Lower prevalence of drug resistance mutations at first-line virological failure to first-line therapy with atripla vs. tenofovir + emtricitabine/ lamivudine + efavirenz administered on a multiple tablet therapy

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Background: Fixed-dose combination antiretroviral therapy administered as a single-tablet regimen (STR) may improve virologic suppression rates. The effect of STRs on development of resistance when virologic failure occurs on STRs is not known.

Objectives: To compare the rate of emergent drug resistance mutations (DRMs) on first-line therapy with coformulated tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC)/ efavirenz (EFV) as an STR versus TDF, lamivudine (3TC) or FTC, and EFV given as non-STR.

Methods: Patients from eight cohorts and four randomized clinical trials who received first-line antiretroviral therapy with Atripla (STR group) or with TDF + FTC/3TC + EFV (non-STR group) were eligible if a genotypic resistance test was available immediately after the first episode of viral failure. The DRM list from the 2013 version of IAS-USA was used.

Results: One hundred and eighty-six patients were included in the final analysis, 122 (65.6%) from eight cohorts and 64 (34.1%) from four randomized clinical trials. The overall proportion of patients with at least one DRM at viral failure was 67.7%, including 53.4% (31 of 58) in the STR group vs. 74.2% (95 of 128) in the non-STR group ($P = 0.005$). Among patients exclusively from cohorts, at least one DRM was detected in 53.4% (31 of 58) in the STR group vs. 78.1% (50 of 64) in the non-STR group ($P = 0.004$). DRMs for individual drugs were: TDF, 15.5 vs. 16.4% ($P = 0.87$); 3TC/FTC, 31 vs. 35.2% ($P = 0.58$); and NNRTI, 51.7 vs. 65.6% ($P = 0.07$). The proportion of patients with an M184V/I among the 128 patients who received FTC was 32.8 vs. 36.2% among the 58 treated with 3TC ($P = 0.65$).

Conclusions: Compared to patients receiving the STR-Atripla, those receiving the same components individually in a non-STR regimen have a statistically significantly increased risk of selecting for DRMs associated with their drugs on failure.

AIDS 2014, **28**:2531–2539

Keywords: antiretroviral treatment, Atripla, HIV-1 resistance, single-tablet regimen

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Received: 29 January 2014; revised: 22 July 2014; accepted: 22 July 2014.

DOI:10.1097/QAD.0000000000000424

Introduction

Combination antiretroviral therapy (cART) has evolved considerably in the past few years and regimens have been greatly simplified. Adherence to cART correlates strongly with HIV viral suppression, reduced rates of resistance, an increase in survival, and an improved quality of life [1,2]. A reduction in pill burden has been associated with improved cART adherence [3,4].

Fixed-dose combinations have been shown to improve adherence to therapy in chronic diseases requiring combination treatments [5,6]. A complete one-pill/once-a-day treatment, also known as a single-tablet regimen (STR), is recommended by the WHO for the simplified treatment of chronic diseases including tuberculosis, hypertension, and HIV [7]. Additionally, STRs make prescribing, dispensing and monitoring treatment easier for nurses and pharmacists. STRs also facilitate the ordering and monitoring of drug stocks by clinics, medicine depots and governments [8].

Atripla (ATR) was the first approved STR [efavirenz (EFV, 600 mg) + emtricitabine (FTC, 200 mg) + tenofovir disoproxil fumarate (TDF, 300 mg)] for the treatment of HIV-1-infected naive adults. Several studies have shown the potential benefits of this STR on outcomes for individuals living with HIV by: reducing pill burden [9]; improving patients' adherence by diminishing the likelihood of taking less than all of the components at any one time (inconsistent adherence, phased prescription refills); improving patients' quality of life [10,11]; and improving the virologic response [11], although a recent study has shown that replacement of the STR TDF/FTC/EFV with its separate components was not associated with risk of virologic failure [12].

We hypothesized that the use of coformulated TDF/FTC/EFV as an STR will reduce the risk of developing HIV-1 drug resistance as it prevents partial noncompliance, which is a relevant concern when a comparable non-STR regimen is used.

Therefore, we conducted the present study to compare the prevalence of drug resistance mutations (DRMs) after virological failure to first-line therapy with ATR, an STR or a non-STR, composed of TDF + FTC/lamivudine (3TC) + EFV.

Patients and methods

The retrospective, nonrandomized study called ATripla RESistance (ATRES) included individuals from clinical cohorts and clinical trials. All researchers invited to participate in this study from clinical cohorts accepted the academic character of this study without any financing except for the statistical analysis. The eight cohorts that accepted to participate in this clinical analysis were the following: Tor Vergata University (Italy), Stanford University (USA), British Columbia University (Canada), the Infectious Disease Program Ponce Clinic at Emory University School of Medicine (USA), RIS (Spain), Guadalajara University (Mexico), and two clinics in Barcelona (see clinical cohorts' description in a supplementary file). We contacted researchers from clinical trials in which one of the combinations of treatment assessed in this trial had been given. Four randomized studies were included in the ATRES study – GS-99-903, GS-01-934, MSD-004, and STARTMRK trials. Eligibility inclusion criteria for the study were: HIV-1 infection with patients at least 18 years old; treatment with TDF + FTC or 3TC + EFV, either as a STR or as multiple tablets (non-STR); no previous cART treatment or no history of resistance mutations or viral failure before changing to one of the combinations assessed in the study; confirmed viral load above 400 HIV RNA copies/ml after a previous viral load below the limit of detection, or confirmed viral load above the limit of detection after 6 months of initiation of ATR or Truvada (TVD) + EFV or TDF + FTC/3TC + EFV from January 2004 to January 2012; genotypic resistance test (GRT) performed within 3 months of viral failure; a viral load at the time of GRT of at least 400 HIV RNA copies/ml; and patient adherence at least 90%.

