National AIDS Treatment Advocacy Project

Perspectives on Viral Load (HIV RNA) and When to Initiate Therapy

Community Education Series on Emerging Issues on HIV/AIDS
--a discussion of data --how to use viral load tests --how to interpret test results
Written and prepared by Jules Levin

TABLE OF CONTENTS

FORWARD

We are entering into a new era for treatment of HIV because of the development of the new class of drugs called protease inhibitors and secondly because of the development of the new technology called viral load testing. On June 3, the FDA granted approval to Roche Diagnostics for their viral load test, AMPLICOR HIV-1 MONITOR test, more commonly known as RT-PCR, viral load or HIV RNA PCR. It is the first test approved by the FDA. Chiron Diagnostic's bDNA test has been submitted to the FDA for consideration. Very recently, two important articles were published about viral load; key information from these articles are addressed here.

One of the two articles present important data from the Pittsburgh portion of MACS (Multi-center AIDS Cohort Study)--"Prognosis in HIV-1 Infection Predicted by the Quantity of Virus in Plasma" (John Mellors et al, May '96); it discusses the correlation between baseline viral load after HIV seroconversion and clinical progression (prognosis)--results say clinical progression can be predicted. The second article--"HIV Viral Load markers in clinical practice" (Mike Saag et al, June '96), discusses the recommended guidelines for using viral load tests by the USA advisory panel to the

International AIDS Society (IAS).

First is a reproduction (transcription) of a presentation by Dr. Robert Coombs on viral load: data related to using viral load measures and its correlation to clinical progression (prognosis); how to properly use the tests and interpret their results, and his comments on the IAS recommended guidelines. It is a mixture of direct quotes from his talk and discussion from this author. Dr. Coombs' talk was presented at the National AIDS Treatment Advocacy Project community treatment forum called: "Protease Inhibitors and Viral Load: Current and Future Use". It took place in Los Angeles on April 13, 1996. A limited supply of videotapes are available of the entire 4-hour forum by contacting NATAP.

Also available is NATAP's 46-page bound booklet--"HIV Protease Inhibitors Report-2nd edition", a comprehensive compilation of available data and information for 5 protease inhibitors. Soon after the Vancouver Conference an updated report will be available, in addition to comprehensive coverage of important breaking information from the Conference reported at our NATAP Internet Web-site

Dr. Robert Coombs, MD, PhD, FRCP(C) Departments of Laboratory Medicine and Medicine University of Washington--Seattle, Washington

INTRODUCTION

The quantitation of plasma viral RNA (commonly referred to as viral load) has provided valuable insights into the pathogenesis of HIV disease and activity of antiviral drugs, including protease inhibitors.

We need to define more clearly the correlates between plasma viral RNA, antiretroviral activity and clinical outcome or efficacy of a therapy. There is some missing information, which will be reviewed today, that we need to better understand the use and interpretation of viral load measurements.

Plasma RNA measurements will have an important role in the clinical treatment decisions doctors and other health providers will be making with HIV infected individuals.

Today, I will give some background and recommendations that are designed to give you some guidelines and guidance to understanding the use and interpretation of viral load measurements.

The following considerations will be addressed today:

- what does an HIV-1 RNA level mean?
- what constitutes a meaningful change in HIV-1 RNA level?
- do changes in HIV-1 RNA level have the same clinical meaning for persons with high compared to those with low HIV-1 RNA levels?
- what are the cost-to-benefit considerations in driving the virus load to "as low as possible?"

Without going into much detail about the technologies involved, there are two current approaches to measuring viral RNA:

- branched DNA (bDNA) assay, which is based on a signal amplification
- technology;
- RT-PCR is based on the reverse transcriptase methodology of amplifying the viral target; the AMPLICOR HIV-I MONITOR test which uses this method is commercially available
- two other methods, the NASBA or QC-PCR assays (quantitative competitive PCR), are available as research tests; they all share a common methodology of amplifying the viral target.

An important principle in this disease is that individuals who become HIV-infected establish an equilibrium between their host immune system and their virus within the first 6 to 12 months of infection. Dr. Coombs described three different groups characterized by the course of the progression of their disease, into one of these three which individuals may fall. Each of the 3 groups was represented on a graph (displayed by Dr. Coombs) depicting the course of that group's progression to AIDS. In the first group, individuals contain the virus effectively, have very low viral load levels (the equilibrium setting their viral load level at well under 10,000 RNA copies within the first 6 to 12 months of infection), and the graphic depiction of the course of their viral load measurements may remain very flat over the course of many years, possibly extending 10 years, and defines a slow-progressing group of patients. The second group does not contain the virus very well and is characterized by having very high viral load levels (100,000 or higher) within the first 6 to 12 months of infection, and they progress very rapidly to an AIDS defining illness, as early as three years after HIV infection.

The third group is in between, with between about 10,000 to 100,000 RNA copies, and patients falling into this group show an intermediate progression rate. The graph line depicting this group slowly ascends from the 6-12 month post sero-conversion period to 7 or 8 years out and depicts this intermediate rate of progression of the disease. As you can see, not all patients start off with low levels and progress to high levels as the disease progresses.

FDA concerns. If you take a patient's viral load measure at any point along the line of the course of their disease to assess their risk of progression, and if you lower their RNA measure with therapy--- the question that is not yet answered is---if you lower that RNA level to say 10,000 do you indeed alter the course of their disease progression? Will the future course of the progression of that person's disease be the same as a person whose viral load was at that level (10,000) prior to therapy? We don't yet know the answer to this question, but we surmise that will occur, i.e. that lowering of viral measure will alter the clinical course of the individual's disease--delay progression and prolong life. The FDA wants a study to address this question. The FDA's very recent approval of the RT-PCR test is for prognosis. In order to approve the tests for "monitoring clinical therapy", the FDA wants a study(s) that examines individuals who make therapy changes vs. those

who don't make therapy changes, after detecting viral load increases. The studies described below do not examine this question, but they study prognosis. However, many doctors and people with HIV/AIDS are using the tests for "monitoring clinical therapy', despite the FDA's limited approval. The FDA stated, in their approval language for the RT-PCR test, --"the test has also been used as an aid in assessing viral response to antiretroviral treatment as measured by changes in HIV-1 RNA levels". A few published papers, in the last year or so, highlight Dr. Coombs' principle. Following is data from a Mellors paper published in the Annals of Internal Medicine 1995; 122: 573-579, that looks at individuals in the Pittsburgh portion of the MACS group.

