatment.

Rapid Achievement of Genetic Diversity

Further evidence of the rapidity of viral turnover has come from genotypic analyses of virus in vivo. Findings made by Dr Saag's group, as well as others, indicate that there is a rapid development of genotypic heterogeneity in the viral population following initial infection. Initially, viral isolates constitute a 'cloud' of genetically distinct, highly related virions, which appear to arise from a common progenitor; over time, with replication and mutation, the population is characterized by quasispecies or 'swarms' of genetically variant virions that change over time under selective pressure.

Dr Saag underlined the rapidity with which HIV develops variants with data from one patient studied by his group: in a patient presenting with symptomatic acute infection, all 44 peripheral blood clones of HIV obtained proved to be genetically identical, indicating a common progenitor; of 21 clones taken 3 weeks later, 11 were genetically distinct. The

ability of the viral population to achieve such great genetic heterogeneity can be explained by high replication and mutation rates. According to Dr Saag, the virus makes on the order of two to three transcription errors per copy; many of these mutations are likely to represent terminal deletions or stop codons in the genetic material, rendering the virus replication-incompetent.

The HIV population rapidly achieves genotypic diversity in vivo

However, some mutations will result in viable virus. It is this ability of the virus to achieve genotypic diversity that appears to underlie the rapid appearance of resistance to some antiretroviral agents. As stated by Dr Saag, given the wide genetic

diversity of the viral population and its rapid turnover rate, resistance appears to occur as a result of selection of preexisting mutants under antiretroviral pressure, with what is initially a minority quasispecies becoming the predominant species over time. He noted that this also implies that resistant mutants are in some sense at a competitive disadvantage to wild type virus. Since resistance is very rarely observed de novo in infected individuals, indicating that any preexist in resistant mutants represent a small minority of the viral population, wild type virus is likely more 'fit' from a Darwinian perspective than is virus with resistance traits. Precisely how much less 'fit' resistant mutants are could have important implications for therapy and requires further study.

Development of Resistant Mutant Population

Figure 9 shows the virologic response in one patient to the addition of nevirapine to zidovudine/didanosine treatment; plasma HIV RNA levels dropped 80% from 103,000 copies/mL at baseline within 14 days and then rapidly returned to the baseline level. Genotypic analyses in patients receiving nevirapine showed that specific mutations were associated with the development of phenotypic resistance and that resistant mutants could be found early after initiation of treatment. Use of an automated DNA sequencing device has permitted assessment of the proportion of isolates in a given sample that possess a particular mutation at different reverse transcriptase codons. Analysis by Dr Saag and colleagues of sequential samples from patients in whom nevirapine was added to existing antiretroviral treatment confirmed that a complete turnover of the plasma viral population to virus with a resistance mutation at a particular codon can occur in a few weeks after the addition of nevirapine.

Dr Saag described data on four patients who had nevirapine added to their regimen showing the proportion of functional clones retrieved from the patients and the proportions of nevirapine-sensitive and nevirapine-resistant clones in plasma and in peripheral blood mononuclear cells (PBMCs) over various

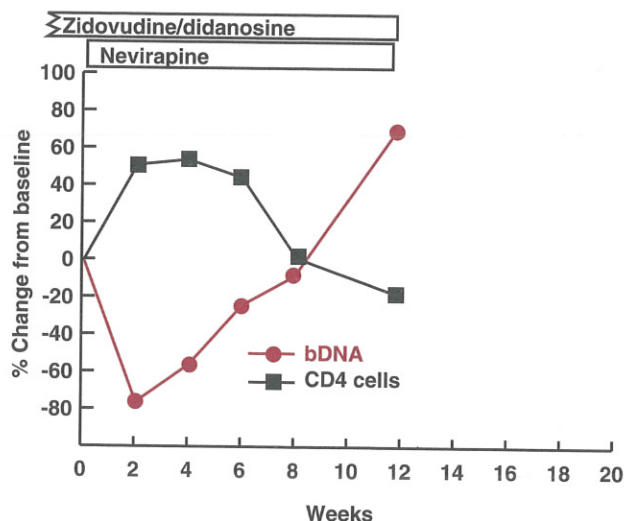


Figure 9. CD4+ cell and plasma HIV RNA responses to addition of nevirapine to existing antiretroviral treatment in a single patient. Baseline CD4+ count was 106 cells/ μ L; baseline bDNA level was 103,000 RNA molecules/mL. Figure courtesy of Dr Saag.