Nucleoside analogue reverse transcriptase inhibitors (NRTI) and nonnucleoside analogue reverse transcriptase inhibitors (NNRTI) DRMs were defined using the 2013 International AIDS Society-United States of America (IAS-USA) list of DRMs. TDF DRMs: 65R, 70E; 3TC/FTC DRMs: 184V/I; and NNRTI DRMs: 90I, 98G, 100I, 101E/P/H, 103N/S, 106A/I/M, 108I, 138A/G/K/Q/R, 179D/F/T/L, 181C/I/V, 188L/C/H, 190A/S, 221Y, 225H, 227C, and 230I/L [13]. We considered other mutations selected at viral failure as a fourth group: 41L, 67N, 70R, 210W, 215Y/F, 219Q/E, 62V, 75I, 77L, 116Y, 151M, 69SSS, 74V, and 115F.

To analyze the effect of the DRMs on the susceptibility to the different antiretrovirals we ran the sequences – or analyzed the set of mutations – from every GRT using the HIVdb Stanford Genotypic Resistance Interpretation Algorithm [genotypic sensitivity score (GSS): 4, sensitive; 3, potential low level; 2, low level; 1, intermediate; 0, resistant] [14]. We defined as resistant all GSS interpretations lower than sensitive or potential low level (sensitive: GSS = 4 or 3; resistant: GSS = 0, 1 or 2).

Patients were divided into two study groups for the main analysis – the STR group (including individuals with ATR) and the non-STR group (including individuals with TVD + EFV, TDF + FTC + EFV, or TDF + 3TC + EFV). They were stratified by: clinical trials (when individuals took the medication as part of a

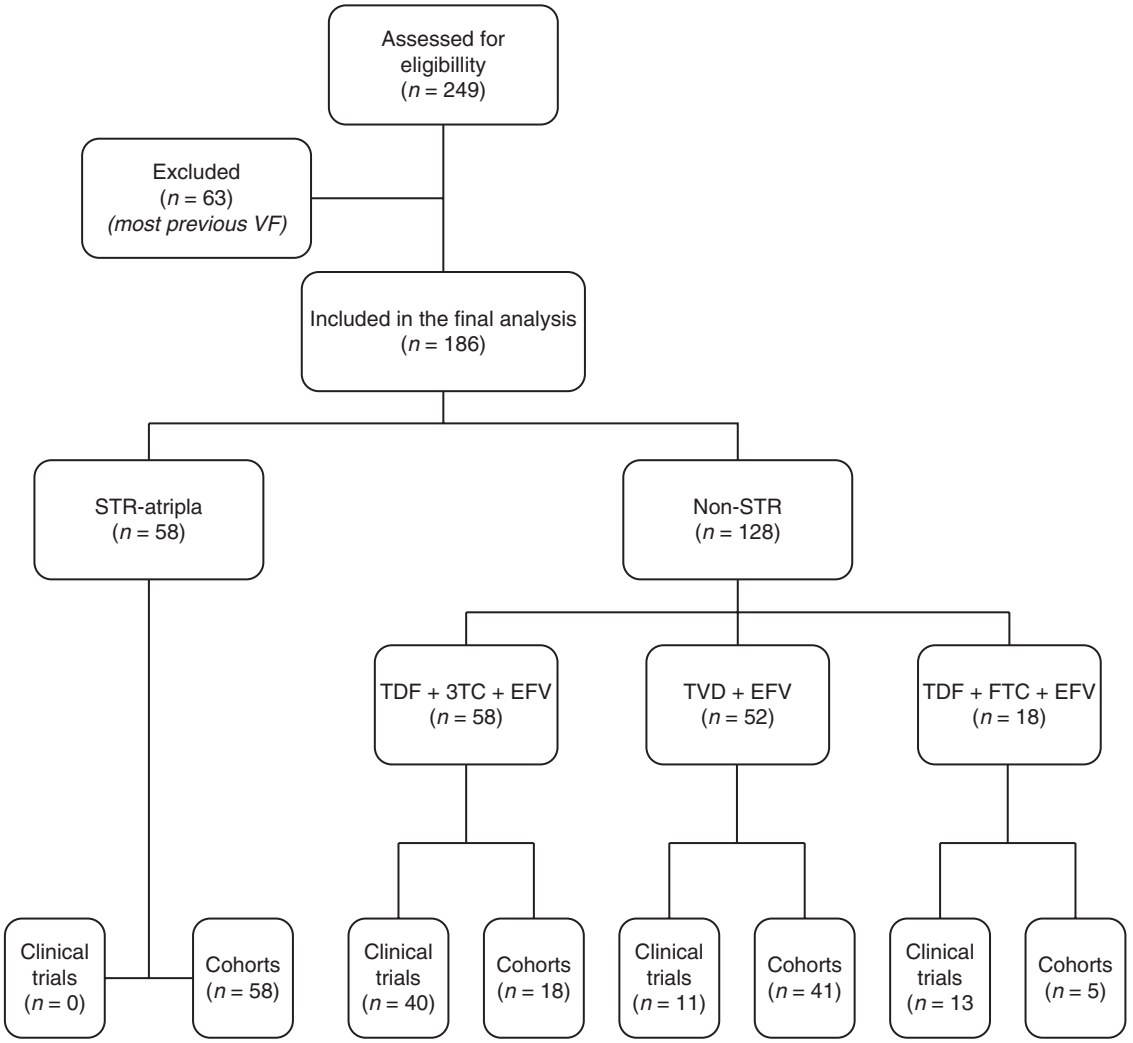

Fig. 1. Patient disposition. Of the 249 individuals initially eligible, 186 were included in the final analysis, 58 in the STR group and 128 in the different non-STR groups. One hundred and twenty-two individuals came from cohort studies (65.6%) and 64 (34.1%) from clinical trials (none of them in the STR group. EFV, efavirenz; FTC, emtricitabine; STR, single-tablet regimen; TDF, tenofovir; TVD, Truvada; 3TC, lamivudine.

clinical trial) vs. no clinical trial (when the individual belonged to a cohort) (stratum 1) (Fig. 1); and naives vs. simplification, according to whether their first treatment was with the study regimen or not (stratum 2). We also grouped individuals according to whether they took FTC or 3TC.

