Hydroxyurea in the Treatment of HIV Infection
Clinical Efficacy and Safety Concerns

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Abstract

Data from basic science and clinical studies suggest that hydroxyurea (hydroxycarbamide)-based regimens are effective treatment options for patients with HIV at various stages of disease. *In vitro* studies of HIV-infected lymphocytes have shown that hydroxyurea: (i) inhibits viral DNA synthesis; (ii) synergistically interacts with nucleoside reverse transcriptase inhibitors (NRTI); and (iii) increases the antiviral activity of didanosine. Clinical studies have confirmed that hydroxyurea in combination with didanosine produces potent and sustained viral suppression in patients with HIV infection. However, some concerns have been recently raised on the use of hydroxyurea in association with NRTI.

Hydroxyurea can cause myelosuppression, skin toxicities, mild gastrointestinal toxicity, and abnormalities of renal and liver functions. In addition, hydroxyurea may accentuate the toxic effects of nucleoside analogues. In fact, some clinical data seem to indicate an increased risk of pancreatitis and neuropathy when hydroxyurea is combined with didanosine and stavudine. Since hydroxyurea-related toxicity is dose dependent, a systematic study of hydroxyurea optimal dosage and schedule was initiated to monitor patients for possible nucleoside toxicity. In the Research Institute for Genetic and Human Therapy (RIGHT) 702 study it was shown that a low, well-tolerated hydroxyurea dose (600mg daily) achieved better antiretroviral activity than higher doses, together with better CD4+ cell count increase and fewer adverse effects.

In this paper the effects of hydroxyurea as salvage therapy for heavily pretreated patients with advanced HIV disease are presented. These studies have shown that some patients with extensive pretreatment experience and advanced disease can respond substantially to the addition of hydroxyurea. The addition of hydroxyurea to didanosine does not prevent the emergence of resistance to didanosine; nonetheless, the efficacy of this therapeutic regimen may not be attenuated by the presence of didanosine-resistant HIV mutants.

Since CD4 T lymphocyte activation is essential for virus replication and CD8 T lymphocyte activation may contribute to pathogenesis, the combination of hydroxyurea with other drugs may lead to the inhibition of HIV, by blocking the 'cell activation-virus production-pathogenesis' cycle. Clinical data indicate that hydroxyurea may play a role in attenuation of viral rebound and immune reconstitution by decreasing CD4 T cell proliferation, as well as preventing the exhaustion of CD8 T cell populations that may result from excessive activation during HIV infection.

While the combination of hydroxyurea with didanosine has provided hope, future studies including those that evaluate optimal dosing and long-term toxicity are needed to define the role for this agent in the treatment of HIV infection.

The ultimate goal of therapy for HIV infection is to eradicate the virus. To date, therapeutic agents have not been able to accomplish this goal, even though select regimens can induce rapid, substantial, and sustained viral suppression in some patients. However, recent evidence suggests that even in patients with undetectable HIV, RNA plasma levels (<50 HIV-RNA copies/mL), latent reservoirs of virus exist that may require at least 7–60 years of therapy in order to completely eradicate the virus.[1,2] Furthermore, current antiretroviral regimens are limited by such problems as drug resistance, difficult-to-adhere-to regimens, and long-term complications.[3] For these reasons, the search for novel therapeutic options and approaches continues.

Hydroxyurea (hydroxycarbamide) is a cytostatic agent that has been used to treat cancer for over 40 years and more recently has been approved for sickle-cell anaemia therapy.[4,5] Because of its ability to deplete intracellular components essential for virus replication,[6] its synergistic antiviral activity with some reverse transcriptase inhibitors,[7] and its cytostatic properties, hydroxyurea has been proposed as a therapeutic option for HIV infection.[8] Hydroxyurea utilises multiple mechanisms of action to impede HIV replication, which have been thoroughly described in the review article by Lori.[9] Principally, hydroxyurea inhibits the cellular enzyme ribonucleotide reductase, thus blocking the transformation of ribonucleotides into deoxy-

ribonucleotides, depleting the intracellular deoxy-nucleotide triphosphate (dNTP) pool, and arresting the cell cycle in the G_1/S phase.[6,10,11] *In vitro* studies on HIV-infected lymphocytes have shown that hydroxyurea strongly inhibits viral DNA synthesis.[6] Synergistic anti-HIV activity has been demonstrated when hydroxyurea is combined with nucleoside reverse transcriptase inhibitors (NRTIs).[7,12-14] This augmentation in antiretroviral activity may be explained by a favourable change in the proportion of activated NRTI (dideoxynucleoside triphosphate, ddNTP) to dNTP and an enhancement of NRTI phosphorylation.[15]

The combination of an agent that inhibits a cellular enzyme (e.g. hydroxyurea) with an agent(s) that inhibits a viral enzyme (e.g. RT inhibitor, protease inhibitor) is the principal rationale for utilising hydroxyurea-based combination regimens to treat HIV infection. This combination would be expected to be highly potent, due to the invocation of synergistic antiviral activity. Furthermore, the inclusion of an agent that inhibits a cellular enzyme might impede drug resistance since such enzymes are not likely to mutate. The use of hydroxyurea in the treatment of HIV infection has other advantages including a simple oral administration schedule, CNS penetration, and relatively low cost. Data are now available on the use of hydroxyurea-based combinations in clinical practice. Clinical trials have shown that hydroxyurea-containing regimens can effectively be utilised in patients with varying degrees of treatment experience at different stages of infection.

In spite of the fact that the toxicity profile of the drug has been well defined during 40 years of clinical experience,[4] special attention should be given to the combination of hydroxyurea with nucleoside analogues, because of the risk of increasing the toxicity of the latter compounds, especially in heavily pretreated patients. Recently, some concerns have been raised on the use of hydroxyurea in association with NRTI. Clinical data seem to indicate an increased risk of pancreatitis and neuropathy when hydroxyurea is combined with didanosine and stavudine,[16,17] suggesting that patients receiving hydroxyurea-containing highly active antiretroviral therapy (HAART) should be closely monitored for possible nucleoside toxicity.

The goal of this review is to present the available clinical data on the use of hydroxyurea for the treatment the HIV infection, in order to assist the readers in comparing and balancing the toxicity concerns and the positive antiviral effects of the hydroxyurea-containing combinations.

1. Clinical Experience

Clinical trials with hydroxyurea have usually paired this agent with didanosine. The rationale for this combination is based on the substantial hydroxyurea-induced shift in the ratio of the didanosine metabolite dideoxyadenosine triphosphate (ddA-TP) to dATP and the resultant enhanced incorporation of ddA-TP into the elongating DNA strand as well as *in vitro* evidence of the antiretroviral activity of this combination.[6,7,15] For the same reason, hydroxyurea also synergises with other adenosine analogues, such as adefovir (9-[2-(phosphonylmethoxy) ethyl] adenine) and tenofovir (9-[2-   (phosphonylmethoxy)   propyl] adenine).[6,7,15,18]

Hydroxyurea alone may have some antiretroviral activity in antigen presenting cells, such as macrophages and dendritic cells,[7,19] whereas protease inhibitors may be less effective in these cell types.[19,20] The combination of hydroxyurea with didanosine has been shown to inhibit HIV replication in both quiescent and activated peripheral blood mononuclear cells (PBMC) *in vitro*.[7] 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.[21-23]

Several pilot trials have been conducted to examine the clinical efficacy and safety of hydroxyurea alone or in combination with various NRTIs (table I). Taken together, these studies have demonstrated that hydroxyurea monotherapy was not effective in decreasing viral load (VL), but that combinations of hydroxyurea with didanosine induced substantial and durable reductions in HIV-RNA levels and were generally well tolerated. The encouraging results of

Table I. Pilot clinical trials of hydroxyurea (hydroxycarbamide) for the treatment of HIV infection

Pilot clinical trial	Significant finding
Biron et al.[29]	HU + ddI reduced VL by 3 months in 12 patients (down to undetectable levels in seven patients). Median increase of CD4+ cell count was +244 cell/mm^3
Clotet et al.[30]	HU + ddI was well tolerated and reduced VL mainly in patients who were naive to ddI
Simonelli et al.[31]	HU monotherapy did not provide therapeutic benefit in 16 heavily pretreated HIV infected patients
Montaner et al.[32]	Addition of HU to ddI substantially decreased VL in 11 patients receiving HU 1000 mg/daily
Foli et al.[33]	HU + ddI (six subjects) was a more potent and less toxic combination than HU + zidovudine (six subjects)
Simonelli et al.[34]	No therapeutic advantage of HU + ddI (six subjects) vs ddI monotherapy (eight subjects) was observed in zidovudine pretreated patients
Foli et al.[35]	Therapeutic advantage of HU + ddI vs ddI monotherapy was observed both in zidovudine pretreated (19 subjects) and in naive patients (18 subjects)

ddI = didanosine; **HU** = hydroxyurea; **VL** = viral load.

some of the pilot clinical trials with hydroxyurea-based combinations have led to the design and implementation of randomised, controlled trials. These larger studies include the Research Institute for Genetic and Human Therapy (RIGHT) 411,[24] the AIDS Clinical Trials Group (ACTG) 307,[25] the Swiss HIV Cohort Study,[26] the Bristol-Myers Squibb (BMS) 055 study,[27] and the ACTG 5025 (table II).[28]

