Evaluation of a 6-Year Highly Active Antiretroviral Therapy in Chinese HIV-1-Infected Patients

Hua-ying Zhou Yu-huang Zheng Yan He Zi Chen Meng Liu Wei Yin
Chun Liu

AIDS Research Laboratory, Department of Infectious Diseases, Second Xiangya Hospital, Central South University, Changsha, China

Key Words

Antiretroviral therapy · Chinese HIV patients · HIV-1 treatment · Long-term antiretroviral therapy · Nevirapine · Tolerability

unexpected long-term toxicity. Though based on a small group, this study demonstrates durable effects of ART in Chinese patients.

Copyright © 2010 S. Karger AG, Basel

Abstract

Objective: To evaluate the long-term efficacy and tolerability of nevirapine (NVP)-based regimens in the treatment of human immunodeficiency virus (HIV)-infected Chinese patients in routine clinical practice. **Methods:** From October 2002 to May 2004, 57 HIV-1-infected patients commenced antiretroviral therapy (ART), and were followed up to December 2008. These antiretroviral-naïve patients, who originally received two nucleoside reverse transcriptase inhibitors and NVP, had HIV RNA levels, T lymphocyte subsets and safety parameters assessed over 6 years. **Results:** Of the 57 patients, 34 patients participated in the long-term follow-up. After 5–6 years, >60% of the patients had HIV RNA levels <50 copies/ μ l, and the median increase in CD4 cell counts from baseline was 329 cells/ μ l. γ -Glutamyl transferase increased in 17 patients (29.8%); serum cholesterol and triglyceride levels were elevated in 15 patients (26.3%), and 25.0% (6/24) of the patients developed lipodystrophy (mainly females). Grade 3/4 adverse events occurred in 3 cases. **Conclusion:** ART with NVP-based regimens suppressed HIV viremia and produced continued CD4 cell increases in a majority of subjects for 6 years. Safety and tolerance were good with no

Introduction

Infection with human immunodeficiency virus (HIV)-1 results in a progressive loss of the number and function of CD4⁺ lymphocytes, which is associated with the risk of developing opportunistic infections and neoplasms. The use of highly active antiretroviral therapy (HAART) as the standard treatment for HIV infection has led to a dramatic decline in HIV-associated morbidity and mortality [1, 2]. The success of current regimens has transformed HIV infection into a chronic condition requiring management over the course of years and decades. Given that HIV infection is chronic and that exposure to HAART is likely to be lifelong, there is a need to evaluate the long-term effects of HAART on this population.

We previously reported the short-term (from 12 to 24 months) effectiveness of HAART including two nucleoside reverse transcriptase inhibitors [NRTIs (stavudine, d4T, plus didanosine, ddI)] and one non-NRTI [NNRTI (nevirapine, NVP)] [3–6]. The data indicated that the regimen had great antiviral potency, immunological bene-

fits and was associated with good tolerance. However, long-term outcomes have not been well described in China. That study was subsequently amended to increase the enrollment, and the duration of treatment was increased to about 6 years. In this paper, we first report the results of the long-term treatment of 57 antiretroviral (ARV) drug-naïve Chinese patients for 6 years.

Patients and Methods

Patients and Control Subjects

From October 2002 to May 2004, 57 HIV-infected patients commenced antiretroviral therapy (ART) and were followed up to December 2008; 39 men and 18 women, aged 12–68 years (average 30.5 years), were treated at the Second Xiangya Hospital, Central South University, Hunan Province, China. They were offered HAART according to the Chinese Guidelines on ART. Thirty-one HIV-seronegative healthy persons participated as control subjects for flow cytometry.

Patients who were pregnant or breastfeeding, or had an absolute neutrophil count $<4.0 \times 10^9/l$, platelet count $<90 \times 10^9/l$, hemoglobin <90 g/l, abnormal γ -glutamyl transferase (GGT) and amylase levels, or severe renal, hepatic or cardiac disease were not included in the study.

To be eligible, HIV-1-infected patients also had to have acquired immunodeficiency syndrome (AIDS)-defining illness and/or CD4+ cell count <350 cells/ μl , and to be naïve for ARV drugs. All patients gave voluntary written informed consent. The protocol and informed consent forms were approved by independent institutional ethics committees or institutional review boards.