Comparison of the prevalence of selection of at least one DRM was analyzed in the overall population and in the population from clinical cohorts between the STR and non-STR groups, and finally between patients who included 3TC vs. FTC in their regimens.

Statistical analysis

Variables are expressed as the mean and SD, median and interquartile range (IQR), or as proportions, as appropriate.

Demographic and clinical characteristics at baseline between patients in the different groups of comparison were compared using the Wilcoxon rank-sum or *t* test for continuous variables, or chi-square or Fisher's exact test for categorical variables. The presence of different groups of mutations, as well as the individual mutations, was compared between groups using the chi-square test or Fisher's exact test.

A multivariate logistic regression model was created after performing a bivariate analysis that identified independent factors associated to present at least one DRM. Predictors associated with a P value less than 0.10 in the bivariate analysis were considered as candidate predictors for the multivariate. Multiple imputations were used to adjust for the effect of missing data in the assessed factors in the logistic regression. A total of 50 imputations via the

Markov Chain Monte Carlo (MCMC) method were tested.

Statistical significance was defined as a bilateral *P* value less than 0.05. All statistical analyses were carried out using the Stata package (release 9.2; Stata Corp., College Station, Texas, USA).

Results

Two hundred and forty-nine individuals were eligible in the initial analysis from the eight clinical cohorts and four clinical trials, which included any of the regimens assessed in our analysis that accepted to participate in the analysis. Sixty-three individuals were excluded from the final analysis because previous lack of viral failure could not be confirmed before starting one of the regimens assessed in the study. One hundred and eighty-six individuals were included in the final analysis: 58 in the STR group and 128 in the non-STR group [TVD + EFV ($n = 52$), TDF + FTC + EFV ($n = 18$), TDF + 3TC + EFV ($n = 58$)]. One hundred and twenty-two individuals came from cohort studies (65.6%) and 64 (34.1%) from clinical trials. From the overall cohort, 12% were women, 56% were MSM, 45% had a previous AIDS diagnosis, and 92% were infected with HIV-1 subtype B. All individuals in the STR group came from the cohorts, whereas in the non-STR group, 64 individuals came from clinical trials and 64 from cohorts. In the STR group, 32 individuals (57%) started from the beginning with the STR, whereas 24 (43%) simplified to STR from other regimens. In the non-STR group, these proportions were 86% (started with the non-STR) and 14% (simplified to the non-STR), respectively.

The median and IQR of plasma viral load and $CD4^+$ T-cell counts before starting cART were 93 705 (36 058–261 000) HIV RNA copies/ml and 140 (33.5–255) cells/μl. At the time of viral failure, median (IQR) viral load and $CD4^+$ cell counts were 7425 (985–68370) copies/ml and 263 (142–512) cells/μl, respectively. The median (IQR) number of days from viral failure to the GRT was 11.5 (0–74.5). Median viral load at the time of GRT was 9662 (IQR 3204–51 507). Baseline GRT was available in 123 of 186 (66%), and reported genotypic changes at positions associated with drug resistance in 26 of 123 individuals (21%). However, only five individuals (4%) had baseline DRMs that were included in the 2009 WHO list of primary resistance mutations: one K219Q in the non-STR group T69D (one in each group), one T215C in the non-STR group, and one V75M in the non-STR group. Only two of these individuals with baseline DRMs selected new DRMs: one individual who had a baseline T69D selected a new K103K/N and another individual with the V75M at baseline selected K65R and G190S [15]. Both individuals were in the non-STR group.

Overall, the number of patients with at least one DRM at viral failure was 126 of 186 (67.7%). The median (IQR) viral load at the time of viral failure in individuals selected compared to those who did not select any DRM was 9602 (16 041–71 318) and 3744 (522–40320) HIV RNA copies/ml ($P = 0.08$), respectively. Analyzing each component separately, the number of individuals with at least one DRM was 30 on TDF (16.1%), 63 on 3TC/FTC (33.8%), and 114 on EFV (61.3%). Regarding the individual mutations selected at viral failure, the most prevalent mutations were: K103N (43.5%), M184V (24.7%), K65R (14%), M184I (10.2%), V108I (8%), P225H (6.9%), and L100I (6.4%) in RT.

Table 1. Factors associated with the selection of at least one drug resistance mutation in bivariate and multivariate analysis.

				Bivariate		Multivariate	
Factor	Descriptive values	*n* (%)	Total	OR not adjusted (95% CI)	*P* value	OR (95% CI)	*P* value
Treatment groups	STR group	31 (53.4%)	58				
	Non-STR group	95 (74.2%)	128	2.51(1.31–4.80)	0.0056	3.520 (1.32–9.9)	0.0026
Stratum 1	Clinical trials	45 (70.3%)	64	1.20(0.62–2.31)	0.5872		
	Cohorts	81 (66.4%)	122				
Stratum 2	Naives	101 (70.1%)	144	1.60(0.78–3.26)	0.1974		
	Simplification	25 (59.5%	42				
Baseline VL	<100 000 copies/ml	46 (59%) 64 (73.6%)	78				
	>100 000 copies/ml		87	1.94 (1.00–3.73)	0.0485		
Baseline $CD4^+$	<200 cells/μl	76 (76%)	100	3.17 (1.61–6.23)	0.0008	3.18 (1.3–8.13)	0.0108
	>200 cells/μl	31 (50%)	62				
VL at VF	<1000 copies/ml	23 (52.3%)	44	0.45 (0.22–0.90)	0.0247		
	>1000 copies/ml	93 (71%)	131				
$CD4^+$ at VF	<200 cells/μl	49 (77.8%)	63	2.20 (1.08–4.51)	0.0308		
	>200 cells/μl	62 (61.4%)	101				
Mean time (days)	No ≥ 1 IAS DRMs	19.24	42		0.0097		
from VF to GRT	Yes ≥ 1 IAS DRMs	38.81	74	1.02 (1.00–1.03)		1.01 (1.005–1.031)	0.022

CI, confidence interval; DRM, drug resistance mutation; GRT, genotypic resistance test; OR, odds ratio; STR, single-tablet regimen; VF, viral failure; VL, viral load. Stratum 1 – stratification by clinical trials vs. no clinical trials. Stratum 2 – stratification by naïves vs. simplification.

