

# Viral Rebound and Emergence of Drug Resistance in the Absence of Viral Load Testing: A Randomized Comparison between Zidovudine-Lamivudine plus Nevirapine and Zidovudine-Lamivudine plus Abacavir

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**Background.** We investigated virological response and the emergence of resistance in the Nevirapine or Abacavir (NORA) substudy of the Development of Antiretroviral Treatment in Africa (DART) trial.

**Methods.** Six hundred symptomatic antiretroviral-naïve human immunodeficiency virus (HIV)–infected adults (CD4 cell count, <200 cells/mm<sup>3</sup>) from 2 Ugandan centers were randomized to receive zidovudine-lamivudine plus abacavir or nevirapine. Virology was performed retrospectively on stored plasma samples at selected time points. In patients with HIV RNA levels >1000 copies/mL, the residual activity of therapy was calculated as the reduction in HIV RNA level, compared with baseline.

**Results.** Overall, HIV RNA levels were lower in the nevirapine group than in the abacavir group at 24 and 48 weeks ( $P < .001$ ), although no differences were observed at weeks 4 and 12. Virological responses were similar in the 2 treatment groups for baseline HIV RNA level <100,000 copies/mL. The mean residual activity at week 48 was higher for abacavir in the presence of the typically observed resistance pattern of thymidine analogue mutations (TAMs) and M184V (1.47 log<sub>10</sub> copies/mL) than for nevirapine with M184V and nonnucleoside reverse-transcriptase inhibitor mutations, whether accompanied by TAMs (0.96 log<sub>10</sub> copies/mL) or not (1.18 log<sub>10</sub> copies/mL).

**Conclusions.** There was more extensive genotypic resistance in both treatment groups than is generally seen in resource-rich settings. However, significant residual activity was observed among patients with virological failure, particularly those receiving zidovudine-lamivudine plus abacavir.

Clinical trials of antiretroviral therapy (ART) in high-income countries routinely use viral load as a key primary end point [1]. Indeed, in these settings, viral load monitoring during ART is universally available, treatment failure is defined virologically, and a switch to a new regimen is recommended as soon as virological

rebound occurs. Therefore, data from genotypic resistance testing of first-line therapy regimens generally relate to early viral rebound. These assays are currently unavailable in most resource-limited countries because of financial and technical constraints [2]. By contrast, ART in resource-limited countries is administered in

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accordance with the World Health Organization (WHO) public health approach to ART [3], which places limited reliance on viral load monitoring and drug resistance testing. Because CD4 cell counts are also not widely available to support patient treatment, treatment failure is often identified on the basis of clinical events, which are used to guide treatment switching. In these settings, therefore, clinical progression will be determined by the dynamics of viral load rebound, the consequential acquisition and evolution of drug resistance, and subsequent reduction in CD4 cell count and increase in risk of clinical disease progression while first-line therapy is continued. To date, there are limited data on these relationships for patients receiving first-line therapy in the absence of monitoring.

The Nevirapine or Abacavir (NORA) substudy of the Development of Antiretroviral Treatment in Africa (DART) trial was a randomized placebo-controlled comparison of abacavir and nevirapine, in combination with zidovudine-lamivudine [4]. In the DART trial, viral load monitoring was not performed in real time, and switches to second-line therapy were guided by clinical monitoring either alone or in conjunction with CD4 cell count monitoring every 3 months, in a randomized comparison [5]. We reported that fewer serious adverse events, regimen changes, and clinical events occurred during the first 48 weeks in the abacavir group than in the nevirapine group [4], despite inferior virological and CD4 cell responses [6]. These results suggest a disconnect between the clinical events and frequently used surrogates of antiviral response, namely, viral load and CD4 cell count.

