RESEARCH ARTICLE

Early assessment of antiretroviral efficacy is critical to prevent the emergence of resistance mutations in HIV-tuberculosis coinfected patients: a substudy of the CARINEMO-ANRS12146 trial [version 1; peer review: 1 approved, 1 approved with reservations]

Elisabeth Baudin[1], Nilesh Bhatt[2], Christine Rouzioux[3], Micaela Serafini[4], Lucas Molfino[5], Ilesh Jani[2], Anne-Marie Taburet[6], Maryline Bonnet[1,7], Alexandra Calmy[8], CARINEMO Study Group

[1]Epicentre, Paris, France
[2]Instituto Nacional de Saúde (INS), Ministry of Health, Maputo, Mozambique
[3]Paris-Descartes University, EA3620, Sorbonne Paris Cite, APHP, Necker Hospital, Paris, France
[4]Médecins Sans Frontières, Geneva, Switzerland
[5]Médecins Sans Frontières, Maputo, Mozambique
[6]Assistance Publique Hôpitaux de Paris, Bicêtre Hospital, and UMR1184 Inserm, CEA, Université Paris-Sud, Paris, France
[7]IRD UMI 233 TransVIHMI - UM – INSERM U1175, Université de Montpellier, Montpellier, France
[8]Geneva University Hospitals, Geneva, Switzerland

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Abstract

Background: In the CARINEMO ANRS 12146 clinical trial, HIV-tuberculosis co-infected patients in Mozambique were randomized to nevirapine (NVP) or to efavirenz (EFV)-based antiretroviral therapy to compare these two non-nucleoside reverse transcriptase inhibitors (NNRTIs) in treatment naïve patients.
Methods: In this sub study, we explored the relationship of NNRTI concentrations with virological escape and the possible emergence of resistance mutations at week 48. The virological escape was defined as an HIV-RNA above 400 copies/m at week 48.
Results: Among the 570 randomized patients, 470 (82%) had an HIV-RNA result at week 48; 54 (12.1%) patients had a viral escape and 35 patients had at least one major resistance mutation detected. Low drug concentration at weeks 12 and 24 (below the 10th percentile) were independently associated with virologic escape at week 48 (adjusted odds ratio [aOR]=2.9; 95% CI: 1.1 -7.2; p=0.0312 and aOR=4.2; 95% CI: 1.8-9.8; p=0.0019, respectively), and independently associated with an increased risk of emergence of resistance mutation

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1. **Conrad Muzoora**, Mbarara University of Science and Technology, Mbarara, Uganda

2. **Gary Maartens**, University of Cape Town, Cape Town, South Africa

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(aOR=4.5; 95% CI: 1.8-14.6; p=0.009 at week 12; aOR=5.1; 95% CI: 1.8-14.6 at week 24). Receiver operating characteristic curves analyses indicated a better predictability of the mid-dose concentration and of the HIV-1 RNA values on resistance mutations in contrast to virological escape.
Conclusions: Very low drug plasma concentrations early after treatment initiation (week 12) were predictive factors of virological escape and the emergence of resistance mutations at week 48, and early monitoring of drug intake may prevent the occurrence of late virological escape and the selection of vial resistance mutations.

Keywords
HIV/TB coinfection, NNRTI concentrations, drug–drug interactions, antiretroviral therapy, resistance, virological escape

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Corresponding author: Elisabeth Baudin (elisabeth.baudin@epicentre.msf.org)

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Introduction

Antiretroviral therapy (ART) aims to sustain virological suppression, which is associated with a clinical benefit and immune recovery. It also prevents HIV transmission and limits the emergence of antiretroviral (ARV) drug resistance. In a recent meta-analysis, Gupta *et al.* reported that East Africa had the highest estimated rate of drug-resistance mutations (29% per year) since the roll-out of ART, with an estimated prevalence of ARV drug resistance of 7.4% at 8 years after rollout[1].

In 2016, 80% of the worldwide prescription of ART-included efavirenz (EFV), a non-nucleoside reverse-transcriptase inhibitor (NNRTI)-class drug[2]. Efavirenz-based ART is also recommended in the context of tuberculosis (TB) coinfection, as drug-drug interactions with rifampicin, a cornerstone anti-TB drug, are limited. However, the risk of central nervous toxicity with EFV may lead to altered adherence to ARTs. Thus, it is important to identify early markers predicting the emergence of new resistance mutations in patients on NNRTI-based ART.

