Impact of HIV drug resistance on virologic and immunologic failure and mortality in a cohort of patients on antiretroviral therapy in China

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Objectives: To study the dynamics of HIV drug resistance (HIVDR) and its association with virologic and immunologic failure as well as mortality among patients on combination antiretroviral therapy (cART) in China.

Design: We recruited 365 patients on cART in two rural Chinese counties in 2003–2004 and followed them every 6 months until May 2010.

Methods: Virologic failure, HIVDR, immunologic failure and death were documented. We used Kaplan–Meier and the proportional hazards models to identify the timing of the events, and risk factors for mortality.

Results: At the end of study, patients had been followed for 1974.3 person-years, a median of 6.1 years. HIVDR mutations were found in 235 (64.4%) patients and 75 died (20.5%, 3.8/100 person-years). Median time from cART to detection of virologic failure was 17.5 months, to HIVDR 36.6 months and to immunologic failure 55.2 months (≈18-month median interval between each adverse milestone). Being male, having a baseline CD4$^+$ cell count of less than 50 cells/μl and HIVDR were associated with higher mortality. Patients who developed HIVDR in the first year of treatment had higher mortality than those developing HIVDR later (adjusted hazard ratio 1.90, 95% confidence interval 1.01–3.48).

Conclusion: HIVDR was common and was associated with higher mortality among Chinese patients on cART, particular when HIVDR was detected early in therapy. Our study reinforces the importance of improving patient adherence to cART in order to delay the emergence of HIVDR and obviate the need to switch to costly second-line drug regimens too early.   © 2013 Creative Common License

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Introduction

Since 1996, combination antiretroviral therapy (cART) has prolonged the lifespan of AIDS patients dramatically and has reduced HIV-related morbidity [1]. Except for a few countries such as Brazil and South Africa with earlier cART introduction [2,3], most developing countries began cART after the 2003 recommendation of the WHO 'Three by Five' goal in the scale-up of national ART programmes [4]. The subsequent rapid emergence of HIV drug-resistant mutants with their potential threat of transmission caused great concern for the long-term benefits and sustainability of cART roll-out in resource-limited countries [5]. However, studies conducted to date in resource-limited settings have studied the emergence of HIV drug resistance (HIVDR) and long-term outcomes only through short-term (typically 12 months) follow-up [6,7].

After a pilot cART programme in 2002, China scaled up the National Free Antiretroviral Treatment Program to provide free cART to HIV-infected patients in 2003 [8]. Initially, most patients entering the program were former plasma donors (FPDs) who acquired HIV infection in the mid-1990s through unhygienic plasmapheresis procedures in central China [9]. Initial first-line regimens consisted of didanosine (ddI) with either zidovudine (ZDV) or stavudine (d4T) as the second drug and nevirapine (NVP) as the third. Didanosine was replaced by lamivudine (3TC) around 2005. Treatment was predominantly conducted in village clinics [8] with almost no access to second-line drugs. In this study, we investigate the emergence of HIVDR and its effect on mortality in the initial group of cART-treated patients in rural, central China followed up to 6 years.

Materials and methods

Study population

An observational cohort study enrolled patients from Queshan County, Henan Province and Fuyang County, Anhui Province from December 2003 to December 2004. Patients who were 18 years of age or older and started cART between 2003 and 2004 were included. All patients approached through informed consent agreed to participate in the study. Patients were followed every 6 months up to 31 May 2010, or to stopping cART, death or loss to follow-up. Data on demographics and risk factors for HIV infection were collected at baseline and information on treatment and outcomes was collected at every follow-up visit, and confirmed with the National cART Database. This study was approved by the institutional review board of the National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Control and Prevention (CDC).

Laboratory tests

Blood samples were collected by the local CDC at enrolment and every follow-up visit. Samples were sent at ambient temperature to the laboratory of NCAIDS in Beijing within 8 h, where a team was on-call 24 h for 7 days a week for receipt; $CD4^+$ cell count, viral load and HIVDR genotyping were performed. $CD4^+$ cell count was conducted using flow cytometry (FACS Calibur, BD Company, Franklin Lakes, New Jersey, USA) within 2 h after arriving at the NCAIDS laboratory. Plasma was isolated and frozen at $-80°C$ until testing for viral load or drug resistance. Plasma HIV RNA was quantified with real-time NASBA (NucliSense Easy Q, bioMérieux, Lyon, France) or with Amplicor HIV-1 monitor test (COBAS, Roche Applied Science, Penzberg, Germany) according to the manufacturers' recommendations. For samples with viral load of at least 1000 copies/ml, HIVDR genotyping was performed at NCAIDS using an in-house PCR protocol as previously described [10]. HIV-1 drug resistance was determined according to the Stanford University's HIV Drug Resistance Database Program version 6.2.0 (http://hivdb.stanford.edu, accessed 2 November 2012). We included all drug resistance mutations that conferred low, intermediate or high-level resistance [11].