Viral load measures just after seroconversion predict disease progression, independent of CD4

The bDNA assay was used for this study. When the analysis was done, only the 1st generation bDNA assay, which measures only as low as 10,000 copies was available. Therefore, the plasma negative group are those individuals that had below 10,000 copies and the plasma positive group is those that had above 10,000 RNA copies. Since this analysis was completed, the 2nd generation bDNA test has become available, which measures as low as 500 copies; and, by looking at additional data, using the more sensitive assay, similar conclusions have been reached (discussed later in this article), that define a lower limit, of 20-30,000 RNA copies, that may indicate a more rapid progression. Baseline RNA measures were those obtained shortly after seroconversion.

Proportion of Patients Developing AIDS

	Plasma Neg. for HIV-1 RNA		Plasma Pos. for HIV-1 RNA	
CD4 Count at Seroconv. Cells/mm3	year 0-1	year 0-2	year 0-1	year 0-2
* >500	11%	6%	33%	45%
* <500	25%	0%	56%	86%

Individuals who had below or above 500 CD4, and had either no virus detected in their plasma (plasma neg.) by the bDNA assay (meaning below 10,000) or who had consistently greater than 10,000 copies (plasma pos.), had very different progression rates

to the development of AIDS. Individuals who consistently had less than 10,000 RNA copies had very low rates (6% and 0%) and individuals consistently above 10,000 had very high rates of progression and these differences were significant.

In fact, this study showed, that using the bDNA assay, individuals with more than 100,000 had a 10 times greater risk of developing AIDS within the next 5 years, than those individuals with under 10,000 copies; and, importantly these conclusions are independent of the CD4 count; that is, whether your CD4 was below or above 500, the rates of progression between the two groups (plasma pos. & plasma neg.) were significantly different.

Variability of viral load measurements.

Dr. Coombs displayed a graph showing only one individual's variability in RNA measures over the course of one week. Although anecdotal, it is intended to illustrate a point. This person's RNA was measured three times (morning, noon, and night) on Monday, Wednesday, Friday and the following Monday. There was a good amount of variability in the scores, both within given days and between different days. (These measures are approx.) On the 1st Monday, the measures were 25,000, 28,000 and 37,000; on Wednesday: 35,000, 37,000 and 35,000; on Friday-- 40,000, 47,000 and 84,000; on the last Monday-- 50,000, 57,000, 65,000, 67,000 and 71,000. There are rises of 2 to 3 fold, all within 1 week. The first measure (25,000) on Monday is more than 3-fold less than the highest measure on Friday (84,000).

There are two points, Dr. Coombs is trying to illustrate.

1. There is variability within individual measurements of the virus. It is
2. generally believed that the biological variability is 3-fold (or 0.5 log). This needs to be taken into consideration when sequentially assessing a patient's RNA measures;
3. Various factors perturb virus level: we are learning that concurrent infection, immunizations, and unexplained events can cause the virus load to jump quite dramatically 2, 3 or more fold. For example, reactivation of genital herpes by itself, which can be clinically innocuous, can result in a significant short-term rise in viral load.

A doctor and the patient need to understand there is variability in measurement and generally it is considered that a 3-fold difference in measures falls with the window of variability we may expect to see.

Commentary: If a 3-fold change is sustained repeatedly over time (sustained means repeating the test a few times with the same result), that difference may not be merely a variability, but may reflect a real change in viral load.

Relationship between disease progression and baseline RNA levels.

An important study you may have heard about is AIDS Clinical Trials Group (ACTG)

175. Dr. Coombs uses some data from this study to illustrate some points about viral RNA that he thinks are important. This study was chosen, to make his points, simply because the plasma was collected specifically for RNA measurement. That is, the proper anti-coagulant was chosen, specimens were processed in a very fixed time period and stored appropriately.

In this study, the RNA was measured by Roche's recently FDA-approved RT-PCR assay. Many of the natural history studies and other studies that have been published use different RNA detection technologies; and, the retrospective studies usually deal with plasma banks that were not initially designed to measure RNA in. Therefore, trying to define what the absolute RNA level is, can be very difficult in these retrospective studies. Furthermore, the different assays have been run without a common standard, and that makes comparison between one assay type and another problematic. For the management of patients, I think we want to get down to the issue of what is the absolute RNA level in the patient and how does that correlate with their overall immune status. It's much more difficult to manage patients based on relative levels of virus, but at the moment we are sort of in the position of having to do that.

[**Commentary**: There are many factors affecting the accuracy and reliability of RNA test results, and we have not yet been able to adequately understand how each of these factors may affect a measure or series of measures.]

ACTG 175 was randomized, double blinded, and involving 2,467 individuals, who were randomized to either AZT, ddI, AZT/ddI, or AZT/ddC. A cohort of 400 individuals from this study had a very detailed virological analysis done: antiretroviral naive 55%, asymptomatic 85%, symptomatic 15%. The mean CD4 count of 343 was relatively high. Plasma was collected and stored specifically for RT-PCR assessment.

Dr. Coombs showed a graph of unpublished data that's been presented at meetings by Dr. David Katzenstein, that shows the relationship between the percent of progression to clinical endpoints and baseline RNA levels {*baseline* means that the RNA measure was before receiving study drug(s)}.

The endpoints are divided into three groups:

- the complete clinical endpoint, which is the combination of a 50% decline
- in CD4 count, the development of AIDS, or death;
- the 2nd group is AIDS or death;
- and the 3rd group is just AIDS alone.