The phase 3 CARINEMO randomized clinical trial enrolled 570 HIV-TB coinfected patients in Mozambique, Africa, and compared the efficacy and safety of two NNRTIs (nevirapine [NVP] and EFV) for ART-naïve patients[3]. In the intent-to-treat population, 64.6% (95% confidence interval (CI): 58.7-70.1%) of patients who received NVP achieved virological suppression at week 48 (defined as HIV-1 RNA <50 copies per ml), compared with 69.8% (95% CI: 64.1-75.1%) of those who received EFV. The evolution of plasma concentrations of NVP and EFV during and after anti-TB therapy, as well as its association with toxicity and virological suppression, has been previously described[4]. The emergence of ARV-resistance mutations was observed during the trial and briefly described, but the relationship between NNRTI plasma concentrations and the emergence of resistance was not investigated. Here, we analyzed subgroup datasets from the CARINEMO trial, which provided a unique opportunity to explore the factors associated with viral replication and the emergence of resistance mutations while on ART. These data also offered the possibility to assess the relationship between viral replication, ARV plasma concentrations and the emergence of resistance mutations. The identification of risk factors of virological escape at week 48 in a well-characterized and homogeneous population is critical to prevent treatment failure in settings where the best timing for routine HIV-RNA still needs to be assessed.

Methods

Trial background

The CARINEMO trial (ClinicalTrials.gov identifier: NCT00495326) was conducted in three health centers located in Maputo, Mozambique, from 2007 to 2011; a full description of the trial is available from Bonnet *et al.*[3]. Participants were randomized to NVP or EFV (without lead-in dose) and received either a fixed-dose combination of NVP (400 mg/day), lamivudine and stavudine (Triomune®) or EFV (600 mg/day) plus lamivudine and stavudine started 4 weeks after anti-TB treatment initiation and for a duration of 48 weeks. In August 2010, stavudine was replaced by zidovudine. For TB, all patients received a fixed-dose combination of isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) for 2 months, followed by 4 months of isoniazid/rifampin.

Four ethics committees approved the study protocol: the Comite Nacional de Bio-Etica para a Saude (Maputo, Mozambique), the Medecins Sans Frontieres Ethics Review Board (Zurich, Switzerland), the Comite de Protection des Personnes (Saint Germain-en-Laye, France), and the Columbia University ethics review committee (New York, NY, USA). All participants provided signed informed consent.

HIV-RNA level measurements and resistance mutations

Plasma HIV-RNA levels were measured at inclusion and then at weeks 12, 24, 36 and 48 using the Roche Cobas Amplicor HIV-1 Monitor Test v1.5 (Roche Diagnostics, Basel, Switzerland) at the molecular biology laboratory of the Instituto Nacional de Saúde, Maputo, Mozambique. Resistance mutations to NRTI and NNRTI were determined in all patients with plasma HIV-1 RNA >400 copies/ml at week 48 by sequencing the reverse transcriptase gene using the consensus technique of the AC11 ANRS Resistance Group (www.hivfrenchresistance.org) at the Department of Virology, Necker Hospital (Paris, France). A patient was defined as having an emergence of resistance mutations at week 48 if at least one (N)NRTI resistance mutation was detected at any level.

Adherence

Adherence counseling on both ART and anti-TB therapies was provided by the study team at the clinics. At each follow-up visit, adherence to both ART and anti-TB treatment was monitored using an analog visual scale, standardized questionnaire administered by a nurse and pill counts. Adherence to ART was calculated for each time point using pills counts only. The number of returned doses during the last 3 months prior to weeks 12, 24, 36 and 48 were compared to the number of doses prescribed and refills. An indicator of compliance was defined by classifying adherence with a threshold of 95%.

Drug concentrations

Pre-dose concentrations of NVP and 12 h after the evening intake of EFV were measured at weeks 12, 24, 36 and 48. Patients for whom the measured concentrations were below the limit of quantification at each measurement were removed from the analysis, assuming the ART was not taken at all.

Statistical analysis

Virological suppression was defined as an HIV-RNA below 400 copies/ml at week 48 and virological escape as an HIV-RNA above 400 copies/ml. Patients switched during follow-up to another ART regimen were excluded from the analysis. Percentiles (P) of drug concentrations were provided for each NNRTI at each time point. The P10, P25, P50, P75 and P95 were calculated and used to categorize drug concentrations. The P10 value was used to classify patients as having low drug concentrations (threshold below which 10% of drug concentrations were measured). Mean changes in HIV-1 RNA values after log transformation at each time point vs. baseline values were com-

pared between patients with and without the emergence of resistance mutations by performing an analysis of covariance at each time point with HIV-1 RNA baseline values (log transformed) and treatment as covariates. Univariate and multivariate logistic regression models were fitted to assess the associations between virological escape and the emergence of resistance mutations at week 48 with drug concentrations at weeks 12 and 24, adherence to ART and other patient-associated factors, such as body mass index, sex, age, CD4 cell counts, as well as the HIV-1 RNA and ART regimen at treatment initiation. For both outcomes, factors associated with a *P*-value <0.20 in univariate analysis were selected for the initial multivariate analysis and a manual backward stepwise approach was used to obtain the final multivariate model. Only factors significantly associated ($P<0.05$) with the outcomes remained in the model and the importance of each in the final model was tested with a likelihood ratio test at the same level of significance (5%). The area under the receiver operating characteristics (ROC) curve was computed to assess the prediction of the low drug concentration on the risk of virological escape and the emergence of resistance mutation. The same analysis was repeated to evaluate the prediction of the HIV 1 RNA at weeks 12 and 24 on the risk of virological escape at week 48 and the emergence of resistance mutation. Other statistical comparisons were performed using the Chi-square test, Fisher's exact test or Student's t-test as appropriate. A *P*-value of ≤0.05 was considered statistically significant. Tests were performed with Stata 14 (StataCorp LP, College Station, TX).

