

1592U89 (Abacavir) Resistance and Phenotypic Resistance Testing

Reported by Jules Levin, Executive Director of NATAP
(3 March 1998)

The following report contains a comprehensive update of the latest information on 1592U89 resistance and cross-resistance. Additionally, two studies [abstracts 686 and 687] reported at Chicago supported the idea that phenotypic resistance at baseline appears to predict an individual's response to 1592. As well, those findings are reported below. In both studies, baseline phenotypic resistance was determined by a Belgian-based company named Virco NV, Belgium. Virco uses their trademarked phenotypic test called Antivirogram. The authors of these abstracts are saying the data in these two studies support that phenotypic testing will predict your susceptibility to 1592. The suggestion is that if you are treatment-experienced you should consider phenotypic testing prior to using 1592.

What is Phenotypic Resistance Testing?

A sample of your blood can be placed in a test tube along with a specific drug. You can see 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 like IC50 or IC90. This is called phenotypic resistance testing. The lab can measure how much drug is required to suppress 90% or 50% of HIV production. 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, than you have 8-fold phenotypic resistance.

A different way to look at resistance is to perform a genotypic analysis, which looks 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 the findings useful for the patient. We are in the early stages of the development of these technologies as we were with viral load testing several years ago. Studies are needed to establish the correlation between genotypic testing results and phenotypic testing results and their predictability of failure or success for a specific drug. Some researchers have expressed doubts that genotypic and phenotypic test results can provide enough information for making reliable treatment decisions. Studies need to be conducted to define the role of these testing technologies in treatment decision making, because there are a number of factors besides resistance that effect drug sensitivity and treatment success or failure.

Commercial Availability of Virco's Phenotypic Test.

An obvious concern is that at this moment phenotypic resistance testing is expensive and not readily available to the general public. However, Virco has plans to make their test commercially available in the USA in the very near future. This may usher in a new era in HIV treatment. By April 1998 Virco will announce arrangements to provide "nationwide access to their technology". It is expected that by May 1998 sites around the USA will be available where blood samples will be collected for testing. These sites will be regularly used "commercial channels". Initially, the collected blood samples will be shipped back to Belgium for processing. But the expectation is that sometime this coming summer arrangements will be made to perform some processing steps in the USA. If demand is high enough, arrangements may be made to complete all the processing in the USA.

Aside from the two studies reported in this report, Virco is conducting and planning a number of studies to try and define the role that phenotypic resistance testing can play in treatment decision making. They will examine if there is a correlation between phenotypic resistance testing results, the prediction of sensitivity to certain drugs, and its predictability for clinical outcome. These studies include looking at phenotypic resistance during primary or acute infection, as well as for making salvage therapy decisions. If phenotypic testing proves reliable, it can be helpful in making salvage-therapy or treatment-switching decisions. After treatment failure, it may be possible to identify which drugs a person has become resistant to, and to which they may remain sensitive. The testing may also be able to predict which drugs the person should switch to if they are sensitive to them by phenotypic testing. Of course, being able to

use the testing in these ways depend upon the results of the studies in predicting drug sensitivity and clinical outcome.

Virco is working with the NIH AIDS Clinical Trials Group (ACTG) and several other organizations in conducting both prospective and retrospective studies. Retrospective studies, where researchers look back at stored blood samples, will attempt to correlate clinical endpoints (AIDS events and death) with phenotypic resistance. These studies include a look at the pharmaco-economic aspects of using phenotypic testing to show that using the technology will be a cost saving to insurers so that monies won't be wasted on ineffective therapies. Currently, total cost for using the test is about \$800 which includes processing and shipping. Future cost will in part depend upon the usefulness of the testing results and demand. Some initial results from the studies outlined above will be available this summer at the International AIDS Conference in Geneva.

Kaiser Permanente, the largest HMO in the USA with over 9 million subscribers, is collaborating with Virco in the clinical study effort to establish the test's utility. Kaiser is collaborating in a study of individuals relatively heavily pre-treated with antiretroviral therapy. Participants will be randomized to new combination therapies. One arm will make switches based on using Virco's phenotypic resistance testing. The other arm will be blinded to the phenotypic resistance test results and will make decisions on treatment switching based on the usual considerations. The study goal is to look at both clinical outcomes (AIDS defining events) and virological outcomes (viral load changes) in assessing the effect of using the test results. In addition, Kaiser will be examining the economic aspects of using this expensive test. They will explore if it saves money over the long run to use this test in identifying appropriate treatments for a particular individual or for specific populations in general.