Although zidovudine-lamivudine plus abacavir had suboptimal virological potency, compared with nonnucleoside reverse-transcriptase inhibitor (NNRTI)-containing regimens in well-resourced settings [7], there remains an interest in triple-nucleoside analogue regimens in resource-limited countries, particularly in the context of human immunodeficiency virus (HIV) and tuberculosis coinfection and treatment [8, 9]. In addition, triple-nucleoside analogue regimens are class-sparing regimens, can be used in women of child-bearing age and during lactation, and are effective against HIV-2 [10]. Therefore, further study of triple-nucleoside reverse-transcriptase inhibitor (NRTI) first-line regimens, particularly with regard to the consequences of viral rebound, is important. Of note, observational studies have reported that the level of virological rebound in patients receiving zidovudine-lamivudine plus abacavir remains low, and virological rebound is frequently associated with the M184V mutation alone, in addition to continued high CD4 cell count [11]. It is therefore likely that this regimen may be appropriate for settings where viral load monitoring is not available. On the other hand, the continual acquisition of nucleoside analogue resistance mutations in the presence of viral load rebound will compromise future NRTI drugs used to support a boosted protease and/or NNRTI in

second-line ART [11]. We performed a detailed retrospective study of participants in the randomized NORA study, to clarify virological response and the emergence of resistance for the 2 drug combinations assessed in the trial.

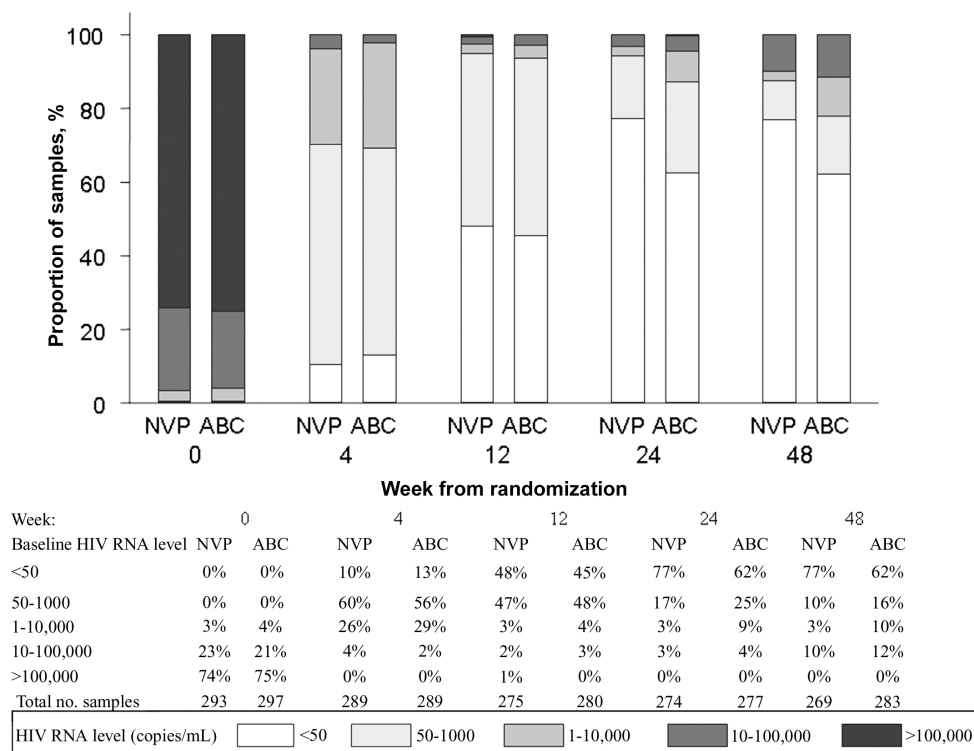
## METHODS

**Ethics statement.** Both the DART study and the NORA substudy received ethics approval in Uganda (Uganda Research Unit on AIDS [UVRI] Science and Ethics Committee) and the United Kingdom (Imperial College).

**Trial design and participants.** The NORA trial [4] was a 24-week randomized double-blind trial conducted at 2 centers in Uganda (Joint Clinical Research Centre, Kampala, and Medical Research Council [MRC]/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda), as a nested substudy in the DART trial (same International Standard Randomized Controlled Trial Number, 13968779) [5]. Six hundred previously untreated symptomatic HIV-infected adults who were beginning ART with CD4 cell counts <200 cells/mm<sup>3</sup> were randomly allocated in a 1:1 ratio to receive coformulated zidovudine-lamivudine plus either (1) abacavir (300 mg) and nevirapine placebo or (2) abacavir placebo and nevirapine (200 mg), twice daily, prescribed for 24 weeks (double dummy design). After 24 weeks, participants continued to receive the open-label equivalent of their blinded drug and continued follow-up in the DART trial.

