Nucleoside Resistance and Cross-Resistance: 1592U89, AZT/3TC, d4T/ddI, d4T monotherapy - Jules Levin, March 26

Not all clinical failure is the result of drug failure and not all drug failure is the result of resistance (Richman DD, *AIDS Res Human Retroviruses*, 1994; 10:901-905). Also, it has been said that if a drug is not potent enough (does not exert much pressure on the virus) mutations may not develop. Following are reports on resistance that may develop from use of d4T/ddI, and cross-resistance to nucleosides that may develop using AZT/3TC. These two reports are based on blood samples taken from individuals who had actually taken these drugs. In vitro experiments do not necessarily predict the outcomes of individuals actually taking the drug in question. There is very little data available yet from treatment-experienced individuals who have subsequently taken 1592U89.

1592U89

Richard Harrigan, of Glaxo Wellcome, reported resistance and cross-resistance data for 1592U89 at the 4th Retrovirus Conference. Participants in a 12 week dose ranging study of 1592 (see NATAP post-Vancouver web report of data), who were essentially treatment-naive, received 4 weeks of 1592 monotherapy. After 4 weeks individuals were randomized to continue 1592 monotherapy or add AZT to the 1592, and to continue for a total of 12 weeks. Baseline CD4 were about 360 cells; baseline RNA was about 31,000 copies for two dose regimen arms and 125,000 for the other dose regimen arm. After 4 weeks, viral load reductions from baseline ranged from 1.4 to 2 log for those receiving 1592 monotherapy. After 12 weeks for those receiving 1592 monotherapy, reductions from baseline were about 1.5 to 2 log. For the different dosing regimens who had AZT added to 1592, the RNA reduction at 12 weeks was at least 1.9 log. For all participants the CD4 increases were about 100 cells (range 79-127) from baseline. For those in the 3 different dose regimens (200 mg tid, 300 mg bid, 600 mg bid) receiving 1592 and at 4 weeks added AZT, 65% had undetectable viral load (<400 copies); for those receiving only 1592 for 12 weeks, 20% were undetectable. In several animal models, 1592 penetrates the CNS well.

At the Retrovirus Conference and at the International Society for Antiviral Research meeting held in New York last week, Harrigan reported that mutations at positions 65, 74 or 184 were observed after 12 weeks of 1592 monotherapy. "The combination of AZT/1592 appeared to prevent the emergence of these mutations over the course of the trial. In vitro the combination of AZT/1592 resulted in the emergence of the 65 mutation, but appeared to delay or prevent the M184V from emerging." >From in vitro experiments (where increasing concentrations of 1592 were used) M184V was the first mutation to develop, but only a moderate 2-3 fold resistance occurred; that is considered a low level

resistance which is not significant. Further passaging of the virus found mutations at 65, 74 and 115. In vitro experiments were conducted with a variety of triple, double and single mutation viruses. They contained different combinations of 65, 74, 115, 184. The 65, 74 and 115 mutations can result from ddI therapy.

Generally, the triple mutations increased resistance about 10-fold; the double mutations increased resistance about 4-8 fold; and, the single mutations only produced modest 2-3 fold increases. Research data so far indicates that a single mutation, whether it is 65, 74 115 or 184, does not appear to be enough to cause resistance.

It may be difficult to judge how an individual will actually respond to therapy based on in vitro mutations. Resistance of 10-fold will not necessarily be enough to cause resistance. Resistance to 3TC after the M184V develops can be 500-fold. Combination therapy with 2 or 3 new drugs including 1592 may be sufficient to suppress 10-fold or higher resistance. Some individuals with extensive nucleoside experience with multiple mutations will be responsive to 1592 and some others also with extensive nucleoside experience and multiple mutations may not be responsive.

As is the case in general in today's new treatment environment, it is be important to select your combination wisely; and, it is important to strategize for future therapies if you need to change what you take first; that is, what's the next combination I'll take if this one fails.

Using three drugs never before used would be the most likely to exert strong enough pressure on the virus to suppress the resistant virus or to delay or prevent the development of resistance. Using 2 new drugs might suffice, but just adding 1592 (or any one new drug) to any regimen is a risky approach. It's been reported that virus is replicating more quickly in those with higher viral burdens, and their virus is more likely to generate mutations faster than those with lower viral loads. The more mutations your virus may have, the more potent the therapy you may need to adequately suppress the virus both initially and to sustain that suppression over time. Individuals with extensive nucleoside experience are more likely to have a more mutant virus and therefore may be more likely to need more potent therapy.

Glaxo said they are gathering data to address this question. Last Fall they initiated study 2003 to evaluate the development of resistance; individuals with a variety of experience with nucleosides added 1592 to their therapy. Individuals may be better armed to make treatment decisions about when and how to use 1592 after more data is available.

