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Long-term zidovudine treatment of asymptomatic HIV-1-infected subjects

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Summary

Eighteen asymptomatic men with persistent human immunodeficiency virus type 1 (HIV-1) p24 antigenemia were treated with zidovudine 250–500 mg (± acyclovir 800 mg) 6-hourly for 4–12 weeks, and thereafter with zidovudine 500 mg (± acyclovir 1600 mg) 12-hourly for 92 weeks. Six additional HIV-1 p24 antigenemic subjects were treated with zidovudine 500 mg 12-hourly for 76 weeks. Disease progression occurred in 4 subjects, despite sustained reduction of serum HIV-1 p24 antigen levels: *Pneumocystis carinii* pneumonia was diagnosed after 60, 80, 90 and 93 weeks, respectively. The median CD4+ cell count of these 4 men at study entry was 0.2×10^9/l, and it declined to 0.07×10^9/l at the moment AIDS was diagnosed. In 20 subjects no disease progression occurred. The median CD4+ cell count of these 20 men at study entry was 0.4×10^9/l and it was 0.45×10^9/l at the end of the study period. Median serum HIV-1 p24 antigen levels at the end of the study period were 42% lower than at study entry in these 20 subjects. In 5/20 men, an initial decline was followed by a rise in antigen levels to above pretreatment value. Treatment with zidovudine was well tolerated. Anemia caused symptoms in 3/24

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men, but prolonged leucopenia or neutropenia did not occur. None developed clinical or convincing biochemical evidence of zidovudine-associated myopathy.

Asymptomatic HIV infection; HIV-1 p24 antigenemia; Zidovudine treatment

Introduction

Treatment with the nucleoside analogue 3′-azido-3′-deoxythymidine (zidovudine or AZT) has delayed death and lessened the frequency of opportunistic infections in selected patients with the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) (Fischl et al., 1987a). However, in such patients clinical and immunological benefits of zidovudine therapy may be of limited duration (Fischl et al., 1987a,b; Dournon et al., 1988; Stambuck et al., 1989; Williams et al., 1989; Pinching et al., 1989). In addition, in these patients zidovudine treatment is associated with considerable hematological toxicity (especially anemia and granulocytopenia), often necessitating dose reductions or drug withdrawal (Dournon et al., 1988; Stambuck et al., 1989; Williams et al., 1989; Pinching et al., 1989; Richman et al., 1987; Gill et al., 1987; Walker et al., 1988).

It is tempting therefore to use zidovudine earlier in the course of the disease – i.e., in symptomless human immunodeficiency virus (HIV) infected patients – in the hope that such treatment will delay or even prevent the progression to symptomatic disease and death, and will be less toxic than in symptomatic patients.

We previously reported on zidovudine (± acyclovir) treatment of 18 asymptomatic HIV-1 infected homosexual men (De Wolf et al., 1988a). In that study population 16–24 weeks of zidovudine in simplified (250 mg 6-hourly, followed by 500 mg 12-hourly) dosage schedules: a) reduced HIV-1 p24 antigenemia, b) was associated with stable or increasing circulating CD4+ cell count numbers, and c) was well tolerated (De Wolf et al., 1988a). We now report 2 years follow-up data on this group and on a group of 14 men with a comparable duration of HIV-1 p24 antigenemia, who were not treated with zidovudine. Also included are results on 6 men who started with zidovudine 500 mg 12-hourly, and were followed for 76 weeks.

