

Original article

HIV-1 drug resistance in antiretroviral-naive individuals with HIV-1-associated tuberculous meningitis initiating antiretroviral therapy in Vietnam

Vu P Thao¹, Thuy Le^{1,2,3*}, Estee M Török⁴, Nguyen TB Yen⁵, Tran TH Chau^{1,6}, Suzanne Jurriaans⁷, Rogier H van Doorn^{1,3}, Menno D de Jong^{1,7}, Jeremy J Farrar^{1,3}, Sarah J Dunstan^{1,3}

¹Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

²Hawaii Center for AIDS, University of Hawaii at Manoa, Honolulu, HI, USA

³Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, UK

⁴University of Cambridge, Department of Medicine, Addenbrooke's Hospital, Cambridge, UK

⁵Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease, Ho Chi Minh City, Vietnam

⁶Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

⁷Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

*Corresponding author e-mail: thuy1@oucru.org

Background: Access to antiretroviral therapy (ART) for HIV-infected individuals in Vietnam is rapidly expanding, but there are limited data on HIV drug resistance (HIVDR) to guide ART strategies.

Methods: We retrospectively conducted HIVDR testing in 220 ART-naive individuals recruited to a randomized controlled trial of immediate versus deferred ART in individuals with HIV-associated tuberculous meningitis in Ho Chi Minh City (HCMC) from 2005–2008. HIVDR mutations were identified by population sequencing of the HIV *pol* gene and were defined based on 2009 WHO surveillance drug resistance mutations (SDRMs).

Results: We successfully sequenced 219/220 plasma samples of subjects prior to ART; 218 were subtype CRF01_AE and 1 was subtype B. SDRMs were identified in 14/219 (6.4%) subjects; 8/14 were resistant to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs;

T69D, L74V, V75M, M184V/I and K219R), 5/14 to non-nucleoside reverse transcriptase inhibitors (NNRTIs; K103N, V106M, Y181C, Y188C and G190A), 1/14 to both NRTIs and NNRTIs (D67N and Y181C) and none to protease inhibitors. After 6 months of ART, eight subjects developed protocol-defined virological failure. HIVDR mutations were identified in 5/8 subjects. All five had mutations with high-level resistance to NNRTIs and three had mutations with high-level resistance to NRTIs. Due to a high early mortality rate (58%), the effect of pre-existing HIVDR mutations on treatment outcome could not be accurately assessed.

Conclusions: The prevalence of WHO SDRMs in ART-naive individuals with HIV-associated tuberculous meningitis in HCMC from 2005–2008 is 6.4%. The SDRMs identified conferred resistance to NRTIs and/or NNRTIs, reflecting the standard first-line ART regimens in Vietnam.

Introduction

HIV is able to develop resistance to all currently licensed antiretroviral drugs, posing threats to the sustainability of antiretroviral therapy (ART) globally. Drug-resistant virus can be transmitted and/or acquired and can persist in the population [1–3], leading to treatment failure, disease progression and death [4–7]. Prevalence of transmitted drug resistance (TDR) in acutely or recently infected individuals in developed countries ranges from 7–24% [8–11]. Baseline HIV drug resistance (HIVDR) testing has

been shown to be cost-effective in the US when TDR prevalence exceeds 5% [12] and is routinely performed to guide initial antiretroviral choices in resource-rich settings [13,14]. As ART is being scaled up in low and middle income countries, where >90% of the world's HIV-infected population reside and ART options are limited, increased surveillance for TDR to inform ART programmes in resource-limited settings is essential.