Drug Treatment

In this original study, 57 HIV-infected patients were assigned to one of three regimens: (i) NVP plus zidovudine (AZT; 300 mg twice daily) with lamivudine (3TC; 300 mg once daily; $n = 9$); (ii) NVP plus d4T (40 mg twice daily) with ddI (200 mg twice daily; $n = 38$); or (iii) NVP plus d4T (40 mg twice daily) with lamivudine (300 mg once daily; $n = 10$). They received NVP (200 mg orally once daily) for the first 2 weeks as the lead-in dose, in the absence of rashes or hepatic damage, followed by 200 mg twice daily. Patients were allowed to change their regimen if the plasma HIV-1 RNA levels were repeatedly $<1,000$ copies/ml or if they proved intolerant. These drugs (single formulations) are manufactured by Desano (Shanghai, China).

The primary end point of the study was defined as virologic suppression, i.e. a plasma viral load <50 copies/ml, and the mean change in CD4+ cell count over the 6-year therapy period. The primary safety criterion was the proportion of patients who experienced an adverse event of grades 2–4.

Patient Visits

Before initiation, patients had the following laboratory tests: chemistry, hematology, a chest radiograph, CD4+ cell count, plasma HIV-1 RNA level and syphilis serology, with the initial group of consecutively screened patients undergoing additional serologic testing for hepatitis B and C, toxoplasmosis, and cytomegalovirus infection or exposure. Patients were also screened for active

opportunistic infections, such as tuberculosis and cytomegalovirus retinitis, if indicated. Patients found to have syphilis, HIV-associated eye disease, or active tuberculosis were treated in accordance with national standards.

All patients were scheduled to return 1 month after HAART initiation for review; when patients missed two consecutive visits, two or more attempts were made to contact them before considering them lost to follow-up.

Medication Adherence

The success of these medications, however, depends on their regular and appropriate use. Multiple factors influence adherence, including adverse effects of the medication, the number of pills required, dosing schedules and costs. Patients suffering from cognitive impairment may simply forget to take their medication on a regular basis. Patients who were qualified for HAART were encouraged to designate a family member or friend as an assistant to aid with ARV medication adherence and toxicity recognition. Once HAART was initiated, patients returned to the hospital at monthly intervals for medical evaluation, adherence education, and medication refills. Adherence to ART was assessed by pill counting.

Quantification of HIV-1 RNA

Plasma HIV-1 RNA viral load was measured by fluorescence quantitative polymerase chain reaction (PCR) before treatment and after 6, 12, 18, 24, 36, 48, 60 and 72 months of treatment. Within 1 h of blood collection in EDTA Vacutainer tubes (BD Diagnostic Systems, Sparks, Md., USA), peripheral blood plasma was separated by routine methods and stored at -80° . HIV-1 viral load was measured in thawed plasma samples by quantitative RT-PCR assay (Shenzhen PG Biotech/Qiagen, Shenzhen, China) according to the protocol provided with the test kit, using a GeneAmp 7300 system (Applied Biosystems, Foster City, Calif., USA). The available range of detection was 50–10,000,000 HIV-1 RNA copies/ml of plasma.

Lymphocyte Subsets

For quantitative determination of CD4+ lymphocytes, CD8+ lymphocytes, naïve lymphocytes (CD4+CD45RA+CD62L+), memory lymphocytes (CD4+CD45RO+) and activated lymphocytes (CD8+CD38+), venous blood samples were collected into EDTA Vacutainer tubes at baseline and 6, 12, 18, 24, 36, 48, 60 and 72 months after the start of therapy. Blood (100 μl) was incubated with 20 μl of CD3 peridinin chlorophyll protein, CD4 phycoerythrin (PE), CD8-PE, CD45RA fluorescein isothiocyanate (FITC), CD45RO-FITC, CD38-FITC or CD62L-cyanine 5 (Cy5) monoclonal antibody (BD Biosciences, San Jose, Calif., USA) for 15 min at 20° in the dark, lysed with BD FACS lysing solution (BD Biosciences) and centrifuged at 1,500 rpm. The cells were washed twice with phosphate-buffered saline and the supernatant was discarded. A two- or three-color protocol was then used to analyze the directly labeled lymphocytes with a BD FACSCalibur flow cytometer (BD Biosciences). At least 10,000 cells were counted. FITC-, Cy5- and PE-conjugated isotypically matched IgGs were used as negative controls.