1.1 Research Institute for Genetic and Human Therapy 411 Trial

RIGHT 411 (table II) was the first randomised, controlled study to compare the use of didanosine monotherapy and the combination of hydroxyurea and didanosine.[24] In this trial, 57 patients with a CD4+ cell count of 250 to 500 cells/mm^3 were randomised to receive didanosine 200mg twice daily alone or didanosine 200mg twice daily plus hydroxyurea 500mg twice daily. In the didanosine group, 19 patients with median VL 50 767 HIV-RNA copies/mL (SD ± 71 404) and median CD4+ cell count 368 cells/mm^3 (SD ± 75) were enrolled, and in the didanosine plus hydroxyurea group 38 patients with median VL 94 028 HIV-RNA copies/mL (SD ± 98 333), and median CD4+ cell count 350 cells/mm^3 (SD ± 78) were enrolled. After 24 weeks, plasma viraemia in the combination group decreased significantly, more than in the didanosine monotherapy group. Patients receiving didanosine and hydroxyurea had an average 1.32 log$_{10}$ decrease from baseline viraemia, while patients receiving on-

ly didanosine had a 0.78 log$_{10}$ decrease (p = 0.0005). Viral rebound (defined as an increase of at least 3-fold over the trough value in two consecutive tests) was observed in 8 of 19 patients in the didanosine monotherapy group and none in the combination group. Furthermore, at week 24, 18.4% of the patients in the didanosine plus hydroxyurea group had a VL below the detection limit (200 HIV-RNA copies/mL) while only 5.3% of patients in the didanosine alone group had a VL below this limit. After discontinuation of the monotherapy arm, continued treatment in the combination group resulted in an average 1.21 log$_{10}$ reduction in plasma viraemia from baseline to week 40. Although CD4+ cell count differences between treatment groups were not statistically significant, they increased more in the didanosine monotherapy arm (+83 CD4+ cells/mm^3) than in the hydroxyurea-didanosine arm (+54 CD4+ cells/mm^3).

1.2 AIDS Clinical Trials Group 307

ACTG 307 (table II) was a placebo-controlled clinical trial designed to randomise patients into multiple treatment arms.[25] Patients were assigned to treatment with either of the following treatment regimens: (i) didanosine 200mg twice daily and hydroxyurea placebo; (ii) didanosine placebo and hydroxyurea 1000mg once daily; (iii) didanosine 200mg twice daily and hydroxyurea 1000mg once daily; (iv) didanosine placebo and hydroxyurea 1500mg once daily; or (v) didanosine 200mg twice daily and hydroxyurea 1500mg once daily. Those

patients randomised to didanosine placebo had didanosine added to hydroxyurea after 4 weeks of hydroxyurea monotherapy. Patients receiving didanosine alone had hydroxyurea (1000 or 1500mg once daily) added after 12 weeks of therapy. A total of 131 patients were enrolled. A preliminary analysis pooled patients into didanosine monotherapy, and didanosine plus hydroxyurea groups. After 8 weeks, mean VL reduction in patients treated with didanosine monotherapy was about half that observed in patients treated with didanosine plus hydroxyurea (-0.83 $\log_{10}$ copies/mL vs -1.57 $\log_{10}$ copies/mL; p = 0.01). At week 24, mean VL reduction in patients initially treated with hydroxyurea monotherapy was -1.05 $\log_{10}$ copies/mL, with didanosine monotherapy -0.79 $\log_{10}$ copies/mL, and with didanosine + hydroxyurea -1.22 $\log_{10}$ copies/mL.

1.3 The Swiss HIV Cohort Study

The Swiss HIV Cohort Study (table II) included 144 HIV-infected patients with a mean VL of 4.53 $\log_{10}$ copies/mL and a mean CD4+ cell count of 370 cells/mm^3.[26] Seventy-two patients were randomised to receive hydroxyurea 500mg twice daily plus didanosine 200mg twice daily plus stavudine 40mg twice daily, while the remaining 72 patients received didanosine 200mg twice daily plus stavudine 40mg twice daily plus hydroxyurea placebo. Seventy-five percent of patients were antiretroviral naive at baseline. After 12 weeks, the intent-to-treat based analysis showed that the VL decreased 2.3 $\log_{10}$ in the hydroxyurea-treated group compared with only 1.7 $\log_{10}$ in the placebo group (p = 0.001). The hydroxyurea-treated group had a CD4+ increase of 28 cells/mm^3 compared with 107 cells/mm^3 in the

Table II. Randomised, controlled clinical trials designed to assess the efficacy and safety of hydroxyurea (hydroxycarbamide)-containing regimens for the treatment of HIV infection

Study	Number of participants	Treatment regimens	Significant trial findings
RIGHT 411[24]	57	First 24 weeks: ddI 200mg twice daily or ddI 200mg twice daily + HU 500mg twice daily. After 24 weeks: ddI 200mg twice daily + HU 500mg twice daily (ddI monotherapy arm interrupted)	HU-containing arm more effective. Blunted increase of CD4+ cell count in the HU-containing arm. HU compensates for ddI resistance
ACTG 307[25]	131	First 12 weeks: ddI 200mg twice daily + HU placebo or ddI placebo + HU 1000mg once daily or ddI 200mg twice daily + HU 1000mg once daily or ddI placebo + HU 1500mg once daily or ddI 200mg twice daily + HU 1500mg once daily	HU-containing arms more effective. 1000mg every day as effective as 1500mg every day and better tolerated
The Swiss HIV Cohort Study[26]	144	First 12 weeks: ddI 200mg twice daily + d4T 40mg twice daily + HU placebo or ddI 200mg twice daily + d4T 40mg twice daily + HU 1000mg twice daily 12–24 weeks: ddI 200mg twice daily + d4T 40mg twice daily + delayed HU or ddI 200mg twice daily + d4T 40mg twice daily + HU 100mg twice daily or ddI + d4T 40mg twice daily	Both HU-containing arms more effective than ddI + d4T arm. Patients who delayed HU therapy until 12 weeks achieved similar virologic response as those who initiated HU immediately, yet achieved a greater CD4+ cell count response
The BMS 055 Study[27]	177	Treatment-naive patients were randomised to: ddI + ZDV + d4T placebo or ddI + d4T + ZDV placebo or ddI + HU + d4T placebo or ddI + HU + d4T[a]	The triple combination of ddI + d4T + HU was superior with regard to virologic response and comparable in CD4+ cell count increase among all treatment arms. The double combination of HU + ddI was as effective as the dual nucleoside analogues combination
The ACTG 5025 Study[28]	202	Patients treated with IDV/ZDV/3TC for at least 6 months and suppressed viral replication were randomised to: ZDV 300mg twice daily; 3TC 150mg twice daily; IDV 800mg q8h; d4T 40mg twice daily; ddI 400mg once daily; HU 600mg twice daily	The addition of HU to potent, suppressive therapy did not enhance the efficacy and was associated with excess toxicity

a Dosages not stated.

3TC = lamivudine; **ACTG =** AIDS Clinical Trials Group; **BMS =** Bristol-Myers Squibb; **d4T** = stavudine; **ddI** = didanosine; **HU** = hydroxyurea; **IDV** = indinavir; **q8h** = every 8 hours; **RIGHT** = Research Institute for Genetic and Human Therapy; **ZDV** = zidovudine.

placebo group (p = 0.001); however, the CD4+/CD8+ ratio increased similarly in both groups, increasing from 0.5 to 0.9 in the placebo group and from 0.7 to 1.1 in the hydroxyurea group. During the first 12 weeks on treatment, three patients in the placebo group and seven in the hydroxyurea group discontinued treatment. Nonresponders in the placebo group (49 patients) either added hydroxyurea to their current regimen (31 patients) during the open-label phase ('delayed hydroxyurea' group) or were discontinued from the study (18 patients). All nonresponders in the hydroxyurea-treated group (26 patients) were discontinued from the study. In the patient group analysed at week 24 on an on-treatment basis, the mean VL reduction was 2.6 $\log_{10}$ in the 34 patients who continuously received hydroxyurea plus didanosine plus stavudine. This value was not significantly different from the 12-week reduction of 2.8 $\log_{10}$ copies/mL. Twenty-seven of these 34 patients (79%) maintained a VL <200 copies/mL, and 17 (63%) had values <20 copies/mL. During this period, five patients discontinued treatment. In the delayed hydroxyurea treatment group, the addition of hydroxyurea to didanosine plus stavudine between weeks 12 and 24 resulted in a significant mean reduction in VL from 1.1 $\log_{10}$ copies/mL to 1.9 $\log_{10}$ copies/mL (p < 0.0001). Patients in this group also experienced a reduction in the increase in CD4+ cell counts from baseline, resulting in a mean increase of 123 cells/mm^3 at week 12, and a mean increase of 86 cells/mm^3 at week 24. The difference in mean CD4+ cell count increase between weeks 12 and 24 was not statistically significant. The minimal increase in CD4+ cells in the hydroxyurea plus didanosine plus stavudine treatment group observed at week 12 (36 cells/mm^3) was maintained through week 24 (31 cells/mm^3). These data suggest that delaying the use of hydroxyurea may provide a useful strategy for improving CD4+ cell count. Further clinical evidence is required, however, to confirm this hypothesis.

1.4 The Bristol-Myers Squibb 055 Study

The multinational, BMS 055 study results (table II) have generally confirmed the findings reported by the Swiss investigators.[27] This was a randomised, double-blind, placebo-controlled trial that included four treatment groups: (i) didanosine plus zidovudine plus stavudine-placebo; (ii) didanosine plus stavudine plus zidovudine-placebo; (iii) didanosine plus stavudine plus hydroxyurea; and (iv) didanosine plus hydroxyurea plus stavudine-placebo. Patients were treatment-naive with a median baseline CD4+ count of approximately 350 cells/mm^3 and a median baseline VL of approximately 4.5 $\log_{10}$ copies/mL.