The primary DART randomization compared clinical monitoring alone with laboratory (including CD4 cell counts) and clinical monitoring. HIV-1 RNA measurements and drug-resistance tests were performed retrospectively for all participants in the NORA substudy (ie, they were not used to guide therapy management in real time).

**Laboratory measurements.** Stored plasma samples obtained at baseline and at 4, 12, 24, and 48 weeks were assayed for HIV-1 RNA at the Joint Clinical Research Centre with use of the Roche Amplicor assay for baseline samples (version 1.5; lower limit of detection, 400 copies/mL) and the Roche ultrasensitive assay for other samples (50 copies/mL). Samples with HIV-1 RNA levels >1000 copies/mL at 48 weeks and baseline samples for this subset of participants were genotyped using reverse-transcription polymerase chain reaction (RT-PCR) and subsequent sequencing of a contiguous region of the *pol* gene, encompassing the whole of the protease gene and codons 1–320 of RT. An in-house sequencing method was used with a Beckman capillary sequencer at the MRC/UVRI Uganda Research Unit on AIDS and an ABI capillary sequencer at University College London. RNA was extracted from plasma (QIAamp viral RNA extraction kit; Qiagen) and reverse transcribed using 1-step RT-PCR (Qiagen). The entire protease and codons 1–320 of RT were amplified from complementary DNA by nested PCR with use of the following primer sets: outer forward, 5' AAT GAT GAC AGC ATG YCA GGG AGT 3'; outer



**Figure 1.** Distribution of human immunodeficiency virus (HIV) RNA, by time since randomization. ABC, abacavir; NVP, nevirapine.

reverse, 5' AGT CTT TCC CCA TAT TAC TAT GCT TTC 3'; inner forward, 5' GGA AAA AGG GCT GTT GGA AAT GTG 3'; and inner reverse, 5' GGC TCT TGA TAA ATT TGA TAT GTC CAT TG3'.

Key mutations were identified by reference to the most recent International AIDS Society–USA classification [12]. Thymidine analogue mutations (TAMs) included those at positions 41, 67, 70, 210, 215, and 219 in RT. Subtype was inferred from the *pol* sequences with use of the REGA HIV subtyping algorithm [13]. Drug resistance sequences from this study were deposited in GenBank with reference numbers GQ409546–GQ409635.

**Statistical analysis.** All analyses of virological response were intention to treat; resistance results at week 48 are presented on both an intention-to-treat and an on-treatment basis. Baseline values were those recorded closest to (but within) 6 weeks after randomization. Statistical tests included Student's *t* test, the  $\chi^2$  test, and the  $\chi^2$  test for trend, as appropriate. A multivariate logistic regression model (backward elimination; exit probability, 0.05) was fitted to identify baseline factors associated with HIV RNA level <50 copies/mL at week 48. All *P* values are 2 sided.

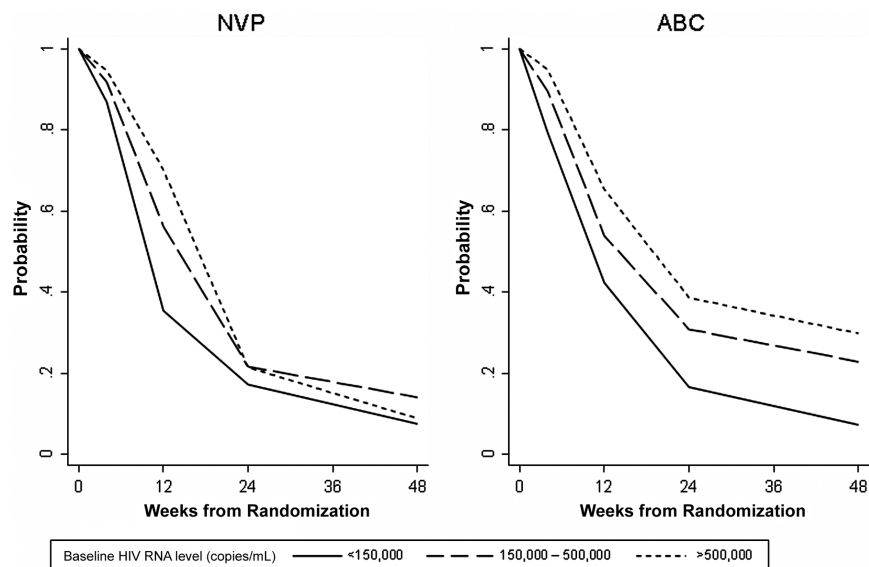
## RESULTS

From January through October 2004, 600 individuals were randomized to receive abacavir ( $n = 300$ ) or nevirapine ( $n = 300$ ). Demographic characteristics and clinical outcome data