Results

Patient characteristics

Of the 570 patients randomized in the CARINEMO trial, 470 had available measurement of HIV-RNA at week 48. Among these, 446 had at least one measure of detectable drug plasma concentrations without being switched during follow-up to another ART regimen (Figure 1). Demographic data and clinical characteristics at baseline and during the 48-week follow-up are summarized in Table 1. De-identified raw data for each patient is available on figshare[5].

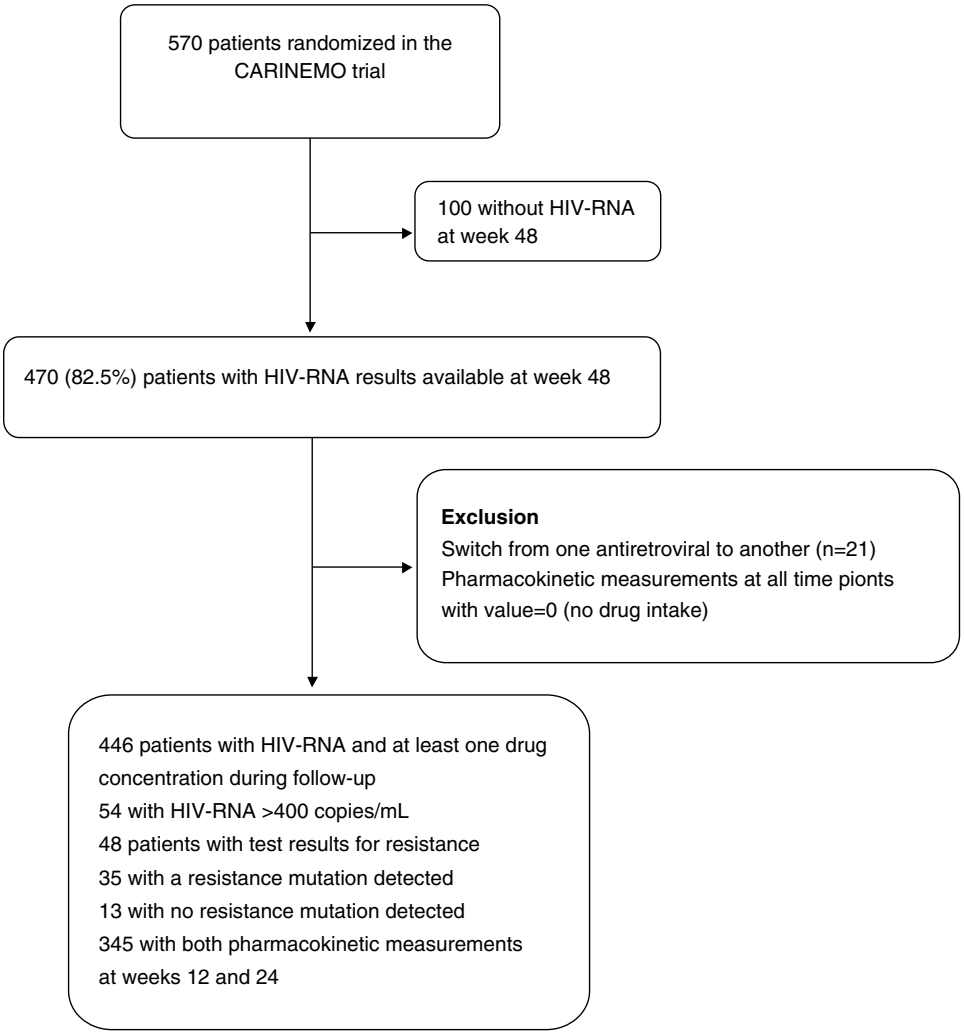

Figure 1. Study flow chart.

ART Adherence

Among the 446 patients an adherence rate less than 95% was observed among 7 (1.6%) patients from enrolment up to week 12, in 11 (2.5%) patients from weeks 12 up to 24, in 11 (2.5%) patients between weeks 24 and 36, and 8 (1.8%) patients between weeks 36 and 48.

Table 1. Patient characteristics at study inclusion.