Study outcomes

Drug resistance was defined as identification of any drug-resistant mutation during cART. We assumed that patients had no transmitted drug-resistant mutations prior to cART initiation because they were among the first group of patients to receive cART in China [12]. The date of drug resistance was defined as the date of initial diagnosis of drug-resistant mutation.

The date of virologic failure was defined as the first recorded date of plasma viral load of more than 5000 copies/ml after 6 months of treatment, as per the 2010 WHO guideline on antiretroviral therapy for HIV infection in adults and adolescents [13]. Similarly, the date of immunologic failure was also defined by WHO criteria [13] as the earliest date of any one of the following after 6 months of treatment: posttreatment $CD4^+$ cell count falling to or below baseline $CD4^+$ cell count; 50% decrease from peak $CD4^+$ cell count; or two consecutive $CD4^+$ cell counts of less than 100 cells/μl or last $CD4^+$ cell count of less than 100 cells/μl. Baseline $CD4^+$ cell count was defined as the last pretreatment count within 6 months of treatment. If no pretreatment $CD4^+$ cell count was done, the earliest count within 1 month of starting treatment was used for the baseline value.

Deaths were recorded by the local clinics. Given the inability of medical facilities in rural areas to determine the cause of death accurately, we assumed all deaths to be HIV-related.

Statistical analysis

We plotted Kaplan−Meier curves to assess the time to virologic failure, drug resistance, immunologic failure and death. We also plotted time from drug resistance to death. Patients were censored if they stopped their cART (typically for intolerance) or were lost to follow-up. Cox proportional hazards regression models were used to explore risk factors for death. For the mortality analysis, we examined risk factors deemed plausible, including original treatment regimen, sex, education, marital status, occupation, baseline CD4$^+$ cell count, baseline viral load and replacing ddI with 3TC and drug resistance as time-dependent covariates.

For the subanalysis of mortality in patients with drug resistance, the start point was changed as the time that drug resistance was first detected, and CD4$^+$ cell count and viral load at the first detection of drug resistance were included, as well as baseline characteristics such as sex, education and marital status. Variables with *P* values less than 0.1 in univariate analyses were entered into the multivariable model. All statistical analyses were performed with SAS 9.1 software (SAS Institute, Cary, North Carolina, USA).

Results

Characteristics of participants

Of the 376 patients initiating ART between 2003 and 2004, 11 patients (2.9%) were excluded because they died (nine within 6 months of ART initiation, two between 6−12 months) with no recorded CD4$^+$ cell count, viral load or drug resistance data. Among the 365 individuals included in the study, 51.2% were from Queshan County in Henan Province and 48.8% from Fuyang County in Anhui Province (Table 1). A majority of individuals were women (59.7%), median age at enrolment was 39 years (range 25−64 years), 72.3% had received primary school education or less, 97.8% were farmers and 84.7% were married or living with their partner. Nearly all the individuals (96.7%) were FPDs.

Baseline CD4$^+$ cell counts and viral load were available from 193 (161 before and 32 within 1 month of starting ART) patients (52.9%) with median CD4$^+$ cell count of 257 cells/μl and median viral load of 4.5 log copies/ml plasma. Of note, 61.1% of patients initiated ART with a CD4$^+$ cell count more than 200 cells/μl (Table 1), despite the treatment guidelines at that time indicating cART to be given when CD4$^+$ cell count was less than 200 cells/μl or with WHO stage III or IV disease.

All patients initially received either ZDV/ddI/NVP (51.0%), or d4T/ddI/NVP (49.0%). By 31 May 2010 or death, whichever came first, 153 out of 365 (41.9%) patients had substituted ddI with 3TC and 212 (58.1%) patients remained on ddI-based regimens. Excluding

Table 1. Characteristics of HIV-infected study population on first-line combination antiretroviral therapy in rural, central China.