for participants enrolled in the NORA trial are described in detail elsewhere [4, 6]. Baseline characteristics were broadly similar between the 2 groups; 430 (72%) of the participants were women, 330 (55%) had WHO stage 3 and 111 (18%) had WHO stage 4 HIV infection, the median age was 37 years (interquartile range, 32–42 years), and the median CD4 cell count was 99 cells/mm<sup>3</sup> (interquartile range, 44–147 cells/mm<sup>3</sup>).

Of 600 participants, 563 (94%; 286 in the abacavir group and 277 in the nevirapine group) completed 48 weeks of follow-up; 25 participants (9 in the abacavir group and 16 in the nevirapine group) died before 48 weeks, most in the first 12 weeks (7 in the abacavir group and 12 in the nevirapine group). Twelve participants (5 in the abacavir group and 7 in the nevirapine group) were unavailable for follow-up. At 48 weeks, 510 participants (91%; 266 [93%] in the abacavir group and 244 [88%] in the nevirapine group) were still receiving their allocated regimens, with substitution of stavudine for zidovudine allowed in the NRTI backbone, mainly because of toxicity. Of the remaining 53 participants, 19 substituted either tenofovir ( $n = 14$ ) or nevirapine ( $n = 5$ ) abacavir, 28 substituted either tenofovir ( $n = 27$ ) or abacavir ( $n = 1$ ) for nevirapine, and 6 stopped ART (1 in the abacavir group and 5 in the nevirapine group) because of toxicity. No participant switched to second-line therapy on the basis of clinical or immunological criteria for treatment failure.

**Virological response.** HIV RNA results were obtained from



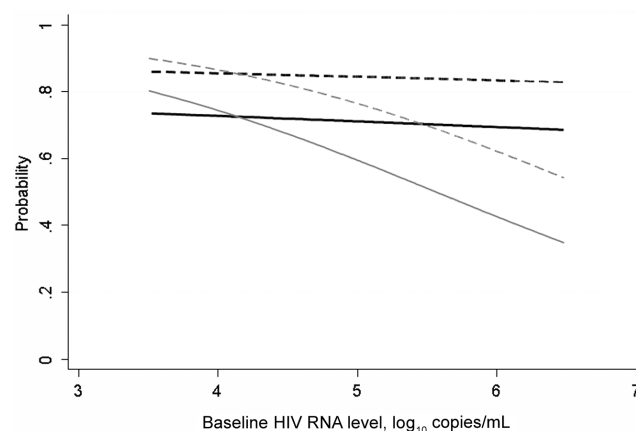
**Figure 2.** Kaplan-Meier estimates of the probability of not achieving virological suppression (human immunodeficiency virus [HIV] RNA, <50 copies/mL), by baseline HIV RNA level and randomized group. Cutoff values (150,000 and 500,000 copies/mL) were chosen to produce 3 groups of approximately equal size (ie, tertiles of distribution). Kaplan-Meier estimates were joined by straight lines rather than the standard step function to aid visual clarity. ABC, abacavir; NVP, nevirapine.

2815 (94%) of 3000 possible samples, with death (76 samples [3%]), missed visits (87 [3%]), or loss to follow-up (22 [1%]) accounting for the missing data. The mean HIV RNA level ( $\pm$  standard deviation [SD]) at baseline was  $5.4 \pm 0.7 \log_{10}$  copies/mL in both the abacavir and the nevirapine groups. The mean reduction in HIV RNA level ( $\pm$  SD) from baseline to 4 weeks was very similar in the abacavir group ( $2.73 \pm 0.78 \log_{10}$  copies/mL) and in the nevirapine group ( $2.70 \pm 0.81 \log_{10}$  copies/mL). There was also no evidence of a difference between groups in HIV RNA level at 12 weeks (Figure 1). However, at 24 weeks, a significantly higher proportion of participants had suppressed HIV RNA levels <50 copies/mL in the nevirapine group (77%) than in the abacavir group (62%;  $P < .001$ ). These proportions were unchanged at week 48, which implies that the number of participants who experienced virological rebound at 24–48 weeks was equal to the number of patients who first achieved suppression during this interval.