Characteristics	Patients (N=446)
Female, n (%)	198 (44.4)
Age (years), median [IQR]	34 [29, 41]
Weight (kg), median [IQR]	52.1 [47.0, 58.0]
Body mass index, kg/m^2, median [IQR]	18.9 [17.4, 20.4]
CD4+ cell count (cells/mm^3), median [IQR]	96 [48, 147]
HIV-1 RNA (log), median [IQR]	5.5 [5.1, 6.0]
Efavirenz-based regimen, n (%)	221 (49.6)
Nevirapine-based regimen, n (%)	225 (50.4)

HIV-1 RNA during the study time points

Among the 446 patients, 54 (12.1%) presented a virological escape; 48 patients (10.8%) had a genotype performed and 35 (7.8%) had at least one major resistance mutation detected on the reverse transcriptase gene. The decrease in HIV-1 RNA levels from baseline was significantly slower in patients in whom resistance mutations were identified at week 48 compared with those with no occurrence of resistance (Table 3).

Drug concentrations during the study time points

Percentile values for NVP and EFV drug concentrations at each time points are presented in Table 2. Values of P10, P25 and P50 at week 12 were 1253 ng/ml, 1784 ng/ml and 2786 ng/ml, respectively, for EFV, and 1893 ng/ml, 2996 ng/ml and 4095 ng/ml, respectively, for NVP. The distribution of drug concentrations using these percentile categories differed statistically between patients with virological suppression and those with virological

Table 2. Values of the percentiles of the drug concentrations in ng/ml for nevirapine (NVP) and efavirenz (EFV) at weeks 12, 24, 36 and 48.

Percentile	EFV week 12	EFV week 24	EFV week 36	EFV week 48	NVP week 12	NVP week 24	NVP week 36	NVP week 48
P10	1253	1134	1399	1112	1893	2496	3080	2903
P25	1784	1561	1869	1743	2996	3845	4334	4509
P50	2786	2542	2655	2450	4095	5269	6016	6095
P75	6965	5423	4223	4098	5522	7091	7892	8534
P95	19604	19977	12545	12760	9122	13185	13985	14215

Table 3. Changes from baseline in log-transformed HIV-RNA at week 12 and 24 for patients with/without the emergence of resistance mutation. Data given as mean (standard deviation).

Time point	Patients without emergence of resistance	Patients with emergence of resistance	*P* value
Baseline			
Log_{10} HIV-1 RNA at baseline, mean	5.55 (0.70)	5.62 (0.73)	NS
Week 12			
Log_{10} HIV-1 RNA	2.07 (0.64)	2.72 (1.36)	
Change from baseline in Log_{10} HIV-1 RNA	-3.48 (0.81)	-2.86 (1.27)	<0.001
Week 24			
Log_{10} HIV-1 RNA	1.81 (0.46)	2.95 (1.24)	
Change from baseline in Log_{10} HIV-1 RNA	-3.73 (0.77)	-2.67 (1.39)	<0.001
Week 36			
Log_{10} HIV-1 RNA	1.79 (0.43)	3.56 (1.09)	
Change from baseline in Log_{10} HIV-1 RNA	-3.77 (0.79)	-2.08 (1.19)	<0.001
Week 48			
Log_{10} HIV-1 RNA	1.76 (0.35)	4.10 (0.89)	
Change from baseline in Log_{10} HIV-1 RNA	-3.79 (0.75)	-1.53 (1.00)	<0.001

NS, not significant.

escape at week 48. At week 12, 28.2% (11/39) of patients with a plasma concentration of the NNRTI-component within the P10 failed to suppress their viral load at week 48 compared with 10.1% (35/348) in those with higher concentrations (p=0.001) similar to week 24 (35% [14/40] vs. 9.2% [32/348], respectively; p<0.001). Among these patients, median concentrations were lower in those with virological escape compared to cases with virological suppression at week 12 for the NVP group and in both the EFV and NVP groups at week 24 (Figure 2, p=NS). The same differences were observed in the distribution of drug concentrations between patients with or without the emergence of resistance mutations. At week 12, 21.2% (8/37) of patients presenting plasma drug concentrations of the NNRTI component within the P10 had resistance mutations at week 48, compared with 5.8% (20/344) in those with higher concentrations (p<0.001), similar to week 24 (26.3% [10/38] vs. 5.2% [18/345], respectively).

Factors associated with virological escape and the emergence of resistance mutation

Multivariate analyses showed that plasma drug concentrations below the P10 threshold at weeks 12 and 24 were independently associated with virological escape at week 48 (adjusted odds ratio [aOR]=2.9; 95% CI: 1.1 -7.2; p=0.0312 and aOR=4.2; 95% CI: 1.8-9.8; p=0.0019, respectively), as well as adherence below 95% at week 24 (aOR=10.5; 95% CI: 1.2-89.8; p=0.044, respectively) (Table 4). There was no influence of the choice of the NNRTI component or the CD4 cell count at baseline on factors associated with virological escape at week 48. Drug concentrations below the P10 threshold at weeks 12 and 24