The progression to these endpoints for each of these 3 groups is compared between 4 different categories of baseline RNA ranges of measures. The first RNA group is those individuals with under 5,000 RNA copies; the second group is individuals between 5,000 and 19,000 copies; the third group is composed of individuals with between 19,000 RNA copies and 54,000; and the fourth group represents those with greater than 54,000 copies.

Those with over 54,000 RNA copies had, by far, the highest progression rates (prognosis), for each of the three clinical endpoint groups: the three markers; or progression to AIDS and death; or just death. Individuals with 19,000 to 54,000 RT-PCR copies had the next highest progression rate, in each of the 3 endpoint groups. Individuals with under 19,000 copies progressed the most slowly in each of the 3 endpoint groups; essentially, those from both groups, under 5,000 and 5,000 to 19,000, were quite similar in their rates of progression, as their was no significant difference. Clearly, in this study, disease is being driven by the people with the higher viral loads, more so with individuals with more than 54,000 copies, but also inclusive of those with more than 20,000 copies.

A 2 log RNA reduction is not the same for everyone.

The issue of relative changes is important. Dr. Coombs displayed a chart of 3 RNA measures: 1,000,000, 100,000 and 10,000. If each had a 2-log reduction in viral load, the 1 million would be reduced to 10,000---the 100,000 would be reduced to 1,000 and the 10,000 would be reduced to 100. The question to us--is the clinical benefit of driving the viral load down 2 logs, the same for individuals in the different ranges of RNA measure? Biologically and intuitively, you can surmise that those with the highest measure at baseline of 1 million, would be the group that would benefit most from driving the viral load down as low as possible.

This is a crucial question because it impacts how aggressively one pursues initiating or changing therapy, and at what cost do we drive the viral load down. Now that we have these very potent drugs--protease inhibitors--the practical use of these drugs is vital to individuals, in the hopes of optimizing and not wasting the potential benefits that may accrue to you from this class of drugs.

Following is a review of data that approaches this concern differently. This data was presented by Upjohn & Pharmacia at the 3rd Conference on Retroviruses and Opportunistic Infections in January 1996. It looks at the relationship in the change in viral load for the patients in their two studies (n=1740) of delavirdine, a non-nucleoside reverse transcriptase inhibitor, as measured by their own in-house assay, . The study participants have been randomized to different treatment regimens, and have been taking study drug for an average of 10 months (range 2 to 18 months).

The patients were grouped into 3 different viral load ranges of measures: greater than 1,000,000 copies; under 100,000 copies; and the middle group of 100,000 to 1 million. We don't know how these numbers correlate with the RT-PCR assay or the bDNA assay because the in-house test used by Upjohn wasn't run with the same set of standards as the other tests.

[**Commentary**: However, Upjohn says their assay was validated and it has the same degree of efficiency and reproducability as the RT-PCR assay; and, the RNA measure is 5 times that of the RT-PCR measure, i.e. a 1 million RNA measure, by the Upjohn assay, is equal to 200,000 RT-PCR copies, 100,000 copies by Upjohn assay is approximately equal to 20,000 RT-PCR copies.]

Individuals were also grouped by changes in viral load, that resulted from the study

therapy to which they were randomized; the 5 groups were: (1) individuals with an increase in RNA; (2) a RNA reduction of a 0.5 log to 0.3 log or 2-3 fold; (3) a reduction of 3 to 5 fold; (4) a decrease of 5 to 10 fold or 1 log; (5) a decrease of more than 1 log.

The graph shown by Dr. Coombs illustrated the point that individuals with the highest viral load (the group with more than 1 million copies), in this study, benefitted the most, as measured by clinical progression. In this group with viral load above 1 million (at baseline), individuals: in group (1) with an increase in viral load (subsequent to initiating therapy), showed the least benefit in clinical progression; there is a linear relationship in the amount of benefit each group had, as measured by clinical progression; that is, the more of a reduction in RNA that individuals had, then the greater their benefit, as measured by clinical progression.

The linear relationship between these groups was clearly evident in the group of individuals with greater than 1 million copies. In the middle group, of individuals with RNA between 100,000 and 1 million, the relationships between the 5 groups weren't quite as linear but, viral load reductions, even modest ones, showed reductions in clinical progression. In the group with under 100,000 RNA copies (20,000 RT-PCR copies), there seemed to be no difference in benefit to clinical progression, between the 5 groups.

Dr. Coombs said, this suggests to him that individuals with higher viral load may receive more benefit than individuals with lower viral loads from therapy that results in similar reductions in viral load. Again, he suggested this may be a factor in how aggressively we use therapy to drive the viral load down. [**Commentary**--However, As I suggest in discussion below, because this group is a healthier population, it may take much longer to detect differences in clinical responses.]

[**Commentary**: His earlier point was--what is the cost/benefit ratio of driving viral load down "as low as possible"? The results of this study indicate that at least among individuals with higher viral load (most likely for those with above 54,000 RNA, and mostly for those above 20,000), the amount of viral load reduction correlates with clinical progression, i.e. even incremental changes (a difference between 3-5 fold and 2-3 fold) in viral load produce differences in clinical progression (prognosis).

The Upjohn studies will not be completed until 1997, but data has been accumulated and analyzed for individuals with an average of 10 months in the trials (range 3-18 months). Although the analysis is preliminary, because the study is still ongoing, you can surmise some confirmation of viral load's correlation with clinical progression (prognosis). There are two studies. For protocol #17, the average CD4 was 135 at study entry, study participants averaged 1 to 1.5 years of AZT-experience. Subjects were randomized to either ddI alone or delavirdine + ddI. For the 2nd study, protocol # 21, subjects averaged 335 CD4, were AZT-naive or with under 6 months experience, and were randomized to either AZT, or AZT+delavirdine (Individuals were randomized to receive 3 different doses of delavirdine: 200, 300 or 400 3X/day).

Proportion of subjects progressing to AIDS or death

	protocol #17	protocol #21
greater than 1 million RNA at baseline	30%	12%
between 100,000 to 1 million	11%	1%
less than 100,000	1%	0%

As you can see, individuals with higher CD4 (335) in #21, progressed more slowly, than those with lower CD4 (135) in #17. These are individuals whose viral load changed as a result of therapy, but therapeutic intervention did not occur because viral load was increasing, for any particular individual (that is the FDA's point). It is only 10 months of follow-up, and we do need longer-term data, but the implications are encouraging.