Characteristics	n (%), N = 365
Site	
Fuyang, Anhui Province	178 (48.8)
Queshan, Henan Province	187 (51.2)
Sex	
Male	147 (40.3)
Female	218 (59.7)
Median age at inclusion, years (range)	39 (25−64)
Education	
Primary school or less	264 (72.3)
Middle school or more	101 (27.7)
Marital status	
Married or living with partner	309 (84.7)
Single, divorced or widowed	56 (15.3)
Occupation	
Farmer	357 (97.8)
Other	8 (2.2)
Risk exposure	
Former plasma donor	353 (96.7)
Other	12 (3.3)
Original combination antiretroviral regimen	
Stavudine, didanosine, nevirapine	179 (49.0)
Zidovudine, didanosine, nevirapine	186 (51.0)
Baseline CD4$^+$ cell count (cells/μl)	
≥500	30 (15.5)
350−499	35 (18.1)
200−349	53 (27.5)
50−199	61 (31.6)
<50	14 (7.3)
Baseline viral load (copies/ml plasma)	
<1000	62 (32.1)
1000−9999	22 (11.4)
10 000−99 999	42 (21.8)
≥100 000	67 (34.7)

baseline values, participants had a median of eight CD4$^+$ cell counts [interquartile range (IQR) 5−10], eight viral load measurements (IQR, 5−10) and eight HIVDR genotypes (IQR, 5−9). At their last visits, 27 patients (7.4%) had stopped cART for at least 6 months. By 2010, 17 patients (4.7%) had been lost to follow-up. Participants were followed for a total of 1974.3 person-years, with a median of 6 years and 1 month (mean 5 years and 5 months) follow-up per patient.

Distribution of time to endpoint events

At the end of the study period, a cumulative 76.4% (279/365) of patients had experienced virologic failure, for an incidence of 14.1 per 100 person-years, and drug-resistant mutations were found in 235 out of 365 (64.4%; 11.9/100 person-years) patients. Immunologic failure was noted in 207 out of 365 (56.7%) patients, for an incidence of 10.5 per 100 person-years, and 75 (20.5%; 3.8/100 person-years) had died. The median times to identification of virologic failure, drug resistance and immunologic failure from the date of cART initiation by Kaplan−Meier plot were 17.5, 36.6 and 55.2 months, respectively, demonstrating a significant increase in median time required from virologic failure to drug resistance, to immunologic failure and to death (*P* < 0.001; Fig. 1a). Among patients with adverse milestones, the given event occurred at a median of nearly 18 months apart.

(a)

Subjects at risk

Death	365	350	332	314	289	274	194
Immunologic failure	365	320	258	226	178	154	101
Drug resistance	365	269	203	169	117	103	74
Virologic failure	365	246	145	127	90	73	50

(b)