The HIV epidemic in Vietnam started in 1990 and is still concentrated amongst the high-exposure risk

groups. The overall HIV prevalence in individuals aged 15–49 years is 0.5%; however, prevalence is 18.4% in injection drug users (IDU), 16.7% in men who have sex with men (MSM) and 3.2% in commercial sex workers (CSW) [15]. ART was initially introduced in Vietnam in the mid-1990s through private donations with intermittent drug supply and through the black market with high prices, so treatment interruption was likely common. In addition, non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) were not available or too expensive during the early 2000s, hence dual therapy regimens with zidovudine and lamivudine or stavudine and didanosine were commonly used (Donn Colby, Harvard Medical School AIDS Initiative in Vietnam, personal communication). The Vietnam National ART programme was commenced in 2005 through international support, primarily from the US President's Emergency Plan for AIDS Relief and the Global Fund to Fight AIDS, Tuberculosis and Malaria. ART coverage has increased from 1% to 53.7% of individuals who meet the Vietnam Ministry of Health criteria to initiate ART from 2003–2009 (CD4⁺ T-cell count <200 cells/ μ l and WHO disease stage III or IV) [15]. Both the history of non-suppressive ART use before 2005 and the rapid ART scale-up since 2005 are expected to be accompanied by the emergence of acquired drug resistance and TDR in Vietnam. From 2003–2009, six studies have reported prevalence of HIVDR in antiretroviral-naive individuals ranging from 2.9–7.6% in Vietnam [16–21]. Differences in reported prevalence may reflect differences in populations and time periods studied, in survey methods and drug resistance algorithms used, and in geographic and sample size factors, thereby making comparison and estimation problematic. For example, small studies using the WHO threshold survey method [22], which sampled relatively lower-HIV-exposure populations such as first-time pregnant women at antenatal clinics or attendees aged <25 years at HIV voluntary counselling and testing (VCT) centres, reported lower prevalence (<5%) [17,19], whereas larger studies that sampled the general population attending HIV clinics reported higher prevalence (>6%) [16,20,21]. Studies from Ha Noi and surrounding provinces tend to report lower prevalence [17,18,20] compared to a study from Ho Chi Minh City (HCMC) [16]. The latter study reported a prevalence of 6.5% of HIVDR mutations amongst 200 ART-naive individuals (43% IDUs and 38% CSWs) from 2001–2002, a period when ART was not yet widely available [16]. In this study we investigate the prevalence and effect of HIVDR mutations in ART-naive individuals who enrolled in a randomized clinical trial comparing immediate versus delayed ART for HIV-associated tuberculous meningitis (TBM) in HCMC from 2005–2008 [23].

Methods

Study setting and population

TBM is the most severe form of extra-pulmonary tuberculosis (TB) and is the most common central nervous system complication in HIV-infected people in Vietnam (Le *et al.* unpublished data). This study was conducted at two specialist centres for TB and HIV: Pham Ngoc Thach Hospital for TB and Lung Disease and Hospital for Tropical Diseases in HCMC. All antiretroviral-naive subjects with HIV-associated TBM who enrolled in a randomized double-blind placebo-controlled trial of immediate versus deferred initiation of ART, were included in this study. Participants were recruited between September 2005 and December 2008 and completed follow-up in December 2008 [23].

Statement of ethics

The trial protocol was approved by the Scientific and Ethical Committees of the two participating hospitals, by HCMC Health Services, and by the Oxford Tropical Research Ethics Committee. Written informed consent was obtained from all subjects or from a relative if the subject was unable to provide consent, according to standard practice in Vietnam. For unconscious subjects with no available relatives, the consent of two independent physicians was considered acceptable.

Treatment

Subjects received standard anti-tuberculosis therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) according to Vietnamese national guidelines. Unless contraindicated, all subjects also received adjunctive dexamethasone at an initial dose of 0.3–0.4 mg/kg/day, according to modified Medical Research Council TBM grade at presentation, which was tapered over 6 to 8 weeks as described elsewhere [24]. Antiretroviral or placebo tablets were commenced as soon as possible after randomization. The ART regimen was zidovudine, lamivudine and efavirenz for all subjects. Medications were administered orally or via nasogastric tube. Subjects received directly observed therapy during their inpatient stay (up to 3 months); administration was supervised by family members after discharge from hospital.

Laboratory procedures

Plasma HIV RNA was measured at baseline and at months 2, 3, 6, 9 and 12 using the Abbott M2000 real-time PCR platform (Abbott Laboratories, Abbott Park, IL, USA). Subjects with confirmed virological failure (VF; HIV RNA >1,000 copies/ml) at months 6, 9 and 12 were selected for drug resistance evaluation. Genotypic drug resistance analysis was performed on all available plasma samples for subjects in this trial at baseline and at the time of VF.

