

Type-1 Thymidine-associated mutations (TAMs) but not K65R mutation play a role in determining virological failure to combined rescue therapy with tenofovir and stavudine

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Abstract

Background: Tenofovir (TDF) and thymidine analogues seem to determine different pathways of viral evolution under selective pressure on HIV reverse transcriptase.

Methods: Multicentric retrospective analysis on patients starting TDF/stavudine (d4T) after HAART failure. Linear regression analyses were used to determine predictors of HIV-RNA change at 6 months of therapy; risk of virological failure (VF = time to first HIV-RNA >500 cp/ml occurred after 3 months of therapy) was calculated using KM estimate, and its determinants by multivariate Cox model.

Results: 172 patients (pts) included: all, but 2, had been previously exposed to thymidine analogues, AZT in 88% and d4T in 77%. The mean viro-immunological values were 4.26 log₁₀/ml and 229 cell/mm³ for HIV-RNA and CD4 cell count, respectively. At baseline, a genotype (GRT) was available in 136 pts; the most frequent mutations were: M184V (76%), T215Y/F (42%), M41L (33%), D67N (29%), L210W (22%); 33% had at least 3 TAMs. Any single type-1 TAM mutation (M41L: $\beta = +0.969$, $P=0.001$; L210W: $\beta = +0.967$, $P=0.003$; T215Y: $\beta = +1.250$, $P<0.0001$) had a negative effect on the change in HIV-RNA at 6 months, whereas among type-2 TAMs mutations, only D67N showed a trend for a negative effect ($\beta = +0.582$, $P=0.051$), both K70R ($\beta = -0.027$, $P=0.934$), and K219Q ($\beta = -0.938$, $P=0.083$) had no effect. Presence of M184V mutation was also related with a greater reduction in HIV-RNA ($\beta = -0.759$, $P=0.019$). The KM estimated risk of VF at 6-months was 22%. Multivariate Cox model confirmed the detrimental effect of type-1 TAMs after adjustment for several more type-1 TAM mutation we found a greater risk of VF (AHR=1.65; 95%CI 1.19-2.29; $P=0.003$), and the presence of the entire pattern had a two-times risk of VF (AHR=2.53; 95%CI 1.01-6.33; $P=0.05$). Conversely, M184V was associated with a protective effect (AHR=0.36%CI 0.15-0.87; $P=0.02$). In 13 GRT at VF, no K65R mutation was detected, whereas a trend for an increasing prevalence of d4T-associated mutations was found (D67N from 23% to 46%; V118I from 15% to 31%; L210W from 31% to 67%; K219Q from 7.7% to 15%).

Conclusions: In pts previously exposed to NRTIs, GRT at baseline may predict virological success to rescue therapy combining d4T with TDF: type-1 TAMs negatively affect virological outcome, while M184V seems to have a favourable impact. Accumulation of TAMs, but not K65R selection, is the most relevant GRT pattern at virological failure to TDF/d4T combination.

Background

- TDF appears to be a useful agent as part of optimised antiviral salvage therapy in treatment-experienced patients
- In all trials conducted to date, TDF has been used in thymidine-analogue sparing regimens (combined with either 3TC or didanosine, or 3TC and ABC)
- Tenofovir (TDF) and thymidine analogues seem to determine different pathways of viral evolution under selective pressure on HIV reverse transcriptase

Objectives

- To evaluate efficacy and safety of d4T/TDF backbone combination in late line antiretroviral therapy
- To assess clinical and virological determinants of treatment success
- To determine evolution of genotypic pattern during drug exposure

Methods

- Multicentric retrospective pooled data analysis (10 Italian centers involved)
- Criteria for inclusion:
 - to start d4T/TDF after virological failure to HAART;
 - To have at least a 1-month evaluation during follow-up
- Demographic, clinical, viro-immunological variables collected at baseline and during follow-up