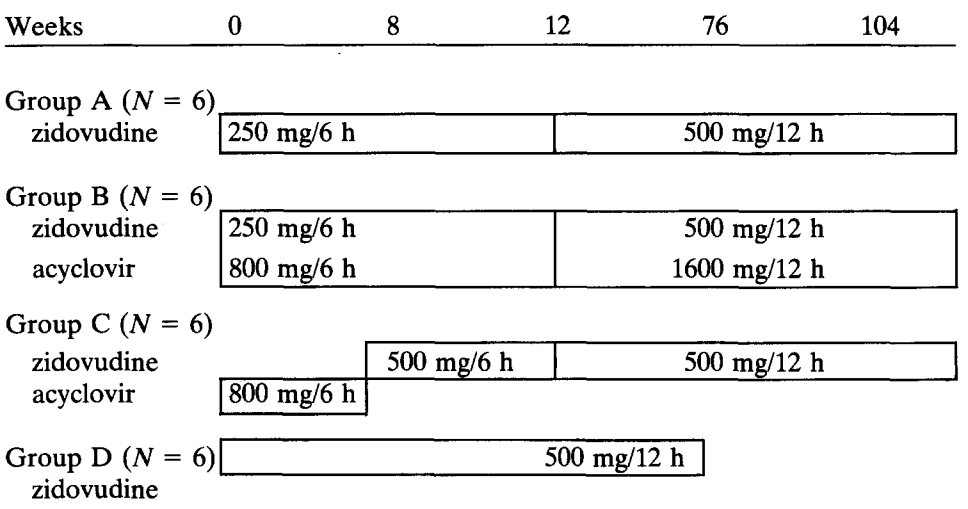
Subjects and Methods

Study population and treatment regimens

Subjects were selected from a previously described cohort of homosexual men (De Wolf et al., 1988b). All were persistently HIV-1 p24 antigenemic, and either without symptoms (CDC group II), or suffering from persistent generalized lymphadenopathy (PGL; CDC group III) only (Centers for Disease Control, 1986). During treatment subjects were seen at least 4-weekly for clinical and laboratory

evaluation, as previously described (De Wolf et al., 1988a). The latest clinical and haematological evaluation was after 104 weeks for groups A, B and C, and at week 76 for group D. Serum HIV-1 p24 antigen levels, circulating CD4+ cell counts, and biochemical parameters were determined at week 96 for groups A, B and C, and at week 72 for group D. In subjects with disease progression the moment of AIDS diagnosis was chosen as last evaluation point.

Treatment strategies were as follows:



Except for insulin, which was administered to two patients, no other chronic medication was recorded.

At the start of the treatment the mean age was 40.6 (range 32–50) years in group A, 35.2 (28–45) in group B, 30.8 (23–42) in group C, and 34.1 (24–43) in group D. The mean duration of HIV antigenemia before treatment was 107.8 (64–124), 86.2 (45–121), 97.5 (35–125) and 64.7 (20–125) weeks in groups A, B, C and D, respectively.

From the same cohort 14 persistently HIV-1 p24 antigenemic men who were classified as CDC II or III at the start of the zidovudine study, were retrospectively used as a control group from week 0–104. At week 0 the mean age was 39.1 (range 32–54) years, and the duration of HIV antigenemia was 80.5 (12–125) weeks. These subjects were seen every 3 months, when blood samples were taken for determination of HIV antigen and CD4+ lymphocyte counts.

Laboratory evaluation

HIV antigen detection Sequential serum samples were tested for HIV-1 p24 antigen (HIV-Ag) by a solid-phase, sandwich-type enzyme immunoassay (Abbott Laboratories, North Chicago, IL, U.S.A.), as previously described (Lange et al.,

1986). Assays were performed at various times throughout the study period with the cut-off varying from 40–65 pg/ml. Samples were also batch-assayed at the end of the study period and the results correlated well with those previously obtained.

Detection of CD4+ lymphocytes Peripheral blood mononuclear cells were isolated from heparinized venous blood by density gradient centrifugation on Ficoll Hypaque. CD4+ lymphocytes were enumerated by an indirect or direct immunofluorescence technique using monoclonal antibodies (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service; Becton Dickinson, Mountain View, CA, U.S.A.) and a flow cytometry system (Coulter EPICS-C, Luton, U.K.).