A preliminary, intent-to-treat analysis of 177 patients treated for 24 weeks demonstrated that the triple combination of didanosine + stavudine + hydroxyurea reduced VL by approximately 2 $\log_{10}$. This reduction was significantly greater than that observed in all other treatment groups. Almost 80% of patients in the triple combination group maintained a VL <400 copies/mL at week 24 (figure 1a). Another important finding of this study was that the combination of hydroxyurea and didanosine inhibited HIV at a rate comparable to the other two dual-nucleoside regimens. Approximately 53% of the patients receiving didanosine plus stavudine, 43% receiving didanosine plus hydroxyurea, and 36% receiving didanosine plus zidovudine maintained a VL <400 copies/mL at week 24 (figure 1a). Similar to the previous studies, absolute CD4+ cell count improvements were lower in the hydroxyurea-containing groups compared with the non-hydroxyurea-containing groups. However, all of the treatment groups experienced a comparable increase in the percentage of CD4+ cells compared with baseline (figure1b).

1.5 AIDS Clinical Trials Group 5025

The ACTG 5025 study (table II) was an 'intensification study' designed to determine whether viral suppression is increased when subjects are switched from a regimen of indinavir 800mg every 8 hours, zidovudine 300mg twice daily and lamivudine 150mg twice daily to a regimen containing indinavir 800mg every 8 hours, didanosine 400mg once daily, stavudine 40mg twice daily and hydroxyurea 600mg twice daily.[28] Primary endpoints were loss of viral
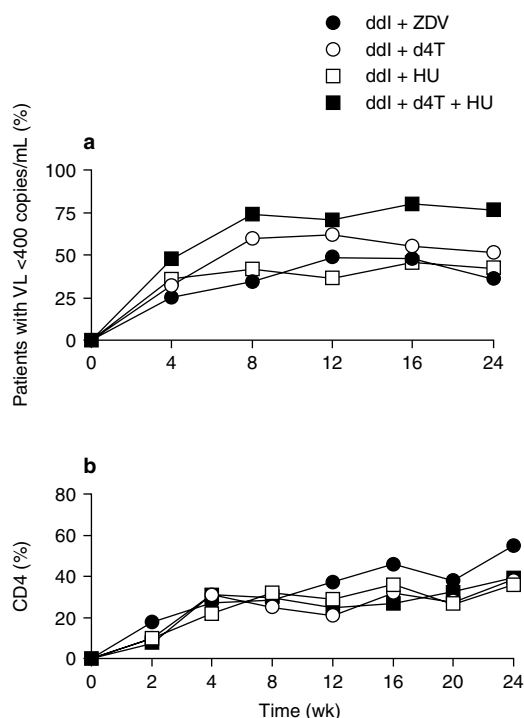
Fig. 1. Preliminary, intention-to-treat analysis of the Bristol-Myers Squibb (BMS) 055 trial.[27] (**a**) Rates of undetectable viraemia after HU/ddI, HU/ddI/d4T and dual nucleoside analogues. (**b**) Treatment with HU-based combinations for 24 weeks resulted in an increase of in the percentage of CD4+ cells compared with baseline. **d4T** = stavudine; **ddI** = didanosine; **HU** = hydroxyurea (hydroxycarbamide); **VL** = viral load; **ZDV** = zidovudine.

suppression (HIV-RNA >200 copies/mL) and drug toxicity. Inclusion criteria were indinavir, zidovudine, and lamivudine for >6 months, HIV-RNA <200 copies/mL, CD4+ cell count >200 cells/mm^3, no prior exposure to didanosine plus stavudine, and no prior exposure to a protease inhibitor other than indinavir. Patients pre-exposed to indinavir, zidovudine and lamivudine were randomised to: (i) continue the same regimen; (ii) switch to indinavir, didanosine, and stavudine; or (iii) switch to indinavir, didanosine, stavudine, and hydroxyurea. The study did not reveal differences in the virologic endpoints; however, the study was terminated since an interim analysis showed a higher percentage of treatment failure (defined as confirmed VL >200 copies/mL or treatment-limiting toxicity) in the hydroxyurea

group compared with the indinavir, zidovudine and lamivudine group.

The antiviral activity of the combination of efavirenz, stavudine, didanosine with or without hydroxyurea were evaluated in the 3D study. This was an international, multicentre, randomised, placebo-controlled trial.[36] This study enrolled 100 HIV-infected, treatment naive patients (VL 46 005 copies/mL, CD4+ cell count 371 cells/mm^3, at baseline), and 49 HIV-infected treatment experienced patients (VL 6882 copies/mL, CD4+ cell count 337 cells/mm^3, at baseline). The data at week 24 available on 91 of 149 patients showed that 90% of the naive and 95% of the experienced patients had a VL <500 copies/mL. CD4+ cell count increased to 48 and 27 cells/mm^3, respectively. In 42 patients a grade 3/4 toxicity was reported. In conclusion, these two combinations seem to be highly effective in controlling viral replication. However, further analyses of the data obtained from the two groups are needed in order to clarify the antiviral activity and the toxicity profile of hydroxyurea.

2. Safety Profile

2.1 Adverse Effects of Hydroxyurea

2.1.1 Bone Marrow Suppression

The principal adverse effect of hydroxyurea is bone marrow suppression leading to neutropenia, anaemia, and thrombocytopenia.[4] For this reason, the use of hydroxyurea with other agents that are also suppressive to the bone marrow, such as zidovudine, results in an increased risk of haematological toxicity.[33] In patients with HIV infection, the incidence of neutropenia is lower than that observed in persons treated with hydroxyurea for leukaemia,[37,38] reflecting the lower dosage used for HIV infection. The risk of neutropenia appears to be greater in persons with an absolute neutrophil count (ANC) of <1700 cells/mm^3. For example, in a study with the triple combination of didanosine plus stavudine plus hydroxyurea in nucleoside-experienced patients, four patients who began treatment with an ANC <1700 cells/mm^3 experienced grade 3 neutro-

penia (e.g. <700 cells/mm³).[39] Neutropenia resolved in these four patients upon therapy discontinuation. At week 24, analysis of the Swiss Cohort study, grade 1 neutropenia occurred in a significantly (p = 0.04) greater portion of the hydroxyurea-treated patients; there was no difference with regard to grades 2 or 3 neutropenia between the hydroxyurea and non-hydroxyurea-treated groups. However, a significantly greater proportion of patients in the hydroxyurea-treated group also experienced more fatigue (p = 0.02).[26] The preliminary analysis of ACTG 307 demonstrated that a 1500mg daily dosage induced significantly more haematological toxicity, principally neutropenia, compared with a 1000mg daily dosage.[25] Bone marrow toxicity induced by hydroxyurea has been described in the past as quickly reversible upon drug suspension.[4] A recent report, however, showed two cases of prolonged myelosuppression after the drug was withdrawn.[40]

2.1.2 Other Adverse Effects

Other rare complications, most commonly associated with the prolonged use of hydroxyurea, include skin toxicities such as alopecia, hyperpigmentation, erythema, and leg ulcers.[4,32,41] Hydroxyurea use has also been associated with mild gastrointestinal toxicity. Nausea, vomiting, diarrhoea, anorexia have been reported frequently; however, they rarely required cessation of therapy.[4] Transient abnormalities in renal functions (elevations of serum urea nitrogen and creatinine, proteinuria, and an active urine sediment) have been observed; however, renal failure and severe prolonged kidney dysfunctions have not been reported.[4] Significant elevation of liver enzymes (ALT, AST) with rare episodes of clinical jaundice have been observed.[4] It is important to stress that hydroxyurea is considered a teratogenic agent,[4] and therefore, should not be used during pregnancy.[42]

2.2 Adverse Effects with Use of Hydroxyurea in Combination with Other Agents

Besides the known adverse events caused by hydroxyurea, particular emphasis must be paid to the possibility that this drug might accentuate the toxic effects of other agents.

2.2.1 Oxidative Phosphorylation Disorders

Nucleoside analogues are known to be toxic to cellular mitochondria.[43] The oxidative phosphorylation disorders consequential to the cumulative damage inflicted on the mitochondrial enzyme gamma-polymerase by nucleoside analogues could be increased by hydroxyurea via the same mechanism by which this drug accentuates the activity of nucleoside analogues on the viral reverse transcriptase. Oxidative phosphorylation disorders reflect a shortage of mitochondrial ATP synthesis. The pyruvate/lactate equilibrium shift in the direction of lactate can lead to functional impairment of the Krebs cycle. When the mitochondrial energy-generating capacity falls below the threshold of an organ (energy-demanding needs of the organ will be more susceptible), sudden cellular failure may occur. Table III illustrates the clinical manifestations of oxidative phosphorylation disorders, some of which have been associated with the use of nucleoside analogues.

2.2.2 Peripheral Neuropathy

Concerns have been raised regarding the potential for increased peripheral neuropathy when hydroxyurea is co-administered with both didanosine and stavudine. In the BMS 055 study, peri-

Table III. Clinical manifestations of oxidative phosphorylation disorders

Disorder	Manifestations
Neurological	Peripheral neuropathy, encephalopathy, dementia, seizures, stroke
Myopathy	Hypotonia, muscle weakness, exercise intolerance
Cardiac	Cardiomyopathy, conduction disorders
Endocrine	Diabetes mellitus
Gastrointestinal	Colonic pseudo-obstruction, exocrine pancreas dysfunction, pancreatitis, hepatomegaly, steatosis, liver failure, lactic acidosis
Nephrological	Nonselective proximal tubular dysfunction with acidaemia, phosphaturia and glucosuria, glomerulopathy
Haematological	Anaemia, thrombocytopenia, pancytopenia
Psychiatric	Depression
General	Multiple systemic lipomas, fatigue

pheral neurological symptoms were reported in 15% (7 of 46) of patients treated with didanosine plus stavudine plus hydroxyurea compared with 9% (4 of 43) of patients treated with didanosine plus hydroxyurea, 11% (5 of 44) of patients treated with zidovudine plus didanosine, and 7% (3 of 44) of patients treated with didanosine plus stavudine.[40] In the Swiss Cohort study, patients randomised to receive hydroxyurea showed a higher incidence of peripheral neurological symptoms.[24,25] Although this difference was not statistically significant (p = 0.09), we have the impression that neurological symptoms were increased by the combination of hydroxyurea and didanosine plus stavudine. This tendency was not observed when hydroxyurea was combined with didanosine without stavudine.[24,25] In addition, a recent publication by Moore et al.[17] has shown that in 1116 patients the relative risk of developing neuropathy in HIV-infected patients treated with didanosine was increased by the addition of stavudine alone, and was further increased by the addition of hydroxyurea to this combination.[17] No data are available on the combination of hydroxyurea and stavudine without didanosine.