Genotypic drug resistance evaluation

HIV RNA was extracted from 200 µl plasma samples using an automated guanidinium-thiocyanate extraction method described elsewhere [25,26]. HIV genotype testing was performed on the ViroSeq platform ($n=23$ samples; ViroSeq HIV-1 genotyping system, Abbott Laboratories) and according to an in-house assay described by Bezemer [27], with the following modifications: the SuperScriptIII RT (Invitrogen, Carlsbad, CA, USA) instead of the MMLV-RT enzyme was used during reverse transcription, 45 instead of 25 cycles and 30 instead of 25 cycles were used during the first- and second-rounds, respectively, of PCR amplification. Approximately 1,200 base-pairs comprising the complete 297-nucleotide protease (PR) region and the first 897 nucleotides in the reverse transcriptase (RT) region of *pol* were checked by agarose gel electrophoresis. Amplification products were purified using QIAquick PCR purification kits (Qiagen, Hilden, Germany) and were subjected to direct sequencing using the ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The gene sequences were analysed using SeqScape (Applied Biosystems). Nucleotide changes were determined by comparison with the consensus sequence pNL4-3 for HIV-1 subtype B (GenBank accession number M19921). HIVDR mutations in baseline samples were determined according to the 2009 WHO drug resistance surveillance mutations (SDRMs) list [28]. Mutations in samples at VF were determined according to the 2010 International AIDS Society (IAS)-USA list [29]. For identification of polymorphisms, the pNL4-3 subtype B amino acid sequence was used as a reference sequence in the PR and RT regions of the 218 CRF01_AE isolates.

HIV-1 subtyping of protease and reverse transcriptase sequences

HIV-1 subtype was determined by phylogenetic analysis of the RT and PR genes using the neighbour-joining algorithm integrated in the Molecular Evolutionary Genetics Analysis (MEGA4) software [30]. Reference sequences of the *pol* gene from all available subtypes were obtained from the Los Alamos National Laboratory database [31]. Phylogenetic analysis was further confirmed by the Stanford database classification system.

Results

Presence of pre-existing HIVDR mutations in chronically infected subjects with HIV-associated tuberculous meningitis

Among the 253 subjects enrolled in the trial, stored baseline plasma samples were available for 220 participants, of whom 90% were male and 84% had history of IDU. The median baseline CD4⁺ T-lymphocyte count

and the median baseline plasma HIV-1 RNA level were 41 cells/µl (IQR 16–104) and 1.56×10^6 copies/ml (IQR 0.85×10^6 – 2.27×10^6), respectively. Both PR and RT regions were successfully sequenced in 219/220 baseline samples. Mutations that confer resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) were found in 8/219 (3.7%) subjects, and NNRTI resistance-conferring mutations in 5/219 (2.3%). Resistance to both NRTIs and NNRTIs was observed in 1/219 (0.5%). No PI resistance mutations were identified (Table 1). Hence, the overall prevalence of pre-existing HIVDR in this cohort in HCMC is 14/219 (6.4%). In 13/14 subjects, the detected mutations confer resistance to the standard first-line antiretrovirals used in Vietnam (stavudine, zidovudine, lamivudine, nevirapine and efavirenz). The remaining subject carried the mutation L74V, which confers resistance to abacavir and didanosine, part of second-line antiretrovirals in Vietnam. In 10/14 subjects the detected mutations are associated with high-level resistance to antiretrovirals according to the Stanford HIV drug resistance database.