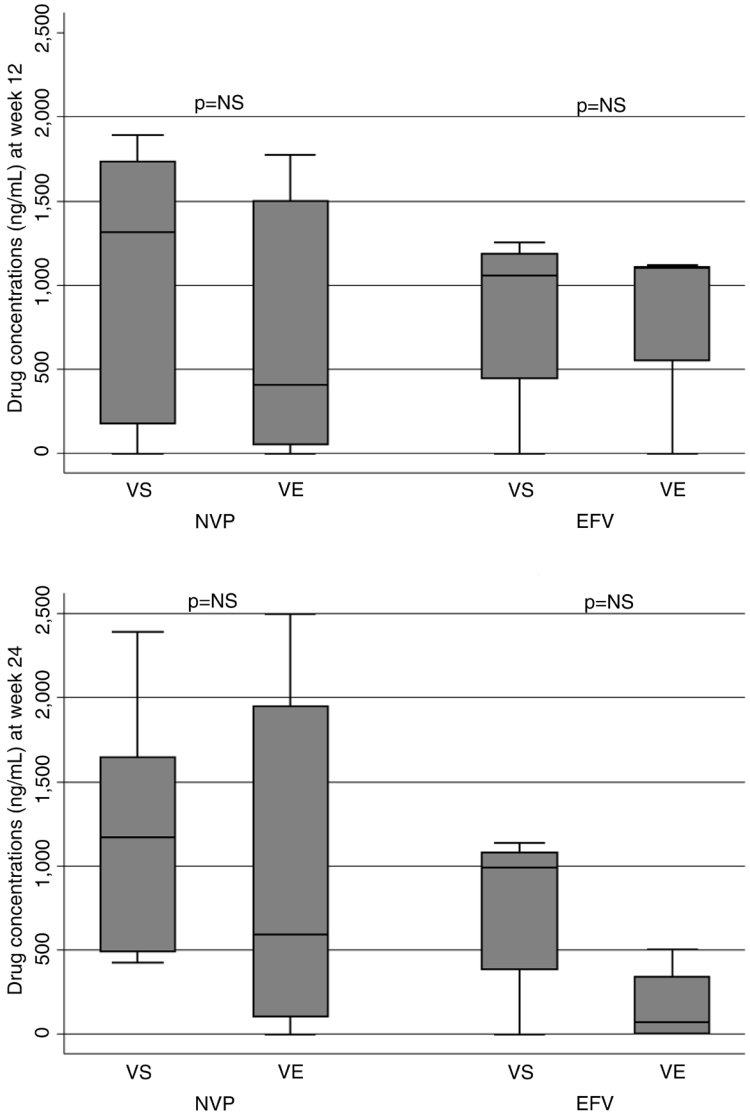

Figure 2. Distribution of the drug concentration (ng/ml) by treatment group (NVP or EFV) for patients with drug concentrations within P10 at weeks 12 and 24 and with either virological suppression (VS) or virological escape (VE).

Table 4. Factors associated with virological escape at week 48 (univariate and multivariate analyses).

Variable	Patients with virological escape, n (%)	Unadjusted odds ratio (95% CI)	*P*-value	Adjusted odds ratio (95% CI)	*P*-value
Sex					
Female	17 (8.6)	1	0.044	1	0.4372
Male	37 (14.9)	1.9 (1.0-3.4)		1.23 (0.53-2.85)	
Age at baseline					
≥34 years	25 (11.1)	1	0.516	1	0.6301
<34 years	29 (13.1)	1.2 (0.7-2.1)		1.2 (0.6-2.5)	
Body mass index at baseline					
≥19 kg/m^2	21 (9.6)	1	0.108	-	-
<19 kg/m^2	33 (14.6)	1.6 (0.9-2.9)		-	
CD4 at baseline					
≤100 cells/mm^3	23 (9.9)	1	0.144	1	0.7415
>100 cells/mm^3	30 (14.4)	1.5 (0.9-2.7)		1.1 (0.5-2.3)	
HIV-1 RNA at baseline					
≤5.5 $\log_{10}$ copies/ml	20 (10.0)	1	0.208	1	0.4172
>5.5 $\log_{10}$ copies/ml	34 (13.9)	1.5 (0.8-2.6)		1.4 (0.6-2.9)	
Antiretroviral treatment					
Efavirenz	18 (8.1)	1	0.012	1	0.1251
Nevirapine	36 (16.0)	2.1 (1.2-3.9)		1.8 (0.8-3.7)	
Adherence to ART at week 12					
≥95%	53 (12.1)	1	0.863	-	-
<95%	1 (14.3)	1.2 (0.1-10.2)		-	
Adherence to ART at week 24					
≥95%	49 (11.3)	1	0.003	1	0.044
<95%	4 (57.1)	10.5 (2.3-48.2)		10.5 (1.2-89.8)	
Drug concentration at week 12					
≥10^{th} percentile	35 (10.1)	1	0.002	1	0.0312
<10^{th} percentile	11 (28.2)	3.5 (1.6-7.7)		2.9 (1.1-7.2)	
Drug concentration at week 24					
≥10^{th} percentile	32 (9.2)	1	0.000	1	0.0019
<10th percentile	14 (35.0)	5.3 (2.5-11.2)		4.2 (1.8-9.8)	

(aOR=4.5; 95% CI: 1.8-14.6; p=0.009 at week 12; aOR=5.1; 95% CI: 1.8-14.6 at week 24) were also independently associated with an increased risk of emergence of resistance mutation as well as the ARV treatment received at initiation (aOR=3.2; 95% CI: 1.1-9.1; p=0.0244), for NVP vs. EFV. Adherence below 95% at week 24 was no longer shown to be associated at the significance level of 5% (p=0.0581) (Table 5).