Side Effects and Adverse Events

During treatment, the authors kept in close contact with the patients. Side effects or opportunistic infections were addressed

Table 1. Description of the study population

Total patients, n	57
Male/female ratio	39/18
Status at HAART initiation	
Median age, years	30.5 (22.5–56.3)
Median known duration of HIV infection, years	3.2 (2.1–6.2)
Median duration of follow-up on HAART, months	64.2 (49.2–69.0)
HAART M0	
Median CD4 count, 10 ⁶ /l	134 (12–247)
Median plasma HIV RNA, log ₁₀ copies/ml	4.57 (3.50–6.02)
Patients with lipodystrophy at HAART M72, %	25.0

Ranges and interquartile ranges (median age) are given in parentheses. M = Month.

and, if necessary, therapy adjusted or terminated. Events noted were gastrointestinal side effects (nausea, vomiting and diarrhea), peripheral neuropathy (paresthesia and pain), skin rash, CNS disorders (dizziness, insomnia and drowsiness) and other side effects such as abdominal pain, fullness or bloating and hair loss. Visits were performed at least every 4 weeks through month 3, every 12 weeks through year 1, every 6 months through year 2 and every 12 months through year 6, obtaining detailed information on gastrointestinal side effects (nausea, vomiting and diarrhea), peripheral neuropathy (paresthesia and pain), various rashes, CNS disorders (dizziness, insomnia and drowsiness) and other side effects such as abdominal pain, fullness, bloating or hair loss, and their degrees. Lipodystrophy was defined as the presence of lipoatrophy of the face, limbs or buttocks, or fat accumulation in the neck, breast or abdominal area assessed both by physician and patient (two items required). Presence of adverse events and the management thereof, as well as adherence to medication, were recorded.

A questionnaire was developed to collect data from subjects who discontinued the study before 6 years, including the last ARV regimen taken, the approximate HIV-1 RNA level and CD4 cell count at the 5- to 6-year assessment, and the date/cause of death (if applicable).

Other Laboratory Tests

Routine blood and urine tests, liver and renal function tests, blood lipid level, amylase and GGT levels were determined 2 weeks before treatment, at baseline, and 2 weeks and 1, 3, 6, 12, 18, 24, 36, 48, 60 and 72 months after starting treatment.

Statistical Analyses

Data were analyzed with SPSS 13.0 (SPSS, Chicago, Ill., USA). Differences between groups (years 5–6 and baseline) were considered significant at $p < 0.05$. Data were expressed as means $\pm$ SD. Group comparisons were made by a paired-sample *t* test.

Results

Patient Evaluation and Treatment

Table 1 shows the characteristics of the 57 study patients on HAART for a median of 64.2 months (range,

48–73). Of the 57 subjects, 34 (60%) patients had been followed for at least 48 months. Initial HAART regimens consisted of two NRTIs with one NNRTI (NVP). During follow-up, 25 patients receiving ddI+d4T+NVP changed their primary regimens as follows: 15 patients had to switch to LAM+AZT+NVP and 10 to LAM+d4T+NVP (median time of switch, 24 months; interquartile range, 16–30), and 3 (5.2%) to a protease inhibitor (Kaletra)-based regimen. Reasons for changing therapy included drug toxicity (ddI) and treatment failure in 3. At the time of analysis, 31 (54.4%) patients were still on the NVP-based regimen. No subjects took IL-2 or other adjunctive immunotherapies. The rate of adherence was >95% in the patients.

During the 6-year follow-up, 8 were transferred to other centers (weeks 98, 101, 117, 124, 130, 140, 148 and 150) and 8 (14%) died, all deaths occurred among severely immunosuppressed patients with baseline CD4 cell counts <50 cells/ μ l, who already had AIDS at inclusion, within the 1st year after enrollment. Seven discontinued study therapy: reasons were loss to follow-up in 3 patients, poor compliance in 2 patients and miscellaneous reasons in another 2 patients.

