

## **NATAP REPORTS**

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**The information in this newsletter is only for educational purposes. Before making any treatment decisions one should consult with their doctor(s).**

### **Newly Uncovered Viral Reservoir**

In a recently published article in Science, Dr Robert Siliciano of Johns Hopkins University discussed his findings of a viral reservoir of post integrated proviral DNA persisting in resting CD4 memory t-cells. These cells are in a reversible state of non-productive infection but can be capable of producing infectious virus if stimulated by antigen. He said that the frequency of these cells and their decay rate are important to evaluating the effect on the potential for eradication.

Using a sensitive assay he found the frequency of these cells to be extremely low. The replication competent integrated proviral DNA was found in less than .01% of resting CD4+ t-cells. He took a lymph node biopsy of 14 individuals and found that the frequency of these cells are not different in the blood and the lymph node. This is important because it may allow researchers to make implications about lymph tissue proviral DNA just by taking a peripheral blood sample. Lymph tissue biopsies can be difficult to perform. He ran an independent assay, a virus culture, because there was thinking that all the DNA was not competent to reproduce virus. His findings were confirmed that some DNA is competent to reproduce virus.

In order to determine the decay rate of these cells, Siliciano entered into a collaboration with clinical researchers to see if this reservoir persists in individuals who have been on long term highly active antiretroviral therapy (HAART). For this study, he selected patients: who were likely to maintain a high level of compliance to the regimens; who were on three or four drugs at least one of which was a protease inhibitor; who had a rapid response to therapy (who became undetectable within 2-3 months); and, who remained undetectable (200 copies/ml) with multiple measurements for the period of study including the times for which they took blood to look for latently-infected cells.

Replication competent proviral DNA was found in resting CD4+ T-cells in essentially all the individuals (18/18), including those who were on therapy for up to 30 months, and it was found that it can persist for a while. In four patients they were unable to get enough resting CD4 T-cells to conduct proper tests. Unfortunately, he concluded that the frequency of these cells in the study individuals was not reduced by being on therapy for a longer period of time. The frequency for those who were on therapy the longest was not much different for those individuals who were on therapy for a short period of time. But, he only took one blood sample from each person. So, he was unable to observe a change in the amount of HIV DNA for an individual. You have to take two blood samples over time to observe a change. He believes that we will have to conduct studies following individuals over time to try and determine the decay rates of these cells.

For comparison sake, it has been generally accepted that free virus only persists for minutes or hours. Cells that produce most of the virus last only a day or two. Cells with unintegrated DNA retain the ability to produce virus for only a few days and extracellular

virus particles bound to follicular dendritic cells (in the lymph tissue) last for about two weeks. Although we have less information on chronically-infected macrophages, their half-life appears to be about two weeks. The half-life of resting CD4+ T-cells with integrated proviral DNA could be 5 to 7 months. This is consistent with the fact that these are memory T-cells and their biological function is to persist. Some researchers have said that memory cells can last for a number of years.

However, after being on HAART for a period of time, an individual may experience a partial restoration of the immune system. If the CD4 cells increase appreciably in conjunction with some immune restoration it is possible that the immune system could control virus that might be produced by the activation of these resting cells. It is also possible that the therapy itself might control a burst of viral replication that could occur from activation of this latent virus. This can only be determined by following individuals over time.

David Ho and the ACTG are planning to conduct studies of strategies designed to target these CD4 memory cells harboring latent virus. Stay tuned.

## **Protease Inhibitors**

The following article reviews the latest updates of data from numerous studies of protease inhibitor therapies reported at recent conferences extending out as far as we now have data, from 1 to 2 years varying by the specific therapy. In general, 80% or more of the study participants were undetectable (by the standard viral load tests, less than 400 or 500 copies/ml). Success with the treatments is associated with continuing to take the regimen, compliance, tolerability of the drugs, and adequate drug blood levels. Also discussed here is the latest treatment strategy information.

Two years is a relatively short period of time. We need to follow individuals taking these treatments for many more years, to monitor how long benefits will last; to evaluate how long viral load reductions and CD4 increases extend life and sustain good health, and to evaluate the tolerability and safety over the long term.

**Treatment Options After Failing a Protease Inhibitor.** Many individuals have developed resistance to a protease inhibitor therapy. If your viral load is stable you may want to not make any changes until more therapies are available. A more durable benefit will occur if you can assemble a potent regimen consisting of 3 drugs each of which can offer you as much antiviral activity as possible. You may want to consider 4 or more drugs for a regimen. Inside this issue is a report by Dr Cassy Workman who used a 6-drug regimen. Failure of any drug in a specific class can reduce the benefit you may receive from a drug in the same class, so a drug may not really be totally new if you've become resistant to a drug in its class. See the [Resistance Supplement](#) below which includes a review of the latest data on 1592U89 resistance and cross-resistance.

Inside this issue we discuss data and information for all the new drugs expected to be available in 1998, as well as information and data on some of the new combinations in research. Combining two protease inhibitors or a protease with a NNRTI can offer potency. Delavirdine is the only NNRTI that significantly increases protease inhibitor

blood levels. Inside this issue is a report on the preliminary effects of delavirdine on the blood levels of certain protease inhibitors. Hydroxyurea in combination with ddl or ddl+d4T can offer a potentially beneficial treatment option.

At this point, most studies attempting salvage therapy after protease failure have produced a small percentage of success. However, usually the regimens used in these studies have not been as potent as they could be. To design a good regimen you will have to use your intuition and knowledge about available treatments. The information in this issue about double protease combinations and NNRTI-protease combinations can be useful. However, I've heard anecdotal reports of 4, 5 or 6 drug regimens being successful in suppressing protease resistant virus. Success may depend upon the specific protease drug you've failed and the level of resistance developed.

The resistance supplement below and the NATAP web site review the studies that have been conducted attempting salvage therapy. There are two studies that offer potential exceptions to the disappointing results from others. At ICAAC, Dr Keith Henry reported preliminary data on a small study of individual who failed nelfinavir+AZT/3TC, had limited nucleoside experience, and were switched to a 4-drug regimen of ritonavir+saquinavir+d4T/3TC. Initially, 12/12 individuals were <500 copies/ml, and at week 16 6/7 were less than 500 copies/ml. The key is the durability. 16 weeks is too soon to make conclusions. An update will be reported at the '98 Human Retrovirus Conference. In ACTG 347, individuals who failed 141W94 monotherapy were switched within two weeks of failure (which maybe a key) to indinavir+nevirapine+d4T/3TC. In the first few months, the group (n=33) has responded well as 29 individuals are less than 500 copies/ml, and 4 persons had viral loads under 2200 copies/ml. It appears as though viral load is trending down. Again this is preliminary as durability is the key. Updates will be reported.

The ICC (Inter Company Collaboration) is a coalition of drug companies that manufacture antiretroviral drugs for HIV. They are planning to start a 6 site study in early '98 for individuals who have failed a protease inhibitor. They have designed a variety of numerous and unique combinations of experimental and approved drugs to use in this study. The study, the combinations, and the sites should be in place by February '98. It's expected that there will be a site in New York City and other major cities. This might be a good opportunity to access a drug combination that you might otherwise not be able to put together. The studies will include new experimental drugs that are not generally available, such as 141W94, adefovir (PMEA) 1592U89, DMP-266, MKC-442 (a new NNRTI). Look for the announcement for the start of the study.

**Monthly Monitoring of Viral Load and Immediately Switching to a Potent Regimen Upon Rebound in Viral Load.** This might be a key factor in potentially avoiding protease inhibitor cross-resistance. In recent months, an increasing number of leading HIV researchers have been supportive of this approach. But at this point it is just a theory because studies have not yet officially addressed the approach. If you monitor your viral load monthly, that would allow you to detect a rebound in viral load quickly. After a second test to confirm that failure, you could immediately change your therapy. By discontinuing the protease inhibitor to which you may now be resistant, you may avoid any further resistance or mutations from further developing in your protease

enzyme. By remaining on a protease after resistance starts and continues to develop, you may be allowing further resistance to develop. If you switch the protease before much resistance has had a chance to develop, you may have a better opportunity to be sensitive to the second protease inhibitor. Thus, you may limit or avoid cross-resistance.

However, this is not black and white. Some individual's viral load can plateau at a detectable level of for example 2,000, 10,000, or 30,000 copies/ml; and their viral load may remain at that level for a period of time. One study suggests that if you can keep viral load below 5,000 copies/ml progression of disease can be maintained. This opinion offers a different approach than full viral load suppression. You should consider the risks and benefits of switching therapy or remaining on the same therapy. Of course, it is difficult to predict whether an individual's viral load will plateau or skyrocket. If a person's viral load appears to be plateauing, it is difficult to predict whether or when it may increase. If a person has few or no adequate treatment options currently available, it may be preferable to closely monitor your viral load and wait until they can start a new regimen consisting preferably of 3 new drugs that will offer adequate potency. Some individuals in this position are considering hydroxyurea therapy as an alternative. See the hydroxyurea section for extensive discussion about its use.

**The Real Deal on the ICAAC Report by Steven Deeks of 53% Protease Inhibitors Failure Rate.** The mainstream press irresponsibly reported a mischaracterization of the information reported by Dr. Deeks. He received a lot of publicity when he reported at ICAAC that patients in his clinic had about a 53% failure rate when using protease inhibitors when he took a look back at their records. In fact, his purpose of reporting this information was to show what happens when protease inhibitors are used incorrectly:

- Many of the patients merely added a protease inhibitor onto current therapy without changing underlying nucleosides, after extensive prior nucleoside experience.
- The compliance rate was not good among these patients.
- Some of the patients had prior saquinavir resistance leading to a measure of cross-resistance to their next protease inhibitor.
- Some individuals had advanced HIV and therefore waited too long to begin therapy; their CD4 was low and/or their viral load was >100,000 copies/ml.
- Because of extensive prior nucleoside treatment, improved regimens could have been designed for some of the individuals including a combination of a protease with a NNRTI or a double protease regimen such as ritonavir+saquinavir with nucleosides.
- For the purposes of the study, failure was defined as <500 copies/ml, or above detection. This means that individuals who may have had a viral load of 1,000 or 800 or 3,000 were defined as failures when in fact that may be a mischaracterization.
- Deeks reported a high success rate of about 80-90% for individuals using protease inhibitors properly.

Deeks study highlights that (1) It is vital to individualize therapy. Every person has a different CD4 count, viral load level, a different background of prior treatment experience, etc. You cannot merely use a single therapy approach for every person such as a protease inhibitor plus two nucleosides. (2) Early aggressive treatment strategies should be considered. Initiating therapy too late in an individual's course of

disease (when their CD4 may be too low or their viral load too high) can cause failure.

**Baseline Viral Load, Nadir of Response, How low should my viral load be?** Many studies have now shown that an individual's viral load before starting therapy predicts how well they will respond to therapy and possibly how durable the response may be. If you begin HAART or for that matter any therapy with a high viral load it is less likely that you may reach undetectable or lower. For example, if your viral load prior to starting therapy is 1,000,000 copies/ml, you are less likely to reach undetectable with HAART. If your viral load is 15,000 copies/ml you are very likely to reach a low viral load and to sustain the viral load suppression. Being more aggressive and starting therapy when your viral load is lower may be beneficial to you. Evaluate your options. If your viral load is high, an extra potent regimen may be necessary.

Some data has been reported by Dale Kempf, PhD of Abbott Labs, suggesting that the nadir of your viral load response will predict the durability of effect from therapy or how long you will sustain your response to therapy. The nadir is the lowest level your viral load reaches from therapy. The lower you can reduce your viral load, the more durable your response will be.

Based on this and other information, a number of researchers have stated their opinion that reducing viral load to <50 copies/ml will probably result in a more durable suppression of viral load than lowering viral load to only 200 or 400 copies/ml. They have said that lowering viral load to as low a level as possible should produce better durability of response. Dr. Doug Richman said recently that the goal of therapy should be to lower viral load to <1 copy/ml. However, actual data supporting this thinking is important (and may be reported at the '98 Human Retroviruses Conference;) Because many individuals may not be able to reach so low, we need to know how crucial it may be. Some individuals may not be able to reach such a low level of viral load without expending several treatment options rather than saving them.

How important is full suppression of viral load to stopping virus replication in the lymph tissue as well as in other potentially protected compartments such as the CSF or brain? The answers to these questions are not yet available. Preliminary research indicates that viral load reduction in plasma causes similar reductions in lymph tissue viral load. But the relationship between plasma viral load reduction and the viral load in the CSF and the effect on the brain is not yet well defined. Considerable additional research to understand these issues well enough to base definitive treatment decisions is required. Partial suppression due to partial penetration may lead to premature resistance in the brain and subsequently in the blood.

**Indinavir.** Dr. Roy Gulick of New York University Medical Center and Bellevue Hospital reported 100 week data for individuals taking indinavir+AZT/3TC in Merck study #035. This was a blinded and randomized study of 97 individuals comparing the triple regimen to AZT/3TC alone, and indinavir monotherapy. Participants were 3TC and protease inhibitor naive, and AZT experienced (average 2.4 years). 80% of participants had taken other nucleosides prior to the study including ddI, ddC or d4T. The median baseline CD4 and viral load were 144 cells and 43,190 copies/ml. **See Table 1**

**Table 1. Week 100 Median CD4 and Viral Load changes from Baseline**

	CD4 inc.	HIV RNA dec.	%<500 copies/ml	%<50 copies/ml
<b>IDV + AZT/3TC</b>	+230	-2.12 log	79% (22/28)	64% (n=25)
<b>IDV mono*</b>	+120	-1.2 log	40% (n=25)	38%
<b>AZT/3TC*</b>	+90	-1.0 log	30% (n=29)	24%

\* It is important to remember that those randomized to indinavir monotherapy added AZT/3TC and those who started with AZT/3TC added indinavir after 6 months on the medications they were originally assigned to. Some of the data in the table are approximations made from visual observations of graph line charts.

Dr Doug Richman, an ACTG investigator in this study, made the following observations about 035 at the recent December ACTG meeting:

- The only reason for failure past 9-12 months should be non-compliance.
- Reconstitution of the immune system appears to be gradually and persistently occurring as CD4 increases from baseline were 225 at week 100.
  - Virus in plasma is a product of virus in lymph tissue.
  - A couple of weeks after discontinuing therapy lymph node viral load comes back towards baseline.
  - Viral RNA nadir predicts duration of maximal response.
- If you can see HIV RNA copies in the blood that means replication is occurring in the lymph tissue.
- The goal of therapy should <1 copy/ml.

**ACTG 320.** This was a randomized and blinded trial of indinavir in combination with 2 nucleosides vs 2 nucleosides alone in 1156 individuals who were AZT-experienced, but 3TC and protease inhibitor naive, with CD4 <200. Most study participants used AZT/3TC but a small percentage used d4T/3TC. The median prior AZT experience was 21 months. This is called a clinical endpoint study and its purpose was to evaluate the effect of indinavir therapy on disease progression and survival. The FDA has declared during this past year that viral load improvements caused by therapy correlate with slowing of disease progression, clinical endpoint studies such as this are no longer required.

The risk of progression to AIDS or death was reduced by about 50% for those receiving indinavir+2 nucleosides compared to those receiving only 2 nucleosides.

The viral load reductions at 6 months from baseline for those receiving the triple regimen were as expected: -2.2 log in the <50 CD4 group, and 3.1 log in the 51-200 CD4 group. However, the preliminary viral load data after 6 months of treatment with the triple regimen showed that only 47% in the <50 CD4 group were <500 copies/ml; and, only 62% in the 51-200 CD4 group were <500 copies/ml. In study 035, 80% at week

100 were <500 copies/ml. At baseline, the individuals in 320 had lower CD4s and higher viral loads than those in 035; although indinavir is a potent protease, this suggests that individuals with more advanced HIV may need more potent regimens than they received in 320 which could be nucleosides in combination with a double protease regimen or with a protease+NNRTI. Resistance testing is planned to try to better understand the results.

**Ritonavir.** In this open-label, non-randomized french study conducted by J. Leibowitz and others, 32 treatment naive participants were initially enrolled to receive ritonavir (600 mg bid) for 14 days followed by the addition of AZT (200 mg tid) plus ddC (0.75 mg tid). The participants' baseline CD4 was between 50 and 250, had a drop of 200 cells to a level of less than 350 over a recent 6 month period, or they had a CD4 count of 250 to 350 with symptoms. The mean baseline CD4 count was 160. Median baseline viral load was about 63,000 copies/ml (4.8 log).

During the first 6 months, 11 participants dropped off the study. Investigators reported 6 of these discontinuations were due to the distasteful solution (liquid) form of ritonavir. When this study started, the ritonavir solution (liquid) formulation was used because the capsule formulation was not yet available. Investigators believed compliance was a problem because of that. After 52 weeks, the capsules were substituted for the liquid formulation, and it appeared as if compliance improved. At about the same time, the mean viral load reduction appeared to improve. In Hamburg, HIV RNA data was reported to 96 weeks. **See Table 2**

**Table 2. Ritonavir+AZT/ddC: Approximate Mean Changes in CD4 and Viral Load from Baseline**

	<b>CD4 inc.</b>	<b>HIV RNA decrease</b>
<b>28 weeks (n=17)</b>	+110 (n=17)	-2.0 log
<b>52 weeks (n=17)</b>	+165 (n=17)	-1.6 log
<b>72 weeks (n=17)</b>	+155 (n=17)	-1.9 log
<b>96 weeks (n=9)</b>	na*	-2.1 log

na = not available

**Ritonavir+AZT/3TC, Naive.** This was an open-label two-armed study where 33 treatment naive individuals were randomized to begin the triple regimen of ritonavir+AZT/3TC simultaneously or to the delayed group where ritonavir was initiated alone followed by the addition of AZT/3TC three weeks later. The immediate vs delayed comparison was undertaken to detect if there is a difference in antiviral effect (or the development of resistance) due to the 3 week delay in adding AZT/3TC. The immediate group (A) had median baseline CD4 and viral load of 177 cells and 5.27 log (about 187,000 copies/ml). The delayed group (B) had a median baseline CD4 and viral load of 134 cells and 5.37 log (about 235,000 copies/ml). **See Table 3**



**Table 3. Ritonavir+AZT/3TC: Changes from Baseline in CD4, viral load & % undetectable**

	CD4 inc.	HIV RNA dec.	% undetectable *	N
Group	A B	A B	A B	A B
<b>week 16</b>	+105 +80	-2.8 -2.8	100% 72%	14 16
<b>week 32</b>	+180 +110	-2.9 -2.9	100% 81%	12 13
<b>week 40</b>	+190 +125	-3.2 -3.0	80% 81%	9 11
<b>week 52</b>	+200 +180	-3.1 -3.1	91% 100%	11 9

\* Undetectable was <230 copies/ml

Values are approximations based on visual observation of graph lines

We know from the results of several studies that side effects from ritonavir can include elevated liver function tests, elevated triglycerides, asthenia (fatigue), diarrhea, nausea, and parasthesia (numbness around mouth or other areas). The dose escalation method and eating hi-fat, hi-calorie meals may help reduce side effects. Over time side effects tend to diminish.

As part of this study, baseline and subsequent tonsil lymph tissue biopsies were conducted. Significant reductions in lymph tissue viral load have been reported. For an extensive discussion of the data published in the May 9 '97, Science, see the NATAP report on our web site - ["Virus Activity in the Lymph Tissue; Reservoirs of virus: provirus DNA and Memory CD4 lymphocytes."](#)

**4-Drug Therapy: Ritonavir+Saquinavir and AZT/3TC.** At ICAAC, Andrew Talal of The Aaron Diamond AIDS Research Center in New York City reported interim findings of a small open label study of 10 chronically infected individuals receiving a 4-drug double protease therapy.

8/10 were treatment naive, 1/10 had prior AZT experience, and 1/10 had AZT and ddC experience. The baseline mean HIV RNA was 5.33 log (215,986 copies/ml) with a range of 8,011-585,068 copies/ml. The baseline mean CD4 count was 385 with a range of 179-673. The Roche Amplicor and Ultrasensitive tests were used to evaluate plasma HIV RNA. Lymph tissue and CSF samples were evaluated, and results are posted to NATAP's website.

**Results.** After 48 weeks, 10 individuals remain on study treatment: the median increase in CD4 from baseline was 105; the median reduction in plasma HIV RNA from baseline was about 3.40 log (using the lower limit of detection of 200 copies/ml); 10/10 have <25 copies/ml; at week 32, the CD8 count decreased about 360 and there was a trend towards normalization of the CD4/CD8 ratio. The study is ongoing and further data will

be reported.