durations of treatment. The proportion of isolates resistant to nevirapine increased quite rapidly and faster in plasma than in the PBMCs. For example, in one patient, nearly two thirds of clones from plasma were nevirapine-resistant by day 14, with 100% being resistant by day 28; for PBMC samples, no resistance was observed over the first 14 days of treatment, half of the clones

were resistant by day 28, and three-quarters were resistant by day 140. As explained by Dr Saag, the proportion of resistant clones in PBMCs has never been observed to reach 100%; this finding suggests that whereas virus in plasma would appear to be

Approximately 30% of virus detectable at a given time was produced within the past 24 hours.

newly produced virus, the circulating PBMCs represent more of a reservoir of past generations of virus, with at least a proportion of surviving cells having been infected with and continuing to produce susceptible progeny.

Rate of Viral Turnover

Detailed kinetic studies of changes in viral load with initiation of nevirapine treatment performed by Dr Saag and colleagues have permitted estimates of the rate of viral turnover. As can be seen from Figure 10, 98% to 99% decreases in plasma viral burden occurred within a few weeks of beginning treatment. The kinetic analyses showed that the half-life of virus in the plasma is approximately two days, indicating that 30% of the virus detected in plasma at any given time was produced within the past 24 hours. As shown in Figure 11, similar findings were

made in patients receiving protease inhibitor monotherapy. As noted by Dr Saag, currently used antiretrovirals act at the reverse transcription stage of replication and have no effect in chronically infected cells that constitutively produce virus. The 98% to 99% decrease in viral load indicates that it is precisely the newly produced virions that are being affected. If a greater proportion of virus was being constitutively produced by chronically infected cells, the drop in viral load in response to current agents would be more gradual.

CD4+ Cell Kinetics in Infection

Kinetic studies of changes in CD4+ lymphocyte counts have indicated that more than 1 billion new cells are produced each day in response to infection. Overall, at a given time, there are approximately one trillion lymphocytes in the lymphoid organs. It can be estimated that some significant proportion—eg, 10% to 25%—are HIV-infected. Thus there are perhaps 100 billion covertly infected lymphocytes (ie, if one tenth of the total are infected), with most being quiescently infected and with some proportion harboring deficient or replication-incompetent virus. Productively infected cells account for approximately 1 billion of the infected cells. It is speculated that the virus from these cells spills out from the lymphoid organs into the plasma, such that measurement of plasma viremia provides an indication of the level of uncontrolled viral replication in the lymphoid tissues. Further, changes in plasma viral levels during treatment provides an indication of the antiretroviral effect of the therapy on active replication.

As elaborated by Dr Saag, the picture that emerges is that of a titanic struggle between the immune system and the virus that begins on day 1 of infection and continues throughout the course

