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Predictors of HIV Virologic Failure And Drug Resistance In Chinese Patients After 48 Months Of Antiretroviral Treatment, 2008-2012

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Running head: Virologic failure and drug resistance among HIV+ Chinese ART patients

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ABSTRACT

Objective: To explore factors associated with HIV virologic failure (VF) and HIV drug resistance (HIVDR) among HIV-positive Chinese individuals four years after initiating first-line 3TC-based antiretroviral treatment (ART) in 2008 at five sentinel sites.

Design: First-line ART initiators who were previously treatment naïve were selected using consecutive ID numbers from the 2008 National Surveillance Database into a prospective cohort study. Questionnaires and Blood samples were collected in 2011 and 2012 to assess the outcomes of interest: VF (defined as viral load ≥ 1000 copies/ml) and HIVDR (defined as VF with genetic drug resistant mutations). Questionnaires and data from National Surveillance Database assessed demographics and drug adherence data.

Results: 536 individuals with HIV were analyzed; the 4-year risk of VF was 63(11.8%) and HIVDR was 27(5.0%). Female participants initiating D4T-based regimens were more susceptible to both VF (adjusted odds ratio, aOR=2.5 95% CI: 1-6.1 P-value=0.04) and HIVDR (aOR=3.6 95% CI: 1 to 12.6 P-value=0.05) versus AZT-based regimens. Male participants missing doses in past month were more susceptible to both VF (aOR=2.8 95% CI: 1.1 to 7.X P-value=0.03) and HIVDR (aOR=9.7 95% CI: 2.1 to 44.1 P-value<0.01). Participants of non-Han nationality were of increased risk for HIVDR (aOR from 4.8-12.2, p<0.05) and non-Han men were at increased risk for VF (aOR = 2.9, p=0.02). All 27 participants detected with HIVDR had non-

nucleoside reverse-transcriptase inhibitor (NNRTI) mutations, 21 (77.8%) also had NRTI mutations, and no protease inhibitor mutations were detected.

Conclusions: Our findings suggest successful treatment outcomes at 4-years for roughly 90% of patients. We found female participants initiating D4T versus AZT-based regimens were more vulnerable to VF and HIVDR, while poor adherence was a risk factor among male participants. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions in China.

Strengths and limitations of this study

- We studied 48-month risk of VF and HIVDR and their associations with demographic and behavioral information among individuals across five sentinel sites.
- Drug adherence and adverse effects influenced VF and HIVDR differently across gender, however, the reasons for the differences were uncertain in the study.
- The outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes.

Key words: HIV, Antiretroviral Treatment, Virological Failure, Drug Resistance, Gender Differences, China

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Introduction

Antiretroviral treatment (ART) has dramatically improved health outcomes and decreased HIV-associated morbidity and mortality through virologic suppression and subsequent CD4 recovery. [1-4] In 2003, China launched a National Free Antiretroviral Treatment Program (NFATP) that includes life-long provision of free ART for people living with HIV who met the national treatment criteria.[5 6] The national treatment criteria from 2008 to 2011 were: (1) CD4 cell count ≤200/mm^3; (2) World Health Organization (WHO) stage III/IV diseases; or (3) willingness to receive ART, regardless of criteria 1 and 2.[7]

The State Council AIDS Working Committee Office and the United Nations Theme Group on AIDS estimated that there were more than 700,000 persons living with HIV in China in 2008, and more than 52,000 individuals with HIV across 31 provinces, autonomous regions, and municipalities had received ART (made freely available by the NAFTP) by August 2008.[8]

With the rapid scale-up of treatment and challenges with adherence, virologic failure (VF) and HIV drug resistance (HIVDR) are ever present and mounting concerns. Incomplete virologic suppression, a major cause of HIVDR, not only compromises therapeutic efficacy for the individual receiving treatment, increasing the risk of viral rebound and opportunistic infections, but also increases the risk of transmitting drug resistant strains to other individuals in the general population.[9 10 11]

Observational studies in China have documented the prevalence of VF and HIVDR strains among treated individuals living with HIV. A cross-sectional study conducted in Yunnan, Guangxi and Xinjiang provinces in 2010 stated that one-year HIVDR prevalence was 75.3% and 4.1%[5]. VF prevalence for sexual transmitted population and intravenous drug users (IDUs) were 8.3% and 19.3%, separately. A 6-year follow-up study in 2010 suggested an incidence of 14.1 per 100 person-year for VF and 11.9 per 100 person-year for HIVDR among former plasma donors in Anhui Province.[12]

NFATP recommended to switch the first-line regimen from Didanosine (DDI) to Lamivudine (3TC) in 2008, and there are few nationwide, prospective studies in China reporting frequency or predictors of VF and HIVDR for people after initiating 3TC based regimens.

The aim of this study is to evaluate predictors of VF and HIVDR in a prospective cohort of Chinese HIV individuals with HIV four years after first initiating first-line 3TC-based ART in 2008 at five sentinel sites. We stratified our analyses by gender based on conflicting findings on gender differences both in virological responses and drug resistance to different ART regimen, as well as gender differences in ART adherence.[13-16] To our knowledge, this is the first long-term study to evaluate VF and HIVDR on 3TC-based regimens.

Ethics Statement

The study was approved by the institutional review board (IRB) of the National Center for AIDS/STD Control and Prevention of the China Center for Disease Control Prevention (NCAIDS, China CDC). All participants provided written informed consent before participation.

Study Design and Data Collection

This study was designed under the WHO Surveillance of HIV drug resistance in adults receiving ART for 48 months. [10 17 18]

Five provinces in China were selected to conduct a prospective cohort study with a follow-up study at 12 months: Guangxi, Henan, Hubei, Xinjiang and Yunnan. Patients were sampled from the 2008 National HIV Surveillance Database. Participant eligibility criteria included being age ≥ 18 years; having initiated NAFTP-sponsored first-line ART in 2008; having been ART-free before 2008; having been on ART for 36±6 months in 2011; and providing consent to participate in the study. Questionnaires administered by trained study personnel using structured interviews collected data in 2011 and 2012. Additional HIV-specific data including route of transmission, initial ART regimen, latest ART regimen, ART distribution location and CD4 cell count were collected from the 2011-2012 National HIV Surveillance Database. There was no missing demographic data, missed questionnaire data was feedback to local CDC for recollection at the time.

Laboratory analysis

Blood specimens were collected from all participants to test CD4 cell count, HIV-1 RNA viral load (VL), and HIV-1 drug resistance mutations in 2011 and 2012. Plasma was isolated and stored at -80°C at a provincial CDC laboratory and then transferred to NCAIDS. CD4 cell count estimation was conducted at CDC laboratories using flow cytometry (FACSC Calibur, BD Company, USA) within 24 hours after specimen collection.

Plasma HIV RNA was quantified with real-time NASBA (NucliSense Easy Q, bioMerieux, France) or COBAS (Roche Applied Biosystems, Germany) according to manufacturer recommendations using in-house PCR (polymerase chain reaction).[19] Virologic failure was defined as VL ≥1000 copies/ml. According WHO protocol, [20] HIVDR tests were performed on samples with VL ≥1000 copies/ml. HIV-1 *pol* gene (protease 1-99 amino acids and part of reverse transcriptase 1-252 amino acids) were amplified, purified and analyzed using the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/). Any low-, intermediate-, or high-level resistance identified was defined as HIVDR.[21-24] HIV VL and drug resistance mutation testing was conducted at NCAIDS.

Data analysis

Questionnaire data were double-entered using Epidata 3.1 (The Epidata Association Odense, Denmark). Statistical Analysis System (SAS 9.4, SAS Institute Inc., Cary, NC, USA) was then used for data cleaning and analyses.

48-month risk for the outcomes of interest was calculated as the proportion of unique persons who had experienced incident VF or HIVDR by the end of follow-up in 2012. Covariates of interest were described using counts and percentages overall and by the outcome of interest, stratified by gender. Univariate logistic regression models were constructed to explore associations between covariates of interest and VF or HIVDR. Odds ratios (OR) and 95% confidence intervals (CIs) are reported. Variables that were significant ($P < 0.05$) in the univariate models were then fit into multivariate logistic regression models assessment for/removal of collinear variables that had the weakest association with the outcome. Adjusted ORs (aOR) and 95% CIs were presented. $P < 0.05$ was defined as statistically significant, and all tests were two sided. Descriptive analysis on HIVDR mutation results was conducted among 27 HIVDR participants, stratified by sex.

Results

1100 subjects were selected using consecutive ID numbers from 2008 National Surveillance Database; of those, 490 were lost follow-up by December, 2012. Among those lost to follow up, 139 died, 55 emigrated, 134 lost contact, 17 refused to participate, 65 stopped ART before 30 months, 36 transferred, 8 were under custody, 6 failed to provide a blood sample, 3 switched from ART to Tangcao tablet (an

antiviral Chinese herbal therapy), 2 became pregnant and switched to other regimens and 1 was paralyzed. After excluding 74 participants for failing the eligibility criteria, 536 participants were included in the final 24-month analysis (Figure 1). The 48-month risk of VF was 11.8% and risk of drug resistance was 5%.

Demographic and ART Information (Tables 1-2)

Of the 536 eligible participants, 51.8% were male; 76.5% were Han majority; 45.0% had an education level of elementary school or less; 56.2% were farmers; and 10.6% were unemployed with the rest having regular income.

All regimens in this cohort were 3TC-based. Initiated Nucleoside Reverse Transcriptase Inhibitors (NRTI) regimens included Zidovudine (AZT) (n=349, 65.1%) or Stavudine (D4T) (n=187, 34.9%). Sixty-six (12.3%) participants later changed to TDF based regimen. Initiated Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) regimens included Nevirapine (NVP) (n=421, 78.5%) or Etravirine (EFV) (n=115, 21.5%). Fifty-five (10.3%) participants later changed to LPV/r based regimen. 169 (31.5%) participants switched the initial ART regimen during 2008-2012, but no statistical significant difference was found in VF and HIVDR risk between participants who switched regimens and participants who did not switch regimens.

We found that 38.4% participants were hesitant to accept ART in the future, 36.8% participants reported doubts whether ART was health promoting and 42.5% participants did not report that poor ART adherence necessarily contributed to

HIVDR. Additionally, 40.5% of participants were not always satisfied with support from friends or relatives. 472 (88.1%) participants reported not missing a dose in the month prior to the date of the survey.

Multivariate model results (Table 3)

As shown in Table 3, minority male participants were at higher risk for both VF (aOR=2.9 95% CI: 1.1 to 7.3 P-value=0.02) and HIVDR (aOR=12.2 95% CI: 1.8 to 84.8 P-value=0.01) compared to Han majority male participants, while female minorities were only at a higher risk for HIVDR (aOR=4.8 95% CI: 1.2 to 19.7 P-value=0.03).

Female participants initiating D4T-based regimens were at a higher risk for both VF (aOR=2.5 95% CI: 1 to 6.1 P-value=0.04) and HIVDR (aOR=3.6 95% CI: 1 to 12.6 P-value=0.05) versus those initiating an AZT-based regimen; interestingly, different from their female counterparts, male participants showed no such association (OR=0.6 95% CI: 0.3 to 1.4 P-value=0.24). Also, female participants had a higher risk of VF given adverse side-effects (aOR=2.7 P-value=0.03). Male participants with missed doses in the month prior to the survey were at a higher risk of both VF (aOR=2.8 95% CI: 1.1 to 7 P-value=0.03) and HIVDR (aOR=9.7 95% CI: 2.1 to 44.1 P-value<0.01) versus those without missed doses in the preceding month. Conversely, missed doses in prior month was not significantly associated with VF or HIVDR for women.

HIV Drug Resistance and Subtype (Table 4)

HIVDR identified in our study was consistence with the NFATP recommended ART regimen. All 27 participants detected with drug resistance had NNRTI mutations, 21 (77.8%) had NRTI mutations. The dominant subtype was CRF07_BC for both males (61.5%) and females (50%). All participants found with HIVDR had developed HIVDR towards NNRT; 85.7% male participants and 69.2% female developed HIVDR toward NRTI; no Protease Inhibitor mutation was detected. There were no CRF08_BC subtypes detected in the study population.

Discussion

The 48-month risk of VF was 11.8% and HIVDR was 5.0%, which indicated relatively good treatment outcomes given meta-analysis suggested a 37-48 months HIVDR prevalence ranging from 6.4%-47.92% in China,[9], similar to a study in China which estimated a one-year VF and HIVDR incidence in 2009 of 3.5%.[25] Our study substantiates the finding that VF and HIVDR largely decreased since the wide-spread of 3TC-based regimens.[26] Studies have shown mixed findings of gender differences on ART adherence and treatment outcomes. [14 16 27 28] In our study, we found male participants had slightly higher risk of VF (12.2% versus 11.2%, P-value = 0.72) but lower risk of HIVDR (2.4% versus 5.4%, P-value= 0.69) than women. Women's risks of VF and HIVDR were not associated with missing doses in the past month, and few

women missed doses relative to men, similar to two other studies in China suggesting women have better adherence behaviors. [29 30]

We found in this study that women, not men, who initiated D4T-based regimens were more susceptible to VF (women vs. men OR=2.3 95% CI: 1.0 to 5.7 P-value= 0.06) and HIVDR (women vs. men OR=3.0 95% CI: 0.8 to 11.3 P-value=0.11), consistent with previous findings that D4T was more likely to increase the risk of mitochondrial toxicity in women. [31 32] Mitochondrial toxicity caused by D4T had been reported to cause many adverse effects such as lactic acidosis, lipodystrophy, and peripheral neuropathy. [33 34] Following the WHO recommendation,[35] the NFATP advocated switching the first-line regimen from D4T to TDF in 2012. The percentage of people living with HIV initiating D4T-based regimen changed from 34.3% in 2010 to10% in 2012 and 0.9% in 2014;[36] however, there were still 29.9% participants in our study who were on D4T-based regimens in 2012. It was noteworthy that we did not see a statistical difference in VF (OR=1.4 95% CI: 0.4 to 4.2 P-value=0.60) and HIVDR (OR=1.0 95% CI: 0.2 to 4.2 P-value=0.98) between women who initiated and remained on D4T-based regimens and those who switched to AZT/TDF based regimens. It is a possible that women switched regimens because of VF; however, further studies need to be done to explore when to switch ART regimen for women receiving D4T-based regimens. It is important to mention that data on ART adherence and adverse effects were collected in 2012, when there were only four female participants still using D4T-based regimens who experienced VF. The sample size

was not sufficient enough to explore whether D4T-based regimens affect drug adherence and adverse effects for women.

Drug adherence and adverse effects influenced our outcomes differently for men compared to women. Male participants were at higher risk of both VF and HIVDR if they reported missed doses. More detailed studies need to be conducted on the frequency and factors associated with missing treatment. However, female participants showed a higher risk of VF if they had adverse effect while men did not. This calls for further researches of what types of adverse effects are occurring and how they affect ART adherence and virological outcomes across gender.

Though not associated with the VF and HIVDR outcomes, 38.4% of study participants reported that they would not 'always' be willing to take ART in the future, 36.8% reported not believing that ART is 'always' health promoting, and 42.5% reported not believing that poor compliance 'always' contributed to HIVDR. As willingness and these knowledge factors may impact more long-term VF and HIVDR outcomes, the motivations behind willingness and knowledge about VF and HIVDR warrant exploration.

Caution is needed when interpreting the study results from multivariate model that older age (>45) was protective for HIVDR in men. There were only 4 male IDU participants with older age (>45) in this study, the amount is not sufficient for us to test for interaction. There is no association between HIVDR and age (OR=6.5 95% CI: 1.1 to 38.1 P-value=0.49) in the sub-analysis we did among participants with age ≤45,

after controlling for variables showed significant in the univariate model. A previous study in HIV positive IDU population in China suggested that there is no association between VF and sex or age.[37] In our study, 61.5% of male participants with HIVDR became HIV infected via IDU, yet there were only 22.7% male IDU participants.

In addition, we found that younger (<45 years) IDU population were more likely to miss doses (18.6%) compared to heterosexual transmission population (8.8%) and blood transfusion transmission population (12.1%). This finding was consistent with studies that implied younger males were at a higher risk of drug abuse.[38 39] This result indicated that younger IDU population could be a main source of VF and HIVDR; therefore they could be future targeted population for behavioral intervention.

The increased risk of VF and HIVDR in non-Han minorities, regardless of gender, may be due to logistical, cultural, or social barriers faced by ethnic minorities which limit their adherence to ART. It has been reported that minorities tend to have lower social economic status than Han majorities, followed by lower education level and fewer access to health facilities.[40] It may be difficult for health professionals to reach for some minorities because of their more remote geographic locations. Additionally, several studies have reported that the percentage of high-risk populations such as female sex workers (FSWs) and IDUs were higher in minorities than in Han majority.[41-43] The causes of this increased VF and HIVDR risk warrants further exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions in China. The finding that higher CD4 cell count at follow-up was

protective for VF and HIVDR was expected. NFATP changed treatment criteria from CD4 cell count ≤200cells/mm^3 to CD4 cell count ≤350cells/mm^3 following the WHO recommendation in 2011, [7 17 44]. Our study indicated that male participants who initiated treatment in 2008 at CD4 cell count >=350 cells/mm^3 were still at higher risk towards VF (aOR=7.1 95% CI: 1.1 to 45.8 P-value=0.04), supporting possible clinical benefits of initiating ART at higher CD4 cell counts, <500 cells/mm^3 as per WHO recommendation in 2013.[45]

Among participants infected by blood transmission, we only found HIVDR subtype B; only one subtype C was found in participants infected with IDU, the dominant subtype was CRF07_BC, found both in participants infected by heterosexual transmission and IDU. The most common NNRTI mutation sites were K103N (40.7%), K101E (22.2%) and V108I (22.2%); the most common NRTI mutation sites were M184V (81.0 %) and K70R (19%). Interestingly, compared to a one-year follow-up study in China with all participants initiated ART in 2011,[46] there is no V108I in their study and we did not find K65R in our study.

Study findings should be interpreted in light of several limitations. Though we did not account for transmitted drug resistance in this study, previous studies have found low transmitted drug resistance risk (<5%) during this period [47-49] in China and we could be fairly certain that participants were outcome free in 2008 as they were new ART initiators. Another limitation of our study is that the outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes. Also, route of

transmission was collected in 2008 when assessing HIV infection among men who have sex with men was not part of data collection instruments; additionally we do not have data on sex worker status. Roughly half of the study participants selected for possible inclusion in the study for having initiated first-line ART in 2008 were lost to follow-up by 2012, creating a possible selection bias for individuals with better ART adherence – this bias may underestimate the true VF and HIVDR risk and also limit the generalizability of our findings to better adherers. Additionally, given the demographic profile of the cohort, our findings are most generalizable to heterosexual Han national who are married/cohabiting and working as agricultural labors in rural areas. Misclassification of self-reported data is possible, though we do not expect this misclassification to be differential by the outcome of interest and thus any such information bias would bias our results toward the null.