Factors associated with the selection of at least one DRM in a bivariate analysis included: non-STR regimens ($P=0.0056$), baseline viral load above 100 000 copies/ml ($P=0.0485$), baseline $CD4^+$ T-cell counts below 200 cells/μl ($P=0.0008$), viral load at viral failure above 1000 copies/ml ($P=0.0247$), $CD4^+$ T-cell counts at the time of viral failure below 200 cells/μl ($P=0.0308$), and days from GRT to viral failure ($P=0.0097$). In the multivariate analysis (Table 1), independent predictors of selection of at least one DRM included: non-STR regimens [3.52, 95% confidence interval (CI) 1.31–9.87, $P=0.0026$); baseline $CD4^+$ T-cell counts below 200 cells/μl (3.18, 95% CI 1.30–8.13, $P=0.0108$); and days from GRT to viral failure (1.02, 95% CI 1.00–1.03, $P=0.022$).

Single-tablet regimen versus nonsingle-tablet regimen

Overall analysis

There were no differences at baseline for viral load, $CD4^+$ cell count, viral load at the time of viral failure, or time from viral failure to GRT between the STR and non-STR groups. However, time [median (IQR)] to viral failure was significantly longer in the STR vs. the non-STR groups – 350 (531–199) vs. 211 (448–126) days, respectively ($P=0.03$).

The overall proportion of patients with at least one DRM was 53.4% (31/58) in the STR group and 74.2% (95/128) in the non-STR group ($P=0.005$) (Fig. 2a). The proportions of patients with drug-specific DRMs did not differ significantly for the individual drug components comparing the STR group to the non-STR group: TDF DRMs, 15.5 vs. 16.4% ($P=0.87$); 3TC/FTC DRMs, 31 vs. 35.2% ($P=0.58$); NNRTI DRMs, 51.7 vs. 65.6% ($P=0.07$); and other DRMs, 5.2 vs. 7% ($P=0.63$). With regards to individual mutations, we did not find any significant difference in the prevalence of any specific mutation between both groups, except for the 100I, with 8% (8/58) vs. 4% (4/128) in the STR vs. non-STR groups, respectively ($P=0.0061$). The prevalence of selection of resistance by the GSS to at least one of the three drugs in the STR vs. the non-STR groups was 30 of 58 (51.7%) vs. 92 to 128 (71.9%) ($P=0.01$), specifically, TDF 13.8% (8/58) vs. 17.2% (22/128) ($P=0.56$); 3TC/FTC 36.2% (21/58) vs. 45.3% (58/128) ($P=0.24$); and EFV 53.4% (31/58) vs. 67.2% (86/128) ($P=0.07$) (Fig. 3a).

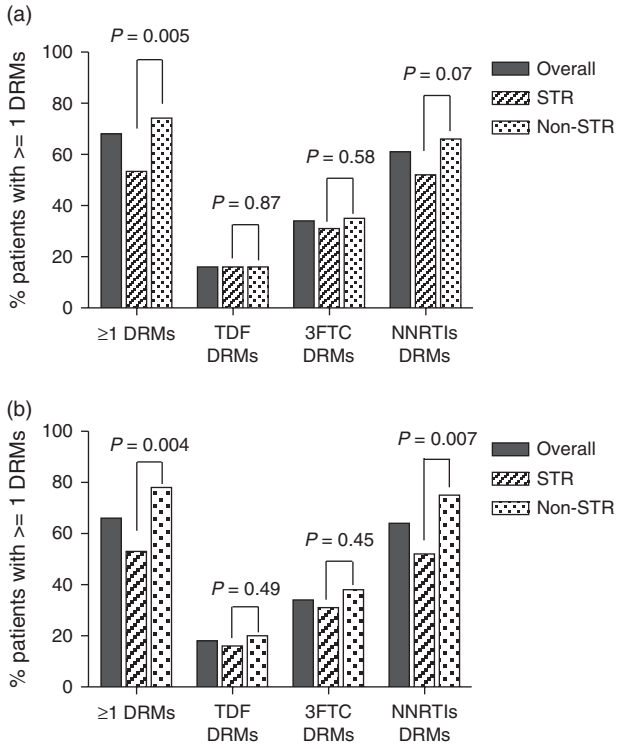

Fig. 2. (a) Proportion of patients with at least one DRM was statistically lower in the STR than in the non-STR in the overall analysis, 53.4 vs 74.2% ($P=0.005$). (b) In the cohort group, the difference in the proportion of patients with at least one DRM was significantly lower in the STR group globally and in the NNRTI. DRM, drug resistance mutation; STR, single-tablet regimen.