2.2.3 Hepatic and Pancreatic Adverse Effects

Increased hepatic and pancreatic toxicities have been correlated with the use of hydroxyurea. In a small study, two patients pre-exposed to antiretroviral drugs experienced hepatitis when their multidrug regimens were switched to new regimens including hydroxyurea and other drugs such as nelfinavir, stavudine, didanosine, ritonavir and saquinavir.[44] In the ACTG 5025 trial the arm containing hydroxyurea (n = 68 patients) was eventually discontinued, due to a higher failure rate.[28] This was mainly due to increased toxicity, which included three deaths. In all of these events, pancreatitis was part of the syndrome. Overall, four episodes of pancreatitis were reported in the hydroxyurea containing arm versus three episodes in the didanosine, stavudine, indinavir arm.

In striking contrast, no episodes of hepatitis, pancreatitis, and/or lactic acidosis were observed in over 500 patients enrolled in the above-mentioned RIGHT 411,[24] ACTG 307,[25] Swiss Cohort Study,[26] and BMS 055[27] trials. Moreover, pancreatic and hepatic enzyme values were not different between the hydroxyurea-containing and non-containing regimens.

2.2.4 Lactic Acidosis

Lactic acidosis is now recognised as a complication of NRTI therapy. In 70 patients receiving antiretroviral therapy, lactic acid and anion gap levels were routinely measured within 60 days after their office visit.[45] In this cohort, 27 patients were also taking hydroxyurea, four of whom experienced both levels above the normal range. Three patients were treated with stavudine, didanosine and lamivudine (two with hydroxyurea), the fourth was treated with stavudine, lamivudine, nelfinavir, and saquinavir. It is unclear whether hydroxyurea increases the risk of hyperlactinaemia because the two subjects experiencing hyperlactinaemia also received stavudine, a drug known to induce high levels of lactic acid.[46] New clinical studies on the combination of hydroxyurea with NRTI should consider monitoring the amount of lactic acid in the serum and/or changes on the mitochondrial DNA, parameters which might predict the level of mitochondrial dysfunction.

2.3 Rationale for Hydroxyurea Toxicity

The unusual findings of the ACTG A5025 study[28] deserve close attention, and yet still lack an explanation. Differences exist between this study and previous ones (table IV):

- patients were heavily pre-exposed to antiretroviral therapy;
- four drugs (including a protease inhibitor), instead of three (hydroxyurea, didanosine, and stavudine), were used. The combination of these two events might have generated 'cumulative toxicity,' a phenomenon related to the length of exposure to drugs and number of drugs used;
- in ACTG 5025 didanosine was administered at 400mg daily, instead of the 200mg twice daily used in previous trials.[24-27] This difference might be relevant, since didanosine-induced pancreatitis is dose dependent.[47-50] Modifying the schedule of the drug could change its bioavailability profile, even if the total daily dose does not

Table IV. Main differences between the AIDS Clinical Trials Group (ACTG) 5025[28] and Research Institute for Genetic and Human Therapy (RIGHT) 411,[24] ACTG 307,[25] Swiss HIV Cohort Study,[26] Bristol-Myers Squibb (BMS) 055[27] studies

ACTG 5025	RIGHT 411, ACTG 307, Swiss Cohort, BMS 055
IDV/ ddl/d4T/HU	HU/ddl or HU/ddl/d4T
Pretreated patient (IDV + ZDV + 3TC)	Naive or not heavily pretreated patients (mainly NRTI)
Viral load <200 copies/mL	Viral load mean: 4.5 log
HU: 600mg twice daily	HU: mainly 500mg twice daily[a]
ddl: 400mg daily	ddl: 200mg twice daily
Peripheral neuropathy	Peripheral neuropathy (only with d4T)
Pancreatitis	No pancreatitis

a   500mg daily in one arm of ACTG 307.

3TC = lamivudine**; d4T** = stavudine; **ddl** = didanosine; **HU** = hydroxyurea (hydroxycarbamide); **IDV** = indinavir; **NRTI** = nucleoside reverse transcriptase inhibitor; **ZDV** = zidovudine

change. In fact, we analysed the *in vitro* mitochondrial toxicity of the combination didanosine plus hydroxyurea in a pancreatic cell line.[51] In this study, didanosine concentrations (1.5 and 10 µmol/L) were chosen to reflect the C_{max} of the drug with the 400mg daily and 200mg twice daily regimens (6.5 ± 3.3 µmol/L and 3.4 ± 1.9 µmol/L, respectively). Hydroxyurea concentrations used represented those attainable *in vivo* (10, 50 and 100 µmol/L). Hydroxyurea alone did not affect mitochondrial functionality. At low didanosine concentrations (1 and 5 µmol/L), there was negligible mitochondrial toxicity. However, at a higher concentration (10 µmol/L) there was a moderate toxic effect. The combination of hydroxyurea to 1 µmol/L didanosine did not affect the mitochondrial functionality. The combination of 5 µmol/L didanosine plus hydroxyurea induced moderate mitochondrial dysfunction which depended on the hydroxyurea concentration. *In vivo* the high C_{max} of the once daily regimen didanosine along with the peak hydroxyurea concentration might facilitate the onset of pancreatitis;

- in ACTG A5025 hydroxyurea was administered at a dosage of 1200 mg/day (600mg twice daily), different from the dosage used in most other trials. Based on these considerations we decided to explore the possibility that lower hydroxyurea doses may be associated with fewer adverse effects. A systematic study for hydroxyurea optimal dosage and schedule was initiated.

3. Hydroxyurea Dosing and Administration in HIV Infection

The optimal dosage of hydroxyurea for the treatment of HIV infection had not been established at the time the trials mentioned so far were carried out; only scattered information was available. A study by Montaner et al. had demonstrated that a 1000 mg/day dosage of hydroxyurea in two divided doses added to didanosine was more effective than a 500 mg/day in terms of VL reduction.[32] The preliminary analysis of ACTG 307 had shown that a 1500 mg/day significantly induced haematological toxicity, principally neutropenia, compared with a 1000 mg/day, without a proportional increase in efficacy.[25] In the same study the addition of hydroxyurea 1000 mg/day to didanosine significantly reduced plasma VL,[25] despite the short half-life of the drug. This was consistent with the *in vitro* demonstration that HIV continues to be inhibited after the drug is eliminated from the supernatant of human macrophages,[7] and such a residual effect may allow for successful twice daily or potentially once daily administration of hydroxyurea.

A study by Foli et al. has shown that the enhancement of mitochondrial toxicity by hydroxyurea is a dose-dependent phenomenon.[51] In this study the *in vitro* effects of didanosine and hydroxyurea, alone and in combination, were analysed on mitochondrial functionality of pancreatic and hepatic human cells, by using a sensitive flow cytometric assay that allows for the analysis of the mitochondrial membrane potential at the single cell level. Pancreatic and

hepatic human cell lines were exposed to several concentrations of hydroxyurea and didanosine, alone or in combination, for as long as 28 days. Mitochondrial functionality was evaluated every 7 days by staining cells with the fluorescent probe JC-1; simultaneously, cell viability was assessed in parallel by cell counting. In the pancreatic cell line, didanosine alone induced a dose-dependent mitochondrial toxicity at concentrations attainable *in vivo*, while none of the concentrations of hydroxyurea attainable *in vivo* had direct effects on mitochondria. Addition of hydroxyurea increased mitochondrial toxicity at high concentration of didanosine in a dose-dependent manner. Only minor effects of the drugs, either alone or in combination, were observed in hepatic cell line mitochondria.

RIGHT 702 was an open-label, 52-week, hydroxyurea dose-regimen study of 115 HIV-infected patients, randomised to one of nine treatment arms in a 3×3 factorial design, to compare safety and efficacy of hydroxyurea administered at three daily doses (600, 800–900, or 1200 mg/day) and at three dosing intervals (once daily, twice daily, or three times daily) in combination with didanosine and stavudine.[52] Mean $\log_{10}$ transformed baseline plasma HIV RNA levels ranged from 4.36 for the 800–900mg group to 4.58 for the 600mg group, and from 4.36 for the three-times-daily group to 4.54 for the daily group. The power of the study was based on adverse event rates, rather than the primary efficacy endpoint (the proportion of patients with plasma HIV-RNA levels <400 copies/mL at week 24 of therapy and no dose intensification before week 24). Patients were randomised to one of nine treatment arms, which were arranged in a 3×3 factorial design, evaluating total daily dose and daily schedule. The total daily doses were either 'very low' (600 mg/day) 'low' (800–900 mg/day), or 'medium' (1200 mg/day). The daily schedules were either once daily, twice daily, or three times daily. Thirteen patients per treatment regimen resulted in 39 patients in each total daily dose group and in each dosing interval group. Analysis was performed on an intention-to-treat basis. When multiple groups were analysed, 600mg was the best of the three total

daily doses for the primary endpoint (plasma HIV-RNA levels <400 copies/mL at week 24 of therapy and no dose adjustment before week 24, the percentages being 76, 51, and 60% for the 600mg group, 800–900mg group, and 1200mg group, respectively) and for all of the other efficacy endpoints. The twice-daily dosing interval schedule was the best of the three dosing intervals for most efficacy endpoints. A pair-wise comparison between 600mg and 800–900mg total daily dose groups revealed statistically significant differences for the proportion of patients with VL <400 copies/mL at week 24 (primary endpoint) [p = 0.027], and week 48 (p = 0.03). The area under the curve (AUC) at week 24 (p = 0.016) and week 48 (p = 0.001) were also lower in the 600mg groups. The twice-daily dose interval groups were superior to the once daily group for all virologic endpoints; however, for the CD4+ cell counts there was a tendency favouring the once daily dose. The most efficacious combination of total daily dose and dose interval for the primary endpoint was 300mg twice daily (p = 0.017). The total daily dose groups and the dosing interval groups were not statistically different with respect to adverse events; however, one case of lethal pancreatitis occurred in the 1200mg hydroxyurea group. Furthermore, there was a tendency for the lowest dose (600mg daily) to be associated with fewer adverse effects and a better CD4+ cell count increase. In conclusion, these surprising results showed that a low, well-tolerated hydroxyurea dose (600mg daily) has better antiretroviral activity than higher doses, together with better CD4+ cell count increase and fewer adverse effects.

