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Occult HIV-1 drug resistance to thymidine analogues following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or efavirenz in sub-Saharan Africa: a retrospective multi-centre cohort study

John Gregson, Pontiano Kaleebu, Vincent C Marconi, Cloete van Vuuren, Nicaise Ndemb, Raph L Hamers, Phyllis Kanki, Christopher J Hoffmann, Shahin Lockman, Deenan Pillay, Tulio de Oliveira, Nathan Clumeck, Gillian Hunt, Bernhard Kerschberger, Robert W Shafer, Chunfu Yang, Elliot Raizes, Rami Kantor, Ravindra K Gupta

Summary

Background HIV-1 drug resistance to older thymidine analogue nucleoside reverse transcriptase inhibitor drugs has been identified in sub-Saharan Africa in patients with virological failure of first-line combination antiretroviral therapy (ART) containing the modern nucleoside reverse transcriptase inhibitor tenofovir. We aimed to investigate the prevalence and correlates of thymidine analogue mutations (TAM) in patients with virological failure of first-line tenofovir-containing ART.

Methods We retrospectively analysed patients from 20 studies within the TenoRes collaboration who had locally defined viral failure on first-line therapy with tenofovir plus a cytosine analogue (lamivudine or emtricitabine) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI; nevirapine or efavirenz) in sub-Saharan Africa. Baseline visits in these studies occurred between 2005 and 2013. To assess between- and within-study associations, we used meta-regression and meta-analyses to compare patients with and without TAMs for the presence of resistance to tenofovir, cytosine analogue, or NNRTIs.

Findings Of 712 individuals with failure of first-line tenofovir-containing regimens, 115 (16%) had at least one TAM. In crude comparisons, patients with TAMs had lower CD4 counts at treatment initiation than did patients without TAMs (60·5 cells per μL [IQR 21·0–128·0] in patients with TAMs vs 95·0 cells per μL [37·0–177·0] in patients without TAMs; p=0·007) and were more likely to have tenofovir resistance (93·8% of 115 patients with TAMs vs 352 [59%] of 597 patients without TAMs; p=0·0001), NNRTI resistance (107 [93%] of 115 patients with TAMs vs 352 [59%] of 597 patients without TAMs; p<0·0001), and cytosine analogue resistance (100 [87%] vs 378 [63%]; p=0·0002). We detected associations between TAMs and drug resistance mutations both between and within studies; the correlation between the study-level proportion of patients with tenofovir resistance and TAMs was 0·64 (p<0·0001), and the odds ratio for tenofovir resistance comparing patients with and without TAMs was 1·29 (1·13–1·47; p<0·0001).

Interpretation TAMs are common in patients who have failure of first-line tenofovir-containing regimens in sub-Saharan Africa, and are associated with multidrug resistant HIV-1. Effective viral load monitoring and point-of-care resistance tests could help to mitigate the emergence and spread of such strains.

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Research in context

Evidence before this study

We did a systematic review using PubMed and Embase, searching from Jan 1, 2000, up to Aug 15, 2016, without language limitations. Manuscripts of interest were also identified from the reference lists of selected papers, clinical trials registries, and abstracts from the Conference on Retroviruses and Opportunistic Infections (CROI) and International AIDS Society (IAS). We used the search terms “HIV” AND “Tenofovir” AND “thymidine analogue” OR “stavudine” OR “zidovudine” OR “AZT” OR “d4T”. We found no studies reporting the implications of previous thymidine analogue use on outcomes following tenofovir-based antiretroviral therapy (ART). One study investigated the implications of transition from thymidine analogue to tenofovir by use of a cross sectional survey in Myanmar before the introduction of tenofovir. The investigators tested viral loads in more than 4000 patients after 12 months of thymidine analogue-based ART to avoid substitutions in viraemic patients. They noted that a substantial proportion of patients were having treatment failure (13% had viral loads>250 copies per mL), in whom direct tenofovir substitution for the thymidine analogue would not be appropriate.

Added value of this study

Our results show that tenofovir-based first-line regimens are failing in a substantial proportion of patients who have evidence of previous exposure and drug resistance to older nucleoside (thymidine) analogues such as zidovudine and stavudine in sub-Saharan Africa. These individuals are likely to have developed drug resistance to the non-nucleoside reverse transcriptase inhibitor as well as the cytosine analogue, and therefore have high-level resistance to at least two of the three drugs present in tenofovir-based first line ART. Our data show that these individuals with thymidine analogue mutations have lower CD4 counts and therefore are at greater risk of clinical complications than are those without previous ART exposure.

