Hydroxyurea with ddI or ddI/d4T: a novel approach to HIV therapy

Results from several hydroxyurea (ddI+hydroxyurea) studies were reported at Vancouver (July `96) and ICAAC (September, `96). Our Vancouver and ICAAC Conference daily highlights (see Conference Reports) contain data and comments about these studies. Hydroxyurea used alone has not been effective against HIV; up until now findings from studies have shown that the combination of hydroxyurea+ddI can lower viral load in blood and in lymph tissue. The combination of AZT+hydroxyurea is not effectual. The accumulation of data so far has come from small pilot studies sponsored mostly by Bristol-Myers Squibb, although the ACTG started a trial in the Summer of `96.

Hydroxyurea has been used as a treatment for several types of cancer for many years, but at significantly higher doses than is being used in its application to HIV disease. How does hydroxyurea work in HIV? Nucleoside analogues such as AZT, d4T, ddI, ddC, 1592U89 and 3TC work by replacing the building blocks for DNA and thereby inhibit the process by which an HIV infected cell reproduces new virus. Hydroxyurea inhibits the building blocks of DNA from being synthesized, thus increasing the opportunity for nucleoside analogues to replace the building blocks and inhibit replication of virus. It appears as though hydroxyurea will be more effective in combination with ddI or ddI/d4T than AZT or ddC. The study discussed below explores its use in combination with ddI/d4T.

The use of hydroxyurea in combination with a nucleoside(s) presents a novel concept to HIV therapy. That is, the simultaneous attack of a host (human) enzyme and a viral enzyme. Nucleoside analogues, non-nucleosides and protease inhibitors attack or exploit a virus enzyme, while hydroxyurea attacks a host enzyme, ribonucleotide reductase; this enzyme regulates the synthesis of the building blocks for DNA allowing for the reproduction information needed for reproducing HIV. The inhibition of this host enzyme increases the chances for nucleosides to incorporate themselves into the DNA and inhibit viral reproduction. As well, Bristol Myers reports that hydroxyurea penetrates the CNS well.

However, there are some considerations. Individuals initiating hydroxyurea therapy with low white blood cell counts (WBC) may not tolerate the therapy well. Second, studies so far conducted have indicated that even when there is a significant viral load reduction, CD4 counts may not show the expected proportional increase. In several studies conducted to date of hydroxyurea+ddI, individuals with lower CD4 counts prior to beginning therapy appear more likely not to experience CD4 increases than those with higher CD4 counts prior to therapy. In fact, in the study discussed below of hydroxyurea+ddI+d4T, the baseline CD4 count for 142 overall study participants was 363, but those receiving hydroxyurea combination therapy exhibit slight CD4 increases. In the Jorge Vila study presented at Vancouver (see report on web site), participants with higher baseline CD4 experienced increases in their CD4 counts, which could have been due to ddI therapy. We do not understand the implications of no increases in CD4 or the

effect of hydroxyurea. The study of this drug is in its early stages, so the amount of available research upon which to base treatment-decisions is limited. Its use in therapy for HIV prior to more extensive research is experimental.

At the recent 4th Retrovirus Conference there were several abstracts related to hydroxyurea. This is the first data reported from the combination of d4T, ddI and hydroxyurea.

Open Label Combination Therapy with d4T, ddI and hydroxyurea in Nucleoside Experienced HIV-1 Patients
authors: R Rossero, M Nokta, L Andron, R Pollard; University of Texas, Galveston

This is a 16 week small open label pilot study of d4T+ddI+hydroxyurea for 35 patients at several sites. The following preliminary data applies to the nineteen patients enrolled at this site. Baseline CD4 was 65 to 374 (mean 226 cells/mm3), and baseline viral load was 18,700 to 374,000 (mean 81,000 copies/ml by bDNA). Participants were treated with full dose of ddI and d4T, and 1 gram/day of hydroxyurea. Prior to the study, seventeen were on antiretroviral monotherapy (AZT, ddI, or d4T) and two were on combination therapy (AZT/ddI, d4T/ddI).

