

Nucleoside Reverse-Transcriptase Inhibitor Resistance Mutations Predict Virological Failure in Human Immunodeficiency Virus-Positive Patients During Lamivudine Plus Dolutegravir Maintenance Therapy in Clinical Practice

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The TANGO trial demonstrated the efficacy of lamivudine plus dolutegravir in virologically suppressed patients without previous virological failures (VFs). In this dataset from clinical practice investigating the impact of past nucleoside reverse-transcriptase inhibitor resistance on this strategy, the combination of M184V/I plus at least 1 thymidine analog-associated mutation significantly increased the risk of VF.

Keywords. dolutegravir; HIV; lamivudine; maintenance therapy; resistance-associated mutations.

After the results of randomized [1, 2] and observational [3] studies, lamivudine (3TC) plus dolutegravir (DTG) dual therapy (DT) represents a recommended maintenance regimen for human immunodeficiency virus (HIV)-positive patients [4, 5]. Due to excellent tolerability, low potential for drug-drug interactions [3], and high virological efficacy even in the setting of naive patients [6], this strategy offers advantages over other DTs based on boosted-protease inhibitors or rilpivirine.

However, long-term follow-up data about the incidence and predictors of virological failure (VF) are still lacking, with some observational reports suggesting worse virological outcomes

for patients starting the DT with a shorter time of virological suppression and the presence of M184V/I at historical genotype [7, 8]. Although M184V/I is well recognized as the key resistance mutation for 3TC, thymidine analog-associated mutations (TAMs) variably decrease susceptibility to all nucleoside reverse-transcriptase inhibitors (NRTIs), including 3TC [9]. However, the role of TAMs in the setting of a maintenance therapy with 3TC-DTG DT has not been explored. The aim of this study was to investigate risk factors of VF, particularly NRTI resistance associated-mutations (RAMs), in a cohort of treatment-experienced, virologically suppressed patients starting 3TC-DTG.

METHODS

An observational cohort from 3 large University hospitals of patients starting 3TC-DTG, with suppressed viral load (HIV-ribonucleic acid [RNA] >50 copies/mL) at the time of switch (baseline) and with no evidence of hepatitis B virus chronic infection, was retrospectively queried. No other eligibility criteria (such as lack of previous VF, a minimum time of continuous virological suppression before switch, and availability of pre-treatment genotype with no documented RAMs) was used for the cohort selection, allowing for the inclusion of patients more representative of clinical practice.

Predictors of time to VF (defined as the first of 2 consecutive HIV-RNA >50 copies/mL or a single HIV-RNA \geq 200 copies/mL) were identified through a multivariable Cox regression model with a stepwise selection of covariates (covariates associated with the outcome at a $P > .05$ were excluded). The 2019 updated IAS-USA drug resistance mutations list was used to identify major and minor NRTI RAMs in all available genotypic resistance tests obtained before baseline. If a specific RAM was detected at least once in the past genotypic tests, the mutation was considered to be present at baseline. Resistance associated-mutations to integrase inhibitors (INIs) could not be considered because of the very limited number of integrase genotypes available.

Five different Cox regression models were generated, exploring the effect of different patterns of NRTI RAMs on VF (predictors other than RAMs were the same in all models). The variables expressing NRTI RAMs were identified as follows: (1) presence versus absence of any NRTI RAM; (2) presence versus absence of at least 1 TAM (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E); (3) presence versus absence of M184V/I (with or without other RAMs); (4) presence versus absence of M184V/I not associated with TAMs; (5) presence versus absence of M184V/I combined with at least 1 TAM. The role of any non-TAM was not specifically studied because of the low number of patients harboring the K65R/E/N mutation (the

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only other non-TAM associated with decreased susceptibility to 3TC) and its collinearity with M184V/I.

Patient Consent Statement

This study was performed in accordance with the principles of the Declaration of Helsinki and received approval from each independent local ethics committee (Study Coordination Site Protocol No. 5284/15). All patients signed informed consent forms.

RESULTS

Six hundred sixty-nine patients were eligible for the study, 338 of whom (50.5%) also had at least 1 previous NRTI genotypic resistance test (GRT). The study population was mostly

composed of men (70.4%), median age of 52 years, and median HIV duration of 15 years. As expected, patients with and without a non-TAM-harboring virus differed for several demographical and viro-immunological factors, as summarized in Table 1.