Implications of all the available evidence

Cheap and effective viral load monitoring, resistance testing, or both could prevent the transition of patients with virological failure onto tenofovir-based first-line ART and also identify individuals with pre-existing drug resistance to first line agents arising from undisclosed prior ART. These individuals could then be treated with second-line regimens.

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Methods

Study population and design

We identified patients from within the TenoRes collaboration, a multicountry retrospective study examining correlates of genotypic drug resistance following failure of tenofovir-containing combination ART. Data in this Article, we characterise the prevalence, determinants, and implications of TAMs in patients after virological failure of tenofovir-containing first-line regimens in sub-Saharan Africa.

### Table 1: Baseline characteristics of patients by region and thymidine analogue mutation status

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of patients</th>
<th>Age at tenofovir initiation (years)</th>
<th>Women</th>
<th>Nevirapine (log10 copies per mL)</th>
<th>Emtricitabine (log10 copies per mL)</th>
<th>Baseline CD4 count (cells per μL)</th>
<th>Baseline viral load (log10 copies per mL)</th>
<th>Year of tenofovir initiation</th>
<th>Length of time on tenofovir-based ART (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eastern Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No TAM</td>
<td>133</td>
<td>36 (30-0-44)</td>
<td>77</td>
<td>(58%)</td>
<td>79 (53%)</td>
<td>44 (33%)</td>
<td>102.5 (40.5-208.5)</td>
<td>5.6 (5.3-5.8)</td>
<td>2011 (2010-2012)</td>
</tr>
<tr>
<td>TAM</td>
<td>26</td>
<td>33.5 (27-0-41)</td>
<td>17</td>
<td>(65%)</td>
<td>20 (77%)</td>
<td>7 (27%)</td>
<td>68.0 (16.5-209.0)</td>
<td>5.4 (5.1-5.8)</td>
<td>2011 (2011-2012)</td>
</tr>
<tr>
<td><strong>Southern Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No TAM</td>
<td>383</td>
<td>34.5 (28-0-41)</td>
<td>225</td>
<td>(59%)</td>
<td>96 (25%)</td>
<td>72 (19%)</td>
<td>98.0 (39-0-167)</td>
<td>4.7 (3-4-5.4)</td>
<td>2011 (2008-2011)</td>
</tr>
<tr>
<td>TAM</td>
<td>78</td>
<td>34.0 (28-4-37)</td>
<td>48</td>
<td>(62%)</td>
<td>13 (17%)</td>
<td>7 (9%)</td>
<td>72.0 (20.0-107)</td>
<td>4.4 (2.9-5.3)</td>
<td>2010 (2010-2011)</td>
</tr>
<tr>
<td><strong>West and central Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No TAM</td>
<td>81</td>
<td>36.1 (31-0-40)</td>
<td>42</td>
<td>(52%)</td>
<td>53 (65%)</td>
<td>65 (80%)</td>
<td>86.5 (30.0-180)</td>
<td>5.2 (4.9-5.6)</td>
<td>2006 (2006-2009)</td>
</tr>
<tr>
<td>TAM</td>
<td>11</td>
<td>36.3 (30-0-42)</td>
<td>4</td>
<td>(36%)</td>
<td>9 (82%)</td>
<td>9 (82%)</td>
<td>58.0 (27.0-143)</td>
<td>4.8 (3.7-5.5)</td>
<td>2006 (2006-2006)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No TAM</td>
<td>597</td>
<td>35.0 (29-0-41)</td>
<td>344</td>
<td>(58%)</td>
<td>228 (38%)</td>
<td>181 (30%)</td>
<td>95.0 (37-0-170)</td>
<td>5.2 (4.5-5.6)</td>
<td>2011 (2008-2011)</td>
</tr>
<tr>
<td>TAM</td>
<td>115</td>
<td>34.0 (28-0-38)</td>
<td>69</td>
<td>(60%)</td>
<td>42 (37%)</td>
<td>23 (20%)</td>
<td>60.5 (21-0-128)</td>
<td>5.1 (4.1-5.6)</td>
<td>2011 (2010-2012)</td>
</tr>
</tbody>
</table>

