A randomized trial to investigate the recycling of stavudine and didanosine with and without hydroxyurea in salvage therapy (RESTART)

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Received 22 October 2003; revised 9 December 2003; accepted 12 December 2003

Background: Treatment failure during highly active antiretroviral therapy (HAART) is ultimately common and associated with the development of resistance mutations to both the specific drug in question and cross-resistance to other available treatment options. In heavily pre-treated patients, the recycling of antiretroviral agents that have been utilized previously may, however, be associated with antiviral efficacy. We therefore conducted an investigation into the concept of recycling stavudine (d4T, Zerit) and didanosine (ddI, Videx) with and without hydroxyurea, in the management of heavily pre-treated HIV-1 infected individuals requiring salvage therapy (RESTART).

Methods: We randomized 21 individuals with treatment failure to receive stavudine and didanosine or stavudine, didanosine and hydroxyurea, for 12 weeks prior to optimizing therapy. Viral load, immunological parameters, genotypic information and the virtual phenotypes were obtained at baseline and at the end of the study.

Results: Significant decreases in viral loads were observed in both groups during a 12 week study period (P = 0.04), the addition of hydroxyurea conferring no additional benefit. This was not predicted by information from genotypes and virtual phenotypes, and these did not reveal sensitive or specific phenotypic cut-offs for those individuals who responded to recycling.

Conclusions: Salvage therapy with didanosine and stavudine can decrease viral loads in heavily pre-treated individuals. Genotypic and virtual phenotype profiles provide little additional information in this setting.

Keywords: recycling, phenotype, nucleoside analogues, HAART

Introduction

In those for whom it is available, highly active antiretroviral therapy (HAART) has reduced short-term mortality and markedly increased quality of life by preventing opportunistic diseases. Despite the initial optimism concerning this selective targeting of the HIV reverse transcriptase and protease, HAART is not able to target the latent reservoir and it is associated with well-known and described clinical side effects and toxicities. Virological failure is also specifically associated with the development of resistance mutations against the agents within received antiretroviral regimens. It has also become clear that there are high levels of cross-resistance within classes when such mutations occur; it is well known, for example, that a resistance to one non-nucleoside reverse transcriptase inhibitor (NNRTI) confers resistance across the class.

Although the nucleoside analogues stavudine (d4T) and didanosine (ddI) had been widely used as the backbone of initial HAART, they are not generally recommended together due to the relatively high levels of toxicity, including pancreatitis, peripheral neuropathy and lactic acidosis. However, both agents may be utilized individually in initial therapy, although stavudine is not recommended in some guidelines due to the development of lipoatrophy.

It has been difficult to delineate precise genotypic patterns of resistance in individuals failing regimens including stavudine or didanosine. Those failing stavudine may develop either the classical codon 75 mutation but also thymidine excision mutations. The impact of these on the potency of stavudine is not fully understood. Similarly, reduced sensitivity to didanosine is associated with mutations at codon 74, although this is rarely observed in those on continu-
Belgium) were performed before study entry and at 12 weeks. CD4 salvage regimens.18,19 Here, it exerts a cytostatic effect through the induction of G1 T lymphocyte cell cycle arrest20 and inhibition of DNA synthesis in infected cells.21,22 As a non-randomized study has shown the antiviral efficacy of stavudine, didanosine and hydroxyurea administered for 12 weeks, prior to optimizing therapy by adding an NNRTI, a protease inhibitor (PI) or both as appropriate. Toxicity was recorded according to standard clinic criteria.

Other agents may confer additional benefit in this situation and hydroxyurea may have particular value in individuals with a low CD4 count.18 This hydroxycarbamide compound has been shown to enhance the antiviral potency of nucleoside analogue-containing salvage regimens.18,19 Here, it exerts a cytostatic effect through the induction of G1 T lymphocyte cell cycle arrest20 and inhibition of DNA synthesis in infected cells.21,22 As a non-randomized study has shown the antiviral efficacy of stavudine, didanosine and hydroxyurea administered for 12 weeks in antiretroviral-experienced patients,19 we randomized individuals to receive stavudine and didanosine plus/minus hydroxyurea for this short time period.