ROC curve

Among the 345 patients with both mid-dose concentrations at weeks 12 and 24, the ROC analysis showed an area under the curve (AUC) at week 12 of 0.62 (95% CI: 0.52-0.72) and 0.67 (95% CI: 0.65-0.82) at week 24 for virological escape. An AUC of 0.76 (95% CI: 0.66 -0.87) and 0.75 (95% CI: 0.63-0.86) at weeks 12 and 24, respectively, was observed for the emergence of

Table 5. Factors associated with the emergence of resistance mutations at week 48 (univariate and multivariate analyses).

Variable	Patients with emergence of resistance, n (%)	Unadjusted odds ratio (95% CI)	*P*-value	Adjusted odds ratio (95% CI)	*P*-value
Sex					
Female	11 (5.6)	1	0.097	1	0.5712
Male	24 (9.9)	1.9 (0.9-3.9)		1.3 (0.5-3.6)	
Age at baseline					
≥34 years	17 (7.6)	1	0.795	1	0.7556
<34 years	18 (8.3)	1.1 (0.5-2.2)		1.2 (0.4-3.0)	
Body mass index at baseline					
≥19 kg/m^2	16 (7.4)	1	0.647	-	
<19 kg/m^2	19 (8.6)	1.2 (0.6-2.4)		-	
CD4 at baseline					
≤100 cells/mm^3	13 (5.6)	1	0.074	1	0.4910
>100 cells/mm^3	21 (10.3)	1.9 (0.9-3.9)		1.4 (0.5-3.7)	
HIV-1 RNA at baseline					
≤5.5 $\log_{10}$ copies/ml	16 (8.0)	1	0.997	1	0.5409
>5.5 $\log_{10}$ copies/ml	19 (8.0)	1.0 (0.5-2.0)		0.7 (0.3-1.9)	
Antiretroviral treatment					
Efavirenz	9 (4.2)	1	0.005	1	0.0244
Nevirapine	26 (11.7)	3.0 (1.4-6.7)		3.2 (1.1-9.1)	
Adherence to antiretroviral treatment at week 12					
≥95%	34 (7.9)	1	0.543	-	
<95%	1 (14.3)	1.9 (0.2-16.6)		-	
Adherence to antiretroviral treatment at week 24					
≥95%	32 (7.4)	1	0.003	1	0.0581
<95%	3 (50.0)	12.4 (2.4-64.1)		20.9 (1.6-280.0)	
Drug concentration at week 12					
≥10th percentile	20 (5.8)	1	0.001	1	0.0090
<10th percentile	8 (21.6)	4.5 (1.8-11.0)		4.5 (1.8-14.6)	
Drug concentration at week 24					
≥10th percentile	18 (5.2)	1	0.000	1	0.0034
<10th percentile	10 (26.3)	6.5 (2.7-15.4)		5.1 (1.8-14.6)	

resistance mutations, thus indicating a better predictability of the mid-dose concentration on resistance mutations in contrast to virological escape. When using the HIV-1 RNA values at weeks 12 and 24 to predict the two outcomes, the ROC analysis showed AUCs of 0.69 (95% CI: 0.60-0.77) and 0.66 (95% CI: 0.55-0.76), respectively, for virological escape and 0.72 (95% CI: 0.63-0.80) and 0.75 (95% CI: 0.65-0.86), respectively, for the emergence of resistance mutations. These results indicate a better predictability of the HIV-1 RNA values on resistance mutations in contrast to virological escape.

Discussion

In the present study, we used the data of a large randomized clinical trial assessing two drugs of the NNRTI class in combination with anti-TB drugs. Our findings showed that very low drug plasma concentrations early after treatment initiation (week 12) were

predictive factors of virological escape and the emergence of resistance mutations at week 48. Low drug concentrations may be explained by a suboptimal adherence or a potent drug interaction when patients receive other drugs such as rifampicin. Recently, it was suggested that the wave of ART treatment failure primarily affecting resource-limited countries should be considered as a fourth epidemic[6]. This epidemic, accompanied by the emergence of ARV drug resistance, could affect 3 to 5 million individuals between 2020 and 2030[7]. Therefore, early predictors of ART failure are critically important. Until low-cost, simple assays for drug monitoring are available, pharmacological drug monitoring cannot be routinely recommended in low-resource settings to trigger drug resistance testing[8,9]. For this reason, we advocate for the development of easy-to-use point-of-care tests for anti-HIV drugs to help monitoring for adequate drug intake and therefore drug exposure during clinic visits. This would allow reducing unnecessary viral load measurements and viral genotype determination and could prevent unnecessary switches to costly and complex salvage ART in contexts where the preservation of future treatment lines is critical.