Conclusions

We found female participants initiating D4T versus AZT-based regimens were more vulnerable to VF and HIVDR, and we suggest future studies on whether and when to change ART regimen for women initiated with D4T-based regimen. Poor adherence was a risk factor among male participants who may benefit from reinforced adherence counseling or social support. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions in China. Finally, this study indicated

that younger men who become infected through IDU may be groups to strategically focus counseling and increased adherence support programs.

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Contributions:

YR, WK, HX and YS designed the study. YR, HX, SL, LL, YM, HT, TZ, GS, HC, WK, XL and PL collected the data. HC, TT and LL conducted laboratory analysis. XL, WK analyzed the data. KW, VM, YR, WK, HX, LL and YS interpreted the data. KW, VM, YR, WK, TT, and YS drafted the report. All authors reviewed, revised, and approved the final report.

Conflicts of interest:

All authors declare that they have no conflicts of interest.

Transparency declarations

Vincent C. Marconi has received fees from ViiV Healthcare.

Figure 1. Figure of exclusion on study cohort

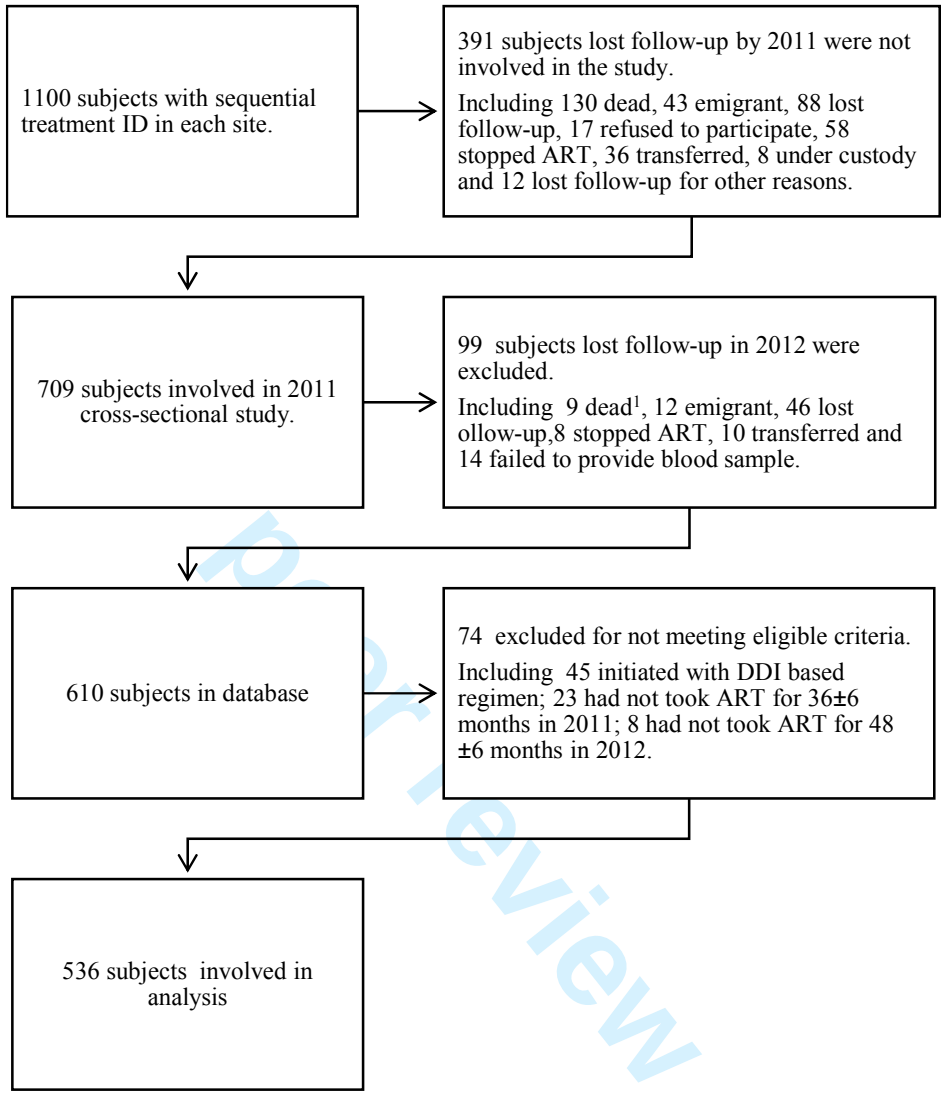

Table 1. Factors associated with virological failure (viral load ≥1000 copies/ml) stratified by sex

Demographic factors								
	Female				Male			
	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value
Total	258	29 (11.2)			278	34 (12.2)		
Ethnicity								
Han nationality	191	19 (9.9)	1		219	20 (9.1)	1	
Other minorities	67	10 (14.9)	1.6 (0.7,3.6)	0.27	59	14 (23.7)	3.1 (1.5,6.6)	<0.01
Education								
Elementary school or less	134	15 (11.2)	1		107	12 (11.2)	1	
Junior school or more	124	14 (11.3)	1 (0.5,2.2)	0.98	171	22 (12.9)	1.2 (0.6,2.5)	0.68
Marital Status								
Single	59	6 (10.2)	1		75	9 (12)	1	
Married or Cohabited	199	23 (11.6)	1.2 (0.4,3.0)	0.77	203	25 (12.3)	1 (0.5,2.3)	0.94
Residence								
Rural	197	19 (9.6)	1		172	17 (9.9)	1	
City	61	10 (16.4)	1.8 (0.8,4.2)	0.15	106	17 (16)	1.7 (0.8,3.6)	0.13
Occupation								
Peasant	163	15 (9.2)	1		138	9 (6.5)	1	
Employee	64	11 (17.2)	2 (0.9,4.7)	0.09	114	17 (14.9)	2.5 (1.1,5.9)	0.03
Unemployed	31	3 (9.7)	1.1 (0.3,3.9)	0.93	26	8 (30.8)	6.4 (2.2,18.6)	<0.01
Age								
<35	73	6 (8.2)	1		53	7 (13.2)	1	

35-45	108	15 (13.9)	1.8 (0.7,4.9)	0.25	119	15 (12.6)	0.9 (0.4,2.5)	0.91
>45	77	8 (10.4)	1.3 (0.4,3.9)	0.65	106	12 (11.3)	0.8 (0.3,2.3)	0.73
Weight (kg)								
<50	89	14 (15.7)	1		49	7 (14.3)	1	
50-70	156	13 (8.3)	0.5 (0.2,1.1)	0.08	193	21 (10.9)	0.7 (0.3,1.8)	0.51
>70	13	2 (15.4)	1 (0.2,4.9)	0.97	36	6 (16.7)	1.2 (0.4,3.9)	0.76
HIV characteristics and treatment factors								
Route of Infection								
Heterosexual Transmission	159	12 (7.5)	1		154	11 (7.1)	1	
Blood Transmission	86	13 (15.1)	2.2 (0.9,5)	0.07	61	10 (16.4)	2.5 (1,6.4)	0.04
Intravenous Drug use	13	4 (30.8)	5.4 (1.5,20.3)	0.01	63	13 (20.6)	3.4 (1.4,8)	0.01
Initial NRTI ART regimen								
AZT based regimen	161	11 (6.8)	1		188	26 (13.8)	1	
D4T based regimen	97	18 (18.6)	3.1 (1.4,6.9)	<0.01	90	8 (8.9)	0.6 (0.3,1.4)	0.24
Latest NRTI ART regimen								
AZT based regimen	181	15 (8.3)	1		195	27 (13.8)	1	
D4T based regimen	77	14 (18.2)	2.5 (1.1,5.4)	0.02	83	7 (8.4)	0.6 (0.2,1.4)	0.21
Switch ART regimen								
No	193	21 (10.9)	1		174	21 (12.1)	1	
Yes	65	8 (12.3)	1.2 (0.5,2.7)	0.75	104	13 (12.5)	1 (0.5,2.2)	0.92
ART drug distribution location								
County hospital or CDC	96	15 (15.6)	1		63	14 (22.2)	1	
Township hospital /village clinic /medication monitor	162	14 (8.6)	0.5 (0.2,1.1)	0.09	215	20 (9.3)	0.4 (0.2,0.8)	0.01
Adverse effects								

No	195	17 (8.7)	1		206	23 (11.2)	1	
Yes	63	12 (19)	2.5 (1.1,5.5)	0.03	72	11 (15.3)	1.4 (0.7,3.1)	0.36
CD4 cell/ml at baseline (2008)								
<350	244	28 (11.5)	1		272	31 (11.4)	1	
≥350	14	1 (7.1)	0.6 (0.1,4.7)	0.62	6	3 (50)	7.8 (1.5,40.2)	0.01
CD4 cell/ml at 36 months (2011)								
0-350	91	18 (19.8)	1		138	21 (15.2)	1	
≥350	167	11 (6.6)	0.3 (0.1,0.6)	<0.01	140	13 (9.3)	0.6 (0.3,1.2)	0.13
CD4 cell/ml at 48 months (2012)								
0-350	81	14 (17.3)	1		122	20 (16.4)	1	
≥350	177	15 (8.5)	0.4 (0.2,1)	0.04	156	14 (9)	0.5 (0.2,1)	0.06
Drug compliance factors								
Missed doses in past month								
No	226	26 (11.5)	1		246	24 (9.8)	1	
Yes	32	3 (9.4)	0.8 (0.2,2.8)	0.72	32	10 (31.3)	4.2 (1.8,9.9)	<0.01
Willing to receive ART in the future								
Always	153	15 (9.8)	1		177	19 (10.7)	1	
Not always	105	14 (13.3)	1.4 (0.7,3.1)	0.38	101	15 (14.9)	1.5 (0.7,3)	0.32
Believe ART is health promoting								
Always	158	16 (10.1)	1		181	19 (10.5)	1	
Not always	100	13 (13)	1.3 (0.6,2.9)	0.48	97	15 (15.5)	1.6 (0.8,3.2)	0.24
Believe poor compliance contribute to HIVDR								
Always	139	16 (11.5)	1		169	19 (11.2)	1	
Not always	119	13 (10.9)	0.9 (0.4,2.1)	0.88	109	15 (13.8)	1.3 (0.6,2.6)	0.53

Degree of satisfaction on support of friends or relatives								
Always satisfied	153	15 (9.8)	1		166	17 (10.2)	1	
Not always satisfied	105	14 (13.3)	1.4 (0.7,3.1)	0.38	112	17 (15.2)	1.6 (0.8,3.2)	0.22
Frequency of taking drugs reminded by friends or relatives								
Often	172	19 (11)	1		168	22 (13.1)	1	
Not often	86	10 (11.6)	1.4 (0.7,3.1)	0.38	110	12 (10.9)	0.8 (0.4,1.7)	0.58
Frequency of taking drugs reminded by doctors								
Often	178	23 (12.9)	1		184	28 (15.2)	1	
Not often	80	6 (7.5)	0.5 (0.2,1.4)	0.19	94	6 (6.4)	0.4 (0.2,1)	0.03

*OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 2. Factors associated with HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

Demographic factors								
	Female				Male			
	Total	HIVDR Risk N (%)	OR (95%CI)	P-value	Total	HIVDR Risk N (%)	OR (95%CI)	P-value
Total	258	14 (5.4)			278	13 (2.4)		
Ethnicity								
Han nationality	191	6 (3.1)	1		219	5 (1.2)	1	
Other minorities	67	8 (11.9)	4.2 (1.4 to 12.5)	0.01	59	8 (6.3)	6.7 (2.1 to 21.4)	<0.01
Education								
Elementary school or less	134	5 (3.7)	1		107	5 (2.1)	1	
Junior school or more	124	9 (7.3)	2 (0.7 to 6.2)	0.22	171	8 (2.7)	1 (0.3 to 3.1)	1
Marital Status								
Single	59	4 (6.8)	1		75	4 (3)	1	
Married or Cohabited	199	10 (5)	0.7 (0.2 to 2.4)	0.60	203	9 (2.2)	0.8 (0.2 to 2.8)	0.75
Residence								
Rural	197	7 (3.6)	1		172	5 (1.4)	1	
City	61	7 (11.5)	3.5 (1.2 to 10.5)	0.02	106	8 (4.8)	2.7 (0.9 to 8.6)	0.09
Occupation								
Peasant	163	5 (3.1)	1		138	0	1	
Employee	64	6 (9.4)	3.3 (1 to 11.1)	0.06	114	9 (5.1)	2 (1 to 12.6)	<0.01
Unemployed	31	3 (9.7)	3.4 (0.8 to 15)	0.11	26	4 (7)	3 (1 to 13.4)	<0.01
Age								
<35	73	5 (6.8)	1		53	6 (4.8)	1	
35-45	108	6 (5.6)	0.8 (0.2 to 2.7)	0.72	119	6 (2.6)	0.4 (0.1 to 1.4)	0.15

>45	77	3 (3.9)	0.6 (0.1 to 2.4)	0.43	106	1 (0.5)	0.1 (0 to 0.6)	0.02
Weight (kg)								
<50	89	8 (9)	1		49	4 (2.9)	1	
50-70	156	4 (2.6)	0.3 (0.1 to 0.9)	0.04	193	7 (2)	0.4 (0.1 to 1.5)	0.19
>70	13	2 (15.4)	1.8 (0.3 to 9.8)	0.47	36	2 (4.1)	0.7 (0.1 to 3.8)	0.64
HIV characteristics and treatment factors								
Route of Infection								
Heterosexual Transmission	159	8 (5)	1		154	2 (0.6)	1	
Blood Transmission	86	4 (4.7)	0.9 (0.3 to 3.2)	0.9	61	3 (2)	3.9 (0.6 to 24.1)	0.14
Intravenous Drug use	13	2 (15.4)	3.4 (0.6 to 18.2)	0.15	63	8 (10.5)	11.1 (2.3 to 53.7)	<0.01
Initial NRTI ART regimen								
AZT based regimen	161	5 (3.1)	1		188	10 (2.9)	1	
bvg based regimen	97	9 (9.3)	3.2 (1 to 9.8)	0.04	90	3 (1.6)	0.6 (0.2 to 2.3)	0.47
Latest ART regimen								
AZT based regimen	181	8 (4.4)	1		195	12 (3.2)	1	
D4T based regimen	77	6 (7.8)	1.8 (0.6 to 5.5)	0.28	83	1 (0.6)	0.2 (0 to 1.5)	0.11
Switch ART regimen								
No	193	10 (5.2)	1		174	6 (1.6)	1	
Yes	65	4 (6.2)	1.2 (0.4 to 4)	0.76	104	7 (4.1)	2 (0.7 to 6.2)	0.22
Adverse side-effect								
No	195	9 (4.6)	1		206	10 (2.5)	1	
Yes	63	5 (7.9)	1.8 (0.6 to 5.5)	0.32	72	3 (2.2)	0.9 (0.2 to 3.2)	0.81
CD4 cell at baseline (2008)								
<350	244	14 (5.7)			272	12 (2.3)	1	
≥350	14	0	-	0.36	6	1 (5)	4.3 (0.5 to 40)	0.2
CD4 cell at 36 months (2011)								

0-350	91	9 (9.9)	1		138	11 (4.8)	1	
≥350	167	5 (3)	0.3 (0.1 to 0.9)	0.03	140	2 (0.7)	0.2 (0 to 0.8)	0.02
CD4 cell at 48 months (2012)								
0-350	81	7 (8.6)	1		122	11 (5.4)	1	
≥350	177	7 (4)	0.4 (0.1 to 1.3)	0.13	156	2 (0.6)	0.1 (0 to 0.6)	0.01
Drug compliance factors								
Missed doses in past month								
No	226	13 (5.8)	1		246	6 (1.3)	1	
Yes	32	1 (3.1)	0.5 (0.1 to 4.2)	0.55	32	7 (10.9)	11.2 (3.5 to 35.9)	<0.01

* ART drug distribution location, Willing to receive ART in the future, Believe ART is health promoting ,Believe poor compliance contribute to HIVDR , Degree of satisfaction on support of friends or relatives, Frequency of taking drugs reminded by friends or relatives, Frequency of taking drugs reminded by doctors are not displayed for no statistical significant difference between categorizes.