Subjects at risk

DR beyond 1st year	133	114	105	87	31	15	
DR within 1st year	102	90	81	69	66	54	17
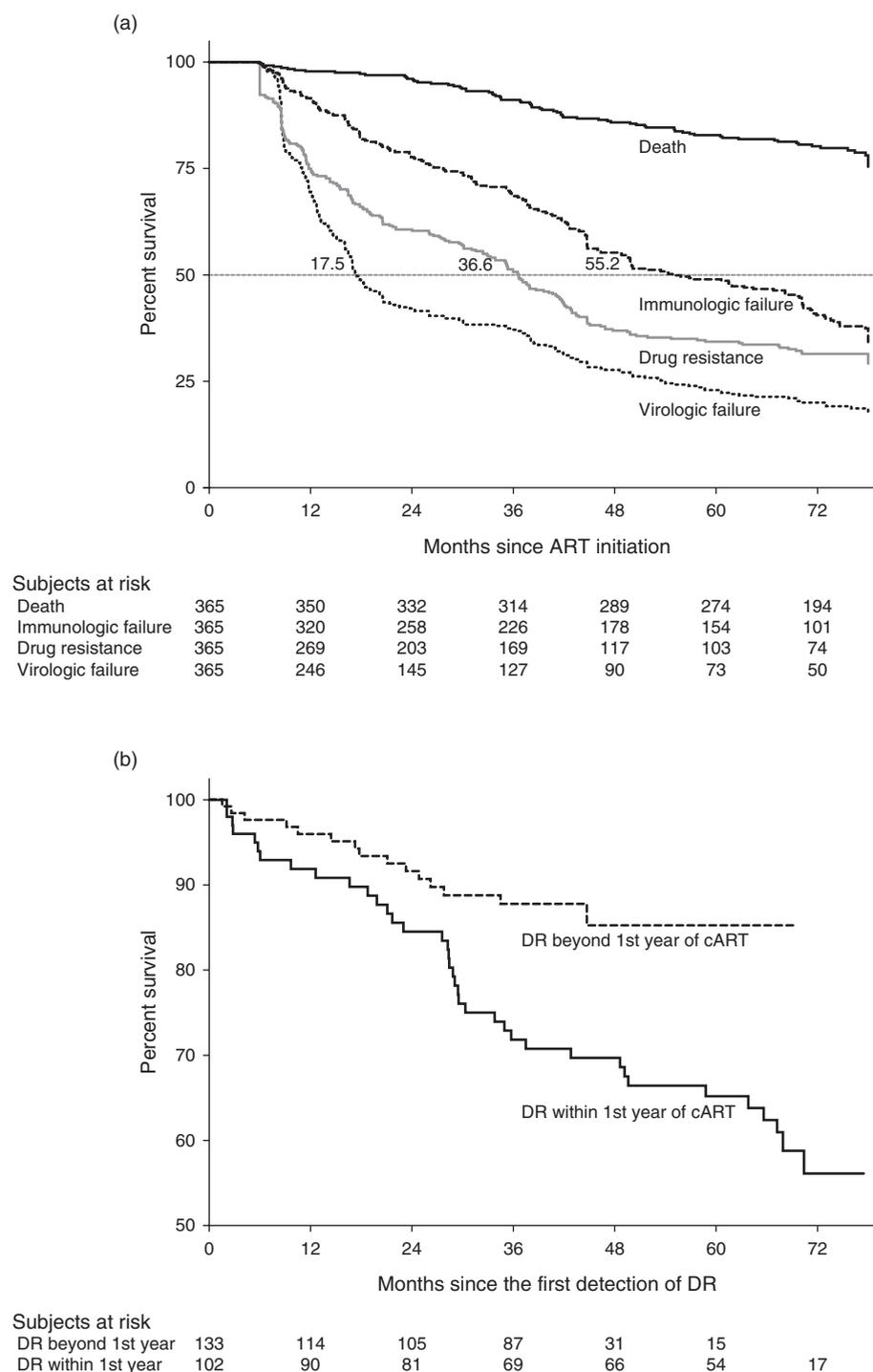
Fig. 1. Kaplan−Meier plot of time to events. (a) From cART initiation to virologic failure, drug resistance, immunologic failure and death ($N = 365$). Dotted line indicates the months on therapy to reach the 50% survival point. (b) From first detection of drug resistance to death among patients with drug resistance ($N = 235$). cART, combination antiretroviral therapy; DR, drug resistance.

Predictors of mortality

In a Cox proportional hazards model (Table 2), factors independently associated with mortality included being males (adjusted hazard ratio 1.9, $P = 0.005$, compared with female patients), baseline CD4$^+$ cell count less than 50 cells/μl (adjusted hazard ratio 4.3, $P = 0.002$, compared with CD4$^+$ cell count >350 cells/μl) and having detected HIVDR (adjusted hazard ratio 3.4, $P < 0.001$). We plotted the Kaplan−Meier life survival curves from the time drug resistance was first detected. Patients with their first detectable drug resistance in the first year of cART had a higher probability of death than

Table 2. Cox proportional hazards regression analysis of risk factors associated with death, all patients (*N* = 365).

