AIDS Rev 2000; 2: 99-104

# The Role of Hydroxyurea in the Treatment of HIV Infection

*Pablo Barreiro[1] and Franco Lori[2]*

[1]*Service of Infectious Diseases. Hospital Carlos III. Instituto de Salud Carlos III. Madrid. Spain.*
[2]*Research Institute for Genetic and Human Therapy (RIGHT) at Washington, D.C., USA, and at Policlinico S. Matteo, Pavia, Italy.*

**Abstract**

**Hydroxyurea (HU) seems to be a unique agent helping current antiretroviral regimens to control HIV replication. On the one hand, it increases the antiviral activity of nucleoside analogues, and on the other hand, it reduces the number of activated CD4 T lymphocytes needed for HIV replication. HU is easy to administer, broadly distributed to different body compartments, relatively well tolerated, and its activity does not select for resistant viruses. Clinical trials have demonstrated substantial and sustained reductions in viral load in patients with primary and chronic HIV infection receiving HU added to regimens containing didanosine. Several studies are currently exploring the role of HU-containing regimens as part of both salvage and simplification strategies. There is also a growing interest in the properties of HU in preserving or eliciting HIV-specific T lymphocyte responses.**

**Key words**

**Hydroxyurea. Didanosine. Antiretroviral therapy. T lymphocytes.**

## Introduction

Hydroxyurea (HU) is a well-known cytostatic drug which has been widely used for the treatment of several neoplasms, mainly those derived from bone-marrow cells[1,2]. As HU reduces the production of natural nucleotides inside the cells, the association of this drug to nucleoside analogs has been proven to enhance HIV suppression[3,4]. Paradoxically, the cytostatic effect of HU could be another helpful tool for the treatment of HIV infection, as the reduction of activated CD4 lymphocytes limits the spread of the virus throughout the immune system[5,6]. These adjuvant properties could make HU specially suitable for the treatment of HIV disease now that the amount of patients in need of salvage regimens is growing. Other provocative indications of HU are as part of simplification strategies in patients with severe toxicities or with significant quality of life impairment due to currently used regimens. The immune-modulatory activity exerted by HU could also be exploited for the preservation of HIV-specific T-lymphocyte response when this drug is added to regimens prescribed during primary or early infection. Furthermore, in chronically HIV-infected patients following simple nucleoside combinations including HU, it might be possible to elicit an anti-HIV cellular response by virtue of the sustained immunological stimulus provided by a low rate of virus replication.

HU is also easy to administer, has good body distribution and a relatively low cost. The efficacy of the drug is not affected by the development of any cellular resistance, and could help to overcome the appearance of mutations to various antirretroviral drugs. Conversely, a significant increase in the appearance of antirretroviral drug toxicities has been described when HU is added to the regimens.

The objective of this review is to offer a broad perspective on the proven benefits and potential

***Correspondence to:***
Pablo Barreiro
Service of Infectious Diseases, Hospital Carlos III
C/ Sinesio Delgado 10
Madrid 28029
Spain

**Table 1.** *Pharmacokinetic parameters of hydroxyurea administered orally at a dose of 500 mg BID.*

| | |
|---|---|
| Peak concentration ($C_{max}$) | 0.135 ± 0.06 mmol/L |
| Trough concentration ($C_{min}$) | 0.0085 ± 0.003 mmol/L |
| Steady state concentrations | 0.045 ± 0.006 mmol/L |
| Drug clearance | 0.18 ± 0.005 L/h/kg |
| Half-life ($T_{1/2}$) | 2.5 ± 0.5 h |

risks associated to the inclusion of HU in the drug armament available for the treatment of HIV infection.

## Pharmacokinetic properties

HU has excellent bioavailability, with a Tmax of 0.85 to 0.96 hours after oral ingestion. The volume of distribution is equivalent to total body water, which means that it is widely distributed. It also has a very good penetration into the central nervous system and lymphoid tissues, as HU enters the cell by passive diffusion[1,7]. HU is eliminated through the kidneys and other non-renal mechanisms. Mean pharmacokinetic parameters in patients who were treated with HU (500 mg BID) are shown in Table 1[8]. The serum levels of HU obtained following this dosage and administration schedule (0.01-0.13 mmol/L) are in the range of the concentration shown to inhibit HIV replication in vitro (0.01-0.1 mmol/L).