Commentary (cont.): This study, in summary, makes 4 points: (1) changes in viral load measures, that occur from therapy correlate with clinical progression, at least at 10 months; but, as mentioned earlier if an individual's viral load is rising and you then intervene with therapy and the viral load declines--will that person's course of disease progression be positively altered? As Dr. Coombs said, we are surmising that it will alter the course of disease progression, but, he and the FDA say we need studies to confirm this; however, the concern is that some individuals in such a study would be randomized to the control group and thereby potentially suffer; (2) together, CD4 and viral load may be more predictive than either alone; (3) individuals with higher viral load may benefit more, from an equal viral load reduction, than those with low viral loads (under 20,000). Although this needs confirmation, it brings into the equation, the consideration of the cost/benefit ratio; (4) each incremental reduction in viral load (for example, a difference between a 2-3 fold and a 3-5 fold reduction) may result in a difference in clinical progression, which again may be more pronounced at higher viral load levels.

When does an individual initiate therapy??

Dr. Coombs is suggesting, that once your viral load is at a certain level or "threshold" (which, he advises, we have not yet been able to establish, and it is suggested from various sources that it could be 20,000, 5,000 or 10,000 again depending also on the assay being used; or, below "detectability" is suggested as a goal by some researchers--which can be below 500, 200 or 25 depending on the test used), then lowering your viral load even more should be weighed by the costs and potential benefits. The cost of

lowering your viral load can include using up treatment options, financial, and side effects. He is suggesting, it may be beneficial to save treatment options, rather than lowering viral load to "as low as possible"?

However, let me make reference to a commentary from below, but relevant to this discussion: "....in patients with more advanced disease (median CD4 cell count, 89/ul), disease progression occurred in up to 30% of patients with fewer than 10,000 HIV RNA copies/ml." --Saag et al, Nature Medicine, vol 2, number 6, June 1996. This progression rate, of 30% appears to be greater than for individuals with under 10,000 RNA copies but higher CD4 counts. Additionally, with reference to the Upjohn study data, the reason that for individuals in the lowest viral load group (under 100,000--or as Upjohn says, the equivalent of 20,000 RT-PCR), there may not be this linear relationship (whereby, those with greatest RNA reduction received the most benefit to clinical progression), is because this arm of the study had fewer participants, their CD4 counts were probably higher, there RNA levels were lower --therefore, they have less risk of progression, less endpoints have so far been accumulated and it should take more time to detect progression and the possibility of development of the linear relationship. Despite what Dr. Coombs says, this may leave open to question, his assertion that it may not be beneficial (cost/benefit ratio) for individuals with RNA below a certain level, say 5-10,000, to initiate protease inhibitor therapy. Research needs to address these type of questions, so we can try to better utilize viral load technology and protease inhibitors. Considering these factors, Dr. Coombs still suggests the cost/benefit ratio factor may limit treatment to individuals in the lower viral load levels.

The more "conservative," cautious, or less aggressive approach, put forward by Dr. Coombs, of when to initiate therapy, represents one school of thinking. Some other leading AIDS researchers are suggesting that the better approach may be to "hit hard and hit early". That is, treat with the most potent therapy and possibly treat as early as possible in an individual's disease stage, unless perhaps if your viral load is already very low (under 5,000 or 10,000) and/or your CD4 may be relatively high. Although, a more aggressive wing of this group may be supporting the notion of "hit hard and hit early" as soon as possible, even soon after sero-conversion--during primary or new infection (after contracting the virus)-- for the purpose of rendering an individual's viral load "undetectable" or as low as possible for as long as possible.

This approach is based in part on early or preliminary data discussed below, which was presented publicly at the Vancouver Int'l. AIDS Conference in July; in these 2 small pilot studies, suppressing virus to "undetectable" levels (in the 2 studies discussed, "undetectable" viral load was defined as below 25 RNA equiv/ml in one study and below 100 RNA equiv/ml in the other--as measured by bDNA) has kept viral replication in check, thereby prevented resistance from emerging, and permitting viral RNA to remain undetectable for a still ongoing period for all study subjects remaining in the trials; (see the NATAP paper Can HIV be eradicated" from the infectedindividual?)

Other trials' results have contributed to this thinking, including: Merck's trial of indinavir/AZT/3TC in AZT-experienced individuals; Abbott's trial of ritonavir/AZT/ddC in treatment-naive individuals. Also a basis for this most aggressive approach to treatment is the Boehringer Ingelheim 1046 study of treatment-naive individuals, which

was only recently (June 7) publicly presented (see Nevirapine article; 80% of the individuals in the group receiving the 3-drug combination of AZT/ddI and nevirapine had their viral load rendered "undetectable" (which in this case was below 200 RNA copies/ml--as measured by PCR); additionally, there is some follow-up out to 18 months where some individuals are still undetectable. In addition, treatment of a small number newborns with potent 3-drug therapy has resulted in similar results. However, it may be advisable to be circumspect about this new information (see discussion below).

There are some differences of opinion about which viral load level is low enough, is it under 25, 100, 200, 5,000 or 10,000? Despite the preliminary results from the pilot studies that are discussed below, researchers need to conduct further studies to confirm which viral load levels should be targeted with therapy. If your viral load is 5,000, should you initiate protease inhibitor therapy now or should you wait? The answer to this question is more clear if your viral load is over 20,000; and, becomes more clear the higher one's viral load climbs; the IAS interim guidelines (discussed below) recommend driving one's viral load as low as possible or to "undetectable levels"; while Dr. Coombs is not convinced that is necessary, for him 5,000 may be acceptable.