Dr David Melnick, of Kaiser, says that in their experience the cost of combination therapy has increased 5-fold but the cost for patient care (number of hospitalizations, and length of stay in hospital) has declined, creating an offset whereby overall costs increase at a rate of about 3% per year. This is in large part due to a cut by 50% of individuals with <50 CD4. Patient care costs are highest for individuals with <50 CD4. In an abstract presented by Dr Melnick at the Chicago Retrovirus Conference, he reported an analysis of patient costs per year based on CD4 count: \$3930 (>500 CD4), \$9238 (200-500 CD4), \$19,541 (<50 CD4). Although Virco's phenotypic test may be costly, it may prove to be cost effective to insurers.

1592U89 Resistance and Phenotypic Resistance Testing.

The authors of the studies reported at the Chicago Retrovirus Conference concluded "high level resistance to 1592 (>8 fold) and/or multiple genotypic mutations known to confer NRTI phenotypic resistance are associated with poor viral load response to 1592. Phenotypic testing seems to be a simple tool for predicting viral load response to 1592, but further clinical trials are required to confirm these findings." For the most part, the authors say the number of NRTI genotypic mutations known to be associated with NRTI resistance also predict the response to 1592, although there was one possible exception (pt# 498, see tables below). Higher levels of dual AZT/3TC phenotypic resistance and baseline phenotypic resistance to other NRTIs also appear to be significant contributing factors to a poor response to 1592.

A more detailed discussion about 1592 resistance based on previous in vitro research is available in the [January 1998 NATAP Reports](#) newsletter, which has been posted to our website or read the article called [1592U89 \(Abacavir\):Efficacy & Resistance Data](#) in the New Articles section of our website. Briefly, resistance to 1592 in vitro is associated with the emergence of the 184 mutation followed by mutations at 65, 74 and 115. Each of these single mutations resulted in no more than a 4-fold increase in the IC50 (phenotypic resistance). Eight-fold or higher resistance occurred only with the emergence of 2 different triple mutations (65, 74, 184 or 74, 115, 184) or with 1 double mutation (74,184).

In the first study presented by Randall Lanier and others of Glaxo Wellcome, they analyzed the baseline and week 24 data for 23 participants in study CNAA2003. These were individuals with prior nucleoside experience who were taking only nucleoside therapy (See our [January 1998 newsletter, NATAP Reports](#), p. 34, or the [NATAP Resistance Supplement](#) in the New Articles section on NATAP's website for more details about the study). For 2003, they continued on their current nucleoside therapy and added only

1592 300 mg bid. In addition to baseline phenotypic resistance, study participants were assessed for their baseline genotypic resistance using the sequencing kit from Applied Biosystems, and the amount of prior nucleoside experience they had.

In the NATAP Report on this study available on our web site or in the printed newsletter, we had week 22 data on 18 individuals. The report in Chicago was on 23 individuals at week 24. At week 24, 9/15 individuals (60%), had >1 log reductions in viral load following the addition of 1592 to their current NRTI therapy. 3/15 had viral load reductions >3 log. These 3 individuals had virtually no 1592 baseline phenotypic resistance. They had prior nucleoside experience but <4 fold baseline phenotypic resistance to other NRTIs (d4T, ddl, 3TC, AZT, ddC).

The study participants were divided into 3 groups, by order of their baseline susceptibility to 1592 as measured by the phenotypic testing: <4 fold resistance to 1592, 4 fold to 7 fold, and (8 fold resistance.

Table 1. <4 fold 1592 Phenotypic Resistance at Baseline

Column 1 is NRTI treatment the person was receiving just prior to the addition of 1592, and the number of months of experience with the treatment. Columns 2-7 give the fold baseline phenotypic resistance to that particular NRTI. For example, in the first row pt# 445 has 1 fold resistance to each of the NRTIs listed. Wherever na is placed that means the information was not available. The last two columns refer to the median viral load changes at weeks 4 and 24. The week 24 columns in all 3 tables also contain the baseline genotypic mutations detected for that individual.