Variable	Number	Death, *n* (/100 person-year)	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
All patients	365	75 (3.8)				
Patients with baseline data	193	37 (3.7)	1.0			
Patients without baseline data	172	38 (3.9)	1.0 (0.7–1.7)	0.8		
Sex						
Female	218	33 (2.7)	1.0		1.0	
Male	147	42 (5.6)	2.0 (1.3–3.2)	0.003	1.9 (1.2–3.1)	0.005
Drug resistance						
No			1.0		1.0	
Yes			3.4 (2.0–6.0)	<0.001	3.4 (1.9–5.9)	<0.001
Baseline CD4$^+$ cell count (cells/µl)						
≥350	65	10 (2.9)	1.0		1.0	
200–349	53	9 (3.3)	1.2 (0.5–2.9)	0.7	1.0 (0.4–2.6)	0.9
50–199	61	10 (3.1)	1.1 (0.5–2.7)	0.8	0.9 (0.4–2.2)	0.8
<50	14	8 (15.2)	5.6 (2.2–14.3)	<0.001	4.3 (1.7–11.0)	0.002
Missing	172	38 (3.9)	1.3 (0.6–2.5)	0.5	1.0 (0.5–2.1)	0.9
Baseline viral load (copies/ml)						
<1000	62	7 (2.2)	1.0			
1000–9999	19	3 (2.7)	1.3 (0.3–5.1)	0.7		
10 000–99 999	42	9 (5.0)	2.0 (0.8–5.4)	0.2		
≥100 000	67	18 (5.6)	2.6 (1.1–6.2)	0.03		
Missing	172	38 (3.9)	1.7 (0.8–3.9)	0.2		

CI, confidence interval; HR, hazard ratio.

those having drug resistance beyond the first year (*P* = 0.002, log rank test; Fig. 1b). In a Cox proportional hazards model using a start point as the time drug resistance was first detected (Table 3), death was associated with having detectable drug resistance within the first year of treatment (adjusted hazard ratio 1.9, *P* = 0.046) after adjusting for CD4$^+$ cell count and viral load at the time of detection of HIVDR.

Emergence of HIV drug resistance

The rate of any nucleoside reverse transcriptase inhibitor (NRTI)–related resistance mutation increased over time from the end of year 1 (9.6%) to the end of year 6 (30.0%, *P* < 0.001 for trend; Fig. s1a, http://links.lww.com/QAD/A337). Among the NRTI-based drug-resistant mutations, T215FSY was the most frequently seen at all time points. M41L, M184V and L210W were also commonly seen, with the rates for these four mutations increasing over time (*P* < 0.001 for trend). Thymidine analogue–associated mutation type 1 (TAM1) mutations (M41L/L210W/T215Y) were more common than TAM2 mutations (D67N/K70R/T215F/K219QE) after 1 year post-cART initiation, and became twice as frequent as the latter from year 4 on. Non–nucleoside reverse transcriptase inhibitor (NNRTI)–related drug-resistant mutations appeared early and peaked in year 4 at 42.2%, finishing at 35.7% in year 6 (Fig. s1b, http://links.lww.com/QAD/A337). The most common NNRTI-resistance mutations detected were K103NS, Y181CI, G190AS and K101EP.

We further analysed mutation patterns when drug resistance was firstly detected in a given year of treatment.

More than 95% of patients had NNRTI-related mutations at the first detection. However, the proportion of NRTI-related mutations was quite different between years (*P* < 0.001; Fig. 2a); persons with detectable drug-resistant mutations within the first year of cART had the lower rate with 23.5% of NRTI-related mutations, whereas those who had drug resistance detected beyond the first year had 50.4% of NRTI-related mutations. The differences were mainly found in the occurrence of TAMs (Fig. s2, http://links.lww.com/QAD/A337), such as M41L (*P* = 0.001), D67N (*P* = 0.001), K70R (*P* = 0.009) and T215Y (*P* = 0.005). When drug resistance was detected for the first time, the median viral load of patients differed significantly depending upon whether HIVDR appeared within the first year of cART (4.9 log copies/ml) or after the first year (4.1 log copies/ml, *P* < 0.001; Fig. 2b).

Discussion

Our cohort study was conducted among patients who were predominantly farmers and FPDs in two counties in central China. They acquired HIV-1 through unhygienic plasmapheresis practices (reinfusion of pooled red blood cells) in the mid-1990s. In 2004, they were the first patients in China to receive free cART through the National Free ART Program. After 6 years of follow-up on first-line cART in our longitudinal study, adverse events had become commonplace: a cumulative three-quarters had failed virologically, nearly two-thirds developed drug resistance mutations, over half had failed

Table 3. Cox proportional hazards regression analysis of risk factors associated with death, patients exhibiting HIV drug resistance at any time in the study (*N* = 235).