## Antiviral activity

HU is a selective inhibitor of intracellular ribonucleotide reductase enzyme, which is responsible for the production of the natural nucleotides required for the elongation of nascent DNA[4,9]. This activity explains that high doses of HU are able to block the proliferation of rapid turn-over cells, as in the case of certain neoplasms[1,10]. The role of HU in the treatment of HIV infection takes advantage of this same mechanism of action, although lower doses than in oncologic patients are normally used.

As HU is able to decrease the intracellular dATP pool, this drug has been shown to enhance the antiretroviral activity of adenosine analogues, mainly didanosine (ddI), but also adefovir dipivoxil (PMEA) and tenofovir disoproxil fumarate (PMPA)[3,4,11-13]. Smaller benefits could be obtained with the association of HU to other nucleoside analogues, as the reduction of paired dNTP levels is less effective than in the case of dATP competitive inhibitors[11,14,15]. At least *in vitro*, HU seems to enhance also thymidine (stavudine, d4T) and cytidine (lamivudine, 3TC) antiviral activity, by increasing the intracellular phosphorylation of these drugs[11,16].

Genotipic resistance to nucleoside analogues is a dynamic process in which the appearance of certain amino-acid substitutions at the reverse transcriptase confer son this enzyme a higher affinity for the natural nucleotides than for the triphosphorylated drug. From this point of view, the addition of HU could help to retain the antiviral activity of adenosine analogues, despite the development of resistance mutations. As HU depletes dATP levels, the natural competitor of ddI at the DNA elongation process, the relative disadvantage of the drug in the presence of resistance substitutions is compensated by the reduction in the chances for dATP to be incorporated[4,17,18].

The addition of HU to a ddI containing regimen does not prevent the development of genotypic resistance to this nucleoside analogue[18]. However, in the presence of HU the appearance of ddI related codon mutations could be overcome, and the amount of virus rebound attenuated[17,18]. This phenomenon has been confirmed by our group in clinical practice. In three patients being treated with HU-ddI for more than 12 months the viral load remained always below 5,000 cop/mL, despite the presence of ddI resistance codon substitutions (T69D, K70R, M184V, L74V). Furthermore, no new mutations where detected with respect to the baseline genotype in those subjects presenting virus rebound over 5,000 cop/mL under HU-ddI. Palmer *et al.* have shown that the *in vitro* antiviral effect of ddI against multidrug resistant isolates was 18 to 22-fold higher when HU was added to the culture[17]. *In vivo*, De Antoni *et al.* found that the association of HU to ddI provides better immunological outcome than ddI alone. This benefit was obtained despite half of the patients receiving HU-ddI presenting mutations conferring ddI resistance[19].

## Immunological activity

The inhibition of ribonucleotide reductase enzyme exerted by HU also has immunological implications. As DNA synthesis is impaired, those cells with high turn over tend to be arrested at a quiescent stage, somewhere between G1 and S phases[20]. This phenomenon explains HU cytotoxic effect, and the frequent appearance of bone marrow toxicities[21]. Paradoxically, the immune activation observed during HIV infection, in which the production of cytokines[22,23] and the increased number of activated T lymphocytes play a major role[24-26], helps to sustain or even increase HIV replication. It is known that HIV is able to infect both latent and activated T lymphocytes, although only in the second subtype of cells the production of infective virions would occur. In this context, the reduction in the number of HIV producing cells provided by the addition of HU to the antiretroviral regimen helps to control virus replication[18,27,28]. This phenomenon is known as the predator-prey hypothesis, in which the lower the number of cells available for HIV to replicate, the easier the viral load would be controlled[29]. Several pilot studies have confirmed this hypothesis in clinical settings, showing that the association of HU plus ddI is able to provide long-term control in virus replication[30-32]. Also, in an observational study, Bar-

rreiro *et al.* have recently reported that in a group of 30 patients switched to HU-ddI after being with undetectable viremia for more than 6 months under a PI-containing regimen, the viral load was sustained below 5,000 cop/mL for more than 12 months in half of them[33].