**Ritonavir+Saquinavir.** During the Spring of '97, 48 weeks of data were reported for this combination. Study #462 was an open label study of several dose regimens for this combination. The study investigators reported that 400 mg ritonavir every 12 hrs + 400 mg saquinavir every 12 hrs produced similar CD4 increases and reductions in viral load at week 48 as the regimen of 600 mg every 12 hrs of ritonavir + 400 mg saquinavir every 12 hrs. They observed a 3.0 to 3.3 log reduction (using a more sensitive measure of HIV RNA reduction) in viral load from baseline, 90% remaining on study drugs were <200 copies/ml, and CD4s increased more than 100 cells. Some participants added d4T+3TC because they had not reached undetectable. After adding d4T/3TC all but one person reached <200 copies/ml and remained there from 4-16 weeks later. Some persons added d4T/3TC just to assure their viral load suppression. An update to 60 or more weeks will be reported at the '98 Human Retroviruses Conference in Chicago in early February.

**Nelfinavir.** Protocol #511 was a randomized and blinded study where 297 treatment naive individuals received either AZT/3TC, nelfinavir 500 mg tid + AZT/3TC, or 750 mg nelfinavir tid + AZT/3TC. About 100 persons were randomized to each arm. After 6 months all individuals were permitted to receive nelfinavir. The mean baseline CD4 and viral load were 288 cells and 153,044 copies/ml. The 750 mg dose was found to be superior in this study and is the only FDA approved dose. **See Table 4**

**Table 4. Mean Changes at Month 12 from baseline in CD4 & Viral Load, and % Undetectable**

	6 months		12 months
	AZT/3TC	750 mg nlf	750 mg nlf
<b>CD4 increase</b>	+90	+155	+180
<b>HIV RNA decrease</b>	-1 to 1.3 log	-2 log	-2 log
<b>Vldec. 50 copy test*</b>	-1.28 log	-2.79 log	na
<b>%&lt;500 copies/ml</b>	25% (n=84)	82% (n=81)	80% (n=69)

\* The Roche Ultrasensitive test with a lower limit of 50 copies/ml was used. The data from this test was not available at month 12.

Some of the numbers in this table were approximated from visual observation of a graph line chart. At month 12 it appeared on the graph as though CD4 was trending upward.

In this study, a 20% rate of diarrhea was reported at 6 months for those taking the 750 mg dose. At month 12, the rate reported was 12%. The reduction may have been due in

part to the use of an over-the-counter antidiarrheal medication which appears to be helpful. Other side effects noted at month 12 were- nausea (7%), abdominal pain (4%), flatulence (2%), rash (4%), asthenia (1%). Nelfinavir is to be taken with food. Without an adequate meal, its absorption and benefit can be significantly reduced.

**Saquinavir SGC (Fortovase), EOF, New Formulation.** The new soft gelatin capsule formulation of (SGC) saquinavir received full FDA approval on Friday November 7, 1997. Invirase is the old formulation of saquinavir (HGC-hard gelatin capsule). Due to the limited bioavailability of HGC saquinavir, the antiviral effect and its durability had limitations. Fortovase is the new formulation and it provides increased exposure (amount) of saquinavir, 8-10 times those that were seen with the HGC saquinavir at its standard dose of 600 mg three times per day. The new SGC saquinavir is prescribed at 1200 mg three times per day.

Roche has explained Fortovase delivers more drug through the body than does Invirase. The HGC took too long to dissolve in the digestive tract and be absorbed, so enzymes had a chance to degrade it, leaving less in the bloodstream. The new formula combines the drug with an oil like substance that is more rapidly digested and allows more drug to reach the bloodstream. The new formulation is more quickly absorbed because small micron sized droplets of medium chain mono- and di-glycerides contain dissolved saquinavir. You now have an oil comprised of these small saquinavir containing droplets which allow for a wider dispersion of the drug which enables it to be absorbed more quickly before degradation from the enzymes.

Roche Labs is transitioning the old formulation of saquinavir off the market. For only 6 months the old version of saquinavir (Invirase) will be available in your pharmacy. If you have been using Invirase and want to continue, Fortovase will be available after 6 months for individuals currently using it in combination with ritonavir or for those patients who through consultation with their doctors have decided to continue taking Invirase.

**Refrigeration.** Your pharmacist will refrigerate saquinavir upon receipt. You can store saquinavir in your fridge. But if you don't refrigerate it, or if you take it out, or in and out of your fridge, you have a total of 3 months of unrefrigerated time. The new saquinavir can be kept out of refrigeration and at room temperature for a total of 3 months.

**Ritonavir+Saquinavir.** Both Roche and Abbott have explained that if you are currently taking the double protease combination of ritonavir+saquinavir it doesn't matter if you use Invirase or Fortovase. The dose will be the same, 400 mg every 12 hours. The drug blood levels of saquinavir hit a maximum peak with ritonavir. Ritonavir increases saquinavir blood levels 20-50 fold. Whether you use Invirase or Fortovase saquinavir, blood levels cannot be higher. Both Abbott and Roche have stated side effects should not be any different.

**Food Effect.** Taking Fortovase with a full meal is recommended because it greatly increases drug levels in studies compared to taking Fortovase without eating and increases drug absorption more than with a light meal.

Taking Fortovase with a light low fat meal is preferable to not eating. Preliminary results

from an ongoing study indicated that blood levels of Fortovase are reduced by about 40% when the drug is taken following a low fat meal compared to it being taken with a high fat meal.

### **Sun Study: Fortovase+AZT/3TC.**

This is an open-label non-comparative examination of the triple regimen of saquinavir SGC (soft-gel capsule, Forto-vase) plus AZT and 3TC. 42 treatment-naive individuals were enrolled with mean baseline and HIV RNA and CD4 of 4.8 log (about 63,000 copies/ml, range 8,951-1,193,168) and 419 cells, respectively. The study is ongoing and the following data is preliminary.

The investigators reported that after 20 weeks:

- The reduction in viral load for 23 evaluable study participants was 3.34 log (range: -4.5 to 2.2 log). However, the investigators used a more sensitive viral load test (20 copies/ml), which can report (accuracy of results using 20 copy test can be inconsistent) viral load reductions down to a lower level than you'll get from using the 400 copy test.
- 91% were <400 copies/ml (undetectable)
- 60% were <20 copies/ml.
- 259 CD4 increase from baseline of 419 cells.
- 19/42 participants had withdrawn from the study by week 20 and were not included in the analysis: 2 due to adverse events, 3 due to non-compliance, 4 due to refusal of treatment, 6 lost to follow-up, 1 missed week 16 visit.

**Safety.** Investigators characterized the triple combination as well-tolerated. The most frequent side effects related to study drug were (>5%): nausea, vomiting, diarrhea, and headaches.

Lab abnormalities: 1 person had a grade III AST/ALT (liver function tests) at week 2 which resolved after discontinuing study treatment. 1 person had a grade IV AST/ALT at week 12 associated with acute hepatitis A. 1 person had a Grade III AST/Grade IV ALT at week 20 associated with acute hepatitis A. An approximate 20% incidence of diarrhea has been reported associated with saquinavir SGC in a different study.

**Cheese Study: SGC Saquinavir vs Indinavir.** In Hamburg, JCC Borleffs of University Hospital Utrecht, and others reported preliminary findings from this 48 week study comparing Fortovase+ AZT/3TC to Crixivan+AZT/3TC. Study participants were protease inhibitor and 3TC naive. They were permitted to be AZT naive or with <12 months AZT experience. Two receiving Fortovase and one receiving Crixivan had <12 months AZT experience. Those with = Grade 3 lab abnormalities were excluded.

At week 8, both groups experienced about a 2 log reduction in viral load. At week 12, viral load was reduced to below detection (400 copies/ml.) using the Amplicor viral load test. Gastrointestinal side effects were the most commonly reported by individuals taking the Fortovase regimen were: diarrhea (6), nausea (2)

Click here to link up to additional Fortovase studies and safety profile data. **See Table 5**

**Table 5. Fortovase (saquinavir): CD4 and Viral Load Changes**

	<b>Baseline</b>	<b>Week 4</b>	<b>Week 12</b>	<b>Week 24</b>
SGC SQV+AZT/3TC CD4 (mean)	310	398	434 (n-16)	563 (253 increase ) n-3
HIV RNA (median)	4.87 log*	3.16 log	2.60 log	2.60 log (decrease 2.27 log)
IDV+AZT/3TC CD4 (median)	296	351	345 (n-14)	505 (209 increase) n-3
CD4 (median)	4.92*	3.12	2.60	2.60 log (dec. 2.32 log)

4.87 log is equal to about 74,000 copies/ml; 4.92 log equals about 83,000 copies/ml.

The number of evaluable participants at week 24 is small (3), so the data could be different after more individuals reach 24 weeks.

**Phase II Dose Ranging Study of 141W94 in combination with AZT/3TC.** Investigators have reported preliminary 12 week data but the study is continuing for 48 weeks. The purpose of this study is to assess safety and tolerability of this triple combination, CD4 and plasma viral load responses; and to make dose selection for studies.

Although participants were required to be 3TC and protease inhibitor naive, they had prior nucleoside experience. The nucleoside experience of the individuals in the different treatment arms of this study varied, and this could confuse trying to discern differences in treatment effect between study arms. Prior nucleoside experience for any given individual in this study may limit the antiviral effect of receiving AZT/3TC in this study.

There are 4 arms in the study: AZT/3TC, and 3 different doses of 141 each with AZT/3TC 900 mg bid (twice daily) 141, 1050 mg bid 141, and 1200 mg bid 141.

It appears as if the 1200 mg dose is the one selected for development. After the first 12 weeks, the CD4 increases were about 100 for those receiving the 1200 mg dose of 141. Their viral load reduction was -2.65 log.

Additional information on 141W94 is available on website- safety and antiviral activity of other doses. **See Table 6**

**Table 6. 141W94: Drug Related Adverse Events in >10% of Patients up to Week 12**

	<b>AZT/3TC</b>	<b>900 mg bid</b>	<b>1050 mg bid</b>	<b>1200 mg bid</b>

<b>N</b>	20	20	20	20
<b>Nausea± vomiting</b>	10	14	13	13 (2)
<b>Diarrhea</b>	6	5	6	7
<b>Gaseous Symptoms</b>	10	4	2	2
<b>Headache</b>	4	4	4	4
<b>Malaise &amp; Fatigue</b>	6	3	6	2
<b>Paresthesias*</b>	-	4	3	6
<b>Skin Rashes</b>	2 (1)	1 (1)	2	9 (4)
<b>Any Grade 3 or 4 Event</b>	2	2	1	3

( ) no. inside parenthesis indicates # of patients who discontinued 141.

\* paresthesia includes a numbness and/or tingling

**Resistance.** Early in vitro research identified a relatively different resistance profile for 141 compared to other available protease inhibitors. 50 was identified as the main mutation. The same in vitro research suggested that saquinavir may resensitize 141 resistant virus to 141. These possibilities remain to be confirmed, although we can be hopeful that protease inhibitor resistant virus may be sensitive to 141. Some expert researchers preliminarily do not believe that 141's resistance profile will be unique enough to suppress protease resistance virus. Essentially, some researchers believe all protease inhibitors will be problematically cross-resistant. Additional information on the resistance profile (mutations) should be available in early 1998. The use of a double protease regimen including 141 with either saquinavir, indinavir, ritonavir or nelfinavir may offer some hope to the potential for suppressing protease inhibitor resistant virus. But, that application has yet to be explored. An initial study of the safety, pharmacokinetics, tolerability and potency of these double protease combinations is ongoing. Data from the study should be available at February '98 Human Anti-retroviral Conference.

Glaxo Wellcome is conducting pediatric studies along side with adult studies. It is expected that FDA approval will be sought at the same time for both adult and pediatric use.

**4-drug Therapy:** 141W94 + 1592U89 + AZT/3TC in 24 individuals- 13 acutely infected and 11 chronically infected. Dr David Ho briefly summarized the preliminary data from a small study conducted at the Aaron Diamond AIDS Research Center in New York. They used a bid regimen, all drugs were taken twice daily. The acutely infected group were within 90 days of infection. All participants received:

- 141W94 1200 mg bid
- 1592U89 300 mg bid
- AZT/3TC 300 mg/150 mg bid

The purpose of the study is to test the antiviral potency of this regimen in blood, CSF and gut-associated lymph tissue. RT and DNA PCR in PBMCs (peripheral blood mononuclear cells), tissues and semen will be conducted. Although a measure of drug activity in the CSF does not necessarily reflect the activity in the brain, AZT and 1592 were selected for their CSF penetration.

**Withdrawals.** 1 person withdrew from the acute group due to GI side effects; additional persons are experiencing nausea. 1 person from the chronic group was lost to follow-up. 22 persons have been treated for 5 to 25 weeks.

**HIV-RNA.** The line graph of the reduction in plasma viral load for the acutely-infected individuals showed a decline of about 3 log by about 10 to 18 weeks. For the chronically-infected individuals, the line graph of the reduction in plasma viral load appeared to be about 2.5 log by week 6 and approached 3 log by about week 20.

At week 8, 8/10 chronically-infected individuals were <100 copies/ml, and 5/6 were <100 copies/ml at week 12. At week 16, 5/5 acutely-infected individuals were <100 copies/ml.

**CD4 cell counts.** The mean baseline CD4 count was about 550 for the acute group. By about 20-26 weeks, the mean increase appeared to be about 100 CD4. In the chronic group, they started off with a mean CD4 count of about 300, and by about week 22 the increase was about 200 CD4.

**CSF.** The antiviral activity in the CSF has been observed in only 6 persons in this study. Additional persons are being studied along with ongoing follow-up for these 6 participants. By measuring free virus in the CSF at baseline and at 1 month on therapy, there is a consistent decrease in HIV-RNA in the CSF for all 6 persons, but only a few have so far reached undetectable.

GI associated lymph tissue. The viral load in lymph tissue was generally declining.

The data reported is preliminary as the study is ongoing. Ho preliminarily concluded that the therapy was reasonably well tolerated, and shows potent antiviral activity in both chronic and acutely infected individuals particularly in the blood and lymph tissue of GALT. The CSF is being assessed.

### **PNU 140690 (protease inhibitor)**

Upjohn conducted and reported at ICAAC preclinical data from studies of their protease inhibitor, PNU 140690 Safety, tolerance and pharmacokinetics were studied in healthy volunteers (n=48) in escalating single doses at doses of 100, 300, 500, 700, 900, 1200, 1600, and 2000 mg.

Investigators characterized PNU 140690 as generally well tolerated for up to the 10

days of study. Gastrointestinal related side effects were the most commonly observed (nausea, diarrhea, vomiting and abdominal cramping). They were mostly mild and occasionally moderate. No serious medical events were reported and no clinically significant lab abnormalities were reported. 140690 showed a modest 30% bioavailability in the dog and rat and was attributed to limited drug absorption. The AUC of various doses suggest dosing at 3 times per day.

Preclinical studies showed a synergy when 140690 was combined with ritonavir vs ritonavir resistant virus. 140690 was active against ritonavir resistant virus. Combining PNU 140690 with ritonavir might improve dosing of PNU to twice daily. Upjohn thinks the resistance profile of 140690 may be sufficiently unique to be effective against protease inhibitor resistant virus. In first quarter of 1998 they plan to explore this potential by initiating a trial treating individuals who've failed indinavir with PNU 140690. Subsequent trials are expected treating individuals who've failed other protease inhibitors.

ABT-378 is a new protease inhibitor being developed by Abbott Labs. See the Double Protease Section for a discussion of this drug.

### **1592U89 (Abacavir)**

**CNA2001: 1592 Study for Treatment Naive Individuals.** This study was for individuals with less than 12 weeks prior experience with AZT and CD4 cell counts between 200 to 500. 80 patients were randomized to one of four dose regimens of 1592.

The study was not designed to determine efficacy.

All patients received 4 weeks of 1592 monotherapy at the dose to which they were randomized. After 4 weeks, individuals were randomized to receive 1592+AZT or 1592+placebo for an additional 8 weeks. There was about a 2 log reduction at week 12 for all the arms (n=15 to 20 individuals per arm). At week 12, about 65% of the individuals who received 1592+AZT were undetectable (<400 copies/ml) while 22% receiving 1592 monotherapy were <400 copies/ml. The CD4 increases ranged from about 70 to 100 cells.

Participants in CNA 2001, described above, were required to stop study medications after 12 weeks. 9 individuals from CNA 2001 participated in a continuation study and received a triple regimen consisting of 1592 300 mg bid, AZT or 3TC, and indinavir or ritonavir.

The preliminary data for 8 participants were reported in Hamburg:

- Baseline HIV RNA (sensitivity limit of 400 copies/ml) was 4.54 log (about 35,000 copies/ml), n=9; after 24 weeks the average viral load reduction was -2.60 log (n=8).
- Baseline CD4 count was 315 cells; after 24 weeks the average CD4 count was 489 (n=8).



No patients discontinued 1592 due to an adverse event. The most commonly reported adverse events were nausea and diarrhea which resolved either spontaneously or upon therapy change. No serious adverse events were observed.

See the Resistance Supplement for [CNA 2003 in Nucleoside Experienced Individuals](#) and for [1592 Resistance](#) information.

**CNAB 2002: 1592 Dose Ranging Study: 44 weeks.** 60 treatment naive individuals with HIV RNA =30,000 copies/ml and CD4 counts =100 cells were randomized to 1592 doses of 100, 300 or 600 mg twice daily (BID). This study was designed to select a dose for future studies, not to measure efficacy. Participants who met switch criteria (<0.7 log decrease at week 4, =5,000 copies/ml after week 4, CD4 count return to baseline, or the occurrence of an AIDS defining event), had the option to switch to open-label 1592 300 mg bid+AZT/3TC. This study was designed to select a dose, not to determine efficacy.

At week 4, all doses showed an antiviral effect by a reduction in HIV RNA:

- 100 mg bid, -0.63 log (-0.52 to -1.54)
- 300 mg bid, -1.55 log (-0.1 to -2.76)
- 600 mg bid, -1.61 log (-0.52 to -2.32)

<0.7 log reduction:

- 9/20 in the 100 mg arm
- 5/20 in the 300 mg arm
- 5/20 in the 600 mg arm

Investigators concluded 300 and 600 mg twice daily doses were superior. 2/20 (10%) and 5/19 (26%) had HIV RNA <400 copies/ml at week 4 in the 300 and 600 mg arms, respectively. The investigators characterized the drug as well tolerated. There were 5 withdrawals before week 24: 4 due to adverse events; 1 lost to follow-up.

For those individuals who added 1592 to AZT/3TC they achieved about a 2 to 2.5 log reduction which was maintained out to 44 weeks. At 24 weeks the N (number of evaluable patients) was 27, at week 36 the N was 6, and at week 44 the N was 3. More than 50 of the original 60 participants are still in the study. Glaxo Wellcome is conducting pediatric studies for 1592. Application for FDA approval for both adult and pediatrics should be submitted together.

## Double Protease Combinations

As you know the first protease inhibitors were approved just about two years ago. We are just beginning to explore some of the different ways they can be used. Combining a protease inhibitor with a NNRTI has begun to be explored but additional research is ongoing and is needed to better understand the effects of combining a given protease inhibitor with a particular NNRTI. Ritonavir+ saquinavir was the first double protease combination to be explored. Studies of a number of other double protease combinations are ongoing or will be evaluated. By this time next year we should have a better

understanding of the potency, tolerability and safety of these combinations. We should have a better understanding of how to use them and in which circumstances they may be most useful. Will they be useful against protease resistant virus? Will they be more potent than a single protease inhibitor? Under which circumstances could 2 be preferable to 1? Raising blood levels of a protease drug by combining it with a NNRTI or other protease may help people who fail protease therapy because they had inadequate blood levels for that drug. These are questions research is addressing.