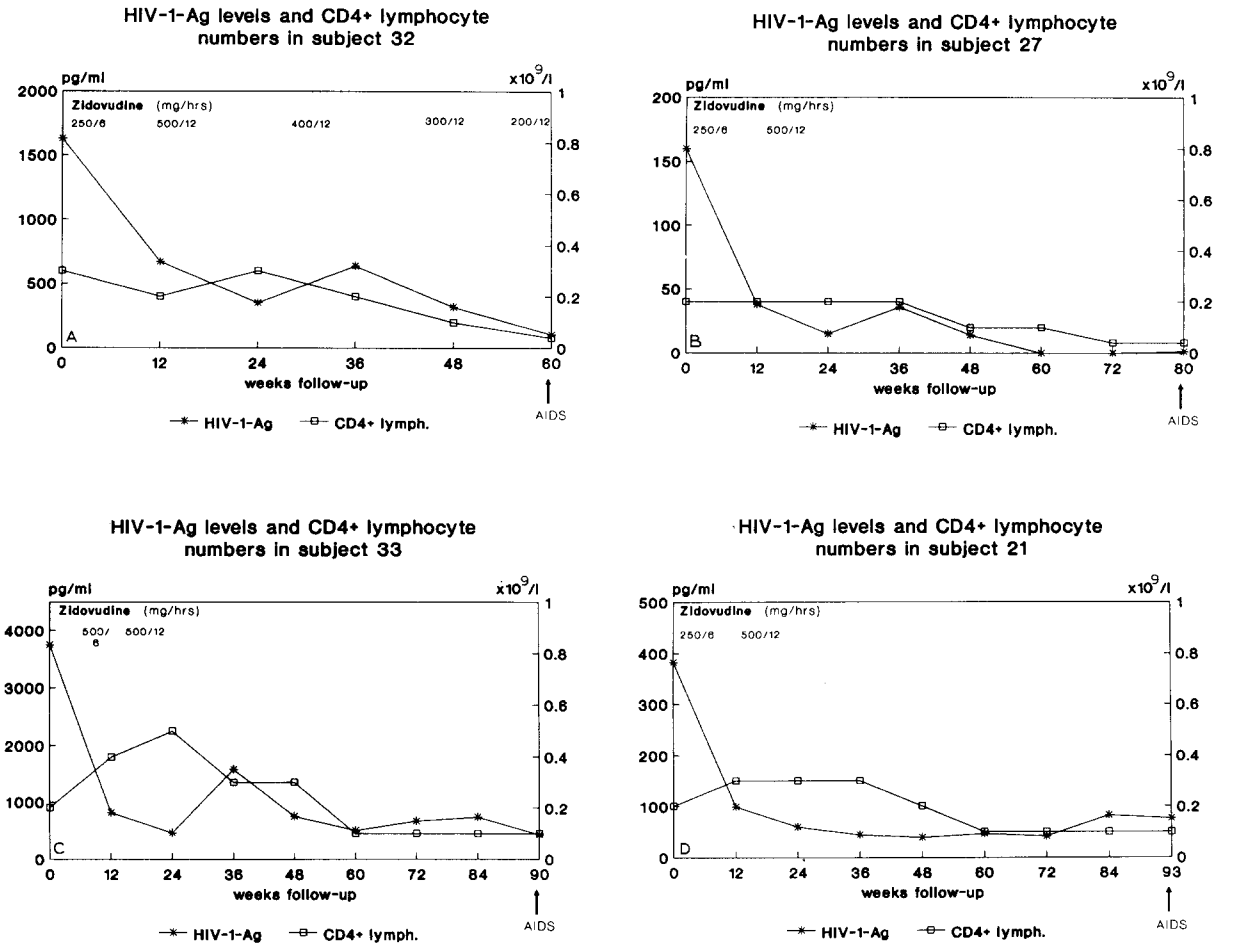


Fig. 1. (A–D) Serum HIV-1 Ag levels and peripheral blood CD4+ lymphocyte counts in 4 treated patients who progressed to AIDS.

Results

Zidovudine-treated subjects

Disease progressors Four of the 24 subjects developed AIDS. Serum HIV-Ag levels and peripheral blood CD4+ cell counts of these subjects are depicted in Fig. 1 A–D and Table 1; hematological parameters in Table 2.

Subject 32 (group A): Pneumocystis carinii pneumonia (PCP) was diagnosed at week 60. At entry this subject had been classified as CDC II. However during the first week of treatment, Candida stomatitis was diagnosed. This subject from the onset frequently mentioned fatigue, and should perhaps therefore be regarded as having early symptomatic disease at entry. He was the only subject in whom disease progression occurred and who developed anemia (which appeared within 12 weeks of zidovudine treatment). He received 3 red cell transfusions, each of 3 units, and zidovudine was dose-reduced thrice.