Among the several immunological dysfunctions observed during HIV infection, CD8 T lymphocytes over-activation is known to occur particularly early, the degree of which predicts a poorer clinical prognosis[34]. The uncontrolled cytotoxic effect exerted by these cells plays a central role in the destruction of readily infected antigen-presenting cells, such as CD4 T lymphocytes or macrophages, being responsible for the subsequent depletion of this subpopulations[6,34-37]. In this context, HU cytostatic effect over CD8 T lymphocytes may help to preserve CD4 T lymphocytes, specially those having been in contact with HIV antigens[38]. There have been several reports of patients being treated during primary infection with HAART regimens including HU, who were able to discontinue all antirretroviral drugs several months later. The preservation of CD4 T lymphocytes with a memory response to HIV antigens provided by the early introduction of HU seems to be implicated in this observation[39-41]. Other studies have shown that the addition of HU to the regimen chosen for the treatment of primary HIV infection helps to normalize some immune parameters and functions. The loss of naive CD4 T lymphocytes was reduced, naive CD8 T lymphocytes recovered more efficiently, and most subjects demonstrated a vigorous HIV-specific T helper response[42].

The combination of HU plus ddI has been reported to help to restore HIV-specific immune responses also in chronically infected patients. Lori *et al.* have communicated that in 12 HIV+ patients treated with HU-ddI for more than two years, the number of naive CD4 and CD8 T lymphocytes became similar to that of uninfected subjects[30]. Also, strong HIV-specific lymphoproliferative responses were detected in half of these patients, which has not been achieved with currently used regimens[43-45]. It is easy to understand that the sustained low rate of virus replication allowed by HU-ddI treatment could help to both elicit and preserve the immune response against HIV antigens. We and others have observed a progressive reduction in the amount of detectable viremia under HU-ddI, in parallel with this enhancement of HIV-specific immune responses[30,31,46,47].

HU could play a role in limiting virus rebound and precluding CD4 T lymphocyte depletion when added to intermittent HAART regimens in structured treatment interruption (STI) strategies. This hypothesis was tested in three patients, two of whom showed a progressive prolongation in the time to virus rebound as cycles of treatment-interruption were performed[48]. As in previous STI studies, prolonged control of virus replication was not achieved, an objective that might require a higher number of STI cycles.

Finally, in a novel approach, we have tested complete treatment withdrawal in two patients achieving ultrasensitive undetectable viremia after more than 12 months under HU-ddI. To date, two months after HU-ddI discontinuation, no virus rebound has been registered.

HU alone may have some antiretroviral activity in macrophages and dendritic cells[4,49], and the combination of HU plus ddI has been shown to inhibit HIV replication in both quiescent and activated peripheral blood mononuclear cells *in vitro*[4]. The inhibition of HIV in quiescent cells is of particular interest, as these cells are thought to represent a latent pool of replication-competent virus[24-26].

## Clinical experience

First observational studies clearly established the inefficacy of HU alone in reducing HIV viral load[50]. However, the addition of HU to ddI monotherapy was shown to produce a significant and prolonged virus suppression, both in naive and ZDV pre-treated patients[15,28,47,51,52]. These preliminary encouraging results invited the design of controlled and randomized trials to better validate the utility of HU as part of antiretroviral regimens.

In the RIGHT 411 study, 57 patients were randomized to receive ddI (200 mg BID) alone or ddI plus HU (500 mg BID)[18]. The decrease in viral load was significantly higher in the HU-ddI arm than in the ddI monotherapy arm at 24 weeks (-1.32 logs versus -0.78 logs). Those patients continuing under HU-ddI obtained up to -1.21 logs reduction in plasma viremia from baseline at 40 weeks. A higher increase in the CD4+ cell count was observed in the ddI monotherapy arm (+83 CD4+ cells/µL) than in the HU-ddI arm (+54 CD4+ cells/µL).

