

Undisclosed Antiretroviral Drug Use in a Multinational Clinical Trial (HIV Prevention Trials Network 052)

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The HIV Prevention Trials Network 052 study enrolled serodiscordant couples. Index participants infected with human immunodeficiency virus reported no prior antiretroviral (ARV) treatment at enrollment. ARV drug testing was performed retrospectively using enrollment samples from a subset of index participants. ARV drugs were detected in 45 of 96 participants (46.9%) with an undetectable viral load, 2 of 48 (4.2%) with a low viral load, and 1 of 65 (1.5%) with a high viral load ($P < .0001$); they were also detected in follow-up samples from participants who were not receiving study-administered treatment. ARV drug testing may be useful in addition to self-report of ARV drug use in some clinical trial settings.

Keywords. HIV; antiretroviral drug; self-report; HPTN 052; Africa; clinical trial.

Many human immunodeficiency virus (HIV) treatment and prevention trials enroll HIV-infected participants who report no history of antiretroviral (ARV) drug use. This information is often obtained by self-report. Undisclosed “off-study” ARV use before enrollment or during a clinical trial may confound study outcomes. The Partners in Prevention HSV/HIV Transmission study observed undisclosed ARV use in 46% of HIV-infected participants who were virally suppressed at enrollment and reported no prior ARV treatment (ART) [1, 2].

The HIV Prevention Trials Network (HPTN) 052 study enrolled 1763 HIV-serodiscordant couples in Africa, Asia, and the Americas (2005–2010) [3]. Eligibility criteria for HIV-infected index participants included a CD4 cell count of 350–550 cells/mm³ and no prior ART; prior receipt of a short-term ARV regimen for prevention of mother-to-child transmission of HIV (pMTCT) was allowed [3]. Index participants were randomized in an unblinded fashion to initiate ART immediately after enrollment irrespective of their CD4 cell count (early ART arm) or to initiate ART once their CD4 cell count reached ≤ 250 cell/mm³ at 2 consecutive measurements (delayed ART arm), or if an AIDS-defining illness developed. Early initiation of ART in HIV-infected index participants was shown to reduce HIV transmission to HIV-uninfected partners by 96% [3].

During an interim review of HPTN 052 by the Study Monitoring Committee, it was noted that some HIV-infected participants were virally suppressed at the time of study enrollment. This finding raised concerns that some participants may have been receiving ART at enrollment and that some participants in the delayed ART arm may have continued off-study ARV use after enrollment. Here, we describe a post hoc analysis of unreported ARV drug use in the HPTN 052 trial.

METHODS

Samples Used for Analysis

Enrollment plasma samples from index participants were tested, including (1) all available samples from participants with viral loads ≤ 400 copies/mL (virally suppressed group), (2) all available samples from participants with viral loads of 401–1000 copies/mL (low viral load group), and (3) samples from a randomly selected subset of participants with viral loads >1000 copies/mL (high viral load group, stratified by site). Follow-up samples

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from a subset of participants were also analyzed. Results from ARV testing were not returned to study sites or study participants. One study site (Pune, India) did not provide approval for ARV testing and was not included in this substudy.

Laboratory Methods

HIV loads and CD4 cell counts were determined in the HPTN 052 trial [3]. The ARV drugs were measured using liquid chromatography–tandem mass spectrometry (API 4000 triple quadrupole mass analyzer; AB SCIEX). This testing was performed at the Clinical Pharmacology Analytical Laboratory (Johns Hopkins University School of Medicine) using assays validated according to the US Food and Drug Administration bioanalytical guidelines. Samples were screened for 16 ARV drugs, including protease inhibitors (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), nucleoside/nucleotide reverse-transcriptase inhibitors (emtricitabine [FTC], lamivudine [3TC], stavudine [d4T], tenofovir [TFV], and zidovudine [ZDV]), and nonnucleoside reverse-transcriptase inhibitors (efavirenz and nevirapine [NVP]). Analytes were extracted from 50 μ L of plasma by protein precipitation with methanol and were separated with reversed-phase liquid chromatography using gradient elution and either a Zorbax Eclipse XDB-C18 column (Agilent Technologies; for FTC, TFV, and ZDV) or an Acquity UPLC BEH C18 column (Waters; for all other drugs). Eluted analytes were subjected to positive electrospray ionization with detection in multiple reaction-monitoring mode. The lower limit of detection was <5 ng/mL for FTC, TFV, and ZDV, 20 ng/mL for 3TC, d4T, and NVP, and 100 ng/mL for the remaining drugs. Results were reported as positive or negative for each analyte.