Adherence is a complex non-steady phenomenon and there is no gold standard or universal tool at present to detect irregular adherence[10–14]. This is particularly true during the first months of treatment initiation in a given population for a given ART treatment, taking into account forgiveness of the combined three ARV drugs[15]. Our study confirms that an adherence rate below 95% is independently associated with an increased risk of virological escape and the emergence of drug resistance[16]. In the absence of an adequate tool, surveillance of plasma concentrations with a simple assay in a subset of randomly selected patients could be a strategy to monitor a given cohort of patients starting ART. Viral load testing and adequate adherence support from the very first weeks of treatment should also be implemented in these settings[17]. The World Health Organization recommends performing the first viral load testing after ARV initiation at 24 weeks. However, some field reports have observed an improvement in long-term virological suppression in patients undergoing 12-week viral load testing[18]. Newly-developed, point-of-care test assays will benefit low-resource settings and help to expand such viral load measurements monitoring[17,19,20].

Our results demonstrated that the early detection of low drug plasma levels of the NNRTI component of the treatment regimen was able to discriminate patients who will later develop a resistance mutation. We showed that low to very low drug concentrations (below P10) in the first months after starting ART were significantly associated with the emergence of later virological escape and drug-resistance mutations. We were surprised by the EFV concentration levels in our study, which triggered a signal for viral escape. Indeed, the P10 at week 12 was 1253 ng/ml for the EFV component, whereas concentrations below 1000 ng/ml were sufficient in earlier studies[21] to predict treatment failure. Furthermore, the ENCORE1 study showed the efficacy of the 400 mg EFV daily dose, suggesting also that the efficacy cut-off might be lower than 1000 ng/ml[22]. We hypothesized that the high frequency of CYP2B6 genetic polymorphism in individuals of African descent may explain a population concentration distribution above that observed in Caucasian patients by Marzolini *et al.*[21].

This study has some limitations. First, in the CARINEMO clinical trial, data were collected at fixed time points and HIV-1 RNA and plasma drug concentrations started to be measured for all patients at week 12. This limited the assessment of earlier effects on virological escape and the emergence of resistance mutations in the very first weeks of treatment initiation. Second, included patients may not be representative of larger coinfected TB/HIV populations. In particular, these patients were closely followed and received support to sustain adherence to the ART and TB drugs. However, the use of pill counts only to calculate the compliance rate may have overestimated adherence. This was shown earlier in other reports[16,23] as observed by the proportion of patients with adherence below 95% and low drug concentrations, even though other factors such as drug genetic polymorphism may have influenced the drug concentrations. Third, the nucleoside analog (NRTI) backbone used in this study is no longer recommended (d4T/lamivudine or zidovudine/lamivudine) and the current use of a backbone such as tenofovir disoproxil fumarate or tenofovir alafenamide, with a longer intracellular half-life, may have changed these results. Although NVP is no longer a preferred first-line therapy and many countries have now transitioned to a dolutegravir-based regimen, we believe that our results remain relevant. Dolutegravir has a shorter half-life than NVP and EFV, and assessing early drug exposure is likely to be extremely critical. In addition, when combined with anti-TB drugs, the dose of dolutegravir needs to be doubled, which supports the use of EFV-based ARV in coinfected TB patients. Fourth, the co-administration of anti-TB drugs with ART may have altered drug concentrations[24] as rifampicin is a known potent inducer of NNRTI metabolism, in particular for NVP-based ART[25]. However, although no treatment effect was shown in our findings on virological escape, we observed a significant treatment effect in the multivariate analyses on resistance mutations, similar to previous trials[26]. ARV concentrations measured 12 h post-dose were previously used to predict virological and resistance outcomes, and were significantly associated with both outcomes at week 48[14,27,28], despite the high inter-individual variability. Finally, the analyses were performed *post hoc* and were not discussed at the time of the initial statistical analysis plan.

In summary, early monitoring of drug intake may prevent the occurrence of late virological escape and the selection of viral resistance mutations. Adherence measurement using solely pill counts does not allow for such a prediction. Indeed, higher concentrations of NNRTI were associated with better virological outcomes. In low-resource settings, implementing routine 12-week HIV-1 viral load and innovative adherence measurements might ensure long-term treatment success and reduce the possibility of the emergence of drug resistance mutations.

Data availability

Raw data associated with this study, including basic demographic information and data on viral load, are available on figshare. DOI: https://doi.org/10.6084/m9.figshare.7655630.v1[5].

Grant information

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The funders had no role in experimental design, data analysis and interpretation, or the decision to submit the work for publication.