Subject 27 (group B): PCP was diagnosed at week 80. At entry this subject had been diagnosed as CDC II. Until shortly before PCP diagnosis he was completely asymptomatic. He had received full dose zidovudine (+ acyclovir) for the entire treatment period.

Subject 33 (group C): PCP was diagnosed at week 90. At entry he had been classified as CDC II. Until PCP occurred he was completely asymptomatic. There had been no zidovudine dose reductions.

Subject 21 (group B): PCP was diagnosed at week 93. At entry he had been classified as CDC III. Until PCP occurred he was completely asymptomatic. There had been no zidovudine dose reductions.

Leucopenia or neutropenia were not observed. At the start of treatment two subjects had platelet counts below normal value, i.e. 114 $\times$ 10^9/l in subject 21, and 116 $\times$ 10^9/l in subject 33. In subject 21 the platelet count subsequently declined to 46 $\times$ 10^9/l at the moment AIDS was diagnosed. None of the other subjects ended with a platelet count below 150 $\times$ 10^9/l.

TABLE 1

Serum HIV-Ag levels and CD4+ cell counts in subjects who showed disease progression (treated N=4, untreated N=6), medians (and ranges)

Subjects	Serum HIV-Ag (pg/ml)			CD4+ cells ($\times$ 10^9/l)		
	Entry	End*	% Change	Entry	End*	% Change
Treated N=4	998 (160–3746)	91 (1–430)	91 ↓	0.2 (0.2–0.3)	0.07 (0.04–0.1)	65 ↓
Untreated N=6	204 (85–504)	319 (108–1166)	56 ↑	0.3 (0.2–0.7)	0.1 (0.03–0.2)	67 ↓

*Time of disease progression to CDC IV.

TABLE 2

Hematological indices in treated subjects who developed AIDS (N=4), medians (and ranges)

	Week 0	End*
Hb (g/dl)	14.9 (13.5–15.3)	12.8 (10.6–13.7)**
MCV (fl)	93.6 (89.9–97.7)	121.7 (111.6–132.6)
Leucocytes ($\times 10^9$/l)	4.8 (4.2–6.1)	4.1 (2.4–5.0)
Neutrophils ($\times 10^9$/l)	1.9 (1.6–3.2)	1.7 (1.3–3.3)
Lymphocytes ($\times 10^9$/l)	2.0 (1.0–3.4)	1.0 (0.7–2.5)
Platelets ($\times 10^9$/l)	133 (114–158)	210 (46–250)

*Time of AIDS diagnosis; **, one subject received 3 red cell transfusions.

Non-progressors In 20/24 subjects no disease progression to CDC IV was noted. Median serum HIV-Ag levels for the different treatment groups are depicted in Fig. 2 and Table 3; CD4+ cell counts in Fig. 3 and Table 3; hematological parameters in Table 4.

Serum HIV-Ag levels were higher at the end of the study period than at entry in 5 subjects. In subject 25, from group A, antigen levels rose after zidovudine dose reduction (see below): serum HIV-Ag was 161 pg/ml at day 0 and 220 pg/ml at week 96. In subject 36, from group A, after an initial decline, the serum HIV-Ag level from week 24 on rose to just above pretreatment level (566 pg/ml) at week 60, and to 4206 pg/ml at week 96 (Fig. 4). This subject professed to be therapy-compliant and pharmacokinetic studies showed a normal uptake of zidovudine (data not shown). The only person to admit poor compliance regarding drug-intake, subject 26 from group B, had a serum HIV-Ag level of 132 pg/ml at moment 0, and of 133 pg/ml at week 96. Subject 22 from group C had a serum HIV-Ag level of 96 pg/ml at moment 0, and of 310 pg/ml at week 96. Subject 46 from group D had a serum HIV-Ag level of 661 pg/ml at moment 0, and of 815 pg/ml at week 72.