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 3. Multivariate Models of Factors associated with virological failure (viral load ≥1000 copies/ml) and HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

	Female				Male			
	Virological failure		HIVDR		Virological failure		HIVDR	
Variables	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Total								
Ethnicity								
Han nationality			1		1		1	
Other minorities			4.8 (1.2 to 19.7)	0.03	2.9 (1.1 to 7.3)	0.02	12.2 (1.8 to 84.8)	0.01
Residence								
Rural			1					
City			2.4 (0.6 to 9.5)	0.22				
Age								
<35							1	
35-45							0.3 (0.1 to 1.4)	0.12
>45							0.03 (0 to 0.6)	0.02
Weight (kg)								
<50			1					
50-70			0.3 (0.1 to 1.1)	0.08				
>70			4.2 (0.6 to 30.0)	0.15				
Route of Infection								
Heterosexual Transmission	1				1		1	
Blood Transmission	1.2 (0.5 to 3)	0.74			1.8 (0.6 to 5.8)	0.33	7 (0.8 to 64.4)	0.09
Intravenous Drug use	4.1 (1 to 17.7)	0.06			2.1 (0.8 to 5.4)	0.12	2.3 (0.3 to 16.1)	0.41
Initial NRTI ART regimen								

AZT based regimen	1		1					
D4T based regimen	2.5 (1 to 6.1)	0.04	3.6 (1 to 12.6)	0.05				
ART drug distribution location								
County hospital or CDC					1			
Township hospital /village clinic /medication monitor					0.5 (0.2 to 1.3)	0.18		
Adverse effect								
No	1							
Yes	2.3 (1 to 5.6)	0.06						
CD4 cell at baseline (2008)								
<350					1			
≥350					7.1 (1.1 to 45.8)	0.04		
CD4 cell at 36 months (2011)								
<350	1		1				1	
≥350	0.4 (0.2 to 1.1)	0.07	0.3 (0.1 to 0.9)	0.04			0.3 (0 to 1.9)	0.2
CD4 cell at 48 months (2012)								
<350	1						1	
≥350	0.6 (0.3 to 1.6)	0.36					0.1 (0 to 1)	0.05
Missed doses in past month								
No					1		1	
Yes					2.8 (1.1 to 7)	0.03	9.7 (2.1 to 44.1)	<0.01
Frequency of taking drugs reminded by doctors								
Often					1			
Not often					0.4 (0.2 to 1.2)	0.12		

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 4. HIV Drug Resistance and Subtype among 27 patients with HIVDR Mutation Detected at 2011 and/or 2012 stratified by sex

	Female (%)	Male (%)	Mutations	N (%)
Overall	14	13		
Subtype				
B	5 (35.7)	3 (23.1)		
C		1 (7.7)		
CRF01_AE	2 (14.3)	1 (7.7)		
CRF07_BC	7 (50)	8 (61.5)		
Antiretrovial Drug				
Non-nucleoside reverse transcpriptase inhibitors (NNRTI,any)	14 (100)	13 (100)	NNRTI Mutations(total)	27
Efavirenz (EFV)	14 (100)	13 (100)	V90I	1 (3.7)
Nevirapine (NVP)	14 (100)	13 (100)	A98G	2 (7.4)
Etravirine (ETR)	6 (42.9)	8 (61.5)	K101E	6 (22.2)
			K103N	11 (40.7)
			V106A	4 (14.8)
			V108I	6 (22.2)
			E138A	1 (3.7)
			V179D/F	3 (11.1)
			Y181C	5 (18.5)
			G190A	5 (18.5)
			H221Y	1 (3.7)
			P225H	3 (11.1)
			F227L	1 (3.7)
			M230L	1 (3.7)

Nucleoside reverse transcpriptase inhibitors (NRTI,any)	12 (85.7)	9 (69.2)	NRTI Mutations(total）	21
Lamivudine (3TC)	12 (85.7)	9 (69.2)	A62V	1 (4.8)
Azidothymidine (AZT)	2 (14.3)	2 (15.4)	D67G	1 (4.8)
Tenofovir (TDF)	1 (7.1)	3 (23.1)	T69N	2 (9.5)
Stavudine (D4T)	3 (21.4)	3 (23.1)	K70R/Q	4 (19)
Didanosine (DDI)	5 (35.7)	3 (23.1)	V75I/M	2 (9.5)
Abcavir (ABC)	12 (85.7)	9 (69.2)	M184V	17 (81.0)
Emtricitabine (FTC)	12 (85.7)	9 (69.2)	T215N	1 (4.8)
			K219E/Q	2 (9.5)
Protease inhibitors (PI,any)	0	0	PI Mutations(total）	0

*NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors; PI: Protease Inhibitor

1. Hong SY, Nachega JB, Kelley K, et al. The global status of HIV drug resistance: clinical and public-health approaches for detection, treatment and prevention. *Infectious disorders drug targets* 2011;11(2):124-33.
2. Gabillard D, Lewden C, Ndoye I, et al. Mortality, AIDS-morbidity, and loss to follow-up by current CD4 cell count among HIV-1-infected adults receiving antiretroviral therapy in Africa and Asia: data from the ANRS 12222 collaboration. *Journal of acquired immune deficiency syndromes* 2013;62(5):555-61. doi: 10.1097/QAI.0b013e3182821821
3. Montaner JS, Lima VD, Harrigan PR, et al. Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and HIV transmission: the "HIV Treatment as Prevention" experience in a Canadian setting. *PloS one* 2014;9(2):e87872. doi: 10.1371/journal.pone.0087872
4. Staszewski S, Morales-Ramirez J, Tashima KT, et al. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. *The New England journal of medicine* 1999;341(25):1865-73. doi: 10.1056/NEJM199912163412501
5. Wang X, Yang L, Li H, et al. Factors associated with HIV virologic failure among patients on HAART for one year at three sentinel surveillance sites in China. *Current HIV research* 2011;9(2):103-11.
6. Zhang F, Dou Z, Ma Y, et al. Five-year outcomes of the China National Free Antiretroviral Treatment Program. *Annals of internal medicine* 2009;151(4):241-51, W-52.
7. Chinese Center of Disease and Control. Manual of the National Free Antiretroviral Treatment, 2007 edition 2007
8. People's Republic of China (2008) UNGASS Country Progress Report. *Beijing: People's Republic of China* 2007
9. Liu H, Ma Y, Su Y, et al. Emerging trends of HIV drug resistance in Chinese HIV-infected patients receiving first-line highly active antiretroviral therapy: a systematic review and meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014;59(10):1495-502. doi: 10.1093/cid/ciu590
10. Djarma O, Nguyen Y, Renois F, et al. Continuous free access to HAART could be one of the potential factors impacting on loss to follow-up in HAART-eligible patients living in a resource-limited setting: N'djamena, Chad. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2014;108(11):735-8. doi: 10.1093/trstmh/tru130
11. DeGruttola V, Dix L, D'Aquila R, et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. *Antiviral therapy* 2000;5(1):41-8.

12. Liao L, Xing H, Su B, et al. Impact of HIV drug resistance on virologic and immunologic failure and mortality in a cohort of patients on antiretroviral therapy in China. *Aids* 2013;27(11):1815-24. doi: 10.1097/QAD.0b013e3283611931
13. Hare AQ, Ordonez CE, Johnson BA, et al. Gender-specific risk factors for virologic failure in KwaZulu-Natal: automobile ownership and financial insecurity. *AIDS and behavior* 2014;18(11):2219-29. doi: 10.1007/s10461-014-0849-1
14. Berg KM, Demas PA, Howard AA, et al. Gender differences in factors associated with adherence to antiretroviral therapy. *Journal of general internal medicine* 2004;19(11):1111-7. doi: 10.1111/j.1525-1497.2004.30445.x
15. Floridia M, Giuliano M, Palmisano L, et al. Gender differences in the treatment of HIV infection. *Pharmacological research* 2008;58(3-4):173-82. doi: 10.1016/j.phrs.2008.07.007
16. Moore AL, Mocroft A, Madge S, et al. Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. *Journal of acquired immune deficiency syndromes* 2001;26(2):159-63.
17. World Health Organization. Surveillance Of HIV Drug Resistance In Adults Receiving Art (Acquired Hiv Drug Resistance). Accessed July 2014. 2014
18. Bennett DE, Myatt M, Bertagnolio S, et al. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral therapy* 2008;13 Suppl 2:25-36.
19. Gaydos CA, Rizzo-Price PA, Balakrishnan P, et al. Impact of international laboratory partnerships on the performance of HIV/sexually transmitted infection testing in five resource-constrained countries. *International journal of STD & AIDS* 2011;22(11):645-52. doi: 10.1258/ijsa.2011.010527
20. Organization WH. World Health Organization Protocol for Population Based Monitoring of HIV Drug Resistance Emerging During Treatment and Related Program Factors at Sentinel Antiretroviral Therapy Clinics.
21. Jiang Y, Qiu M, Zhang G, et al. Quality assurance in the HIV/AIDS laboratory network of China. *International journal of epidemiology* 2010;39 Suppl 2:ii72-8. doi: 10.1093/ije/dyq224
22. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2006;42(11):1608-18. doi: 10.1086/503914
23. Yan H, Yu H, Xing W, et al. Development of a proficiency testing program for the HIV-1 BED incidence assay in China. *Scientific reports* 2014;4:4512. doi: 10.1038/srep04512
24. Zhong P, Pan Q, Ning Z, et al. Genetic diversity and drug resistance of human immunodeficiency virus type 1 (HIV-1) strains circulating in Shanghai. *AIDS*

research and human retroviruses 2007;23(7):847-56. doi: 10.1089/aid.2006.0196

25. Xing H, Wang X, Liao L, et al. Incidence and associated factors of HIV drug resistance in Chinese HIV-infected patients receiving antiretroviral treatment. *PloS one* 2013;8(4):e62408. doi: 10.1371/journal.pone.0062408
26. Xing H, Ruan Y, Hsi JH, et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003–12. *Journal of Antimicrobial Chemotherapy* 2015:dkv078.
27. Nicastri E, Leone S, Angeletti C, et al. Sex issues in HIV-1-infected persons during highly active antiretroviral therapy: a systematic review. *The Journal of antimicrobial chemotherapy* 2007;60(4):724-32. doi: 10.1093/jac/dkm302
28. Puskas CM, Forrest JI, Parashar S, et al. Women and vulnerability to HAART non-adherence: a literature review of treatment adherence by gender from 2000 to 2011. *Current HIV/AIDS reports* 2011;8(4):277-87. doi: 10.1007/s11904-011-0098-0
29. Dou Z, Xu J, Jiao JH, et al. Gender difference in 2-year mortality and immunological response to ART in an HIV-infected Chinese population, 2006-2008. *PloS one* 2011;6(8):e22707. doi: 10.1371/journal.pone.0022707
30. Sabin LL, Desilva MB, Hamer DH, et al. Barriers to adherence to antiretroviral medications among patients living with HIV in southern China: a qualitative study. *AIDS care* 2008;20(10):1242-50. doi: 10.1080/09540120801918651
31. Wester CW, Okezie OA, Thomas AM, et al. Higher-than-expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana: preliminary results from a large randomized clinical trial. *Journal of acquired immune deficiency syndromes* 2007;46(3):318-22. doi: 10.1097/QAI.0b013e3181568e3f
32. Goedecke JH, Micklesfield LK, Levitt NS, et al. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS research and human retroviruses* 2013;29(3):557-63. doi: 10.1089/aid.2012.0252
33. Feleke Y, Fekade D, Mezegebu Y. Prevalence of highly active antiretroviral therapy associated metabolic abnormalities and lipodystrophy in HIV infected patients. *Ethiopian medical journal* 2012;50(3):221-30.
34. Moyle GJ, Nelson MR, Hawkins D, et al. The use and toxicity of didanosine (ddI) in HIV antibody-positive individuals intolerant to zidovudine (AZT). *The Quarterly journal of medicine* 1993;86(3):155-63.
35. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva2013.
36. Dou Z, Zhang F, Zhao Y, et al. Progress on China' s national free antiretroviral therapy strategy in 2002-2014. *Chinese Journal Epidemiology* 2015;36(12):1345-50.

37. Leng X, Liang S, Ma Y, et al. HIV virological failure and drug resistance among injecting drug users receiving first-line ART in China. *BMJ open* 2014;4(10):e005886.
38. Wu Z, Zhang J, Detels R, et al. Characteristics of risk-taking behaviors, HIV and AIDS knowledge, and risk perception among young males in southwest China. *AIDS education and prevention : official publication of the International Society for AIDS Education* 1997;9(2):147-60.
39. Yang H, Li X, Stanton B, et al. Heterosexual transmission of HIV in China: a systematic review of behavioral studies in the past two decades. *Sexually transmitted diseases* 2005;32(5):270-80.
40. Myers Jr SL, Xiaoyan G, Cruz BC. Ethnic minorities, race, and inequality in China: A new perspective on racial dynamics. *Review of Black Political Economy* 2013;40(3):231.
41. Zoufaly A, Jochum J, Hammerl R, et al. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. *The Journal of antimicrobial chemotherapy* 2015;70(3):922-5. doi: 10.1093/jac/dku470
42. Shou L, WANG QX, Lei N, et al. The changing trends of HIV/AIDS in an ethnic minority region of China: modeling the epidemic in Liangshan prefecture, Sichuan Province. *Biomedical and Environmental Sciences* 2013;26(7):562-70.
43. Zhang L, Zhu J, Rui B, et al. High HIV risk among Uigur minority ethnic drug users in northwestern China. *Tropical Medicine & International Health* 2008;13(6):814-17.
44. Chinese Center for Disease Control and Prevention. Manual of the National Free Antiretroviral Treatment, third edition. Accessed 2013 Jun 12. . 2013
45. World Health Organization. Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach: World Health Organization 2013.
46. Xing H, Ruan Y, Hsi JH, et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003-12. *The Journal of antimicrobial chemotherapy* 2015;70(7):2097-103. doi: 10.1093/jac/dkv078
47. Liao L, Xing H, Shang H, et al. The prevalence of transmitted antiretroviral drug resistance in treatment-naive HIV-infected individuals in China. *Journal of acquired immune deficiency syndromes* 2010;53 Suppl 1:S10-4. doi: 10.1097/QAI.0b013e3181c7d363
48. Zhao K, Kang W, Liu Q, et al. Genotypes and transmitted drug resistance among treatment-naive HIV-1-infected patients in a northwestern province, China: trends from 2003 to 2013. *PloS one* 2014;9(10):e109821. doi: 10.1371/journal.pone.0109821

49. Su Y, Zhang F, Liu H, et al. The prevalence of HIV-1 drug resistance among antiretroviral treatment naive individuals in mainland China: a meta-analysis. *PloS one* 2014;9(10):e110652. doi: 10.1371/journal.pone.0110652

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page	Recommendation
Title and abstract	1	2	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		2-3	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
			Introduction
Background/rationale	2	5	Explain the scientific background and rationale for the investigation being reported
Objectives	3	6	State specific objectives, including any prespecified hypotheses
			Methods
Study design	4	7	Present key elements of study design early in the paper
Setting	5	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	7	**(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	8-9	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	9	Describe any efforts to address potential sources of bias
Study size	10	9	Explain how the study size was arrived at
Quantitative variables	11	9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	9	(*a*) Describe all statistical methods, including those used to control for confounding
		9	(*b*) Describe any methods used to examine subgroups and interactions
		7,9-10	(*c*) Explain how missing data were addressed
		9-10	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Results			
Participants	13*	9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		9-10	(b) Give reasons for non-participation at each stage
		19	(c) Consider use of a flow diagram
Descriptive data	14*	10	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
		10	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*10*	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	11,9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		8	(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	14-15	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	12	Summarise key results with reference to study objectives
Limitations	19	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	12-16	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	17	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	18	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Predictors of HIV Virologic Failure And Drug Resistance In Chinese Patients After 48 Months Of Antiretroviral Treatment, 2008-2012 : A Prospective Corhort Study

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Public health
Keywords:	Antiretroviral Treatment, Virological Failure, Drug Resistance, Gender Difference, HIV & AIDS < INFECTIOUS DISEASES>

Predictors of HIV Virologic Failure And Drug Resistance In Chinese Patients After 48 Months Of Antiretroviral Treatment, 2008-2012 : A Prospective Corhort Study

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Running head: Virologic failure and drug resistance among HIV+ Chinese ART patients

Body: 3,111

ABSTRACT

Objective: To explore factors associated with HIV virologic failure (VF) and HIV drug resistance (HIVDR) among HIV-positive Chinese individuals four years after initiating first-line 3TC-based antiretroviral treatment (ART) in 2008 at five sentinel sites.

Design: First-line ART initiators who were previously treatment naïve were selected using consecutive ID numbers from the 2008 National Surveillance Database into a prospective cohort study. Questionnaires and Blood samples were collected in 2011 and 2012 to assess the outcomes of interest: VF (defined as viral load ≥ 1000 copies/ml) and HIVDR (defined as VF with genetic drug resistant mutations). Questionnaires and data from National Surveillance Database assessed demographics and drug adherence data.

Results: 536 individuals with HIV were analyzed; the 4-year risk of VF was 63(11.8%) and HIVDR was 27(5.0%). Female participants initiating D4T-based regimens were more susceptible to both VF (adjusted odds ratio, aOR=2.5, 95% CI: 1-6.1, P-value=0.04) and HIVDR (aOR=3.6, 95% CI: 1 to 12.6, P-value=0.05) versus AZT-based regimens. Male participants missing doses in past month were more susceptible to both VF (aOR=2.8, 95% CI: 1.1 to 7, P-value=0.03) and HIVDR (aOR=9.7, 95% CI: 2.1 to 44.1, P-value<0.01). Participants of non-Han nationality were of increased risk for HIVDR (aOR from 4.8-12.2, P-value<0.05) and non-Han

men were at increased risk for VF (aOR=2.9, 95% CI: 1.1 to .3, P-value=0.02). All 27 participants detected with HIVDR had non-nucleoside reverse-transcriptase inhibitor (NNRTI) mutations, 21 (77.8%) also had NRTI mutations, and no protease inhibitor mutations were detected.

Conclusions: Our findings suggest successful treatment outcomes at 4-years for roughly 90% of patients. We suggest conducting further study on whether and when to change ART regimen for women initiated with D4T-based regimen, and reinforcing adherence counseling for men. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions.

Strengths and limitations of this study

- We studied 48-month risk of VF and HIVDR and their associations with demographic and behavioral information among individuals across five sentinel sites.
- Study found drug adherence and adverse effects influenced VF and HIVDR differently across gender..
- The outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes.

Key words: HIV, Antiretroviral Treatment, Virological Failure, Drug Resistance, Gender Differences, China

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Introduction

Antiretroviral treatment (ART) has dramatically improved health outcomes and decreased HIV-associated morbidity and mortality through virologic suppression and subsequent CD4 recovery. [1-4] In 2003, China launched a National Free Antiretroviral Treatment Program (NFATP) that includes life-long provision of free ART for people living with HIV who met the national treatment criteria.[5, 6] The national treatment criteria from 2008 to 2011 were: (1) CD4 cell count ≤200/mm^3; (2) World Health Organization (WHO) stage III/IV diseases; or (3) willingness to receive ART, regardless of criteria 1 and 2.[7]

The State Council AIDS Working Committee Office and the United Nations Theme Group on AIDS estimated that there were more than 700,000 persons living with HIV in China in 2008, and more than 52,000 individuals with HIV across 31 provinces, autonomous regions, and municipalities had received ART (made freely available by the NAFTP) by August 2008.[8]

With the rapid scale-up of treatment and challenges with adherence, virologic failure (VF) and HIV drug resistance (HIVDR) are ever present and mounting concerns. Incomplete virologic suppression, a major cause of HIVDR, not only compromises therapeutic efficacy for the individual receiving treatment, increasing the risk of viral rebound and opportunistic infections, but also increases the risk of transmitting drug resistant strains to other individuals in the general population.[9 10, 11]

Observational studies in China have documented the prevalence of VF and HIVDR strains among treated individuals living with HIV. A cross-sectional study conducted in Yunnan, Guangxi and Xinjiang provinces in 2010 stated that one-year HIVDR prevalence was 4.1%[5]. VF prevalence for sexual transmitted population and intravenous drug users (IDUs) were 8.3% and 19.3%, separately. A 6-year follow-up study in 2010 suggested an incidence of 14.1 per 100 person-year for VF and 11.9 per 100 person-year for HIVDR among former plasma donors in Anhui Province.[12] NFATP recommended to switch the first-line regimen from Didanosine (DDI) to Lamivudine (3TC) in 2008, and there are few nationwide, prospective studies in China reporting frequency or predictors of VF and HIVDR for people after initiating 3TC based regimens.

The aim of this study is to evaluate predictors of VF and HIVDR in a prospective cohort of Chinese HIV individuals with HIV four years after first initiating first-line 3TC-based ART in 2008 at five sentinel sites. We stratified our analyses by gender based on conflicting findings on gender differences both in virological responses and drug resistance to different ART regimen, as well as gender differences in ART adherence.[13-16] To our knowledge, this is the first long-term study to evaluate VF and HIVDR on 3TC-based regimens.