Glaxo is planning a limited expanded access program for adults to begin this Summer; they are considering a program for individuals with below 50 CD4 and few if any remaining treatment options. They are planning a larger and less restrictive expanded access program for the Fall. An expanded access program for pediatrics is planned to begin this Spring.

D4T/DDI

Last year preliminary results were reported for a randomized, double-blinded pilot study of d4T+ddI by Richard Pollard, MD, of the University of Texas. Eighty-five treatment-naive participants, with median baseline CD4 and HIV RNA of 330 cells and 31,600 copies/ml, were randomized to one of five dosing combinations of the two drugs; this included the maximum dose used of 200 mg bid ddI and 40 mg bid d4T. The preliminary data available for all the dosing regimens combined showed reductions in viral load of about 1.20 log at 8 weeks and 1.40 log at 52 weeks; at 52 weeks, CD4 changes varied from -22 cells in the lowest dosing regimen to +141 cells in the highest dosing regimen (200mg bid ddI+40 mg bid d4T). The original more in-depth report of the data from the Pollard study is available on the NATAP web site under the Post Vancouver section of the Drug Development page. There were two cases of peripheral neuropathy and 8 cases of elevated liver enzymes. The investigators said the sustained RNA reduction after 52 weeks may be at least partially due to the slow development of resistance to both drugs, that ddI resistance develops slowly, and d4T resistance has been difficult to demonstrate (Lin et al, Jnl of Infect Diseases, 1994;170: 1157-64). Final analysis of the full set of data is being collected and will be reported soon.

Commentary: I suspect there may be additional as yet unidentified factors other than simply mutation changes (genotypic mutations) when a person develops resistance to a particular drug; I'm not referring to malabsorption, non-compliance or these types of concerns.

Baseline and on-treatment isolates(blood samples) from the above described Pollard study, who had remained on study treatment for at least 26 weeks (26-43), were tested for phenotypic resistance and biological phenotyping (SI/NSI). Data for the 20 patients so far analyzed from this ongoing study were presented at the 4th Retrovirus Conference and is summarized below.

Commentary: following the report on d4T/ddI resistance is a report on cross-resistance that can develop subsequent to AZT/3TC therapy. The immediately following d4T/ddI data indicates low level resistance that can result from taking d4T/ddI for individuals in this study. The AZT/3TC report indicates a low level and incidence of the development of cross-resistance to ddC, ddI, or d4T following AZT/3TC therapy for individuals in this study. But, let's put that in context. Although these reports are of interest, it is generally accepted that following extensive nucleoside experience efficacy is reduced for subsequent use of even never before used nucleosides.

In today's treatment environment, it is recommended that nucleoside monotherapy no longer be used. In some instances ddI monotherapy may still be used based on the results of ACTG 175. Increasingly nucleosides will not be

used without being accompanied in a combination with one or two protease inhibitors and/or a NNRTI. Mutation and resistance profiles that emerge from combination nucleoside therapy may be different than from those resulting from having used monotherapy. Future testing and research will explore the patterns of resistance that emerge from multi-drug combination therapy. Predicting an individual's response to therapy based on genotypic or even phenotypic resistance is not an exact science. Since the framework within which we are using drugs is changing, will our understanding of resistance change also? Will we begin to see a variety of new mutation profiles subsequent to taking different multi-drug combinations?

Susceptibilities of HIV-1 Isolates Derived from Patients Treated with ddI and d4T in Combination

authors: E Coakley, J Gillis, L Pednault*, L Dunkle*, S Hammer--*Bristol-Myers Squibb

Commentary: following is an expansive discussion of this study and related subject matter. If you only want to know the study goal and its result explained simply and briefly without reading any further, here it is: investigators studied patients (treatment-naive, CD4 of 330) from the Pollard study, who received d4T/ddI for at least 26 weeks (26-43), for the development of resistance. Of the 20 patients so far studied the data indicates essentially no resistance was detected.

The genotypic mutation associated with diminished susceptibility (resistance) to ddI in clinical isolates (blood samples from humans) is "primarily characterized by" a mutational change of L74V; although less frequently, mutation changes (codon alteration is also used to describe this change) K65R, V75T and M184V have been reported. "The genotype (mutation) associated with phenotypic resistance to d4T remains unclear."

Commentary: it is generally accepted that resistance (phenotypic) to AZT follows the emergence of mutations that have been identified to occur in a fairly predictable pattern.