HIV-1 subtyping and polymorphisms not associated with drug resistance in Ho Chi Minh City, Vietnam

Phylogenetic analysis and subtype classification according to MEGA4 and the Stanford HIV drug resistance database revealed that 218/219 (99.5%) subjects were infected by HIV-1 subtype CRF01_AE; the remaining one by subtype B. Non-synonymous polymorphisms were identified at all 15 drug resistance positions described in the 2009 WHO SDRM list and were more prevalent in the RT than PR gene of the 218 CRF01_AE isolates (Table 2). Of note, some natural polymorphisms were present at higher frequencies in this HCMC cohort compared to a study in northern Vietnam; both were overwhelmingly dominated by CRF01_AE subtype [18], that is, T69N (9.2% versus 0.4%), V106I (8.2% versus 1.5%) and V179I (17.4% versus 0%). The polymorphism L63C, which requires changes in all three nucleotides, was present in 45.4% of samples in this cohort. This substitution was present in 27.2% of the prior 200-subject cohort in HCMC [16] but has not been reported in northern Vietnam. The two polymorphisms V106I and V179D occurred in 18/219 (8.2%) and 5/219 (2.3%) subjects, respectively. These polymorphisms are not considered TDR mutations according to the WHO SDRM list and thus were not included in our resistance prevalence analysis. However, V106I and V179D have been associated with resistance to etravirine (a second-generation NNRTI available only in resource-rich countries) and are included in the 2010 IAS-USA mutation list [29]. If the IAS-USA algorithm is used, the prevalence of pre-existing HIVDR in our study

Table 1. Pre-existing 2009 WHO surveillance drug resistance mutations in individuals with HIV-associated tuberculous meningitis in Ho Chi Minh City from 2005–2007

Subject	HIV RNA, copies/ml	Drug resistance-associated mutations		
		NNRTI	NRTI	PI
1	904,780	K103N, V106M, G190A	–	–
2	609,200	Y181C	–	–
3	95,730	Y181C	–	–
4	1,604,510	–	V75M	–
5	5,600	–	M184V	–
6	57,235	Y181C	D67N	–
7	64,020	–	M184V	–
8	34,465	–	K219R	–
9	4,889,900	K103N, Y181C	–	–
10	333,130	–	L74V	–
11	315,310	–	V75M	–
12	1,359,330	K103N, Y181C	–	–
13	4,637,185	–	M184I	–
14	282,230	–	T69D	–

NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Table 2. Non-synonymous polymorphisms at 2009 WHO surveillance drug resistance mutation sites in 218 HIV-1 CRF01_AE isolates from Ho Chi Minh City

Drug resistance positions ^a	Amino acid in B reference	Amino acid in CRF01_AE reference	Amino acid substitution in this cohort	Subjects <i>n</i> (%)
Protease				
82	V	V	I	24 (11.0)
88	N	N	K	1 (0.5)
Reverse transcriptase				
67	D	D	N	1 (0.5)
69	T	T	D	1 (0.5)
–	–	–	N	20 (9.2)
–	–	–	A	4 (1.8)
–	–	–	S	3 (1.4)
74	L	L	V	1 (0.5)
75	V	V	M	2 (0.9)
–	–	–	L	2 (0.9)
101	K	K	R	1 (0.5)
103	K	K	N	3 (1.4)
–	–	–	R	1 (0.5)
106	V	V	M	1 (0.5)
–	–	–	I	17 (7.8)
179	V	V	I	38 (17.4)
–	–	–	D	5 (2.3)
–	–	–	A	2 (0.9)
–	–	–	E	2 (0.9)
–	–	–	N	1 (0.5)
181	Y	Y	C	4 (1.8)
184	M	M	V	2 (0.9)
190	G	G	A	1 (0.5)
210	L	L	M	7 (3.2)
–	–	–	F	1 (0.5)
219	K	K	R	1 (0.5)
–	–	–	T	2 (0.9)

^aDrug resistance positions were based on 2009 WHO surveillance drug resistance mutation list. Amino acids displayed in bold font are associated with drug resistance.