Statistical Analysis

We used χ^2 and Fisher's exact tests to compare differences in proportions of categorical variables and the Wilcoxon rank sum test to compare differences in medians for continuous variables between individuals in the virally suppressed group with or without ARV drugs detected. Virologic failure was defined as 2 consecutive viral load measurements >1000 copies/mL \geq 16 weeks after ART initiation. Analyses were performed using SAS software, version 9.2.

Ethical Considerations

Written informed consent was obtained from all participants in the HPTN 052 trial. The trial was approved by institutional review boards or ethics committees at each participating institution.

RESULTS

Detection of ARV Drugs in Enrollment Samples

Enrollment samples from 209 HIV-infected index participants were tested for ARV drugs (Supplementary Figure 1). None of

the women in this substudy reported receiving ARV drugs for pMTCT within the month before enrollment. Forty-eight participants (23.0%) had \geq 1 ARV drug(s) detected: 45 of 96 (46.9%) in the virally suppressed group, 2 of 48 (4.2%) in the low viral load group, and 1 of 65 (1.5%) in the high viral load group ($P < .0001$). The following drugs were detected in the virally suppressed group: NVP + 3TC + d4T ($n = 21$), NVP + 3TC ($n = 14$), NVP alone ($n = 4$), NVP + ZDV + 3TC ($n = 2$), NVP + d4T ($n = 2$), NVP + ZDV + d4T ($n = 1$), and ZDV + d4T ($n = 1$). The drugs detected in the low viral load group were NVP + 3TC ($n = 1$) and ZDV alone ($n = 1$). Only 1 (1.5%) of the 65 samples from the high viral load group had an ARV drug detected (3TC only).

The 48 participants who had ARV drugs detected (including 45 in the virally suppressed group; Tables 1 and 2), were enrolled at 6 study sites in 5 countries; 22 were randomized to the early ART arm, and 26 to the delayed ART arm. None of these 48 participants transmitted HIV to his or her partner, and only 2 of 161 participants who did not have ARV drugs detected at enrollment transmitted HIV to their partners.

Factors Associated With Detection of ARV Drugs at Enrollment

We examined the association of demographic and clinical factors with detection of ARV drugs at enrollment among the 96 participants in the virally suppressed group. In this group, ARV drugs were detected in 41 of 73 samples (56.2%) from Africa, 4 of 12 (33.3%) samples from Asia, and none of 11 samples from the Americas ($P = .001$). ARV drug detection by country is shown in Table 1. CD4 cell count at enrollment was lower among participants who had ARV drugs detected than among those who did not have (median, 403 vs 472 cells/mm³; $P < .001$). There was no significant association of ARV drug detection with age, sex, report of previous ARV use for pMTCT, or self-reported condom use (Table 2). Among 51 participants in the immediate ART study arm, there was no significant difference in frequency of virologic failure during the first year of study-administered ART between those who did and those who did not have ARV drugs detected at enrollment (1 of 19 [5.3%] vs none of 32, respectively; $P = .37$). Viral load data from follow-up visits is presented in Supplementary Figure 2.

Detection of ARV Drugs in Follow-up Samples

We next examined whether participants who had ARV drugs detected at enrollment continued to use ARV drugs off study after enrollment. This analysis was limited to the 16 participants in the virally suppressed group who were randomized to the delayed ART arm and had \geq 1 follow-up visit with a viral load <1000 copies/mL before initiation of study-administered ART. At least 1 ARV drug was detected in 46 of 47 samples tested (median, 3 samples per participant); the drugs detected were NVP + 3TC + d4T ($n = 35$), NVP + 3TC ($n = 8$), NVP + ZDV + 3TC ($n = 2$), and NVP alone ($n = 1$). In 12 participants,

Table 1. Detection of ARV Drugs at Study Enrollment by Country and Region Among Index Participants With a Viral Load ≤ 400 Copies/mL at Study Enrollment