Ethics Statement

The study was approved by the institutional review board (IRB) of the National Center for AIDS/STD Control and Prevention of the China Center for Disease Control

Prevention (NCAIDS, China CDC). All participants provided written informed consent before participation. Signed informed consent was obtained from each of the participants prior to the interviews and blood collection.

Methods

Study Design and Data Collection

This study was designed under the WHO Surveillance of HIV drug resistance in adults receiving ART for 48 months. [10, 17, 18] Five provinces in China with highest rate of transmission were selected to conduct a prospective cohort study with a follow-up study at 12 months: Guangxi, Henan, Hubei, Xinjiang and Yunnan. Participants were sampled from the 2008 National HIV Surveillance Database through sequential sampling from each province. Participant eligibility criteria included being age ≥ 18 years; having initiated NAFTP-sponsored first-line ART in 2008; having been ART-free before 2008; having been on ART for 36±6 months in 2011; and providing consent to participate in the study.

Questionnaires administered by trained study personnel from provincial CDC in private rooms using structured interviews collected data in 2011 and 2012. Each study participant was assigned a confidential identification number used to label questionnaire and blood specimen. Additional HIV-specific data including route of transmission, initial ART regimen, latest ART regimen, ART distribution location and CD4 cell count were collected from the 2011-2012 National HIV Surveillance

Database. There was no missing demographic data, missed questionnaire data was feedbacked to local CDC for recollection at the time.

Laboratory analysis

Blood specimens were collected from all participants to determine CD4 cell count, HIV-1 RNA viral load (VL), and HIV-1 drug resistance mutations in both 2011 and 2012. CD4 cell count estimation was conducted at provincial CDC laboratories using flow cytometry (FACSC Calibur, BD Company, USA) within 24 hours after specimen collection. Plasma was isolated and stored at -80°C at a provincial CDC laboratory and then transferred to NCAIDS for testing HIV VL and drug mutation. Plasma HIV RNA was quantified with real-time NASBA (NucliSense Easy Q, bioMerieux, France) or COBAS (Roche Applied Biosystems, Germany) according to manufacturer recommendations using in-house PCR (polymerase chain reaction), both of the assays were performed automatically.[19] Virologic failure was defined as VL ≥1000 copies/ml. According WHO protocol, [20] HIVDR tests were performed on samples with VL ≥1000 copies/ml. HIV-1 *pol* gene (protease 1-99 amino acids and part of reverse transcriptase 1-252 amino acids) were amplified, purified and analyzed using the Stanford HIV Drug Resistance Database (https://hivdb.stanford.edu/hivdb). Levels of HIVDR were classified according to the algorithm of Stanford HIVdb program. The scores are the sum of each mutation penalty score for a drug. Scores less than 10 indicate susceptible; scores between 10 and 14 indicate potential low-level resistance; scores between 15 and 29 indicate low-level resistance; scores

between 30 and 59 indicate intermediate resistance. Scores of 60 or greater indicate high-level resistance. Any low-, intermediate-, or high-level resistance identified was defined as HIVDR.[21-25]

Data analysis

Questionnaire data were double-entered using Epidata 3.1 (The Epidata Association Odense, Denmark). Statistical Analysis System (SAS 9.4, SAS Institute Inc., Cary, NC, USA) was then used for data cleaning and analyses.

48-month risk for the outcomes of interest was calculated as the proportion of unique persons who had experienced incident VF or HIVDR by the end of follow-up in 2012. Covariates of interest were described using counts and percentages overall and by the outcome of interest, stratified by gender. Univariate logistic regression models were constructed to explore associations between covariates of interest and VF or HIVDR. Odds ratios (OR) and 95% confidence intervals (CIs) are reported. Variables that were significant ($P < 0.05$) in the univariate models were then fit into multivariate logistic regression models assessment for removal of collinear variables that had the weakest association with the outcome. Adjusted ORs (aOR) and 95% CIs were presented. $P < 0.05$ was defined as statistically significant, and all tests were two sided. Descriptive analysis on HIVDR mutation results was conducted among 27 HIVDR participants, stratified by sex.

Results

1100 subjects were selected using consecutive ID numbers from 2008 National Surveillance Database; of those, 490 were lost follow-up by December, 2012. Among those lost to follow up, 139 died, 55 emigrated, 134 lost contact, 17 refused to participate, 65 stopped ART before 30 months, 36 transferred, 8 were under custody, 6 failed to provide a blood sample, 3 switched from ART to Tangcao tablet (an antiviral Chinese herbal therapy), 2 became pregnant and switched to other regimens and 1 was paralyzed. After excluding 74 participants for failing the eligibility criteria, 536 participants were included in the final 24-month analysis (Figure 1). The 48-month risk of VF was 11.8% and risk of drug resistance was 5%.

Demographic and ART Information (Tables 1-2)

Of the 536 eligible participants, 51.8% were male; 76.5% were Han nationality; 45.0% had an education level of elementary school or less; 56.2% were farmers; and 10.6% were unemployed with the rest having regular income.

All regimens in this cohort remained 3TC-based from 2008 to 2012. Initiated Nucleoside Reverse Transcriptase Inhibitors (NRTI) regimens included Zidovudine (AZT) (n=349, 65.1%) or Stavudine (D4T) (n=187, 34.9%). 66 (12.3%) participants later changed to TDF based regimen. Initiated Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) regimens included Nevirapine (NVP) (n=421, 78.5%) or Etravirine (EFV) (n=115, 21.5%). 55 (10.3%) participants later changed to LPV/r based regimen. 169 (31.5%) participants switched the initial ART regimen during 2008-2012, but no statistical significant difference was found in VF (p=0.74) and

HIVDR (p=0.29) risk between participants who switched regimens and participants who did not switch regimens.

We found that 38.4% participants were hesitant to accept ART in the future, 36.8% participants reported doubts whether ART was health promoting and 42.5% participants did not report that poor ART adherence necessarily contributed to HIVDR. Additionally, 40.5% of participants were not always satisfied with support from friends or relatives. 472 (88.1%) participants reported not missing a dose in the month prior to the date of the survey.

Multivariate model results (Table 3)

As shown in Table 3, minority male participants were at higher risk for both VF (aOR=2.9, 95% CI: 1.1 to 7.3, P-value=0.02) and HIVDR (aOR=12.2, 95% CI: 1.8 to 84.8, P-value=0.01) compared to Han nationality male participants, while female minorities were only at a higher risk for HIVDR (aOR=4.8, 95% CI: 1.2 to 19.7, P-value=0.03).

Female participants initiating D4T-based regimens were at a higher risk for both VF (aOR=2.5, 95% CI: 1 to 6.1, P-value=0.04) and HIVDR (aOR=3.6, 95% CI: 1 to 12.6, P-value=0.05) versus those initiating an AZT-based regimen; interestingly, different from their female counterparts, male participants showed no such association (OR=0.6, 95% CI: 0.3 to 1.4, P-value=0.24). Also, female participants had a higher risk of VF given adverse side-effects (aOR=2.3, 95% CI: 1 to 5.6, P-value=0.06).

Male participants with missed doses in the month prior to the survey were at a higher

risk of both VF (aOR=2.8, 95% CI: 1.1 to 7, P-value=0.03) and HIVDR (aOR=9.7, 95% CI: 2.1 to 44.1, P-value<0.01) versus those without missed doses in the preceding month. Conversely, missed doses in prior month was not significantly associated with VF or HIVDR for women.

HIV Drug Resistance and Subtype (Table 4)

HIVDR identified in our study was consistence with the NFATP recommended ART regimen. All 27 participants detected with drug resistance had NNRTI mutations, 21 (77.8%) had NRTI mutations. The dominant subtype was CRF07_BC for both males (61.5%) and females (50%). All participants found with HIVDR had developed HIVDR towards NNRTI; 85.7% male participants and 69.2% female developed HIVDR toward NRTI; no Protease Inhibitor mutation was detected. There were no CRF08_BC subtypes detected in the study population.

Discussion

The 48-month risk of VF was 11.8% and HIVDR was 5.0%, which indicated relatively good treatment outcomes given meta-analysis suggested a 37-48 months HIVDR prevalence ranging from 3.04%-47.92% in China,[9] similar to stdies in China which estimated a one-year HIVDR incidence of 3.5% in 2009 and 2.1% in 2012 .[26, 27] Our study substantiates the finding that VF and HIVDR largely decreased since the wide-spread of 3TC-based regimens.[28] Studies have shown mixed findings of gender differences on ART adherence and treatment outcomes. [14, 16, 29, 30] In our study, we found male participants had slightly higher risk of VF (12.2% versus 11.2%, P-

value=0.72) but lower risk of HIVDR (2.4% versus 5.4%, P-value=0.69) than women. Drug adherence and adverse effects influenced our outcomes differently for men compared to women. Women's risks of VF and HIVDR were not associated with missing doses in the past month, also, few women missed doses relative to men, similar to two other studies in China suggesting women have better adherence behaviors. [31, 32] Male participants were at higher risk of both VF and HIVDR if they reported missed doses. More detailed studies need to be conducted on the frequency and factors associated with missing treatment. However, female participants showed a higher risk of VF if they had adverse effect while men did not. This calls for further research of what types of adverse effects are occurring and how they affect ART adherence and virological outcomes across gender.

We found in this study that women, not men, who initiated D4T-based regimens were more susceptible to VF (women vs. men OR=2.3 95% CI: 1.0 to 5.7 P-value=0.06) and HIVDR (women vs. men OR=3.0 95%, CI: 0.8 to 11.3, P-value=0.11), consistent with previous findings that D4T was more likely to increase the risk of mitochondrial toxicity in women. [33, 34] Mitochondrial toxicity caused by D4T had been reported to cause many adverse effects such as lactic acidosis, lipodystrophy, and peripheral neuropathy. [35, 36] Following the WHO recommendation,[37] the NFATP advocated switching the first-line regimen from D4T to TDF in 2012. The percentage of people living with HIV initiating D4T-based regimen changed from 34.3% in 2010 to10% in 2012 and 0.9% in 2014;[38] however, there were still 29.9% participants in our study

who were on D4T-based regimens in 2012. It was noteworthy that we did not see a statistical difference in VF (OR=1.4 95%, CI: 0.4 to 4.2, P-value=0.60) and HIVDR (OR=1.0, 95% CI: 0.2 to 4.2, P-value=0.98) between women who initiated and remained on D4T-based regimens and those who switched to AZT/TDF based regimens. It is possible that women switched regimens because of VF; however, further studies need to be done to explore when to switch ART regimen for women receiving D4T-based regimens. It is important to mention that data on ART adherence and adverse effects were collected in 2012, when there were only four female participants still using D4T-based regimens who experienced VF. The sample size was not sufficient to explore whether D4T-based regimens affect drug adherence and adverse effects for women.

Though not associated with the VF and HIVDR outcomes, 38.4% of study participants reported that they would not 'always' be willing to take ART in the future, 36.8% reported not believing that ART is 'always' health promoting, and 42.5% reported not believing that poor compliance 'always' contributed to HIVDR. As willingness and these knowledge factors may impact more long-term VF and HIVDR outcomes, the motivations behind willingness and knowledge about VF and HIVDR warrant exploration.

Caution is needed when interpreting the study results from multivariate model that older age (>45) was protective for HIVDR in men. There were only 4 male IDU participants with older age (>45) in this study, the number is not sufficient to test for

interaction. There is no association between HIVDR and age (OR=6.5, 95% CI: 1.1 to 38.1, P-value=0.49) in the sub-analysis we did among male IDU participants , after controlling for variables showed significant in the univariate model. A previous study in HIV positive IDU population in China suggested that there is no association between VF and sex or age.[39] In this study, 61.5% of male participants with HIVDR became HIV infected via IDU, yet there were only 22.7% male IDU participants.

In addition, we found that younger (<45 years) IDU population were more likely to miss doses (18.6%) compared to heterosexual transmission population (8.8%) and blood transfusion transmission population (12.1%). This finding was consistent with studies that implied younger males were at a higher risk of drug abuse.[40, 41] This result indicated that younger IDU population could be a main source of VF and HIVDR; therefore they could be future targeted population for behavioral intervention.

The increased risk of VF and HIVDR in non-Han minorities, regardless of gender, may be due to logistical, cultural, or social barriers faced by ethnic minorities which limit their adherence to ART. It has been reported that minorities tend to have lower social economic status than Han majorities, followed by lower education level and fewer access to health facilities.[42] It may be difficult for health professionals to reach for some minorities because of their more remote geographic locations. Additionally, several studies have reported that the percentage of high-risk populations such as female sex workers (FSWs) and IDUs were higher in minorities than in Han nationality. [43-45] The causes of this increased VF and HIVDR risk warrants further

exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions in China. The finding that higher CD4 cell count at follow-up was protective for VF and HIVDR was expected. NFATP changed treatment criteria from CD4 cell count ≤200cells/mm^3 to CD4 cell count ≤350cells/mm^3 following the WHO recommendation in 2011, [7, 17, 46]. Our study indicated that male participants who initiated treatment in 2008 at CD4 cell count >=350 cells/mm^3 were still at higher risk towards VF (aOR=7.1, 95% CI: 1.1 to 45.8, P-value=0.04), supporting possible clinical benefits of initiating ART at higher CD4 cell counts, <500 cells/mm^3 as per WHO recommendation in 2013.[47]

Among participants infected by blood transmission, we only found HIVDR subtype B; only one subtype C was found in participants infected with IDU, the dominant subtype was CRF07_BC, found both in participants infected by heterosexual transmission and IDU. The most common NNRTI mutation sites were K103N (40.7%), K101E (22.2%) and V108I (22.2%); the most common NRTI mutation sites were M184V (81.0 %) and K70R (19%). Interestingly, compared to a one-year follow-up study in China with all participants initiated ART in 2011,[48] there is no V108I in their study and we did not find K65R in our study.

Study findings should be interpreted in light of several limitations. Though we did not account for transmitted drug resistance in this study, previous studies have found low transmitted drug resistance risk (<5%) during this period [49-51] in China and we could be fairly certain that participants were outcome free in 2008 as they were new ART

initiators. Another limitation of our study is that the outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes. Also, route of transmission was collected in 2008 when assessing HIV infection among men who have sex with men was not part of data collection instruments; additionally we do not have data on sex worker status. Roughly half of the study participants selected for possible inclusion in the study for having initiated first-line ART in 2008 were lost to follow-up by 2012, creating a possible selection bias for individuals with better ART adherence – this bias may underestimate the true VF and HIVDR risk and also limit the generalizability of our findings to better adherers. Additionally, given the demographic profile of the cohort, our findings are most generalizable to heterosexual Han national who are married/cohabiting and working as agricultural labors in rural areas. Misclassification of self-reported data is possible, though we do not expect this misclassification to be differential by the outcome of interest and thus any such information bias would bias our results toward the null.

Conclusions

We found female participants initiating D4T versus AZT-based regimens were more vulnerable to VF and HIVDR, and we suggest future studies on whether and when to change ART regimen for women initiated with D4T-based regimen. Poor adherence was a risk factor among male participants who may benefit from reinforced adherence counseling or social support. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important

group to tailor adherence-focused interventions in China. Also, this study indicated that younger men who become infected through IDU may be groups to strategically focus counseling and increased adherence support programs.

Funding

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Contributions

YR, WK, HX and YS designed the study. YR, HX, SL, LL, YM, HT, TZ, GS, HC, WK, XL and PL collected the data. HC, TT and LL conducted laboratory analysis. XL, WK analyzed the data. KW, VM, YR, WK, HX, LL and YS interpreted the data. KW, VM, YR, WK, TT, and YS drafted the report. All authors reviewed, revised, and approved the final report.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Transparency Declarations

Vincent C. Marconi has received fees from ViiV Healthcare.

Conflicts of Data Sharing Statement

No additional data available.

Table 1. Factors associated with virological failure (viral load ≥1000 copies/ml) stratified by sex

DEMOGRAPHIC FACTORS	Female				Male			
	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value
Total	258	29 (11.2)			278	34 (12.2)		
Ethnicity								
Han nationality	191	19 (9.9)	1		219	20 (9.1)	1	
Other minorities	67	10 (14.9)	1.6 (0.7,3.6)	0.27	59	14 (23.7)	3.1 (1.5,6.6)	<0.01
Education								
Elementary school or less	134	15 (11.2)	1		107	12 (11.2)	1	
Junior school or more	124	14 (11.3)	1 (0.5,2.2)	0.98	171	22 (12.9)	1.2 (0.6,2.5)	0.68
Marital Status								
Single	59	6 (10.2)	1		75	9 (12)	1	
Married or Cohabited	199	23 (11.6)	1.2 (0.4,3.0)	0.77	203	25 (12.3)	1 (0.5,2.3)	0.94
Residence								
Rural	197	19 (9.6)	1		172	17 (9.9)	1	
City	61	10 (16.4)	1.8 (0.8,4.2)	0.15	106	17 (16)	1.7 (0.8,3.6)	0.13
Occupation								
Peasant	163	15 (9.2)	1		138	9 (6.5)	1	
Employee	64	11 (17.2)	2 (0.9,4.7)	0.09	114	17 (14.9)	2.5 (1.1,5.9)	0.03
Unemployed	31	3 (9.7)	1.1 (0.3,3.9)	0.93	26	8 (30.8)	6.4 (2.2,18.6)	<0.01
Age								

<35	73	6 (8.2)	1		53	7 (13.2)	1	
35-45	108	15 (13.9)	1.8 (0.7,4.9)	0.25	119	15 (12.6)	0.9 (0.4,2.5)	0.91
>45	77	8 (10.4)	1.3 (0.4,3.9)	0.65	106	12 (11.3)	0.8 (0.3,2.3)	0.73
Weight (kg)								
<50	89	14 (15.7)	1		49	7 (14.3)	1	
50-70	156	13 (8.3)	0.5 (0.2,1.1)	0.08	193	21 (10.9)	0.7 (0.3,1.8)	0.51
>70	13	2 (15.4)	1 (0.2,4.9)	0.97	36	6 (16.7)	1.2 (0.4,3.9)	0.76
HIV CHARACTERISTICS AND TREATMENT FACTORS								
Route of Infection								
Heterosexual Transmission	159	12 (7.5)	1		154	11 (7.1)	1	
Blood Transmission	86	13 (15.1)	2.2 (0.9,5)	0.07	61	10 (16.4)	2.5 (1,6.4)	0.04
Intravenous Drug use	13	4 (30.8)	5.4 (1.5,20.3)	0.01	63	13 (20.6)	3.4 (1.4,8)	0.01
Initial NRTI ART regimen								
AZT based regimen	161	11 (6.8)	1		188	26 (13.8)	1	
D4T based regimen	97	18 (18.6)	3.1 (1.4,6.9)	<0.01	90	8 (8.9)	0.6 (0.3,1.4)	0.24
Latest NRTI ART regimen								
AZT based regimen	181	15 (8.3)	1		195	27 (13.8)	1	
D4T based regimen	77	14 (18.2)	2.5 (1.1,5.4)	0.02	83	7 (8.4)	0.6 (0.2,1.4)	0.21
Switch ART regimen								
No	193	21 (10.9)	1		174	21 (12.1)	1	