Figure 1. Non-synonymous polymorphisms at positions not associated with drug resistance based on the 2009 WHO surveillance drug resistance mutation list in 218 HIV-1 CRF01_AE isolates from Ho Chi Minh City

PR position ^a	L10	I13	K14	I15	G16	K20	E35	M36	N37	R41	K43	K45	R57	Q61	L63
CRF01_AE	I (49) V (25) M (1)	V (148)	R (12) N (1)	V (42)	E (107) A (38) A/S (1)	R (37) I (3)	D (197) D/N (2)	I (217)	D (54) D/E (3) S (1)	K (213)	R (15)	R (15)	K (20)	E (8) H (2)	C (99) P (20) S (3)
PR position ^a	I64	H69	K70	I72	T74	V77	L89	M93	F99						
CRF01_AE	L (1) M (1) V (1)	K (214) Q (1) T (1)	R (76)	V (5) R (1) T (1)	S (1)	I (3)	M (205) M/I (3) I (1)	L (41) V (2) M (1)	L (3)						
RT position ^a	E6	K11	V35	T39	K43	V60	Q102	K122	D123	I135	S162	K173	Q174	D177	I178
CRF01_AE	D (191) N (4) K/N (1)	T (142)	T (211) A (1) I (1) R (1)	K (100) E (63) N (30)	E (71) R (3) Q (4) A (1)	I (32)	K (184) R (4) K/R (5) T (1)	E (212) E/V (2)	S (162) N (13) N/S (37)	T (28) V (2) L (1) R (1)	C (159)	I (125) T (33) R (26) M (10)	K (109) R (12)	E (109)	M (126)
RT position ^a	G196	T200	I202	Q207	R211	K238	V245	A272	V276	R277	K281	T286	E291	V292	
CRF01_AE	E (12)	I (55) A (51)	V (24)	A (182) S (15) D (12)	S (208)	R (188)	E (205)	P (61)	I (34)	K (210)	R (32)	A (179)	D (187)	I (193)	

^aProtease (PR) and reverse transcriptase (RT) positions are based on pNL4-3 reference. Values shown in parenthesis represent number of isolates carrying a certain amino acid substitution.

increases from 6.4% to 16%. Other non-synonymous polymorphisms at positions not associated with the 2009 WHO SDRMs are listed in Figure 1; similar polymorphism frequencies have been described in subtype CRF01_AE in other studies in Asia [18,32].

HIV drug resistance development in subjects with virological failure

The mortality rate of TBM in HIV-infected individuals is twice as high compared to HIV-uninfected individuals [33]. Due to the early and high mortality of subjects in the trial (58%) [23], the effect of pre-existing drug resistance mutations on virological and clinical outcome could not be accurately assessed. Amongst the 219 trial subjects included in this study, only 69 survived and completed follow-up at month 6, 45 at month 9, and 14 at month 12. After 6 months follow-up, 8/69 surviving subjects had protocol-defined VF. Major 2010 IAS-USA mutations were detected in five of these subjects (Table 3). All five had mutations that confer high-level resistance to NNRTI (K103N, Y188L, P225H and/or M230L), and three additionally had mutations that confer high-level resistance to NRTI (M184V and/or T215Y/F/I/S). Two subjects had pre-existing mutations prior to ART initiation and accumulated further resistance mutations on non-suppressive therapy (subjects 3 and 7). Amongst the eight subjects with VF, three had virus containing V106I polymorphism both at baseline and at time of VF. Virus containing V106I and/or V179D did not develop on ART in any of the eight subjects with VF.

Discussion

We report a prevalence of 6.4% of pre-existing HIVDR in 219 ART-naive chronically HIV-infected individuals with TBM in HCMC from 2005–2007 using the 2009 WHO SDRM list. NRTI mutations were most commonly identified, occurring in 4.1% of subjects. Most observed mutations conferred resistance to NRTIs in the standard first-line ART regimens in Vietnam, while one subject had a mutation (L74V) that confers resistance to abacavir and didanosine used in second-line ART regimens in Vietnam. NNRTI mutations were identified in 2.8% of subjects, and all confer high-level resistance to nevirapine and efavirenz. No PI mutations were identified.