In ACTG 307 a total of 131 patients were randomized to ddI (200 mg BID), HU (1,000 mg QD), HU (1,500 mg QD), ddI-HU (1,000 mg QD) and ddI-HU (1,500 mg QD)[53]. In those patients receiving HU monotherapy, ddI was added 4 weeks later, and patients on ddI monotherapy had HU (1,000 mg or 1,500 mg) added 12 weeks after randomization. The combination of HU plus ddI provided a 2-fold reduction in viral load compared with that obtained under ddI monotherapy at 8 weeks (-1.57 logs versus -0.83 logs). At week 24, mean viral load reduction in patients initially treated with HU monotherapy was -1.05 logs, with ddI monotherapy it was -0.79 logs, and with ddI-HU was -1.22 logs.

The Swiss HIV Cohort Study randomized 144 patients, one half receiving HU (500 mg BID) plus ddI (200 mg BID) plus d4T (40 mg BID) and the other half ddI plus d4T[27]. The reduction in viral load at 12 weeks was significantly higher with the addition of HU to the dual nucleoside combination (-2.3 logs versus -1.7 logs). Also, the proportion of patients achieving undetectable viremia was 2-fold higher in the HU-containing arms (54 versus 28% for < 200 copies/mL limit of detection, and 19 versus 8% for < 20 copies/mL limit of detection). Finally, the HU-treated group had a CD4+ cell increase of 28 CD4+ cells/µL compared with 107 CD4+ cells/µL in the placebo group ($p = 0.001$). At 24 weeks the proportion of patients receiving HU with viral load below

20 and 200 copies/mL rose to 63 and 79%, respectively, and a mean viral load reduction of 2.6 logs was attained. The increase in CD4+ cells at 12 weeks was poorer in patients receiving HU (+36 CD4+ cells/µL versus +123 CD4+ cells/µL).

The BMS 055 study was a randomized, double-blind, placebo-controlled trial that included 177 patients in four different treatment groups: ddI plus ZDV, ddI plus d4T, ddI plus HU, and ddI plus d4T plus HU[53]. In an intent-to-treat analysis, 80% of patients in the triple drug combination achieved viral load < 400 copies/mL at 24 weeks, which constituted a 2 logs mean viral load reduction. Interestingly, the proportion of patients with viral load below 400 copies/mL at 24 weeks in the HU-ddI group was comparable to that observed under dual nucleoside combinations (HU-ddI: 43%, ddI-d4T: 53%, and ddI-ZDV: 36%). Similarly to previous studies, absolute CD4 count improvements were lower in the HU-containing groups compared to the others.

In the ACTG 5025 study, patients receiving IDV, ZDV, and 3TC were randomized to continue the same regimen or to change the nucleoside backbone to ddI and d4T, with or without HU[55]. No virological or immunological differences were found, although the HU containing arm was burdened with a higher incidence of toxicities, including two deaths caused by pancreatitis.

HU has also been included in studies exploring strategies for the treatment of early stage HIV-infection. For instance, 24 patients received HU-ddI and one PI during the first weeks after seroconversion[39]. Plasma viremia became undetectable in all patients within 16 weeks and remained undetectable for up to 21 months of treatment. *In situ* hybridization revealed no HIV RNA in lymph node specimens from 6 out of 7 subjects and no HIV DNA in 2 out of 6 patients. Another 16 subjects with primary HIV infection and 20 patients with relatively early HIV infection received HU-ddI, d4T and NFV[56]. With this regimen, undetectable viral load was achieved and sustained in 95% of patients with early infection and 88% of patients with primary infection. During the follow up the percentage of CD4+ cells increased, and little modification in the absolute CD4 count was observed. In another study comparing standard HAART with HU-ddI and IDV, although similar virological and immunological outcome was observed, the number of CD8+ cells decreased faster in the HU-containing arm[38].

The combination of HU-ddI has also been tested as a maintenance regimen after prolonged and intense virus suppression under a PI-containing regimen[33]. Sixty HIV infected patients under HAART, with undetectable viral load (< 50 copies/mL) for more than 6 months, were randomly assigned to continue with the current regimen or to switch to HU plus ddI. In the later group HAART was planned to be resumed as soon as viral load rose over 5,000 copies/mL. After 12 months, half of the patients simplified to HU-ddI remained below 5,000 copies/mL, and 14% discontinued this treatment due to adverse events. During this period of time, a mean loss of 200 CD4+ cells was observed, despite no change in the percentage of this population of lymphocytes being observed.