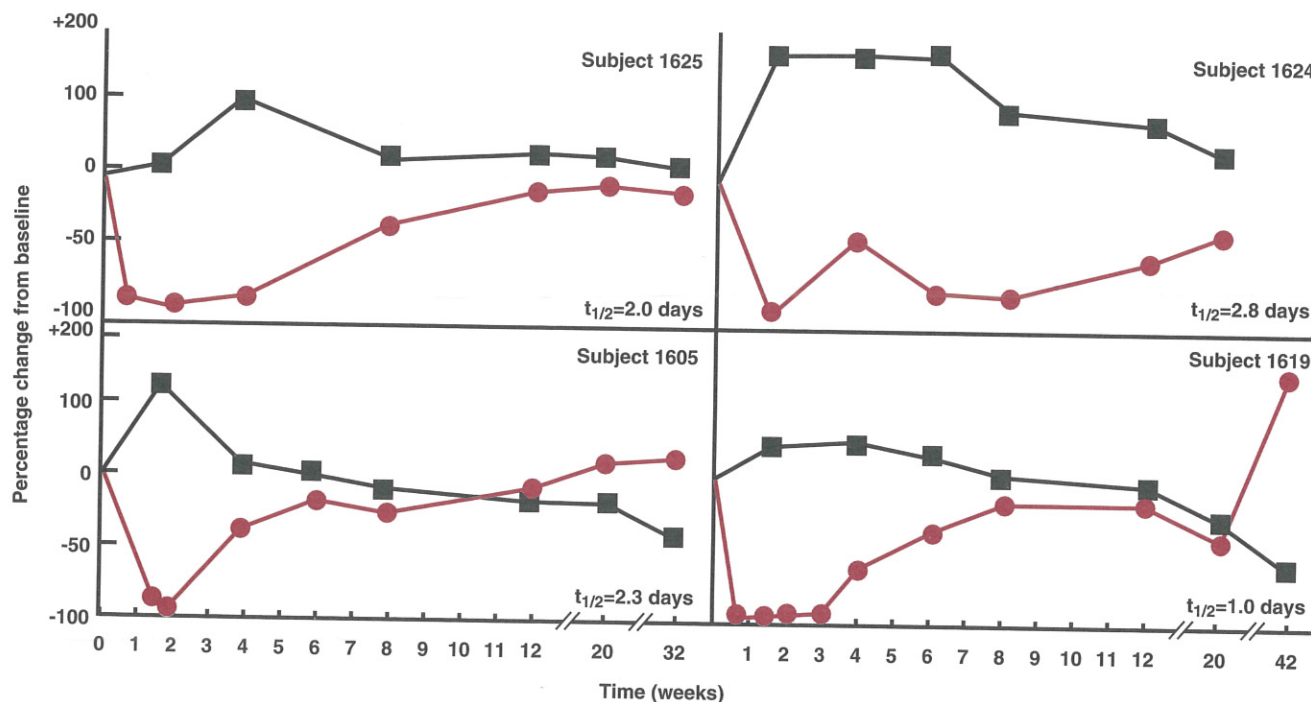


Figure 10. Plasma HIV kinetics in four subjects initiating nevirapine treatment. Shown are CD4+ cell response (■) and plasma viral RNA (●) as percentage change from baseline and computed half-life of viral population. Adapted from Wei X, et al. Nature 1995.