Participant Category	No. (%) of Participants Enrolled									
	Africa					Asia		Americas		Total
	Botswana	Kenya	Malawi	South Africa	Zimbabwe	India	Thailand	Brazil	United States	
Enrolled in HPTN 052	77 (4.4)	60 (3.4)	481 (27.3)	96 (5.5)	240 (13.6)	425 (24.1)	106 (6)	276 (15.7)	2 (0.1)	1763
Enrolled in this study ^a	77 (100)	60 (100)	481 (100)	96 (100)	240 (100)	250 (58.8)	106 (100)	276 (100)	2 (100)	1588
Viral load data available ^b	76 (4.8)	60 (3.8)	476 (30.1)	96 (6.1)	240 (15.2)	250 (15.8)	106 (6.7)	276 (17.5)	2 (0.1)	1582
Virally suppressed ^c	5 (5.2)	1 (1)	34 (35.4)	11 (11.5)	22 (22.9)	11 (11.5)	1 (1)	11 (11.5)	0	96
ARV drug(s) detected	0	1 (2.2)	20 (44.4)	1 (2.2)	19 (42.2)	4 (8.9)	0	0	0	45
Virally suppressed/ No. tested ^d	5/76 (6.6)	1/60 (1.7)	34/476 (7.1)	11/96 (11.5)	22/240 (9.2)	11/250 (4.4)	1/106 (0.9)	11/276 (4.0)	0/2	96/1582 (6.1)
ARV drug(s) detected/ No. tested ^e	0/76	1/60 (1.7)	20/476 (4.2)	1/96 (1.0)	19/240 (7.9)	4/250 (1.6)	0/106	0/276	0/2	45/1582 (2.8)

Abbreviation: ARV, antiretroviral.

^a A total of 1763 couples were enrolled in HPTN 052. This substudy included 12 of the 13 HPTN 052 study sites (see Methods).

^b Six index participants (1 from Botswana, 5 from Malawi) did not have viral load data from the enrollment visit.

^c Virally suppressed was defined as a viral load ≤ 400 copies/mL at the enrollment visit.

^d For these data, the denominator (number tested) indicates the number of samples with viral load data available from the region indicated in the column header.

^e For these data, the denominator (number tested) indicates the number of samples tested for the presence of ARV drugs from the region indicated in the column header.

the drugs detected in the follow-up samples were also detected in the corresponding enrollment sample; in 4 participants, d4T was detected in follow-up samples but not at the enrollment visit.

DISCUSSION

This study examined off-study ARV drug use in HIV-infected index participants in HPTN 052. All index participants enrolled in the trial denied prior or current ART at the time of study enrollment. Off-study ARV use was documented in approximately half of the participants with viral suppression (overall, 45 of 96 [46.9%]; in Africa, 41 of 73 [56.2%]). This group represented 2.8% of the 1582 enrolled index participants for whom viral load data were available from the enrollment visit. ARV drugs were also detected in a small number of participants with higher viral loads (2 of 48 participants [4.2%] with viral loads 401–1000 copies/mL; 1 of 65 randomly selected participants [1.5%] with viral loads >1000 copies/mL). In the virally suppressed group, detection of ARV drugs at enrollment was associated with a lower baseline CD4 cell count.

Off-study ARV use at enrollment did not seem to affect the response to study-administered ART. Among participants who were randomized to the early ART arm of the trial, detection of

ARV drugs at enrollment was not associated with a lack of viral suppression or treatment failure during the first year of study-administered ART. Among participants in the delayed ART arm of the trial, viral suppression during the first year of the trial in the absence of study-administered ART was more common among participants who had ARV drugs detected at enrollment. In many cases, those participants continued to use off-study ARV drugs after enrollment, which was not disclosed to study staff.

The findings of this study and the report from the Partners in Prevention HSV/HIV Transmission trial [2] highlight the limitations of using self-report to gather information about past or current ARV drug use. There are many reasons why participants may have chosen not to disclose ARV drug use at enrollment in HPTN 052. Disclosure of past or current ART would have excluded them from enrollment and the trial's potential benefits (eg, perceived opportunity for improved routine care, access to study-administered ART, possible prevention benefits for partners, and reimbursement for travel). Furthermore, some HIV-infected individuals may have not disclosed their HIV status to their partner before enrollment (fearing stigma, discrimination, or partner abandonment); they may have preferred to disclose their HIV status to their partner at enrollment

Table 2. Factors Associated With Detection of ARV Drugs in Index Participants With a Viral Load ≤ 400 copies/mL at Study Enrollment

Factor	Detection of Undisclosed ARV Drugs		
	Yes (n = 45)	No (n = 51)	P Value ^a
Age, median (IQR), y	33.0 (29.0–38.0)	33.0 (27.0–40.0)	.96
Sex			
Male	18 (40.0)	21 (41.2)	.91
Female	27 (60.0)	30 (58.8)	
Report of previous ARV drug use during pregnancy, No. (%) ^b			
Yes	4 (14.8)	9 (32.1)	.13
No	23 (85.2)	19 (67.9)	
Report of 100% condom use, No. (%) ^b			
Yes	40 (88.9)	46 (90.2)	>.99
No	5 (11.1)	5 (9.8)	
Baseline CD4 cell count, median (IQR), cells/mm ³	403 (387–455)	472 (440–530)	<.001

Abbreviations: ARV, antiretroviral; IQR, interquartile range.