The prevalence of pre-existing HIVDR mutations in this study appears higher than in two previous studies, which used the WHO method for surveillance of TDR during 2003–2008, both of which showed TDR prevalences <5% [17,19]. However, aside from the small sample sets ($n=63$ and $n=49$), these studies surveyed relatively low-HIV-exposure populations (first-time pregnant women in antenatal clinics and individuals aged <25 years from VCT centres) compared to the population making up the concentrated HIV epidemic in Vietnam, where 52% are IDUs and 4% are CSWs [34]. By nature of the risk behaviors and social stigmatism, these high-HIV-exposure individuals are less likely to attend VCT centres and antenatal clinics. The WHO threshold survey method, which was designed to exclude ART-experienced individuals to improve the accuracy of

Table 3. Genotypic drug resistance profile of five individuals with virological failure and major 2010 International AIDS Society-USA mutations

Time, months	Subject 3		Subject 7		Subject 15		Subject 16		Subject 17	
	HIV RNA ^a	Genotype	HIV RNA ^a	Genotype	HIV RNA ^a	Genotype	HIV RNA ^a	Genotype	HIV RNA ^a	Genotype
0	76,500	Y181C	64,020	M184V	56,310	wt	495,5200	wt	29,230	wt
1	150	-	1,540	-	490	-	4,870	-	-	-
2	150	-	37,300	M184V, K103N	3545	Y188L	150	-	150	-
3	-	-	152,755	M184V, K103N, V108I	700	-	395	-	-	-
4	150	-	-	-	150	-	150	-	450	-
5	150	-	-	-	-	-	-	-	2,335	K103N, M184V, Y188HLY
6	-	-	47,910	M184V, K103N, V108I, T215F/I/S, P225H, M230L	-	-	-	-	215	-
7	41,325	V179D	-	-	205	-	150	-	2,605	K103N, M184V, Y188L
8	1,295	K103N, V106I	-	-	1,975	M184V, Y188L	-	-	-	-
9	-	-	51,850	M184V, K103N, V108I, T215F, M230L	1,825	M184V, Y188L	132,495	K103N	-	-
10	-	-	-	-	-	-	95,885	K103N, P225HP	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	44,940	M184V, K103N, V108I, T215F/I/S, P225H, M230L	-	-	-	-	-	-

^aCopies/ml. wt, wild type.

TDR estimation, may thereby be counter-intuitive and underestimate TDR in countries where HIV epidemics are concentrated in high-HIV-exposure populations, such as Vietnam. This study gives a specific example of the deficiencies of the WHO antenatal and VCT clinic-based screening method outside of sub-Saharan Africa – where the at-risk population is often made up of IDUs, CSWs and MSM. The resistance prevalence in our study is similar to studies that surveyed a more representative population attending HIV treatment clinics who report no prior use of ART, with resistance prevalence ranging from 6.2–7.6% [16,20,21]. Our resistance prevalence falls into the range of these data during the period of 2002–2009, suggesting that TDR has remained relatively stable despite the rapid scale-up of ART in Vietnam over the past 5 years [15]. Although TDR prevalence in Vietnam is at the level of which routine baseline resistance testing is considered cost-effective in resource-rich countries [12,35], cost-effectiveness studies of drug resistance testing and continuing surveillance of TDR targeting representative HIV-infected populations in resource-poor countries are clearly needed.

Non-synonymous polymorphisms were observed at all 15 drug resistance foci listed in the 2009 WHO SDRM. The V106I and V179D polymorphisms, both considered minor mutations associated with resistance to efavirenz according to the 2010 IAS-USA algorithm

[29], were observed in 8.2% and 2.3% of subjects, respectively. If the IAS-USA algorithm is used for TDR analysis, the resistance prevalence in our study would increase from 6.4% to 16%; however, this would be misleading. An example of such an analysis using the IAS-USA algorithm was recently published by the multicentre TREAT Asia study reporting a HIVDR prevalence of 13.8% in 682 ART-naive chronically infected individuals from Hong Kong, Malaysia and Thailand from 2007–2009 [36]. In that study, 77.7% of subjects were infected with CRF01_AE subtype, and the HIVDR prevalence of 13.8% included viral isolates containing the V106I (1.9%) and V179D (3.2%) polymorphisms. The resistance prevalence in this study would have substantially decreased if the WHO SDRM list was used, which would have better reflected the burden of primary resistance in the Asia-Pacific region. Our study highlights the importance of appropriate use of available HIVDR algorithms. The IAS-USA mutation list was not developed to be used in epidemiologic analyses to identify TDR but rather for listing relevant mutations or polymorphisms that are associated with a reduced susceptibility to anti-retroviral drugs [29]. The WHO SDRM algorithm is a parsimonious list of rigorously-defined mutations only at non-polymorphic positions in 8 major HIV subtypes and should be the most appropriate algorithm

for estimation and comparison of TDR in different regions and different times [28].