Data are median (IQR) or n (%) unless specified otherwise. Tenofovir=tenofovir disoproxil fumarate. ART=antiretroviral therapy.
Leu210Trp mutation has also been shown to compromise more TAMs inclusive of either the Met41Leu or reverse transcriptase. Although the presence of three or Lys65Arg/Asn or Lys70Glu/Gly/Gln mutations in absence of TAMs at the resistance test.

was collected on patients irrespective of the presence or first-line patients, although identical information concerns that they might represent pre-treated rather than first-line patients, although identical information was collected on patients irrespective of the presence or absence of TAMs at the resistance test.

We defined tenofovir resistance as the presence of Lys65Arg/Asn or Lys70Glu/Gly/Gln mutations in reverse transcriptase. Although the presence of three or more TAMs inclusive of either the Met41Leu or Leu210Trp mutation has also been shown to compromise tenofovir clinically, no individuals in this study had such a profile. TAMs were defined as Met41Leu, Asp67Asn, Lys70Arg, Leu210Trp, Thr215Phe/Tyr, or Lys219Gln/Glu. Our definition of TAMs also included the revertant mutations Thr215Ser/Cys/Asp/Ile/Val, although only two patients presented with such a mutation without the presence of at least one other TAM. TAM revertants are indicative of previous TAM treatment failure if a thymidine analogue drug is used. no TAMs have occurred to put an upper limit on the estimated prevalence.

We interpreted drug resistance mutations using the Stanford HIV Drug Resistance Algorithm version 70. We restricted our analysis to study sites from sub-Saharan Africa because we specifically wanted to investigate the large-scale programmatic shifts in tenofovir use that are currently occurring in this region in the absence of intensive viral load monitoring and baseline resistance testing. Studies were included if they had resistance data on ten or more patients, although in sensitivity analyses that included all available data, the conclusions were not altered (appendix).

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We assessed 34 studies and excluded 14 because they contained fewer than ten patients (56 patients excluded). We identified 712 patients who had viral failure with WHO-recommended, tenofovir-based first-line regimens in 20 studies across sub-Saharan Africa (table 1; appendix). Most (461 [65%]) patients were from southern Africa, with 159 (22%) patients from eastern Africa and 92 (13%) from west and central Africa. 481 (68%) of 712 infections were with HIV-1 subtype C (appendix). Median age at baseline was 35·0 years (IQR 28·8–40·7) and 413 (58%) patients were women. The median year of initiation was 2011, and patients were followed up for a median of 18 months (12–27). Where available, the overall median baseline CD4 count was 92 cells per μL (34–169) and median viral load was log10 5·23 copies HIV-1 RNA (4·5–5·6) per mL. Patient characteristics were broadly similar between patients with and without TAMs, with the exception of baseline CD4 count, which was roughly 30 cells per μL lower in patients with TAMs in all regions (p<0·001). We noted that usage of emtricitabine was 10% lower in patients with TAM compared to those without. 33 (16%) of 209 women with available data on single-dose nevirapine had known previous exposure to single-dose nevirapine. Prevalence of NNRTI resistance was 88% (29 of 33 patients) in patients with single-dose nevirapine exposure and 82% (378 of 462 patients overall or 142 [81%] of 176 women) in those without single-dose nevirapine exposure (p=0·38). For many patients, it was not known whether or not they had received single-dose nevirapine, including men, for whom single dose nevirapine use was always answered as no.

TAMs were detected in 115 (16%) of 712 patients (figure 1A). The prevalence of TAMs was similar in eastern Africa (26 [16%] of 158), southern Africa (78 [17%] of 461 patients), and west and central Africa (11 [12%] of 92 patients). TAMs were less common in patients with HIV-1 subtype D than in patients with other subtypes (appendix). Despite individual studies tending to have only a small number of patients, all but four of the 20 included studies reported a prevalence of TAMs between 5% and 25% (figure 1A). Asp67Asn was the most common TAM and was present in 50 (7%) of 712 patients; it was more common in southern (41 [9%] of 461 patients) and eastern Africa (eight [5%] of 159 patients) than in west and central Africa (one [1%] of 91 patients; p=0·015). The next most common TAMs were Lys219Glu (46 [6%] of 712 patients) and Met41Leu (20 [3%] patients; figure 1B). 20 (3%) patients had two or more TAMs and seven (1%) patients had three or more TAMs.