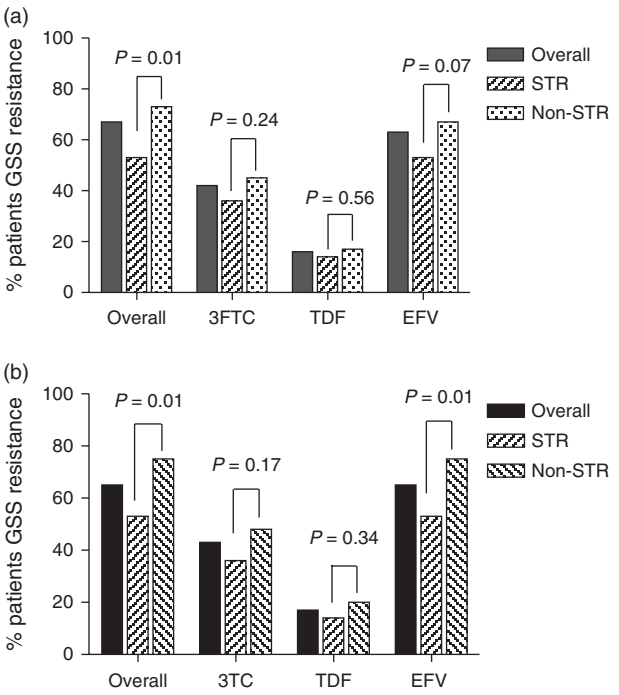

Fig. 3. (a) In the overall analysis similar results were observed in the prevalence of selection of resistance to the different drugs by the genotypic sensitivity score (GSS) as in the analysis of atleast 1DRM, although these differences did not reach statistically significant. (b) The impact of genotypic resistance reached statistically significant in the GSS of the NNRTI in the cohort groups.

When comparing STR vs. non-STR only in regimens that included FTC (TVD + EFV vs. TDF + FTC + EFV), the selection of at least one DRM was lower in the STR vs. non-STR groups – 31/58 (53.4%) vs. 56/70 (80%) ($P = 0.001$), respectively (data not shown). With regards to DRMs for individual antiretrovirals in the FTC group, the selection of at least one DRM was lower for all three antiretrovirals with statistical significance for the reduced selection of NNRTI DRMs [30/58 (51.7%) vs. 52/70 (74.3%); $P = 0.008$].

Time to viral failure was significantly longer in the STR with a median (IQR) of 350 days (531–199) vs. 211 (448–126) in the non-STR regimens ($P = 0.03$).

Cohort analysis

No significant differences were detected between individuals in the two groups [STR ($n = 58$) vs. non-STR ($n = 68$)] with regards to baseline viral load or $CD4^+$ cell count, viral load at the time of viral failure, time to viral failure, or time from viral failure to GRT.

The proportion of patients in the cohort group with at least one DRM was significantly lower in the STR [53.4% (31/58)] vs. non-STR [78.1% (50/64)] groups ($P = 0.004$). Regarding specific drug DRMs, TDF 15.5% (9/58) vs. 20.3% (13/64) ($P = 0.49$); 3TC/FTC 31% (18/64) vs. 37.5% (24/64) ($P = 0.45$); and EFV 51.7% (30/58) vs. 75% (48/64) ($P = 0.007$) (Fig. 2b). As in the overall analysis, no significant differences were found when individual DRMs were compared. The prevalence of selection of resistance by the GSS, comparing the STR vs. the non-STR groups, was: 51.7% (30/58) vs. 75% (48/64) ($P = 0.01$). Regarding the selection of genotypic resistance to at least one drug, the following comparisons were found: TDF 13.8% (8/58) vs. 20.3% (13/64) ($P = 0.34$); 3TC/FTC 36.2% (21/59) vs. 48.4% (31/64) ($P = 0.17$); and EFV 53.4% (31/58) vs. 75% (48/64) ($P = 0.01$) (Fig. 3b).

There were no individuals in the STR group belonging to clinical trials, so the comparison between STR and non-STR in the strata of clinical trials could not be performed.

Lamivudine versus emtricitabine

In the cohort groups, when the non-STR only included regimens with FTC (TVD + EFV and TDF + FTC + EFV), the selection of at least one DRM was lower in the STR vs. non-STR groups – 31 of 58 (53.4%) vs. 36 of 46 (78.3%) ($P = 0.008$), respectively (data not shown).

A comparison was also made between patients taking FTC versus 3TC in their regimen regardless of use of STR. The number of individuals who selected the M184I/V was 42 (32.8%) in those who received FTC (ATR, TVD + EFV or TDF + FTC + EFV, $n = 128$) vs. 21 (36.2%) in those who received 3TC ($n = 58$) ($P = 0.65$). The overall proportion of patients with at least one DRM to TDF was 16 of 128 (12.5%) vs. 14 of 58 (24.1%) ($P = 0.04$) and to EFV, 82 of 128 (64.1%) vs. 32 of 58 (55.2%) ($P = 0.25$), in the FTC vs. 3TC groups, respectively (Fig. 4a).

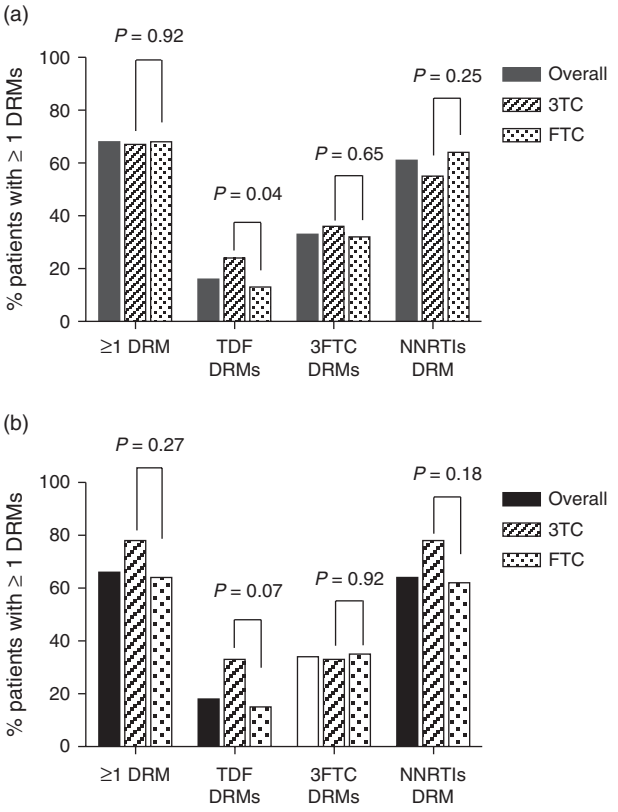

Fig. 4. (a) There was a trend to a greater selection of 3TC and TDF DRM in the 3TC vs. FTC group in the overall analysis. (b) In the cohort groups the trend to a greater selection of at least one DRM with 3TC compared to FTC was observed for TDF and NNRTI. 3TC, lamivudine; DRM, drug resistance mutation; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