Variable	Number	Death no. (/100 person-year)	Crude HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
All patients with drug resistance	235	55 (6.6)				
Sex						
Female	136	24 (4.7)	1.0			
Male	99	31 (9.7)	2.0 (1.2–3.5)	0.01		
DR firstly being detected						
Late onset[a]	133	15 (3.7)	1.0		1.0	
Early onset[a]	102	40 (9.2)	2.5 (1.4–4.6)	0.004	1.9 (1.0–3.5)	0.05
CD4$^+$ cell count level at first DR detection (cells/µl)						
≥350	70	7 (2.5)	1.0		1.0	
200–349	65	9 (3.9)	1.8 (0.6–5.1)	0.3	1.4 (0.5–4.1)	0.5
50–199	85	29 (10.0)	4.9 (2.0–11.8)	<0.001	3.8 (1.6–9.3)	0.003
<50	15	10 (27.7)	12.7 (4.6–35.1)	<0.001	11.5 (4.0–32.6)	<0.001
Viral load at first DR detection (copies/ml)						
1000–9999	80	7 (2.6)	1.0		1.0	
10 000–99 999	81	13 (4.3)	1.7 (0.7–4.2)	0.3	1.1 (0.4–2.9)	0.9
≥100 000	74	35 (13.3)	5.0 (2.2–11.4)	<0.001	3.3 (1.4–7.6)	0.005

Early onset: Less than or equal to 1 year after commencing combination antiretroviral therapy. CI, confidence interval; DR, drug resistance; HR, hazard ratio.
[a]Late onset: More than 1 year after commencing combination antiretroviral therapy.

immunologically and one-fifth had died. For patients with adverse events, median intervals of about 18 months each separated virologic failure (at 17.5 months) from HIVDR (at 36.6 months) from immunologic failure (at 55.2 months). Hence, if a patient is suboptimally virally suppressed, our real-world experience in a poor, rural region of China suggests about a year and half between each of these three adverse events: virologic failure, HIVDR and immunologic failure. Our disappointing drug resistance finding (a cumulative 64.4% of the cohort) highlights the challenges found with an unfamiliar chronic disease receiving lifelong treatment in a subsistence farming culture.

Many studies have examined the relationship between virologic failure and drug resistance [14–16], virologic failure and immunologic failure [17–19], virologic failure and death [17,19,20], drug resistance and death [21], immunologic failure and resistance [22], and immunologic failure and death [19,20]. Our study has the advantage of having examined thoroughly these relationships in one single cohort analysis; our median clinical follow-up of over 6 years in the absence of widely available second-line treatment offers data unique in the literature. It is notable that individuals experienced virologic failure before the emergence of drug resistance in the cohort study. Reasons for such a sequence may include a relatively low adherence or insufficient dosing with which drug concentration could not reach the threshold for drug resistance viruses outgrowing wild viruses [23]. The limitation of consensus genotypic testing inhibits the detection of minor viral quasispecies with an abundance of less than 10–20% [24]. Nevertheless, our results underscore the importance of viral load measurement as the primary marker of treatment efficacy and suggest the importance of switching cART

regimens when there is evidence of virologic failure, to prevent the accumulation of drug resistance.

The cumulative rate of drug resistance was 24.2% at 12 months of treatment and 37.9% at 24 months, higher than the rates reported in other low and middle-income countries scaling-up cART. Malawi, for example, had 12% drug resistance in patients on treatment for more than 6 months [25]. Cameroon had resistance levels of 4.4% after 12 months of treatment and 16.9% at 24 months [26]. The reasons for these differences are likely multifactorial and may be related to provider experience, structural factors such as convenience of medication refills, patient adherence, stigma and levels of patient disclosure to others, and initial treatment regimen used, including drug side effects and tolerance [27,28]. In a multicentre study among antiretroviral-naive patients in China, persons on a 3TC-based treatment regimen had much higher rates of virologic suppression (less than 50 copies/ml) than those on a ddI-based regimen (68.2 vs. 39.7%, *P* < 0.001) [29].