Virologic Outcomes

In the intention-to-treat analysis, HIV-1 RNA levels were <50 copies/ml in 72.3% at 1 year, 56.1% at 2 years, 55.6% at 3 years, 55.9% at 4 years, 62.5% at 5 years and 75.0% at 6 years. The HIV-1 RNA viral load decreased from a mean of 5.37 log copies/ml at baseline to 2.01 log copies/ml after 12 months, 2.14 log copies/ml after 24 months, 2.57 log copies/ml after 36 months, 2.35 log copies/ml after 48 months, 2.13 log copies/ml after 60 months and 2.17 log copies/ml after 72 months on HAART ($p = 0.003$; table 2). In total, 3 subjects had virological failure: 1 in year 2 and 2 in year 4. Genotypic testing was obtained

Table 2. Efficacy of 6-year ART

	Baseline	Month 6	Month 12	Month 18	Month 24	Month 36	Month 48	Month 60	Month 72	Control	p value years 5–6 vs. baseline
Patients studied, n	57	52	47	43	41	36	34	24	12	31	
HIV RNA, log copies/ml	5.37	2.23	2.01	1.95	2.14	2.57	2.35	2.13	2.17	–	0.003
<50 copies/ml	0	36 (69.2%)	34 (72.3%)	26 (60.5%)	23 (56.1%)	20 (55.6%)	19 (55.9%)	15 (62.5%)	9 (75.0%)	–	0.001
CD3+CD4+, cells/ μ l	134 $\pm$ 84	225 $\pm$ 137	289 $\pm$ 113	311 $\pm$ 174	337 $\pm$ 135	363 $\pm$ 133	401 $\pm$ 127	436 $\pm$ 135	463 $\pm$ 149	863 $\pm$ 263	0.005
CD3+CD8+, cells/ μ l	911 $\pm$ 427	823 $\pm$ 294	765 $\pm$ 317	758 $\pm$ 297	769 $\pm$ 303	793 $\pm$ 207	785 $\pm$ 127	779 $\pm$ 167	783 $\pm$ 127	512 $\pm$ 195	0.0015
CD4+CD45RA+62L+ cells/ μ l	49 $\pm$ 21	87 $\pm$ 27	123 $\pm$ 35	129 $\pm$ 44	137 $\pm$ 65	147 $\pm$ 33	161 $\pm$ 27	179 $\pm$ 57	183 $\pm$ 38	404 $\pm$ 95	0.0022
CD4+CD45RO+, cells/ μ l	107 $\pm$ 57	149 $\pm$ 64	178 $\pm$ 77	193 $\pm$ 66	205 $\pm$ 85	257 $\pm$ 71	283 $\pm$ 63	312 $\pm$ 58	325 $\pm$ 67	428 $\pm$ 149	0.0013
CD8+CD38+, cells/ μ l	611 $\pm$ 297	403 $\pm$ 175	416 $\pm$ 155	391 $\pm$ 117	381 $\pm$ 131	365 $\pm$ 145	372 $\pm$ 119	383 $\pm$ 107	379 $\pm$ 113	212 $\pm$ 94	0.0027
CD8+CD38+/CD3+CD8+	(68.5%)	(51.2%)	(53.7%)	(51.7%)	(48.9%)	(46.3%)	(47.5%)	(49.7%)	(48.3%)	(40.8%)	

Lymphocyte subpopulations (means $\pm$ SD) and HIV-1 RNA after 5–6 years of treatment; 57 patients received HAART, and 23 discontinued study therapy before 5 years for the following reasons: transferral to other centers (n = 8), miscellaneous reasons (n = 2), unknown (n = 3), poor compliance (n = 2) and death (n = 8). Controls are HIV-seronegative health workers.

for 2 of the 3 patients who experienced virological failure; both demonstrated emergence of the resistance mutation for NRTI (mutation K70R and L210W in reverse transcriptase).