Finally, HU has been studied as part of salvage regimens for heavily pretreated patients[57-61]. In one study, a total of 38 heavily pretreated patients received a salvage regimen including HU. One month later 45% of them attained viral load below 500 copies/mL[28]. Another study including 49 patients who were treated with a Mega-HAART regimen including HU showed a significant reduction in viral load (-1.7 logs) and remarkable increase in the CD4+ cells count (+95 CD4+ cells/µL) after 6 months[62].

## Side effects

Due to HU cytostatic activity, the main toxicity encountered in clinical practice is bone marrow suppression, which could lead to neutropenia, anemia and/or thrombocytopenia[1]. This myelosuppressive effect could be enhanced if HU is associated to other hematotoxic drugs, such as ZDV or cothrimoxazole[15]. However, the incidence of these adverse events is much lower in HIV infected subjects than in oncologic patients, who receive higher doses of the drug[63,64]. In this regard it was established in the ACTG 307 study that the optimal dose of HU for the treatment of HIV infection was 1,000 mg daily[54]. Although two cases of prolonged myelosuppression after HU discontinuation have been communicated[65], this adverse effect is often easily reversible upon drug discontinuation[1].

Other rare complications include alopecia, hyperpigmentation, erythema, leg ulcers, nausea, vomiting, diarrhea, anorexia, transient and mild abnormalities of renal function, and elevations of liver enzymes[1,28,62]. HU should not be used during pregnancy as it has teratogenic activity[66].

Following the same mechanism by which HU facilitates the inhibition of the reverse transcriptase (see above), this drug could enhance the mitochondrial toxicity inflicted by nucleoside analogs by virtue of γ-DNA polymerase inhibition[67]. A recent study has found a higher incidence of peripheral neuropathy when HU was added to d4T and/or ddI containing regimens[68]. Moreover, increased hepatic and pancreatic toxicities have been correlated with the use of HU[55,69].

It is important to note that pancreatic toxicity can manifest several months after therapy starts. Therefore, close monitoring of lipase and/or amylase values together with a thorough medical evaluation of potential symptoms of pancreatitis (abdominal pain, etc.) is strongly advised.

## Future perspectives

Hydroxyurea has come to represent a glimmer of hope for millions of HIV-infected individuals around the globe. We firmly believe that further clinical testing is warranted in order to maximize the efficacy and minimize the toxicity of hydroxyurea in combination with other anti-HIV drugs.