We only found a trend in the cohort analysis between the FTC and the 3TC groups regarding the differences in the number of TDF DRMs, 16 of 104 (15.4%) vs. six of 18 (33.3%) ($P = 0.07$); M184I/V, 36 of 104 (34.6%) vs. six of 18 (33.3%) ($P = 0.92$); or NNRTI DRMs, 64 of 104 (61.5%) vs. 14 of 18 (77.8%) ($P = 0.18$) (Fig. 4b).

Discussion

The overall proportions of individuals who selected at least one DRM after viral failure was statistically lower in the STR than in the non-STR groups in both the overall analysis and the analysis of cohorts (there were no patients in clinical trials on an STR). This difference, favorable to

the STR, was also found in other analyses when the STR was compared to the non-STR regimens: non-STR combinations that only included FTC (TVD + EFV and TDF + FTC + EFV) in both the overall analysis ($P = 0.0014$) and the cohort analysis ($P = 0.008$); or non-STR combinations that only included regimens with the three components individually (TDF + FTC + EFV and TDF + 3TC+EFV) ($P = 0.076$) (data not shown). One potential explanation for our findings is that partial adherence is not possible in those receiving an STR. Compared to an STR, a non-STR regimen includes: a higher pill burden with more pill bottles; a higher complexity in taking the medication; greater possibility of patient fatigue; and a higher possibility of errors from the prescribing provider and the pharmacies, all factors associated with a lower adherence to an HIV regimen [16]. In a recent retrospective analysis of medical and pharmacy claims from a large commercially insured population of HIV+ patients treated in the United States (LifeLink database), different non-STRs were associated with a partial nonadherence of between 7 and 11%, and an additional risk of hospitalization ranging from 43 to 54%, compared to an STR [17]. A similar analysis could not be done in individuals from the four clinical trials because no one in the STR group came from the clinical trials, that is, the overall analysis did not include patients on an STR from clinical trials.

The percentage of individuals with at least one DRM at viral failure was 67.7% in our analysis, similar to that observed in clinical trials, ranging from 55 to 68.4% in the analyses of viral failure [18–21]. Regarding the different drugs, the rate of selection of DRMs was also similar to previous observations, with a greater rate of NNRTI resistance (61.3% in the ATRES study vs. from 55 to 68.4% in the clinical trials), followed by 3TC/FTC (33.8% in ATRES vs. from 10.5 to 38% in the clinical trials), and finally TDF (16.1% in ATRES vs. from 0 to 17% in the clinical trials). It is important to note that these results differ partially from those presented at the XIII International HIV Drug Resistance Workshop [18] due to the prior analysis not including mixed populations of mutant and wild type.

Of the 186 episodes of viral load analyzed in the study, 64 are episodes of virologic failures detected in patients recruited in formal clinical trials from 2000 to 2008. The total number of patients was 871. The remaining 122 episodes of viral failure were detected in prospective clinical cohorts from 2000 to 2010. The total number of patients exposed to study medications considered in the study during same period were not formally collected in report forms. From the data of five of these eight cohorts, approximately 4800 patients were estimated to have rates of virologic failures of 18% with ATR, 22% with TVD + FTC, 19% with TDF + EFV + FTC, and 20% with TDF + EFV + 3TC.

Given the very low rate of first-line virologic failures, and even fewer who develop DRMs at viral failure, to do this analysis we needed to look retrospectively at a large number of cohorts and clinical trials. For this analysis, we considered the regimen that the individual was taking at the time of viral failure, and looked independently at the regimens previously taken. As in most clinical trials [19–22], we only included GRTs from individuals with viral load at least 400 HIV RNA copies/ml at failure. It has been recently shown that resistance genotyping performed on samples with above 400 copies/ml yield 90% successful results and the results are predictive of the risk of treatment failure [23,24]. To make our analysis as robust as possible, we excluded from this analysis 63 individuals for whom we had no confirmation of lack of prior viral failure. In most of these cases, the GRT at viral failure included mutations that are not usually selected by their current drug regimen, which suggested prior viral failure to other drugs.

Non-STR regimens were an independent predictor of selection of at least one DRM in the multivariate analysis, as well as baseline $CD4^+$ T-cell counts below 200 cells/μl as well as time from viral failure to GRT. However, potential colinearity between the time between viral failure and GRT and the treatment group was assessed and only a minor trend was detected – the STR group showed a lower median time between viral failure and GRT compared to the non-STR group, but it was not statistically significant ($P = 0.2$).

The analysis of time to viral failure was also noteworthy, in that time to viral failure was significantly longer in the STR than in the non-STR regimens. This represents an additional signal that supports the higher efficacy of the STR compared to the non-STR regimens.