Statistical Analysis

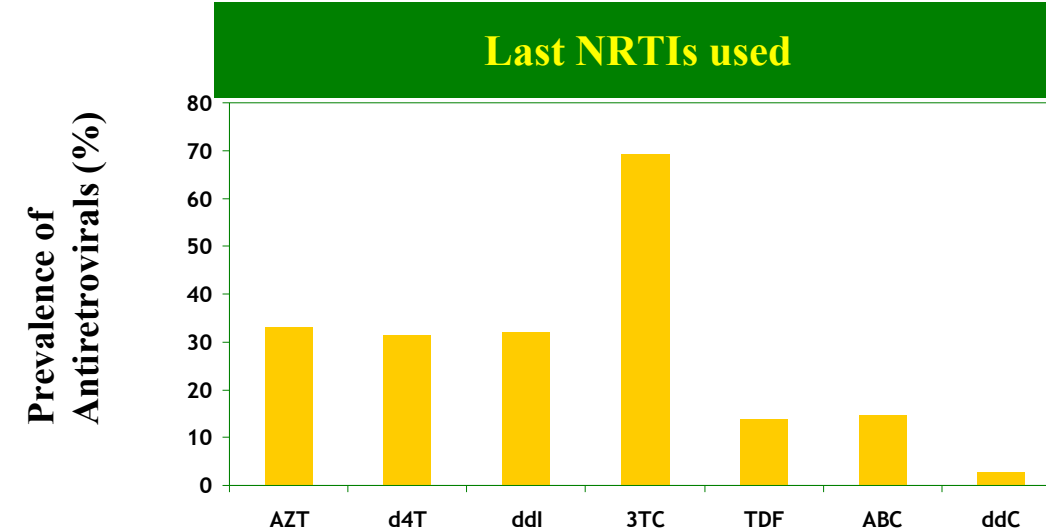
- HIV-RNA and CD4 count mean changes from baseline at various times during follow-up
- Linear regression analyses were used to determine predictors of HIV-RNA change at 6 months of therapy
- KM estimates of virological failure (time to first HIV-RNA >500 cp/ml occurred after 3 months of therapy)
- Independent predictors of virological failure by multiple Cox regression

General characteristics

Patients (n=172)	
Age (years), mean (range)	41 (20-70)
Previous AIDS, n (%)	42 (24.4)
HIV-RNA log ₁₀ /ml, median (IQR)	4.26 (3.65-4.97)
CD4 cell count/mm ³ , median (IQR)	229 (116-383)
Number of previous HAART failures, median (IQR)	2 (1-3)
Time (months) on HAART, median (IQR)	56 (33-72)

Previous NRTI exposure

Type and Time of previous NRTI exposure	Patients (n=172)
AZT previous exposure, n (%)	152 (88.4)
Mean time on AZT (months), median (IQR)	29 (13-49)
3TC previous exposure, n (%)	166 (96.5)
Mean time on 3TC (months), median (IQR)	36 (20-53)
ddI previous exposure, n (%)	123 (71.5)
Mean time on ddI (months), median (IQR)	19 (7-33)
d4T previous exposure, n (%)	133 (77.3)
Mean time on d4T (months), median (IQR)	36 (17-51)
ddC previous exposure, n (%)	40 (23.3)
Mean time on ddC (months), median (IQR)	8 (4-18)
ABC previous exposure, n (%)	57 (33.1)
Mean time on ABC (months), median (IQR)	9 (4-15)
TDF previous exposure, n (%)	36 (20.9)
Mean time on TDF (months), median (IQR)	7 (2-11)



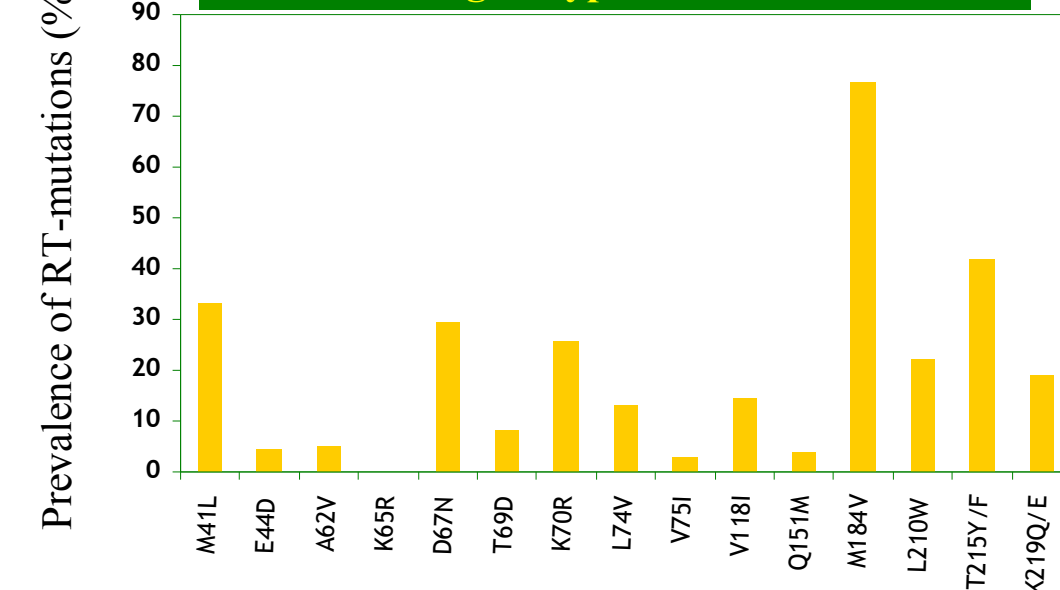
Most frequent Antiretrovirals Combined with d4T/TDF