## References


1. Donehower R. An overview of the clinical experience with hydroxyurea. Sem Oncol 1992; 19: 11-9.
2. Charache S, Terrin M, Moore R, *et al*. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Engl J Med 1995; 333: 1317-22.
3. Gao W, Cara A, Gallo R, *et al*. Low levels of deoxynucleotides in peripheral blood lymphocytes: A strategy to inhibit human immunodeficiency virus type 1 replication. Proc Natl Acad Sci USA 1993; 90: 8925-8.
4. Lori F, Malykh A, Cara A, *et al*. Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. Science 1994; 266: 801-5.
5. Lori F, Gallo R. Hydroxyurea and AIDS: An old drug finds a new application? AIDS Res Hum Retroviruses 1995; 11: 1149-51.
6. Lori F. Hydroxyurea and HIV: 5 years later from antiviral to immune-modulating effects. AIDS 1999; 13: 1433-42.
7. Gwilt P, Tracewell W. Pharmacokinetics and pharmacodynamics of hydroxyurea. Clin Pharmacokinet 1998; 34: 347-58.
8. Villani P, Maserati R, Regazzi M, *et al*. Pharmacokinetics of hydroxyurea in patients infected with human. J Clin Pharmacol 1996; 36: 117-21.
9. Meyerhans A, Vartanian J, Hultgren C, *et al*. Restriction and enhancement of human immunodeficiency virus type 1 replication by modulation of intracellular deoxynucleoside triphosphate pools. J Virol 1994; 68: 535-40.
10. Rocha B, Freitas A, Coutinho A. Population dynamics of T lymphocytes. Renewal rate and expansion in the peripheral lymphoid organs. J Immunol 1983; 131: 2158-64.
11. Gao W, Johns D, Chokekuchai S, *et al*. Disparate actions of hydroxyurea in potentiation of purine and. Proc Natl Acad Sci USA 1995; 92: 8333-7.
12. Back D, Kewn S, Hoggard P, *et al*. Enhanced activation of nucleoside analogues in the presence of hydroxyurea *in vitro*. Geneva: 12th World AIDS Conference 1998. Abstract 42262.
13. Wainberg M, Miller M, Quan Y, *et al*. *In vitro* selection and characterization of HIV-1 with reduced susceptibility to PMPA. Antivir Ther 1999; 4: 87-94.
14. Malley S, Grange J, Hamedi-Sangsari F, *et al*. Synergistic anti-human immunodeficiency virus type 1 effect of hydroxamate compounds with 2',3'-dideoxyinosine in infected resting human lymphocytes. Proc Natl Acad Sci USA 1994; 91: 11017-21.
15. Foli A, Lori F, Tinelli C, *et al*. Hydroxyurea and didanosine as a more potent combination than hydroxyurea and zidovudine. Antiviral Ther 1997; 2: 33-40.
16. Palmer S, Cox S. Increased activation of the combination of 3'-azido-3'-deoxythymidine. Antimicrob Agents Chemother 1997; 41: 460-4.
17. Palmer S, Shafer R, Merigan T. Hydroxyurea enhances the activities of didanosine, 9-[2-(phosphonylmethoxy) ethyladenine, and 9-[2- (phosphonylmethoxy) propyl adenine against drug-susceptible and drug- resistant human immunodeficiency virus isolates. Antimicrob Agents Chemother 1999; 43: 2046-50.
18. Lori F, Malykh A, Foli A, *et al*. Combination of a drug targeting the cell with a drug targeting the virus controls human immunodeficiency virus type 1 resistance. AIDS Res Hum Retroviruses 1997; 13: 1403-9.
19. De Antoni A, Foli A, Lisziewicz J, *et al*. Mutations in the *pol* gene of human immunodeficiency virus type 1 in infected patients receiving didanosine and hydroxyurea combination therapy. J Infect Dis 1997; 176: 899-903.
20. Maurer-Schultze B, Siebert M, Bassukas I. An *in vivo* study on the synchronizing effect of hydroxyurea. Exp Cell Res 1988; 174: 230-43.
21. Necas E, Hauser F. Analysis of the effect of hydroxyurea on stem cell (CFU-s) kinetics. Cell Tissue Kinetics 1982; 15: 39-47.
22. Fauci A. Host factors and the pathogenesis of HIV-induced disease. Nature 1996; 384: 529-34.
23. Vicenzi E, Biswas P, Mengozzi M, *et al*. Role of pro-inflamatory cytokines and beta-chemokines in controlling HIV replication. J Leukoc Biol 1997; 62: 34-40.
24. Zagury D, Bernard J, Leonard R, *et al*. Long-term cultures of HTLV-III-infected T cells: A model of cytopathology of T-cell depletion in AIDS. Science 1986; 231: 850-3.