Yes	65	8 (12.3)	1.2 (0.5,2.7)	0.75	104	13 (12.5)	1 (0.5,2.2)	0.92
ART drug distribution location								
County hospital or CDC	96	15 (15.6)	1		63	14 (22.2)	1	
Township hospital /village clinic /medication monitor	162	14 (8.6)	0.5 (0.2,1.1)	0.09	215	20 (9.3)	0.4 (0.2,0.8)	0.01
Adverse effects								
No	195	17 (8.7)	1		206	23 (11.2)	1	
Yes	63	12 (19)	2.5 (1.1,5.5)	0.03	72	11 (15.3)	1.4 (0.7,3.1)	0.36
CD4 cell/ml at baseline (2008)								
<350	244	28 (11.5)	1		272	31 (11.4)	1	
≥350	14	1 (7.1)	0.6 (0.1,4.7)	0.62	6	3 (50)	7.8 (1.5,40.2)	0.01
CD4 cell/ml at 36 months (2011)								
0-350	91	18 (19.8)	1		138	21 (15.2)	1	
≥350	167	11 (6.6)	0.3 (0.1,0.6)	<0.01	140	13 (9.3)	0.6 (0.3,1.2)	0.13
CD4 cell/ml at 48 months (2012)								
0-350	81	14 (17.3)	1		122	20 (16.4)	1	
≥350	177	15 (8.5)	0.4 (0.2,1)	0.04	156	14 (9)	0.5 (0.2,1)	0.06
DRUG COMPLIANCE FACTORS								
Missed doses								

in past month								
No	226	26 (11.5)	1		246	24 (9.8)	1	
Yes	32	3 (9.4)	0.8 (0.2,2.8)	0.72	32	10 (31.3)	4.2 (1.8,9.9)	<0.01
Willing to receive ART in the future								
Always	153	15 (9.8)	1		177	19 (10.7)	1	
Not always	105	14 (13.3)	1.4 (0.7,3.1)	0.38	101	15 (14.9)	1.5 (0.7,3)	0.32
Believe ART is health promoting								
Always	158	16 (10.1)	1		181	19 (10.5)	1	
Not always	100	13 (13)	1.3 (0.6,2.9)	0.48	97	15 (15.5)	1.6 (0.8,3.2)	0.24
Believe poor compliance contribute to HIVDR								
Always	139	16 (11.5)	1		169	19 (11.2)	1	
Not always	119	13 (10.9)	0.9 (0.4,2.1)	0.88	109	15 (13.8)	1.3 (0.6,2.6)	0.53
Degree of satisfaction on support of friends or relatives								
Always satisfied	153	15 (9.8)	1		166	17 (10.2)	1	
Not always satisfied	105	14 (13.3)	1.4 (0.7,3.1)	0.38	112	17 (15.2)	1.6 (0.8,3.2)	0.22
Frequency of taking drugs reminded by friends or								

relatives								
Often	172	19 (11)	1		168	22 (13.1)	1	
Not often	86	10 (11.6)	1.4 (0.7,3.1)	0.38	110	12 (10.9)	0.8 (0.4,1.7)	0.58
Frequency of taking drugs reminded by doctors								
Often	178	23 (12.9)	1		184	28 (15.2)	1	
Not often	80	6 (7.5)	0.5 (0.2,1.4)	0.19	94	6 (6.4)	0.4 (0.2,1)	0.03

*OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 2. Factors associated with HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

DEMOGRAPHIC FACTORS	Female				Male			
	Total	HIVDR Risk N (%)	OR (95%CI)	P-value	Total	HIVDR Risk N (%)	OR (95%CI)	P-value
	258	14 (5.4)			278	13 (4.7)		
Ethnicity								
Han nationality	191	6 (3.1)	1		219	5 (2.3)	1	
Other minorities	67	8 (11.9)	4.2 (1.4 to 12.5)	0.01	59	8 (13.6)	6.7 (2.1 to 21.4)	<0.01
Education								
Elementary school or less	134	5 (3.7)	1		107	5 (4.7)	1	
Junior school or more	124	9 (7.3)	2 (0.7 to 6.2)	0.22	171	8 (4.7)	1 (0.3 to 3.1)	1
Marital Status								
Single	59	4 (6.8)	1		75	4 (5.3)	1	
Married or Cohabited	199	10 (5)	0.7 (0.2 to 2.4)	0.60	203	9 (4.4)	0.8 (0.2 to 2.8)	0.75
Residence								
Rural	197	7 (3.6)	1		172	5 (2.9)	1	
City	61	7 (11.5)	3.5 (1.2 to 10.5)	0.02	106	8 (7.5)	2.7 (0.9 to 8.6)	0.09
Occupation								
Peasant	163	5 (3.1)	1		138	0	1	
Employee	64	6 (9.4)	3.3 (1 to 11.1)	0.06	114	9 (7.9)	2 (1 to 12.6)	<0.01
Unemployed	31	3 (9.7)	3.4 (0.8 to 15)	0.11	26	4 (15.4)	3 (1 to 13.4)	<0.01

Age								
<35	73	5 (6.8)	1		53	6 (11.3)	1	
35-45	108	6 (5.6)	0.8 (0.2 to 2.7)	0.72	119	6 (5)	0.4 (0.1 to 1.4)	0.15
>45	77	3 (3.9)	0.6 (0.1 to 2c.4)	0.43	106	1 (0.9)	0.1 (0 to 0.6)	0.02
Weight (kg)								
<50	89	8 (9)	1		49	4 (8.2)	1	
50-70	156	4 (2.6)	0.3 (0.1 to 0.9)	0.04	193	7 (3.6)	0.4 (0.1 to 1.5)	0.19
>70	13	2 (15.4)	1.8 (0.3 to 9.8)	0.47	36	2 (5.6)	0.7 (0.1 to 3.8)	0.64
HIV CHARACTERISTICS AND TREATMENT FACTORS								
Route of Infection								
Heterosexual Transmission	159	8 (5)	1		154	2 (0.6)	1	
Blood Transmission	86	4 (4.7)	0.9 (0.3 to 3.2)	0.9	61	3 (2)	3.9 (0.6 to 24.1)	0.14
Intravenous Drug use	13	2 (15.4)	3.4 (0.6 to 18.2)	0.15	63	8 (10.5)	11.1 (2.3 to 53.7)	<0.01
Initial NRTI ART regimen								
AZT based regimen	161	5 (3.1)	1		188	10 (2.9)	1	
D4T based regimen	97	9 (9.3)	3.2 (1 to 9.8)	0.04	90	3 (1.6)	0.6 (0.2 to 2.3)	0.47
Latest ART regimen								
AZT based regimen	181	8 (4.4)	1		195	12 (3.2)	1	
D4T based regimen	77	6 (7.8)	1.8 (0.6 to 5.5)	0.28	83	1 (0.6)	0.2 (0 to 1.5)	0.11
Switch ART regimen								

No	193	10 (5.2)	1		174	6 (3.4)	1	
Yes	65	4 (6.2)	1.2 (0.4 to 4)	0.76	104	7 (6.7)	2 (0.7 to 6.2)	0.22
Adverse effect								
No	195	9 (4.6)	1		206	10 (4.9)	1	
Yes	63	5 (7.9)	1.8 (0.6 to 5.5)	0.32	72	3 (4.2)	0.9 (0.2 to 3.2)	0.81
CD4 cell at baseline (2008)								
<350	244	14 (5.7)			272	12 (2.3)	1	
≥350	14	0	-	0.36	6	1 (5)	4.3 (0.5 to 40)	0.2
CD4 cell at 36 months (2011)								
0-350	91	9 (9.9)	1		138	11 (4.8)	1	
≥350	167	5 (3)	0.3 (0.1 to 0.9)	0.03	140	2 (0.7)	0.2 (0 to 0.8)	0.02
CD4 cell at 48 months (2012)								
0-350	81	7 (8.6)	1		122	11 (5.4)	1	
≥350	177	7 (4)	0.4 (0.1 to 1.3)	0.13	156	2 (0.6)	0.1 (0 to 0.6)	0.01
DRUG COMPLIANCE FACTORS								
Missed doses in past month								
No	226	13 (5.8)	1		246	6 (2.4)	1	
Yes	32	1 (3.1)	0.5 (0.1 to 4.2)	0.55	32	7 (21.9)	11.2 (3.5 to 35.9)	<0.01

* ART drug distribution location, Willing to receive ART in the future, Believe ART is health promoting ,Believe poor compliance contribute to HIVDR , Degree of satisfaction on support of friends or relatives, Frequency of taking drugs reminded by friends or relatives, Frequency of taking drugs reminded by doctors are not displayed for no statistical significant difference between categorizes.

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 3. Multivariate Models of Factors associated with virological failure (viral load ≥1000 copies/ml) and HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

	FEMALE				MALE			
	Virological Failure		HIVDR		Virological Failure		HIVDR	
Variables	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Total								
Ethnicity								
Han nationality			1		1		1	
Other minorities			4.8 (1.2 to 19.7)	0.03	2.9 (1.1 to 7.3)	0.02	12.2 (1.8 to 84.8)	0.01
Residence								
Rural			1					
City			2.4 (0.6 to 9.5)	0.22				
Age								
<35							1	
35-45							0.3 (0.1 to 1.4)	0.12
>45							0.03 (0 to 0.6)	0.02
Weight (kg)								
<50			1					
50-70			0.3 (0.1 to 1.1)	0.08				
>70			4.2 (0.6 to 30)	0.15				
Route of Infection								
Heterosexual Transmission	1				1		1	
Blood Transmission	1.2 (0.5 to 3)	0.74			1.8 (0.6 to 5.8)	0.33	7 (0.8 to 64.4)	0.09

Intravenous Drug use	4.1 (1 to 17.7)	0.06			2.1 (0.8 to 5.4)	0.12	2.3 (0.3 to 16.1)	0.41
Initial NRTI ART regimen								
AZT based regimen	1		1					
D4T based regimen	2.5 (1 to 6.1)	0.04	3.6 (1 to 12.6)	0.05				
ART drug distribution location								
County hospital or CDC					1			
Township hospital /village clinic /medication monitor					0.5 (0.2 to 1.3)	0.18		
Adverse effect								
No	1							
Yes	2.3 (1 to 5.6)	0.06						
CD4 cell at baseline (2008)								
<350					1			
≥350					7.1 (1.1 to 45.8)	0.04		
CD4 cell at 36 months (2011)								
<350	1		1				1	
≥350	0.4 (0.2 to 1.1)	0.07	0.3 (0.1 to 0.9)	0.04			0.3 (0 to 1.9)	0.2
CD4 cell at 48								

months (2012)						
<350	1				1	
≥350	0.6 (0.3 to 1.6)	0.36			0.1 (0 to 1)	0.05
Missed doses in past month						
No			1		1	
Yes			2.8 (1.1 to 7)	0.03	9.7 (2.1 to 44.1)	<0.01
Frequency of taking drugs reminded by doctors						
Often			1			
Not often			0.4 (0.2 to 1.2)	0.12		

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 4. HIV Drug Resistance and Subtype among 27 patients with HIVDR Mutation Detected at 2011 and/or 2012 stratified by sex

	FEMALE (%)	MALE (%)	MUTATIONS	N (%)
Overall	14	13		
Subtype				
B	5 (35.7)	3 (23.1)		
C		1 (7.7)		
CRF01_AE	2 (14.3)	1 (7.7)		
CRF07_BC	7 (50)	8 (61.5)		
Antiretrovial Drug				
Non-nucleoside reverse transcpriptase inhibitors (NNRTI,any)	14 (100)	13 (100)	NNRTI Mutations(total)	27
Efavirenz (EFV)	14 (100)	13 (100)	V90I	1 (3.7)
Nevirapine (NVP)	14 (100)	13 (100)	A98G	2 (7.4)
Etravirine (ETR)	6 (42.9)	8 (61.5)	K101E	6 (22.2)
			K103N	11 (40.7)
			V106A	4 (14.8)
			V108I	6 (22.2)
			E138A	1 (3.7)
			V179D/F	3 (11.1)
			Y181C	5 (18.5)
			G190A	5 (18.5)
			H221Y	1 (3.7)
			P225H	3 (11.1)
			F227L	1 (3.7)
			M230L	1 (3.7)

Nucleoside reverse transcpriptase inhibitors (NRTI,any)	12 (85.7)	9 (69.2)	NRTI Mutations(total）	21
Lamivudine (3TC)	12 (85.7)	9 (69.2)	A62V	1 (4.8)
Azidothymidine (AZT)	2 (14.3)	2 (15.4)	D67G	1 (4.8)
Tenofovir (TDF)	1 (7.1)	3 (23.1)	T69N	2 (9.5)
Stavudine (D4T)	3 (21.4)	3 (23.1)	K70R/Q	4 (19)
Didanosine (DDI)	5 (35.7)	3 (23.1)	V75I/M	2 (9.5)
Abcavir (ABC)	12 (85.7)	9 (69.2)	M184V	17 (81.0)
Emtricitabine (FTC)	12 (85.7)	9 (69.2)	T215N	1 (4.8)
			K219E/Q	2 (9.5)
Protease inhibitors (PI,any)	0	0	PI Mutations(total）	0

*NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors; PI: Protease Inhibitor

References

1. Hong SY, Nachega JB, Kelley K et al. The global status of HIV drug resistance: clinical and public-health approaches for detection, treatment and prevention. *Infectious disorders drug targets* 2011; **11**: 124-33.
2. Gabillard D, Lewden C, Ndoye I et al. Mortality, AIDS-morbidity, and loss to follow-up by current CD4 cell count among HIV-1-infected adults receiving antiretroviral therapy in Africa and Asia: data from the ANRS 12222 collaboration. *Journal of acquired immune deficiency syndromes* 2013; **62**: 555-61.
3. Montaner JS, Lima VD, Harrigan PR et al. Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and HIV transmission: the "HIV Treatment as Prevention" experience in a Canadian setting. *PloS one* 2014; **9**: e87872.
4. Staszewski S, Morales-Ramirez J, Tashima KT et al. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. *The New England journal of medicine* 1999; **341**: 1865-73.
5. Wang X, Yang L, Li H et al. Factors associated with HIV virologic failure among patients on HAART for one year at three sentinel surveillance sites in China. *Current HIV research* 2011; **9**: 103-11.
6. Zhang F, Dou Z, Ma Y et al. Five-year outcomes of the China National Free Antiretroviral Treatment Program. *Annals of internal medicine* 2009; **151**: 241-51, W-52.
7. Chinese Center of Disease and Control. Manual of the National Free Antiretroviral Treatment, 2007 edition 2007.
8. People's Republic of China (2008) UNGASS Country Progress Report. *Beijing: People's Republic of China* 2007.
9. Liu H, Ma Y, Su Y et al. Emerging trends of HIV drug resistance in Chinese HIV-infected patients receiving first-line highly active antiretroviral therapy: a systematic review and meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **59**: 1495-502.
10. Djarma O, Nguyen Y, Renois F et al. Continuous free access to HAART could be one of the potential factors impacting on loss to follow-up in HAART-eligible patients living in a resource-limited setting: N'djamena, Chad. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2014; **108**: 735-8.
11. DeGruttola V, Dix L, D'Aquila R et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. *Antiviral therapy* 2000; **5**: 41-8.

12. Liao L, Xing H, Su B et al. Impact of HIV drug resistance on virologic and immunologic failure and mortality in a cohort of patients on antiretroviral therapy in China. *Aids* 2013; **27**: 1815-24.
13. Hare AQ, Ordonez CE, Johnson BA et al. Gender-specific risk factors for virologic failure in KwaZulu-Natal: automobile ownership and financial insecurity. *AIDS and behavior* 2014; **18**: 2219-29.
14. Berg KM, Demas PA, Howard AA et al. Gender differences in factors associated with adherence to antiretroviral therapy. *Journal of general internal medicine* 2004; **19**: 1111-7.
15. Floridia M, Giuliano M, Palmisano L et al. Gender differences in the treatment of HIV infection. *Pharmacological research* 2008; **58**: 173-82.
16. Moore AL, Mocroft A, Madge S et al. Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. *Journal of acquired immune deficiency syndromes* 2001; **26**: 159-63.
17. World Health Organization. Surveillance Of HIV Drug Resistance In Adults Receiving Art (Acquired Hiv Drug Resistance). Accessed July 2014. 2014.
18. Bennett DE, Myatt M, Bertagnolio S et al. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral therapy* 2008; **13 Suppl 2**: 25-36.
19. Gaydos CA, Rizzo-Price PA, Balakrishnan P et al. Impact of international laboratory partnerships on the performance of HIV/sexually transmitted infection testing in five resource-constrained countries. *International journal of STD & AIDS* 2011; **22**: 645-52.
20. Organization WH. World Health Organization Protocol for Population Based Monitoring of HIV Drug Resistance Emerging During Treatment and Related Program Factors at Sentinel Antiretroviral Therapy Clinics.
21. Jiang Y, Qiu M, Zhang G et al. Quality assurance in the HIV/AIDS laboratory network of China. *International journal of epidemiology* 2010; **39 Suppl 2**: ii72-8.
22. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2006; **42**: 1608-18.
23. Yan H, Yu H, Xing W et al. Development of a proficiency testing program for the HIV-1 BED incidence assay in China. *Scientific reports* 2014; **4**: 4512.
24. Zhong P, Pan Q, Ning Z et al. Genetic diversity and drug resistance of human immunodeficiency virus type 1 (HIV-1) strains circulating in Shanghai. *AIDS research and human retroviruses* 2007; **23**: 847-56.
25. !!! INVALID CITATION !!! .
26. Xing H, Wang X, Liao L et al. Incidence and associated factors of HIV drug resistance in Chinese HIV-infected patients receiving antiretroviral treatment. *PloS one* 2013; **8**: e62408.