In crude comparisons across the entire study population, patients with TAMs were more likely to have tenofovir resistance (p<0·0001), as well as resistance to cytosine analogues (100 [87%] patients with TAMs vs 378 [63%] of patients without TAMs; p<0·0002) and nevirapine or efavirenz (107 [93%] of 115 patients with TAMs vs 462 [77%] of 597 without TAMs; p<0·0001), with consistent findings in all regions (figure 2). Of the 115 patients with TAMs, 93 (81%) had Lys65Arg/Asn or Lys70Glu/Gly/Gln, whereas in the remaining 597 patients without TAMs, 352 (59%) patients had these tenofovir resistance mutations (p<0·001). Tenofovir resistance mutations at Lys65 or Lys70 were present in 92 (86%) of 107 patients with TAM mutations without Thr215Phe/Tyr, and one (13%) of eight patients with TAM mutations with Thr215Phe/Tyr (p<0·0001).

**Role of the funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RKG and JG had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

We pooled estimates across studies using fixed-effects weighting to assess the strength of association between the two. Third, we calculated odds ratios for drug-resistance mutations in patients with and without TAMs. We pooled estimates across studies using fixed-effects weighting works well in scenarios with zero-cell counts. All analyses were done with STATA version 11.2.

**Figure 2: Estimated prevalence of drug resistance mutations**

Prevalence of resistance to nevirapine or efavirenz (NNRTIs), tenofovir, and cytosine analogue by presence or absence of TAM mutations. TAM=thymidine analogue mutation. Tenofovir=tenofovir disoproxil fumarate. NNRTI=non-nucleoside reverse transcriptase inhibitor.
We found a significant association between TAMs and tenofovir resistance both at the study-level and the individual-level. Studies with the highest prevalence of TAMs tended to also have the most tenofovir resistance (figure 3A, Spearman’s ρ of study-level resistance was 0.64, p<0.0001). For example, in the ten studies in which less than 15% of patients had TAMs, tenofovir resistance was present in 112 (52%) of 216 patients, whereas in the ten studies with more than 15% of patients with TAMs, tenofovir resistance was present in 333 (67%) of 496 patients (p<0.0001). We found similar associations for other drug resistance mutations, such as higher levels of nevirapine or efavirenz resistance and cytosine analogue resistance in patients with TAMs (appendix).

Within the study, patients with a TAM were more likely to also have tenofovir resistance (odds ratio 1.29, 95% CI 1.16–1.43; figure 3B). The association was maintained among patients stratified by co-administered cytisine analogue, co-administered nevirapine or efavirenz, sex, baseline viral load (<log_10,5 copies per mL vs ≥log_10,5 copies per mL), or baseline CD4 count (<100 cells per μL vs ≥100 cells per μL; figure 4). Notably, OR for tenofovir resistance was not affected by the possibility of within study drug substitution of thymidine analogue for tenofovir (figure 4). We found similar, although slightly weaker, within-study associations of TAM mutations with both nevirapine or efavirenz resistance and cytosine analogue resistance (appendix).

We assessed studies for potential within-programme drug substitutions and whether viral load confirmation was sought beforehand (table 2). We found that thymidine analogue substitution for tenofovir had occurred and that suppression was rarely confirmed before the change in treatment. Three studies implemented resistance testing before initiating tenofovir, although none excluded patients with drug resistance from initiating first line ART.

Discussion

We found TAMs that are specifically selected by zidovudine or stavudine in roughly 16% of patients with failure of tenofovir-based first-line antiretroviral regimens. TAMs were associated with greater drug resistance to all components of WHO recommended, tenofovir-containing first-line treatment. The prevalence of resistance to tenofovir reached 80% in individuals with TAMs, a result that is concerning and very much more unexpected given that the tenofovir mutation Lys65Arg and TAMs are thought to be antagonistic to one another.17 Patients with TAMs tended to have lower CD4 counts than did patients without TAMs, which is consistent with longer duration of infection or faster disease progression.