Results: The mean increase in CD4 at 12 weeks for 6 patients was 12 cells/mm3. Viral load decreased by 1.5 log at 2 weeks, "a little more than 1 log at 4 weeks", and for 5 patients at 12 weeks there was a 2 log reduction from baseline. Pretreatment circulating tumor necrosis factor alpha (TNF a) levels of 25-114 (mean 54 pg/ml) decreased by more than 2 fold in five patients during treatment.

Side effects: "Neutropenia to absolute neutrophil count (ANC) below 700/ul developed in four patients entering study with ANC baselines below 1,700/ul. Neutropenia was reversible upon withdrawal of hydroxyurea and ANC was maintained at pretreatment levels during continued ddI and d4T dosing in three patients".

The investigators concluded "combination therapy with d4T, ddI and hydroxyurea decreases viral load in nucleoside experienced individuals but may induce neutropenia in those with low entry ANC."

Commentary: Although preliminary data indicate an enhanced antiviral effect by adding hydroxyurea to d4T/ddI therapy, there is some concern about the effect of hyroxyurea on overall HIV disease because CD4 counts do not increase. Hydroxyurea is cytostatic at the doses being investigated for HIV disease; that is, it keeps cells from replicating. HIV infected as well as non-infected cells that are activated and ready to replicate are shut down but not eliminated.

ddI+d4T + hydroxyurea in Moderately Immunosuppressed HIV-Infected Patients
authors: Rutschmann OT, Opravil M, Iten A, Malinverni R, Vernazza P, Bucher H, Bernasconi E, Vincent-Suter S, Gabriel V, Yerly S, Perrin L, Hirschel B, and the Swiss HIV Cohort Study

Investigators said:

- ddI+d4T have shown synergy in vitro and in vivo without evidence of additive toxicity

Commentary: See the NATAP report (Drug Development) of Pollard's pilot study of d4T+ddI using a variety of dosing combinations in treatment-naive individuals. Neuropathy does not appear to develop for study participants, but they are relatively healthy group without prior drug experience. Neuropathy appears to become more of a problem for individuals with more advanced HIV and/or more extensive drug experience. However, in the Pollard study, significant CD4 increases and viral load reductions (an average reduction of about 1.3 log from baseline for all the different dosing groups) were sustained out to 50 weeks. For a comprehensive discussion of the data and see the NATAP report mentioned above.

- Adding hydroxyurea to ddI resulted in total suppression of HIV replication in resting lymphocytes in vitro and caused profound decreases in virus in vivo.

Inclusion criteria:

- CD4 - 200-500
- RNA - >1,000 copies/ml
- d4T and hydroxyurea naive
- ddI naive or ddI use for <6 months but2 months

This 12 week study randomized 142 individuals to d4T (40 mg bid)+ ddI (200 mg bid) + hydroxyurea (500 mg bid) or d4T (40 mg bid)+ ddI (200 mg bid)+ placebo.

Baseline characteristics:	
sex	male 75% female 25%
CDC 1993 stage	A1 1% A2 58% B2 35% C2 6%
treatment-naive	yes 80% no 20%
mean HIV RNA mean CD4 mean CD4% mean CD8 mean total lymphocytes	4.52 (about 33,100 copies/ml) 363 21.7 1032 1803

mean CD4/CD8 ratio	0.43

Changes in Viral Load:			
HIV RNA	week 4	week 12 % undetectable	p value at week 12 placebo vs Hydrea
200 copy test	placebo -1.6 log hydrea -1.5 log	placebo -1.6 log 32% hydrea -1.9 log 55%%	0.06
20 copy test	placebo -1.6 log hydrea -1.5 log	placebo -1.8 log na hydrea -2.2 log na	0.04

When using the more sensitive Roche PCR test (20 copies), the log reduction is greater because the limit of detection is lower. By direct visual observation of graph, it appeared as though there were about 8-10 individuals below 20 copies.