Resistance testing is now widely used for choice of new therapy in individuals failing HAART and here two types of resistance tests are available—genotypic and virtual phenotype. Both tests have been associated with improvements in future antiretroviral success when accurately interpreted.4 We investigated the use of these resistance tests in combination with the efficacy of recycling the nucleoside analogues—didanosine and stavudine—in heavily pre-treated HIV-1-infected individuals. In particular, we wished to discover whether the genotype and virtual phenotype of heavily pre-treated HIV-1-infected individuals influenced virological outcome in the context of stavudine and didanosine recycling.

### Methods

We recruited 21 individuals with prior experience (for at least 8 weeks) of zidovudine (AZT), stavudine (d4T), didanosine (ddI) and lamivudine (3TC), and evidence of virological failure (HIV-1 RNA > 5000 copies/mL). All existing therapy was discontinued and patients were randomized on a 1:1 basis to receive stavudine (40 mg twice daily) and didanosine (400 mg once daily) with or without hydroxyurea (500 mg twice daily) for 12 weeks, prior to optimizing therapy by adding an NNRTI, a protease inhibitor (PI) or both as appropriate. Toxicity was recorded according to standard clinic criteria.

Genotypic and VirtualPhenotype tests (Virco NV, Mechelen, Belgium) were performed before study entry and at 12 weeks. CD4 subset analysis was performed at this time using whole blood stained with murine anti-human monoclonal antibodies to CD4 (TetraOne, Beckman Coulter, High Wycombe, UK) and was evaluated on an Epics XL-MCL (Beckman Coulter) flow cytometer. Viral load in patient plasma was measured using the Quantiplex HIV RNA 3.0 (Chiron bDNA) assay with a lower limit of detection of 50 HIV-1 copies/mL (Chiron Diagnostics, Halstead, UK). All patients were men who have sex with men, written informed consent was provided and the study received appropriate ethical approval in accordance with the declaration of Helsinki.

Non-parametric statistical methods were used for between-group comparisons (Mann–Whitney U-test) and data are presented as medians with interquartile ranges. Within-group differences were also calculated using the paired t-test for repeated measures. Data were further analysed for trends over time using repeated measures ANOVA with the MIXED procedure in SAS. All P values presented are two sided.

### Results

In the 21 individuals recruited, a statistically significant decrease in viral load (P = 0.04) was observed; the addition of hydroxyurea did not confer increased antiviral efficacy.

Table 1 demonstrates the patient characteristics and changes in virological and immunological parameters during the study. All patients were men who have sex with men. There were no statistically significant differences between either group at baseline and all patients had a viral load of >12 500 copies/mL. Although the CD4 counts did not show significant increases during the study period, the HIV-1 viral load decreased in both groups, with a greater decrease occurring in the patients not randomized to receive hydroxyurea (P = 0.02 versus P = 0.05). For all 21 patients, the median HIV-1 viral load decreased from 68 060 to 33 339 RNA copies/mL and CD4 counts increased from 79 to 101 cells/mm³. There were no reports of neuropathy during the trial and pancreatitis was not observed (serum amylases remained in the normal range).