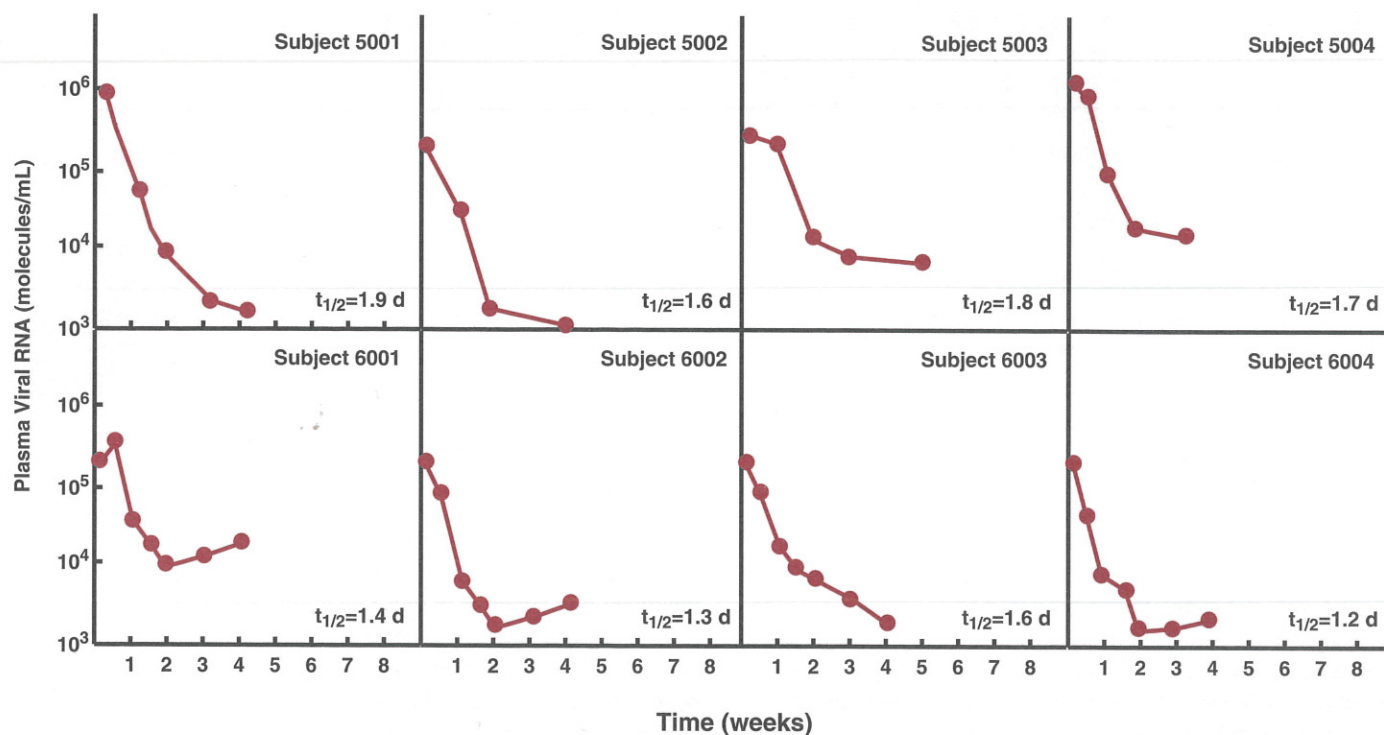


Figure 11. Plasma HIV kinetics in eight subjects initiating protease inhibitor treatment. Shown are log-scale changes in plasmaviral RNA and computed half-life of viral population. Adapted from Wei X, et al. *Nature* 1995.

of disease. The immune system creates a hostile environment that the virus attempts to overcome through sheer force of numbers. Dr Saag noted that although the goal of reducing viral production in plasma to zero has not been achieved with current antiretroviral therapies, reductions of from 10^6 to 10^3 copies/mL have been accomplished. Dr Saag suggested that the remaining virus represents

production from the pool of cells that are chronically infected and constitutively producing virus, and noted that this component of the viral population will not be affected until drugs active against integrated provirus are available. He also stressed, however, that the ability to reduce viral burden from 1

million to 1000 virions/mL and to maintain it at this reduced level for years would constitute a significant therapeutic achievement.

Treatment Implications

Dr Saag maintained that the advances in understanding of viral dynamics in vivo motivate a shift in the paradigm of antiretroviral therapy to one of treatment being tailored to the individual patient. Clinical studies provide a good idea of drug safety and perhaps of the relative activity of different regimens, but they do not provide guidance on how the individual patient should be treated. Each patient is host to a unique population of viruses and

exhibits a unique immune response. As an example of the failure of clinical trials to provide a good idea of how the individual is best treated, Dr Saag posited the administration of identical regimens to two patients for a 3 year period, with one patient developing resistant virus within weeks of beginning treatment and the other harboring virus that remains sensitive to the treatment for the entire course of the study. As related by Dr Saag, treatment in former is essentially equivalent to providing a patient with hypertension treatment for several weeks, after which the patient becomes refractory, and then following the patient for more than 2 years with essentially no treatment.

The ability to assess viral burden and effect of treatment on viral burden may allow individualization of therapy; Dr Saag identified the relating of viral load to clinical outcome as a primary research task in the coming year.

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