4. Salvage Therapy for Advanced HIV Infection

Hydroxyurea has been studied as salvage therapy for heavily-pretreated patients with advanced HIV disease.[53-57] These studies have shown that some patients with extensive pretreatment experience and advanced disease can respond substantially to the addition of hydroxyurea, although there is concern that toxicity may be more severe compared with

patients with less-advanced disease, similar to what has been observed with other drug combinations.

In a study of patients who had been exposed to a median of seven antiretroviral agents for 45 months, 17 of 38 patients (45%) who had completed 1 month of therapy that included hydroxyurea achieved a VL of <500 copies/mL.[32] Youle and colleagues reported that patients whose disease failed to respond to protease inhibitor-containing therapy exhibited marked initial responses in HIV-RNA levels and CD4+ cell count when treated with multidrug regimens (e.g. five to seven drugs) with a backbone of efavirenz, hydroxyurea, didanosine, ritonavir, and indinavir.[41] This study included 49 patients who, at the time of treatment switch, had been taking protease inhibitor-containing regimens for more than 6 months, had a median baseline VL of 5.81 $\log_{10}$ copies/mL and a CD4+ cell count of 97 cells/mm³. After a median follow-up of 12 weeks, there were significant reductions in HIV RNA (median –1.7 $\log_{10}$ copies/mL per 6-month period; p = 0.021) and increases in CD4+ cell count (median +95 cells/mm³ per 6-month period; p = 0.003) compared with baseline. Of the 15 patients who had been treated for at least 24 weeks at the time of this report, nine (60%) had HIV RNA levels <400 copies/mL. In a study presented by Miles et al., 17 HIV-infected patients with clinical and genotypic resistance to stavudine and lamivudine were treated for at least 4 weeks with the combination stavudine, lamivudine and hydroxyurea.[55] Before starting hydroxyurea treatment, median VL was 5.6 $\log_{10}$, all patients carried the 184V mutation, and most of them carried also the 75 mutation. After 4 weeks on therapy the median drop in VL was 1.7 $\log_{10}$, suggesting a potent antiretroviral role for hydroxyurea. In addition, 23 patients receiving didanosine plus hydroxyurea in combination with an antiretroviral after virologic failure were studied in a retrospective study.[56] Before starting didanosine plus hydroxyurea treatment patients' median VL was 4.6 $\log_{10}$, and median CD4+ cell count was 270 cells/mm³. In this population 52% of the patients had been previously exposed to didanosine. A new antiretroviral agent was added to 83% of the didanosine plus hydroxy-

urea patients' regimen. After 28 weeks on therapy the mean VL drop was 0.7 $\log_{10}$ along with a mean CD4+ cell count drop of 34 cells/mm³. The drop in VL in the subjects with previous didanosine experience was similar to that observed in patients with no prior didanosine exposure. Also, the subjects receiving an additional new antiretroviral had a comparable reduction in VL compared with the patients who did not. The authors suggest that this study seems to indicate a limited efficacy of the didanosine plus hydroxyurea combination in salvage therapy. Grunke et al. had successfully used hydroxyurea as part of salvage therapy when the drug was used in combinations containing several new and non-cross-resistant antiviral drugs.[57]

Recently, the effects of hydroxyurea addition to a quadruple combination of reverse transcriptase inhibitors in 69 patients not responding to PI-based HAART has been reported.[58] These patients were randomised to receive stavudine, didanosine, efavirenz and abacavir, or the identical regimen plus hydroxyurea, or plus interleukin (IL)-2. After 48 weeks on therapy the proportion of patients reaching a plasma level <50 HIV-RNA copies/mL was significantly higher in the hydroxyurea-containing groups compared with the group not receiving hydroxyurea. Patients receiving the four RTI plus plus hydroxyurea experienced a drop in CD4+ cell counts, while in the patients treated with the four drug regimen alone or plus hydroxyurea and IL-2 the CD4+ cell count increased substantially. Overall the adverse events were more frequent in the hydroxyurea-treated groups.

5. Evidence for Diminished Resistance to Nucleoside Reverse Transcriptase Inhibitors

Tissue culture work has shown that the presence of hydroxyurea can slow the development of resistance to didanosine (Wainberg et al., unpublished observation). However, the addition of hydroxyurea to didanosine does not prevent the emergence of resistance to didanosine;[24] nonetheless, the efficacy of this therapeutic regimen may not be attenuated by

the presence of didanosine-resistant HIV mutants.[13,24]

The combination of hydroxyurea plus didanosine appears to overcome resistance to didanosine, possibly due to the depletion of cellular dATP, which may favour the incorporation of the active metabolite of didanosine (ddA-TP) into the replicating HIV DNA strand.[7,13,24] The favourable ratio of ddNTP to dNTP (particularly dATP) that is induced by the presence of hydroxyurea permits continued phenotypic sensitivity by genetically resistant viral isolates with mutations at codons 74 and 184. In the presence of a resistant HIV mutant, the nucleoside analogue may not compete efficiently with endogenous nucleotides (i.e. dATP) for incorporation into the nascent DNA chain. However, if the concentration of the competitor is reduced, or the concentration of the phosphorylated form of the nucleoside analogue is sufficiently increased, such as occurs when hydroxyurea is added,[7,10] the relative efficacy of the nucleoside analogue may be augmented without modification of the viral reverse transcriptase. This represents a novel approach to overcoming resistance.

This phenomenon was first described in the RIGHT 411 trial.[24,59] Viral RNA sequencing was conducted using plasma samples from 11 patients in the hydroxyurea plus didanosine group and eight patients in the didanosine monotherapy group. Two of eight didanosine-treated patients (25%) displayed mutations known to induce resistance to didanosine (e.g. codons 65 and 74), and 6 of 11 in the hydroxyurea plus didanosine group (55%) displayed such mutations (e.g. codons 74 and 184). Nonetheless, VL was reduced to a significantly greater extent in the hydroxyurea plus didanosine group (mean 93% below baseline) compared with didanosine monotherapy (mean 53% below baseline) after 24 weeks of therapy (AUC, U test of Mann-Whitney; p = 0.0005). None of the 38 patients who received hydroxyurea plus didanosine experienced viral rebound (e.g. an increase of at least 3-fold over the trough value for at least two consecutive tests) compared with eight of 19 patients (42%) in the didanosine monotherapy group. An in vitro evaluation of HXB2D clones with K65R or L74V mutations showed that 50 μmol/L of hydroxyurea reduced the concentration required to inhibit 50% (IC_{50}) of didanosine to levels comparable to those needed to suppress the wild type clone in the absence of hydroxyurea. The concentration of hydroxyurea used (50 μmol/L) is readily achievable in vivo.

Palmer et al.[13] and Wainberg et al.[18] have confirmed these data with didanosine and also demonstrated similar activity by combining hydroxyurea with two novel adenosine analogues, adefovir and tenofovir, in a variety of clinical isolates that displayed resistance to these compounds (table V). For example, the addition of hydroxyurea 50 μmol/L to didanosine resulted in an 18-fold reduction in the IC_{50} for a multidrug resistant (MDR) isolate containing six reverse transcriptase (RT) mutations (e.g. 41L, 67N, 184V, 210W, 215Y, 219N) and a 22-fold reduction in the IC_{50} for a multinucleoside-resistant isolate containing four RT mutations (e.g. 75I, 77L, 116Y, 151M).

Table V. Inhibition of HIV mutants resistant to nucleoside analogues by hydroxyurea[13]

RT mutation in HIV isolate	Fold change in IC_{50}					
	ddI		adefovir		tenofovir	
	HU–	HU+	HU–	HU+	HU–	HU+
Reference isolate	–	>8	–	↓8	–	↓>26
65	↑5.8	↓>46	↑4.2	↓130	↑3.7	↓60
74	↑3.0	↓>24	↑1.3	↓13	↑1.3	↓ >34
75, 77, 116, 151	↑95	↓22	↑2.8	↓5.0	↑4.9	↓32

HU = hydroxyurea (hydroxycarbamide); **IC$_{50}$** = concentration that produces 50% inhibition; **65** = reverse transcriptase (RT) mutation conferring resistance to didanosine (ddI); **74** = RT mutation conferring resistance to ddI, adefovir and tenofovir; **75, 77, 116, 151** = RT mutations conferring multidrug resistance; ↑ = increase; ↓ = decrease.