**Primer on a Drug's Levels in Blood or Pharmacokinetics.** In the articles in this section, the NNRTI section, and other parts of the newsletter you will see references to the pharmacokinetics (PK) of a drug. The PK of a drug refers to the concentration or levels of a drug in your blood. This explanation should help you better understand the discussions. The AUC, Cmin, and Cmax are referred to as the pharmacokinetic parameters or measures of drug levels in the blood. They are considered an important factor in a drug's antiviral potency and durability. The Cmin, which is also called the trough, is the lowest blood level of a drug during a fixed dosing period (for example, 8 hours for indinavir, or 12 hours for ritonavir); it is generally believed that the trough level for a drug should be above a certain level to adequately suppress virus replication. If the trough is too low, resistance may begin to develop. There may be a difference of opinion about the importance of the Cmin in certain circumstances. The AUC (area under the curve) is the total exposure of a drug in your blood over a fixed dosing period (e.g., 8 or 12 hours). The Cmax is the highest or peak level achieved by the drug in your blood over the fixed dosing period. The significance of Cmax is uncertain. Some experts believe a higher Cmax can be associated with more severity of side effects and that a lower Cmax may be associated with less severity, if the toxicity is drug concentration dependent. Some experts say that a higher Cmax may be beneficial to penetration of virus reservoirs and have additional benefits related to antiviral effects. Both may be true.

**Indinavir (IDV)+ Nelfinavir (NLF).** Researchers reported preliminary or interim analysis of 1000mg indinavir (IDV) q12 + 750 nelfinavir (NLF) q12 (every 12 hrs) saying that the trough level of nelfinavir at 12 hours was too low. They are concerned about longer term efficacy and resistance. So they are planning to explore the combination using higher doses of nelfinavir. It is uncertain how much of an increase in nelfinavir dose will be necessary to raise the trough to a level where their concerns are alleviated. As you raise the dose of any drug, potential side effects and toxicities are a concern. But in the study of NLF bid dosing reported by Dr Michael Sension, the 1250 mg bid dose appeared to be tolerable in that small short term study. See results in the article on "Double Protease Combinations".

Investigators reported that coadministration of IDV 1000mg every 12 hours with 750mg NLF every 12 hours resulted in Cmax, AUC and trough (blood levels) similar to IDV at 800 every 8 hours without NLF. But IDV had no obvious effect on NFV steady state blood levels resulting in low trough levels. They also concluded that the combination was generally well tolerated and had a substantial antiviral effect.

At week 8, 7/10 study participants were <500 copies/ml; 9/10 were <2,500 copies/ml; for 9/10, the CD4 increase was +156 cells. 1 patient discontinued due to rash. Other

adverse events were: diarrhea/loose stools (6), bloating (2), nephrolithiasis (1). By visual observation of the graph in the abstract of the individual viral load reductions for the evaluable study participants it appeared as if all but one person at week 8 achieved a reduction of between -1.7 to -2 log from baseline. But again, the primary concern is the prevention of resistance and the durability of the antiviral effect.

The judgement of the researchers is that higher doses of NLF need to be explored to assure durability.

**Indinavir+Ritonavir.** In this study, investigators explored different dosing combinations of the two drugs in healthy individuals to see the effect on different measures of blood levels or pharmacokinetics (AUC, Cmax, and Cmin or trough). The drugs were taken under non-fasting conditions (370 kcal, 5% fat) and every 12 hours. The optimal doses have not been defined. Further studies are planned which if successful should produce guidelines for using the combination.

Study results showed that ritonavir can substantially increase indinavir AUC, Cmax, Cmin and half-life. Investigators reported that "across groups, ritonavir increased indinavir AUC up to 480%, and Cmax up to 110%. The mean apparent half-life was increased from about 2 hours to 5.8 hours." They found no effect of indinavir on ritonavir blood levels.

Investigators explored 4 different combination regimens in this study and said, if given every 12 hours they will likely give higher trough, lower Cmax and similar renal clearance of indinavir compared with the standard dose of 800 mg every 8 hours. But each dose regimen had varying effects on indinavir blood levels. There will be a number of considerations in determining an effective, tolerable and safe dosing regimen. Therefore, it is premature to experiment using these drugs together until further studies have been conducted.

Investigators concluded all regimens were well tolerated overall, and all adverse events were minor. They reported that mild circumoral parasthesia, a tingling or numbness around the mouth which can occur as a side effect to ritonavir, diminished with time, and that no new side effects were observed.

**Nelfinavir+Saquinavir.** Two studies preliminarily address the potential use of this combination. The first explores the pharmacokinetics (PK) or interaction between the two drugs when they are used together. 14 individuals enrolled in study who were protease inhibitor naive and were either stable on nucleosides, washed out from the nucleosides they were on or in the case of 1 person was nucleoside naive.

Investigators said that "multiple doses of SGC SQV 1200 mg did not have an effect on nelfinavir PK. Multiple doses of nelfinavir, however, increased SQV single dose mean AUC by 4.9 times and Cmax by 2.8 times. An 800 mg tid dose of SQV in combination with NFV 750 mg tid provides similar exposure (drug levels) to the 1,200 mg dose given alone. The SQV PK is non-linear, which was factored into the decision to decrease SQV dose in combination dosing by only 33% rather than 4 fold. Steady state blood levels of SQV SGC at 800 mg tid and NFV 750 tid in combination at 4 weeks were virtually

identical to levels of each at 2 weeks (at 2 weeks SQV SGC was given at 1200 mg tid) when they were given alone." **See Table 7**

**Table 7. Nelfinavir+Saquinavir: Median Viral Load and CD4 Changes from Baseline**

11/14 had prior nucleoside experience and 3 were treatment naive.

	Base	1 mo	N	4 mo	N	6 mo	N	11 mo	N
<b>CD4 increase</b>	327	+80	14	+50	14	+200	12	+175	10
<b>Viral load decrease</b>	39,917	-2.25 log	14	-2.1 log	14	-2 log	12	-2.3 log	10
<b>%&lt;500 copies/ml</b>		65%	na	61%	13	60%	na	90%*	3

Baseline CD4 range was 19 to 621 cells. Baseline viral load was 39,917 copies/ml and the range was from 19,847 to 109,064 copies/ml.

\* The %<500 at 11 months is based upon only 3 patients and may not be predictive of the actual % when more individuals are evaluated.

The second trial is called the SPICE study and compared 4 treatment regimens:

- nelfinavir+saquinavir + 2 nucleosides
- nelfinavir + 2 nucleosides

The new formulation of saquinavir called Fortovase was used in this study. The dosing regimen used was 750 mg nelfinavir tid and 800 mg saquinavir tid. This 48 week study was not designed to compare the efficacy of the 4 regimens. It was designed to explore pharmacokinetics and preliminary antiviral activity. Some study participants were nucleoside naive and some were nucleoside experienced. The number of naive and experienced were not necessarily evenly divided between the 4 treatment arms, which is why you cannot compare the CD4 increases and viral load decreases between the 4 arms. However, the efficacy and safety data of two regimens nelfinavir+saquinavir+2 nucleoside regimen and nelfinavir+saquinavir are useful. Data is extended to only 16 weeks, so it is preliminary.

53% of participants in this arm were treatment naive. 25% who were treatment experienced switched to 2 new nucleosides. The baseline CD4 and viral load for this group was 300 and about 50,000 copies/ml. **See Table 8**

**Table 8. Nelfinavir+Saquinavir**

<b>NFV+SQV+2 nukes</b>	<b>week 16</b>
treatment exp, %< 400 copies/ml	75% (n=20)
treatment naive, %<400 copies/ml*	90% (n=23)
group as a whole, %<400 copies/ml	84%
group as a whole, <50 copies, Ultrasensitive test	49%
VL decrease , Amplicor 400 copy test*	-1.95 log
VL decrease , Ultrasensitive 50 copy test*	-2.57 log (n=43)
CD4 increase	+100

\* This is not an intent-to-treat analysis. It includes only individuals who remained on study treatment. If a person withdrew because of side effects, no antiviral response, etc. they were not included in this data. If they were included the percentage would likely be lower.

Reported side effects considered at least possibly related or of unknown relationship to study drug and moderate, severe or life threatening in degree included: diarrhea (35%), nausea (8%), abdominal pain (2%), vomiting (4%), asthenia (4%), arthralgia (2%). There were 2 discontinuations for adverse events or intercurrent illnesses.

For the 2-drug regimen of nelfinavir+ saquinavir taken without nucleosides baseline CD4 and viral load were 301 and about 63,000 copies/ml; 56% were treatment naive. **See Table 9**

**Table 9. Nelfinavir + Saquinavir**

<b>nelfinavir+saquinavir</b>	<b>Week 16</b>
treatment exp, % <400 copies/ml	55% (n=21)
treatment naive, %<400 copies/ml*	55% (n=26)
group as a whole, <50 copies, Ultrasensitive test	25%
VL decrease, Amplicor 400 copy test*	-1.58 log
VL decrease, Ultrasensitive 50 copy test*	-1.96 log (n=45)
CD4 increase	+110

Side effects included: diarrhea (46%), abdominal pain (7%), vomiting (2%), fatigue (6%), asthenia (2%), arthralgia (2%). 2 individuals discontinued study treatment.

**ABT-378 + Ritonavir.** ABT-378 is a second generation protease inhibitor in an early stage of development. In preclinical studies, ABT-378 has shown it was significantly more potent than ritonavir, has a different resistance profile, has an ability to suppress ritonavir resistant virus, and significantly less than standard doses of ritonavir greatly enhanced the blood drug levels of ABT-378. Abbott Labs, the developer of ABT-378, has reported that the side effects so far seen have been favorable compared to those seen for ritonavir at this stage of its development.

The first clinical trial in HIV infected humans started in November '97. Based upon information emerging from this study, it is expected that additional studies will begin in early '98 including exploration of the potential for the combination of ABT-378/ritonavir in individuals for whom prior protease therapy has failed.

The pattern for the development of resistance to ABT-378 was studied in vitro and reported at ICAAC. As occurs with other protease inhibitors, mutations appeared in a sequential or step wise way with increasing concentrations of ABT-378 : I84V, L10F, M46I, T91S, V32I, I47V. Selection at the final concentration of ABT-378 resulted in a secondary mutation of V47A, and a reversion at 32 back to wild-type. You will note the potentially significant absence of a mutation at position 82. A mutation at this position is key to ritonavir and indinavir resistance. Based upon the in vitro cross-resistance data reported at ICAAC, it appears as though ABT-378/ritonavir may not be able to suppress all levels of ritonavir resistance. High level ritonavir resistance may not be able to be suppressed by ABT-378/ritonavir. However, lower levels extended to possibly 10-20 fold resistance or a little higher may be able to be suppressed because of the potency and antiviral activity expected due to the high drug level concentrations that appear achievable in the study described in the following paragraph (see commentary below).

Abbott researchers reported the effects (pharmacokinetics) of combining varying single doses of ABT-378 and ritonavir in both a non-fasting and fasting state to measure the effect of food. 12 different combinations of the two drugs were explored using doses of ABT-378 ranging from 100 to 800 mg, and using ritonavir in doses ranging from 50 to 300 mg. The AUC (drug blood levels) of ABT-378 was increased 100-300 fold when taken with ritonavir, and blood levels achieved were significantly higher than the IC50 for wild-type virus. The IC50 is the inhibitory concentration necessary to suppress 50% of virus replication. It is a standard research method for testing antiviral potency of a drug. It did not appear as though ABT-378 significantly altered ritonavir blood levels.

Investigators concluded that with single-dose coadministration of the two drugs, no subject withdrew due to an adverse event; 378/ritonavir was generally well-tolerated; one subject taking the dosing combination of 800 mg 378 and 200 mg ritonavir had a grade 2 elevation of SGOT/SGPT (liver function tests) and one subject receiving placebo had a grade 2 SGOT elevation. Food did not effect the pharmacokinetics of the combination; and, the high levels of drug concentration achieved can be expected to be highly suppressive of HIV. As stated above, several dose combinations are being explored in treatment naive HIV infected individuals including 200 mg 378+100 mg ritonavir and 400 mg 378+100 mg ritonavir in a twice daily dosing regimen which will include d4T+3TC. The PK of once daily dosing is still being researched by Abbott.

**Ritonavir+Nelfinavir.** Preliminarily, it appears as though ritonavir increases nelfinavir blood levels about 2 to 2.5 fold. Two small studies are under way exploring two different bid regimens: 750 nelfinavir bid + 400 bid ritonavir; 1000 nelfinavir bid + 400 mg bid ritonavir. Data is expected to be reported in February 1998 at the Human Retroviruses Conference. Again, it is premature to experiment with these combinations until more information is available.

### **NNRTI - Non-nucleoside Reverse Transcriptase Inhibitor Section**

DMP-266 (efavirenz, Sustiva-brandname). DMP-266 is a new NNRTI (non-nucleoside reverse transcriptase inhibitor). The efavirenz pivotal studies from which data is used by the FDA for consideration of approval are ongoing. An expanded access program started in October for individuals with <50 CD4. On December 16, DuPont Merck announced new expanded eligibility criteria: a person is eligible if at any time they have had a CD4 count <400, if they are failing or intolerant to their current treatment, and their physician is unable to assemble a treatment combination without Sustiva that is likely to produce a sustained reduction of virus in the blood.

In ongoing studies, efavirenz is being taken once a day at a dose of 600 mg with or without food. The long half-life of the drug (40-52 hrs) lends itself to once-daily dosing. The most common side effects are CNS symptoms and are associated with taking the 600 mg dose: lightheadedness or dizziness, and antihistamine-like effects have been seen when using the higher doses. Company officials say experience so far indicates these symptoms usually last several hours and seem to dissipate within a few weeks after starting medication. Company officials say these side effects can be addressed by adjusting the dosing regimen by taking the 600 mg dose before bedtime, by taking 300 mg twice daily, or if that's not tolerable by lowering the dose to 400 mg once per day. But they believe the 600 mg dose may be more potent in the long term. Rash has also been seen when treated with efavirenz, but the company reports that in most cases it resolves without requiring any change in efavirenz treatment.

A pediatric study using nelfinavir+266 is just beginning using 50 and 75 mg capsules. Children who are capable of swallowing a capsule will be eligible. A liquid formulation is being developed.

Efavirenz should not be used as monotherapy, as resistance can develop rapidly. It should only be used as part of a combination therapy well designed to adequately suppress viral load.

The original dose used in earlier studies was 200 mg once a day. A key mutation in the development of resistance to efavirenz is K103N. DuPont Merck increased the dose to 600 mg daily hoping it will well suppress this mutation; and, it was also chosen to suppress other single mutations associated with NNRTI resistance. Company officials say the dose increase is likely to cause increased antiviral activity of DMP-266. In a 14-day monotherapy study where the 200 mg dose was compared to placebo the peak reduction in viral load was 1.7 log when using the Roche Amplicor 400 copy test.

**CSF.** In a small study of 3 individuals, investigators reported that efavirenz penetrated the CSF in all three. The 3 patients were receiving DMP-266 200 mg once a day in

combination with indinavir in a study for 28 days when samples were taken. Plasma (blood) and CSF samples were both obtained at the same time point. **See Table 10.**

**Table 10. Results - DMP - 266 CSF Drug Levels**

	<b>Time Post</b>			<b>CSF/Plasma</b>
<b>pt.</b>	Dose (hr)	CSF (nM)	Plasma (nM)	Ratio(%)
<b>1</b>	1.2	26.4	2213	1.19
<b>2</b>	4	34.6	3329	1.04
<b>3</b>	22	16.9	2606	0.65

**Indinavir+Efavirenz.** Study participants were randomized to either indinavir monotherapy or indinavir+efavirenz. Baseline CD4 and viral load are in table 11. After it was determined that efavirenz decreased indinavir AUC (indinavir concentration in the blood over the 8-hour dosing period) by about 35%, study participants who were taking 800 mg indinavir with efavirenz increased their indinavir dose to 1000 mg every 8 hours by about 12 weeks into the study. Also, after 12 weeks individuals receiving indinavir monotherapy were permitted to add d4T+efavirenz. If taking indinavir with efavirenz, DuPont Merck recommends 1000 mg indinavir every 8 hours. The dose of efavirenz used in this study was initially 200 mg once per day but was raised after a minimum of 36 weeks to 600 mg once a day. More detailed data on side effects are available on NATAP website.

**\*\*NOTE:** Based on the following information study investigators believe that the more you lower your viral load the more durability you will obtain. Investigators used the Amplicor 400 copy test to see if there was any virus signal. They found that they were unable to detect virus signal from 80% of the individuals in the efavirenz+indinavir arm using the 400 copy test. This does NOT mean viral load was not present in the blood.

Study investigators said, the individuals in this study for whom they found no viral load signal were better able to remain below detection by the standard Amplicor test (<400 copies/ml). Their viral load reduction was more durable. For those for whom no signal was found about 90% had less than 400 copies/ml at 48 weeks. For those for whom a signal was detected, at week 48 about 60% of these individuals remained less than 400 copies/ml. From a mathematical model used to analyze the results, they concluded those who had no signal were 8 times more likely to remain less than 400 copies/ml at 48 weeks than those for whom they detected a signal but had less than 400 copies/ml. See Tables 11 and 12

**Table 11. DMP-266+Indinavir**

	<b>Efavirenz+indinavir</b>	<b>indinavir*(d4T+266)</b>
<b>Baseline</b>		



- N (# of individuals)	59	42
-CD4	283	284
-HIV RNA, approx	123,000 copies/ml	104,710
-Prior Therapy	64%	81%
At Week 48		
-N	37	22
-VL dec., 400 copy test	-2.4 log	-1.9 log
-% <400 copies/ml	90%	65%
-No VL signal**	80%	55%
-CD4 inc.	+250 CD4	+150 CD4

**Table 12. This is a Summary of Rashes**

Toxicity Grade	DMP-266+indinavir	Indinavir
I	19/84 (22.6%)	11/42 (26.2)
II	9/84 (10.7%)	1/42 (2.4%)
III	0/84	1/42
<b>total rashes</b>	<b>28/84 (33.3%)</b>	<b>13/42 (31%)</b>

There has been 1 case of Stevens Johnson Syndrome (serious rash) out of 1300 individuals who've received efavirenz, which investigators think was due to another medication the person was taking.

**Pharmacokinetics (PK) for efavirenz+nelfinavir.** A PK interaction study has been conducted for efavirenz taken with nelfinavir in healthy volunteers. Investigators reported that with coadministration of both drugs (750 mg nelfinavir 3x/day and 400 mg efavirenz once-daily), and after steady state blood levels were reached for both drugs, nelfinavir AUC (overall blood levels over a fixed time period) increased 15% and the Cmax (peak blood level of drug) of nelfinavir was increased 30%. Investigators reported there was no significant effect on efavirenz AUC or Cmax by nelfinavir. Further studies are in progress to determine proper dosing, and explore safety and efficacy. These results are different than when combining efavirenz with indinavir. Indinavir blood levels are decreased 30-35%, and so DuPont Merck recommends the dose of indinavir should

be increased to 1000 mg every 8 hrs.

**Efavirenz+AZT/3TC.** This study explores the safety and efficacy of three different doses of DMP-266 + AZT/3TC vs AZT/3TC alone in treatment naive individuals who are asymptomatic or mildly symptomatic, a relatively healthy group. It also explores an unusual and potentially useful therapeutic approach of using efavirenz+2 nucleosides as a first line treatment (rather than a protease regimen), thereby strategically preserving the protease inhibitor option for a future time. The preliminary follow-up data extends only to 16 weeks but the study is ongoing and additional information will be reported. The four treatment arms are:

- 200 mg efavirenz once-daily +AZT/3TC
- 400 mg efavirenz once-daily +AZT/3TC
- 600 mg efavirenz once-daily +AZT/3TC

See Table 13

**Table 13. DMP-266+AZT/3TC**

	<b>AZT/3TC</b>	<b>200mg 266</b>	<b>400mg 266</b>	<b>600mg 266</b>
<b>N</b>	33	36	34	34
<b>Baseline</b>				
<b>-CD4</b>	397	337	359	394
<b>-VL approx</b>	45,700 copies/ml	61,650	58,880	43,650
<b>Early Discontinuation</b>	4	4	7	8
<b>-adverse event (a/e)</b>	1	0	1	4
<b>-noncompliance</b>	1	1	0	2
<b>-withdrew consent</b>	0	0	1	0
<b>-lost to followup</b>	2	3	4	1
<b>Week 16</b>				
<b>-mean CD4 increase approx, (n)</b>	+115 CD4 (22)	+125 (21)	+125 (21)	+155 (17)
<b>-mean VL decrease (400 copy test), (n)</b>	-1.4 log (26)	-2.1 log (28)	-2.1 log (24)	-1.9 log (25)

<b>-% &lt;400 copies/ml</b>	44%	94%	89%	82%
<b>Ultrasensitive test - &lt;40 copies/ml</b>	4 (15%)	23 (74%)	17 (63%)	16 (64%)

**Delavirdine.** In October Upjohn & Pharmacia called a small community meeting to report preliminary data from a new study of a triple regimen containing delavirdine and to discuss their development plans for their proteaseinhibitor (PNU140690). The delavirdine study compared AZT+3TC+delavirdine, AZT+3TC, and delavirdine+AZT. The study has not yet been presented at an AIDS scientific meeting. Although the study participants were not AZT naive, they were required to have less than 6 months AZT experience. 80% had no prior AZT experience.