At week 48, fewer patients in the nevirapine group ( $n = 34$ ) than in the abacavir group ( $n = 62$ ) had HIV RNA levels >1000 copies/mL; however, for patients with levels above this threshold, the mean HIV RNA level was significantly higher in the nevirapine group ( $4.44 \pm 0.57 \log_{10}$  copies/mL;  $n = 34$ ) than in the abacavir group ( $4.19 \pm 0.68 \log_{10}$  copies/mL;  $n = 62$ ;  $P = .06$ ). For patients with HIV RNA levels >50 copies/mL, however, the mean HIV RNA level ( $\pm$  SD) did not differ significantly between the 2 groups ( $3.36 \pm 1.30 \log_{10}$  copies/mL in the nevirapine group [ $n = 62$ ] and  $3.36 \pm 1.12 \log_{10}$  copies/mL in the abacavir group [ $n = 107$ ];  $P = .96$ ).

Figure 2 shows time to virological suppression (HIV RNA

level, < 50 copies/mL), stratified by baseline HIV RNA level. In the nevirapine group, the probability of achieving suppression by 48 weeks did not depend on baseline HIV RNA level, although suppression was achieved more slowly in patients with higher baseline values. In contrast, in the abacavir group, this probability decreased as the baseline HIV RNA level increased, from 93% (95% confidence interval [CI], 87%–97%) for baseline HIV RNA level <150,000 copies/mL to 77% (95% CI, 68%–



**Figure 3.** Estimated probability of virological suppression (human immunodeficiency virus [HIV] RNA, <50 copies/mL) at 48 weeks, by baseline HIV RNA level, for baseline CD4 cell counts of 50 and 150 cells/mm<sup>3</sup>. Black lines represent the nevirapine group, gray lines represent the abacavir group, solid lines represent patients with CD4 counts of 50 cells/mm<sup>3</sup>, and dashed lines represent patients with CD4 cell counts of 150 cells/mm<sup>3</sup>.

**Table 1. Resistance Mutations at 48 Weeks**

Variable	All patients, no. (%)		Excluding patients with therapy substitutions before 48 weeks, no. (%) <sup>a</sup>	
	Abacavir arm (n = 56)	Nevirapine arm (n = 29)	Abacavir arm (n = 50)	Nevirapine arm (n = 28)
Any International AIDS Society major mutation	50 (89)	24 (83)	44 (88)	23 (82)
Any TAM	30 (54)	8 (28)	25 (50)	7 (25)
M41L	4 (7)	2 (7)	4 (8)	1 (4)
D67NG	21 (38)	5 (17)	18 (36)	4 (14)
K70R	24 (43)	2 (7)	20 (40)	1 (4)
L210W	1 (2)	0 (0)	1 (2)	0 (0)
T215FY	12 (21)	4 (14)	10 (20)	3 (11)
K219QEN	8 (14)	0 (0)	7 (14)	0 (0)
Any NNRTI-associated mutation	5 (9)	21 (72)	3 (6)	21 (75)
K103N	4 (7)	7 (24)	3 (6)	7 (25)
V106AM	0 (0)	2 (7)	0 (0)	2 (7)
Y181CI	0 (0)	5 (17)	0 (0)	5 (18)
Y188CLH	1 (2)	1 (3)	0 (0)	1 (4)
G190AS	1 (2)	8 (28)	0 (0)	8 (29)
Any lamivudine- or abacavir-associated mutation	50 (89)	21 (72)	44 (88)	20 (71)
M184V <sup>b</sup>	50 (89)	21 (72)	44 (88)	20 (71)
K65R	2 (4)	0 (0)	1 (2)	0 (0)
Y115F	1 (2)	0 (0)	1 (2)	0 (0)
Permutations of mutations <sup>c</sup>				
TAMs only	0 (0)	0 (0)	0 (0)	0 (0)
M184V only	19 (38)	2 (8)	19 (44)	2 (9)
NNRTI only	0 (0)	3 (12)	0 (0)	3 (13)
TAMs and M184V	26 (52)	1 (4)	22 (51)	0 (0)
TAMs and NNRTI	0 (0)	0 (0)	0 (0)	0 (0)
M184V and NNRTI	1 (2)	11 (46)	0 (0)	11 (48)
TAMs, M184V, and NNRTI	4 (8)	7 (29)	3 (7)	7 (30)