6. Role in Immune System Reconstitution

Virus-specific immune responses, such as those by CD8 cytotoxic and CD4 T helper lymphocytes, are critical for effective control of infection.[60-65] The clinical course of HIV infection is typified by a progressive loss of these important components of the immune system.[60-65] Despite a blunt in increase of CD4+ cell count,[24,26,27,32] the results of studies of hydroxyurea-based combinations suggest that these regimens may attenuate the loss of immune system components or permit immune reconstitution, both during acute and chronic infection.[66-68]

In an interim assessment of 12 chronically infected patients treated for an average of 122 weeks with hydroxyurea plus didanosine. These patients belonged to a larger cohort of 57 HIV-1 infected patients described elsewhere.[22] At the end of the study (week 40), 12 patients of the hydroxyurea plus didanosine group elected to continue this therapy; even though new drugs (such as protease inhibitors) had become available. In this group of patients, average baseline viraemia was 51 795 (SD = 66 869) and CD4+ cell count average was 376 cells/mm^3 (SD = 72). After 122 weeks of therapy the mean level of naive CD4 T cells (54.1%) and naive CD8 T cells (39.8%) were significantly higher than in untreated patients (23.8% with p = 0.00003 and 9.8% with p = 0.000002, respectively) and were similar to uninfected, healthy subjects.[67] In addition, vigorous HIV-specific CD4 T helper responses, measured as described elsewhere,[60] were detected in 6 of 12 patients (50%). This is in striking contrast with the lack of anti-HIV T helper responses observed in chronically infected patients after HAART treatment;[69-71] none of these six patients could be classified as long-term non-progressors according to current definitions[72,73] in that they had high VL and low CD4+ cell counts at baseline.

Immunophenotypic markers and function were evaluated in a group of eight hydroxyurea plus didanosine plus indinavir-treated patients with early HIV infection (median, 9.5 months since infection).[67] Compared with an untreated control group, patients treated for a median of 4.5 months had higher proliferative responses to allo and flu anti-

gens;[74] higher CD3-zeta expression in CD4 (p = 0.004) and CD8 cells (p = 0.002); and higher levels of naive (CD45RA+CD62L+) CD4 (p = 0.02) and CD8 (p = 0.01) cells. Furthermore, patients treated with hydroxyurea plus didanosine plus indinavir also had lower levels of CD8 T lymphocyte activation (HLA-DR+CD38+; p = 0.004), which has been suggested as a marker of poor prognosis.[75] While not significantly different, hydroxyurea plus didanosine plus indinavir-treated patients had higher levels of CD28+ expression on CD8 T lymphocytes, a co-stimulatory molecule essential for lymphocyte proliferation. A similar increase of the CD28+ expression on CD8 T lymphocytes has been recently observed in patients treated with non-hydroxyurea containing HAART.[76,77]

In another study,[68] patients were treated during acute HIV infection, i.e. prior to complete Western blot seroconversion, with the combination of hydroxyurea, didanosine and indinavir. This treatment was associated with the normalisation of some immune parameters and functions. No loss of naive CD4 T lymphocytes was observed, and a recovery of naive CD8 T lymphocytes proportion up to 35% occurred within a few weeks. A vigorous HIV-specific CD4 T helper response (stimulation index >9) was observed in seven of eight patients treated before complete Western blot seroconversion, and only in one out of five control patients treated after seroconversion. Furthermore, a limited size of the latent viral reservoir (ranging between <0.02 and 0.5 infectious units per million cells analysed) was documented in quiescent peripheral blood lymphocytes after treatment initiation before complete Western blot seroconversion.

Recently, Ogorman et al. presented preliminary immunological data on the 3D study in which the effects of the combination of efavirenz, stavudine, didanosine with and without hydroxyurea on the CD4 and CD8 T cell subsets were evaluated.[78] In this sub-study, 41 treatment-naive patients (21 in the hydroxyurea group, and 20 in the non-hydroxyurea group) were enrolled. After 48 weeks of treatment in the hydroxyurea group a non-significant decrease in the number of CD4 T cells was noticed, along with a

significant increase in naive CD4 T cells. The absolute number of the CD8 T cells did not change significantly in both the hydroxyurea and non-hydroxyurea group, while the number of CD8 naive T-cells increased only in the non-hydroxyurea group. There was a significant decrease in the CD4 and the CD8, CD38+ T cells in both hydroxyurea and non-hydroxyurea groups. The increase of CD4 naive T cells without a parallel increase in total CD4 T cell counts might indicate a selective loss of memory CD4 cells, and suggest that hydroxyurea can preserve the naive T cell subset, which represents the reservoir for immune response.

The paradox of immune restoration by hydroxyurea, despite a blunted CD4+ cell count increase might be explained by the cytostatic properties of this drug. Indeed, by exerting its cytostatic effect on CD4 and CD8 T lymphocytes,[79] hydroxyurea may reduce HIV replication by decreasing CD4 T cell proliferation as well as prevent the exhaustion of CD8 T cell populations that may occur as a result of excessive activation in the context of HIV infection.[9,75,80,81] Cell activation is essential for HIV infection. Since CD4 T lymphocyte activation allows for virus replication and CD8 T lymphocyte activation may contribute to pathogenesis, the combination of hydroxyurea with other drugs may lead to the inhibition of HIV, by blocking the 'cell activation-virus-production-pathogenesis' cycle.

7. Evidence for Attenuated Viral Rebound

The durability of the reduction in HIV-RNA was documented by the follow-up of the RIGHT 411 trial.[24] As stated above (see section 6) 12 patients following 40 weeks of didanosine plus hydroxyurea treatment, agreed to continue this therapeutic regimen.[82] At mean 122 weeks of treatment, the mean HIV-RNA levels decreased from 51 795 copies/mL (range 602–199 256) to mean 186 copies/mL (range <50–915). Between week 40 and 122, plasma viraemia continued to decrease, achieving an additional $\log_{10}$ reduction by week 122 (figure 2). These data suggest that significant and durable viral suppression may occur over time, and that the rate at
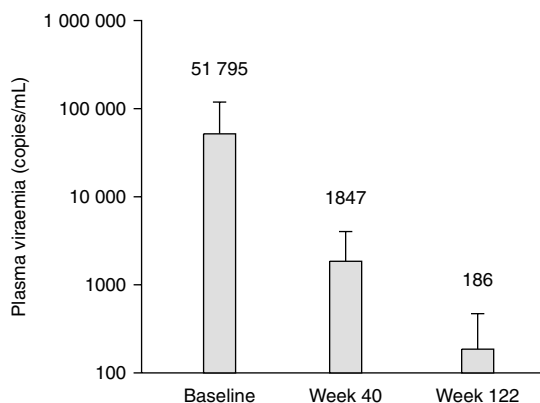

Fig. 2. Durability of the reduction in HIV-RNA in the follow-up of the RIGHT 411 trial.[24] Treatment with hydroxyurea (hydroxycarbamide) plus didanosine for a mean of 122 weeks resulted in continued reductions of plasma HIV-1 ribonucleic acid (RNA) levels over time.

which this occurs may not be as rapid as has been shown with other potent antiretroviral regimens.[83] However, the end result remains durable viral suppression.

Also, during this study period, there was a moderate increase in the mean CD4+ cell count which increased from 376 cells/mm^3 at baseline (range 277–493) to 406 cells/mm^3 after a mean of 122 weeks of treatment. However, the mean CD4/CD8 cell ratio significantly increased from 0.56 at baseline (range 0.4–0.9) to 0.83 (range 0.5–1.4).

Some of the patients were followed up for longer. All patients were offered the option to interrupt treatment, nine agreed to interrupt therapy and were compared with seven patients receiving HAART during an 8-week treatment interruption. Both groups had similar baseline VL, CD4+ cell count, and length of treatment. Treatment was resumed if viral rebound >10 000 copies/mL (virological failure) or CD4+ cell count decreased below 200 cells/mm^3 (immunological failure) occurred in two consecutive measurements. Therapy in none of the 12 patients who remained on dual therapy failed. Viral rebound was spontaneously contained, and CD4+ cell count remained stable. In four of seven patients in the HAART group HIV was not controlled by week 6 and these patients had to restart therapy due to either viraemia rebound or CD4+ cell count de-

crease (figure 3). Before therapy interruption, the 12 patients who remained on dual therapy had a vigorous HIV-specific T cell immune response (median CD4VIR 1.2%), while the HAART-treated patients did not (median CD4VIR 0.2%). CD4VIR represents the percentage of HIV-specific CD4 subpopulation expressing interferon (IFN)-γ within the total CD4 population (CD3+, CD4+, IFN+). This difference was statistically significant (p =.002). This study showed that HIV can be controlled during hydroxyurea-containing therapy interruption in patients with established infection, and that control of viral replication correlates with vigorous anti-HIV-specific immune response.

Evidence suggesting that hydroxyurea-based regimens attenuate viral rebound when therapy is discontinued has already been presented. In the study originally reported by Biron et al.,[29] 12 antiretroviral-naive patients experienced reductions in VL following treatment with hydroxyurea and didanosine.[29] After 1 year of treatment and the addition of other patients to the study, 10 of 20 patients had undetectable VL.[84] Eight of these patients had a lymph node biopsy at day 360, and seven of these patients had no evidence of infectious virus in the lymph nodes. Two patients agreed to stop treatment.[85] One patient had initiated therapy 3 months postinfection (pre-treatment CD4+ cell count 1452

cells/mm^3; VL 676 copies/mL); the other patient had initiated therapy 12 months postinfection (pre-treatment CD4+ cell count 874 cells/mm^3; VL 1120 copies/mL). One year after therapy discontinuation, there was no detectable HIV-RNA in plasma or lymph nodes,[84] although integrated proviral DNA was found in lymph node mononuclear cells in both patients, and in the PBMC of one patient, at a rate of 4 copies/10^6 cells.[85] The authors suggested that the proviral DNA was incompetent for transcription since no infectious virus was obtained upon culture of lymph node mononuclear cells and peripheral purified CD4 T cells. It is important to consider the possibility that these two patients may have been long-term non-progressors who would have exhibited these characteristics whether or not they received hydroxyurea-based therapy.