This relates directly to the question--when should an individual initiate therapy, and with which drugs?? If your viral load is 5,000, should you initiate protease inhibitor (or nevirapine) therapy now or should you wait? The IAS guidelines described below recommend initiating therapy if your viral load is above 5-10,000 and your CD4 status suggests progression. In light of the new data revealed in Vancouver, the authors of the IAS guidelines may or may not revise their recommendations. I think that for doctors and their patients, there will be no clear recommendations forthcoming, because the interpretation of this data will probably remain controversial for a good while.

The subject of cross-resistance between protease inhibitors is relevant to this discussion. Will treatment with a particular protease inhibitor cause any or significant cross-resistance to another protease inhibitor? We do not yet adequately understand the cross-resistance relationships between different protease inhibitors. If your viral load is relatively low, by waiting until we better understand cross-resistance between protease inhibitors, you may be better able to optimize your results from therapy. But, we may not have information for a good while (1 year maybe), that is adequate to understand cross-resistance sufficiently to answer these questions (see Pre-Vancouver Protease Inhibitor Update for a discussion of ACTG 333, the first study of protease cross-resistance). Also, there are many individual and theoretical factors that can be considered in making these decisions. Does one want to use the best available therapy early in their disease stage, rather than save it for later? The aggressive treaters say yes, and some of the underpinning for their thinking is discussed below. They say, there will be other therapies available. Other treaters are more cautious about spending options. The answers to many of these questions are not yet clear.

The 2 studies presented in Vancouver are ongoing small pilot trials meant to test specific hypothesis'. Twelve therapy-naive individuals were recruited who were chronically infected (HIV+ for a while with some progression), and were treated with open-label nelfinavir (750 mg 3x/day) and AZT/3TC. In the 2nd study, 12 individuals who had recently sero-converted were recruited and treated with open-label ritonavir/AZT/3TC.

The principal investigators of both studies, David Ho and Martin Markowitz of the Aaron Diamond AIDS Research Center in New York City, wanted to explore the extent to which these therapies in these populations could turn off viral replication and the longer-term implications of that. In the sero-converter, trial the investigators also wanted to explore the possibility of "eradicating" HIV-1 in a recently infected person with a relatively intact immune system.

In Vancouver, only 4 months of data was available for presentation for the treatment-naive, chronically infected group treated with nelfinavir/AZT/3TC; and, the data so far available and presented in Vancouver, for the sero-converter study of ritonavir/AZT/3TC, extends only to between 4 and 10 months for the study subjects. Again, all study subjects, in both trials, remaining on therapy had their viral load rendered "undetectable."

The meaning and interpretation of these study results, and their potential application has created much controversy in the AIDS research and medical community. Many feel it is pre-mature to draw strong conclusions from these preliminary results; and that it is pre-mature to begin treating sero-converters and treatment-naive individuals in this way, i.e. "hit hard and early" with the most potent therapy at the earliest possible time of intervention. However, some researchers disagree and expressed that we should treat individuals with this "hit hard and early" approach to therapy.

The potential benefits of a potent 3 or 4 drug therapy may be different for individuals who have extensive prior drug experience with nucleosides (AZT, ddI, ddC, 3TC, d4T). These individuals may not be as responsive as those who are treatment-naive, and of course as those who are recently infected. If you merely add a protease inhibitor to other drugs you have been taking for a while, you may not be able to reduce your viral load to undetectable; or, if you are able to reduce it to undetectable, it may not be as likely to remain at that level and it could begin to rebound. For this group of individuals, the set of issues are considerably different, more difficult and cannot be bunched together in a discussion with treatment-naive or newly infected. However, there are promising treatment approaches that can be utilized by this group. Protease inhibitors now in human trials that are not yet approved are Agouron's nelfinavir and Glaxo Wellcome's141W94; as well, Glaxo Wellcome's 1592U89 is a potent NRTI (nucleoside reverse transcriptase inhibitor) which is also in early human trials. There are real prospects for sequencing from one of the currently approved protease inhibitors onto a different one after they become available. Other antiretroviral drugs are in either available or in human studies, including DMP-266 (NNRTI), nevirapine, (NNRTI), and delavirdine (NNRTI), which will be useful in designing treatment strategies for those who are drug-experienced. I am just concerned that all the attention appears to me to be focused on the treatment-naive and newly infected populations without adequate attention to those who are treatment-experienced.

Clearly, there are many remaining questions: how durable will the responses be of the individuals in the 2 aforementioned studies (treatment-naive and newly infected)? What will the findings be of residual virus in other "compartments"--CSF, lymph nodes, testes, etc.? Only a small number of individuals (24) were studied, what follow-up studies do we need? Individuals with advanced HIV (low CD4s) may not respond as well to treatment as those who are seroconverters or treatment-naive. What treatment strategies will be best

for individuals with moderate or advanced disease who may have exhausted most if not all previously available treatment options? How will protease inhibitor cross-resistance effect treatment strategies for all of these different types of individuals?

A number of discussions are onging to devise future studies to address some of these remaining questions. Studies are being planned for individuals with greater than 500 CD4. Four-drug combination studies are being planned, to begin shortly, for treatment-naive and possibly for treatment-experienced individuals. One proposal currently under discussion is devising a study to allow individuals to begin stopping part or all of their therapy after being "undetectable" for a prolonged period of time, to see if they remain undetectable. Other unique approaches to study design are being discussed and planned.

Cautious approach to "new paradigm".

It is very exciting that we can suppress virus to "undetectable" levels, and that viral levels can remain undetectable for a prolonged period, but there are reasons to consider for not rushing too quickly to accepting these new approaches to treatment of HIV, i.e. "hit hard and early" in primary infection, with treatment-naive individuals and with all others as well. It may be premature to design a new approach to treatment of HIV based on such a small body of research. Some observers say, a potential problem could be-- how long will viral load stay undetectable for some individuals, and what options remain for those individuals if and when their viral load rises? Dr. Coombs contends that these approaches, such as treating acute infection or early intervention (individuals with "higher" CD4), and using 4-drug combinations are promising theories, that need to be adequately researched before we put too much stock into them. Without more extensive studies of treatment intervention in primary (new) infection and treatment-naive individuals, doctors may not be willing to utilize such approaches to treatment. However, it may be helpful for you and your doctor to be informed and educated about these issues, and to discuss treatment strategies with a knowledgeable physician(s).