Our drug resistance prevalence estimates represent prevalence for participants with documented virological failure. Although it is important to know the prevalence of drug resistance among all participants treated with first-line therapy, this was not possible, mostly because of the absence of a clear denominator in many sites. A large

Figure 3: Study-level prevalence of TAMs and association with tenofovir resistance

(A) Scatter plot of study-level prevalence of tenofovir resistance and prevalence of TAMs by region. Markers are weighted by study size. (B) Meta-analysis of odds ratios for tenofovir resistance in participants with TAMs versus those without TAMs within individual studies. TAM=thymidine analogue mutation. Tenofovir=tenofovir disoproxil fumarate.
double mutation (L90M and T215Y) or combinations involving TAMs.

With a median follow-up of 1 year, a total of 540 patients was included in the study. The overall prevalence of TAMs was 6·6% (95% CI 5·4–8·0). Within this cohort, the most prevalent TAMs were L74V (3·3%) and M184V (2·6%).

The most prevalent double mutations were L74V and M184I (0·8%), while the most prevalent triple mutation was L74V M184I K219E (0·2%).

The study found that patients with TAMs had a significantly higher risk of virological failure compared to those without TAMs. The risk was highest for patients with both TAMs and NNRTI mutations, followed by patients with TAMs alone, and then patients with NNRTI mutations alone.

The study also found that patients with TAMs had a significantly higher risk of resistance to thymidine analogues, tenofovir, and NNRTIs, compared to patients without TAMs.

The study concluded that patients with TAMs have a significantly higher risk of virological failure and drug resistance. Therefore, it is recommended that patients with TAMs should be closely monitored for virological failure and drug resistance during treatment with tenofovir.
We have previously identified key mutations that could be used in such assays, including Lys65Arg, Lys103Asn, Val106Met, Tyr181Cys, Gly190Ala, and Met184Val, and on the basis of the present study, Asp67Asn and Lys219Gln/Glu could be added to this list. If HIV-1 drug resistance is detected with such assays, second-line ART could be initiated, taking into account the mutations identified. If they become sufficiently cheap and reliable, drug resistance assays could be used in place of viral load monitoring at treatment initiation or switches.

Our study has some limitations. The sampling was not systematic and therefore prevalence estimates might not be fully representative of countries and regions. Our drug resistance prevalence estimates represent prevalence for participants with documented virological failure. We can only estimate the overall number initiating treatment, because it was not systematically assessed. Using data from WHO and Uganda on the prevalence of virological failure, we calculate that if 15% of people initiating ART have failure at 1 year (on treatment analysis), then our data represent about 4750 patients initiating tenofovir-based first-line ART.

Although none of the studies overtly used targeted viral load testing in individuals suspected of having treatment failure, such targeting might have occurred at the clinical level, potentially biasing our estimates of TAM resistance upwards. Conversely, Sanger sequencing can miss drug resistance mutations in 30% or more of patients. Additionally, we did not assess thymidine analogue resistance conferred by mutations in the connection domain between HIV-1 reverse transcriptase and RNAseH that are known to be selected by zidovudine, leading to further underestimation of drug resistance.

Notably, stavudine selects not only for TAMs, but also for Lys65Arg in up to 20% of patients who have failure of stavudine. However, given that TAM and Lys65Arg are not selected together by a single stavudine-based regimen, exposure to stavudine would probably not explain the genotypes with both TAM and Lys65Arg that were seen in our study.

This study has important policy implications for the limitation of drug resistance as tenofovir becomes more widely used both as treatment and pre-exposure prophylaxis. First, a single point-of-care viral load test could be implemented to prevent substitution of first line zidovudine for tenofovir in patients with virological failure. Regular viral load monitoring has been advocated in the past for treatment monitoring and could identify early virological failure in patients with previously undisclosed ART and drug resistance. However, this regular monitoring might be less cost effective than...
targeted viral load measurement. Second, simple resistance test kits could both assist in screening for drug resistance before ART initiation and also contribute to population level surveillance of HIV-1 drug resistance in both treated and untreated populations—a priority in sub-Saharan Africa given the substantial mortality now recognised to be associated with HIV-1 drug resistance. These proposals should be part of a multipronged approach and subjected to cost effectiveness assessment in the wider context of other interventions that aim to limit burden of the HIV epidemic.

**References**