**NOTE.**Analysis excludes 4 patients with baseline resistance. NNRTI, nonnucleoside reverse-transcriptase inhibitor; TAM, thymidine analogue mutation

<sup>a</sup> Other than stavudine substitutions for zidovudine.

<sup>b</sup> M184I was not observed.

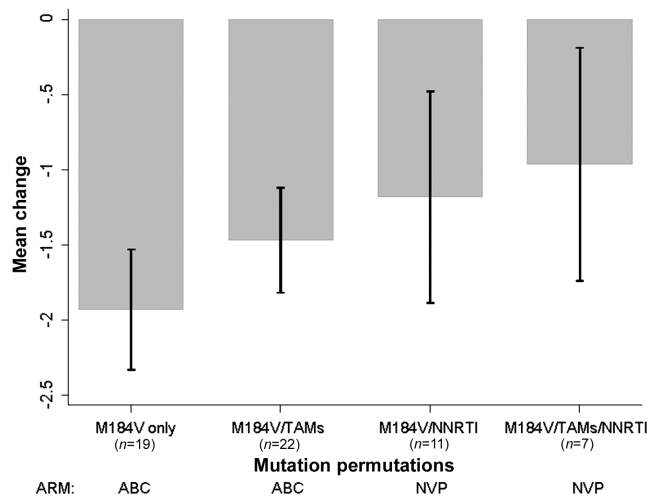
<sup>c</sup> Percentage expressed relative to number of patients with  $\geq 1$  mutation.

85%) for HIV RNA level of 150,000–500,000 copies/mL and 70% (95% CI, 61%–79%) for HIV RNA level >500,000 copies/mL. Of note, time to suppression was similar in both groups for participants with baseline HIV RNA levels <150,000 copies/mL. Virological response was less durable in the abacavir group; of 206 participants who achieved HIV RNA levels <50 copies/mL before 48 weeks, 58 (28%) rebounded to higher levels at week 48, compared with 17% (39 of 223 patients) in the nevirapine group ( $P = .005$ , by Mantel-Haenszel test stratified by time of suppression).

A multivariate logistic regression model to predict HIV RNA levels <50 copies/mL at 48 weeks revealed no association with sex ( $P = .6$ ), age ( $P = .8$ ), or WHO stage ( $P = .5$ ) at ART initiation. Conversely, baseline CD4 cell count (odds ratio, 1.49 per 50 cells/mm<sup>3</sup> higher; 95% CI, 1.26–1.76;  $P < .001$ ), baseline HIV RNA level ( $P = .003$ ), and treatment group ( $P < .001$ ) were

strongly predictive. Figure 3 shows predicted response over the range of observed baseline HIV RNA values, for baseline CD4 cell counts of 50 and 150 cells/mm<sup>3</sup> (approximate quartiles). The probability of virological suppression at week 48 was similar in the 2 treatment groups when baseline HIV RNA level was less than  $\sim 5 \log_{10}$  (100,000) copies/mL, but the abacavir group had an inferior response at higher levels ( $P = .04$ , by test for interaction). Virological suppression in the nevirapine group was largely unaffected by baseline HIV RNA level, whereas suppression decreased markedly with higher baseline level in the abacavir group. In a sensitivity analysis, similar results were seen for HIV RNA levels <1000 copies/mL (data not shown).