In our analysis of risk factors associated with mortality, advanced immunosuppression at treatment initiation was the strongest risk factor, consistent with global experiences [8,30]. Our study also demonstrated an association between HIVDR and mortality, consistent with previous studies [21,31]. In addition, patients who developed HIVDR during treatment were nearly two-fold more likely to die than those who developed HIVDR later. The need for early adherence education and monitoring when starting ART must be stressed, as it was the earliest treatment failures who suffered the most adverse clinical outcomes. It also underscores the WHO HIVDR monitoring strategy, putting an emphasis on the first year of cART.
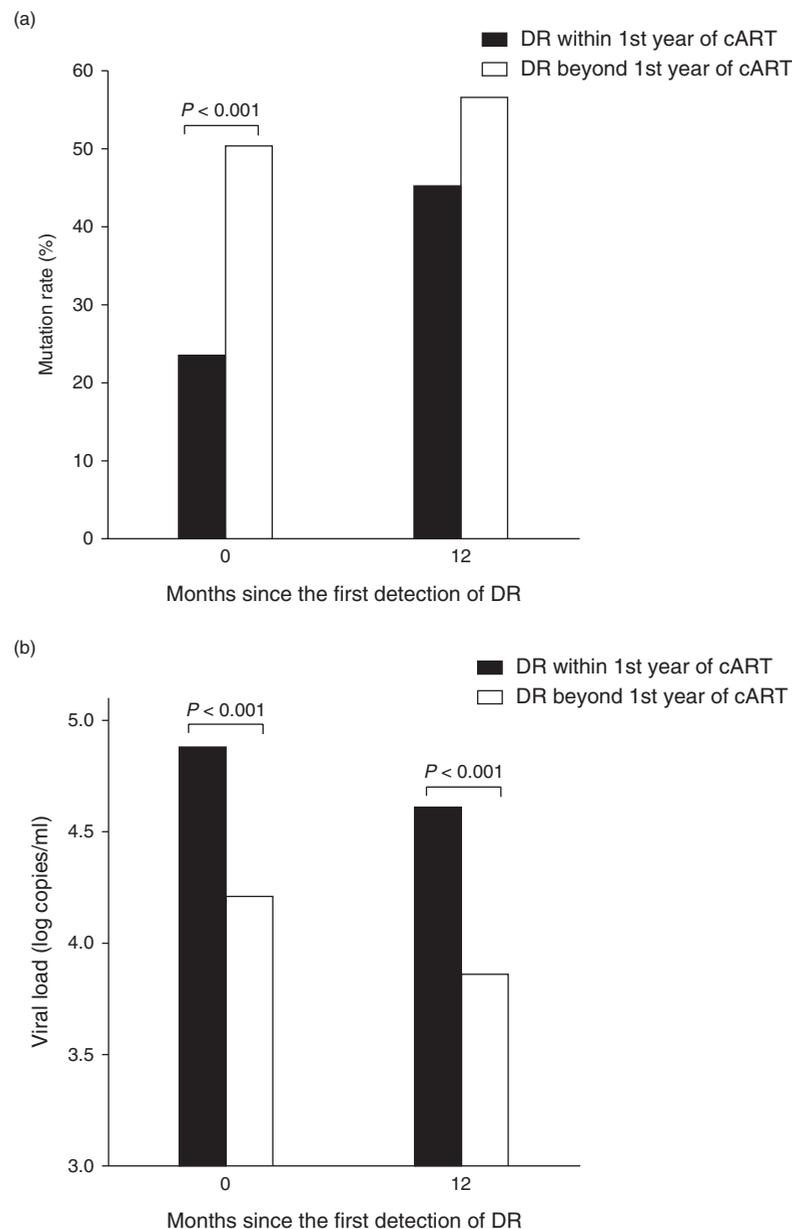
Fig. 2. **Mutation rate and viral load in patients with early or later onset of drug resistance.** (a) The rate of nucleoside reverse transcription inhibitor (NRTI)-related mutations. (b) Plasma viral load. Chi-square or Mann–Whitney test was used to calculate P value. cART, combination antiretroviral therapy; DR, drug resistance.

When HIVDR viruses were first detected, we found that drug resistance mutation patterns were quite different between patients acquiring detectable HIVDR in the first year and those who developed HIVDR later. Three-quarters of people developing HIVDR in their first year of cART had solitary NNRTI-resistant mutations, whereas half of the patients with later onset of HIVDR had both NRTI and NNRTI-resistant mutations. Some NRTI-resistant mutations, especially TAMs including M41L, K70R, T215FY and L210W, can reduce viral replicative fitness [32]. Even though we do not know whether replicative fitness differences are an important contributor to the higher mortality seen, there was significantly higher viral load in the group with detectable HIVDR in the first year of treatment than the later onset HIVDR group. In individuals receiving NNRTI-based cART, persons with lower (<75%) medication adherence had a higher risk of HIVDR than those with moderate-to-high (75−100%) level of adherence [33]. Early onset of drug resistance suggests poor adherence and hence incomplete viral suppression, likely from near the beginning of cART use, and persons with early HIVDR consequently have a higher risk of other adverse events and death.