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CARINEMO - ANRS 12146 clinical trial study group

Ilesh V. Jani MD PhD, Nádia Sitoe Bsc, Adolfo Vubil Bsc MSc, Maria Nhadzombo, Fernando Sitoe, Delário Nhumaio, Odete Bule (Instituto Nacional de Saúde, Mozambique); Rui Bastos MD and Elizabete Nunes MD (Hospital Central, Maputo, Mozambique); Paula Samo Gudo MD MPH (National Tuberculosis Control Program, Mozambique); Josué Lima MD and Mie Okamura (International Center for AIDS Care and Treatment Programs, Mozambique); Laura Ciaffi MD, Agnès Sobry MD, Mariano Lugli and Bruno Lab (Médecins Sans Frontières - Switzerland, Mozambique); Avertino Barreto MD (Mozambique National AIDS Service Organisation, Mozambique); Christophe Michon MD (Regional Hospital, Annecy, France); Alexandra Calmy MD PhD (Médecins Sans Frontières; Division of Infectious Diseases, Geneva University Hospital, Geneva, Switzerland); Alpha Diallo (ANRS pharmacovigilance unit, France); Christine Rouzioux PharmD PhD (Paris-Descartes University, EA3620, Sorbonne Paris Cite, APHP, Necker Hospital, Paris, France).

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Reviewer Report 23 April 2019

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?

Gary Maartens
Division of Clinical Pharmacology, University of Cape Town, Cape Town, South Africa

This is a post hoc study from the CARINEMO trial, which shows the relationship between NNRTI concentrations & virologic failure & resistance, which is an important addition to the literature showing the value of ARV concentrations as objective adherence measures. In general, the article is well written.

Specific comments:

1. Title: The title doesn't reflect what was actually done - I suggest something like: "Efavirenz and nevirapine concentrations as predictors of virologic failure & resistance: a substudy..."

2. Abstract, results: in last sentence "mid-dose concentration" is used - this is true for EFV, but not NVP (it's a trough) - rather use C_{12} throughout. Mid-dose concentration is also incorrectly used elsewhere in the paper. Clarify that in ROC analyses VL was done at week 12 & 24.

3. Introduction: 2nd paragraph, 1st sentence: delete hyphen in "ART-included"

4. Methods:
- It's clear from the CONSORT diagram that participants with undetectable NNRTI concentrations at all time points were excluded (incidentally the number is missing from the CONSORT diagram, please correct) - this exclusion criterion should be in the methods (I would personally not have excluded them, but no need to re-analyse).
- Clarify in adherence section that pill counts were used as the measure in analyses, not the other two measures.
- I object to the categorisation of adherence using the threshold of 95% as this is not evidence-based - an early study (Ann Intern Med. 2000;133:21-30[1]) of mostly unboosted PIs suggested this threshold, but that was a small study (n=99) of ARVs that are no longer relevant - despite this the threshold remains inappropriately used by many researchers. Numerous studies (e.g. Ann Intern Med. 2007;146:564-573[2]) have shown a relatively smooth dose response relationship between NNRTI adherence & virologic outcomes. I suggest

change the analysis plan based on the distribution of their pill count data (e.g. lowest quartile/tertile or the above/below median).
- The use of the term "virologic escape" is eccentric - this term is usually used to reflect detectable virus in a compartment (typically the CNS) despite undetectable plasma VL. I suggest use either virologic failure or categorise as suppressed/unsuppressed.
- It's unclear if undetectable NNRTI concentrations were included in the P10 group (they should have been). Also, please state how undetectable NNRTI concentrations were handled in the ROC analyses (usual to take mid-point between zero & the limit of quantification of the assay).

5. Results:
- It's unacceptable to use P = NS; the calculated value should be given.
- I found the statement below surprising given the findings of the main trial: "There was no influence of the choice of the NNRTI component or the CD4 cell count at baseline on factors associated with virological escape at week 48." The aOR was 1.8 for virologic failure in those randomised to NVP - although 95% CIs did cross 1 & P was >0.05; this does not mean there is no effect - the statement should be modified.

6. Discussion: Their statement "Our study confirms that an adherence rate below 95% is independently associated with an increased risk of virologic escape..." is not borne out by their data as they did not explore other thresholds.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I have had experience researching ARV drug concentrations as objective adherence measures & other adherence measures

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 27 March 2019

https://doi.org/10.5256/f1000research.19436.r44251

Conrad Muzoora
Department of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda

This is a sub-study of an already published major study (CARINEMO ANRS 12146) that initially evaluated the efficacy of Niverapine- and Efavirenz-based Antiretroviral therapy (ART) in patients on concomitant anti-tuberculous therapy.

This sub-study utilized viral load, resistance and drug level data to answer an important question about the relationship between low drug levels in the early phase of ART and week 48 virological escape/possible emergence of resistance.

This is a relatively understudied area and the authors provide very useful data that has otherwise been unavailable in published literature.

The manuscript is well written and easy to understand with sound statistics, straight forward results and justifiable conclusions.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: HIV and co-infections: Cryptococol Meningitis and Tuberculosis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.