**Resistance findings.** Genotypic results were obtained for 88 samples (92%) from baseline and 89 samples (93%) from week 48 for the 96 participants with HIV RNA levels >1000 copies/mL at week 48. Of the 89 participants whose viral sub-



**Figure 4.** Mean change in log<sub>10</sub> human immunodeficiency virus (HIV) RNA level from baseline to week 48, by main category of mutational permutations. Error bars represent 95% confidence intervals. Thymidine analogue mutations (TAMs) and nonnucleoside reverse-transcriptase inhibitor (NNRTI)-associated mutations are as listed in Table 1. ABC, abacavir; NVP, nevirapine.

type was determined, 51 (57%) were infected with subtype A virus, 25 (28%) with subtype D, 4 (5%) with subtype C, 5 (6%) with a C/D recombinant, and 4 (5%) with an A/D recombinant. Four participants (3 in the abacavir group and 1 in the nevirapine group) had complex mutational patterns involving NRTI and NNRTI mutations in their baseline sample, probably indicative of prior undisclosed therapy; these participants were excluded from analyses of the week 48 resistance data. Observed resistance patterns are shown both including and excluding 7 participants (6 in the abacavir group and 1 in the nevirapine group) who had made substitutions to their randomized therapy before 48 weeks (Table 1); further description of findings is based on the latter analysis.

One or more International AIDS Society-USA major resistance mutations was detected in 88% of samples in the abacavir group, compared with 82% in the nevirapine group ( $P = .5$ ) (Table 1). Among such samples, the M184V mutation was present in all but 3 patients in the nevirapine group who had NNRTI resistance only. TAMs were significantly more common ( $P = .04$ ) in the abacavir group (25 [50%] of 50) than in the nevirapine group (7 [25%] of 28), with substitutions at codons 67 and 70 (TAM II pathway) being the most common. In addition, the number of TAMs per sample was significantly higher in the abacavir group than in the nevirapine group ( $P = .005$ , by test for trend): in the abacavir and nevirapine groups, respectively, 24% and 71% had 1 TAM, 44% and 29% had 2, 16% and 0% had 3, and 16% and 0% had  $\geq 4$ . NNRTI resistance was observed in viruses from three-quarters of participants in the nevirapine group and manifested as a single mutation (mainly

at codons 103, 181, or 190), except for 2 cases of dual mutations. Apart from M184V, mutations selected for by abacavir were rare, with only 1 case of K65R and 1 case of Y115F.

The residual activity of the 2 regimens, defined as the difference between HIV RNA levels at week 48 and baseline, was determined for the main categories of mutational permutations in each treatment group in patients with viral loads  $>1000$  copies/mL at week 48 (Figure 4). The mean residual activity ( $\pm$  standard error [SE]) of the abacavir-containing regimen was  $1.93 \pm 0.19$  log<sub>10</sub> copies/mL in the presence of M184V alone and  $1.47 \pm 0.17$  log<sub>10</sub> copies/mL in the presence of M184V plus TAMs. Surprisingly, there was no evidence of an effect of the number of TAMs (reduction in residual activity,  $0.04$  log<sub>10</sub> copies/mL per additional TAM; 95% CI,  $-0.27$  to  $0.35$ ;  $P = .8$ ). The mean residual activity ( $\pm$  SE) of nevirapine-containing regimens was lower:  $1.18 \pm 0.32$  log<sub>10</sub> copies/mL when the virus harbored M184V and NNRTI mutations and  $0.96 \pm 0.32$  log<sub>10</sub> copies/mL when all 3 component drugs were compromised. Because genotyping was attempted only in week 48 samples with HIV RNA levels  $>1000$  copies/mL, these estimates may be biased downward; some unsequenced samples may have harbored resistance mutations. Analyses comparing the 2 main categories of mutational permutations within each treatment group failed to identify an association with either baseline HIV RNA level or HIV RNA area under the curve between baseline and 24 weeks (data not shown), although these analyses have limited statistical power.