117 individuals were randomized to one of the 3 arms. The baseline CD4 counts and viral load were about 350 and 31,500 copies/ml. An interim analysis after 32 weeks of a 1 year study showed that those receiving delavirdine plus AZT/3TC had a CD4 increase of 96, a 2.0 log reduction in viral load when using a more sensitive test (40 copies/ml), a 1.5 log reduction when using the standard 400 copies/ml test, 50% were <40 copies/ml, and 63% were <400 copies/ml (n=45).

By comparison, at week 32, those receiving AZT/3TC had a CD4 increase of 70 cells, a 1.3 log reduction from baseline, 31% were <400 copies/ml, and 12% were <40 copies/ml. Those receiving delavirdine+AZT had a 20 CD4 increase, 0.5 log reduction in viral load, 5% were <400 copies/ml, and 0% were <40 copies/ml.

**Preliminary interaction when combining Nelfinavir with Delavirdine.** It is uncertain which doses of the two drugs should be used when taken together. Studies are planned or already ongoing to explore the various dosing combination of the two drugs. ACTG 359 is for individuals who have failed indinavir; it started in November '97 and is exploring use of 3 and 4 drug regimens including nelfinavir+saquinavir+delavirdine 600 mg bid; adefovir (new nucleotide) will also be used in this study. Initial interaction (PK) data should be available soon. As well, Upjohn has a study exploring different dose regimens for using nelfinavir with delavirdine. Two doses of delavirdine (400 or 600 mg tid) will be explored with nelfinavir 750 mg tid in combination with d4T and ddI. Experimenting using drugs before proper dosing is identified can result in either one of two potentially harmful outcomes. The blood concentration of a drug could be too low resulting in resistance developing; or, blood levels of a drug could be too high resulting in toxicities.

Upjohn conducted an initial study exploring interactions between delavirdine and nelfinavir in 24 healthy volunteers. Group A received nelfinavir 750 mg every 8 hrs for 1 week followed by concomitant treatment with nelfinavir 750 mg every 8 hrs plus delavirdine 400 mg every 8 hrs. Group B received delavirdine 400 mg every 8 hrs for 1 week followed by concomitant treatment with delavirdine 400 mg every 8 hrs plus nelfinavir 750 mg every 8 hrs. All doses were taken with food. **See Tables 14 and 15.**

**Table 14. Mean Changes in Nelfinavir Pharmacokinetic (blood levels)**

Values obtained from 12 individuals in group A

	NLF alone	NLF+DLV	Mean Change
NLF AUC (ug h/mL, 0-8 hrs)	26	50	+113% ( $\pm$ 25%)
NLF Cmax (ug/mL)	4.2	7.6	+93% ( $\pm$ 24%)
NLF Cmin (ug/mL)	2.2	4.9	+146% ( $\pm$ 37%)

**Table 15. Mean Changes in Delavirdine Pharmacokinetic Values**

The following data was obtained from 7 individuals in group B

	DLV alone	DLV+NLF
DLV AUC (uM h, 0-8)	210 ( $\pm$ 112)	122 ( $\pm$ 24)
DLV Cmax (uM)	32 ( $\pm$ 16)	21 ( $\pm$ 5)
DLV Cmin (uM)	21 ( $\pm$ 13)	10 ( $\pm$ 3)

**Safety.** 4/24 individuals discontinued the study due to neutropenia. Two subjects experienced serious (grade 3/4) neutropenia as a result of the combination. In each case, the neutropenia resolved after stopping both drugs. Investigators said except for the neutropenia the combination was well tolerated. If using these two drugs together, absolute neutrophil count should be frequently monitored.

As you can see the AUC of delavirdine was reduced by about 40% in this study; the Cmax was reduced by about 34%; and the important Cmin, also called the trough, was reduced by about 50%. Delavirdine and nelfinavir concentration data were available for 20 patients in a community practice setting where individuals received the two drugs at their normally recommended doses for over 1 month. Blood samples were obtained between 6 to 9 hrs after the doses of both drugs. The median Cmin or trough concentration of delavirdine was about 40% lower than when taking delavirdine in combination with nucleosides. The reduced Cmin or trough of delavirdine is the reason that a higher dose of delavirdine of 600 mg tid will be explored in the above mentioned clinical study where nelfinavir will be combined with delavirdine and d4T and ddl in 3 and 4 drug combinations. Trough levels will be evaluated. Again, it may be preferable to wait until proper dosing regimens have been identified before using these combinations.

**Preliminary PK of Combining Ritonavir with Delavirdine.** This was a small preliminary interaction study (n=12). 10/12 individuals had AIDS. The baseline CD4 counts were about 163 with a wide variability among the participants. 600 mg bid of ritonavir was administered for at least 14 days with nucleosides. Delavirdine 400 mg tid was added. Participants had prior ritonavir experience of 1 to 12 months. There was no group who started with delavirdine and then added ritonavir after steady state of delavirdine was reached. They used historical PK values of delavirdine, and PK values observed in a previous PK study using ritonavir at 300 mg bid. Based on these previous observations and other information, Upjohn does not expect ritonavir will have a

significant effect on delavirdine blood levels.

ACTG 359 is just beginning. The regimens available in the study will include ritonavir+saquinavir+delavirdine; adefovir will also be used in the study. In this study delavirdine dosing will be 600 mg bid. Investigators will be analyzing interaction data in the initial stage of the study. It is expected that saquinavir should not effect delavirdine blood levels, but this study will explore 3 drugs used together all of which effect the CYP450 liver enzyme system.

In the interaction (PK) study, delavirdine increased overall ritonavir blood levels by 70%. **See Table 16** for the effects on specific PK parameters.

**Table 16. Delavirdine effect on ritonavir PK**

	RTV alone	RTV+DLV	Mean increase (range)
RTV AUC (ugxhrs/mL, 0-12)	68	111	inc. 60% (22-90%)
RTV Cmax (ug/mL)	10.7	17.7	inc. 66% (24-109)
RTV Cmin (ug/mL)	2.6	4.7	inc. 84% (41-127)

**Safety.** There was 1 grade 4 elevation of triglycerides; 1 grade 3 elevation of LFTs; 1 grade 1 rash; and 1 grade 2 thrombocytopenia. All of these side effects may have been experienced by one person.

**Indinavir + Delavirdine.** As explained in the [July issue of NATAP Reports](#), delavirdine increases indinavir blood levels. Please refer to that article for detailed discussion. A trial will be conducted exploring two indinavir doses (400 mg tid or 600 mg tid) with 400 mg tid delavirdine. As well, a 2 times per day dose regimen study is planned: 800 or 1000 mg bid indinavir plus 600 mg bid delavirdine.

**Nevirapine + Indinavir.** 24 individuals enrolled in what was originally designed as an open label study to determine the effects of pharmacokinetics (PK) or blood levels of these two drugs when used together. Participants were protease inhibitor naive but were permitted to continue their background NRTI therapy. This report is of the long term extension of the study. 19 participants completed the PK part of the study. 17 enrolled and 12 remain in the extension. Reasons for dropout throughout the trial were: failure to return to clinic/lost to follow-up (6); adverse events - rash (2), kidney stones (1); viral load failure (2); consent withdrawal (1).

The median baseline CD4 was 434; the median baseline viral load was low at 3.5 log (about 3,100 copies/ml). 53% of the participants had prior nucleoside experience. One half were on NVP+IDV and half were on NVP+IDV+nucleosides. As reported by NATAP more extensively in our [July issue of NATAP Reports](#), there was a statistically significant reduction in indinavir AUC (-28%), Cmin (-38%) and Cmax (-11%) when taken with nevirapine. There was no significant effect of indinavir on nevirapine blood levels. Despite the PK effect of indinavir on nevirapine, indinavir was given at its usual

dose of 800 mg every 8 hrs in this study. Some doctors are using 1000 mg of indinavir every 8 hours. Alex Dusek, of Boehringer Ingelheim, reported in Hamburg that after 60 weeks 92% (n=12) were below 400 copies/ml, and their median CD4 increase was 250. The mean viral load reduction was 1.9 log at 32 weeks; and, 9/12 participants had >100 CD4 increases at week 32. All patients under 400 were also under 20 copies per ml. It is important to remember that the median baseline viral load was low at 3,100 copies/ml, making it easier to potentially suppress viral load and to sustain it.

### **Laboratories That Offer the Ultra-sensitive PCR Test to Individuals.**

The Roche Ultra-sensitive test measures to 50 copies/ml, while the standard approved tests used commonly to measure viral load are the Roche Amplicor 400 copy test and the Chiron bDNA 500 copy test. Many individuals want to use the Ultrasensitive test but may not know where its available. Previously the test was only a research tool and not available to the general public. NATAP has assembled a list of labs that offer the test to treating physicians and individuals. Click on "Baseline Viral Load, Nadir of Response, How low should my viral load be?" for more discussion.

There are several points about the test results to bear in mind. The test is not yet FDA approved, although the manufacturer has applied for approval. Different labs may give you varying results because their methods can be different. You should use the same lab when retesting.

Each laboratory listed below has told me they validate their testing in house. You may want to speak to the lab yourself or ask your doctor to call to ask about their testing as there can be differences between labs in the reliability or accuracy of the results they obtain. You want a lab which has validated its testing adequately so the results will be reliable.

**Test Results Variability.** As with the standard viral load tests, although not common, test results can have variability because of several reasons. From 50 copies/ml and above you can see a 2-fold variability within a given lab just based on the performance of the procedures for the test. You can see a 2-3 fold biological variability due to your own changes within your body (fluctuations in immune system, colds, flus, or vaccines); as you may know you can get different CD4 counts from one day to the next. All together, it is estimated that a 5-fold variability may fall within normal test result variability.

**Insurance reimbursement and cost.** NATAP conducted a phone survey of the labs. Most labs said private insurers were paying for the test. New York State medicaid is not yet generally paying for the test, but Specialty Lab said they are receiving and accepting NYS medicaid reimbursement. Immediately below is a table for contact information. Some of the labs listed below may be more convenient for an individual than other labs based on location. If you are paying cash, pricing can vary between labs, so it is recommended to discuss cost with the lab; you can negotiate.

Some of the labs use the Roche test while other labs use a what they call a "home brew," where they have put together their own test. Based on my research, using the

Roche test kit may produce more consistently reliable results but a "home brew" can be just as accurate and reliable. This is another question you may want to ask the lab and your doctor about. You can request the Roche test.

Some labs report it to you if they cannot find any virus at all. If you request, some labs will give you your absolute count (eg, 13 copies/ml). Remember, the accuracy of test results are most reliable when reported as either below or above 50 copies/ml. You can test the same sample today and not find any virus at all, but if you conduct the same test, in the same lab the next day you may find 20 copies/ml. So not finding virus does not mean virus is not present in the blood.

LabCorp and Quest are national with a number of locations. Consolidated is an independent lab and previously only performed viral load testing for drug research studies. It is based in LA but has an office in NYC for testing. You can call Richard Fung at Manhattan Medical Care (1780 Broadway, 12th floor; ph: 212 787-0900).

### **CSF Penetration by Protease Inhibitors**

**BID Dosing.** Merck, Agouron and Roche are researching their protease inhibitors in a twice-a-day regimen. Merck reported preliminary 24 week data was encouraging, but they caution it is premature to experiment using indinavir twice a day. They are conducting additional research with indinavir 1200 mg every 12 hours in combination with nucleosides.

Preliminary 16 week data was reported at Hamburg for nelfinavir 1250 mg twice-a-day in combination with d4T and 3TC. Again, the data is encouraging but it may be premature to experiment; a larger study is ongoing in Europe to evaluate the bid regimen. Roche is just starting its trial to see if Fortovase, the new formulation of saquinavir, can be dosed twice a day. See NATAP's web site for the actual data from these studies.

**Effect of Indinavir on CSF.** At the 8th European Congress of Clinical Microbiology and Infectious Diseases in May '97, Courtney Fletcher (University of Minnesota, PhD), reported findings from evaluating indinavir pharmacokinetics (PK) in HIV infected children receiving indinavir with ddl+d4T. Indinavir penetrated the CSF in 4 children measured with concentrations of 151-977 ng/mL.

Dr. Anne Collier of the University of Washington reported at IDSA in early September '97 her preliminary findings from two groups of study participants who underwent lumbar punctures. Investigators found that indinavir penetrated the CSF in study participants. Investigators measured actual indinavir drug levels at various time points. An important point is that individuals in group B were not taking indinavir at baseline but then added indinavir. So the effect of indinavir on the CSF could be evaluated by comparing the CSF HIV RNA before and after starting indinavir therapy. In group A, all individuals had been on indinavir therapy before the CSF HIV RNA was measured, so there was no pretreatment measure of CSF HIV RNA for comparison.

Of the 10 individuals in group A, 7 were taking indinavir+2 NRTIs, 1 was taking indinavir + 1 NRTI, 2 were taking indinavir alone. 9/10 individuals had <200 copies/ml CSF HIV

RNA. Only 4/10 had plasma HIV RNA <200 copies/ml but the median plasma HIV RNA was 2.4 log (about 250 copies/ml, ranging from <200 to 5.4 log- 250,000 copies). The median CD4 count was 328.

In group B, 9 individuals had CSF and plasma viral load measures at baseline prior to taking indinavir therapy and at week 8 after all 9 started indinavir therapy. 7/9 were taking 2 nucleosides at baseline, 1 was taking 1 RTI and 1 person was not taking any therapy. Then, 5 individuals simply added indinavir to their current regimen, 2 changed 1 RTI, and 1 person started 3 new drugs - 2 new RTIs and indinavir. **See Table 17**

**Table 17. Group B changes in CSF HIV RNA and Plasma HIV RNA**

<b>N=9, Group B</b>	<b>Week 0</b>	<b>Week 8</b>
<b>Plasma HIV RNA</b>		
<b>median</b>	5.1 log (125,800 copies/ml)	2.9 log (794 copies/ml)
<b>range</b>	3.9 -5.9 log (7,943-794,000)	<200 to 4.4 log (25,000)
<b>&lt;200 copies/ml</b>	0/9	1/9
<b>CSF HIV RNA</b>		
<b>median</b>	2.7 log (500 copies/ml)	<2.3 log (200 copies/ml)
<b>range</b>	<200-5.1 log (126,000)	<200-3.6 log (4,000)
<b>&lt;200 copies/ml</b>	2/9	6/8

Most individuals in group B were not taking optimal therapy as we know it today since 5 simply added indinavir to current NRTI therapy, etc.

The results from this study as well as those from the ritonavir/saquinavir study (below) are preliminary and need further study for confirmation. Collier said this was an initial observational pilot study but results suggest that indinavir penetrates into the CSF of adults. There are several confounding factors making it difficult to determine the absolute effect of indinavir on CSF HIV RNA.

Some researchers postulate that the absolute drug concentration in the CSF is more important than the CSF-plasma ratio of the drug. In this study only one drug level measure was taken for each participant, but they were taken at different time points during the dosing cycle. Essentially, the absolute drug levels of indinavir were found to be above the IC95 level identified in vitro to be 100 nM of indinavir at all these various time points. The IC95 drug level is the amount of drug necessary to inhibit 95% of virus replication. In one case the blood level was less than 100 nM, but investigators believed because there is no protein binding in the CSF, the drug level in that case would be adequate.



Median indinavir CSF ratios ranged from 2.2 to 76%, with the highest ratios seen near the end of the dosing period. The CSF to plasma ratio of HIV RNA was found to be higher later in the dosing interval of 8 hours. That is because the plasma indinavir levels decline towards 8 hours but the CSF indinavir level remains the same, so the percentage changes.

It is uncertain if CSF penetration by a drug means that it penetrates the brain and effects HIV brain-related disease. Studies to explore this question are planned. Individuals with signs of early HIV related-brain disease will be treated with a potent protease inhibitor regimen to examine the effect of therapy. Dr. Justin McArthur, a leading researcher in this field at Johns Hopkins, has stated that HIV brain disease is reversible if detected and treated early enough (prior to neuron death), but at some point (after neuronal death starts) it becomes irreversible. So, you need to evaluate individuals with early disease to detect therapy effect.

Another way in which we should be able to determine the effect of HAART on brain disease, and this is also ongoing, is simply by following individuals receiving HAART over long periods of time and to see if less HIV brain-related disease develops.

**CSF Study: Ritonavir+Saquinavir+D4T vs Ritonavir+Saquinavir.**

We have been receiving results of ongoing research on the effect of HAART on opportunistic infections and viral load in lymph tissue. Now, research is beginning to turn to the effect of HAART on the CSF and the brain. I think determining this effect may be the most difficult. But some of the initial preliminary research findings are reported and reviewed in this section. In our July issue we reviewed nevirapine and nucleoside CSF penetration. Ongoing and future research will address how HAART affects viral load in the CSF and HIV related brain disease.

At Hamburg, data was reported from the Prometheus Study on the first 18 weeks of treatment for 104 individuals randomized (open-label) to ritonavir+saquinavir or the triple regimen of d4T combined with ritonavir+saquinavir. Aside from evaluating serum HIV RNA and the peripheral CD4 responses to therapy, there was a substudy to look at the effect of study treatments on CSF HIV RNA, and to measure levels of the drugs in serum and CSF. Participants were protease inhibitor and d4T naive.

All study medications were taken every 12 hours. If serum viral load was not undetectable by week 18, participants were permitted to add NRTIs. **See Tables 18, 19, 20, & 21.**

**Table 18. Ritonavir+Saquinavir: Baseline characteristics**

	<b>RTV+SQV</b>	<b>d4T+RTV/SQV</b>
<b>Number of participants (N)</b>	54	50
<b>Prior treatment experience*</b>	69%	64%

<b>Mean CD4</b>	289	247
<b>%&lt;100 CD4</b>	17%	20%
<b>Mean HIV RNA**</b>	4.1 log (12,500 copies/ml)	4.2 log (15,800 copies/ml)

\*Individuals who had NRTI treatment experience were pretreated for a median of 28 months.

\*\* These are relatively low baseline viral loads

**Table 19. HIV RNA and CD4 changes at Week 18 from Baseline**

	<b>RTV+SQV</b>	<b>d4T+RTV/SQV</b>
<b>HIV RNA decrease (Roche Amplicor)</b>	-1.43 log	-1.75 log
<b>% Undetectable at wk 18*</b>	60%	76%
<b>CD4 increase</b>	150 cells	150 cells

\* undetectable is <400 copies/ml. At week 18 the graph line for the number of participants with undetectable HIV RNA was heading upward.

**Table 20. Ritonavir+Saquinavir - Week 0 vs Week 12 changes in serum and CSF viral load**

<b>P t</b>	<b>Regimen</b>	<b>Serum wk 0</b>	<b>Serum wk 12</b>	<b>CSF wk 0</b>	<b>CSF wk 12</b>
1	RTV/SQV	1,596 copies/ml	<590	<209	<515
2	RTV/SQV	883,318	624	24,118	<248
3	RTV/SQV	2,019	<197	<234	<632
4	RTV/SQV	190,461	3,435	1,645	11,536
5	d4T/R+S	7,645	<149	18,149	<362
6	d4T/R+S	27,907	<167	13,788	<146
7	d4T/R+S	25,6662	<158	<408	<315
8	d4T/R+S	64,175	<130	3,872	1,233
9	d4T/R+S	89,544	325	868	<301

**Table 21. Ritonavir and Saquinavir absolute serum and CSF drug levels**

(concentration in ng/ml)

Pt	Study arm	SQV serum	SQV CSF	RTV serum	RTV CSF	Ratio (%)
2	R+S	95	<LLQ*	6,200	<LLQ	-
6	d4T/R+S	171	<LLQ	2,497	26	1.04
8	d4T/R+S	285	<LLQ	3,359	57	1.70

LLQ is below the level of quantification (25 ng/ml)

Two participants who intensified their study regimen of ritonavir+saquinavir with 3TC/d4T because of a detectable viral load at week 18 had an undetectable viral load weeks after intensification.