The two polymorphisms V106I and V179D were identified in 8.2% and 2.3% of subjects in the current study, and have been associated with etravirine resistance. Although etravirine is not yet available outside of America and Western Europe, V106I in combination with V179D have recently been identified as a new pattern of mutations conferring resistance to nevirapine and efavirenz [37], which are the most common drugs used in first-line ART regimens globally. Our data is consistent with a study by Scherrer *et al.* [38] that found a 3.2% prevalence of a similar etravirine-associated-resistance mutation V179T in treatment-naive patients infected with subtype CRF01_AE. That study found that most etravirine resistance-associated mutations in drug-naive patients are polymorphisms and not TDR mutations, and that these polymorphisms are significantly more or less common in different HIV subtypes [38]. This study highlights the need for studies to evaluate the prevalence and effect of subtype-specific polymorphisms on ART outcome and whether subtype-specific pathways to resistance occur.

Due to the early and high mortality rate in subjects with HIV-associated TBM in this study (58%), it was not possible to assess the effect of pre-existing SDRMs and identified polymorphisms on clinical or virological outcome. Amongst the eight subjects who met protocol-defined VF, HIVDR mutations were detected in five. All failed in the presence of major NNRTI mutations K103N and/or Y188L, and three failed with M184V mutations. Four subjects showed a pattern of accumulating resistance mutations. Interestingly, one subject had a pre-existing Y181C mutation, which disappeared while the K103N emerged by month 8 on ART. Switching from Y181C to K103N may occur as K103N carrying variants have better fitness compared to Y181C carrying variants in subtype B virus [39]. A shift in resistance pattern from Y181C to K103N has been described in women receiving a single dose of nevirapine to prevent mother-to-child transmission in Uganda [40].

An inherent limitation of a study method using a chronically infected population to estimate TDR is the possibility of over-estimating resistance prevalence. In this study, the ART history was based on patients' self-report, and some subjects may not have revealed their true previous ART exposure in order to be eligible for the trial. By contrast, an opposing limitation of using a chronically infected population to estimate TDR is the possibility of under-estimating resistance prevalence as the Sanger sequencing method used to detect HIVDR does not reliably detect HIV variants present at frequencies <15–25% of viral quasispecies [41]. It is generally known that TDR strains in the absence of antiretroviral selection pressure will over time revert

to wild-type virus, and thus minority resistant variants may be missed by standard genotyping methods. Nevertheless, compared to the population recommended by the WHO threshold survey method, the population in this study is more representative of the general HIV-infected population attending HIV clinics in Vietnam [15,34]. Although it remains important to distinguish between TDR from acquired resistance for epidemiological purposes, particularly during the scale-up of ART in the developing world, the implications of TDR and/or acquired resistance for the individual patient and/or for public health in light of ART outcome and further spread of drug-resistant virus is the same.

In summary, using the 2009 WHO SDRM algorithm not in combination with the WHO threshold survey method, we report a 6.4% prevalence of pre-existing HIVDR in ART-naive chronically infected individuals with TBM in HCMC, Vietnam, from 2005–2007. The identified HIVDR mutations conferred resistance to NRTIs and/or NNRTIs that are part of the standard first-line ART in Vietnam. Our HIVDR resistance data is comparable to other studies using similar sampling methodology in the general populations seen in HIV clinics and suggests that HIVDR has remained relatively stable despite the rapid scale-up of ART in Vietnam.

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Disclosure statement

The authors declare no competing interests.

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