^a Fisher's exact or χ^2 tests were used for categorical variables, and the Wilcoxon rank sum test for continuous variables.

^b Data on previous ARV use during pregnancy and 100% condom use are from the enrollment visit. Two female participants did not answer the question about pregnancy.

with the help of a trained couples counselor and may therefore have chosen not to disclose past or current ART.

It is important to note that undisclosed ART use in HPTN 052 could have reduced the apparent impact of the study intervention: early ART for prevention of HIV transmission. This is because participants in the delayed ART arm (control study arm) who were virally suppressed from off-study ART would have been less likely to transmit HIV to their partners. Therefore, it is notable that the remarkable effect of the study intervention (a 96% reduction in HIV transmission with early ART) was apparent, even though some study participants were taking ART outside of the study [3].

The frequency of undisclosed off-study ARV drug use among HIV-infected participants with undetectable viral loads at enrollment was similar in HPTN 052 and the Partners in Prevention HSV/HIV Transmission study [2] (46.9% and 46.0%, respectively). In HPTN 052, off-study ARV drug use was most common at the African study sites and was also observed in Asia but not in the Americas. The HIV-infected individuals with undetectable viral loads and no evidence of ARV drug use may have been elite controllers. In HPTN 052, these individuals accounted for 51 (3.2%) of the 1582 participants included in this substudy. This indicates that the presence of an undetectable viral load in the absence of study-administered ART does not necessarily indicate off-study ARV drug use. Further studies are needed to evaluate the potential impact of enrolling elite controllers in different clinical trial settings.

It is important to emphasize that ARV drug screening was performed only in a subset of participants in HPTN 052; only 209 (11.9%) of the 1763 index participants in HPTN 052 were tested. The finding of off-study ARV use in some participants emphasizes the potential value of ARV testing in a many different types of clinical trials. Further studies are needed to determine the frequency of undisclosed ARV use in different clinical and research settings and to understand the reasons that a study participant may chose not to disclose use of ARV drugs. Evaluation of off-study ARV use may also be important in studies that enroll HIV-uninfected individuals, because ARV drugs are also used for preexposure prophylaxis [4–7] and may be used as recreational drugs [7–10]. ARV drug testing may also be important for studies of elite controllers, because some individuals receiving ART may not disclose their HIV infection status. Recent development of a low-cost, high-throughput ARV screening assay [11] may facilitate this type of analysis in future studies.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. All authors contributed to manuscript preparation. Additional author roles are listed below (listed sites are all HPTN sites). J. M. F. drafted the manuscript, reviewed and analyzed study data; L. W., statistician for HPTN 052, performed statistical analyses; T. L. P. performed ARV drug testing; S. S. O., data analyst for HPTN 052, performed statistical analyses; E. P. M., HPTN QA/QC representative for HPTN 052, coordinated sample management; Y. C., statistician for HPTN 052, reviewed statistical analysis; V. O. M., pharmacist for the Kisumu, Kenya, site; M. C. H., lead investigator for the Lilongwe, Malawi, site; J. K., lead investigator for the Blantyre, Malawi, site; J. G. H., lead investigator for the Harare, Zimbabwe, site; S. C., lead investigator for the Chiang Mai, Thailand, site; R. P., lead investigator for the Soweto, South Africa, site; I. S., lead investigator for the Johannesburg, South Africa, site; N. K., lead investigator for the Chennai, India, site; B. J., lead investigator for one of the sites in Rio de Janeiro, Brazil; J. M., lead investigator for the Gabarone, Botswana, site; J. P., lead investigator for another of the sites in Rio de Janeiro; B. R. S., lead investigator for the Porto Alegre, Brazil, site; K. H. M., lead investigator for the Boston, Massachusetts, site; M. M. and T. G., senior study managers for HPTN 052; N. B. coordinated ARV drug testing and reviewed test results; C. W. H., pharmacologist for this substudy, contributed to study design, drug testing, and interpretation of ARV drug data; M. S. C., principal investigator for HPTN 052; S. H. E., protocol virologist for HPTN 052, designed the study, analyzed the data, and drafted and finalized the manuscript.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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