A Spanish group analysed the association of hydroxyurea as an immune-system modulator, with HAART.[86] Twenty chronically infected patients were randomised into two groups receiving stavudine plus didanosine plus indinavir plus hydroxyurea (hydroxyurea group), with baseline CD4+ cell count 695 cells/mm^3, VL 46 000 copies/mL, or stavudine plus didanosine plus indinavir (ART group), with baseline CD4+ cell count 710 cells/mm^3, VL 45 000 copies/mL. Patients underwent five consecutive cycles of structured treatment interruption, which involved 2-month treatment periods, in the first treatment-interruption period, HAART was reinitiated whenever viraemia was >200 copies/mL (median duration 3 weeks). Interruptions 2, 3, and 4 were fixed-schedule interruptions lasting 2 weeks each. The fifth interruption was continued as long as the set point was reached. Hydroxyurea was discontinued during the first three interruption periods along with all other drugs, but continued during the last two cycles despite interruption of other drugs. Immune responses were evaluated by analysing cytotoxic T lymphocyte activity and lymphocyte proliferation and were found to increase in both groups after the first three interruption periods. Interestingly, viral control (VL below 5000 copies/mL) in the absence of drug therapy began to emerge in the hydroxyurea group when hydroxyurea was
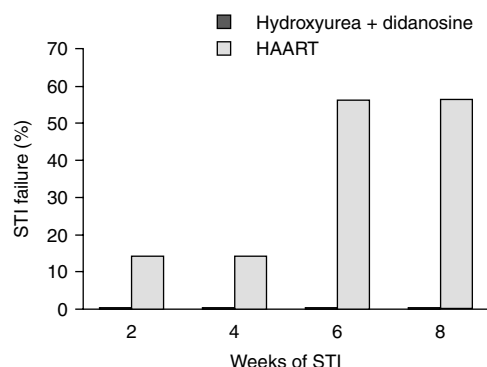
Fig. 3. Percentages of treatment interruption failure in patients treated with hydroxyurea (hydroxycarbamide) plus didanosine and patients treated with highly active antiretroviral therapy (HAART). Treatment failure was defined as viral rebound >10 000 copies/mL (in two consecutive tests) or CD4+ cell count decrease <200 cells/mm^3.[24] **STI** = structured treatment interruption.

continued during the interruption periods. At the end of the study, T lymphocyte proliferative responses were analysed in 11 of 20 patients. One of six patients in the ART group and all patients in the hydroxyurea group had CD4 T lymphocyte proliferative responses specific to the HIV protein p24.

8. Conclusions

Hydroxyurea is a unique agent that augments the activity of nucleoside analogues and offers an effective alternative for treatment of patients with HIV infection. Targeting a cellular rather than a viral enzyme may permit long-term use of this agent without the induction of resistance or cross-resistance to other agents. Hydroxyurea has been mainly tested as part of multiple-drug regimens always also containing didanosine, because of the proven synergistic antiviral effects of the two drugs. Several randomised, controlled studies have produced consistent results showing that hydroxyurea-based regimens induced substantial and sustained reduction in VL in HIV infected patients. However, high doses hydroxyurea might be associated with serious adverse events. Using low doses hydroxyurea is a promising alternative. Future research of hydroxyurea-based regimens is needed to establish the appropriate dosage and administration schedule, evaluate the potential for intermittent therapy, and determine the efficacy and safety in combination with other agents, and in paediatric patients. Further research is also warranted to assess the role of hydroxyurea-based regimens toward attenuation of loss of immune function or to induce immune reconstitution.

Acknowledgements

References

1. Zhang L, Ramratnam B, Tenner-Racz K, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. N Engl J Med 1999; 340 (21): 1605-13
2. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med 1999; 5 (5): 512-7
3. Carr A, Samaras K, Thorisdottir A, et al. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 1999; 353 (9170): 2093-9
4. Donehower RC. An overview of the clinical experience with hydroxyurea. Semin Oncol 1992; 19 (3 Suppl. 9): 11-9
5. Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med 1995; 332 (20): 1317-22
6. Gao WY, Cara A, Gallo RC, et al. Low levels of deoxynucleotides in peripheral blood lymphocytes: a strategy to inhibit human immunodeficiency virus type 1 replication. Proc Natl Acad Sci U S A 1993; 90 (19): 8925-8
7. Lori F, Malykh A, Cara A, et al. Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. Science 1994; 266 (5186): 801-5
8. Lori F, Gallo RC. Hydroxyurea and AIDS: an old drug finds a new application? AIDS Res Hum Retroviruses 1995; 11 (10): 1149-51
9. Lori F. Hydroxyurea and HIV: 5 years later: from antiviral to immune-modulating effects. AIDS 1999; 13 (12): 1433-42
10. Gao WY, Johns DG, Mitsuya H. Anti-human immunodeficiency virus type 1 activity of hydroxyurea in combination with 2′,3′-dideoxynucleosides. Mol Pharmacol 1994; 46 (4): 767-72
11. Johns DG, Gao WY. Selective depletion of DNA precursors: an evolving strategy for potentiation of dideoxynucleoside activity against human immunodeficiency virus. Biochem Pharmacol 1998; 55 (10): 1551-6
12. Malley SD, Grange JM, 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 U S A 1994; 91 (23): 11017-21
13. Palmer S, Shafer RW, Merigan TC. Hydroxyurea enhances the activities of didanosine, 9-[2- (phosphonylmethoxy) ethyl]adenine, and 9-[2- (phosphonylmethoxy)propyl] adenine against drug-susceptible and drug-resistant human immunodeficiency virus isolates. Antimicrob Agents Chemother 1999; 43 (8): 2046-50
14. Lori F, Lisziewicz J. Hydroxyurea: mechaninsms of HIV-1 inhibition. Antivir Ther 1998; 3 Suppl. 4: 25-33
15. Gao WY, Johns DG, Chokekuchai S, et al. Disparate actions of hydroxyurea in potentiation of purine and pyrimidine 2′,3′-dideoxynucleoside activities against replication of human immunodeficiency virus. Proc Natl Acad Sci U S A 1995; 92 (18): 8333-7
16. Moore RD, Keruly JC, Chaisson RE. Incidence of pancreatitis in HIV-infected patients receiving nucleoside reverse transcriptase inhibitor drugs. AIDS 2001; 15 (5): 617-20
17. Moore RD, Wong WM, Keruly JC, et al. Incidence of neuropathy in HIV-infected patients on monotherapy versus those on combination therapy with didanosine, stavudine and hydroxyurea. AIDS 2000; 14 (3): 273-8

18. Wainberg MA, Miller MD, Quan Y, et al. In vitro selection and characterization of HIV-1 with reduced susceptibility to PMPA. Antivir Ther 1999; 4 (2): 87-94

19. Piccinini G, Foli A, Comolli G, et al. Complementary antiviral efficacy of hydroxyurea and protease inhibitors in human immunodeficiency virus-infected dendritic cells and lymphocytes. J Virol 2002; 76 (5): 2274-8

20. Perno CF, Newcomb FM, Davis DA, et al. Relative potency of protease inhibitors in monocytes/macrophages acutely and chronically infected with human immunodeficiency virus. J Infect Dis 1998; 178 (2): 413-22

21. 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 (4740): 850-3

22. Zack JA, Arrigo SJ, Weitsman SR, et al. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell 1990; 61 (2): 213-22

23. Stevenson M, Stanwick TL, Dempsey MP, et al. HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO J 1990; 9 (5): 1551-60

24. Lori F, Malykh AG, 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 (16): 1403-9

25. Frank I. Clinical use of hydroxyurea in HIV-1 infected patients. J Biol Regul Homeost Agents 1999; 13 (3): 186-91

26. Rutschmann OT, 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 (8): F71-7

27. Federici ME, Lupo S, Cahn P, et al. Hydroxyurea in combination regimens for the treatment of antiretroviral naive, HIV-infected adults[abstract no. 287/12235]. Proceedings of the 12th World AIDS Conference 1998 Jun 28-Jul 3; Geneva; 12: 58-9

28. Havlir DV, Gilbert PB, Bennett K, et al. Effects of treatment intensification with hydroxyurea in HIV-infected patients with virologic suppression. AIDS 2001; 15 (11): 1379-88

29. 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 (1): 36-40

30. Clotet B, Ruiz L, Cabrera C, et al. Short-term anti-HIV activity of the combination of didanosine and hydroxyurea. Antivir Ther. 1996 Aug; 1 (3): 189-93

31. Simonelli C, Nasti G, Vaccher E, et al. Hydroxyurea treatment in HIV-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol. 1996 Dec 15; 13 (5): 462-4

32. Montaner JS, Zala C, Conway B, et al. A pilot study of hydroxyurea among patients with advanced human immunodeficiency virus (HIV) disease receiving chronic didanosine therapy: Canadian HIV trials network protocol 080. J Infect Dis 1997; 175 (4): 801-6

33. Foli A, Lori F, Maserati R, et al. Hydroxyurea and didanosine as a more potent combination than hydroxyurea and zidovudine. Antivir Ther 1997; 2 (1): 33-40

34. Simonelli C, Comar M, Zanussi S, et al. No therapeutic advantage from didanosine (ddI) and hydroxyurea versus ddI alone in patients with HIV infection. AIDS. 1997 Aug; 11 (10): 1299-300

35. Foli A, Maserati R, Minoli L, et al. Therapeutic advantage of hydroxyurea and didanosine combination therapy in patients previously treated with zidovudine. AIDS. 1998 Jun 18; 12 (9): 1113-4