Two subjects developed symptomatic anemia. In subject 25, from group A, the

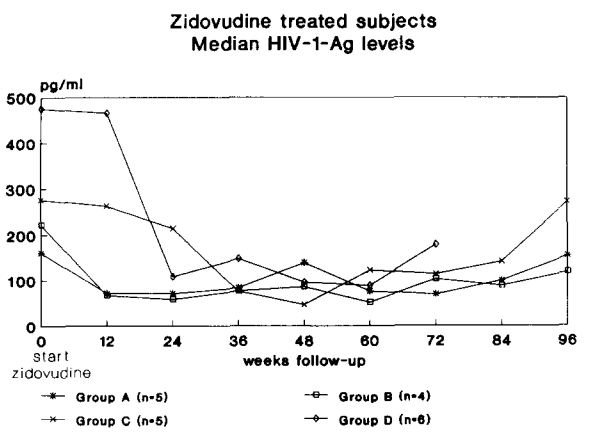


Fig. 2. Median serum HIV-1 Ag levels in 20 treated subjects who did not progress to AIDS.

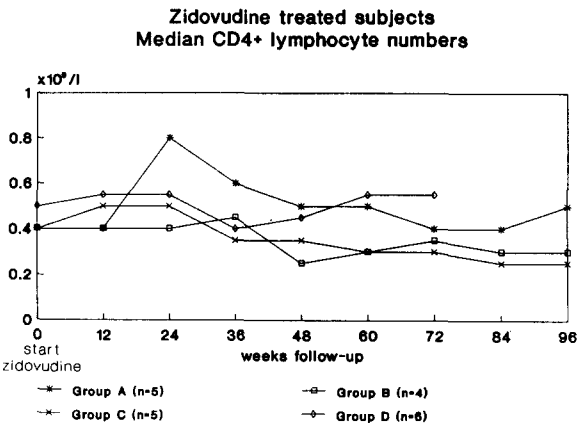


Fig. 3. Median peripheral blood CD4+ lymphocyte counts in 20 treated subjects who did not progress to AIDS.

anemia surfaced after a year of treatment. Treatment was interrupted for 2 weeks at week 59, and subsequently the dose was reduced to 400 mg/12 h. In subject 30, from group B, the anemia surfaced within 6 weeks. This patient received 4 transfusions of 3 units of red cells, zidovudine was dose-reduced five times (to a minimum of 200 mg/12 h from week 27 on), and interrupted at week 25 and 49, for two weeks on each occasion. Subject 30 had previously required blood transfusion for no obvious reason other than hepatitis B infection in 1981. In both subjects dose reductions and interruptions were followed by rises in serum HIV-Ag levels.

In most subjects leucocyte and neutrophil counts declined, but none repeatedly had leucocyte counts $<2.0 \times 10^9$/l or neutrophil counts $<1.0 \times 10^9$/l. Platelet counts at week 0 were $\geqslant 150 \times 10^9$/l in 17/20 subjects and >100 in all. At the end of the study period platelets were > 150 in 18 and > 100 in 19 subjects.

Non-hematological side effects In none of the 24 subjects (disease progressors included) did the treatment have an obvious effect on serum biochemical indices (creatinine, urea, electrolytes, liver enzymes). Serum folate levels did not change substantially during treatment. Vitamin B_{12} levels declined by more than 100 pg/ml in 12 subjects; in 5 cases vitamin B_{12} declined to levels around 150 pg/ml. Although none developed anemia at this level intramuscular vitamin B_{12} was given.

Beside symptoms of anemia none of the 24 men mentioned subjective adverse experiences of more than 2 weeks duration (De Wolf et al., 1988a). None developed clinical evidence of zidovudine-associated myopathy (Pinching et al., 1989; Helbert et al., 1988; Fischl et al., 1989) and except for subject 44, from group D, none ended with an elevated plasma level of creatinine phosphokinase (CPK). The plasma CPK level in subject 44 at week 72 was 124 U/l (normal value < 120 U/l). No CPK values had been obtained at the start of the study.