The genotypic resistance profiles of all patients in our small study are shown in Table 2. Notably, there were few changes during the 12 weeks of this study and only one patient who did not have the L74V mutation prior to nucleoside analogue recycling developed this. From these data, it is difficult to draw conclusions regarding restoration of genotypic sensitivity to other antiretrovirals by recycling nucleosides, a conclusion also found in one other study.23

### Table 1. Patient characteristics and changes in HIV-1 viral load and CD4 count during the study. The median is shown (with interquartile)

<table>
<thead>
<tr>
<th></th>
<th>Stavudine/didanosine (n = 10)</th>
<th>Stavudine/didanosine + hydroxyurea (n = 11)</th>
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<tbody>
<tr>
<td>Median age (years)</td>
<td>44.6</td>
<td>38.1</td>
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<tr>
<td>Previous drug experience</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 NRTI/NNRTI/PI</td>
<td>1</td>
<td>2</td>
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<tr>
<td>4 NRTI/NNRTI</td>
<td>1</td>
<td>2</td>
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<tr>
<td>5 NRTI/NNRTI/PI</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Baseline HIV-1 viral load (RNA copies/mL)</td>
<td>103 090 (53 246–361 326)</td>
<td>49 135 (13 855–104 192)</td>
</tr>
<tr>
<td>Viral load at week 12</td>
<td>26 619 (7016–42 169)</td>
<td>40 059 (3507–13 4750)</td>
</tr>
<tr>
<td>P value for change in viral load</td>
<td>P = 0.042 (Mann–Whitney U-test)</td>
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<tr>
<td>Baseline CD4 count (cells/mm³)</td>
<td>78 (48 to 107)</td>
<td>79 (16 to 215)</td>
</tr>
<tr>
<td>CD4 count at week 12</td>
<td>103 (101 to 127)</td>
<td>60 (22 to 229)</td>
</tr>
<tr>
<td>P value for change in CD4 count</td>
<td>P = 0.581 (Mann–Whitney U-test)</td>
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NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
The recycling of nucleoside analogues

There were no statistically significant differences in changes in viral load or CD4 count between those patients with a codon 74 mutation (n = 3; didanosine resistance) and those without (n = 18). In all patients studied, the change in viral load did not correlate with the number of nucleoside analogue mutations (or thymidine analogue mutations), or with the virtual phenotypic resistance profiles to either stavudine or didanosine (Figure 1). We then combined patterns of resistance to stavudine and didanosine and grouped all 21 individuals into four categories according to virtual phenotypic values (Table 3). Using non-parametric tests (Kruskal–Wallis), there were no statistically significant differences between those individuals who were sensitive and/or resistant to stavudine and didanosine (and vice versa). Interestingly, the seven patients classified as being resistant to stavudine and didanosine (last row, Table 3) had a greater decrease in viral load than those who were classified as being sensitive to both, although the ‘resistant to both’ group of patients were the only individuals who failed to achieve an overall increase in median CD4 counts.

We failed to demonstrate a phenotypic ‘cut-off’ at which point didanosine and/or stavudine would or would not achieve a decrease or increase in viral load (this includes using a cut-off for normal susceptible range of IC50 of 1.8 for didanosine and 1.7 for stavudine). Only weak correlations were observed between an increase in viral load and higher values of virtual phenotypic resistance (fold change in IC50 of greater than 3) to didanosine (Pearson’s correlation coefficient, r = 0.18) and stavudine (r = 0.28). No correlations were observed with patterns of resistance to other antiretrovirals, and the virtual phenotype at the end of the study period demonstrated no notable changes. In addition, previous specific NNRTI or PI-based HAART regimens did not predict results.

Discussion

In individuals with limited antiretroviral options, recycling of previously utilized drugs is a common therapeutic strategy. Both clinical and biological cut-offs of phenotypic resistance for stavudine and didanosine in combination are poorly understood and this small study gives no indication that such cut-offs will be useful in interpreting the future response to these nucleoside analogues in combination, in heavily pre-treated patients requiring salvage therapy. Although a statistically significant decrease of 0.31 log10 in viral load was observed in both groups (P = 0.04), overall there were no statistically significant differences between specific characteristics in those patients who demonstrated a decrease in viral load and those who did not.