Changes in CD4 and CD8:			
	week 4	week 12	p value at week 12, placebo vs Hydrea
CD4 -placebo -hydrea	 +104 +43	 +91 +10	0.003
CD4% -placebo -hydrea	 +2.1 +1.6	 +2.8 +3.4	0.5
CD8 -placebo -hydrea	 +61 -32	 -54 -183	0.1
Total lymphocytes -placebo -hydrea	 +164 +20	 +69 -205	0.02
CD4/CD8 ratio -placebo -hydrea	 0.05 0.07	 0.11 0.09	0.7

Take note of the small increase in CD4 at week 12 for those taking hydrea vs those not taking hydrea.

The authors concluded that the lack of effect on CD4 counts by hydroxyurea combination therapy can be due to hydroxyurea induced lymphopenia.

Commentary: In the study reported at Vancouver and conducted by Jorge Vila 25 individuals receiving hydroxyurea+ddI experienced increases in CD4 of about 163 cells (from 482 to 575). It is always difficult to compare results between studies. But, in the Swiss study above the baseline CD4 was 363, 80% were treatment experienced (in the Vila study, participants were treatment-naive).

Hydroxyurea and Resistance

The following 2 studies (hydroxyurea+ddI) are related and address issues of resistance. The first study discovered the development of ddI-related genotypic (mutation) changes in a subset of patients in the study. The second study presents the investigators conclusions as to why viral load remains suppressed despite the development of genotypic mutations.

Analysis of Mutations of HIV-1 Reverse Transcriptase after Therapy with ddI+ Hydroxyurea
authors: Anna De Antoni, Andrea Foli, Julianna Lisziewicz and Franco Lori

The investigators studied 12 out of 40 patients treated in a trial with hydroxyurea+ddI and 8 out of 8 treated with ddI monotherapy, after 24 weeks as part of an ongoing trial. Participants taking hydroxyurea/ddI exhibited a more potent viral suppression than those taking ddI, which was statistically significant after 4 weeks. The viral suppression was consistent and sustained without virus rebound. DDI mutations emerged in both groups, but in a higher frequency in the combination (58.3%) than in the monotherapy arm (37.5%). For the exact mutations, you can read the abstract. The authors suggest perhaps it is due to greater selective pressure in the presence of hydroxyurea.

Overcoming Drug Resistance to HIV-1 by the Combination of Cell and Virus Targeting
authors: F Lori, AG Malykh, A Foli, R Maserati, A De Antoni, MA Wainberg

Combination therapy using nucleosides, NNRTIs and/or protease inhibitors targets the virus (viral proteins). It aims to inhibit viral replication by interfering with the activity of the viral enzyme (reverse transcriptase enzyme, protease enzyme). The use of an agent (hydroxyurea) which targets a cellular protein in combination with a drug(s) that targets a viral one is an alternative therapeutic option. Again, a consistent and sustained viral suppression was achieved for many of the participants in this study without rebound for over 1 year, despite the development of ddI-related genotypic mutations. These in vivo results were consistent with their in vitro observations: "HIV-1 molecular clones resistant to ddI were rendered sensitive to ddI (at concentrations routinely achievable in vivo) after addition of hydroxyurea. Hydroxyurea lowered the levels of the cellular

competitor of ddI, namely dATP, and this favored the incorporation of ddI, even if the reverse transcriptase was resistant to this nucleoside analogue." The authors concluded that the use of hydroxyurea with nucleosides may generate treatments which are simple, well-tolerated and inexpensive.

Commentary: It appears as though the development of resistance is a barrier which prevents antiretroviral therapy from more profound and sustainable viral suppression. The authors are suggesting the possibility that combining an agent that inhibits a cellular (host) protein with a therapy inhibiting a viral protein (enzyme) will sustain the suppression of viral burden despite the emergence of viral resistance. Preliminary results from the Vila study presented at Vancouver found that 5/6 receiving hydroxyurea/ddI had undetectable viral load in their lymph tissue after 1 year of therapy.

A question I would raise is-- after discontinuing hydroxyurea/ddI or ddI/d4T/hydroxyurea therapy, will nucleoside resistance remain and be problematic?

Existing drugs may be discovered that might be more suitable in targeting a host enzyme. As well, there are remaining questions to be answered about hydroxyurea's potential.