Of the 8 patients transferred to other centers, 4 subsequently had HIV-1 RNA <50 copies/ml at 5–6 years on other ARV regimens; 2 patients had HIV-1 RNA between 50 and 1,000 copies/ml and 2 had no virological data available. In the analysis of all 57 subjects combining on-study and 8 post-study data (5- to 6-year follow-up for 24 and last available measurement for 6), 19 (63.3%) had HIV-1 RNA levels <50 copies/ml.

Immune Restoration

The CD4+ T-cell count continued to increase over time, with a mean gain of 155, 177, 203, 229, 267, 302 and 329 cells/ μ l at the 12-, 18-, 24-, 36-, 48-, 60- and 72-month follow-up, respectively. From baseline to year 2, CD4+ T-cell counts increased an average of 102 cells/ μ l per year. Notably, the median change in the CD4+ T cell count from baseline to year 1 was +155, whereas the average yearly change from year 2 to year 6 was +32 cells/ μ l ($p < 0.05$). During the study period, no subject had a confirmed CD4+ T-cell count >900 cells/ μ l; 3 subjects had confirmed CD4+ T-cell counts >700 cells/ μ l. Total CD8+ T-cell counts rapidly decreased from 911 $\pm$ 427 cells/ μ l at baseline to 765 $\pm$ 317 cells/ μ l after 12 months, 769 $\pm$ 303 cells/ μ l after 24 months, 793 $\pm$ 207 cells/ μ l after 36 months, 785 $\pm$ 127 cells/ μ l after 48 months, 779 $\pm$ 167 cells/ μ l after 60 months and 783 $\pm$ 127 cells/ μ l after 72 months of HAART ($p = 0.0015$; table 2). There is a sig-

nificant difference between groups (years 5–6 and control subjects; $p < 0.001$).

The number of peripheral blood memory (CD45RO+) CD4+ lymphocytes increased from baseline (107 $\pm$ 57 cells/ μ l) to year 2 (205 $\pm$ 85 cells/ μ l) and year 6 (325 $\pm$ 67 cells/ μ l; $p = 0.0013$). In addition, the number of naïve (CD45RA+CD62L+)CD4+ T lymphocytes increased significantly ($p = 0.0022$); the median year-3 to year-6 increases were 98 and 134 cells/ μ l, respectively (table 2). The proportion of activated CD8+ T lymphocytes (CD38+) significantly decreased: at baseline, the mean proportion of activated CD8+ T lymphocytes exceeded 60% (68.5%) and gradually decreased over 72 months, reaching 48.3% ($p = 0.0027$).

Safety and Tolerance

Of the 57 patients, 32 (56%) HIV-infected patients developed various drug-related side effects (tables 3, 4). The incidence, frequency and severity of adverse events were consistent with those established for the study drugs. The incidence of treatment-related adverse events (grade 2 or higher) within the first 24 weeks of therapy was highest in patients receiving ddI+d4T+NVP (>50%). After 24 weeks, the incidence of these symptoms was comparable in all groups. The most common adverse event was nausea, followed by vomiting and diarrhea. Other events were insomnia, headache, rashes, fatigue, increased GGT and other impairments. After 1 month, 2 patients experienced slight baldness without folliculitis. It improved on ART without additional therapy or change in HAART regimen. Serum cholesterol and triglyceride elevations

Table 3. Main side and adverse effects during 6 years of HAART

	Gastro-intestinal	Peripheral neuropathy	Rash	Abdominal pain/bloating	CNS disorders	Fever	Baldness
Grades 1–2, n	17	8	7	3	3	5	2
Grade 3, n	3	2	1	1	0	0	0
Incidence, %	55.5	27.8	22.2	11.1	8.3	13.9	5.6

A total of 2 patients permanently discontinued study medication because of peripheral neuropathy.