Careful selection of the proper initial first-line regimen can improve both effectiveness and sustainability of

cART. This is especially crucial for the public health scale-up of cART, wherein large numbers of patients are on the same regimens and there are fewer monitoring resources for follow-up of individuals and for community-wide assessments. Different levels of antiretroviral drug toxicity, drug–drug interactions, varying pharmacokinetics and pharmacogenomics, and socioeconomic limitations all affect cART adherence dramatically [23]. At the early stage of China's National Free Antiretroviral Treatment Program, suboptimal regimens were chosen due to limited resources and accessibility of the best antiretroviral drugs. The initially used regimens, NVP and ddI along with ZDV or d4T, may have less potency and more side effect than other later regimens (TDF and an NNRTI along with 3TC, for example). A lack of optimized regimens likely contributes to lower medication adherence. In addition, NVP has a much longer half-life than other drug components; low adherence may result in NVP monotherapy and predispose to virologic failure and drug resistance.

Countries with limited resources may still want to use more potent and less toxic first-line cART regimens, as the long-term costs may be offset by maximizing the effects and sustainability of the first-line treatment, minimizing or delaying emergence of HIVDR, and easing the burden on intensive treatment monitoring. When to change virologically failing treatment regimens in resource-limited settings with limited second-line treatment options is a challenging question, given realities of drug availability and cost [34]. Our study explored the time to virologic failure, drug resistance, immunologic failure and death in a remote rural area before the availability of second-line cART regimens. Because drug-resistant mutations impair viral fitness and replication, thereby partially suppressing plasma viral load [32,35,36], there may be benefit of continuing failing treatment regimens in patients when they have no access to second-line treatment options, especially in those with advanced HIV. Our study suggested as much, as patients who had detectable drug resistance beyond the first year of cART (later onset) seemed to benefit from partially active first-line regimens, experiencing reduced mortality compared with persons with earlier onset of HIVDR (within the first year of cART). However, our study also showed that the continuation of a virologically failing regimen was associated with an increased risk of resistance mutation accumulation. The decision of when to change regimens needs to balance these risks and benefits, as well as consider economic factors and long-term effects of first-line and second-line treatment [37].

The principal strength of our study is the long 6-year follow-up of our patients for the continuum of adverse virologic, immunologic and clinical outcomes in the context of HIVDR; other cohorts have been followed for only 12−24 months to study emergence of HIVDR. We also had a follow-up rate of more than 95% over 6 years and were able to assess laboratory specimens quickly, despite the remote rural setting. There are two notable limitations to our study. The first is that our study has uncertain generalizability, as it was conducted in two locations among FPDs. It is likely that this cohort is representative of FPDs because they generally had similar demographics and were infected with the same strain of HIV-1 at a short window period of blood contamination between 1993 and 1995 [9,38]. Furthermore, a recent analysis of HIV mortality in China found similar mortality risks among a variety of at-risk cohorts [30]. Although adherence rates may vary among different populations and therefore the specific timing of HIVDR emergence may vary, the overall sequence of events from virologic failure to death is consistent with the current literature. A second limitation is that about half of the patients were missing baseline CD4$^+$ cell count and viral load data due to the sudden start of cART to save lives, even before laboratory services were established. However, an analysis between those with and without baseline data showed no significant differences in either demographics or mortality rate; so, biases should be minimal (Table 2).

In conclusion, our clinical cohort study examined the frequency, sequence and timing of the emergence of virologic failure, genotypic resistance, immunologic failure and death in rural Chinese farmers on cART over 6 years. Failing treatment regimens were not changed due to limited resources and availability of second-line drugs. The cohort was stopped when second-line drugs became available and the seriousness of their unavailability was made clear from the data that emerged from our study. The median timing of these events was about one and half years between adverse milestones, but the interval times between these benchmarks would differ, we believe, on the basis of specific population characteristics. Still, this timetable of adverse outcomes may be helpful for healthcare practitioners and planners. Prevention of drug resistance in the very first year of treatment is important to lower long-term mortality, especially in resource-limited settings in which second-line therapies are limited. It remains a high research priority to optimize the timing of a switch to second or even third-line treatment regimens in the setting of limited resources.

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Conflicts of interest

All authors have no conflicts of interest to declare.

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