Although generally the comparison of the proportion of patients with at least one DRM is a standardized way to assess differences regarding the risk of developing resistance at viral failure between different regimens, this analysis may have some limitations. All of the IAS-USA N(t)RTI DRMs to TDF and 3TC/FTC are tightly associated with phenotypic resistance, whereas some IAS-USA NNRTI DRMs (i.e. 90I, 98G, 108I, 138A/G/K/Q/R, 179D/F/T/L, and 221Y) have a low effect on NNRTI phenotypic sensitivity. Therefore, considering mutations with a high and a low value as being equal in this analysis of 'percentage of individuals with at least one DRM' could lead to misinterpretation. Moreover, the selection of resistance to one antiretroviral vs. more than one is not equivalent. We therefore performed another analysis assessing the effect of all mutations selected at viral failure using GSS according to the 'weighted' HIVdb Stanford Genotypic Resistance Interpretation Algorithm, and we compared the selection of resistance to the different antiretrovirals of the regimen. We observed

similar results in the prevalence of selection of resistance to the different drugs by the GSS as in the analysis of at least one DRM. The selection of genotypic resistance to each drug was always greater in the non-STR group than in the STR group, although these differences only reached statistical significance with NNRTIs in the cohort groups ($P = 0.01$). A worse impact on NNRTI genotypic resistance in the non-STR group was also reflected in a higher, although not statistically significant, prevalence of resistance to second-generation NNRTIs (ETV and RPV), 5.2% (3/58) vs. 7% (9/128) in the STR and non-STR groups, respectively ($P = 0.63$) (data not shown).

Emtricitabine has greater in-vitro and in-vivo potency than 3TC [25,26]; greater synergy when combined with TDF [27,28]; and a longer intracellular half-life [29,30]. Regarding resistance selection, there are little data directly comparing these two drugs. However, looking at different studies, selection of M184I/V with 3TC at viral failure in the GS-99-903 study (3TC + TDF + EFV) was greater than with FTC in the GS-01-934 study (FTC + TDF + EFV), 38% (18/47) vs. 10.5% (2/19), respectively (17,18). This trend to a greater selection of M184V with 3TC compared to FTC was also seen in ACTG 5202 when ABC/3TC was compared with TDF/FTC (both with EFV, 18.4 vs. 12.1%), respectively [20]. In the ATRES study, we continued to see this trend of a greater selection of not only the M184V/I but also TDF mutations in the 3TC vs. FTC groups, in both the overall and cohort group analysis.

There are several limitations to our study. The data sources are quite heterogeneous due to this analysis including individuals from multinational cohorts and clinical trials. As is done in many clinical trials, in order to assess the rate of DRM selection, we considered individuals with viral failure and GRT performed with at least 400 copies/ml. Adherence was assessed only via medical report and not by any standardized and uniform method.

In summary, compared with patients receiving the STR, those receiving the individual components in a non-STR regimen have a statistically significantly shorter time to viral failure and an increased risk of selecting DRMs associated with the development of viral failure. These results are timely, as several HIV drugs approach patent expirations, and many are speculating that this may allow the replacement of STRs with comparable generic non-STR combinations of drugs as a cost-saving strategy despite a slightly lower efficacy and higher rate of resistance [31]. Apart from representing a step backwards in the achievement of many therapeutic advances regarding patients' quality of life, simplicity, adherence and efficacy, our results show that STR use is associated with longer time to viral failure and reduced rate of DRMs.

Acknowledgements

We thank Rob Camp for his critical reading and the English language editing of the manuscript, and to Iñaki Perez for his statistical support.

Supported for the statistical analysis only by research grants from Gilead Sciences, who did not participate in the study design, analysis, or writing.

This paper is in memory of Iñaki Perez, a great colleague and friend who died recently (1966–2014).

J.L.B. and J.M.G. designed the study. J.L.B. undertook the statistical analysis. All authors were involved in the interpretation of data. J.L.B. wrote the manuscript. All authors critically reviewed and subsequently approved the final version.

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Conflicts of interest

The following authors have received research funding, consultancy fees or lecture sponsorships: J.L.B. – Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp and Dohme, and ViiV Healthcare; J.M.G. – Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck Sharp and Dohme, and ViiV Healthcar, Theratechnologies, and Tibotec; M.D.M. – Employee and stock share holder of Gilead Sciences; M.M.S. – Abbott, Bristol Myers Squibb, Merck Sharp; R.P. – Merck-Sharpe & Dohme, ViiV Healthcare, Gilead Sciences, Bristol Myers Squibb, Pfizer, Roche Diagnostics, SL; C.F.P. – Abbott, Bristol Myers Squibb, Gilead, Merck Sharp & Dohme, Janssen, Pfizer, Roche, and ViiV Healthcare; M.L.N. – Tibotec and Dohme, Janssen, and ViiV Healthcare; R.H., ViiV Healthcare, Tobira Therapeutics, Selah Genomics, Quest Diagnostics and Merck Sharp and Dohme.