TABLE 3

Serum HIV-Ag levels and CD4+ cell counts in subjects who remained CDC II/III (treated $N=20$, untreated $N=8$), medians (and ranges)

	Serum HIV-Ag (pg/ml)			CD4+ cells ($\times 10^9$/l)		
	Entry	End*	% Change	Entry	End*	% Change
Group A	161	154	4 ↓	0.4	0.5	25 ↑
$N=5$	(66–566)	(46–4206)		(0.3–0.8)	(0.3–0.5)	
Group B	211	119	44 ↓	0.4	0.3	25 ↓
$N=4$	(96–465)	(55–194)		(0.2–0.5)	(0.2–0.5)	
Group C	276	272	1 ↓	0.4	0.25	37.5 ↓
$N=5$	(96–2336)	(53–489)		(0.2–0.7)	(0.05–0.4)	
Group D	475	189	60 ↓	0.5	0.55	10 ↑
$N=6$	(245–1890)	(69–815)		(0.3–1.4)	(0.1–1.0)	
All treated subjects	287	167	42 ↓	0.4	0.45	12.5 ↑
$N=20$	(66–2336)	(46–4206)		(0.2–1.4)	(0.05–1.0)	
Untreated subjects	81	105	30 ↑	0.6	0.45	25 ↓
$N=8$	(56–1700)	(54–2288)		(0.1–0.9)	(0.1–1.0)	

*Week 96 for groups A–C, week 72 for group D and week 92 for untreated subjects.

Non-treated subjects

In 6/14 subjects disease progression to CDC IV was noted. Four developed *Pneumocystis carinii* pneumonia at week 5, 72, 83 and 94 respectively, one AIDS-related complex (CDC IV-A) at week 60, and one severe HIV-related psoriasis (CDC IV-E) at week 31, for which he was treated with zidovudine; this last subject subsequently developed cryptococcal meningitis at week 102. Median serum HIV-Ag levels and CD4+ cell counts of these 6 progressors are depicted in Table 1. Median serum HIV-Ag levels and CD4+ cell counts of the non-progressors are depicted in Table 3.

TABLE 4

Hematological indices in treated subjects who remained CDC II/III ($N=20$), medians (and ranges)

	Week 0	End of study period*
Hb (g/dl)	14.8 (12.7–16.4)	14.3 (9.7–16.1)**
MCV (fl)	93.5 (83.5–97.9)	120.5 (95.4–132.8)
Leucocytes ($\times 10^9$/l)	5.8 (4.1–12.0)	4.9 (1.7–8.3)
Neutrophils ($\times 10^9$/l)	2.8 (1.6–7.8)	2.0 (0.8–5.8)
Lymphocytes ($\times 10^9$/l)	1.9 (1.0–7.1)	1.9 (0.5–4.6)
Platelets ($\times 10^9$/l)	191 (112–296)	203 (95–676)

*End of study period is week 104 for groups A–C and week 76 for group D; **, one subject who received 4 red cell transfusions included.

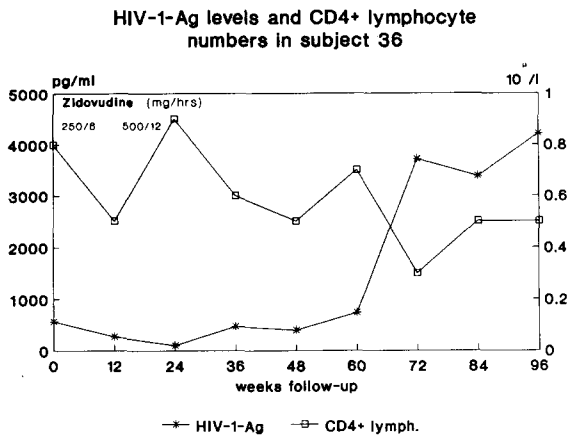


Fig. 4. Serum HIV-1 Ag levels and peripheral blood CD4+ lymphocyte counts in one treated subject (No. 36) who did not progress to AIDS.

Discussion

Whether zidovudine is effective in delaying disease progression in subjects with asymptomatic HIV infection can only be answered by large, randomised, placebo-controlled studies. A comparison of the disease progression rate of the treated with that of the untreated subjects in the current study suggests a beneficial effect of zidovudine, but these findings are of limited value as the study was not designed as an efficacy trial.