**Safety.** Discontinuations of study medications were permitted for up to 2 months. 7 participants permanently discontinued study medications because of clinical (4) or laboratory (3) toxicities: elevated liver enzymes (2), 1 with grade 4; gastrointestinal side effects (2), nausea/diarrhea; peripheral neuropathy (1); skin rash (1); thrombocytopenia grade 4 (1). Main laboratory abnormalities: elevation of liver enzymes - 10% of patients had a grade 3 or 4 elevation of SGOT any time during the study; elevation of triglycerides - 25% of patients had a grade 3 or 4 toxicity.

**CSF Sub Study.** 9 study participants volunteered for lumbar punctures, which were performed at weeks 0 and 12. HIV RNA was measured at both time points and drug levels in CSF were measured at week 12. One person (Pt#2, RTV/SQV) had a partial serum response (detectable) and a complete CSF response (undetectable). Patient #8 (d4t/RTV/SQV) reached undetectable serum HIV RNA but not in CSF HIV RNA. 64-69% of the overall study population had a mean duration of prior therapy of 28 months but were protease and d4T naive.

The authors stated that the reason for the low drug levels in CSF (and the low ratios) are because of the high protein binding of ritonavir and saquinavir in serum. Nevertheless, RTV/SQV ( $\pm$  d4T) appears "highly effective in the central nervous system." Abbott officials say, the IC<sub>50</sub> of ritonavir in the absence of protein is 42 ng/ml. Since there is very little protein in the CSF, one would expect that low levels of ritonavir or saquinavir would have antiviral effect. The IC<sub>50</sub> is the amount of drug (the inhibitory concentration) needed to inhibit 50% of virus replication.

One concern is that although a PI may penetrate the CSF, will the amount of drug that penetrates be adequate to achieve the suppression necessary to prevent replication and resistance? Some researchers have raised the concern that if inadequate amount of drug penetrates and there isn't enough overall viral suppression from the combination regimen resistance mutations could develop.

### Hydroxyurea as a Novel Approach to HIV Therapy

The use of hydroxyurea (HU) has been receiving increased attention. This article updates new information and data which appeared in [NATAP Reports July 1997 issue](#). Although, hydroxyurea in combination with ddI or ddI/d4T has been shown to significantly decrease viral load, there are some concerns about its effects. In some studies where viral load was appreciably reduced when using HU with ddI or ddI+d4T, the CD4 counts did not proportionately increase; although, in some other studies CD4 increases were larger. Individuals with higher CD4 prior to using HU therapy may be more likely to achieve a better CD4 increase. HU acts in a way that may prevent CD4s from increasing. The mechanism by which that may occur was described in the article on HU in our July issue. HU therapy also may cause bone marrow suppression and neutropenia, a reduction in white blood cell count, which appears to be reversible upon stopping of therapy. Following are several reports issued over the last several months, including data from additional studies.

In the August 30, 1997 issue of Lancet, Dr. Jorge Vila reported in a research letter about a study of hydroxyurea+ddI addressing the issue of induction/maintenance, whether or not the proviral DNA they detected is infectious, the combination's effect on a variety of virus activity measures in plasma and lymph nodes, and the combination's activity in resting cells as a key to its benefit. It discussed the use of hydroxyurea+ddI as a first line therapy for treatment naive individuals, as an eradication therapy (if eradication is possible; it has not yet been proven), or as a therapy that may be useful during primary infection. The recently reported findings by Dr. Robert Siliciano has created doubt that eradication is possible.

You may have read press coverage about these 2 study participants, whose plasma viral load was undetectable (>200 copies/ml) after one year of therapy with ddI+hydroxyurea. Both agreed to completely stop therapy and remained undetectable at 1 year of follow-up. These two patients started therapy at 3 and 12 months, respectively, after HIV infection. Seropositivity was confirmed by ELISA and western blot. Neither patient had symptoms after infection. However, there may be reasons not to have overly high expectations from these developments. The baseline viral loads for these 2 persons were low (676 and 1120 copies/ml) and their CD4s were normal prior to study treatment, they were recently just infected, and I have heard a number of other anecdotal reports of individuals stopping a potent protease therapy and remaining undetectable.

In this study conducted by Vila, 25 ddI-naive individuals without symptoms with CD4 >200 were treated for up to one year with 200 mg ddI twice daily, and 500 mg hydroxyurea twice daily. Baseline HIV RNA was 29,396 copies/ml. Eight individuals received lymph node biopsies. Investigators said treatment was well tolerated. There were no treatment interruptions due to side effects. There were a few minor transient symptoms such as digestive disorders, and an increase in mean corpuscular volume. After one year, leucopenia (reduction in WBCs) was seen in four patients (three grade 1, and one patient Grade II). No changes in platelets, hemoglobin, amylases, lipases, or LFTs were recorded.

**Results.** 13/24 and 10/20 individuals suppressed their plasma viral load to undetectable (<200 copies/ml) at 6 months and one year, respectively. Most of these undetectable

individuals at one year also had no detectable infectious virus in their lymph node MNCs and CD4s after one year's therapy. The average CD4 increased from 525 to 601 cells, although in other studies the CD4 increase can be slight because hydroxyurea inhibits cell replication. Of the 8 individuals who had their lymph node biopsied, 1/8 had a detectable plasma viral load (1017 copies/ml), undetectable virus in lymph node mononuclear cells (MNCs), but had detectable infectious virus in their lymph node CD4s; 5/8 had undetectable plasma viral load, and no infectious virus in both their lymph node MNCs and CD4s; 1/8 had undetectable plasma viral load, but had detectable infectious virus in both the lymph node MNCs and CD4s; data was not available for one person.

For the two individuals undetectable after stopping therapy, they both reached undetectable in their plasma HIV RNA by one year on therapy and remained undetectable one year after stopping therapy. In addition, extracellular RNA was undetectable after 2 years in both plasma and lymph nodes; and intracellular HIV RNA was below the level of detection (undetectable) in PBMC (peripheral blood mononuclear cells) and LNMC (lymph node mononuclear cells). Culture yielded no infectious virus from either LNMC or purified CD4 t-cells.

At both 1 and 2 years proviral DNA was non-quantifiable in both patients who remained undetectable for a year after stopping therapy. Qualitative evaluation of proviral DNA at 2 years in PBMC showed low levels for both patients but Vila said it "seems" to be non-infectious or incompetent for the release of infectious virions. Siliciano's findings are that there is a small pool of proviral DNA that is replication competent and persists or will be long lasting. Some researchers have suggested that Vila just may have not found the replication competent proviral DNA.

Investigators said that "an explanation for the absence of viral rebound (for the 2 patients who stopped therapy) could be that this combination may exercise its anti-HIV activity in resting cells." But according to anecdotal reports other PWAs on treatment without hydroxyurea may have also remained undetectable after stopping therapy. Resting cells are an important reservoir for proviral DNA. It has been suggested that ddl, as well as 3TC and ddC, work in resting cells, while d4T and AZT work in activated cells. Hydroxyurea's mechanism of action may, theoretically, increase the chances of ddl in terminating the successful growth of proviral DNA competent to produce infectious virus. Furthermore, the authors say these findings suggest the combination of ddl and hydroxyurea may be applied for potential eradication in early disease, although Siliciano's findings might suggest otherwise.

Bristol Myers Squibb has started three trials exploring hydroxyurea in HIV treatment. A large trial comparing ddl+ d4T to hydroxyurea+ddl+d4T in 200 individuals with 200-500 CD4s started in December 1996. The second trial of 20 individuals will explore a 4-drug regimen of nelfinavir+ddl+d4T+hydroxyurea in individuals with >500 CD4. The third trial will explore the potential for eradication in 20 individuals during primary infection with the same 4-drug regimen:nelfinavir+ddl+d4T+hydroxyurea.

**DDI Resistance.** The authors stated that cellular proteins are less prone to mutations than viral proteins and that resistance of tumor cells to HU has not been reported after

35 years of clinical experience.

Investigators in the study found that virus with genotypic resistance to ddl were suppressed when HU was used with ddl. If resistance to HU does not develop and the development of genotypic resistance to an accompanying drug in a regimen does not effect the combination's efficacy, then it may not be necessary to suppress viral load to below detection to prevent the development of resistance from causing therapy failure.

### **Hydroxyurea + d4T/ddl vs d4T/ddl.**

In Hamburg, OT Ruschmann, Bernard Hirschel and others from the Swiss HIV Cohort Study reported findings from this 24 week study comparing the triple regimen of hydroxyurea (HU) and d4T/ddl to the double nucleoside regimen of d4T+ddl. The purpose as defined by the investigators of the study was to determine the short term effects of the HU triple regimen.

144 participants were d4T naive, ddl naive or with <6 months prior experience. They were randomized to HU+d4T/ddl or d4T/ddl. The dosing regimens were: HU- 500 mg bid, ddl - 200 mg bid, d4T- 40 mg bid. After 12 weeks, those receiving ddl/d4T were given the option of adding HU.

72 individuals were randomized to each group. Prior to reaching 12 weeks 7 discontinued from the HU group (1 due to an adverse event); and 3 discontinued from the d4T/ddl group (none due to adverse event). At week 12, there were 65 evaluable participants remaining in the HU arm and 69 in the placebo arm. **See Table 22 and 23**

**Table 22. Hydroxurea - d4T/ddl: Results - Adverse Events**

	<b>d4T+ddl</b>	<b>HU + d4T/ddl</b>
<b>Diarrhea</b>	9	15
<b>Nausea/vomiting</b>	7	11
<b>Pancreatitis</b>	1	0
<b>Fatigue</b>	2	10
<b>Neuropathy grade 1</b>	6	9
<b>grade 2</b>	4	6
<b>grade 3</b>	0	3
<b>Neutropenia grade 1</b>	3	11
<b>grade 2</b>	0	2
<b>grade 3</b>	0	1

Elevated lipase	3	5
Elevated ALT/AST (LFTs)	34	36

**Table 23. Week 12 Changes from Baseline**

	<b>d4T/ddl</b>	<b>HU+ d4t/ddl</b>
<b>CD4</b>	+107	+28
<b>CD4%</b>	+2.5	+3.0
<b>CD8</b>	+5	-124
<b>Total lymphocytes</b>	+196	-124
<b>HIV RNA (200 copy test)</b>	-1.5 log	-1.9 log
<b>HIV RNA (20 copy test)</b>	-1.7 log	-2.3 log
<b>%&lt;200 copies/ml</b>	29% (20/69)*	60% (39/65)*
<b>%&lt;20 copies/ml</b>	8% (6/72)*	19% (14/72)*

\* These percentages are based on the number of evaluable participants. It is not an intent-to-treat analysis which would base the percentage on the 72 patients that started in each arm. But, the %<20 copies/ml is based upon the entire 72 patient groups that started the study. In the HU arm 26 had >200 copies/ml and in the placebo arm 49 had > 200 copies/ml. These individuals had viral loads ranging from 201 copies/ml on up.

Investigators reported 27 participants withdrew at week 12 due to nausea (8), neuropathies (4), depression (3), lost to follow-up (8), and patient choice (4). Adverse events were more frequent in the HU arm ( $p < 0.05$ ).

The mean baseline CD4 and viral load were 367 cells and 4.53 log (about 33,900 copies/ml). The mean baseline CD8 was 1017, the total lymphocytes were 1774.

**After 24 weeks.** At week 24, there were 19 evaluable patients still in the d4T/ddl arm, 24 in the group that added HU to d4T/ddl at week 12, and 34 who started and were continuing the triple regimen of HU+d4T/ddl (the dropouts were mostly individuals who did not reach <200 copies/ml, 34/39 who reached <200 copies/ml stayed on HU triple therapy.)

Those who had a limited reduction in viral load from d4T/ddl were characterized as poor responders, and added HU at week 12. They were able to produce a nice decrease in viral load approaching those observed in patients initially randomized to the HU arm. But only 55% of these individuals who added HU had <200 copies/ml at week 24.

Generally, those initially randomized to the HU arm were able to sustain their viral load reductions at week 24. 84% who started the HU regimen and stayed with it remained <200 copies/ml at week 24.

Generally, the lower a person's viral load was at baseline, the more likely they were to reach undetectable. From a graph shown by the investigators it appeared as though most of those individuals with between 1,000 and 10,000 copies/ml at baseline were able to reach <20 copies/ml. However, those with >100,000 copies/ml at baseline were less likely to reach <200 copies/ml (and more unlikely to reach the 20 copy test) than those with <100,000 copies/ml viral load at baseline. Commentary - This may be a factor to consider when deciding when to start antiretroviral therapy. If you accept the idea that lowering viral load to undetectable or to as low as possible should be the goal of therapy (when possible), beginning therapy when one's viral load is no more than about 10,000 copies/ml may be a superior approach because it could be easier to reach <20 copies/ml and possibly easier to retain that reduction over the longer term.

**Safety and Antiviral Activity of Hydroxyurea (HU) with ddl.** 80 individuals without prior ddl, ddC and 3TC experience were enrolled in this multi-center, randomized, open-label AMFAR study in which participants were randomized to receive ddl alone for the first 12 weeks or ddl+HU. Individuals randomized to ddl alone were permitted to add HU after week 12. The delayed HU group received ddl alone for 12 weeks and the immediate HU group received HU+ddl from the start of the study. Participants were stratified by baseline CD4 (50-300, 301-600) to detect if there were differences in CD4 responses to HU therapy based on baseline CD4, since prior HU studies have shown that despite viral load reductions CD4 increases may not be proportionate and sometimes there were only slight increases in CD4 counts.

The HU dose was 500 mg bid (twice daily); the ddl dose was adjusted for body weight greater than or equal to 60 kg, 2 100 mg tablets bid; less than 60kg 125 mg bid (a 100 mg tablet and a 25 mg tablet). Plasma HIV RNA was evaluated by Chiron's bDNA assay. Exclusion criteria included: absolute neutrophil count less than 1500 cells/mm cubed; SGOT, SGPT, alkaline phosphatase, or total bilirubin less than 2.5 times upper limit of normal; evidence of pancreatitis (assessed by serum amylase, pancreatitis amylase isoenzyme, and/or serum lipase); evidence of peripheral neuropathy of grade 2 (moderate) or higher.

**Safety.** Discontinued prior to week 12: 2/38 in the immediate HU group; 8/42 in the delayed HU group (the dropouts in the delayed group were primarily due to suboptimal plasma HIV RNA suppression and persisting gastrointestinal adverse events.)

**Discontinuations weeks 13-24:** 14 individuals in the immediate group dropped out primarily characterized as due to physician/participant decision; 6 discontinued during weeks 13-24 in the delayed group also primarily due to physician/participant decision.

A total of 12 serious adverse events were reported but only 3 were determined as possibly study drug related.

Thrombocytopenia was reported in 1% of patients receiving ddl in phase I studies.



Thrombocytopenia (less than 50,000/uL) was reported in 1-4% of patients receiving recommended or high dose ddI in ACTG 116B/117 and 116A. Thrombocytopenia has been reported as associated with HU and is usually preceded by leukopenia. See Table 24.

**Table 24. Hydroxyurea - ddI:  
Week 12 HIV RNA and CD4 changes from Baseline by CD4 Strata**

	Immediate HU		Delayed HU	
	50-300	301-600	50-300	301-600
<b>CD4 strata</b>	50-300	301-600	50-300	301-600
<b>N=68</b>	18	12	18	17
<b>median base VL</b>	68,545	44,665	153,400	34,890
<b>median wk 12 VL</b>	4,861	1,054	31,735	2,394
<b>mean wk 12 log decrease</b>	-1.1 log	-1.2 log	-0.6 log	-1.1 log
<b>wk 12 less than 500 copies/ml</b>	6 (27%)	7 (58%)	2 (11%)	8 (53%)
<b>CD4 (n=68)</b>	18	13	20	15
<b>median base CD4</b>	244	385	158	424
<b>median wk 12 CD4</b>	232	409	191	485
<b>median wk 12 CD4 change</b>	+7	+61	+27	+42

**Week 24.** After week 12 HU was added to ddI monotherapy and resulted in a mean 0.4 log reduction in HIV RNA beyond that seen with ddI alone (n=33). At week 24 the HIV RNA reduction for the delayed HU group was similar to that seen at week 24 for the immediate HU group (n=30).

#### **Protease Inhibitor + HU and ddI**

Franco Lori, Heiko Jessen and others reported in Hamburg, results from a group of 17 individuals receiving indinavir or nelfinavir plus HU and ddI for an average of 8 months (range 1-12 months). Five were treated before sero-conversion; 5 within one year after sero-conversion; and 7 more than 1 year after sero-conversion. The investigators mentioned an appeal for this combination was that HU and ddI inhibit HIV in macrophages (resting cells), while protease inhibitors are effective in stimulated cells.

The average viral load and CD4 before treatment was reported to be 698,000 copies/ml and 450 cells. Investigators reported 17/17 achieved undetectable plasma viral load; semen viral load undetectable in 6/6; CD4 increases were 159 cells; treatments were

reported as well as tolerated, 7/8 became undetectable for HIV RNA in lymph nodes. However, as I mentioned above, a question that remains, do they have latently infected integrated proviral DNA?

Investigators reported that for individuals treated before sero-conversion Western Blot remained negative during treatment. One of these patients discontinued treatment while their plasma viral load was undetectable. Seven months later they were still undetectable and their Western Blot was still negative. As mentioned earlier, there have been other anecdotal reports of remaining undetectable after stopping treatment with HAART. Several immune system tests suggested the patients immune system may have improved. Partial immune system restoration has also been observed for some individuals following successful treatment with HAART (potent protease inhibitor+two nucleosides) which doesn't include HU.

### Conference Briefs

**Salvage Therapy with 6 Drugs.** Dr. Cassy Workman of Australia presented this abstract in Hamburg saying that because many individuals have failed protease inhibitor therapy salvage therapies need to be identified. A strategy is to recycle nucleosides for individuals who have been heavily pretreated with all available nucleosides. This study explores that concept for 12 heavily pretreated individuals who have failed previous treatment regimens. Following is preliminary 12 week data.

All 12 individuals had been exposed to and failed regimens including all available nucleosides and 3 protease inhibitors (indinavir, ritonavir and saquinavir). The 12 were started on an open-label combination of 6 drugs:

- |    |            |      |    |         |
|----|------------|------|----|---------|
| 1. | d4T        | 40   | mh | bid     |
| 2. | 3TC        | 150  | mg | bid     |
| 3. | ddl        | 400  | mg | per day |
| 4. | nevirapine | 200  | mg | bid     |
| 5. | nelfinavir | 1000 | mg | tid     |
6. saquinavir HGC 600 mg tid (old formulation of saquinavir)

D4T, 3TC, ddl and saquinavir were recycled for all 12 individuals. All 12 were naive to nevirapine and nelfinavir. The mean baseline viral load was 364,877 copies/ml and the median was 41,787 copies/ml. The range was 1,200 to 2,487,483 copies/ml.

9 patients remained on all 6 drugs and reached undetectable by week 12. The patients need to continue to be followed because 12 weeks is not long enough to conclude this approach worked for them. 3 who had been intolerant to components previously were again intolerant and are marked below. Compliance data were recorded. **See Table 25.**

**Table 25. 6 Drug Salvage Therapy: Results**

Pt	Prior Treatment	Base VL	Wk 12 VL	Base CD4	Wk 12 CD4	Compliance
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1	10 yrs, 6 months	11,400	<400	560	+30	>90%
2	8 yrs, 4 mos	43,790	<400	135	+30	100%
3	6 yrs, 3 mos	13,500	<400	310	+150	100%
4	6 yrs, 4 mos	33,900	<400	140	+220	95%
5	4 yrs, 3 mos	41,787	<400	110	+30	90%
6*	2 yrs, 1 mo	221,749	463,550	90	+50	100%
7*	9 yrs, 6 mos	1,422,293	517,941	90	-30	90%
8	5 yrs, 4 mos	6,500	<400	220	+80	100%
9	3 yrs, 7 mos	1,200	<400	750	-50	100%
10	5 yrs, 5 mos	47,300	<400	290	+80	100%
11	5 yrs, 4 mos	3,500	<400	290	+80	100%
12*	9 yrs, 3 mos	2,487,489	577,171	10	+10	100%

\*These were the 3 individuals who were intolerant to components of the regimen.