In the following tables you will see genotypic mutations. It is helpful to know for which NRTI(s) these mutations can contribute to resistance:

- 41, 67, 70, 215, 219 (AZT)
- 184 (3TC, possibly ddl and ddC)
- 74 (ddl, ddC)
- 101, 103 (NNRTI mutations)
- 115 (has been identified as a mutation that can infrequently cause multi-drug resistance, and has been identified as a mutation occurring associated with in vitro 1592 resistance)

| NRTI exp (mo exposure) | Pt ID# | 1592 | 3TC | AZ T | d4T | ddl | dd C | Wk4 VL Ch | Wk24 VL Ch |
|------------------------|--------|------|-----|------|-----|-----|------|-----------|---------------|
| AZT (13) | 445 | 1 | 1 | 1 | 1 | 1 | 1 | -3.34 | na |
| AZT(15) | 465 | 1 | 2 | 1 | 1 | 1 | 1 | -2.31 | -2.38 log |
| AZT(24) | 442 | 1 | 1 | 1 | 1 | 1 | 1 | -2.52 | -3.05 |
| AZT(33) | 507 | 1 | 1 | 1 | 1 | 1 | 1 | -0.26 | na |
| AZT(38) | 482 | 1 | 1 | 1 | 1 | 1 | 1 | -0.52 | -1.18 |
| AZT/ddl(0/64) | 498 | 1 | 2 | 30 | 1 | ? | 1 | -1.53 | na |
| | | | | | | | | | 41,67,70?,74? |
| | | | | | | | | | 115,215 |
| d4T(12) | 496 | 1 | 2 | 1 | 1 | 1 | 1 | -0.96 | -3.49 |
| d4T(6) | 458 | 1 | 1 | 1 | 2 | 1 | 1 | -2.48 | -3.05 |

| | | | | | | | | | |
|----------------|-----|---|------|----|---|---|---|--------------|-------------------|
| d4T(7) | 443 | 1 | 1 | 1 | 1 | 1 | 1 | -2.25 | na(-1.72@wk22) |
| d4T(8) | 457 | 1 | 22x | 1 | 1 | 2 | 2 | -0.56 | -0.74 |
| | | | | | | | | | 184 |
| AZT/3TC(19/12) | 517 | 2 | 1 | 1 | 2 | 1 | 1 | 0.14 | na |
| AZT/ddI(0/64) | 447 | 2 | 5 | 1 | 3 | 1 | 1 | -1.11 | na(-0.74@wk22) |
| | | | | | | | | | 101,103 |
| AZT/3TC(37/11) | 497 | 3 | 109x | 10 | 2 | 1 | 1 | -0.07 | na(-0.56@wk22) |
| | | | | | | | | | 67,70,184,215 |
| | | | | | | | | | 219 |
| AZT/3TC(52/10) | 441 | 3 | 109x | 1 | 1 | 3 | 2 | -2.32 | -1.78 |
| | | | | | | | | | 70,184 |
| median | | | | | | | | -1.32 | -2.38 log* |

? mixture of wildtype (no mutations) and resistance mutations. That is, investigators found both, virus with no resistance and virus with mutations

* the median applies only to week 24 values. Where week 22 but not week 24 values are reported, those values weren't figured into the median.

In Table 1, only one person (pt# 497) had dual AZT/3TC resistance. All others had <4 fold 1592 baseline phenotypic resistance and resistance to at the most only 1 other NRTI. The higher the levels of dual AZT/3TC resistance the less likely a person is to respond well to 1592. However, having used AZT previously for a longer period of time does not necessarily predict a higher level AZT resistance.