Table 4. Main laboratory parameter changes in the study patients during 6 years of HAART

	WBC	Hb	Pt	Trans.	T _{bil}	GGT	Amy.	BUN	TG	Chol.
Grade 1, n	11	14	8	8	5	14	0	2	9	8
Grade 2, n	7	5	6	2	0	3	2	1	6	4
Grade 3, n	0	0	0	0	0	0	1	0	1	0

Amy = Amylase; BUN = blood urea nitrogen; Chol. = cholesterol; Hb = hemoglobin; Pt = prothrombin time; T_{bil} = total bilirubin; TG = triglycerides; Trans. = transaminase. Most laboratory parameters were grade 1 or 2. One patient discontinued study medication because of suspected lactic acidosis (GGT and amylase elevation).

occurred in 15 patients (26.3%); 25.0% (6/24) of the patients developed lipodystrophy, mainly female patients. GGT increased in 17 patients (29.8%). Most adverse events were mild to moderate (grade 1 or 2), occurring most often within the first 24 weeks of therapy. Symptoms were usually transient and not limiting treatment. Grade 3/4 adverse events occurred in 3 cases taking ddl+d4T+NVP (peripheral neuropathy after 5 and 6 months in 2 patients and suspected lactic acidosis after 8 months in 1 patient). One subject experienced grade 3 rash (after 4 months) and was treated symptomatically with antihistamine without discontinuation of NVP.

Discussion

Sustained virological and immunological responses are key goals of ART. HIV is a chronic disease that is likely to require prolonged treatment with ART. Assessment of durability of ART and long-term safety have been published in some countries [7–13]. However, to date, reports on the long-term efficacy and tolerance of HAART in Chinese patients are scarce [4]. Here we report the 6-year results from the longest prospective follow-up of Chinese HIV-infected patients taking potent ART.

Our study demonstrated the long-term efficacy of NVP-based HAART in HIV-infected Chinese patients, with CD4 counts still increasing beyond 6 years of treatment; 54.5% of patients were still treated on NVP-based HAART regimen after 4–6 years, and >60% had a plasma viral load <50 copies/ml after 5–6 years. These data support the World Health Organization recommendation to use the NNRTI-based HAART regimen as a first-line regimen in resource-limited settings [14]. This study was not designed to compare the three treatment regimens. Our results are consistent with other reports. In a study of 33 patients followed for a median period of 6 years on the triple combination of indinavir, zidovudine and lamivudine, combining on-study and post-study data (5–6 years), 58% had HIV-1 RNA levels <50 copies/ml [15].

The results from this study imply that the number of CD4+ T lymphocytes will continue to increase for at least 6 years after starting HAART. After an initial biphasic effect, the CD4 cell count continues to rise at a steady rate, but the rate of this increase slowed after 2 years, and the CD4+ cell reached a plateau, in agreement with other published results [16, 17]. Hunt et al. [18] presented strong evidence that CD4+ counts continue to increase up to 4 years after initiation of HAART. However, the rate of CD4+ recovery in adults is slow and a steady state is not

usually reached after 4 years most likely because the thymus is less functional in adults than in children [19]. It is notable, however, that despite improvements in absolute, naïve and memory CD4 cell counts and percentages, in the majority of subjects CD4 cell subset levels were below those seen in HIV-negative subjects.

In this study, 8 of the 57 subjects (14%) died. All deaths occurred within the 1st year after enrollment among severely immunosuppressed patients with baseline CD4 cell counts <50 cells/ μ l who already had AIDS at study inclusion, demonstrating that patients with low baseline CD4 counts are at increased risk of acute morbidity and mortality. Patients who survive the initial months of ART and fully suppress the viral load have good chances of immunological recovery during the 1st year.

Drug-related toxicities continue to be an important issue. In our study, 32 (56%) HIV-infected patients developed various drug-related side effects. Other clinical trials reported that 7% of patients discontinued NVP treatment following rashes. Our study also showed that the main adverse events associated with the NVP-based regimen are gastrointestinal symptoms and rashes, but the rate of NVP-associated rash was relatively low, and did not result in discontinuation of treatment. After 4 months, 1 patient experienced a grade-3 rash and was treated symptomatically with antihistamine but did not discontinue NVP. It is possible that this was not an NVP-related rash, but an immune reconstitution phenomenon of dermatitis or pruritic papular eruptions. At the 1-month follow-up, 2 patients experienced baldness. According to the Chinese description of NNRTI, baldness can occur during treatment with 3TC, and it has been reported in China previously [20]. Since it improved on ART, it is possible that this was HIV related. Of the patients, 25.0% (6/24) demonstrated lipodystrophy at 5–6 years, being lower than generally observed among HAART-treated patients from developed countries [13]. Lipodystrophy rates of up to 83% have been reported [21]. In fact, such differences may be related to differences in the study populations and regimens. Grade 3/4 adverse events occurred in 3 cases taking ddI+d4T+NVP (peripheral neuropathy after 5–6 months, $n = 2$, and suspected lactic acidosis after 8 months, $n = 1$), and treatment was changed to AZT+3TC+NVP. We diagnosed lactic acidosis mainly through gastrointestinal symptoms, increased lactate dehydrogenase, amylase, pH and GGT, as lactate tests were not available at the time. One of the patients developed suspected lactic acidosis and discontinued medication; treatment was changed to AZT+3TC+NVP. Because of the increasing recognition of adverse events on ddI+d4T,