27. Wang J, He C, Hsi JH et al. Virological outcomes and drug resistance in Chinese patients after 12 months of 3TC-based first-line antiretroviral treatment, 2011-2012. *PLoS One* 2014; **9**: e88305.
28. Xing H, Ruan Y, Hsi JH et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003–12. *Journal of Antimicrobial Chemotherapy* 2015: dkv078.
29. Nicastri E, Leone S, Angeletti C et al. Sex issues in HIV-1-infected persons during highly active antiretroviral therapy: a systematic review. *The Journal of antimicrobial chemotherapy* 2007; **60**: 724-32.
30. Puskas CM, Forrest JI, Parashar S et al. Women and vulnerability to HAART non-adherence: a literature review of treatment adherence by gender from 2000 to 2011. *Current HIV/AIDS reports* 2011; **8**: 277-87.
31. Dou Z, Xu J, Jiao JH et al. Gender difference in 2-year mortality and immunological response to ART in an HIV-infected Chinese population, 2006-2008. *PloS one* 2011; **6**: e22707.
32. Sabin LL, Desilva MB, Hamer DH et al. Barriers to adherence to antiretroviral medications among patients living with HIV in southern China: a qualitative study. *AIDS care* 2008; **20**: 1242-50.
33. Wester CW, Okezie OA, Thomas AM et al. Higher-than-expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana: preliminary results from a large randomized clinical trial. *Journal of acquired immune deficiency syndromes* 2007; **46**: 318-22.
34. Goedecke JH, Micklesfield LK, Levitt NS et al. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS research and human retroviruses* 2013; **29**: 557-63.
35. Feleke Y, Fekade D, Mezegebu Y. Prevalence of highly active antiretroviral therapy associated metabolic abnormalities and lipodystrophy in HIV infected patients. *Ethiopian medical journal* 2012; **50**: 221-30.
36. Moyle GJ, Nelson MR, Hawkins D et al. The use and toxicity of didanosine (ddI) in HIV antibody-positive individuals intolerant to zidovudine (AZT). *The Quarterly journal of medicine* 1993; **86**: 155-63.
37. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva, 2013.
38. Dou Z, Zhang F, Zhao Y et al. Progress on China' s national free antiretroviral therapy strategy in 2002-2014. *Chinese Journal Epidemiology* 2015; **36**: 1345-50.
39. Leng X, Liang S, Ma Y et al. HIV virological failure and drug resistance among injecting drug users receiving first-line ART in China. *BMJ open* 2014; **4**: e005886.
40. Wu Z, Zhang J, Detels R et al. Characteristics of risk-taking behaviors, HIV and AIDS knowledge, and risk perception among young males in southwest China.

AIDS education and prevention : official publication of the International Society for AIDS Education 1997; **9**: 147-60.

41. Yang H, Li X, Stanton B et al. Heterosexual transmission of HIV in China: a systematic review of behavioral studies in the past two decades. *Sexually transmitted diseases* 2005; **32**: 270-80.

42. Myers Jr SL, Xiaoyan G, Cruz BC. Ethnic minorities, race, and inequality in China: A new perspective on racial dynamics. *Review of Black Political Economy* 2013; **40**: 231.

43. Zoufaly A, Jochum J, Hammerl R et al. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. *The Journal of antimicrobial chemotherapy* 2015; **70**: 922-5.

44. Shou L, WANG QX, Lei N et al. The changing trends of HIV/AIDS in an ethnic minority region of China: modeling the epidemic in Liangshan prefecture, Sichuan Province. *Biomedical and Environmental Sciences* 2013; **26**: 562-70.

45. Zhang L, Zhu J, Rui B et al. High HIV risk among Uigur minority ethnic drug users in northwestern China. *Tropical Medicine & International Health* 2008; **13**: 814-7.

46. Chinese Center for Disease Control and Prevention. Manual of the National Free Antiretroviral Treatment, third edition. Accessed 2013 Jun 12. . 2013.

47. World Health Organization. *Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*: World Health Organization, 2013.

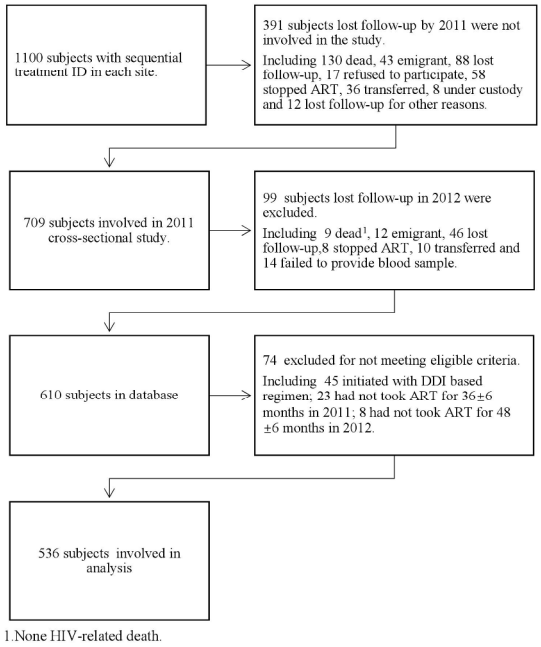
48. Xing H, Ruan Y, Hsi JH et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003-12. *The Journal of antimicrobial chemotherapy* 2015; **70**: 2097-103.

49. Liao L, Xing H, Shang H et al. The prevalence of transmitted antiretroviral drug resistance in treatment-naive HIV-infected individuals in China. *Journal of acquired immune deficiency syndromes* 2010; **53 Suppl 1**: S10-4.

50. Zhao K, Kang W, Liu Q et al. Genotypes and transmitted drug resistance among treatment-naive HIV-1-infected patients in a northwestern province, China: trends from 2003 to 2013. *PloS one* 2014; **9**: e109821.

51. Su Y, Zhang F, Liu H et al. The prevalence of HIV-1 drug resistance among antiretroviral treatment naive individuals in mainland China: a meta-analysis. *PloS one* 2014; **9**: e110652.

Figure 1. Figure of exclusion on study cohort



1.None HIV-related death.

215x279mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page	Recommendation
Title and abstract	1	2	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		2-3	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
			Introduction
Background/rationale	2	5	Explain the scientific background and rationale for the investigation being reported
Objectives	3	6	State specific objectives, including any prespecified hypotheses
			Methods
Study design	4	7	Present key elements of study design early in the paper
Setting	5	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	7	**(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	8-9	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	9	Describe any efforts to address potential sources of bias
Study size	10	9	Explain how the study size was arrived at
Quantitative variables	11	9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	9	(*a*) Describe all statistical methods, including those used to control for confounding
		9	(*b*) Describe any methods used to examine subgroups and interactions
		7,9-10	(*c*) Explain how missing data were addressed
		9-10	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Results			
Participants	13*	9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		9-10	(b) Give reasons for non-participation at each stage
		19	(c) Consider use of a flow diagram
Descriptive data	14*	10	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
		10	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*10*	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	11,9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		8	(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	14-15	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	12	Summarise key results with reference to study objectives
Limitations	19	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	12-16	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	17	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	18	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Predictors of HIV Virologic Failure And Drug Resistance In Chinese Patients After 48 Months Of Antiretroviral Treatment, 2008-2012 : A Prospective Cohort Study

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Public health
Keywords:	Antiretroviral Treatment, Virological Failure, Drug Resistance, Gender Difference, HIV & AIDS < INFECTIOUS DISEASES>

Predictors of HIV Virologic Failure And Drug Resistance In Chinese Patients After 48 Months Of Antiretroviral Treatment, 2008-2012 : A Prospective Corhort Study

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Running head: Virologic failure and drug resistance among HIV+ Chinese ART patients

Body: 3,111

ABSTRACT

Objective: To explore factors associated with HIV virologic failure (VF) and HIV drug resistance (HIVDR) among HIV-positive Chinese individuals four years after initiating first-line 3TC-based antiretroviral treatment (ART) in 2008 at five sentinel sites.

Design: First-line ART initiators who were previously treatment naïve were selected using consecutive ID numbers from the 2008 National Surveillance Database into a prospective cohort study. Questionnaires and Blood samples were collected in 2011 and 2012 to assess the outcomes of interest: VF (defined as viral load ≥ 1000 copies/ml) and HIVDR (defined as VF with genetic drug resistant mutations). Questionnaires and data from National Surveillance Database assessed demographics and drug adherence data.

Results: 536 individuals with HIV were analyzed; the 4-year risk of VF was 63(11.8%) and HIVDR was 27(5.0%). Female participants initiating D4T-based regimens were more susceptible to both VF (adjusted odds ratio, aOR=2.5, 95% CI: 1-6.1, P-value=0.04) and HIVDR (aOR=3.6, 95% CI: 1 to 12.6, P-value=0.05) versus AZT-based regimens. Male participants missing doses in past month were more susceptible to both VF (aOR=2.8, 95% CI: 1.1 to 7, P-value=0.03) and HIVDR (aOR=9.7, 95% CI: 2.1 to 44.1, P-value<0.01). Participants of non-Han nationality were of increased risk for HIVDR (aOR from 4.8-12.2, P-value<0.05) and non-Han

men were at increased risk for VF (aOR=2.9, 95% CI: 1.1 to .3, P-value=0.02). All 27 participants detected with HIVDR had non-nucleoside reverse-transcriptase inhibitor (NNRTI) mutations, 21 (77.8%) also had NRTI mutations, and no protease inhibitor mutations were detected.

Conclusions: Our findings suggest successful treatment outcomes at 4-years for roughly 90% of patients. We suggest conducting further study on whether and when to change ART regimen for women initiated with D4T-based regimen, and reinforcing adherence counseling for men. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions.

Strengths and limitations of this study

- We studied 48-month risk of VF and HIVDR and their associations with demographic and behavioral information among individuals across five sentinel sites.
- Study found drug adherence and adverse effects influenced VF and HIVDR differently across gender..
- The outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes.

Key words: HIV, Antiretroviral Treatment, Virological Failure, Drug Resistance, Gender Differences, China

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Introduction

Antiretroviral treatment (ART) has dramatically improved health outcomes and decreased HIV-associated morbidity and mortality through virologic suppression and subsequent CD4 recovery. [1-4] In 2003, China launched a National Free Antiretroviral Treatment Program (NFATP) that includes life-long provision of free ART for people living with HIV who met the national treatment criteria.[5, 6] The national treatment criteria from 2008 to 2011 were: (1) CD4 cell count ≤200/mm^3; (2) World Health Organization (WHO) stage III/IV diseases; or (3) willingness to receive ART, regardless of criteria 1 and 2.[7]

The State Council AIDS Working Committee Office and the United Nations Theme Group on AIDS estimated that there were more than 700,000 persons living with HIV in China in 2008, and more than 52,000 individuals with HIV across 31 provinces, autonomous regions, and municipalities had received ART (made freely available by the NAFTP) by August 2008.[8]

With the rapid scale-up of treatment and challenges with adherence, virologic failure (VF) and HIV drug resistance (HIVDR) are ever present and mounting concerns. Incomplete virologic suppression, a major cause of HIVDR, not only compromises therapeutic efficacy for the individual receiving treatment, increasing the risk of viral rebound and opportunistic infections, but also increases the risk of transmitting drug resistant strains to other individuals in the general population.[9 10, 11]

Observational studies in China have documented the prevalence of VF and HIVDR strains among treated individuals living with HIV. A cross-sectional study conducted in Yunnan, Guangxi and Xinjiang provinces in 2010 stated that one-year HIVDR prevalence was 4.1%[5]. VF prevalence for sexual transmitted population and intravenous drug users (IDUs) were 8.3% and 19.3%, separately. A 6-year follow-up study in 2010 suggested an incidence of 14.1 per 100 person-year for VF and 11.9 per 100 person-year for HIVDR among former plasma donors in Anhui Province.[12] NFATP recommended to switch the first-line regimen from Didanosine (DDI) to Lamivudine (3TC) in 2008, and there are few nationwide, prospective studies in China reporting frequency or predictors of VF and HIVDR for people after initiating 3TC based regimens.

The aim of this study is to evaluate predictors of VF and HIVDR in a prospective cohort of Chinese HIV individuals with HIV four years after first initiating first-line 3TC-based ART in 2008 at five sentinel sites. We stratified our analyses by gender based on conflicting findings on gender differences both in virological responses and drug resistance to different ART regimen, as well as gender differences in ART adherence.[13-16] To our knowledge, this is the first long-term study to evaluate VF and HIVDR on 3TC-based regimens.

Ethics Statement

The study was approved by the institutional review board (IRB) of the National Center for AIDS/STD Control and Prevention of the China Center for Disease Control

Prevention (NCAIDS, China CDC). All participants provided written informed consent before participation. Signed informed consent was obtained from each of the participants prior to the interviews and blood collection.

Methods

Study Design and Data Collection

This study was designed under the WHO Surveillance of HIV drug resistance in adults receiving ART for 48 months. [10, 17, 18] Five provinces in China with highest rate of transmission were selected to conduct a prospective cohort study with a follow-up study at 12 months: Guangxi, Henan, Hubei, Xinjiang and Yunnan. Participants were sampled from the 2008 National HIV Surveillance Database through sequential sampling from each province. Participant eligibility criteria included being age ≥ 18 years; having initiated NAFTP-sponsored first-line ART in 2008; having been ART-free before 2008; having been on ART for 36±6 months in 2011; and providing consent to participate in the study.

Questionnaires administered by trained study personnel from provincial CDC in private rooms using structured interviews collected data in 2011 and 2012. Each study participant was assigned a confidential identification number used to label questionnaire and blood specimen. Additional HIV-specific data including route of transmission, initial ART regimen, latest ART regimen, ART distribution location and CD4 cell count were collected from the 2011-2012 National HIV Surveillance

Database. There was no missing demographic data, missed questionnaire data was feedbacked to local CDC for recollection at the time.

Laboratory analysis

Blood specimens were collected from all participants to determine CD4 cell count, HIV-1 RNA viral load (VL), and HIV-1 drug resistance mutations in both 2011 and 2012. CD4 cell count estimation was conducted at provincial CDC laboratories using flow cytometry (FACSC Calibur, BD Company, USA) within 24 hours after specimen collection. Plasma was isolated and stored at -80°C at a provincial CDC laboratory and then transferred to NCAIDS for testing HIV VL and drug mutation. Plasma HIV RNA was quantified with real-time NASBA (NucliSense Easy Q, bioMerieux, France) or COBAS (Roche Applied Biosystems, Germany) according to manufacturer recommendations using in-house PCR (polymerase chain reaction), both of the assays were performed automatically.[19] Virologic failure was defined as VL ≥1000 copies/ml. According WHO protocol, [20] HIVDR tests were performed on samples with VL ≥1000 copies/ml. HIV-1 *pol* gene (protease 1-99 amino acids and part of reverse transcriptase 1-252 amino acids) were amplified, purified and analyzed using the Stanford HIV Drug Resistance Database (https://hivdb.stanford.edu/hivdb). Levels of HIVDR were classified according to the algorithm of Stanford HIVdb program. The scores are the sum of each mutation penalty score for a drug. Scores less than 10 indicate susceptible; scores between 10 and 14 indicate potential low-level resistance; scores between 15 and 29 indicate low-level resistance; scores

between 30 and 59 indicate intermediate resistance. Scores of 60 or greater indicate high-level resistance. Any low-, intermediate-, or high-level resistance identified was defined as HIVDR.[21-25]

Data analysis

Questionnaire data were double-entered using Epidata 3.1 (The Epidata Association Odense, Denmark). Statistical Analysis System (SAS 9.4, SAS Institute Inc., Cary, NC, USA) was then used for data cleaning and analyses.

48-month risk for the outcomes of interest was calculated as the proportion of unique persons who had experienced incident VF or HIVDR by the end of follow-up in 2012. Covariates of interest were described using counts and percentages overall and by the outcome of interest, stratified by gender. Univariate logistic regression models were constructed to explore associations between covariates of interest and VF or HIVDR. Odds ratios (OR) and 95% confidence intervals (CIs) are reported. Variables that were significant ($P < 0.05$) in the univariate models were then fit into multivariate logistic regression models assessment for removal of collinear variables that had the weakest association with the outcome. Adjusted ORs (aOR) and 95% CIs were presented. $P < 0.05$ was defined as statistically significant, and all tests were two sided. Descriptive analysis on HIVDR mutation results was conducted among 27 HIVDR participants, stratified by sex.

Results

1100 subjects were selected using consecutive ID numbers from 2008 National Surveillance Database; of those, 490 were lost follow-up by December, 2012. Among those lost to follow up, 139 died, 55 emigrated, 134 lost contact, 17 refused to participate, 65 stopped ART before 30 months, 36 transferred, 8 were under custody, 6 failed to provide a blood sample, 3 switched from ART to Tangcao tablet (an antiviral Chinese herbal therapy), 2 became pregnant and switched to other regimens and 1 was paralyzed. After excluding 74 participants for failing the eligibility criteria, 536 participants were included in the final 24-month analysis (Figure 1). The 48-month risk of VF was 11.8% and risk of drug resistance was 5%.

Demographic and ART Information (Tables 1-2)

Of the 536 eligible participants, 51.8% were male; 76.5% were Han nationality; 45.0% had an education level of elementary school or less; 56.2% were farmers; and 10.6% were unemployed with the rest having regular income.

All regimens in this cohort remained 3TC-based from 2008 to 2012. Initiated Nucleoside Reverse Transcriptase Inhibitors (NRTI) regimens included Zidovudine (AZT) (n=349, 65.1%) or Stavudine (D4T) (n=187, 34.9%). 66 (12.3%) participants later changed to TDF based regimen. Initiated Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) regimens included Nevirapine (NVP) (n=421, 78.5%) or Etravirine (EFV) (n=115, 21.5%). 55 (10.3%) participants later changed to LPV/r based regimen. 169 (31.5%) participants switched the initial ART regimen during 2008-2012, but no statistical significant difference was found in VF (p=0.74) and

HIVDR (p=0.29) risk between participants who switched regimens and participants who did not switch regimens.

We found that 38.4% participants were hesitant to accept ART in the future, 36.8% participants reported doubts whether ART was health promoting and 42.5% participants did not report that poor ART adherence necessarily contributed to HIVDR. Additionally, 40.5% of participants were not always satisfied with support from friends or relatives. 472 (88.1%) participants reported not missing a dose in the month prior to the date of the survey.

Multivariate model results (Table 3)

As shown in Table 3, minority male participants were at higher risk for both VF (aOR=2.9, 95% CI: 1.1 to 7.3, P-value=0.02) and HIVDR (aOR=12.2, 95% CI: 1.8 to 84.8, P-value=0.01) compared to Han nationality male participants, while female minorities were only at a higher risk for HIVDR (aOR=4.8, 95% CI: 1.2 to 19.7, P-value=0.03).

Female participants initiating D4T-based regimens were at a higher risk for both VF (aOR=2.5, 95% CI: 1 to 6.1, P-value=0.04) and HIVDR (aOR=3.6, 95% CI: 1 to 12.6, P-value=0.05) versus those initiating an AZT-based regimen; interestingly, different from their female counterparts, male participants showed no such association (OR=0.6, 95% CI: 0.3 to 1.4, P-value=0.24). Also, female participants had a higher risk of VF given adverse side-effects (aOR=2.3, 95% CI: 1 to 5.6, P-value=0.06).