Table 2. 4-fold to 7-fold 1592 Baseline Phenotypic Resistance

As you can see, individuals with higher baseline phenotypic 1592 resistance (5-7 fold) have more phenotypic resistance to AZT, 3TC and/or other NRTIs. Several individuals have enough d4T resistance that you might expect to see a d4T related mutation; although the 75 mutation has been observed to be associated with d4T use, usually no resistance mutation has been observed to be associated with d4T failure. You can see in this table that every person has at least 2 NRTI mutations, while in Table 1 only 4/14 individuals had genotypic mutations.

| Prior Treat (mo exp) | Pt ID# | 1592 | 3TC | AZT | d4T | ddl | ddC | VL Ch wk 4 | VL Ch wk 24 |
|----------------------|--------|------|-----|-----|-----|-----|-----|------------|-------------|
| AZT (30) | 495 | 5 | 13 | 99 | 6 | 1 | 2 | -0.36 | -1.72 log |

| | | | | | | | | | |
|--------------------|-----|---|------|-----|---|---|---|-----------|-----------------|
| | | | | | | | | | 67,70, 215, 219 |
| AZT/3TC (12/12) | 515 | 5 | 3 | 15 | 7 | 3 | 1 | -0.33 | -2.34@wk22 |
| | | | | | | | | | 41,215 |
| AZT/ddI (21/13) | 508 | 5 | 9 | 27 | 1 | 1 | 2 | -0.72 | -0.31 |
| | | | | | | | | | 41,115 |
| AZT (14) | 491 | 7 | 109x | 1 | 4 | 4 | 3 | -1.18 | -1.28 |
| | | | | | | | | | 74,184 |
| d4T (10) | 513 | 7 | 13 | 126 | 9 | 4 | 4 | -1.17 | -1.65 |
| | | | | | | | | | 41, 215 |
| median | | | | | | | | -0.72 log | -1.47 log |

Table 3. 8 Fold 1592 Phenotypic Resistance at Baseline

| | Pt ID# | 1592 | 3TC | AZT | d4T | ddl | ddC | VL Ch wk4 | VL Ch wk24 |
|--------------------|--------|------|------|-------|-----|-----|-----|-----------|----------------------|
| AZT/3TC (37,37) | 460 | 8 | 30x | 1636x | 5 | 6 | 3 | 0.24 | 0.03 |
| | | | | | | | | | 41,67,215 |
| AZT/3TC (19,10) | 473 | 9 | 109x | 11 | 5 | 5 | 4 | -0.07 | 0.43 |
| | | | | | | | | | 41,67,70,184 |
| | | | | | | | | | 215 |
| AZT/3TC (13,13) | 516 | 10 | 109x | 29 | 4 | 2 | 1 | -0.23 | -0.36 |
| | | | | | | | | | 41,67,74,184, 215 |
| AZT (18) | 490 | 47 | 109x | 8 | 6 | 4 | 5 | 0.41 | 0.09 |
| median | | | | | | | | 0.09 | 0.06 |

3/4 individuals had no VL decrease in response to 1592. The other person had a small response (-0.36). All 4 of these individuals had high level dual AZT/3TC resistance, accompanied with some resistance to other NRTIs. As you can see there is a trend towards decreased response to 1592 at higher levels of baseline phenotypic resistance to 1592.

Table 4. Overall Week 24 % Viral Load Responses

| 1592 fold Resist | >0.5 log drop | >1.0 log drop | >1.5 log drop | >2.0 log drop |
|------------------|---------------|---------------|---------------|---------------|
| <4 fold (n=7) | 100% | 86% | 71% | 57% |
| 4-7 fold (n=14) | 75% | 75% | 50% | 0% |
| >7 fold (n=4) | 0% | 0% | 0% | 0% |

The factors that appear to effect response to 1592 are:

- the level of baseline phenotypic 1592 resistance (<4 fold, 4-8 fold, or >8 fold)
- NRTI mutations
- higher levels of dual AZT/3TC resistance appear associated with reduced response to 1592
- baseline phenotypic resistance to other NRTIs appears associated with response to 1592

Preliminary 1592 Expanded Access Results.

In Chicago, Glaxo Wellcome held a community meeting separate from the Conference where they told us that of the first 200 participants in the 1592 expanded access program, only 25% had >0.5 log drop; and, only 40% of these 25% (10% overall) had >1.0 log drop at month 2. The individuals who did best added a NRTI which they had not taken in last year. 1500 individuals have enrolled in the program and they said these first 200 were representative of the remaining individuals. But it is important to keep these results in perspective. The criteria for entry into the program was a CD4 count <50, so these were individuals with advanced HIV and few unexhausted treatment options. They probably had extensively used all NRTIs and failed protease inhibitor therapy. But, if you are treatment-experienced, you should be cautious in deciding how to use 1592 so that you can maximize its benefit to you. The information in this report should be very helpful. Additional information should be forthcoming in the near future.