25. Zack J, Arrigo S, Weitsman S, Go A, Haislip A, Chen I. HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. Cell 1990; 61: 213-22.
26. Stevenson M, Stanwick T, Dempsey M, *et al*. HIV-1 replication is controlled at the level of T cell activation and proviral integration. The Embo Journal 1990; 9: 1551-60.
27. Rutschmann O, Opravil M, Iten A, *et al*. A placebo-controlled trial of didanosine plus stavudine, with and without hydroxyurea, for HIV infection. The Swiss HIV Cohort Study. AIDS 1998; 12: F71-7.
28. Montaner J, Zala C, Conway B, *et al*. A pilot study of hydroxyurea among patients with advanced HIV disease receiving chronic didanosine therapy: Canadian HIV trials network protocol 080. J Infect Dis 1997; 175: 801-6.
29. De Boer R, Boucher C, Perelson A. Target cell availability and the successful suppression of HIV by hydroxyurea and didanosine. AIDS 1998; 12: 1567-70.
30. Lori F, Rosenberg E, Lieberman J, *et al*. Hydroxyurea and didanosine long-term treatment prevents HIV breakthrough and normalizes immune parameters. AIDS Res Hum Retroviruses 1999; 15: 1333-8.
31. Vila J, Biron F, Nugier F, *et al*. One-year follow-up of the use of hydroxycarbamide and didanosine in HIV infection. Lancet 1996; 348: 203-4.
32. Jessen H, Foli A, Lisziewicz J, *et al*. Long-term suppression of HIV-1 by hydroxyurea and didanosine. JAMA 1997; 277: 1437-8.
33. Barreiro P, de Mendoza C, Soriano V, *et al*. Hydroxyurea plus didanosine as maintenance therapy after 1 year on highly active antiretroviral therapy. AIDS 2000; 14: 207-8.
34. Giorgi J, Liu Z, Hultin L, Cumberland W, Hennessey K, Detels R. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: Results of 6 years of follow-up. J Acquir Immun Defic Syndr 1993; 6: 904-12.
35. Zinkernagel R, Hengartner H. T-cell-mediated immunopathology versus direct cytolysis by virus: Implications for HIV and AIDS. Immunol Today 1994; 15: 262-8.
36. Zinkernagel R, Hengartner H. Correlates of protective viruses damaging to HIV infection. Science 1996; 272: 1362.
37. Zinkernagel R. Immunology thaugh by viruses. Science 1996; 271: 173-8.
38. Ravot E, Tambussi G, Jessen H, *et al*. Effect of hydroxyurea on T cell count changes during primary HIV infection. AIDS 2000; 14: 619-22.
39. Lisziewicz J, Jessen H, Finzi D, *et al*. HIV-1 suppression by early treatment with hydroxyurea, didanosine, and a protease inhibitor. Lancet 1998; 352: 199-200.
40. Vila J, Nugier F, Bargues G, *et al*. Absence of viral rebound after treatment of HIV-infected patients with didanosine and hydroxycarbamide. Lancet 1997; 350: 635-6.
41. Lisziewicz J, Rosenberg E, Lieberman J, *et al*. Control of HIV despite the discontinuation of antiretroviral therapy. N Engl J Med 1999; 340: 1683-4.
42. Lori F, Jessen H, Lieberman J, *et al*. Treatment of human immunodeficiency virus infection with hydroxyurea, didanosine, and a protease inhibitor before seroconversion is associated with normalized immune parameters and limited viral reservoir. J Infect Dis 1999; 180: 1827-32.
43. Autran B, Carcelain G, Li T, *et al*. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997; 277: 112-6.
44. Plana M, García F, Gallart T, *et al*. Lack of T-cell proliferative response to HIV-1 antigens after 1 year of highly active antiretroviral treatment in early HIV-1 disease. Lancet 1998; 352: 1194-5.
45. Pitcher C, Quittner C, Peterson D, *et al*. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat Med 1999; 5: 518-25.
46. Weverling G, Lange J, Jurriaans S, *et al*. Alternative multidrug regimen provides improved suppression of HIV-1 replication over triple therapy. AIDS 1998; 12: F117-22.
47. Biron F, Lucht F, Peyramond D, *et al*. Anti-HIV activity of the combination of didanosine and hydroxyurea in HIV-1-infected individuals. J Acquir Immune Defic Syndr Hum Retrovirol 1995; 10: 36-40.