Male participants with missed doses in the month prior to the survey were at a higher

risk of both VF (aOR=2.8, 95% CI: 1.1 to 7, P-value=0.03) and HIVDR (aOR=9.7, 95% CI: 2.1 to 44.1, P-value<0.01) versus those without missed doses in the preceding month. Conversely, missed doses in prior month was not significantly associated with VF or HIVDR for women.

HIV Drug Resistance and Subtype (Table 4)

HIVDR identified in our study was consistence with the NFATP recommended ART regimen. All 27 participants detected with drug resistance had NNRTI mutations, 21 (77.8%) had NRTI mutations. The dominant subtype was CRF07_BC for both males (61.5%) and females (50%). All participants found with HIVDR had developed HIVDR towards NNRTI; 85.7% male participants and 69.2% female developed HIVDR toward NRTI; no Protease Inhibitor mutation was detected. There were no CRF08_BC subtypes detected in the study population.

Discussion

The 48-month risk of VF was 11.8% and HIVDR was 5.0%, which indicated relatively good treatment outcomes given meta-analysis suggested a 37-48 months HIVDR prevalence ranging from 3.04%-47.92% in China,[9] similar to stdies in China which estimated a one-year HIVDR incidence of 3.5% in 2009 and 2.1% in 2012 .[26, 27] Our study substantiates the finding that VF and HIVDR largely decreased since the wide-spread of 3TC-based regimens.[28] Studies have shown mixed findings of gender differences on ART adherence and treatment outcomes. [14, 16, 29, 30] In our study, we found male participants had slightly higher risk of VF (12.2% versus 11.2%, P-

value=0.72) but lower risk of HIVDR (4.7% versus 5.4%, P-value=0.69) than women. Drug adherence and adverse effects influenced our outcomes differently for men compared to women. Women's risks of VF and HIVDR were not associated with missing doses in the past month, also, few women missed doses relative to men, similar to two other studies in China suggesting women have better adherence behaviors. [31, 32] Male participants were at higher risk of both VF and HIVDR if they reported missed doses. More detailed studies need to be conducted on the frequency and factors associated with missing treatment. However, female participants showed a higher risk of VF if they had adverse effect while men did not. This calls for further research of what types of adverse effects are occurring and how they affect ART adherence and virological outcomes across gender.

We found in this study that women, not men, who initiated D4T-based regimens were more susceptible to VF (women vs. men OR=2.3 95% CI: 1.0 to 5.7 P-value=0.06) and HIVDR (women vs. men OR=3.0 95%, CI: 0.8 to 11.3, P-value=0.11), consistent with previous findings that D4T was more likely to increase the risk of mitochondrial toxicity in women. [33, 34] Mitochondrial toxicity caused by D4T had been reported to cause many adverse effects such as lactic acidosis, lipodystrophy, and peripheral neuropathy. [35, 36] Following the WHO recommendation,[37] the NFATP advocated switching the first-line regimen from D4T to TDF in 2012. The percentage of people living with HIV initiating D4T-based regimen changed from 34.3% in 2010 to10% in 2012 and 0.9% in 2014;[38] however, there were still 29.9% participants in our study

who were on D4T-based regimens in 2012. It was noteworthy that we did not see a statistical difference in VF (OR=1.4 95%, CI: 0.4 to 4.2, P-value=0.60) and HIVDR (OR=1.0, 95% CI: 0.2 to 4.2, P-value=0.98) between women who initiated and remained on D4T-based regimens and those who switched to AZT/TDF based regimens. It is possible that women switched regimens because of VF; however, further studies need to be done to explore when to switch ART regimen for women receiving D4T-based regimens. It is important to mention that data on ART adherence and adverse effects were collected in 2012, when there were only four female participants still using D4T-based regimens who experienced VF. The sample size was not sufficient to explore whether D4T-based regimens affect drug adherence and adverse effects for women.

Though not associated with the VF and HIVDR outcomes, 38.4% of study participants reported that they would not 'always' be willing to take ART in the future, 36.8% reported not believing that ART is 'always' health promoting, and 42.5% reported not believing that poor compliance 'always' contributed to HIVDR. As willingness and these knowledge factors may impact more long-term VF and HIVDR outcomes, the motivations behind willingness and knowledge about VF and HIVDR warrant exploration.

Caution is needed when interpreting the study results from multivariate model that older age (>45) was protective for HIVDR in men. There were only 4 male IDU participants with older age (>45) in this study, the number is not sufficient to test for

interaction. There is no association between HIVDR and age (OR=6.5, 95% CI: 1.1 to 38.1, P-value=0.49) in the sub-analysis we did among male IDU participants , after controlling for variables showed significant in the univariate model. A previous study in HIV positive IDU population in China suggested that there is no association between VF and sex or age.[39] In this study, 61.5% of male participants with HIVDR became HIV infected via IDU, yet there were only 22.7% male IDU participants.

In addition, we found that younger (<45 years) IDU population were more likely to miss doses (18.6%) compared to heterosexual transmission population (8.8%) and blood transfusion transmission population (12.1%). This finding was consistent with studies that implied younger males were at a higher risk of drug abuse.[40, 41] This result indicated that younger IDU population could be a main source of VF and HIVDR; therefore they could be future targeted population for behavioral intervention.

The increased risk of VF and HIVDR in non-Han minorities, regardless of gender, may be due to logistical, cultural, or social barriers faced by ethnic minorities which limit their adherence to ART. It has been reported that minorities tend to have lower social economic status than Han majorities, followed by lower education level and fewer access to health facilities.[42] It may be difficult for health professionals to reach for some minorities because of their more remote geographic locations. Additionally, several studies have reported that the percentage of high-risk populations such as female sex workers (FSWs) and IDUs were higher in minorities than in Han nationality. [43-45] The causes of this increased VF and HIVDR risk warrants further

exploration, and ethnic minorities may be an important group to tailor adherence-focused interventions in China. The finding that higher CD4 cell count at follow-up was protective for VF and HIVDR was expected. NFATP changed treatment criteria from CD4 cell count ≤200cells/mm^3 to CD4 cell count ≤350cells/mm^3 following the WHO recommendation in 2011, [7, 17, 46]. Our study indicated that male participants who initiated treatment in 2008 at CD4 cell count >=350 cells/mm^3 were still at higher risk towards VF (aOR=7.1, 95% CI: 1.1 to 45.8, P-value=0.04), supporting possible clinical benefits of initiating ART at higher CD4 cell counts, <500 cells/mm^3 as per WHO recommendation in 2013.[47]

Among participants infected by blood transmission, we only found HIVDR subtype B; only one subtype C was found in participants infected with IDU, the dominant subtype was CRF07_BC, found both in participants infected by heterosexual transmission and IDU. The most common NNRTI mutation sites were K103N (40.7%), K101E (22.2%) and V108I (22.2%); the most common NRTI mutation sites were M184V (81.0 %) and K70R (19%). Interestingly, compared to a one-year follow-up study in China with all participants initiated ART in 2011,[48] there is no V108I in their study and we did not find K65R in our study.

Study findings should be interpreted in light of several limitations. Though we did not account for transmitted drug resistance in this study, previous studies have found low transmitted drug resistance risk (<5%) during this period [49-51] in China and we could be fairly certain that participants were outcome free in 2008 as they were new ART

initiators. Another limitation of our study is that the outcomes were measured in 2011 and 2012, and thus we may be missing transient VF outcomes. Also, route of transmission was collected in 2008 when assessing HIV infection among men who have sex with men was not part of data collection instruments; additionally we do not have data on sex worker status. Roughly half of the study participants selected for possible inclusion in the study for having initiated first-line ART in 2008 were lost to follow-up by 2012, creating a possible selection bias for individuals with better ART adherence – this bias may underestimate the true VF and HIVDR risk and also limit the generalizability of our findings to better adherers. Additionally, given the demographic profile of the cohort, our findings are most generalizable to heterosexual Han national who are married/cohabiting and working as agricultural labors in rural areas. Misclassification of self-reported data is possible, though we do not expect this misclassification to be differential by the outcome of interest and thus any such information bias would bias our results toward the null.

Conclusions

We found female participants initiating D4T versus AZT-based regimens were more vulnerable to VF and HIVDR, and we suggest future studies on whether and when to change ART regimen for women initiated with D4T-based regimen. Poor adherence was a risk factor among male participants who may benefit from reinforced adherence counseling or social support. Increased VF and HIVDR risk among non-Han minorities warrants further exploration, and ethnic minorities may be an important

group to tailor adherence-focused interventions in China. Also, this study indicated that younger men who become infected through IDU may be groups to strategically focus counseling and increased adherence support programs.

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Contributions

YR, WK, HX and YS designed the study. YR, HX, SL, LL, YM, HT, TZ, GS, HC, WK, XL and PL collected the data. HC, TT and LL conducted laboratory analysis. XL, WK analyzed the data. KW, VM, YR, WK, HX, LL and YS interpreted the data. KW, VM, YR, WK, TT, and YS drafted the report. All authors reviewed, revised, and approved the final report.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Transparency Declarations

Vincent C. Marconi has received fees from ViiV Healthcare.

Conflicts of Data Sharing Statement

No additional data available.

Table 1. Factors associated with virological failure (viral load ≥1000 copies/ml) stratified by sex

DEMOGRAPHIC FACTORS	Female				Male			
	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value	Total	Virological failure Risk, N (%)	OR (95%CI)	P-value
Total	258	29 (11.2)			278	34 (12.2)		
Ethnicity								
Han nationality	191	19 (9.9)	1		219	20 (9.1)	1	
Other minorities	67	10 (14.9)	1.6 (0.7,3.6)	0.27	59	14 (23.7)	3.1 (1.5,6.6)	<0.01
Education								
Elementary school or less	134	15 (11.2)	1		107	12 (11.2)	1	
Junior school or more	124	14 (11.3)	1 (0.5,2.2)	0.98	171	22 (12.9)	1.2 (0.6,2.5)	0.68
Marital Status								
Single	59	6 (10.2)	1		75	9 (12)	1	
Married or Cohabited	199	23 (11.6)	1.2 (0.4,3.0)	0.77	203	25 (12.3)	1 (0.5,2.3)	0.94
Residence								
Rural	197	19 (9.6)	1		172	17 (9.9)	1	
City	61	10 (16.4)	1.8 (0.8,4.2)	0.15	106	17 (16)	1.7 (0.8,3.6)	0.13
Occupation								
Peasant	163	15 (9.2)	1		138	9 (6.5)	1	
Employee	64	11 (17.2)	2 (0.9,4.7)	0.09	114	17 (14.9)	2.5 (1.1,5.9)	0.03
Unemployed	31	3 (9.7)	1.1 (0.3,3.9)	0.93	26	8 (30.8)	6.4 (2.2,18.6)	<0.01
Age								

<35	73	6 (8.2)	1		53	7 (13.2)	1	
35-45	108	15 (13.9)	1.8 (0.7,4.9)	0.25	119	15 (12.6)	0.9 (0.4,2.5)	0.91
>45	77	8 (10.4)	1.3 (0.4,3.9)	0.65	106	12 (11.3)	0.8 (0.3,2.3)	0.73
Weight (kg)								
<50	89	14 (15.7)	1		49	7 (14.3)	1	
50-70	156	13 (8.3)	0.5 (0.2,1.1)	0.08	193	21 (10.9)	0.7 (0.3,1.8)	0.51
>70	13	2 (15.4)	1 (0.2,4.9)	0.97	36	6 (16.7)	1.2 (0.4,3.9)	0.76
HIV CHARACTERISTICS AND TREATMENT FACTORS								
Route of Infection								
Heterosexual Transmission	159	12 (7.5)	1		154	11 (7.1)	1	
Blood Transmission	86	13 (15.1)	2.2 (0.9,5)	0.07	61	10 (16.4)	2.5 (1,6.4)	0.04
Intravenous Drug use	13	4 (30.8)	5.4 (1.5,20.3)	0.01	63	13 (20.6)	3.4 (1.4,8)	0.01
Initial NRTI ART regimen								
AZT based regimen	161	11 (6.8)	1		188	26 (13.8)	1	
D4T based regimen	97	18 (18.6)	3.1 (1.4,6.9)	<0.01	90	8 (8.9)	0.6 (0.3,1.4)	0.24
Latest NRTI ART regimen								
AZT based regimen	181	15 (8.3)	1		195	27 (13.8)	1	
D4T based regimen	77	14 (18.2)	2.5 (1.1,5.4)	0.02	83	7 (8.4)	0.6 (0.2,1.4)	0.21
Switch ART regimen								
No	193	21 (10.9)	1		174	21 (12.1)	1	

Yes	65	8 (12.3)	1.2 (0.5,2.7)	0.75	104	13 (12.5)	1 (0.5,2.2)	0.92
ART drug distribution location								
County hospital or CDC	96	15 (15.6)	1		63	14 (22.2)	1	
Township hospital /village clinic /medication monitor	162	14 (8.6)	0.5 (0.2,1.1)	0.09	215	20 (9.3)	0.4 (0.2,0.8)	0.01
Adverse effects								
No	195	17 (8.7)	1		206	23 (11.2)	1	
Yes	63	12 (19)	2.5 (1.1,5.5)	0.03	72	11 (15.3)	1.4 (0.7,3.1)	0.36
CD4 cell/ml at baseline (2008)								
<350	244	28 (11.5)	1		272	31 (11.4)	1	
≥350	14	1 (7.1)	0.6 (0.1,4.7)	0.62	6	3 (50)	7.8 (1.5,40.2)	0.01
CD4 cell/ml at 36 months (2011)								
0-350	91	18 (19.8)	1		138	21 (15.2)	1	
≥350	167	11 (6.6)	0.3 (0.1,0.6)	<0.01	140	13 (9.3)	0.6 (0.3,1.2)	0.13
CD4 cell/ml at 48 months (2012)								
0-350	81	14 (17.3)	1		122	20 (16.4)	1	
≥350	177	15 (8.5)	0.4 (0.2,1)	0.04	156	14 (9)	0.5 (0.2,1)	0.06
DRUG COMPLIANCE FACTORS								
Missed doses								

in past month								
No	226	26 (11.5)	1		246	24 (9.8)	1	
Yes	32	3 (9.4)	0.8 (0.2,2.8)	0.72	32	10 (31.3)	4.2 (1.8,9.9)	<0.01
Willing to receive ART in the future								
Always	153	15 (9.8)	1		177	19 (10.7)	1	
Not always	105	14 (13.3)	1.4 (0.7,3.1)	0.38	101	15 (14.9)	1.5 (0.7,3)	0.32
Believe ART is health promoting								
Always	158	16 (10.1)	1		181	19 (10.5)	1	
Not always	100	13 (13)	1.3 (0.6,2.9)	0.48	97	15 (15.5)	1.6 (0.8,3.2)	0.24
Believe poor compliance contribute to HIVDR								
Always	139	16 (11.5)	1		169	19 (11.2)	1	
Not always	119	13 (10.9)	0.9 (0.4,2.1)	0.88	109	15 (13.8)	1.3 (0.6,2.6)	0.53
Degree of satisfaction on support of friends or relatives								
Always satisfied	153	15 (9.8)	1		166	17 (10.2)	1	
Not always satisfied	105	14 (13.3)	1.4 (0.7,3.1)	0.38	112	17 (15.2)	1.6 (0.8,3.2)	0.22
Frequency of taking drugs reminded by friends or								

relatives								
Often	172	19 (11)	1		168	22 (13.1)	1	
Not often	86	10 (11.6)	1.4 (0.7,3.1)	0.38	110	12 (10.9)	0.8 (0.4,1.7)	0.58
Frequency of taking drugs reminded by doctors								
Often	178	23 (12.9)	1		184	28 (15.2)	1	
Not often	80	6 (7.5)	0.5 (0.2,1.4)	0.19	94	6 (6.4)	0.4 (0.2,1)	0.03

*OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 2. Factors associated with HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

DEMOGRAPHIC FACTORS	Female				Male			
	Total	HIVDR Risk N (%)	OR (95%CI)	P-value	Total	HIVDR Risk N (%)	OR (95%CI)	P-value
	258	14 (5.4)			278	13 (4.7)		
Ethnicity								
Han nationality	191	6 (3.1)	1		219	5 (2.3)	1	
Other minorities	67	8 (11.9)	4.2 (1.4 to 12.5)	0.01	59	8 (13.6)	6.7 (2.1 to 21.4)	<0.01
Education								
Elementary school or less	134	5 (3.7)	1		107	5 (4.7)	1	
Junior school or more	124	9 (7.3)	2 (0.7 to 6.2)	0.22	171	8 (4.7)	1 (0.3 to 3.1)	1
Marital Status								
Single	59	4 (6.8)	1		75	4 (5.3)	1	
Married or Cohabited	199	10 (5)	0.7 (0.2 to 2.4)	0.60	203	9 (4.4)	0.8 (0.2 to 2.8)	0.75
Residence								
Rural	197	7 (3.6)	1		172	5 (2.9)	1	
City	61	7 (11.5)	3.5 (1.2 to 10.5)	0.02	106	8 (7.5)	2.7 (0.9 to 8.6)	0.09
Occupation								
Peasant	163	5 (3.1)	1		138	0	1	
Employee	64	6 (9.4)	3.3 (1 to 11.1)	0.06	114	9 (7.9)	2 (1 to 12.6)	<0.01
Unemployed	31	3 (9.7)	3.4 (0.8 to 15)	0.11	26	4 (15.4)	3 (1 to 13.4)	<0.01

Age								
<35	73	5 (6.8)	1		53	6 (11.3)	1	
35-45	108	6 (5.6)	0.8 (0.2 to 2.7)	0.72	119	6 (5)	0.4 (0.1 to 1.4)	0.15
>45	77	3 (3.9)	0.6 (0.1 to 2c.4)	0.43	106	1 (0.9)	0.1 (0 to 0.6)	0.02
Weight (kg)								
<50	89	8 (9)	1		49	4 (8.2)	1	
50-70	156	4 (2.6)	0.3 (0.1 to 0.9)	0.04	193	7 (3.6)	0.4 (0.1 to 1.5)	0.19
>70	13	2 (15.4)	1.8 (0.3 to 9.8)	0.47	36	2 (5.6)	0.7 (0.1 to 3.8)	0.64
HIV CHARACTERISTICS AND TREATMENT FACTORS								
Route of Infection								
Heterosexual Transmission	159	8 (5)	1		154	2 (0.6)	1	
Blood Transmission	86	4 (4.7)	0.9 (0.3 to 3.2)	0.9	61	3 (2)	3.9 (0.6 to 24.1)	0.14
Intravenous Drug use	13	2 (15.4)	3.4 (0.6 to 18.2)	0.15	63	8 (10.5)	11.1 (2.3 to 53.7)	<0.01
Initial NRTI ART regimen								
AZT based regimen	161	5 (3.1)	1		188	10 (2.9)	1	
D4T based regimen	97	9 (9.3)	3.2 (1 to 9.8)	0.04	90	3 (1.6)	0.6 (0.2 to 2.3)	0.47
Latest ART regimen								
AZT based regimen	181	8 (4.4)	1		195	12 (3.2)	1	
D4T based regimen	77	6 (7.8)	1.8 (0.6 to 5.5)	0.28	83	1 (0.6)	0.2 (0 to 1.5)	0.11
Switch ART regimen								

No	193	10 (5.2)	1		174	6 (3.4)	1	
Yes	65	4 (6.2)	1.2 (0.4 to 4)	0.76	104	7 (6.7)	2 (0.7 to 6.2)	0.22
Adverse effect								
No	195	9 (4.6)	1		206	10 (4.9)	1	
Yes	63	5 (7.9)	1.8 (0.6 to 5.5)	0.32	72	3 (4.2)	0.9 (0.2 to 3.2)	0.81
CD4 cell at baseline (2008)								
<350	244	14 (5.7)			272	12 (2.3)	1	
≥350	14	0	-	0.36	6	1 (5)	4.3 (0.5 to 40)	0.2
CD4 cell at 36 months (2011)								
0-350	91	9 (9.9)	1		138	11 (4.8)	1	
≥350	167	5 (3)	0.3 (0.1 to 0.9)	0.03	140	2 (0.7)	0.2 (0 to 0.8)	0.02
CD4 cell at 48 months (2012)								
0-350	81	7 (8.6)	1		122	11 (5.4)	1	
≥350	177	7 (4)	0.4 (0.1 to 1.3)	0.13	156	2 (0.6)	0.1 (0 to 0.6)	0.01
DRUG COMPLIANCE FACTORS								
Missed doses in past month								
No	226	13 (5.8)	1		246	6 (2.4)	1	
Yes	32	1 (3.1)	0.5 (0.1 to 4.2)	0.55	32	7 (21.9)	11.2 (3.5 to 35.9)	<0.01

* ART drug distribution location, Willing to receive ART in the future, Believe ART is health promoting ,Believe poor compliance contribute to HIVDR , Degree of satisfaction on support of friends or relatives, Frequency of taking drugs reminded by friends or relatives, Frequency of taking drugs reminded by doctors are not displayed for no statistical significant difference between categorizes.