From our results it can be gathered that tolerance of zidovudine in asymptomatic HIV-infected subjects is such that long-term treatment is justifiable. Hematological toxicity compared favorably to that encountered in AIDS and ARC patients treated with zidovudine (Dournon et al., 1988; Stambuck et al., 1989; Williams et al., 1989; Pinching et al., 1989; Richman et al., 1987; Gill et al., 1987; Walker et al., 1988). Moreover, in no subject was clinical or convincing biochemical evidence of zidovudine-associated myopathy seen, whereas this has been reported to occur in 18–53% of AIDS and ARC patients who have been treated for more than 240 days (Pinching et al., 1989; Helbert et al., 1988; Fischl et al., 1989).

Reductions in serum HIV-Ag levels attained in the present study with four- and bi-daily dosing are similar to those attained with the currently recommended 4-hourly dosing schedules in AIDS and ARC patients (Dournon et al., 1988; Stambuck et al., 1989; Williams et al., 1989; Pinching et al., 1989; Chaisson et al., 1986; Jackson et al., 1988; Chaisson et al., 1988). It thus appears that both 250 mg 6-hourly and 500 mg 12-hourly are effective zidovudine starting doses, and that 500 mg 12-hourly is an acceptable maintenance dose.

The observation that with prolonged treatment serum HIV-Ag levels tend to rise again has also been made in AIDS and ARC patients with more frequent zidovudine dosing (Williams et al., 1989; Reiss et al., 1988). Recently it has been re-

ported that HIV isolates of AIDS and ARC patients who had received zidovudine for 6 months or longer showed reduced sensitivity to this drug (Larder et al., 1989). Although in that study appearance of such isolates with reduced sensitivity was not associated with a consistent increase in serum HIV-Ag levels, numbers were too small for definitive conclusions. It may be that the rising serum HIV-Ag levels observed in the present study do reflect the emergence of HIV strains with reduced sensitivity to zidovudine. After an initial decline, levels of unintegrated viral DNA in peripheral blood mononuclear cells from a number of these subjects, both with stable and with rising serum HIV-Ag levels, had returned to pretreatment values at week 72 (S. Jurriaans et al., submitted for publication), suggesting a decreased efficacy of zidovudine in inhibiting reverse transcriptase.

Disease progression occurred in 4/24 treated subjects, despite sustained reduction of serum HIV-Ag levels in these 4 men. Such reduction can thus not be equaled to clinical efficacy. The median CD4+ cell count at study entry in the 4 disease progressors was 0.2×10^9/l (range 0.2–0.3 $\times 10^9$/l), whereas it was 0.4×10^9/l (range 0.2–1.4 $\times 10^9$/l) in the 20 non-progressors. Whether this means that zidovudine is most efficacious in preventing disease progression in those with higher CD4+ cell counts is unclear, since without treatment the disease progression rate would also be expected to be highest in those with the lowest CD4+ cell numbers (De Wolf et al., 1988b; Moss et al., 1988), as was indeed the case in the untreated group.

The limited duration of clinical and immunological benefits of zidovudine in AIDS and ARC patients (Fischl et al., 1987a; Fischl et al., 1987b; Dournon et al., 1988; Stambuck et al., 1989; Williams et al., 1989; Pinching et al., 1989), and the disease progression observed in the present study could hypothetically be due to the emergence of zidovudine-resistant HIV strains. This also implies that in order to maintain the activity of zidovudine the emergence of drug-resistant virus variants should be suppressed. This goal might be obtained by combination therapy (Larder et al., 1989). One of the agents found to potentiate the anti-HIV activity of zidovudine in vitro is the anti-herpetic drug acyclovir (Mitsuya et al., 1987). In the present study there was no obvious virological or clinical benefit obtained with the addition of acyclovir. Acyclovir alone does not exhibit anti-retroviral activity (Mitsuya et al., 1987). Future clinical trials in both symptomatic and asymptomatic patients should focus on more promising additive or synergistic combinations of anti-retroviral drugs, as any effective agent when given alone might eventually lead to the emergence of resistance.

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