Twenty-three VFs were detected over 1.9 years of median follow-up time (1.6 per 100 patient-years of follow-up). In the overall population, the estimated probability of VF was 1.6% (95% confidence interval [CI], 0.8–3.0), 4.0% (95% CI, 2.5–6.2), and 5.4% (95% CI, 3.5–8.1) at 1, 2, and 3 years, respectively.

Starting from a model that included risk factor for HIV infection, age, detectable HIV-RNA at baseline, presence of TAMs at last GRT, nadir CD4 and zenith HIV-RNA, viral subtype and HIV-RNA at last GRT, time of virological suppression, and previous VF with an INI-containing regimen, the presence of any

Table 1. Characteristics of the Study Population, Overall and Separately According to Presence or Absence of at Least on NRTIs-RAM

Variables	All Population (n = 669)	Presence of RAMs at Historical Genotype (n = 75)	Absence of RAMs at Historical Genotype (n = 263)	PValue
Age (years)	52 (45–59)	54 (51–59)	49 (40–56)	<.001
Male gender	474 (70.9)	53 (70.7)	195 (74.1)	.548
Caucasian ethnicity	611 (91.3)	69 (92.0)	242 (92.0)	.997
Risk Factor for HIV				<.001
Heterosexual	286 (42.8)	28 (37.3)	113 (43.0)	
MSM	270 (40.3)	24 (32.0)	128 (48.6)	
IDUs	95 (14.2)	20 (26.7)	17 (6.5)	
Other	18 (2.7)	3 (4.0)	5 (1.9)	
Anti-HCV serostatus	127 (19.0)	20 (26.7)	29 (11.0)	.001
Zenith HIV-RNA				.843
≤10 ⁵ cp/mL	344 (51.4)	39 (52.0)	133 (50.6)	
>10 ⁵ and ≤5 × 10 ⁵ cp/mL	193 (28.9)	26 (34.7)	85 (32.3)	
≥ 5 × 10 ⁵ cp/mL	92 (13.8)	10 (13.3)	44 (16.7)	
Unknown	40 (6.0)	0 (0.0)	1 (0.4)	
Nadir CD4 ≤200 cells/μL	291 (45.8)	42 (56.8)	94 (36.0)	.001
CDC stage C (n = 426)	121 (28.4)	17 (22.7)	33 (12.6)	.011
Time since HIV diagnosis (years)*	15 (8–22)	24 (19–28)	9 (5–14)	<.001
Time of cumulative exposure to ARVs*	12 (6–19)	20 (18–22)	7 (4–11)	<.001
Time of viral suppression (years)*	8 (4–11)	8 (4–12)	5 (3–8)	<.001
HIV-RNA at last genotype (cp/mL)*	21 450 (3300–88 118)	3733 (431–15 220)	35 383 (6759–126 465)	<.001
Detectable baseline HIV-RNA**	218 (32.6)	26 (34.7)	99 (37.6)	.638
ARV therapy before switch:				.013
2 NRTIs+3rd drug	410 (61.3)	34 (45.3)	166 (63.1)	
Two-drug regimen	231 (34.5)	35 (46.7)	88 (33.5)	
Other combinations	28 (4.2)	6 (8.0)	9 (3.4)	
Reasons for switch to 3TC+DTG				.783
Proactive switch	243 (36.3)	26 (34.7)	93 (35.4)	
Toxicity	214 (32.0)	24 (32.0)	93 (35.4)	
Other/unknown	212 (31.7)	25 (33.3)	77 (29.2)	
HIV Viral Subtype	n = 316	n = 68	n = 247	.085
B	262 (82.9)	63 (92.7)	198 (80.3)	
A	7 (2.2)	0	7 (2.8)	
C	9 (2.9)	0	9 (3.6)	
CRF	23 (7.3)	1 (1.5)	22 (8.9)	
F	11 (3.4)	3 (4.4)	8 (3.2)	
Other	2 (1.3)	1 (1.5)	3 (1.2)	