References

1. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. [Accessed 11 September 2013]
2. Chesney MA. **The elusive gold standard. Future perspectives for HIV adherence assessment and intervention.** *J Acquir Immune Defic Syndr* 2006; **43**:S149–S155.
3. Maggiolo F, Ripamonti D, Arici C, Gregis G, Quinzan G, Camacho GA, *et al*. **Simpler regimens may enhance adherence to antiretrovirals in HIV infected patients.** *HIV Clin Trials* 2002; **5**:371–378.
4. Stone VE, Jordan J, Tolson J, Miller R, Pilon T. **Perspectives on adherence and simplicity of HIV-infected patients on antiretroviral therapy. Self-report of the relative importance of multiple attributes of Highly Active Antiretroviral Therapy (HAART) regimens in predicting adherence.** *J Acquir Immune Defic Syndr* 2004; **36**:808–816.
5. Gradman AH, Basile JN, Carter BL, Bakris GL, Materson BJ, Black HR, *et al*. **Combination therapy in hypertension.** *J Am Soc Hypertens* 2010; **4**:90–98.
6. Moulding T, Dutt AK, Reichman LB. **Fixed-dose combinations of antituberculous medications to prevent drug resistance.** *Ann Intern Med* 1995; **122**:951–954.
7. Bangalore S. **Fixed-dose combinations improve medication compliance: a meta-analysis.** *Am J Med* 2007; **120**:713–719.
8. http://www.tac.org.za/community/node/3299. [Accessed 6 June 62012]
9. Sax PE, Meyers JL, Mugavero M, Davis KL. **Adherence to antiretroviral treatment and correlation with risk of hospitalization among commercially insured HIV patients in the United States.** *PLoS One* 2012; **7**:e31591.
10. Airoldi M, Zaccarelli M, Bisi L, Bini T, Antinori A, Mussini C, *et al*. **One pill once-a-day HAART: a simplification strategy that improves adherence and quality of life of HIV-infected subjects.** *Patient Prefer Adherence* 2010; **4**:115–125.
11. Bangsberg DR, Ragland K, Monk A, Deeks SG. **A single tablet regimen is associated with higher adherence and viral suppression than multiple tablet regimens in HIV+ homeless and marginally housed people.** *AIDS* 2010; **24**:2835–2840.
12. Engsig F, Gerstotoft J, Helleberg M, Nielsen L, Konbrog G, Mathiesen L, *et al*. **Effectiveness of antiretroviral therapy in individuals who for economic reasons were switched from a once-daily single-tablet regimen to a triple-tablet regimen.** *J Acquir Immune Defic Syndr* 2014; **66**:407–413.
13. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer RW, *et al*. **2013 Update of the drug resistance mutations.** *Top Antivir Med* 2013; **21**:6–14.
14. http://sierra2.stanford.edu/sierra/servlet/JSierra. [Accessed 12 June 2013]
15. Bennett DE, Camacho RJ, Oteleas D, Kuritzkes DR, Fleury H, Kiuchi M, *et al*. **Drug resistance mutations for surveillance of transmitted HIV-1 drug resistance: 2009 update.** *PLoS One* 2009; **4**:e4724.
16. Juday T, Gupta S, Grimm K, Wagner S, Kim E, *et al*. **Factors associated with complete adherence to HIV combination antiretroviral therapy.** *HIV Clin Trials* 2011; **12**:71–78.
17. Cohen C, Davis KL, Meyers JL. **Partial adherence to antiretroviral therapy and hospitalization risk in an HIV population. Poster H-211.** *52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)*. 9–12 September 2012, San Francisco, CA, USA.
18. BlancoJL J, Montaner J, Branstetter D, Santoro MM, Campos-Loza AE, Shafer RW, *et al*. **Drug resistance prevalence after failing antiretroviral therapy (ART) with tenofovir (TDF) + emtricitrabine/lamivudine (3TC/FTC) + efavirenz (EFV) vs. the single tablet regimen (STR) Atripla: the ATRES study.** *Antiviral Ther* 2012; **17 ((Suppl 1))**:A115.
19. Margot NA, Lu B, Cheng A, Miller MD, *et al*. **Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903.** *HIV Med* 2006; **7**:442–450.
20. Margot NA, Enejosa J, Cheng AK, Miller MD, McColl DJ, *et al*. **Development of HIV-1 drug resistance through 144 weeks in antiretroviral-naive subjects on emtricitabine, tenofovir disoproxil fumarate, and efavirenz compared with lamivudine/zidovudine and efavirenz in study GS-01-934.** *J Acquir Immune Defic Syndr* 2009; **52**:209–221.
21. Lennox JL, Dejesus E, Berger DS, Lazzarini A, Pollard RB, Ramalho Madruga JV, *et al*. **Raltegravir versus efavirenz regimens in treatment-naive HIV-1-infected patients: 96-week efficacy, durability, subgroup, safety, and metabolic analyses.** *J Acquir Immune Defic Syndr* 2010; **55**:39–48.
22. Sax PE, Tierney C, Collier AC, Daar ES, Mollan K, Budhathoki C, *et al*. **Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results.** *J Infect Dis* 2011; **204**:1191–1201.
23. Swenson LC, Gonzalez-Serna A, Min J, Woods CK, Li JZ, Harrigan PR, *et al*. **HIV drug resistance occurring during low-level viraemia is associated with subsequent virologial failure.** *Antiviral Ther* 2013; **18 ((Suppl 1))**:A40.
24. Li JZ, Gallien S, Do TD, Martin JN, Deeks S, Kuritzkes DR, *et al*. **Prevalence and significance of HIV-1 drug resistance mutations among patients on antiretroviral therapy with detectable low-level viremia.** *Antimicrob Agents Chemother* 2012; **56**:5998–6000.
25. Schinazi RF. **Assessment of the relative potency of emtricitabine and lamivudine.** *J Acquir Immune Defic Syndr* 2003; **34**:243–245.
26. Rousseau FS, Wakeford C, Mommeja-Marin H, Sanne I, Moxham C, Harris J, *et al*. **Prospective randomized trial of emtricitabine versus lamivudine short-term monotherapy in human immunodeficiency virus-infected patients.** *J Infect Dis* 2003; **188**:1652–1658.
27. Borroto-Esoda K, Vela JE, Myrick F, Myrick F, Rauy AS, Miller MD. **In vitro evaluation of the anti-HIV activity and metabolic interactions of tenofovir and emtricitabine.** *Antivir Ther* 2006; **11**:377–384.
28. Feng JY, Ly JK, Myrick F, Goodman D, White KL, Svaroskaia ES, *et al*. **The triple combination of tenofovir, emtricitabine and efavirenz shows synergistic anti-HIV-1 activity in vitro: a mechanism of action study.** *Retrovirology* 2009; **6**:44.
29. Anderson PL, Kakuda TN, Kawle S, Fletcher CV. **Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals.** *AIDS* 2003; **17**:2159–2168.
30. Wang LH, Wiznia AA, Rathore MH, Chittick GE, Bakshi SS, Emmanuel PJ, *et al*. **Pharmacokinetics and safety of single oral doses of emtricitabine in human immunodeficiency virus-infected children.** *Antimicrob Agents Chemother* 2004; **48**:183–191.
31. Walensky RP, Paltiel AD, Schackman BR. **Cost-effectiveness of generic antiretroviral therapy: in response.** *Ann Intern Med* 2013; **158**:776–777.