Resistance is measured by researchers by genotypic resistance and phenotypic resistance. Phenotypic resistance results when one or more mutations decrease the susceptibility of HIV to an antiretroviral drug. Susceptibility is most often measured *in vitro* (in the test tube) by the concentration (amount) of drug necessary to inhibit 50% of viral replication (IC_{50}). If the amount of drug necessary doubles that is called a 2-fold decrease in susceptibility or a 2-fold increase in phenotypic resistance. A low IC_{50} means susceptibility and a high IC_{50} means loss of susceptibility or phenotypic resistance.

It is generally accepted that phenotypic change (resistance) is caused because of one or more genotypic mutations. For example, a genotypic change(mutation) at position 82 has been associated with phenotypic resistance to indinavir and also ritonavir. The emergence of this 82 mutation in conjunction with the emergence of several other mutations has been associated with the rebound in viral load for individuals taking indinavir or ritonavir.

Although it appears to be uncommon, mutation changes for d4T occur at position 75 (V75T) and 50 (I50T); they have been seen by *in vitro* testing, and resulted in 7- and 30-fold resistance, respectively. A variety of different mutations have been described but no consistent profile is apparent (Genotypic and Phenotypic Analysis of HIV-1 Isolates from Patients on prolonged D4T Therapy, Lin et al, Jnl of Infec Diseases, 1994, 170:1157-64).

SI vs NSI virus. An individual's virus can be either a synctium inducing (SI) virus or a non-synctium inducing (NSI) virus. The presence of SI virus causes infected cells to stick together in clusters with non-infected cells; once it clumps with t-helper cells (CD4s), these t-helper cells are prevented from acting in their usual role as a defense mechanism against outside pathogens. In ACTG 175, the presence of the NSI type of virus at baseline was predictive of decreased disease progression as compared to the presence of SI at baseline (Katzenstein et al,NEJM, Oct 96, v335, n15, 1091-1097). When an individual's NSI virus changes to an SI virus disease progression may increase.

In this study, investigators tested the 20 samples from treatment-naive individuals who were treated for at least 26 (range 26-52 weeks) weeks with d4T/ddI to identify changes in susceptibility to the therapy.

Commentary: some researchers refer to a 10-fold decrease in susceptibility or increase in IC_{50} as the cutoff where a higher number means partial or low-level resistance while lower than 10-fold means the virus (or isolate) remains susceptible; others have said with regards to protease inhibitors that >4-fold decrease in susceptibility causes resistance.

The participants in the Pollard study were randomized to 5 different dosing regimens; one of those five was the full recommended doses of both drugs (400mg bid ddI/40 mg bid d4T). In this study, the isolates (blood samples) which exhibited changes in susceptibility were distributed across the different dosing regimens except for the high dose combination; for the high dose combination, so far in the analysis only 2 pairs of isolates (pre and during treatment) have been studied, and neither of these isolates displayed any decrease in susceptibility (resistance).

Preliminary Changes in susceptibility (resistance)--analysis is ongoing: the baseline viral loads are relatively comparable for the 20 patients in this study, the 86 patients in the overall d4T/ddI Pollard study, for the 8 isolates with a 3-fold decrease in susceptibility to one or both drugs, and for those with a 3-fold decrease. Sixteen of the 20 pretreatment isolates were NSI at baseline, which is

reflective of the intermediate stage of disease of the individuals in the study.

- 8/20 isolates demonstrated a 3-fold decrease in d4T and/or ddI susceptibility (increase in resistance)
- 5/20 isolates showed 4-fold decrease in susceptibility
- 0/20 isolates showed 8-fold decrease in susceptibility
- one isolate demonstrated the maximum decrease in susceptibility for d4T with a 7.5 fold decrease
- a different isolate exhibited the maximum decrease in susceptibility for ddI with a 7.3 fold decrease in susceptibility
- the mean fold decrease in susceptibility for the 20 participants studied is 1.8 for d4T, as well as 1.8 for ddI
- the frequency of a dual decrease in susceptibility to both d4T and ddI occurred in 1/20 isolate pairs

The study is ongoing. Of the isolates not yet fully analyzed, 3 pretreatment isolates converted from NSI to SI during treatment, and no isolate converted from SI to NSI.

No definite relationship was discerned between biological phenotype (NSI/SI changes) from baseline to during treatment, but the limited number of baseline SI isolates limited findings.

The study is ongoing with plans for phenotypic testing for additional isolate pairs, genotyping for those patients for whom viral load has rebounded, and for those demonstrating 3-fold decrease in susceptibility.

Commentary: after at least 26 weeks (26-43) of treatment there were few instances of resistance, although many of the study participants were not taking the full doses of either medications. Of the 20 subjects studies only 2 displayed

4-fold resistance to ddI (5.7, 7.3) and only two displayed 4-fold resistance to d4T (4. 7.5). The study investigators concluded that the low level of resistance to either drug individually or to dual resistance displayed in this study may be the reason that CD4 increases remain sustained out to 52 weeks, and peak initial viral load reductions appear to be sustained as far out as 52 weeks.