48. Lori F, Maserati R, Foli A, Seminari E, Timpone J, Lisziewicz J. Structured Treatment Interruptions to Control HIV. Lancet 2000; 355: 287-8.
49. Piccinini G, Foli A, Giuditta C, *et al*. Antithetical antiviral efficacy of hydroxyurea and protease inhibitors in lymphocytes and in dendritic cells. Florence: 4th International Workshop on HIV, Cells of Macrophage Lineage, and other Reservoirs. 1999.
50. Simonelli C, Nasti G, Vaccher E, *et al*. Hydroxyurea treatment in HIV-infected patients. J Acquir Immun Defic Syndr Hum Retrovirol 1996, 13: 462-4.
51. Foli A, Maserati R, Minoli L, *et al*. Therapeutic advantage of hydroxyurea and didanosine combination therapy in patients previously treated with zidovudine. AIDS 1998; 12: 1113-4.
52. Clotet B, Ruiz L, Cabrera C, *et al*. Short-term anti-HIV activity of the combination of didanosine and hydroxyurea. Antiviral Ther 1996; 1: 189-93.
53. Federici M, Lupo S, Cahn P, *et al*. Hydroxyurea in combination regimens for the treatment of antiretroviral naive, HIV-infected adults. Geneva: 12th World AIDS Conference:1998. Abstract 287/12235.
54. Frank I, Boucher H, Fiscus S, *et al*. Phase I/II dosing study of once-daily hydroxyurea (HU) alone vs. didanosine (ddI) alone vs. ddI+HU. Chicago: 6th CROI 1999. Abstract 402.
55. Havlir D, Gilbert P, Bennett K, *et al*. Randomized trial of continued indinavir (IDV)/ZDV/3TC vs. switch to IDV/ddI/d4T or IDV/ddI/d4T + hydroxyurea in patients with viral suppression. San Francisco: 7th CROI 2000. Abstract 456.
56. Kahn J, Cone R, Hecht F, *et al*. Treatment of d4T+ddI+nelfinavir+hydroxyurea for early or recent primary HIV infection. Chicago 1999. 6th CROI: Abstract 702.
57. Federici M, Lupo S, Cahn P, *et al*. Hydroxyurea in combination regimens for the treatment of antiretroviral naive HIV-infected adults. Geneva: 12th World AIDS Conference. 1998. Abstract 287/12235.
58. Youle M, Mocroft A, Johnson M, *et al*. Surrogate marker responses to multidrug combinations comprising hydroxyurea, efavirenz, double protease inhibitors and nucleoside analogues in protease inhibitor failures. Chicago: 6th CROI 1999. Abstract 400.
59. Miles S, Winters R, Ruane P. Salvage of multi-drug resistant HIV infection with D4T/3TC/hydroxyurea. Geneva 1998: 12th World AIDS Conference. Abstract 288/12205.
60. Cadden J, Dube M. Hydroxyurea (HU) plus ddI as Salvage Therapy in HIV. San Diego: 38th ICAAC 1998. Abstract I-202.
61. Grunke M, Dechant C, Low P, Rascu A, Kalden J, Harrer T. Hydroxyurea as part of a salvage regimen for heavily pretreated patients with advanced HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 1999; 21: 424-6.
62. Lori F, Lisziewicz J. Targeting HIV reservoirs and reconstituting the immune system. AIDS Res Hum Retroviruses 1999: 1597-617.
63. Thurman W, Bloedow C, Howe C, *et al*. A phase I study of hydroxyurea. Cancer Chemother Rep 1963; 29: 103-7.
64. Griffith K. Hydroxyurea (NSC 32065): Results of a phase I study. Cancer Chemother Rep 1964; 40: 33-3.
65. Goodrich J, Khardori N. Hydroxyurea toxicity in human immunodeficiency virus-positive patients. Clin Infect Dis 1999; 29: 692-3.
66. Wilson J, Scott W, Ritter E, *et al*. Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. Teratology 1975; 11: 169-78.
67. Brinkman K, ter Hofstede H, Burger D, *et al*. Adverse effects of reverse transcriptase inhibitors: Mitochondrial toxicity as common pathway. AIDS 1998; 12: 1735-44.
68. Moore R, Wong W, Keruly J, *et al*. Incidence of neuropathy in HIV-infected patients on monotherapy versus those on combination therapy with didanosine, stavudine and hydroxyurea. AIDS 2000; 14: 273-8.
69. Weissman S, Sinclair G, Green C, *et al*. Hydroxyurea-induced hepatitis in human immunodeficiency virus-positive patients. Clin Infect Dis 1999; 29: 223-4.