Susceptibility Profile of 943 Clinical HIV-1 Isolates to 1592U89.

A second study using the Virco phenotypic assay technology called Antivirogram was reported by Dr John Mellors and others. The assay was performed on 943 patient blood samples obtained from clinical trials (eg, CEASAR) or from individual patient monitoring. Phenotypic resistance was determined to the following NRTIs:

- 1592
- AZT
- 3TC
- ddI
- ddC
- d4T

As in the previous study patients were grouped by 3 categories of resistance: <4 fold, 4 to 8, and >8 fold.

The authors concluded the Antivirogram can identify patients who are most likely to benefit from 1592.

Table 5. 1592 Susceptibility (or resistance) of 943 Isolates with Differing Resistance to AZT, 3TC, AZT+3TC, and other NRTIs

This chart seems complicated to understand but I will explain it. If you don't want to read the explanations, you can jump ahead to the conclusions by the authors at the very end of this section.

In column 2, 379 isolates were not resistant to AZT and 3TC. 368 isolates were non-resistant to AZT or 3TC and were not resistant to the NRTIs tested. 100% of the 368 isolates were susceptible or non-resistant (<4 fold resistance) to 1592. 100% of 11 isolates were non-resistant to AZT or 3TC but were

resistant (>10 fold resistant) to 0, 1, 2, or 3 other NRTIs. If there was no AZT or 3TC resistance, none of the isolates had >4 fold 1592 resistance even if they had >4 fold resistance to other NRTIs.

In column 3, all isolates (n=125) were >10 fold resistant to AZT but were not 3TC resistant. 111 isolates or 96% had <4 fold resistance to other NRTIs and were <4 fold resistant to 1592. 5 isolates had <4 fold resistance to other NRTIs but had intermediate (4 to 8 fold) resistance to 1592. Of the 19 isolates with >10 fold AZT resistance and >4 fold resistance to one additional NRTI, 13 (68%) had <4 fold 1592 resistance, and 6 (32%) had intermediate (4 to 8 fold) 1592 resistance. None of the isolates in column 3, with >10 fold AZT resistance (no 3TC resistance) had >8 fold 1592 resistance. In summary, isolates with only AZT resistance were 96% sensitive (<4 fold resistance) to 1592. Resistance to increasing numbers of NRTIs increases resistance to 1592. This pattern also occurs in the remaining data.

In column 4, a total of 196 isolates had >10 fold 3TC resistance but were not AZT resistant. 98% with no resistance to other NRTIs were sensitive or <4 fold resistant to 1592; 2% had intermediate (4 to 8 fold) resistance to 1592. If you look back to Table 2, you can see the median viral load response to 1592 treatment for 5 individuals with intermediate phenotypic baseline 1592 resistance was -1.47 log at week 24. If you look back to Table 1 you will see the median viral load reduction for 14 individuals (in study CNA2003) at week 24, with <4 fold baseline phenotypic 1592 resistance, was -2.38 log. However, the same pattern occurs when there is resistance to increasing numbers of other NRTIs in addition to 3TC resistance: 60% of isolates with resistance to one NRTI in addition to 3TC are sensitive (<4 fold) to 1592 while 36% of isolates are sensitive to 1592 which have resistance to 2 NRTIs in addition to 3TC.

The authors say the overall pattern emerging is that resistance to 1592 increases when there is resistance to increasing numbers of NRTIs.

Column 5 addresses isolates with dual >10 fold AZT/3TC resistance. 100 isolates with >10 fold dual AZT/3TC resistance have <4 fold 1592 resistance. 71% with dual AZT/3TC resistance but no resistance to other NRTIs are sensitive to 1592. With resistance to increasing numbers of NRTIs sensitivity to 1592 declines: 40% of isolates have <4 fold resistance to 1592 when there is resistance to AZT/3TC and 1 additional NRTI; 13% of isolates have <4 fold resistance to 1592 when there is resistance to 2 additional NRTIs and AZT/3TC; and strikingly, 0% of isolates have <4 fold resistance to 1592 when there is resistance to 3 NRTIs in addition to AZT/3TC dual resistance.