36. Murphy R, Katlama C, Autran B, et al. The effects of hydroxyurea or placebo combined with efavirenz, didanosine, and stavudine in treatment naive and experienced patients: preliminary 24 week results from the 3rd study [abstract no.WeOrB603]. The XIII International AIDS Conference; 2000 Jul 9-14; Durban, South Africa

37. Thurman WG, Bloedow C, Howe CD, et al. A phase I study of hydroxyurea. Cancer Chemother Rep 1963; 29: 103-7

38. Griffith K. Hydroxyurea (NSC 32065): results of a phase I study. Cancer Chemother Rep 1964; 40: 33-8

39. Rossero R, McKinsey D, Green S, et al. Open label combination therapy with stavudine, didanosine, and hydroxyurea in nucleoside experienced HIV-1 infected patients [abstract 653]. The 5th International Conference on Retroviruses and Opportunistic Infections; 1998 Feb 1-5; Chicago (IL)

40. Goodrich J, Khardori N. Hydroxyurea toxicity in human immunodeficiency virus-positive patients. Clin Infect Dis 1999; 29 (3): 692-3

41. Lori F, Lisziewicz J. Targeting HIV reservoirs and reconstituting the immune system. The 2nd Annual Meeting of the Research Institute for Genetic and Human Therapy; 1999; Washington, DC. AIDS Res Hum Retroviruses 1999 Dec 10; 15 (18): 1597-617

42. Wilson JG, Scott WJ, Ritter EJ, et al. Comparative distribution and embryotoxicity of hydroxyurea in pregnant rats and rhesus monkeys. Teratology 1975; 11 (2): 169-78

43. Brinkman K, ter Hofstede HJ, Burger DM, et al. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 1998; 12 (14): 1735-44

44. Weissman SB, Sinclair GI, Green CL, et al. Hydroxyurea-induced hepatitis in human immunodeficiency virus-positive patients. Clin Infect Dis 1999; 29 (1): 223-4

45. Harris M, Tesiorowski A, Montaner JS. Screening for nucleoside-associated lactic acidosis [abstract TuPpB1233]. The XIII International AIDS Conference; 2000 Jul 9-14; Durban, South Africa

46. Cote HC, Brumme ZL, Craib KJ, et al. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV- infected patients. N Engl J Med 2002; 346 (11): 811-20

47. Alpha International Coordinating Committee. The Alpha trial: European/Australian randomized double-blind trial of two doses of didanosine in zidovudine-intolerant patients with symptomatic HIV disease. AIDS 1996; 10 (8): 867-80

48. Maxson CJ, Greenfield SM, Turner JL. Acute pancreatitis as a common complication of 2′,3′-dideoxyinosine therapy in the acquired immunodeficiency syndrome. Am J Gastroenterol 1992; 87 (6): 708-13

49. Jablonowski H, Arasteh K, Staszewski S, et al. A dose comparison study of didanosine in patients with very advanced HIV infection who are intolerant to or clinically deteriorate on zidovudine. German ddI Trial Group. AIDS 1995; 9 (5): 463-9

50. Nguyen BY, Yarchoan R, Wyvill KM, et al. Five-year follow-up of a phase I study of didanosine in patients with advanced human immunodeficiency virus infection. J Infect Dis 1995; 171 (5): 1180-9

51. Foli A, Benvenuto F, Piccinini G, et al. Direct analysis of mitochondrial toxicity of antiretroviral drugs. AIDS 2001; 15 (13): 1687-94

52. Lori F, Pollard R, Shalit P, et al. A low hydroxyurea dose (600mg daily) is found optimal in a randomized, controlled

trial (RIGHT 702) [abstract TuPeB4465]. The XIV International AIDS Conference; 2002 Jul 7-12; Barcelona, Spain

53. Montaner J, Jahnke N, Yip B, et al. Multi-drug rescue therapy (MDRT) for HIV-infected individuals with prior virologic failure to multiple regimes. Preliminary results [abstract 22495]. Proceedings of the 12th World AIDS Conference 1998 Jun 28-Jul 3; Geneva, Switzerland

54. 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 failure [abstract 400s]. The 6th Conference on Retroviruses and Opportunistic Infections; 1999 Jun 28-Jul 3; Chicago (IL)

55. Miles S, Winters RE, Ruane P. Salvage of multi-drug resistant HIV infection with D4T/3TC/hydroxyurea [abstract #288/12205]. The 12th World AIDS Conference; 1998, Geneva, Switzerland

56. Cadden JJ, Dube MP. Hydroxyurea (HU) plus ddI as salvage therapy in HIV [abstract I-202]. The 38th International Conference on Antimicrobial Agents and Chemotherapy; 1998 Sep 24-27; San Diego (CA)

57. Grunke M, Dechant C, Low P, et al. Hydroxyurea as part of a salvage regimen for heavily pretreated patients with advanced HIV infection. J Acquir Immune Defic Syndr 1999; 21 (5): 424-6

58. Solas C, Lafeuillads A, Halfon P, et al. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. Antimicrob Agent Chemother 2003; 47 (1): 238-43

59. 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 (4): 899-903

60. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997; 278 (5342): 1447-50

61. Ogg GS, Kostense S, Klein MR, et al. Longitudinal phenotypic analysis of human immunodeficiency virus type 1- specific cytotoxic T lymphocytes: correlation with disease progression. J Virol 1999; 73 (11): 9153-60

62. Schwartz D, Sharma U, Busch M, et al. Absence of recoverable infectious virus and unique immune responses in an asymptomatic HIV+ long-term survivor. AIDS Res Hum Retroviruses 1994; 10 (12): 1703-11

63. Pontesilli O, Carotenuto P, Kerkhof-Garde SR, et al. Lymphoproliferative response to HIV type 1 p24 in long-term survivors of HIV type 1 infection is predictive of persistent AIDS-free infection. AIDS Res Hum Retroviruses 1999; 15 (11): 973-81

64. Goulder PJ, Bunce M, Krausa P, et al. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. AIDS Res Hum Retroviruses 1996; 12 (18): 1691-8

65. Harrer E, Harrer T, Barbosa P, et al. Recognition of the highly conserved YMDD region in the human immunodeficiency virus type 1 reverse transcriptase by HLA-A2-restricted cytotoxic T lymphocytes from an asymptomatic long-term nonprogressor. J Infect Dis 1996; 173 (2): 476-9

66. Galpin JE, Lori F, Globe DR, et al. Improvement in CD4 cell diversity during 7-month trial of hydroxyurea in combination with ddI or ddI and d4T [abstract 657]. The 5th International Conference on Retroviruses and Opportunistic Infections; 1998 Feb 5, Chicago (IL)

67. Lori F, Jessen H, Lieberman J, et al. Immune restoration by combination of a cytostatic drug (hydroxyurea) and anti-HIV drugs (didanosine and indinavir). AIDS Res Hum Retroviruses 1999; 15 (7): 619-24

68. 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 (6): 1827-32

69. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997; 277 (5322): 112-6

70. Plana M, Garcia 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. Immunology Study Group of Spanish EARTH-1 Study. Lancet 1998; 352 (9135): 1194-5

71. Pitcher CJ, Quittner C, Peterson DM, 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 (5): 518-25

72. Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. N Engl J Med 1995; 332 (4): 209-16

73. Cao Y, Qin L, Zhang L, et al. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N Engl J Med 1995; 332 (4): 201-8

74. Clerici M, Stocks NI, Zajac RA, et al. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients: independence of CD4+ cell numbers and clinical staging. J Clin Invest 1989; 84 (6): 1892-9

75. Giorgi JV, Liu Z, Hultin LE, et al. 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. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 1993; 6 (8): 904-12

76. Plana M, Martinez C, Garcia F, et al. Immunologic reconstitution after 1 year of highly active antiretroviral therapy, with or without protease inhibitors. J Acquir Immune Defic Syndr 2002; 29 (5): 429-34

77. Lange CG, Lederman MM, Madero JS, et al. Impact of suppression of viral replication by highly active antiretroviral therapy on immune function and phenotype in chronic HIV-1 infection. J Acquir Immune Defic Syndr 2002; 30 (1): 33-40

78. Ogorman M, Alatrakchi N, Belsey E, et al. Hydroxyurea therapy spares the naive T cell compartment in patients treate with antiretroviral drugs: the 3D Study. The 1st IAS Conference on HIV Pathogenesis and Treatment; 2001 Jul 8-11; Buenos Aires, Argentina

79. Ravot E, Tambussi G, Jessen H, et al. Effects of hydroxyurea on T cell count changes during primary HIV infection. AIDS 2000; 14 (5): 619-22

80. Zinkernagel RM, Hengartner H. T-cell-mediated immunopathology versus direct cytolysis by virus: implications for HIV and AIDS. Immunol Today 1994; 15 (6): 262-8

81. Zinkernagel RM, Hengartner H. Correlates of protective viruses damaging to HIV infection. Science 1996; 272 (5266): 1362

82. 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 (15): 1333-8

83. Weverling GJ, Lange JM, Jurriaans S, et al. Alternative mul-
    tidrug regimen provides improved suppression of HIV-1 repli-
    cation over triple therapy. AIDS 1998; 12 (11): F117-22
84. Vila J, Biron F, Nugier F, et al. 1-year follow-up of the use of
    hydroxycarbamide and didanosine in HIV infection. Lancet
    1996; 348 (9021): 203-4
85. 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 (9078): 635-6
86. Plana M, Lopalco L, Garcia F, et al. Effect of associating a
    cytostatic drug + HAART and holding the cytostatic drug after
    STI and definitive interruption of HAART on HIV-1 specific
    immune response [abstract 535-Ms]. The 9th Conference on
    Retroviruses and Opportunistic Infections; 2002 Feb 24-28;
    Seattle (WA)

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