The author concluded that prior protease inhibitor experience may have caused some cross-resistance to nelfinavir, and considering that resistance to nevirapine can develop quickly, the results are surprising. They suggest that recycling may be effective if enough agents are used. This approach should be explored with further study. Failure in this group was not correlated with non-compliance but with inability to tolerate all drugs in the regimen.

**In Vitro Effect of HIV Protease Inhibitors on Methadone Metabolism.** When taken together methadone and a protease inhibitor can cause a drug interaction. Methadone is eliminated by P4503A4. Protease inhibitors and NNRTIs are eliminated through the same liver process. Healthy liver microsomes were used for this study. To predict the clinical relevance of the inhibitory effect of the protease inhibitor on methadone pharmacokinetics,  $K_i$  were compared to mean plasma concentrations of protease inhibitor measured in HIV-infected individuals after administration of standard doses. Assuming that the same concentrations are present in the hepatocytes, a 2-fold increase in methadone AUC can be expected when co-administered with ritonavir; coadministration with indinavir should lead to a 30% increase in methadone AUC, and no interaction is expected with saquinavir. Authors said a human study should be conducted to validate this data.

**Impact of HIV Protease Inhibitors on HCV Viremia.** The authors of this study said, co-infection with HIV and HCV is associated with increased HCV RNA. However, the

authors of abstract I-167 said, some studies have suggested that chronic hepatitis C could be more severe in HIV infected individuals and others have failed to find evidence to support this hypothesis.

The aim of this study was to explore the effect of protease therapy on HCV viremia. 19 IVUDs coinfectd with HIV and HCV were prospectively followed. All had progressive HIV and were treated with a protease inhibitor (ritonavir-9, indinavir-7, ritonavir+saquinavir-3) and 2nucleosides.HIVRNA (Roche Amplicor), CD4, CD8, and HCV RNA were measured at baseline and weeks 6, 17, and 32.

Authors concluded that although there was an initial increase in HCV viremia noted at week 6, there was little change in HCV viremia in subsequent follow-up to 32 weeks. Authors noted there was no decrease on HCV viremia despite the improvement of immune functions. See Table 26.

**Table 26. On HCV viremia despite the improvement of immune functions.**

	Baseline	wk 6	wk 17	wk 32	P wk 6 vs base	P wk 32 vs base
<b>HCV RNA</b>	5.3	+0.4	-0.2	-0.1	.003	.52
<b>HIV RNA</b>	5.0	-2.1	-2.4	-2.6	.0001	.0003
<b>CD4</b>	63	+73	+89	+115	.002	.001
<b>CD8</b>	456	+295	+224	+193	.001	.05

Baseline and changes in HCV and HIV RNA are expressed in log. 5.3 log is about 199,000 copies/ml; 5.0 log is 100,000 copies/ml.

**Effect of Infuenza Vaccination on HIV RNA.** 25 HIV-infected individuals were randomized to receive the Connaught whole virion influenza vaccine (n=9), the Connaught subvirion influenza vaccine (n=10), or a saline control (n=6). The baseline median CD4 count was 162 cells (range 1-788); the median viral load was 1750 copies/ml by RT-PCR (range 20-191,600). All but 4 individuals were receiving antiretroviral therapy. Viral load was determined at 1, 2, 5, 7, 14, 21, 28 and 60 days.

**Results.** The influenza vaccine was associated with a >3 fold increase in plasma viral load in 42% (8) of the vaccinated individuals. The increase was transient, occurred at a median of 21 days, and was of "limited magnitude" (maximum 8-fold increase over baseline). 5 of the individuals with a rise in viral load received the subvirion flu vaccine and 3 received the whole virion flu vaccine. 7/19 vaccinated individuals had a >4 fold increase in antibody titer to the flu vaccine. Investigators reported there was a positive association between response to flu vaccine and rise in viral load. Viral load increases occurred even in persons on regimens containing a protease inhibitor. Authors said, the clinical significance of the "burst" in viral load during immune stimulation requires further investigation.

**T-20.** At the September '97 IDSA meeting, preliminary data was reported from a phase I/II dose escalating study of T-20. T-20 inhibits fusion of HIV with host cells (CD4s). Although the following data is encouraging, it is preliminary from early stages of research in human. As well, current administration of T-20 is through an IV. Further studies are needed to evaluate T-20's efficacy and safety. Fusion inhibition's approach to HIV therapy is different than any other currently approved HIV antiviral.

16 treatment-naive or experienced (off drugs for 15 days prior to starting T-20) individuals received doses of 3, 10, 30 or 100 mg of T-20 every 12 hours for 14 days.

T-20 was administered by bolus intravenous infusion.

Investigators reported no drug-associated adverse events and a dose dependent decrease in plasma viral load and increase in CD4. The investigators concluded they saw significant anti-HIV activity. **See Table 27.**

**Table 27. T-20 - DOSE Mean Viral Load Mean CD4**

DOSE	Mean Viral Load			Mean CD4		
	Day 0	D 14	change	D 0	D 14	change
3 mg	4.82	4.71	-0.11	248	207	-41
10 mg	5.12	5.06	-0.06	357	344	-13
30 mg	4.95	4.47	-0.48	410	431	+21
100 mg	4.20	<2.70*	-1.50	322	374	+52

All 4 participants receiving the 100 mg dose were <500 copies/ml (undetectable).

Follow-up studies are necessary to confirm preliminary findings. Sub-cutaneous administration with an implant is expected to be explored in a forthcoming study. Hopefully, the company can develop an alternative way to administer T-20, otherwise it may only be acceptable for individuals with no other treatment options.

**Adefovir Dipivoxil (PMEA).** The [NATAP Reports July issue](#) contains a lengthy article about this nucleotide. At this point the information and data available for PMEA are preliminary, based on a small number of individuals. But the limited data so far suggests: (1) viral load reductions of about 0.5 log; (2) the potential appeal for PMEA is that it may have limited cross-resistance with nucleosides, so if you've had extensive nucleoside experience PMEA may still be effective; (3) the limited data suggests that

resistance and consequent diminished antiviral effectiveness may not develop very easily. Additional data is expected in February 1998 from study #408, which may be revealing in how PMEA may be helpful.

**MKC-442.** MKC-442 is a new NNRTI in an earlier stage of development by Triangle Pharmaceuticals. The company has been conducting a dose ranging study of 48 HIV infected individuals. Previously, they reported a median decrease in viral load of 1.18 log after 8 days for those taking 500 mg bid. In Hamburg, they reported median reductions of -1.41 log and -1.30 log at 8 and 15 days, respectively. After 1 week, those on 750 mg bid had their dose raised to 1000 mg bid for 3 additional weeks. The results from the 1000 mg dose are not yet available. Preliminary pharmacokinetics predicts a half-life of 6-8 hours for MKC-442. But, the company is also experimenting with a dose of 500 mg Tid which might be useful for individuals with a shorter half-life than 6-8 hours who thereby might have a lower trough. Clinical trials have recently started using a dose of 750 mg bid. When resistance develops from drug failure they have observed a key mutation at 103; K103N is also a key mutation for DMP-266. Investigators reported the drug as well tolerated with 10/44 experiencing headache, 5/55 nausea/vomiting, 2/44 experiencing a mild grade 2 rash, and modest elevations in GGT which is a liver related lab measure.

**D4T/3TC and AZT/3TC.** The sequencing of nucleoside combinations has become an issue now that data from a number of studies released in the past year have shown that several 2 drug nucleoside combinations (AZT/3TC, d4T/3TC, d4T/ddI, AZT/ddC) may be reasonable choices for use in an individual's first line therapy, which could be a 3 or 4-drug regimen including a protease inhibitor(s). Sequencing may depend on resistance and cross-resistance between nucleosides. See enclosed Resistance Supplement for a discussion of this subject.

Preliminary data from several studies have suggested that d4T/3TC is equivalent to AZT/3TC in treatment naive individuals, in terms of CD4 and viral load responses. In Hamburg, interim data from two studies were reported suggesting that for treatment naive individuals d4T+ddI or d4T+3TC in combination with indinavir appear to be as efficacious as AZT+3TC in combination with indinavir. Dr. Trip Gulick reported 6 month data from an interim analysis comparing d4T+3TC+ indinavir to AZT/3TC+indinavir. About 44 individuals were randomized to each arm in this open label study. At week 12, the number of evaluable patients was almost as large as the number starting the study and changes in viral load and CD4 were about equal for both groups. At month 6, the number of evaluable patients was only 15 and the d4T group CD4 increase was greater for the d4T group than for the AZT group. But it is too soon to say if there was a true difference in CD4.

Dr Robert Murphy reported 6 month data from an interim analysis comparing d4T/ddI+indinavir to AZT/3TC+indinavir. About 45 individuals were randomized to each of the two arms in this open label study. As was reported by Dr. Gulick at 12 weeks, where the number of evaluable patients was almost equal to the the starting number, there was no difference between the two groups with regards to CD4 or viral load. At week 24, there was a trend towards a higher CD4 increase in the d4T group (350 CD4 increase in the d4T group vs about 75 CD4 increase in the AZT group); but, the number

of evaluable patients was only 10 in each arm and too small to draw a conclusion. Both Drs Gulick and Murphy said that based on this interim analysis d4T/ddl and d4T/3TC appeared about equivalent to AZT/3TC in terms of efficacy. But, both cautioned this is an interim analysis and follow-up data may be more revealing. An ultrasensitive viral load test will be used in follow-up analysis. When deciding which nucleoside combination to select, its resistance implications should also be considered. See the Resistance Supplement in this issue.

### **Protease Inhibitors & Pediatrics**

Therapeutic options for children are more limited than for adults. The pathogenesis or course of disease for children is different than for adults. Children born with HIV do not have a developed immune system to fight HIV. An adult has a developed and intact immune system when HIV is contracted. Therefore, the disease can progress quickly in children. It may be more crucial for children to initiate therapy earlier in the course of disease progression than for adults. Although not yet widely accepted, some experts counsel that a potent therapy capable of maximal suppression of viral load should be considered as soon as possible after HIV infection.

### **SGC Saquinavir+Nucleosides.**

Fourteen children from 3-13 yrs old who were able to swallow adult capsules started treatment with SGC SQV (initial dose, 33 mg/kg tid) and 2 nucleosides of choice. Because a child's organ system is not fully developed and constantly quickly evolving the pharmacokinetics (blood levels) of a drug become more of a concern than for an adult. Selecting adequate dosing for children is more complicated and difficult. Dosing of any HIV drug may need to be more individually assessed than for adults.

The study is assessing tolerability, efficacy, and pharmacokinetics. Participants will be followed for a minimum of 24 weeks with intensive pharmacokinetic (PK) sampling at week 0 (single dose profile), week 4 (steady state), and as necessary thereafter. Sparse PK sampling is scheduled at every study visit after week 4.

12/14 children had prior nucleoside experience with a mean duration of 4 years. The nucleoside combinations selected for this study were: d4T/3TC (11), d4T/ddl (2), or AZT/3TC (1).

**Safety and tolerability.** No serious adverse events have been reported. 1 child with pre-existing thrombocytopenia had a marked decrease in platelet count study week 4. There was no interruption or discontinuation of therapy.

**Preliminary results.** The mean baseline CD4 and viral load were 446 cells and 4.6 log (about 39,000 copies/ml.) Because of the different course of disease progression in children compared to adults, the CD4 and viral levels have a different significance. Investigators reported by week 4, CD4 increased by 95 cells, and mean viral load declined by 2.1 log. At weeks 4 and 8, 5/12 and 7/7 children were <400 copies/ml. By visual observation of the line graphs it appeared that viral load at week 8 for 6/8 children was steady or declining further compared to the week 4 reduction. One child's viral load reduction was about 1 log at week 4 and the same at week 8. One child who had been

on study medications for 12 weeks had sustained about a 2.2 log reduction at week 12.

The authors concluded the PK characteristics are consistent with expectations. And the drugs were generally well tolerated.

**Indinavir PK in Children.** At the 8th European Congress of Clinical Microbiology and Infectious Diseases in May '97, Courtney Fletcher from the University of Minnesota, PhD, reported findings from evaluating indinavir pharmacokinetics (PK) in HIV-infected children receiving indinavir with ddl+d4T. Indinavir penetrated the CSF in 4 children measured with concentrations of 151-977 ng/mL.

In their PK evaluation of 12 children taking indinavir+ddl/d4T indinavir doses were adjusted if trough concentrations were <100 ng/mL. 9/12 children required adjustment of their indinavir regimen to q6h as trough values were <100 ng/mL. Investigators concluded indinavir PK in these children were highly variable. With a regimen of 500 mg/m<sup>2</sup> q8h, 9/12 children had trough values <100 ng/mL. Fletcher said concentrated guided dosing of indinavir may be necessary for an optimal anti-HIV effect, and can be used since intraindividual variability was modest.

### **Ritonavir + Nucleosides**

At ICAAC, 12 weeks data were reported for a randomized open-label study (ACTG 338) comparing AZT/3TC, d4T+ritonavir, and AZT/3TC+ritonavir. 298 children enrolled had a median age of 7.1 years (range 2-17 yrs), baseline viral load of 4.32 log (21,000 copies/ml). An interim analysis at week 12 showed for those in the AZT/3TC arm 6/43 (14%) were <500 copies/ml. Children in both ritonavir containing arms achieved about 60% undetectable (500 copies/ml.) At week 12, of those children on a ritonavir containing regimen 57% were on full dose ritonavir, and 10% permanently discontinued ritonavir. Those not responding well to AZT/3TC (viral load>10,000 copies/ml) were offered ritonavir+d4T+nevirapine. At week 24, the Data Safety Monitoring Board examined safety data and did not detect any safety problems for those taking ritonavir. The important messages are that no safety concerns were detected at week 24; and if a child is taking nucleoside therapy, switching to a protease inhibitor combination should be considered; switching to AZT/3TC was inferior to the two ritonavir regimens. The study is ongoing and 48 week data will be reported this Summer.

### **NATAP Resistance Supplement**

#### **Part I**

In October '97, NATAP held a Resistance Forum at NYU Medical Center. The morning session was a discussion of drug resistance. The afternoon session was a discussion on genotypic and phenotypic resistance testing. This first report reviews the discussions by 4 expert speakers in the morning session on drug resistance. A second report on the afternoon session is being prepared.

1. [Introduction by Dr. Roy Gulick, NYU/Bellevue Medical Center](#)
2. [Nucleoside Resistance by Dr. Daniel Kuritzkes, University of Colorado](#)
3. [Protease Inhibitor Resistance by Dr. Jody Lawrence, Stanford University](#)



#### 4. [1592U89 Resistance by Dr. Marty St Clair, Glaxo Wellcome](#)

The NATAP Resistance Forum was organized to discuss resistance and cross-resistance to drugs used for treatment of HIV, because resistance can be the most important reason for failure of a therapy; therefore, it is crucial to try and prevent it, and in many cases it is preventable. We will discuss ways in which you can act to help prevent resistance.

In previous years when treatment consisted of AZT, ddI or d4T monotherapy treatment choices were so limited that the development of resistance was not important because you could not prevent it for a long period of time. Although using two nucleosides in combination such as AZT/3TC or d4T/3TC could delay resistance better than monotherapy, it was still limited. Now, by using potent 3 or 4 drug combinations, viral load can be suppressed to below detection, and resistance can be prevented or at least significantly delayed. The suppression of HIV viral load is the key to preventing or delaying resistance; and, the prevention of resistance is the key to keeping viral load suppressed. The suppression of viral load can delay disease progression, that is the delay or prevention of the development of opportunistic infections and the preservation of your immune system. Following is a detailed reproduction of each speaker's discussion, which can be very helpful for you in understanding and preventing resistance.

#### **Introduction by Dr. Roy Gulick**

Dr. Gulick said the new goal of antiretroviral therapy is to suppress HIV replication as much as possible for as long as possible. The achilles heel of therapy, up until the development of the new treatments, has been the development of drug resistance. A simple definition of resistance is a change in the virus such that it is allowed to grow in the presence of an antiretroviral drug. A loss of drug sensitivity or susceptibility defines resistance. An increase in the viruses' fitness occurs. Fitness means the ability of the virus to duplicate itself in the presence of drug defines drug resistance.

Although many people are doing very well with the new treatments why is it failing others? There are multiple reasons why not everyone is responding well:

- Baseline resistance and cross resistance which will be the focus of today's discussion.
- The use of less potent regimens.
- Sequential monotherapy, which can mean starting one drug, adding a second drug weeks or months later and then adding a third weeks or months after that; adding them in piecemeal does not constitute triple drug therapy.
- Non-adherence or non-compliance
- Drug levels in blood can vary between individuals; drug interactions can effect blood levels of a drug.
- Tissue reservoir penetration by a drug such as the brain and genital tract; a drug may not suppress viral load in the brain as well as the blood, and consequent resistance could develop in the brain.
- Other unknown reasons.

Despite this long list of reasons, many of them lead to the development of resistance; and so, resistance is the main culprit. Resistance is the reason that many people are not doing well on today's new treatments. Today we'll be talking about baseline resistance, which refers to having resistance before even trying drugs, and cross resistance. Cross resistance refers to when a person takes one drug and fails, has a rebound in viral load, may not be very sensitive or susceptible to a second drug even if they've never taken the second drug before. Taking one drug will diminish the effect of other drugs in the same class taken down the road. Resistance to a nucleoside will not cause crossresistance to a protease inhibitor. But resistance to one protease inhibitor can cause cross resistance to another protease inhibitor.

It helps to understand resistance by understanding how HIV duplicates itself. In the HIV Life Cycle diagram, HIV is over on the left and the human T-cell to the right of it. HIV specifically recognizes the T-cell and binds to it through a couple of different proteins, which have been relatively recently identified. It loses its outer protein coat and its inner protein coat and exposes its genes in the form of RNA inside the host T-cell. It brings with it and makes its own protein called reverse transcriptase (RT). RT changes the viral RNA into viral DNA which develops a complex which enters into the nucleus of the cell and integrates or combines with the cell DNA. At the point of integration the cell is infected for the life of the cell. Although research is addressing this issue, we know of no way yet of removing the viral DNA once it has integrated.

After a possible period of being quiet the process turns back on and DNA is used to make RNA. RNA is used to make proteins which all assemble and are specifically cut by an enzyme or protein called the HIV protease. After that occurs the virus is fully mature and buds off from the cell. Each infected T-cell can make up to a thousand or perhaps many thousands of new viral particles which have bud off from the cell. Many of these will be fully capable of starting the process all over again and infecting other T-cells. After enough of these viral particles have budded off from the cell, the membrane

or the outer coating of the T-cell is compromised and the cell dies. After enough cells die the immune system becomes compromised and people get sick.

A goal of understanding the process by which HIV reproduces itself is to try and figure ways to interfere with it. Most of the drugs that we have either interfere with the reverse transcriptase step, and we call them reverse transcriptase or RT inhibitors, or the step very late in the life cycle where the proteins are cut by the HIV protease, and drugs which work at that step, and we call them protease inhibitors.

There are research efforts ongoing to develop other drugs that are targeted to work at the initial step of recognition and binding by a virus particle and the T-cell, and at the step of integration (integrase inhibitors). Research for these drugs is not very far along in development. Today's discussion will focus on the available RTIs and protease inhibitors.

### **Why Does Resistance Occur?**

It has to do with factors that we've come to understand only in the last couple of years. Most individuals have a high viral burden (load). It is believed that a person has billions of viral particles (viruses) being produced and cleared every day. So, there is an enormous amount of duplication (replication) of the virus on a daily basis throughout the whole course of HIV infection. There is also a high rate of mutation. Every time HIV duplicates itself it makes errors or mistakes in the genes. On average, it makes one or several mistakes every time it duplicates its own genes. And, many T-cells are made and lost on a daily basis.

The many different mutations that occur every time the virus duplicates itself causes genetic diversity. That is, a person is infected with many different viruses, different because they contain different combinations of mutations. Drugs select certain viruses to suppress. The fittest viruses may survive and go on to duplicate themselves, unless you can fully suppress replication including all or almost all viruses. It appears as though an effective triple drug regimen when it is successful is suppressing all or almost all viruses. The fit viruses can more easily evade suppression when therapy is not potent enough to fully suppress viral replication and sustain it for a relatively long period of time. Certainly, that is why viral load will usually rebound relatively quickly after using therapy consisting of one RT inhibitor.