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 3. Multivariate Models of Factors associated with virological failure (viral load ≥1000 copies/ml) and HIVDR (viral load ≥1000 copies/ml with drug resistance) stratified by sex

	FEMALE				MALE			
	Virological Failure		HIVDR		Virological Failure		HIVDR	
Variables	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Total								
Ethnicity								
Han nationality			1		1		1	
Other minorities			4.8 (1.2 to 19.7)	0.03	2.9 (1.1 to 7.3)	0.02	12.2 (1.8 to 84.8)	0.01
Residence								
Rural			1					
City			2.4 (0.6 to 9.5)	0.22				
Age								
<35							1	
35-45							0.3 (0.1 to 1.4)	0.12
>45							0.03 (0 to 0.6)	0.02
Weight (kg)								
<50			1					
50-70			0.3 (0.1 to 1.1)	0.08				
>70			4.2 (0.6 to 30)	0.15				
Route of Infection								
Heterosexual Transmission	1				1		1	
Blood Transmission	1.2 (0.5 to 3)	0.74			1.8 (0.6 to 5.8)	0.33	7 (0.8 to 64.4)	0.09

Intravenous Drug use	4.1 (1 to 17.7)	0.06			2.1 (0.8 to 5.4)	0.12	2.3 (0.3 to 16.1)	0.41
Initial NRTI ART regimen								
AZT based regimen	1		1					
D4T based regimen	2.5 (1 to 6.1)	0.04	3.6 (1 to 12.6)	0.05				
ART drug distribution location								
County hospital or CDC					1			
Township hospital /village clinic /medication monitor					0.5 (0.2 to 1.3)	0.18		
Adverse effect								
No	1							
Yes	2.3 (1 to 5.6)	0.06						
CD4 cell at baseline (2008)								
<350					1			
≥350					7.1 (1.1 to 45.8)	0.04		
CD4 cell at 36 months (2011)								
<350	1		1				1	
≥350	0.4 (0.2 to 1.1)	0.07	0.3 (0.1 to 0.9)	0.04			0.3 (0 to 1.9)	0.2
CD4 cell at 48								

months (2012)						
<350	1				1	
≥350	0.6 (0.3 to 1.6)	0.36			0.1 (0 to 1)	0.05
Missed doses in past month						
No			1		1	
Yes			2.8 (1.1 to 7)	0.03	9.7 (2.1 to 44.1)	<0.01
Frequency of taking drugs reminded by doctors						
Often			1			
Not often			0.4 (0.2 to 1.2)	0.12		

* OR: odds ratio; CI: confidence interval; HIVDR: HIV drug resistance; ART: antiretroviral treatment; AZT: Zidovudine; D4T: Stavudine

Table 4. HIV Drug Resistance and Subtype among 27 patients with HIVDR Mutation Detected at 2011 and/or 2012 stratified by sex

	FEMALE (%)	MALE (%)	MUTATIONS	N (%)
Overall	14	13		
Subtype				
B	5 (35.7)	3 (23.1)		
C		1 (7.7)		
CRF01_AE	2 (14.3)	1 (7.7)		
CRF07_BC	7 (50)	8 (61.5)		
Antiretrovial Drug				
Non-nucleoside reverse transcpriptase inhibitors (NNRTI,any)	14 (100)	13 (100)	NNRTI Mutations(total)	27
Efavirenz (EFV)	14 (100)	13 (100)	V90I	1 (3.7)
Nevirapine (NVP)	14 (100)	13 (100)	A98G	2 (7.4)
Etravirine (ETR)	6 (42.9)	8 (61.5)	K101E	6 (22.2)
			K103N	11 (40.7)
			V106A	4 (14.8)
			V108I	6 (22.2)
			E138A	1 (3.7)
			V179D/F	3 (11.1)
			Y181C	5 (18.5)
			G190A	5 (18.5)
			H221Y	1 (3.7)
			P225H	3 (11.1)
			F227L	1 (3.7)
			M230L	1 (3.7)

Nucleoside reverse transcpriptase inhibitors (NRTI,any)	12 (85.7)	9 (69.2)	NRTI Mutations(total）	21
Lamivudine (3TC)	12 (85.7)	9 (69.2)	A62V	1 (4.8)
Azidothymidine (AZT)	2 (14.3)	2 (15.4)	D67G	1 (4.8)
Tenofovir (TDF)	1 (7.1)	3 (23.1)	T69N	2 (9.5)
Stavudine (D4T)	3 (21.4)	3 (23.1)	K70R/Q	4 (19)
Didanosine (DDI)	5 (35.7)	3 (23.1)	V75I/M	2 (9.5)
Abcavir (ABC)	12 (85.7)	9 (69.2)	M184V	17 (81.0)
Emtricitabine (FTC)	12 (85.7)	9 (69.2)	T215N	1 (4.8)
			K219E/Q	2 (9.5)
Protease inhibitors (PI,any)	0	0	PI Mutations(total）	0

*NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors; PI: Protease Inhibitor

References

1. Hong SY, Nachega JB, Kelley K et al. The global status of HIV drug resistance: clinical and public-health approaches for detection, treatment and prevention. *Infectious disorders drug targets* 2011; **11**: 124-33.
2. Gabillard D, Lewden C, Ndoye I et al. Mortality, AIDS-morbidity, and loss to follow-up by current CD4 cell count among HIV-1-infected adults receiving antiretroviral therapy in Africa and Asia: data from the ANRS 12222 collaboration. *Journal of acquired immune deficiency syndromes* 2013; **62**: 555-61.
3. Montaner JS, Lima VD, Harrigan PR et al. Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and HIV transmission: the "HIV Treatment as Prevention" experience in a Canadian setting. *PloS one* 2014; **9**: e87872.
4. Staszewski S, Morales-Ramirez J, Tashima KT et al. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. *The New England journal of medicine* 1999; **341**: 1865-73.
5. Wang X, Yang L, Li H et al. Factors associated with HIV virologic failure among patients on HAART for one year at three sentinel surveillance sites in China. *Current HIV research* 2011; **9**: 103-11.
6. Zhang F, Dou Z, Ma Y et al. Five-year outcomes of the China National Free Antiretroviral Treatment Program. *Annals of internal medicine* 2009; **151**: 241-51, W-52.
7. Chinese Center of Disease and Control. Manual of the National Free Antiretroviral Treatment, 2007 edition 2007.
8. People's Republic of China (2008) UNGASS Country Progress Report. *Beijing: People's Republic of China* 2007.
9. Liu H, Ma Y, Su Y et al. Emerging trends of HIV drug resistance in Chinese HIV-infected patients receiving first-line highly active antiretroviral therapy: a systematic review and meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **59**: 1495-502.
10. Djarma O, Nguyen Y, Renois F et al. Continuous free access to HAART could be one of the potential factors impacting on loss to follow-up in HAART-eligible patients living in a resource-limited setting: N'djamena, Chad. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2014; **108**: 735-8.
11. DeGruttola V, Dix L, D'Aquila R et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. *Antiviral therapy* 2000; **5**: 41-8.

12. Liao L, Xing H, Su B et al. Impact of HIV drug resistance on virologic and immunologic failure and mortality in a cohort of patients on antiretroviral therapy in China. *Aids* 2013; **27**: 1815-24.
13. Hare AQ, Ordonez CE, Johnson BA et al. Gender-specific risk factors for virologic failure in KwaZulu-Natal: automobile ownership and financial insecurity. *AIDS and behavior* 2014; **18**: 2219-29.
14. Berg KM, Demas PA, Howard AA et al. Gender differences in factors associated with adherence to antiretroviral therapy. *Journal of general internal medicine* 2004; **19**: 1111-7.
15. Floridia M, Giuliano M, Palmisano L et al. Gender differences in the treatment of HIV infection. *Pharmacological research* 2008; **58**: 173-82.
16. Moore AL, Mocroft A, Madge S et al. Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. *Journal of acquired immune deficiency syndromes* 2001; **26**: 159-63.
17. World Health Organization. Surveillance Of HIV Drug Resistance In Adults Receiving Art (Acquired Hiv Drug Resistance). Accessed July 2014. 2014.
18. Bennett DE, Myatt M, Bertagnolio S et al. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral therapy* 2008; **13 Suppl 2**: 25-36.
19. Gaydos CA, Rizzo-Price PA, Balakrishnan P et al. Impact of international laboratory partnerships on the performance of HIV/sexually transmitted infection testing in five resource-constrained countries. *International journal of STD & AIDS* 2011; **22**: 645-52.
20. Organization WH. World Health Organization Protocol for Population Based Monitoring of HIV Drug Resistance Emerging During Treatment and Related Program Factors at Sentinel Antiretroviral Therapy Clinics.
21. Jiang Y, Qiu M, Zhang G et al. Quality assurance in the HIV/AIDS laboratory network of China. *International journal of epidemiology* 2010; **39 Suppl 2**: ii72-8.
22. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2006; **42**: 1608-18.
23. Yan H, Yu H, Xing W et al. Development of a proficiency testing program for the HIV-1 BED incidence assay in China. *Scientific reports* 2014; **4**: 4512.
24. Zhong P, Pan Q, Ning Z et al. Genetic diversity and drug resistance of human immunodeficiency virus type 1 (HIV-1) strains circulating in Shanghai. *AIDS research and human retroviruses* 2007; **23**: 847-56.
25. !!! INVALID CITATION !!! .
26. Xing H, Wang X, Liao L et al. Incidence and associated factors of HIV drug resistance in Chinese HIV-infected patients receiving antiretroviral treatment. *PloS one* 2013; **8**: e62408.

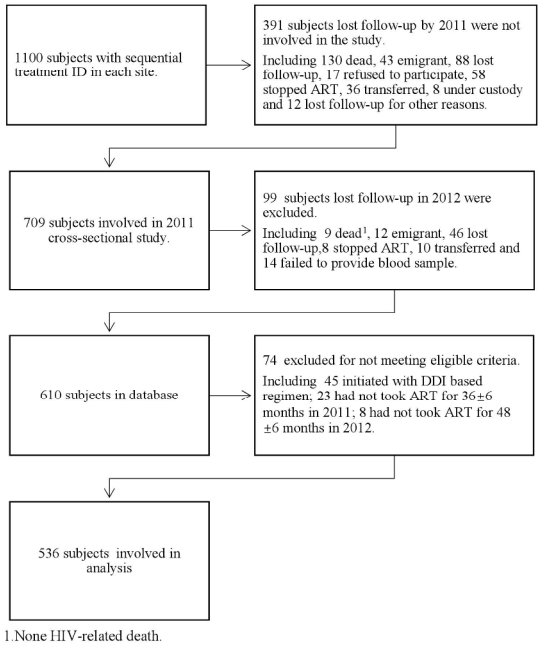
27. Wang J, He C, Hsi JH et al. Virological outcomes and drug resistance in Chinese patients after 12 months of 3TC-based first-line antiretroviral treatment, 2011-2012. *PLoS One* 2014; **9**: e88305.
28. Xing H, Ruan Y, Hsi JH et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003–12. *Journal of Antimicrobial Chemotherapy* 2015: dkv078.
29. Nicastri E, Leone S, Angeletti C et al. Sex issues in HIV-1-infected persons during highly active antiretroviral therapy: a systematic review. *The Journal of antimicrobial chemotherapy* 2007; **60**: 724-32.
30. Puskas CM, Forrest JI, Parashar S et al. Women and vulnerability to HAART non-adherence: a literature review of treatment adherence by gender from 2000 to 2011. *Current HIV/AIDS reports* 2011; **8**: 277-87.
31. Dou Z, Xu J, Jiao JH et al. Gender difference in 2-year mortality and immunological response to ART in an HIV-infected Chinese population, 2006-2008. *PloS one* 2011; **6**: e22707.
32. Sabin LL, Desilva MB, Hamer DH et al. Barriers to adherence to antiretroviral medications among patients living with HIV in southern China: a qualitative study. *AIDS care* 2008; **20**: 1242-50.
33. Wester CW, Okezie OA, Thomas AM et al. Higher-than-expected rates of lactic acidosis among highly active antiretroviral therapy-treated women in Botswana: preliminary results from a large randomized clinical trial. *Journal of acquired immune deficiency syndromes* 2007; **46**: 318-22.
34. Goedecke JH, Micklesfield LK, Levitt NS et al. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS research and human retroviruses* 2013; **29**: 557-63.
35. Feleke Y, Fekade D, Mezegebu Y. Prevalence of highly active antiretroviral therapy associated metabolic abnormalities and lipodystrophy in HIV infected patients. *Ethiopian medical journal* 2012; **50**: 221-30.
36. Moyle GJ, Nelson MR, Hawkins D et al. The use and toxicity of didanosine (ddI) in HIV antibody-positive individuals intolerant to zidovudine (AZT). *The Quarterly journal of medicine* 1993; **86**: 155-63.
37. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach. Geneva, 2013.
38. Dou Z, Zhang F, Zhao Y et al. Progress on China' s national free antiretroviral therapy strategy in 2002-2014. *Chinese Journal Epidemiology* 2015; **36**: 1345-50.
39. Leng X, Liang S, Ma Y et al. HIV virological failure and drug resistance among injecting drug users receiving first-line ART in China. *BMJ open* 2014; **4**: e005886.
40. Wu Z, Zhang J, Detels R et al. Characteristics of risk-taking behaviors, HIV and AIDS knowledge, and risk perception among young males in southwest China.

AIDS education and prevention : official publication of the International Society for AIDS Education 1997; **9**: 147-60.
41. Yang H, Li X, Stanton B et al. Heterosexual transmission of HIV in China: a systematic review of behavioral studies in the past two decades. *Sexually transmitted diseases* 2005; **32**: 270-80.
42. Myers Jr SL, Xiaoyan G, Cruz BC. Ethnic minorities, race, and inequality in China: A new perspective on racial dynamics. *Review of Black Political Economy* 2013; **40**: 231.
43. Zoufaly A, Jochum J, Hammerl R et al. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. *The Journal of antimicrobial chemotherapy* 2015; **70**: 922-5.
44. Shou L, WANG QX, Lei N et al. The changing trends of HIV/AIDS in an ethnic minority region of China: modeling the epidemic in Liangshan prefecture, Sichuan Province. *Biomedical and Environmental Sciences* 2013; **26**: 562-70.
45. Zhang L, Zhu J, Rui B et al. High HIV risk among Uigur minority ethnic drug users in northwestern China. *Tropical Medicine & International Health* 2008; **13**: 814-7.
46. Chinese Center for Disease Control and Prevention. Manual of the National Free Antiretroviral Treatment, third edition. Accessed 2013 Jun 12. . 2013.
47. World Health Organization. *Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*: World Health Organization, 2013.
48. Xing H, Ruan Y, Hsi JH et al. Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003-12. *The Journal of antimicrobial chemotherapy* 2015; **70**: 2097-103.
49. Liao L, Xing H, Shang H et al. The prevalence of transmitted antiretroviral drug resistance in treatment-naive HIV-infected individuals in China. *Journal of acquired immune deficiency syndromes* 2010; **53 Suppl 1**: S10-4.
50. Zhao K, Kang W, Liu Q et al. Genotypes and transmitted drug resistance among treatment-naive HIV-1-infected patients in a northwestern province, China: trends from 2003 to 2013. *PloS one* 2014; **9**: e109821.
51. Su Y, Zhang F, Liu H et al. The prevalence of HIV-1 drug resistance among antiretroviral treatment naive individuals in mainland China: a meta-analysis. *PloS one* 2014; **9**: e110652.

Figure Legends

Figure 1. Figure of exclusion on study cohort
1.Non-HIV related death

Figure 1. Figure of exclusion on study cohort



1.None HIV-related death.

215x279mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page	Recommendation
Title and abstract	1	2	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		2-3	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
			Introduction
Background/rationale	2	5	Explain the scientific background and rationale for the investigation being reported
Objectives	3	6	State specific objectives, including any prespecified hypotheses
			Methods
Study design	4	7	Present key elements of study design early in the paper
Setting	5	7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	7	**(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	8-9	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	9	Describe any efforts to address potential sources of bias
Study size	10	9	Explain how the study size was arrived at
Quantitative variables	11	9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	9	(*a*) Describe all statistical methods, including those used to control for confounding
		9	(*b*) Describe any methods used to examine subgroups and interactions
		7,9-10	(*c*) Explain how missing data were addressed
		9-10	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Results			
Participants	13*	9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		9-10	(b) Give reasons for non-participation at each stage
		19	(c) Consider use of a flow diagram
Descriptive data	14*	10	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
		10	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*10*	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	11,9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		8	(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	14-15	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	12	Summarise key results with reference to study objectives
Limitations	19	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	12-16	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	17	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	18	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.