Table 1. Continued

Variables	All Population (n = 669)	Presence of RAMs at Historical Genotype (n = 75)	Absence of RAMs at Historical Genotype (n = 263)	PValue
NRTIs-RAMs				na
TAMs			na	
M41L	31 (9.2)	31 (9.2)		
D67N	22 (6.5)	22 (6.5)		
K70R	20 (5.9)	20 (5.9)		
L210W	14 (4.1)	14 (4.1)		
T215YF	30 (8.9)	30 (8.9)		
K219QE	20 (5.9)	20 (5.9)		
Non-TAMs				
M184V/I	48 (14.2)	48 (14.2)		
K65R/E/N	4 (1.2)	4 (1.2)		
K70E	1 (0.3)	1 (0.3)		
L74V	4 (1.2)	4 (1.2)		
Y115F	1 (0.3)	1 (0.3)		
Other NRTIs-RAMs				
T69Ins	0 (0)	0 (0)		
A62V	4 (1.2)	4 (1.2)		
V75I	1 (0.3)	1 (0.3)		
F77L	1 (0.3)	1 (0.3)		
F116Y	1 (0.3)	1 (0.3)		
Q151M	1 (0.3)	1 (0.3)		
Previous failure with an INI-based regimen	14 (2.1)	2 (2.7)	9 (3.4)	.745
Previous failure with a 3TC/FTC-based regimen	287 (42.9)	62 (82.7)	74 (28.1)	<.001

Abbreviations: ARVs, antiretrovirals; CDC, Centers for Disease Control and Prevention; DTG, dolutegravir; FTC, emtricitabine; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug user; INI, integrase inhibitor; MSM, men who have sex with men; na, not applicable; NRTI, nucleoside reverse-transcriptase inhibitor; RAM, resistance associated-mutation; RNA, ribonucleic acid; TAM, thymidine analog mutation; 3TC, lamivudine.

NOTES: Values within brackets are expressed as percentages except for * median values (interquartile range). Bold test refers to statistically significant differences between the two groups.

**Detectable HIV-RNA indicates any value measured below 50 copies/mL.

NRTIs-RAM (Cox model 1) was not predictive of VF, and this variable was therefore excluded from the model ($P = .118$); similarly, the presence of at least 1 TAM (Cox model 2) and the presence of M184V/I in the absence of TAMs (Cox model 4) were not associated with the outcome ($P = .243$ and $P = .693$, respectively). In contrast (Cox model 3), the presence of M184V/I (versus its absence; adjusted hazard ratio [aHR], 3.31; 95% CI, 1.02–10.74; $P = .046$) and, particularly (Cox model 5), the combination of M184V/I plus at least 1 TAM (versus no M184V/I plus TAMs; aHR, 4.63; 95% CI, 1.19–17.94; $P = .027$) predicted time to VF. In all 5 models, duration of viral suppression and previous VF with INIs were also significantly associated with VF (Table 2 summarizes results of the Cox models). Because genotypic resistance tests for INIs were not available for a substantial number of patients (648 of 669, 96.8%), no association between INI-RAMs and VF could be assessed; a minor mutation was detected (T97A) in only 3 patients in a previous genotypic test, including 1 patient who subsequently experienced VF.

The effect size of M184V/I with and without TAMs on VF was also estimated: compared with patients with no RAMs, those harboring a virus with the M184V/I but no TAMs had no increased risk of VF (aHR, 1.88; 95% CI, 0.23–15.07; $P = .554$), whereas the association of M184V/I and at least 1 TAM

confirmed a significant association with the outcome (aHR, 4.40; 95% CI, 1.12–17.24; $P = .034$), after adjusting for previous VF with INIs (aHR, 6.03; 95% CI, 1.29–28.25; $P = .023$) and time of virological suppression (>2 versus ≤ 2 years; aHR, 0.27; 95% CI, 0.09–0.82; $P = .021$).

Since a potential interaction between time of virological suppression at baseline and RAMs has been previously suggested [6, 7], a sensitivity analysis was performed to estimate the effect of M184V/I with and without TAMs in the subgroups of patients with ≤ 7 and >7 years of virological suppression (median value in our population). Whereas in the group with longer time of virological control no association between RAMs and outcome was detected, patients with shorter duration of virological suppression had a higher risk of VF when M184V/I and TAMs were simultaneously present (compared with absence of RAMs; aHR, 11.56; 95% CI, 2.22–60.08; $P = .004$), but not when M184V/I was present without TAMs (compared with absence of RAMs; aHR, 4.90; 95% CI, 0.58–41.79; $P = .146$).