A challenge of a different sort is compliance with adhering to the regimens for taking protease inhibitor drugs in 3- or 4-drug combinations (i.e., taking the fully recommended doses, at all recommended times, and not taking reduced doses or missing doses---non-compliance can cause resistance). This challenge may be a formidable one, as many individuals are already non-compliant. If a large body of people are not compliant, we could end up with a large pool of protease inhibitor resistant people, which could be transmissable. For individuals with early disease and no signs of sickness, will they be compliant with the rigorous demands of taking 3 or 4-drug protease inhibitor therapy? Of course, this shouldn't deter researchers and doctors from recommending the utilization of protease inhibitors and multi-drug combinations in the way that will optimize benefits for individuals that will be compliant. It is the responsibility of society to devise a strategy for dealing with non-compliance: the drug companies, federal public health and research officials, the medical community, and the "activist" community.

As mentioned earlier, one of the newly suggested approaches to HIV treatment is, if an individual remains "undetectable" (below the level of detection by laboratory RNA tests capable of measuring as low as 10 RNA copies, or is under 100 or 200 copies adeaquate?) for 18 months or 2 years, can that individual begin to stop taking 1, 2 or all

of the medications he was taking? Is that person's virus "eradicated"? Will their virus remain in "remission"? HIV may be present in a number of "compartments" in the body besides the blood.

The belief that the virus can be "eradicated" or that lowering viral load to "undetectable" may not be true, if the virus and its replication is driven by the "reservoir" hidden in these compartments. Does protease inhibitor or nevirapine therapy accompanied by 2 or 3 additional drugs affect the virus hidden in these compartments? Or, will this hidden virus emerge after an individual may begin to stop taking 1 or 2 drugs of their combination therapy? And of course, there is the possibility that virus can be driven from these other "compartments. Continuing studies are necessary to explore this unknown.

Despite these difficult questions and obstacles, it is important to remember we are in fact entering into a new and exciting era for the treatment of HIV. Never before have we been in a position to ask some of these questions---such as, can we "eradicate HIV from an infected person? But, we need to remain circumspect, to encourage continuing research to address the unanswered questions, and not to allow ourselves to become overconfident or complacent.

It may be however important to better understand the thinking of those who are supporting the notion of being aggressive with new approaches to HIV treatment. Aside from the early new data from these pilot trials, a reason some experts recommend "hit hard and hit early" is, because the earlier it is in an individuals disease progression, their virus has not had as much opportunity to replicate and therefore mutate. The virus will tend to be more homogenous; the contention is that a homogenous (the virus hasn't had ample opportunity yet to mutate very much) virus is more receptive to treatment and less likely to develop resistance to the treatment as quickly as an individual's virus that is more heterogenous from replication and mutation.

Additionally, it is widely accepted that disease progression and consequent sickness and death is a result of a depleted immune system. Presumably, the earlier treatment begins, the more intact an individuals immune system will be at the start of therapy; and, hopefully therapy will maintain the healthy status of the immune system, or at least maintain it for a longer period of time.

This thinking is at least partially based on the recent research developments from David Ho, George Shaw and others. Prior thinking was that there was a prolonged period of relative virus latency; this has been replaced with the thinking that ongoing, high-level viral replication takes place from the time of initial infection. This research says, as many as 10 billion new HIV virions are produced per day, with a half-life in plasma of 6 hours. CD4 cells, a principle target for the virus responsible for viral replication, are also produced in high number, and once productively infected, have a half-life of 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.

Additionally, prior to now the only available drugs for treatment of HIV were of very moderate benefit (AZT, ddI, etc.). Now, we have much more potent drugs-- protease inhibitors, nevirapine--with which to treat HIV, and we've found that resistance can be

suppressed for a prolonged period of time by utilizing a protease inhibitor or nevirapine as part of a potent 3-drug combination; also, we now have available the viral load test by which to measure antiviral activity. Of course, researchers are as yet uncertain of the durability of the suppression of virus resulting from potent 3-drug therapy.

Some of these experts are comparing treatment of HIV with treatment of cancer and tuberculosis, by saying the traditional or the classic approach to treatment of cancer and TB is to hit "early and hard" with combination therapy. However, can it be compared to cancer and TB? Again, they postulate, that if treated early enough in the disease with potent therapy, an individual may be better able to, in a sense, put the virus in remission; that is, lower the viral load to an adequately "undetectable" level (undetectable does not mean virus isn't present--how low is enough?), which will hopefully stop viral replication and prevent the emergence of resistance; or, possibly "eradicate" the virus from the blood or the system. The objections to pre-maturely accepting this thinking are outlined above; it may be advisable to remain at once excited, skeptical and circumspect until we have more data from studies implemented to explore and confirm these new approaches to treating HIV.

Nonetheless, the ACTG 175, Upjohn and MACS research, discussed above, appear to indicate that reductions in viral load can alter the course of clinical progression. Although, reductions in RNA were mostly modest in the 175 and Upjohn studies, the drug therapies used in the study did not include any protease inhibitors. In the group with over 1 million viral load, a 1 log or greater reduction in RNA produced the most benefit to clinical progression, when compared to individuals, in the other groups with less viral load reductions. As you know, successful protease inhibitor therapy, which utilizes effective multi-drug combinations, has produced in some instances, significantly more than 1 log decreases in viral load. Importantly, still to be confirmed, is the durability of these reductions in viral load.] *end of commentary*

Treatment effects are not totally explained by CD4 and RNA.

Dr. Coombs displayed a graph of data from Bill O'Brien's VA study (O'Brien et al, NEJM 1995) which indicated that individuals with both an increase in CD4 and a reduction in viral load clinically progressed more slowly than individuals who had neither and individuals who had only a CD4 increase or a viral load reduction. Dr. Coombs said, that looking at combinations of responses to drugs, both immunologic (CD4) and virologic (RNA) markers are more beneficial than looking at one or the other alone. The data from the Upjohn studies support CD4 and viral load together are more predictive.