As you can see (8 fold resistance to 1592 occurred (34/35 isolates) almost exclusively when there was dual AZT/3TC resistance (>10 fold) accompanied by >4 fold resistance to additional NRTIs. And, the more NRTIs an isolate had resistance to the greater the % of isolates with (8 fold 1592 resistance: 4% with dual AZT/3TC resistance (>10 fold) and no resistance to other NRTIs had (8 fold 1592; 11% of isolates had (8 fold 1592 resistance when they had >10 fold AZT/3TC resistance and resistance to 1 additional NRTI; 22% of isolates with AZT/3TC resistance and resistance to 2 additional NRTIs had resistance to 1592; and 71% of isolates were (8 fold 1592 resistant when that had >4 fold resistance to 3 NRTIs and dual AZT/3TC resistance.

| >10 fold rest to- | Non-Res to AZT or 3TC | AZT | 3TC | AZT+3TC | total |
|-------------------------------|-----------------------|-------------|---------|-----------|-------|
| # of other (0-3) | 0 1-3 | 0 1 2 | 0 1 2 | 0 1 2 3 | |
| NRTIs with >4 fold resistance | | | | | |
| Susc to 1592: | | | | | |
| susc (n) | 368 11 | 111 13 1 | 183 9 4 | 73 23 4 0 | 800 |

| | | | | | |
|-------------------|--------------|-------------|-------------|----------------|-----|
| (<4 fold) (%) | 100% 100% | 96 68 50 | 98 60 36 | 71 40 13 0 | 85 |
| Intermediate (n) | 0 0 | 5 6 1 | 3 6 6 | 26 28 20 7 | 108 |
| (4 to 8 fold) (%) | 0 0 | 4 32 50 | 2 40 55 | 25 49 65 29 | 11 |
| Resistant (n) | 0 0 | 0 0 0 | 0 0 1 | 4 6 7 17 | 35 |
| (>8 fold) (%) | 0 0 | 0 0 0 | 0 0 9 | 4 11 22 71 | 4 |

A flaw with the above analysis is the categorization of all isolates as having greater than or less than 10 fold resistance to AZT, 3TC, or AZT/3TC. This distinction overlooks the situation where a person or isolate has 11-fold resistance to AZT & 3TC compared to an isolate or person with 100-fold 3TC resistance and 11-fold AZT resistance. In the above analysis these two situations are categorized together.

Conclusions by the authors:

- If a person is found to be sensitive to 1592 by using the Virco Antivirogram phenotypic resistance test, they are likely to benefit from 1592 treatment. The most sensitivity is defined as having <4 fold baseline phenotypic resistance to 1592. Intermediate sensitivity is defined as 4 to 8 fold 1592 resistance. If a person has >8 fold 1592 resistance, that is predictive of minimal responses to 1592.
- It is not known whether there is a clinically significant difference between isolates with <4 fold resistance and isolates with 4 to 8 fold resistance.
- For those isolates with AZT/3TC resistance (>10 fold) and no resistance to additional NRTIs, 71% have <4 fold resistance to 1592.
- Over 95% of those isolates with >10 fold resistance to AZT or 3TC alone remain sensitive to 1592 (<4 fold resistance).

If resistance to AZT, 3TC or AZT+3TC is associated with other NRTI resistance, sensitivity to 1592 decreases. The sensitivity declines as there are increasing numbers of NRTIs that the isolate is resistant to. None of the isolates with dual AZT/3TC resistance and resistance to 3 additional NRTIs were <4 fold resistant to 1592, but 29% (n=7) had intermediate (4-8 fold resistance to 1592; and 71% of these isolates had (8 fold 1592 resistance. However, "it remains to be determined whether reduced 1592 sensitivity results mainly from exposure to AZT/3TC or whether it is a more general phenomenon resulting from regimens of NRTIs."

[Back to top](#)