Eleven approved antiretroviral drugs:

#### **• Nucleoside RT inhibitors:**

AZT,	zidovudine	(ZDV),	Retrovir
ddl,		didanosine,	Videx
ddC,		zalcitabine,	HIVID
d4T,		stavudine,	Zerit
3TC, lamivudine, Epivir			

#### **• Non-Nucleoside RT inhibitors:**

delavirdine, Rescriptor	nevirapine,	Viramune
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• **Protease Inhibitors:**

	indinavir, ritonavir,	Crixivan
Fortovase, Invirase, nelfinavir, Viracept	saquinavir hard gel	Norvir capsule saquinavir
	soft gel capsule	

New drugs in development:

• **Nucleoside Analogue RT Inhibitors:**

adefovir dipivoxil	1592U89	(abacavir)
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• **Non-Nucleoside RT Inhibitors:**

MKC-442	DMP-266,	efavirenz,	Sustiva
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• **Protease Inhibitors:**

ABT-378	142W94,	VX-478
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Currently, there are 3 ways to test for HIV resistance: genotypic resistance, phenotypic resistance and clinical resistance. In a genotypic resistance test, you are looking at the genes or genetic material of the virus itself. Such a test can be performed before starting a drug(s), while taking a drug(s), or after stopping the drug(s). You are looking to identify changes in the genes (mutations). In using a phenotypic resistance test, a person's virus can be taken into the lab. They are not simply looking at the genes. The virus is grown in the lab in a culture in the presence of drug to see how much drug may be necessary to suppress the virus. If more drug is required than the amount used in recommended doses, that means resistance has developed. A measure of how much more drug is required than the recommended dose reflects the amount of phenotypic resistance. Phenotypic resistance can occur as a result of changes or mutations in the genes (genotypic mutations).

Clinical resistance is reflected by viral load test results. Most doctors who take care of patients use this approach. After starting a drug regimen viral load can go down below detection and stay down. This might mean there is no resistance developing. After starting therapy, viral load might not go down much or at all, or it might go down and then come back up. This could mean resistance has developed. If a person is completely not taking the drugs prescribed, of course no change in viral load should occur, which means there is no resistance. Clinical resistance may not correlate with genotypic or phenotypic resistance; you may not be able to detect geno- or phenotypic

resistance, although clinical resistance or failure occurs.

The use of geno and phenotypic resistance tests may be effective tools in the future, but some people are using them now to try and figure out which drugs may or may not work for a person and which drugs they could successfully switch to.

**How do you prevent resistance?** You can maximize antiviral activity of the drugs you are taking by selecting the most efficacious or potent combination regimen. These days a number of drugs are available to put together such a regimen. Another way is to maximize the genetic barrier to resistance. Combine drugs which have no overlapping resistance mutations. Generally, choose drugs that need more than one mutation for resistance to develop. Choose drugs that the person has never taken before. Do not treat sequentially. That is, do not add drugs to a regimen one at a time spaced out over a period of weeks or months. When switching a regimen because a person is failing the regimen they are taking, the US Public Health Service Treatment Guidelines recommends changing to at least two new drugs. It is preferable, if possible, to start therapy with 3 new drugs.

### **Reverse Transcriptase Inhibitor (RTI, nucleoside) Resistance by Dr. Daniel Kuritzkes**

There are many causes for failure of drug therapy, as Dr Gulick outlined. Possibly the most important cause today is problems of adherence to therapy which leads to suboptimal treatment which leads inevitably to resistance to drug therapies. Its important to realize the presence of reservoirs (e.g., brain) where drugs may not penetrate well where viruses may find themselves uninhibited or only partially inhibited by the drugs. As well, differences of metabolism within a cell may play an important role in whether a drug is effective or not. The nucleosides are actually prodrugs. The drugs you actually take are not themselves effective against the reverse transcriptase. They must be activated by the cells. They are activated by a process called phosphorylation, in which phosphate groups are added one at a time until there are three of them and the drugs are then in their triphosphate form. It is the triphosphate which is now a nucleotide that is recognized by reverse transcriptase and is able to inhibit reverse transcriptase by competing with the naturally occurring triphosphate nucleotides inside the cell.

Different kinds of cells and cells at different stages in their lifecycle have a different ability to activate these drugs because the enzymes that are responsible for activating the nucleosides are expressed differently in cells that are resting or cells that are activated. For example, we know that AZT is activated or phosphorylated best in T-cells that are in an active state and is phosphorylated less extensively in resting T-cells. Whereas, ddI appears to be phosphorylated equally well in resting and active cells. It is unclear what the clinical relevance of this information is, but it is important to keep in mind that the differences in the ability of the cells to phosphorylate and activate the nucleosides could potentially have some implications.

Perhaps the clearest example we have to date is that drugs that compete for the same enzymes for phosphorylation may antagonize each other in their ability to inhibit HIV. D4T has a very similar structure to AZT and uses the same enzymes as AZT.

Preliminary data from ACTG study #290 suggests that using AZT and d4T together does not work well clinically, supporting research from lab studies suggesting antagonism between these two drugs. Individuals in study 290 who had been on long term AZT and added d4T to AZT had a more rapid decline in CD4 count than those who simply switched to d4T or those who stayed on AZT. The study was stopped as a result and more complete data will be presented at '98 Human Retroviruses Conference. This is an example potentially of intracellular metabolism being the cause not of resistance as we understand it, but of metabolic failure.

As Dr. Gulick explained earlier, HIV has a high replication and mutation rate which leads to genetic diversity; because of that, there are mutations pre-existing even before starting any drug therapy. These mutations may by themselves cause resistance to a drug, but they appear rarely. Treatment by a single drug may inhibit virus that do not have mutations while those with mutations may not be inhibited. These mutated viruses may continue to replicate and resistance to the drug may develop over a period of time causing drug failure. The longer this drug continues to be used the more resistance will develop as the mutant viruses replicate more.

If you use two drugs, some of the viruses may be resistant to one drug but not the other and some viruses may be resistant to both drugs. But you've created a higher genetic barrier to the development of resistance. That is, resistance to the two drug regimen should develop, but it is more difficult to develop and should occur more slowly. So, you have mutant viruses that may have been pre-existing or might emerge on therapy. Where only a single mutation is needed for resistance to one of the drugs, it is highly likely for the mutation to be present. If more mutations are necessary for resistance to a drug it is much less likely that there are pre-existing viruses containing these multiple mutations.

Using a potent triple drug combination has been shown to be more successful at inhibiting virus replication and therefore the development of mutations causing resistance. An extremely potent two drug combination which requires multiple mutations for resistance to develop may be just as effective. By potent inhibition of virus replication the opportunities for the emergence of virus with mutations is much more limited. The 1 and 2 year results from several studies show that with proper adherence 80-90% of the study participants were able to sustain viral load below detection by standard commercially available tests.

An extremely potent triple drug combination has created a high genetic barrier. There may be rare pre-existing viruses resistant to one or even two of the drugs, but there are no rare mutant viruses that are resistant to all the drugs. So, in the short term (1-2 years) we do not yet see viruses escaping inhibition by the triple drug therapy.

(Commentary - Some researchers believe that low level virus replication is ongoing even in the face of so-called complete viral suppression. Some believe eventually resistance will develop to the potent triple drug regimens. They believe that lowering viral load to as low as possible may be the best approach to dealing with that potential problem. At the December '97 ACTG meeting, Doug Richman, MD, a leading AIDS researcher from UCSD, said the goal of therapy should be to lower viral load to <1

copy/ml. The results of two studies suggest lowering viral load to <20 copies/ml may increase durability of viral suppression. However, some researchers suggest that it may be possible to delay disease progression if you can maintain viral load at a low but still detectable level. One study suggested that keeping viral load below 5,000 copies/ml using just two nucleosides as an initial therapy could delay disease progression for a year. The criticism of that approach is that resistance will develop to the drugs used possibly within a year or less, and that could limit subsequent choices for constructing effective combinations. After developing resistance to nucleoside(s), it appears that benefit will diminish from other nucleosides used afterwards.)

Dr. Kuritzkes went on to discuss two concrete examples of resistance. First he discussed nevirapine (Viramune) which is a NNRTI; this was followed by a discussion using AZT and 3TC as an example of a NRTI. A single mutation can be enough for development of resistance to nevirapine. Resistance is the loss of ability to suppress virus. Although it's rare, the nevirapine single mutation resistant virus can pre-exist in a person even if they've never before taken the drug. If nevirapine is used alone, resistance would usually develop within weeks. The overgrowth of pre-existing mutants, viruses already resistant to nevirapine, emerge very rapidly leading to a complete loss of antiviral activity because only one drug was used. However, in Boehringer Ingelheim study #1046 which studied individuals with no prior drug therapy experience, nevirapine was effective in inhibiting or suppressing viral replication in a triple regimen with two nucleosides, but a two drug regimen consisting of nevirapine plus just 1 nucleoside did not adequately inhibit virus replication. So, resistance can develop quickly if you use a drug alone which needs only one resistance mutation for a loss of viral load suppression.

A different and more complicated situation can occur with the use of nucleoside RT inhibitors (NRTI). For example, although a drug such as 3TC can develop high level resistance very quickly with a single mutation, it can interact in a complicated way with AZT. In Glaxo Wellcome study NUCA 3001, when 3TC was used alone a potent inhibition of viral replication occurred but resistance developed fairly quickly accompanied by a rebound in viral load. The development of a single mutation, such as with nevirapine, caused high level 3TC resistance and a loss of antiviral activity. But, in the NUCA 3001 study viral load did not rebound to its level just prior to starting 3TC. Although high level resistance was detected viral load did not go back to baseline, there was a little persisting antiviral activity. When AZT and 3TC were combined you can observe a persisting greater additive or possibly synergistic antiviral effect, which may in part be due to 3TC preventing AZT resistance mutations.

It has been found that in some patients 3TC reverses and delays AZT resistance until possibly a large number of AZT mutations have accumulated, and some other so far poorly defined mutations accumulated. But, in a greater number of patients, in whom 3TC does not have that effect, it is not understood why viral load reduction persist despite the presence of the 3TC 184 resistance mutation. And 3TC resistance develops within 6 weeks even when taken with AZT. At week 52, viral load inhibition was sustained in the study, although after one year some study participants started to see a rise in viral load as viruses resistant to both drugs emerged. In this study for those

taking AZT/3TC, it can take many months for enough AZT mutations to accumulate for viruses to become highly AZT resistant. Over the course of the first year despite the accumulation of one or just a few mutations in about a 1/3 of patients their viruses by and large remained highly sensitive to AZT although they were resistant to 3TC.

**Geno and Phenotype Testing.** You can measure in the lab how much drug is needed to inhibit HIV by either 50% or 90%. When you talk about the amount of drug required to do this you are talking about the inhibitory concentration (IC), so you'll hear terms such as IC50 or IC90. This is called phenotypic resistance testing. If you need more drug to suppress the virus than is normally needed that means there is phenotypic resistance. If the amount of drug necessary to inhibit virus replication is 8 times more, then you have 8 fold phenotypic resistance. A different way to look at resistance is to perform a genotypic analysis, where you are looking at the sequence of the genes in the virus itself. You are looking for changes in the genes (mutations); but you have to look for mutations that have been proven to result in drug resistance, to make your findings useful for the patient.

Commentary - There are some concerns I have about genotypic testing. Will your genotypic analysis detect a MDR (multi-drug resistant) mutation which is discussed below? You can fail d4T without any mutation being detectable (discussed below)? If you stopped taking a drug, after a while a resistance mutation may not be detectable although it might have been detectable while on therapy.

**Nucleoside Resistance Mutations Primer.** This is a review of different kinds of mutations that are responsible for resistance to various drugs with a discussion of how resistance to one drug can overlap with or cause resistance to another drug (cross-resistance). The principle AZT mutations that lead to AZT resistance are 41, 67, 70, 215, and 219. But, we've recently learned that there are additional minor mutations that continue to accumulate even after the first 4 or 5 have accumulated. By themselves these mutations do not give rise to broad cross-resistance. But clearly patients that have failed AZT and have highly resistant viruses do not respond as well to subsequent (nucleoside) therapy. What we don't know for sure is if its due to true cross-resistance at the level of the virus or is it because people who are failing therapy have been selected in a sense and are going to do poorly whichever (nucleoside) therapy they are given. We do not yet have adequate data for individuals who were treated first with other nucleosides and subsequently switched to AZT+3TC, to know if individuals who failed ddl or d4T would have the same or worse subsequent outcome.

The principal mutation that can occur from ddl therapy is 74. This mutation is shared by ddC and is also potentially shared by 1592U89. Although we don't know yet what happens in people who fail ddl (how well will they respond to other nucleosides?), it's clear that people who are resistant to ddl are not going to respond to ddC; and, people who are ddC resistant are not going to respond to ddl. (Commentary- there is some suspicion that 3TC and ddl may have some cross-resistance).

D4T is a real puzzle. Although a mutation at 75 has been found in the lab, there is little convincing evidence that patients treated with d4T show resistance to d4T. Clearly, prolonged d4T therapy leads eventually to d4T failure, but there are no virologic



markers that we can measure identifying viruses that are no longer responding to d4T. But, there is a curious mutation that gives rise to resistance to many of the nucleosides. This is a mutation, called the multi-drug resistant mutation (MDR), that can emerge from using the combination of AZT+ddl. There are several that can emerge (62, 75, 77, 115), but the principal one is 151. When a virus accumulates a MDR mutation plus others you can get virus that is highly resistant to AZT, ddl, ddC+d4T and perhaps to 3TC (if 184 is also present), and we don't know yet about 1592. About 10% of patients using the combination of AZT+ddl can get the MDR mutation, so you should be concerned about using this combination certainly as an initial therapy.

**Selecting Treatment Based on Resistance.** Each treatment option carries with it the risk of certain resistance and cross-resistance patterns (and certain side effects). At the present time it may be difficult to say that any one specific set of treatments is clearly better than an equally potent set of treatments in terms of what the downstream options are going to be. But, whichever choices are made initially it carries important implications for what options remain because of the patterns of resistance and cross-resistance described above. (commentary - The results of several studies revealed in the past year suggested that d4T+3TC had similar effects as AZT+3TC, in terms of CD4 increases and viral load reductions for individuals who had never before taken therapy. Some doctors are using d4T/3TC as a first line nucleoside option).

Several studies have shown that patients who failed AZT do not respond as well to subsequent (nucleoside) treatments. But, to be fair we are not sure if this is due to cross-resistance due specifically to AZT resistance, or people who fail an initial nucleoside therapy are not going to respond well to any subsequent nucleoside therapy.

We don't have data yet on individuals failing d4T or ddl first and then switching to AZT. As mentioned above ddl failure clearly leads to ddC failure. It is yet uncertain of the effect of a ddl failure on subsequent 1592 treatment in patients. Although the MDR mutation that can result from AZT+ddl combination therapy can be uncommon, the potential problem is severe enough that you should avoid using it as an initial regimen. 3TC may have some degree of cross-resistance with ddl and ddC, but there is little data from patients on the use of ddl after 3TC failure. It seems less likely that 3TC resistance will play a major role in cross-resistance to 1592. (Commentary- having dual resistance to AZT and 3TC may play a more significant role in causing cross-resistance to 1592. See the 1592 resistance report later in this section).

### **Strategies for Preventing Resistance**

- Raise the genetic barrier to resistance; that is, select drugs for your regimen wisely in order to choose drugs that require complex patterns of mutations; you don't want to use many drugs together each of which needs only one mutation that will lead to resistance; that would make it easier to select from the pre-existing pool of mutations that would cause a drug to fail more quickly
- You want to use drugs with non-overlapping patterns of resistance such as AZT/3TC or d4T/3TC.
- We need better drugs that are easier to take and better tolerated; by making drugs that have characteristics making it easier to take, compliance will improve; for

example, a once-a-day regimen should be easier to take for many individuals. The current crop of available drugs do have limitations; increasing the amount of a certain drug may increase its effectiveness, but you can't do that because using higher concentrations of the current drugs would cause safety problems.

### **Protease Inhibitor Resistance by Dr. Jody Lawrence**

Additional protease inhibitors in development:

- Two protease inhibitors from Bristol Myers Squibb, one is in a human study
- PNU 140690 from Upjohn & Pharmacia in phase I human study
- DuPont Merck 850 & 851 about to enter phase 1 human study.
- Parke Davis has several protease inhibitors in early development.

Although protease inhibitors are potent and can lead to success, they must be taken as prescribed (compliance) to have prolonged suppression. Drug levels in the blood must be kept above a certain level. If by missing a dose or not taking full doses (poor adherence), or due to high metabolism you drop down below that level needed to suppress the virus, it allows the virus to mutate. If the mutated virus replicates enough and resistance develops, the drug will not be potent enough to suppress viral replication any longer.

The approved protease inhibitors have overlapping resistance mutations. That is, they have some of the same mutations in common for resistance to that drug to develop. The major or primary mutations for indinavir and ritonavir for patients taking the drugs are almost identical: 82, 84, 54, 46. The major mutations reported for saquinavir are 48 and 90. It's been reported that the major mutation for nelfinavir is 30; and for 141W94, 50 appears to be the major mutation. If you concentrate only on the major mutations it looks simpler than it is. It may appear as though there aren't as many overlapping mutations and possibly you could switch from one protease inhibitor in some situations to another. But a number of other mutations develop with time on treatment and many of them are overlapping. There appears to be much more cross-resistance between these drugs than we originally thought.

(Commentary - For example, additional indinavir mutations found in humans include 71, 90, 10, 20, 32, 64, and 63. Additional ritonavir mutations found in humans during ritonavir therapy include 20, 33, 36, 71, 63 and 90. Additional mutations that have been identified with saquinavir include 10, 46, 54, 63, 71, 82 and 84. Although Agouron says that only a mutation at 30 causes resistance and that these other mutations observed are not relevant to resistance to nelfinavir, these have been observed - 71, 77, 88, 46, and 84. For 141W94, 46 and 47 are additional mutations that have been observed. These additional mutations are usually referred to as secondary mutations. Some combination of the major mutations and/or the secondary mutations can cause resistance to a specific protease inhibitor.)

As you can see, although the major mutations may not be as overlapping, the secondary mutations can be. This is a major factor in the development of cross-resistance.

For both ritonavir and indinavir it has been uniformly reported by both manufacturers, Merck and Abbott, that an accumulation of several mutations is required for resistance to emerge. Merck has reported it takes an accumulation of three or more mutations for resistance to begin to develop to indinavir. Merck has reported that mutations at 46+63+82 produced a 4-fold resistance when tested in the test tube or lab.

They also reported that mutations at 46+63+82+84 produced an 8-fold increase in resistance to indinavir in the lab. Merck has stated that the accumulation of mutations causing resistance to indinavir do not necessarily occur in an ordered fashion. In other words, it may be a random accumulation of several different mutations. However, Abbott has said that the mutations for ritonavir occur in a step wise and ordered way, possibly with 82 occurring first followed by specific other mutations.

Abbott has reported that a mutation at 82 has been observed in the lab to confer 2.5 fold resistance; that mutations of 82+54+71+20+36 observed in the lab caused an 8 fold increase in resistance to ritonavir. These examples stated above for ritonavir and indinavir are only isolated examples for specific situations. Individuals can experience different genotypic resistance mutation profiles.

Dr. Lawrence showed an example of one person's experience in developing resistance to indinavir. This person at baseline, before taking indinavir, had a few mutations that sometimes are referred to as polymorphisms or as we described earlier pre-existing mutations. At week 12, the person picked up a couple more genotypic mutations but no phenotypic resistance was detected. In this Resistance Supplement, Dr. Gulick defines phenotypic resistance. At week 24, an 82 mutation was observed along with a 63 mutation, and now a 4 fold increase in phenotypic resistance was detected. With further therapy additional mutations (84, 71, 24) were seen at week 40 accompanied by about an additional 4 fold increase in phenotypic resistance. By week 52, additional mutations were observed (20, 54) and phenotypic resistance at least doubled from its level at week 40. A rebound in an individual's viral load may or may not occur at the same time as genotypic or phenotypic resistance is observed. A person's viral load may rebound before these changes are seen.

A more detailed discussion about protease inhibitor resistance and mutations accompanied with tables is available on the NATAP web site ["Protease Inhibitors: Success and Resistance"](#).