DISCUSSION

Lamivudine-DTG DT has demonstrated efficacy comparable to 3-drug regimens in both naive [6, 10] and treatment-experienced [1–3] patients, but previous VFs could potentially

Table 2. Predictors of Virological Failures According to Different Cox Regression Models

Exposure Variable	Models 1, 2, 4* aHR (95% CI)	PValue	Model 3** aHR (95% CI)	PValue	Model 5*** aHR (95% CI)	PValue
M184V/I (presence vs absence)			3.31 (1.02–10.74)	.046		
M184V/I with TAMs (presence vs absence)					4.63 (1.19–17.94)	.027
Previous failure on an INI-based regimen (at least 1 vs none)	5.84 (1.28–26.64)	.025	6.41 (1.36–30.18)	.019	5.51 (1.15–26.50)	.033
Time of virological suppression (>2 years vs ≤2)	0.29 (0.10–0.86)	.023	0.27 (0.09–0.80)	.018	0.23 (0.08–0.74)	.013

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; INI, integrase inhibitor; TAM, thymidine analog mutation.

NOTES: All Cox models were built after a stepwise selection of the following covariates (variables associated with the outcome at a $P > .05$ were excluded at each step): age, ethnicity, risk factor for HIV infection, detectable HIV-RNA at baseline, nadir CD4 and zenith HIV-RNA, viral subtype and HIV-RNA at last genotypic resistance test, time of viral suppression at baseline and previous virological failure (VF) with an INI-containing regimen. The variables representing RAMs were different according to the 5 models.

*In model 1, we used “presence of any NRTI-RAM versus none”; in model 2, “presence of at least 1 TAM versus none”; in model 4, “presence of M184V/I without TAMs versus all other combinations of RAMs or no RAMs.” Because none of these variables was associated with VF, they were not included in their respective Cox models.

**Model 3 included the “presence of M184V/I versus absence of M184V/I.”

***Model 5 included “presence of M184V/I plus at least 1 TAM versus all other combinations of RAMs or no RAMs.”

limit the feasibility of this strategy in a substantial proportion of patients. In a recent study from clinical practice [11], patients starting 3TC-DTG with and without TANGO’s enrollment criteria had comparable outcomes. In addition, a recent pilot study [12] reported no VF in patients on 3TC-DTG, regardless of previous 3TC resistance. However, previous observational studies [7, 8] suggested an increased risk of VF during 3TC-based DT when M184V/I was present in combination with a shorter time of viral suppression.

Our study showed that a shorter duration of virological suppression and the presence of M184V/I were independent predictors of VF; however, M184V/I alone was less accurate in predicting the outcome than its combination with TAMs. Indeed, M184V/I and TAMs appear to synergize with each other since TAMs alone were not associated with VF. In addition, the effect was stronger in the subgroup of patients with ≤7 years of virological suppression. Whether duration of viral suppression is a proxy for the size of the reservoir of resistant viral quasispecies or for patient’s adherence, or conversely it represents a real confounder in the causal path between RAMs and VF, is not clear at this time; however, these variables deserve attention when switching a patient to 3TC-DTG DT.

Another predictor of VF found in the present study was a history of VF with any INI. In our cohort, genotypic resistance test for INIs was rarely performed, mainly because of the low number of patients failing during a previous INI-containing regimen (14 patients) but also for unavailability of this test until a more recent calendar year. As expected, no major INIs RAMs were detected, and only a minor mutation, T97A, was detected in 3 patients, one of whom subsequently experienced VF. Because no impact on failure of first-generation INI has been reported [13], a causal relationship of T97A alone on VF is debatable. A previous failure with an INI could therefore represent a proxy for reduced treatment adherence; however, this observation deserves further clarifications.

Our work clearly has some limitations, including the retrospective nature of the study and the lack of previous GRT for a significant number of patients. Moreover, given the potential

differences among clinical centers in choosing simplification regimens or timing of follow-up visits, other predictors of VF could be detected in other cohorts. However, after stratifying the Cox regression for clinical center (data not shown), predictors of VF were confirmed across the different centers, underlining the generalizability of our findings at least within this data set.

Our study has the strengths of a long follow-up period and a large sample size. The use of multiple multivariable models and sensitivity analysis, as well as the biological plausibility of the predictors analyzed, also increase the reliability of our findings.

CONCLUSIONS

In conclusion, the possibility of a reduced efficacy of the 3TC-DTG DT should be kept in mind when previous virological failures are present and past TAMS and M184V/I are documented, particularly for patients with limited duration of virological suppression. In the absence of randomized studies comparing DT and 3-drug regimens in virologically suppressed patients with past drug resistance and/or treatment failures, it remains to be established whether these factors represent an absolute contraindication to switching to 3TC-DTG DT, but caution is recommended not to jeopardize future treatment options.

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