e.g. mitochondrial toxicity, lipodystrophy, peripheral neuropathy and hepatotoxicity, d4T plus ddI is no longer recommended as first-line regimen in China. During follow-up, patients receiving ddI+d4T+NVP changed their primary regimens as follows: 15 patients had to switch to LAM+AZT+NVP, and 10 patients to LAM+d4T+NVP (median time of switch, 24 months; interquartile range, 16–30).

There are limitations to this study, namely selection bias and the small number of patients. Also, some information regarding minor toxicity, concentration of ARV drugs and adherence to therapy might not have been recorded with the highest accuracy. Despite these limitations, the current study provides significant data on the long-term benefits and risks of ART in Chinese patients.

In conclusion, this study demonstrates extended virologic and immunologic responses to NVP-based HAART in Chinese patients. As in western countries, HAART prolongs survival in HIV-1-infected patients. In our cohort, approximately >60% of the patients had undetectable HIV-1 RNA levels throughout the whole follow-up period, and CD4 values recovered and remained stable, too. However, in China, large-scale studies assessing the effectiveness of HAART in the long term are further required to demonstrate definitively the feasibility of these treatments.

Acknowledgments

The authors acknowledge the participation of the following members of the Division of Infectious Diseases: Huang Li, Tang Jiang-se and Zhang Jin, who helped collect the case data and blood samples in the experiments.

This study was supported by Grants from the Ministry of Health and the National AIDS Research Foundation of China (WA2003_15).

References

- 1 Murphy EL, Collier AC, Kalish LA, Assmann SF, Para MF, Flanigan TP, Kumar PN, Mintz L, Wallach FR, Nemo GJ: Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. *Ann Intern Med* 2001;135:17–26.
- 2 Van Sighem AI, Van De Wiel MA, Ghani AC, Jambroes M, Reiss P, Gyssens IC, Brinkman K, Lange JM, de Wolf: Mortality and progression to AIDS after starting highly active antiretroviral therapy. *AIDS* 2003;17:2227–2236.