He went on to say, we still have a lot to learn about what exactly are the factors that define the treatment effect. At the moment, we are trying to understand how much of the treatment effect can be explained by changes in RNA and/or CD4, and it's clear that even together they explain only part of it, but not all of it. Other studies have illustrated that other factors are relevant, such as properties of the virus and other additional properties of the host, that are extremely important in defining the types of response that individuals will experience from changes in viral load alone. So, it is much more complicated than we are currently appreciating, to better understand an individual's response to treatment. At the moment, we are left with more simple markers of the disease, for example CD4

and viral load, to help make clinical decisions about what does or doesn't constitutes effective therapy. In future clinical studies, it would be helpful if we are able to factor in other properties or disease markers.

Guidelines for using viral load testing and interpreting measurements.

The discussion so far by Dr. Coombs is helpful in placing into better context the following part of his discussion. The International AIDS Society (IAS) convened a USA advisory panel, who, after careful deliberation, have assembled a list of interim recommendations related to viral load measurement and the use of test results. The panel consists of:

- Michael Saag, MD, University of Alabama-Birmingham
- Mark Holodney, MD, Stanford University
- William A. O'Brien, MD, UC-Los Angeles
- Robert W. Coombs, MD, PhD, University of Washington
- Margaret E. Poscher, MD, UC-San Francisco
- Donna M. Jacobson, BS, IAIDS Society-USA
- George M. Shaw MD, PhD, University of Alabama-Birmingham
- Douglas D. Richman, MD, UC-San Diego
- Paul A. Volberding, MD, UC-San Francisco
- Daniel R. Kuritzkes, MD, University of Colorado

In fact, these recommendations have just been published (Nature Medicine, volume 2, number 6, June 1996). The recommendations are characterized as interim, because more information is important to better understanding how to use the tests, the meaning and interpretation of test results, and how to better apply these results. Currently, individuals and their doctors are utilizing the test and its results in managing HIV, and in fact it is widely believed that proper use of the test and its results can be helpful in clinical practice. These recommendations are intended to provide helpful guidelines for patients and doctors.

The following questions are addressed by the recommendations:

1. Where to initiate therapy?
2. What target level of HIV RNA should we look for after initiating therapy?
3. What is the minimum decrease in HIV RNA indicative of antiviral activity and hopefully efficacy of the drug or therapy?
4. What is the change in RNA level that suggests drug treatment failure?

5. How frequently should we monitor RNA?

6. What are the optimum methods of specimen processing?

With each IAS recommendation, Dr. Coombs has added his own editorial, sometimes differing with the recommendation.

(1) Where to initiate therapy?
panel recommendation: More than between 5,000-10,000;

Dr. Coombs-- editorialized that 5-10,000 applies when using the bDNA test, but if using RT-PCR, he recommends greater than 20,000, because from his experience the RT-PCR can run twice as high as bDNA.

----The panel strongly encourages therapy at greater than 25,000; Dr. Coombs says 25,000 by bDNA and 50,000 by RT-PCR. In clinical and natural history studies reported to date, some people with higher than 25,000 (bDNA) or 50,000 (PCR) are in the group associated with higher disease progression rates.

Dr. Coombs: Although RT-PCR and bDNA correlate well in their results, there are no common RNA assay standards; therefore, values from the bDNA assay cannot be readily translated into values from the RT-PCR assay. One common recommendation is if you are being monitored by one assay type, you should continue using it, and not switch from one assay to another, until we have common assay standards.

(2) Target level of RNA after initiating therapy

The panel recommends--"undetectable levels of plasma HIV RNA should be sought... under 5,000 would be acceptable." (Dr. Coombs says--5,000 by bDNA is the equivalent of 10,000 by RT-PCR). Panel: "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" having been used. "Prospective clinical trials are urgently needed to address this question."

Dr. Coombs: Under 10,000 (bDNA) or 20,000 (RT-PCR) RNA copies is probably a reasonable target, based on that individuals with these measures appear to have a very slow clinical progression rate. We don't yet know how much benefit accrues from driving the viral load much lower. Since this is a slow progressing group, establishing the clinical benefit to them from a log or more change, will take some time to sort out.

[Commentary: Dr. Coombs' approach is cautionary. But, more information to better understand if it is beneficial to lower one's viral load to 500 rather than 4,000 would be helpful. Or more to the point of the discussion, is there a difference between 10,000 and 5,000? Dr. Coombs' earlier discussion of the cost/benefit ratio of therapy decisions is applicable to this situation.]

[commentary: MACS--correlation of viral load measurement with clinical progression. At this time, the subject of viral load has taken center stage, as the FDA recently reviewed and approved Roche's application for their RNA test. An article has just been

published, "Prognosis in HIV-1 Infection Predicted by the Quantity of Virus in Plasma," (Mellors et al, Science, vol 272, 24 May 1996). It is fitting to mention here some results, because the study (the Pittsburgh portion of the MACS trial), which this article is about, offers data about clinical progression to AIDS and survival for groups of individuals with viral load under 4,430, of between 4,531 and 13,020, and over 13, 021. Above, Dr. Coombs refers to the lack of data regarding the benefit of driving viral load much below 10,000--- is there a benefit to lowering viral load from 4,000 to 500? What is the cost/benefit ratio? Data from this retrospective study is related this discussion.

One hundred and eighty seropositive study participants enrolled in MACS in 1984 and 1985. Their blood specimens were taken at study entry and every 6 months and stored. RNA measures were taken and analyzed retrospectively, from the stored samples. Individuals were followed for progression to AIDS (1987 CDC definition) and death. Based on their retrospective baseline RNA value, individuals were divided into the following ranges of RNA: under 4,530; 4,531-13,020; 13,021-36,270; over 36,271. For this analysis, HIV-1 RNA was measured by the new 2nd generation bDNA assay; the 1st assay upon which the first MACS data analysis was based (discussed earlier in this article) is limited, in that it measures down to 10,000 RNA copies; this 2nd generation assay is more sensitive, as it measures as low as 500 copies. Since, we are discussing whether it may be beneficial to reduce one's viral load from 10,000 to 5,000 or lower, the data from this study has some relevance.