The lack of efficacy of including hydroxyurea in this setting is consistent with data from previous non-randomized trials in which it did not enhance the antiviral efficacy of tenofovir24 or contribute to the antiviral potency of nevirapine, stavudine and didanosine in primary HIV infection.25 In one 12 week non-randomized trial of the triple combination of didanosine, stavudine and hydroxyurea in a similar patient population, a 1.3 log10 decrease in viral load was observed at 12 weeks.19 One randomized study has also shown a benefit to its longer use at 48 weeks. In that study, only 25% of patients receiving antiretrovirals alone had plasma HIV-1 RNA <200 copies/mL versus 59.1% in the group receiving hydroxyurea, and 56.5% in the group receiving hydroxyurea and interleukin-2 (intent-to-treat; P <0.01).26

The largest study recruited individuals with a low viral load (<200 copies/mL), a CD4 cell count of >200 cells/mm3, who had received prior treatment with indinavir, zidovudine and lamivudine. They were randomized to stay on this regimen or receive indinavir, didanosine and stavudine plus or minus hydroxyurea (600 mg twice daily). Treatment failure occurred more frequently in subjects randomised to the hydroxyurea-containing arm than the other arms, time
to treatment failure was significantly shorter in those receiving hydroxyurea ($P < 0.05$), and pancreatitis was more common in those who received didanosine, stavudine and hydroxyurea. Their results suggest caution in using this combination.

A further recent trial utilized 4 week cycles of high dose stavudine (280 mg daily) in 11 asymptomatic patients who had previously received an average of 6 years of nucleoside reverse transcriptase inhibitors (NRTIs). Participants received stavudine for the first 4 weeks, after which it was discontinued for 4 weeks. Additional 4 week drug cycles were given if plasma HIV-1 RNA levels increased to at least 75% of baseline values. Stavudine was well tolerated and there was a median 0.65 log_{10} reduction in viral load as well as a median increase in the CD4 cell count of 110 cells/mm$^3$ at the end of the cycles. However, plasma viraemia increased and CD4 cell counts decreased between cycles. The study also suggested that increasing viral resistance was not a significant problem, as reflected by the acquisition of only one new nucleoside reverse transcriptase mutation among the participants. Significant relationships between stavudine exposure and changes in plasma HIV RNA levels were observed. Taking these results together, a similar approach might be considered, using more potent regimens containing both stavudine and didanosine for patients in whom resistance to nucleosides is a partial mechanism.

New antiretrovirals with novel mechanisms of action are now being used in salvage regimens. It has been shown that there are increased rates of virological failure unless other agents with antiviral efficacy are used, although this study has shown a reduction in viral load in heavily pre-treated HIV-1-infected individuals, in a group randomized to receive only the combination stavudine and didanosine. These results need to be interpreted with caution due to the small sample size: larger studies may reveal virtual phenotypic cut-off: sensitive ≤1 resistant >1

<table>
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<tr>
<th>Virtual phenotypic cut-off: sensitive ≤1 resistant &gt;1</th>
<th>Change from baseline to week 12: median (interquartile range)</th>
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<tr>
<td></td>
<td>didanosine</td>
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<td>sensitive</td>
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### Table 3. Changes in viral load and CD4 count in individuals with different combinations of stavudine and didanosine resistance, according to virtual phenotype. A cut-off of 1 indicates the sensitivity in the virtual phenotypic assay with >1 indicating resistance, ≤1 indicating sensitivity.

Can be recycled in salvage therapy (although they should not be given to females who may become pregnant). Whereas these medications are considered the cheapest and most readily available of antiretrovirals, we caution against the addition of hydroxyurea in such salvage therapy. We observed in this randomized trial that this did not provide any further benefit and other studies have shown that it may cause harm.

### References


25. Zala, C., Salomon, H., Ochoa, C. et al. (2002). Higher rate of toxicity with no increased efficacy when hydroxyurea is added to a regimen of stavudine plus didanosine and nevirapine in primary HIV infection. Journal of Acquired Immune Deficiency Syndromes 29, 368–73.