Lack of Emergence of Genotypic Resistance to D4T after Two Years Monotherapy

authors: The D4T Spanish Team (V Soriano and U Dietrich on behalf of the team)

Twenty-four individuals with mild immunological deterioration at baseline (CD4 below 500), who had more than 16 weeks previous AZT experience, received monotherapy d4T for two years (either 30 or 40 mg bid). They were unable to detect the 75 or 50 mutations, previously reported to confer resistance to d4T, in any of the individuals after 2 years. Despite that all had stopped taking AZT 2 years ago, 20/24 still had AZT mutations. Viral load using the NASBA method was undetectable in the 4 subjects who did not have any AZT resistance mutations. However, subjects with more than 2 AZT-related mutations had the highest viral loads. The authors concluded that prolonged treatment with d4T doesn't appear to be associated with the emergence of d4T resistance, and this could explain apparent more sustained benefit than with other drugs.

Commentary: Just because a mutation was not detected in this study does not mean that d4T treatment will not fail. We don't know the lower level of detection for the viral load test used in this case. As far as I know the NASBA test does not measure as low as the Roche or Chiron test. So these subjects may not be undetectable by the standards we are accustomed to. As I stated earlier, I suspect there are other factors involved in a decrease in efficacy (CD4 decrease and/or viral load rebound) besides the detection of or lack of detection of mutations. Predicting how effective a therapy will be when an individual actually uses it based on (genotypic) mutations is not an exact science and is not necessarily reliable. The viral loads and CD4 were not available from the abstract either at baseline or after 2 years of therapy.

Lack of M184V Mediated Cross-Resistance to ddI and ddC as a Result of 3TC Therapy

authors: V Miller, S Staszewski, M Sturmer, B Morgenstern, R Pauwels, M-P DeBethune, K Hertoos, C Van Eynde, P Schel, A Van Cauwenberge, BA Larder, PR Harrigan, M Tisdale, SD Kemp, S Bloor, C Stone, A Kohle, R Myers

Investigators examined the incidence and level of phenotypic (for a discussion about what phenotypic and genotypic resistance mean see article above) resistance to AZT, 3TC, ddC, and ddI in individuals treated with AZT/3TC. 117 samples from 87 individuals with a history of AZT/3TC experience were analyzed; All 117 samples have phenotypic resistance to 3TC; 53/117 samples have AZT/3TC experience only, while the other 64/117 have had RTI experience in addition to AZT/3TC. The authors concluded that the incidence of cross-resistance to ddI, d4T and ddC subsequent to AZT/3TC treatment was low and that the level of cross-resistance was also low. Those samples that were 3TC-resistant (M184V mutation present), but AZT-sensitive were not cross-resistant; but, all cross-resistant samples were both AZT-resistant (intermediate-high) and 3TC- resistant.

Effect of AZT Resistance vs No AZT Resistance

	ddC	ddI	d4T
pts w/ 3TC rx			
(117 samples)			
AZT rx >10x (84)	16 (19%)	7 (8.3%)	17 (20.2%)
AZT rx <10x (33)	0	0	0
AZT/3TC only			
(53 samples)			
AZT rx >10x (35)	8 (15%)	2 (3.7%)	4 (7.5%)
AZT rx <10x (18)	0	0	0

rx- resistance

The first group's additional RTI experience may reflect the higher incidence of cross-resistance than the second group having only AZT/3TC experience. Of those with AZT/3TC experience only (and both AZT and 3TC resistance), 15%, 3.7% and 7.5% of the samples displayed low level resistance (3- to 8-fold) to ddC, ddI and d4T, respectively. There was 1 patient who displayed >10-fold resistance to d4T following AZT/3TC therapy, although its not clear if they were just AZT resistant or resistant to both AZT and 3TC. Again, those with 3TC resistance only, and <10-fold AZT-resistance did not display any cross-resistance to ddC, ddI, d4T.

I would again refer you to my commentary at the beginning of the report where I discuss the limitations to all of the study results discussed in this.

Commentary: it appears to me that phenotypic and genotypic resistance testing is an indication of what may occur but in the final analysis response to therapy is individual and it may be difficult to make conclusions without observing an individual's actual response to therapy.

With regards to RTIs, generally, <10-fold resistance is not considered a concern for cross-resistance. 5-fold or less is considered low level, and cross-resistance is considered less of a concern. As you can observe from the table, the percent of individuals with > 10-fold resistance is minimal (0-3.4%), and the percent of individuals with >5-fold varies from 2.3-6.9%. Generally, 2-3 fold resistance is not considered a concern.