It is in context to quote from the Nature Medicine article--"Higher HIV RNA levels correlate with lower baseline CD4 counts, more rapid declines in CD4 counts, and more rapid disease progression....maintenance of plasma HIV RNA levels below 10,000 in early HIV disease appears to be associated with decreased risk of progression to AIDS. However, in patients with more advanced disease (median CD4 counts, 89/ul), disease progression occurred in up to 30% of patients with fewer than 10,000 HIV RNA copies/ml." -----The cost/benefit ratio concept is importantly relevant to this last sentence. If individuals with lower CD4 counts have more potential to progress in the under 10,000 copy range, than those with higher CD4 counts but also with under 10,000 RNA, then this may dictate a different and more aggressive approach (for individuals with lower CD4 and under 10,000 RNA) to the question---when should an individual initiate or change therapy?

"For the 4 groups, ranging from the lowest through the highest viral load, the proportion of subjects who progressed to AIDS by 5 years after study entry were 8, 26, 49 and 62%. The median times to development of AIDS for subjects in these 4 groups were greater than 10, 7.7, 5.3, and 3.5 years. (Remember, the data collection was only out to 10 years). For the 4 groups, ranging from the lowest through the highest viral load, the proportions of subjects who died within 5 years were 5, 10, 25 and 49%. The median estimated survival times in these 4 groups, ranging from the lowest viral load to the highest were, greater than 10, 9.5, 7.4 and 5.1". These results are mostly independent of CD4 values.

Although it is not clearly defined, you may be able to infer, from these study results, that lowering viral load from 13,021 to under 4,530 may have value. However, if you factor in the cost/benefit ratio considerations, the value of such a reduction in RNA is more

complicated to determine.] *end of commentary.*

(3) Minimal decrease in HIV RNA indicative of antiviral activity and hopefully efficacy

Panel recommends: at least a 0.5 log reduction that is sustained (3-fold or greater), based on our understanding of factors affecting variability (biological and within-assay variability). "It is likely that the clinical benefits of antiretroviral therapy are related to the duration as well as to the magnitude of HIV suppression...., although the precise duration of HIV suppression necessary to result in measurable clinical benefits still needs to be clearly defined."

Dr. Coombs: Sustained decreases of as little as 2-fold, in clinical studies, have been associated with clinical benefit. Just looking at the amount of RNA reduction (0.5) may not be adequate to judge the prospect of clinical benefit. As discussed earlier, a 1 log decrease for an individual with a viral load of 1 million copies may be more beneficial, to that individual, than a 1 log reduction for an individual with 20,000 RNA copies. For example, a 1 log reduction for an individual with 1 million RNA copies will lower his viral load to 100,000 copies, and 1 log reduction for an individual with 20,000 RNA copies will lower their viral load to 2,000 RNA copies.

(4) Change in HIV RNA that suggests drug treatment failure

Panel: "The return of HIV RNA levels to pre-treatment (or to within 0.3-0.5 log of the pre-treatment value) values, confirmed by at least two measurements, is indicative of drug failure and should prompt considerations of alternative treatment regimens".

Dr. Coombs: A return to baseline is probably an indication that the viral load is no longer reflecting antiviral activity. Again, this change is very dependent on starting RNA level. For example, a return to baseline, if baseline is less than 10,000 (bDNA) or 20,000 (RT-PCR) may not be so bad, and addresses the issue of the cost/benefit ratio of driving viral load lower, without evidence of clinical benefit, which we urgently need to obtain.

(5) Suggested frequency of HIV RNA measurement

Panel: At baseline: 2 measurements, 2 to 4 weeks apart to assess the inherent variability. Subsequently, measurements might be obtained along with the CD4 count every 3 to 4 months, since serial determination of both markers simultaneously provide useful information. Shorter intervals may be appropriate as critical decision points--such as the return of the viral load to baseline values--are approached. RNA levels should be measured 3-4 weeks after initiating or changing therapy to determine antiviral activity, before waiting to see if the CD4 count reflects a change.

(6) Optimal methods of specimen processing

Panel: Optimal procedures for storage, handling, and processing samples have yet to be fully defined. Each provider should adopt consistent procedures for handling specimens, including using the same collection tube and anticoagulant, processing techniques, transport and storage procedures. To minimize HIV RNA 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. Consistent use of the same assay in an individual patient is very important.

Dr. Coombs: Use the recommended anticoagulant, as it varies, depending on which assay you are using. Prompt processing and freezing of the specimen within 2-4 hours, or at least within 6 hours of phlebotomy.

Conclusion by Dr. Coombs at the forum: In the long term, only carefully controlled trials will prove to us the utility of HIV RNA measurement for routine clinical management of HIV-1 infected individuals. Many vital questions remain to be addressed for us to understand how to best utilize the new potent therapies; drug companies, our federal government and academic researchers must be held accountable for properly conducting exhaustive research until we have the answers we need.

In this discussion, varying points of view on different issues are presented for the purpose of objectivity and to convey a better understanding of the issues. In the end, the intent of this paper is to assist individuals and medical care providers in making more informed treatment decisions.

About The National AIDS Treatment Advocacy Project (NATAP): NATAP is a New York State non-profit corporation dedicated to facilitating the effort for development of effective treatment for HIV. We advocate on treatment and policy issues for people with HIV and AIDS, with drug companies, researchers, government officials (including the FDA) and other treatment and policy advocates. We are equally committed to disseminating important information about these treatments to concerned people. NATAP is committed to the concept of, at least, making HIV manageable.

About the author: Jules Levin is the Executive Director of NATAP, based in Brooklyn, N.Y.

For additional copies of the report, please contact: National AIDS Treatment Advocacy Project, Inc. 72 Orange Street, #3C, Brooklyn, N.Y. 11201 Telephone: 718-624-8541, Fax: 718-624-8399 Internet Treatment Web page address: http://www.aidsnyc.org/natap