Dr. Lawrence then went on to discuss some of the studies presented at recent conferences. The study presented by Dr. Steven Deeks caught the headlines in the mainstream press. He reported that 53% of patients in his clinic had failed protease inhibitor therapy when he looked back at their charts. But, his purpose was to show how protease inhibitors should not be used. The mainstream press misinterpreted his point. The high failure rate was due to using protease inhibitors improperly. In fact, he reported that individuals using them properly had 80-90% success rates. Deeks pointed out that only 1/13 individuals who were drug naive had drug failure. Non-adherence was a problem among the group with a high failure rate. They had high baseline viral load and low baseline CD4 counts meaning that therapy should be started before an individual reaches such an advanced stage. Some of them merely added a protease

inhibitor to their current nucleoside therapy. As well, some had previously taken saquinavir so may have had some cross-resistance.

### **Protease Inhibitor Salvage Therapy**

Dr. Lawrence reviewed several studies for individuals who failed indinavir and then were switched to a ritonavir+saquinavir regimen. Although initial viral load reductions may have been as much as 1.5 log, which is still not as much as seen when an individual is naive to protease inhibitors, the reductions were generally short lived. By week 24 or week 16, the percentage of individuals who were undetectable (<500 copies/ml) was low ranging from 10% and 25% to 37%.

She reviewed several studies of individuals who failed saquinavir therapy and then were switched to an indinavir regimen. The results were a little bit better but not very much. She reviewed one study of individuals who failed saquinavir and were switched to ritonavir+saquinavir. Individuals received an initial 1.52 log reduction in viral load but by week 16 viral load rebounded.

She discussed a study she is conducting where individuals who failed saquinavir were first switched to a nelfinavir regimen. NNRTIs were not used in this study because it was before we knew how to use NNRTIs with protease inhibitors. The individuals were mostly heavily pretreated with nucleosides so when they switched from saquinavir to nelfinavir only 30% were able to change to new nucleosides. Some individuals recycled old nucleosides. After an initial nice viral load reduction for some individuals, by week 12 only 2/16 were still undetectable. Subsequently, individuals stopped nelfinavir and added indinavir+nevirapine to their unchanged nucleoside therapy. There was a nice initial viral load reduction that lasted a little longer but over time the durability appears to be fading.

(Commentary - One strategy to deal with protease cross-resistance is discussed in more detail in the Protease Inhibitor Update in the section called "[Monthly Monitoring of Viral Load](#)." Essentially, if you monitor your viral load monthly and change to a potent therapy possibly consisting of 4 or even 5 drugs as soon as you detect a rebound in viral load, you may be able to limit cross-resistance).

Dr. Lawrence then went on to discuss two reports from ICAAC in October '97 where individuals who failed nelfinavir were switched to other therapies to assess their response. The prospective study conducted and reported by Keith Henry showed more optimistic results. Individuals were divided into two small but different groups in this study. The first group of 12 individuals had limited prior nucleoside experience, CD4s of about 200, and viral load of about 60,000 copies/ml. They failed the regimen they were taking in nelfinavir study #511 of nelfinavir+AZT+3TC; they were drug naive prior to enrolling in 511. They were switched to ritonavir+saquinavir+d4T/3TC. Initially, 12/12 (100%) were <500 copies/ml and at week 16 6/7 (85%) were still <500 copies/ml, which is encouraging. But this is still only 16 weeks. The key is to see how durable the effect will be. A follow-up report will be presented at the '98 Human Retroviruses Conference in February.

Henry took a group of 7 individuals from nelfinavir study #525 who had much more nucleoside experience, protease naive, and had significantly lower CD4s (65) and higher viral load (233,000 copies/ml). Only 3/7 were able to achieve a viral load <500 copies/ml. The other study was a retrospective study (looked back at patient charts). 6 went on indinavir therapy and 6 went on ritonavir+saquinavir therapy, after failing nelfinavir therapy. Only 3/12 individuals had a good response.

Dr. Lawrence suggested that if you can detect viral load rebound before it develops too much (as discussed in the article, Monthly Monitoring of Viral Load), a quick switch to another potent regimen may work. If you delay switching therapy, over time the number of mutations will build up for all the protease inhibitors with cross-resistance developing.

(Commentary - She suggested one way to increase the efficacy and durability of protease inhibitor therapy is to increase the trough levels of a drug. This is the subject of ongoing research. The trough level of a drug is the amount of drug in the blood at the end of the dosing period: for example, 8 hours for indinavir and 12 hours for ritonavir. Some individuals fail therapy because they have low trough levels due to high metabolism or taking a dose too late. Combining 2 protease inhibitors or a protease inhibitor with a NNRTI may raise the trough level if the drugs are dosed properly and if the right drugs are selected. This may prevent an individual from having inadequate blood levels of a drug. However, sometimes when you raise blood levels you can increase side effects. Several protease-protease combinations are being explored with this idea in mind: indinavir-ritonavir, indinavir-nelfinavir, ABT-378/ritonavir, ritonavir-nelfinavir. As well, delavirdine used in combination with other protease inhibitors may be useful to apply this concept because delavirdine is the only NNRTI that significantly increases protease inhibitor blood levels. However, the proper dosing regimens must be identified before experimenting with these combinations, and ongoing studies are exploring this now. With the exception of ritonavir+saquinavir, where dosing regimens have been identified.)

Dr. Lawrence was asked a question from the audience, is nelfinavir a preferable choice for a person's first protease therapy? She said, that a key question now is should a person start with nelfinavir or indinavir? On the one hand, the preliminary Keith Henry data may suggest, if the data holds up beyond 4 months, that you may have more leeway for bringing on other drugs for salvage therapy if you fail nelfinavir as a first line therapy. Although individuals with more extensive prior nucleoside experience did not do as well. However she said, we don't know if you might develop resistance to nelfinavir more rapidly if you started on that drug, than for indinavir if you started on indinavir first. She said we need head to head comparisons of the two drugs to determine which may be a better first line protease inhibitor.

### **1592U89 Resistance by Dr. Marty St Clair**

New data and resistance information about the use of 1592U89 has been recently reported at the 6th European Conference on Clinical Aspects and Treatment of HIV Infection in Hamburg, Germany (Oct 11-15,1997), and at the NATAP Resistance community education forum at NYU Medical Center (Oct. 25, 1997). Some of the information was previously reported, but much of it is brand new. If you do not want to

read all the information in detail, first is a brief summary of the report.

Treatment-naive individuals in studies have achieved about a 2 log reduction in viral load due to 1592. For individuals who are nucleoside experienced the benefit can be less. Based on in vitro data and preliminary data from 22 week results of a small human study (CNA 2003, page 35), individuals with extensive experience with nucleosides and/or resistance to a broad number of nucleosides may or may not respond well to 1592. The participants have viral load reductions at week 22 ranging from none to -2.44 log. It appears too early to predict exactly how an individual may respond, but Table 3 below lists HIV RNA responses for the 18 study participants and other related information, and the data in the table may offer some suggestions on how 1592 will effect treatment experienced individuals. Glaxo Wellcome believes, and the preliminary data supports this thinking, that individuals with extensive experience and/or resistance to a number of nucleosides will be less likely to respond well to 1592. However, from examination of the data in Table 3 of the 18 study participants, some individuals who you might expect not to respond well to 1592 do in fact get a good response to 1592; as well some individuals you might expect to respond well do not. As study results become available over time, analysis will reveal more helpful information. In an animal study, 1592 penetrated the brain and CSF well. An ongoing dementia study using 1592 should be more revealing. For more information on the CSF animal data, see the article "[The CSF and Indinavir, Ritonavir+Saqinavir, DMP-266, 1592U89, AZT vs d4T](#)".

**Emerging Resistance Patterns.** A more comprehensive review and discussion of the in vitro resistance data is available in an article posted to the NATAP web site several months ago called [1592U89 Cross-Resistance](#).

One of the first things researchers do with a new drug which is expected to be used by humans is to passage virus in the presence of the new drug to see if mutations could be found that might render the virus resistant to the drug. Researchers took a wild type virus (no resistance mutations) and put it into the test tube with 1592; 4 mutations emerged. The first to emerge was the M184V mutation which is the 3TC mutation followed by 65R, 74V and 115F upon further passage. Each of these 4 mutations individually result in 2, 3 or 4 -fold increase in resistance. In more scientific terminology, each of these single mutations resulted in no more than a 4-fold increase in the IC50. The IC50 is the inhibitory concentration of drug that will block replication of virus by 50%. This is a standard test used by researchers.

The Glaxo Wellcome researchers believe that a greater than approximately 8-fold increase in resistance is necessary to cause a concerning reduction in sensitivity to 1592. In the research reported here, a 10 fold or higher resistance to 1592 occurred only with the presence of three mutations; except, one double mutation caused an 8.5 fold increase in resistance to 1592.

The Fold Increase in IC50 (or, increase in resistance) The following increases in IC50 have been reported for these listed single, double and triple mutations: **See Table 1**

**Table 1**

<b>wild type virus</b>	<b>1.0</b>
65R	3.10-fold
74V	3.73
115F	2.06
184V	2.92
65R/74V	3.56
65R/184V	6.88
65R/74V/184V*	10.15
74V/184V*	8.50
74V/115F	3.44
115F/184V	6.78
74V/115F/184V*	10.99

As you can see in table 1, one double mutation (74V/184V) and two triple mutations have >8 fold increase in IC50 (or, increase in resistance) to 1592. It is important to remember that this is an in vitro experiment. That is, it was conducted in the laboratory. Lab experiments do not always predict responses to a drug by an individual.

Researchers conducted an additional in vitro experiment. **See Table 2.** They took viruses which were resistant to nucleosides and measured the fold increase in IC50 for 1592. In other words, the amount of resistance to 1592 a virus had if it were resistant to another nucleoside.

**Table 2.**

<b>Virus</b>	<b>Known resistance</b>	<b>Fold increase in IC50 for 1592</b>
Wild type	none	1.0
mutations- 67N/70R/215F/219 Q	AZT (120 fold)	2.1
mutation- 74V	ddl (5-10 fold)	3.6

	ddC (18 fold)	
mutation- 181C	NNRTI (>100 fold)	1.7
mutation- 184V	3TC (500 fold),	2.2
	ddl/ddC (2-10 fold)	

The wild type virus had no resistance to any nucleoside and was not resistant to 1592. The next virus had 4 AZT associated mutations and was very resistant to AZT (120 fold). The third virus only had one mutation (74V) but was 5-10 fold resistant to ddl and 18 fold resistant to ddC. The fourth virus had a NNRTI mutation and possesses some NNRTI resistance. The fifth virus was extensively 3TC resistant (500 fold) with 2-10 fold resistance for both ddl and ddC. In each case for each of the viruses listed in the table, the amount of resistance to 1592 (or the increase in IC50) was less than 4 fold.

The question remains, how will 1592 perform for individuals who have had varying degrees of experience with different nucleosides; and, have varying degrees of levels of resistance associated with those nucleosides. Keep reading.

Glaxo Wellcome researchers have referred to one patient in the treatment-naive study (CNA 2001). This patient entered the study with a 184V mutation although 3TC experience was prohibited by study design. The person achieved about a 1 log drop in viral load after 4 weeks of 1592 monotherapy. For weeks 4 through 12 of the study the person received 1592+AZT and achieved a viral load reduction at week 12 of about 2 logs. Despite their 3TC resistance the person responded to 1592, but of course this is only one person.

**CNA 2003: Treatment Experienced.** In this study 18 individuals with greater than 6 months nucleoside experience with AZT, AZT/3TC, d4T, and ddl, but who had not taken protease inhibitors received 1592U89. The therapies were all failing because all the study participants were required to have >10,000 copies/ml of viral load. In this study, 1592 was merely added to their current therapy; no other component of the therapy was changed. By merely adding one drug onto a failing regimen, you may be limited in the capacity to capture the individual's full ability to respond to the newly added drug.

The average reduction in viral load from baseline for the 18 individuals was at:

- week 4: -1.11 log
- week 22: -1.30 log

For those who did not have the 3TC M184V mutation at baseline (prior to receiving 1592), their reduction in viral load from baseline was:



- week 4: -1.49 log
- week 22: -1.48 log

Other individuals who did have the M184V mutation at baseline, some of whom also had additional mutation(s), their reduction in viral load was:

- week 4: -0.52 log
- week 22: -0.97 log

See below - More on Emerging Resistance Patterns. Generally, it appears as if 3TC resistance should not cause cross-resistance to 1592; but, dual AZT/3TC resistance may or may not cause cross-resistance to 1592.

These averages can be misleading because each of the 18 study participants had varying baseline characteristics and varying prior nucleoside experience. Phenotypic resistance and genotypic mutations to 1592, 3TC and AZT were measured at baseline. As well, the changes in viral load were measured at week 4 and week 22. The 18 participants had varying baseline phenotypic resistance to 1592, 3TC and AZT, varying genotypic mutations and varying viral load responses. The viral load responses at week 22 did not always correlate in a way you might expect with the baseline genotype or phenotype data. At the NATAP community forum, Dr. St Clair presented all the data for each of the 18 participants which follows in Table 3. A closer review of the individual data may be more helpful in understanding the treatment effect of 1592 for treatment experienced individuals. But, data from a larger set of individuals is needed in order to better understand how to use 1592 in treatment experienced individuals. Glaxo has said they will be compiling data on treatment response to 1592 from their ongoing trials for treatment experienced individuals. This data will be helpful in deciding how to best use 1592 for a given individual.

Glaxo researchers, Lanier at Hamburg and St Clair at the NATAP meeting, concluded treatment experienced individuals would generally be less responsive to 1592, if:

- their phenotypic resistance to 1592 at baseline tended to be at a higher level
- the more nucleoside associated genotypic mutations one has, the less responsive to 1592 you should be
- Dr St Clair said they believe phenotypic resistance >8 fold to 1592 may be the cutoff above which individuals may not respond well or at all to 1592.

The following table was presented by Dr. Marty St. Clair, of Glaxo Wellcome, at the NATAP community education forum on October 25, 1997 at NYU Medical Center. See Table 3.

**Table 3. Viral Load Changes and Baseline Genotypic and Phenotypic 1592 Resistance for the 18 CNA 2003 (treat. exp.) Study Participants**

Pt ID #	Prior Th	mos.	1592	STC	AZT	wk 4	wk22	bsl geno mt
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442	AZT	18	0.41	0.68	0.93	-2.34	-2.44	wt
445	AZT	12	1.03	1.33	1.11	-3.15	-1.81	wt
465	AZT	15	0.83	1.52	0.76	-2.33	-2.03	wt
482	AZT	38	0.70	1.21	0.67	-0.28	+0.07	wt
490	AZT	18	47.00	100.7 2	7.46	+0.42	-0.72	41, 74, 184, 210, 215
491	AZT	14	7.01	100.7 2	1.30	-1.19	-2.26	74, 184
495	AZT	30	5.39	13.12	98.56	-0.34	-0.92	67, 70, 215, 219
441	AZT/3 TC	52/10	3.22	109.7 2	1.07	-2.35	-	184
473	AZT/3 TC	19/10	9.41	109.7 2	11.50	-0.08	+0.25	41, 70, 184
497	AZT/3 TC	12/11	3.11	109.7 2	9.56	-0.00	-0.56	184
515	AZT/3 TC	12/12	4.68	3.27	15.26	-0.38	-2.34	67, 184, 210, 215
516	AZT/3 TC	12/12	9.82	109.7 2	28.92	-0.05	+0.17	41, 67, 74, 184, 210, 215
517	AZT/3 TC	19/12	1.59	1.01	0.97	+0.07	-0.55	wt
447	ddl/AZ T	48/0	1.46	4.55	0.51	-1.01	-0.74	wt
443	d4T	9	0.58	0.34	0.95	-2.16	-1.72	65
459	d4T	6	1.02	1.10	0.79	-2.53	-1.99	wt
496	d4T	11	1.18	1.62	0.85	-1.11	-2.33	wt
513	d4T	10	7.32	12.45	125.77	-1.19	-1.86	41, 210,

								215
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**Analysis of Table 3.** The mean viral load reduction at week 22 for the 8 individuals with no baseline genotypic mutations (wild type) is -1.49 log. Surprising to me, PT# 482 had no genotypic mutations at baseline and had virtually no phenotypic resistance to 1592, AZT and 3TC at baseline; but had 38 months of prior AZT experience. PT# 482 had virtually no response to 1592 at week 22. In contrast, pts 442, 445, and 465 also had no genotypic mutations at baseline, virtually no phenotypic resistance to 1592, AZT and 3TC at baseline, but had less prior experience with AZT (18, 12 and 15 months, respectively). The viral load reductions for pts 442, 445 and 465 at week 22 are -2.44 log, -1.81 log, and -2.03 log, respectively.

PT# 447 had 48 months of prior ddl experience, also had no genotypic mutations identifiable at baseline, and had relatively low phenotypic resistance to 1592, AZT and 3TC; this person had a limited response at week 22 with a -0.74 reduction in viral load. A possible explanation for the results discussed here are that the actual length of time of prior nucleoside experience may be a factor regardless of a lack of identified geno- or phenotypic resistance.

Pts 473, 497 and 516 had 109-fold baseline phenotypic 3TC resistance, and >9 fold AZT resistance; their viral load responses were limited or none at week 22 ( +0.25 log, -0.56 log, and +0.17 log, respectively).

4/6 individuals with 3 or more genotypic mutations at baseline had a limited response to 1592 at week 22 (pt 513, -1.86; pt 490, -0.72 log; pt 495, -0.92 log; pt 473, +0.25 log; pt 515, -2.34 log; pt 516, +0.07 log).

**More on Emerging Resistance Patterns.** In Hamburg, Randall Lanier reported the findings from an in vitro experiment looking at viruses with phenotypic resistance to 3TC but phenotypically AZT sensitive, and separately to viruses with dual phenotypic resistance to AZT/3TC. The fold increase in 1592 IC50 was measured.

**3TC Resistance.** All 25 clinical isolates (individual's blood samples) with 3TC resistance but AZT sensitivity had less than 8-fold phenotypic resistance to 1592. 10 of the 25 isolates had between 5 and 7 fold resistance. 10/25 had approximately 3 fold or less phenotypic resistance. The median was 4.4 fold phenotypic resistance to 1592. In other words, 3TC phenotypic resistance caused a median 4.4 fold increase in 1592 resistance which means that 3TC resistance by itself may not cause resistance to 1592.

**AZT/3TC dual resistance.** 13 clinical isolates had dual phenotypic resistance to AZT/3TC. 5/13 had > 8 fold phenotypic resistance to 1592. 8/13 had <8 fold resistance to 1592. Remember, the Glaxo researchers believe that >8-fold phenotypic resistance to 1592 may predict a poor response to 1592 or no response at all. The median response for the 13 isolates was a 6.7 fold increase in phenotypic resistance. The phenotypic resistance for 6 of the isolates was approximately equal to or less than 5-fold. 2 of the isolates had between 8 and 10 fold resistance, and 3 of the isolates were about 12 fold or higher.

This in vitro data suggests that some individuals with AZT/3TC resistance may not respond well to 1592, but that some individuals with AZT/3TC resistance may be able to respond well to 1592. In fact, the Glaxo Wellcome researchers said they believe all the data compiled above suggests that failure to respond to 1592 is more likely to occur when an individual has broad and/or extensive resistance to nucleosides (AZT, 3TC, ddl, d4T, ddC). A close look at Table 3 shows how 18 individuals with a variety of different nucleoside experience and resistance actually responded to 1592.

**Expanded Access Tips.** Here are a few words about how to best use the 3 drugs available through expanded access. If you have extensive nucleoside experience including use of AZT/3TC, ddl, and d4T, you may or may not get a good response to 1592U89. As you can see from the results of CNA 2003, there were a variety of viral load responses from no response at all to a reduction of 2.44 log. Anecdotally, I have heard from several individuals who have received good responses to 1592 despite extensive nucleoside experience. However, when planning your regimen you should consider the possibility that you may not respond well to 1592. If you have not yet tried a NNRTI you should respond well to efavirenz which is a potent NNRTI, but resistance to a NNRTI can develop quickly. A NNRTI must be used in a regimen well designed to adequately suppress viral load in order to prevent resistance. Based on preliminary data a 0.5 log reduction in viral load is average for PMEA. However, the preliminary data suggests those with extensive AZT experience may not respond as well. If possible, your goal should be suppression of viral load to below detection. You may need additional therapy to accomplish this. It is a personal choice and everyone's situation is different, but sometimes it may be preferable to delay changing your regimen until you can assemble a regimen you feel more confident in.

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