- 3 Zhou HY, Zheng YH, Yang X, Gong GZ, Chang HW, Zhong FY, Zou W: A Twenty-four week clinical trial using didanosine, stavudine and nevirapine for highly active antiretroviral therapy. *J Chin Physician* 2003;5:1592-1595.
- 4 Zhou HY, Zheng YH, Zhang CY, Ding PP, Zou W: A one-year clinical trial using didanosine, stavudine and nevirapine for highly active antiretroviral therapy. *Chin Med J (Engl)* 2005;118:609-611.
- 5 Zhou HY, Zheng YH, Zhang CY, et al: Evaluation of human immunodeficiency virus type 1-infected Chinese patients treated with highly active antiretroviral therapy for two years. *Viral Immunol* 2007;20:180-187.
- 6 Zheng YH, Cheng J, Lei JH, Lai LY, Zhu YH, He Y, Luo HY, Zhou HY, Ding PP, Zhang CY, Li H: Immunological change in HIV-1-infected patients treated with highly active antiretroviral therapy. *Chin J Dermatol* 2006;39:42-46.
- 7 Valdez H, Connick E, Smith KY, Lederman MM, Bosch RJ, Kim RS, St Clair M, Kuritzkes DR, Kessler H, Fox L, Blanchard-Vargas M, Landay A: Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS* 2002;16:1859-1866.
- 8 Ungsedhapand C, Srasuebku P, Cardiello P, Ruxrungtham K, Ratanasuwan W, Kroon ED, Tongtalung M, Juengprasert N, Ubo-lyam S, Siangphoe U, Emery S, Lange JM, Cooper DA, Phanuphak P: Three year durability of dual-nucleoside versus triple-nucleoside therapy in a Thai population with HIV infection. *J Acquir Immune Defic Syndr* 2004;36:693-701.
- 9 Gulick RM, Mellors JW, Havlir D, Eron JJ, Meibohm A, Condra JH, Valentine FT, McMahon D, Gonzalez C, Jonas L, Emini EA, Chodakewitz JA, Isaacs R, Richman DD: Three-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. *Ann Intern Med* 2000;133:35-39.
- 10 Resino S, Resino R, Micheloud D, Gurbindo Gutiérrez D, León JA, Ramos JT, Ciria L, de José I, Mellado J, Muñoz-Fernández A: Long-term effects of highly active antiretroviral therapy in pretreated, vertically HIV type 1-infected children: 6 years of follow-up. *Clin Infect Dis* 2006;42:862-869.
- 11 Viard JP, Burgard M, Hubert JB, Aaron L, Rabian C, Pertuiset N, Lourenço M, Rothschild C, Rouzioux C: Impact of 5 years of maximally successful highly active antiretroviral therapy on CD4 cell count and HIV-1 DNA level. *AIDS* 2004;18:45-49.
- 12 Smith K, Aga E, Bosch RJ, Valdez H, Connick E, Landay A, Kuritzkes D, Gross BH, Francis IR, McCune JM, Kessler H, Lederman M: Long-term changes in circulating CD4 T lymphocytes in virologically suppressed patients after 6 years of highly active antiretroviral therapy. *AIDS* 2004;18:1953-1956.
- 13 Patel K, Hernán MA, Williams PL, Seeger JD, McIntosh K, Van Dyke RB, Seage GR 3rd: Long-term effectiveness of highly active antiretroviral therapy on the survival of children and adolescents with HIV infection: a 10-year follow-up study. *Clin Infect Dis* 2008;46:507-515.
- 14 World Health Organization (WHO): Scaling Up Antiretroviral Therapy in Resource-Limited Settings: Treatment Guidelines for a Public Health Approach - 2006 revision. Geneva, WHO, 2006. <http://www.who.int/hiv/pub/guidelines/art/en/index.html> (accessed November 9, 2006).
- 15 Gulick RM, Meibohm A, Havlir D, Eron JJ, Mosley A, Chodakewitz JA, Isaacs R, Gonzalez C, McMahon D, Richman DD, Robertson M, Mellors JW: Six-year follow-up of HIV-1-infected adults in a clinical trial of antiretroviral therapy with indinavir, zidovudine, and lamivudine. *AIDS* 2003;17:2345-2349.
- 16 Kaufmann GR, Bloch M, Finlayson R, Zaunders J, Smith D, Cooper DA: The extent of HIV-1-related immunodeficiency and age predict the long-term CD4 T lymphocyte response to potent antiretroviral therapy. *AIDS* 2002;16:359-367.
- 17 Tarwater PM, Margolick JB, Jin J, Phair JP, Detels R, Rinaldo C, Giorgi J, Muñoz A: Increase and plateau of CD4 T-cell counts in the 3(1/2) years after initiation of potent antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001;27:168-175.
- 18 Hunt PW, Deeks SG, Rodriguez B, Valdez H, Shade SB, Abrams DI, Kitahata MM, Krone M, Neilands TB, Brand RJ, Lederman MM, Martin JN: Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. *AIDS* 2003;17:1907-1915.
- 19 Franco JM, Leon-Leal JA, Leal M, Cano-Rodriguez A, Pineda JA, Macias J, Rubio A, Rey C, Sanchez B, Lissen E: CD4+ and CD8+ T lymphocyte regeneration after anti-retroviral therapy in HIV-1-infected children and adult patients. *Clin Exp Immunol* 2000;119:493-498.
- 20 Shu-Yun Wu: Seborrheic baldness due to lamivudine. *Adverse Drug Reactions J* 2005;3:230.
- 21 Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA: Diagnosis, prediction, and natural course of HIV-1 protease inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999;353:2093-2099.