## DISCUSSION

Our results are broadly consistent with data from the AIDS Clinical Trials Group (ACTG) 5095 trial that demonstrated inferior virological response to zidovudine-lamivudine plus abacavir, compared with a zidovudine-lamivudine plus NNRTI regimen during a median follow-up of 32 weeks, although the NNRTI studied in this trial was efavirenz rather than nevirapine [7]. A surprising finding in our study was the similar initial response during the first 12 weeks in the 2 groups; we interpret these data as suggesting equivalent intrinsic antiviral activity (as opposed to longer-term durability), contradicting the frequently held view that triple-NRTI regimens are less potent than protease inhibitor- or NNRTI-containing regimens [14]. The virological difference between the 2 groups that emerged at later time points was attributable to a combination of more late suppression, mainly at 12–24 weeks, and lower rates of viral rebound among participants who were prescribed nevirapine. The reasons for this are unclear, but one hypothesis is that by 12 weeks, in some patients, resistance had already developed to  $\geq 1$  drug in the zidovudine-lamivudine plus abacavir regimen. As an aside, we note that the virological failure rates reported for the ACTG 5095 study may be artificially high, because treatment failure was defined as HIV RNA level  $\geq 200$

copies/mL at 16 weeks [7], whereas our study indicated that viral suppression is often first achieved later than this.

One important difference between the NORA and ACTG 5095 trials was the effect of baseline viral load on subsequent virological response. In the ACTG 5095 study, a suboptimal response with zidovudine-lamivudine plus abacavir was observed at all values of baseline viral load; in contrast, in the NORA study, a virological difference between the regimens occurred only in participants with a baseline viral load >150,000 copies/mL. The results of the NORA study are similar to findings from an earlier trial that compared zidovudine-lamivudine plus abacavir with zidovudine-lamivudine plus indinavir [15]. Finally, the low mean baseline CD4 cell count in the NORA study (median, 99 cells/mm<sup>3</sup>), combined with evidence of a strong association between this parameter and virological response (for both drug combinations), implies that we may have underestimated the virological response that could be achieved with these regimens in less severely immunocompromised clinical populations.

The most common resistance pattern was G190A/S or K103N with M184V in patients with nevirapine failure and M184V with or without TAMs in those with abacavir failure. The extent of resistance in both groups was higher than observed in studies from resource-rich settings, presumably because treatment was not changed at the time of rebound [16, 17]. This allowed us to explore the relationship between the emergence of resistance and viral rebound in more detail. Of interest, patients in whom triple-nucleoside therapy failed had more TAMs than those who experienced nevirapine failure, despite a lower mean viral load at week 48 (among patients who experienced treatment failure and had viral loads >1000 copies/mL). It is difficult to interpret this finding without also considering mutations other than TAMs; therefore, we analyzed the residual activity (ie, viral load relative to baseline value) of the drug regimens according to the main mutational patterns. The most common set of mutations in the zidovudine-lamivudine plus nevirapine group, namely, NNRTI mutation(s) with M184V, was associated with less residual activity of the drug regimen than the common patterns emerging with abacavir, namely, M184V alone or with TAM(s). This may have been a consequence of a lack of NNRTI activity in the context of key NNRTI mutations, as has been demonstrated elsewhere [18]. We did not demonstrate a reduction in residual activity with increasing number of TAMs, although there were relatively few patients with ≥4 mutations, a threshold thought to confer clinical resistance to abacavir [19]. Indeed, the limited number of TAMs, which were generally within the TAM 2 pathway ( 67, 70 215E, 219) implies that susceptibility to second-line nucleoside or nucleotide analogues may be retained. Nevertheless, we recognize that continual virological failure beyond 48 weeks will lead to further acquisition of RT mutations, thus further compromising a sec-

ond-line regimen including nucleoside or nucleotide analogues. A full phenotypic analysis is being undertaken.

It is important to recognize that our study extended only to 48 weeks and that no participant met the clinical or immunological criteria for treatment failure in the DART trial [5]. The continual evolution of resistance during therapy, including emergence of compensatory mutations, may either reduce or widen the differences observed between drug groups over time. Longer-term follow-up data from the NORA study will shed light on this issue, although the fact that some participants were also randomized to structured treatment interruptions after week 48 will complicate the interpretation of this analysis [20]. The crucial clinical question relates to the response to second-line therapy. On the basis of the 48-week data, it is reasonable to assume that first-line zidovudine-lamivudine plus abacavir therapy will eventually lead to extensive nucleoside analogue resistance, implying that a second-line regimen comprising a boosted protease inhibitor and an NNRTI may represent the most active combination. Further research is required to optimize first- and second-line therapies in resource-limited settings.

## DEVELOPMENT OF ANTIRETROVIRAL TREATMENT IN AFRICA (DART) VIROLOGY GROUP AND TRIAL TEAM

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