Patients (n=172)	
PI-containing regimen, n (%)	
- LPV/r	71 (41.3)
- NFV	18 (10.5)
NNRTI-containing regimen, n (%)	
- NVP	10 (5.8)
- EFV	11 (6.4)
NRTI-containing regimen, n (%)	
- 3TC	21 (47.7)
- ddI	14 (31.8)
- ABC	3 (6.7)

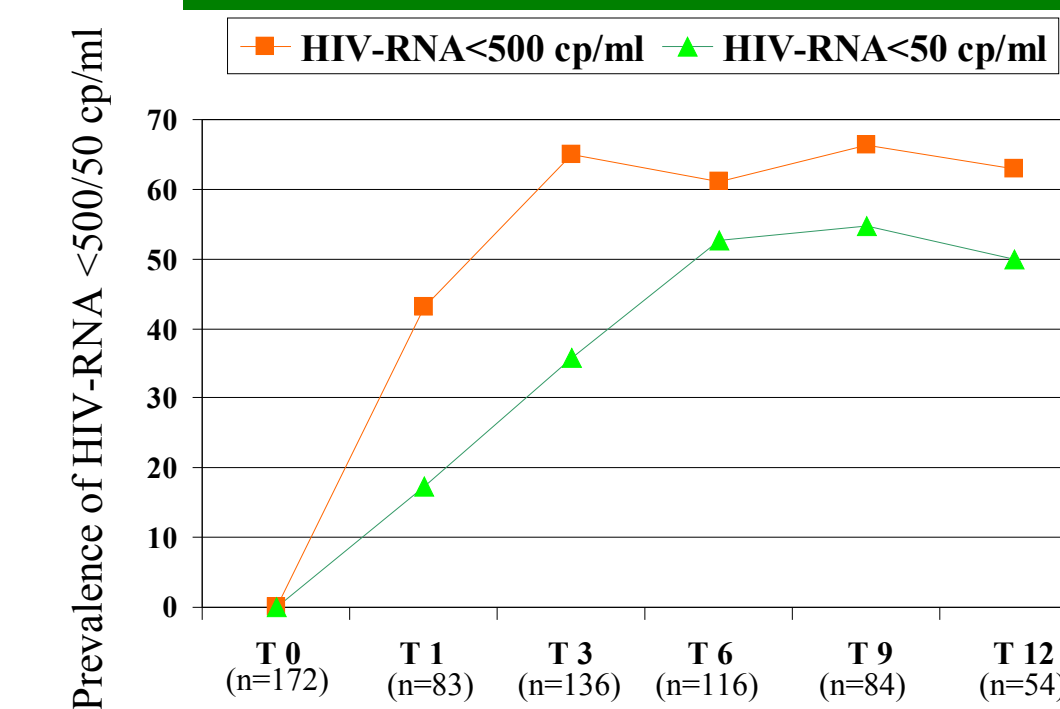
Genotypic mutation pattern at baseline

Number of PI mutations, mean (range)	1.61 (0-12)
Number of NNRTI mutations, mean (range)	0.73 (0-4)
Number of TAMs, mean (range)	1.91 (0-7)
At least 3 TAMs, n (%)	47 (34.6)
Type 1 TAMs (M41L, L210W, T215Y), n (%)	26 (19.1)
Type 2 TAMs (D67N, K70R, K219Q), n (%)	14 (10.3)

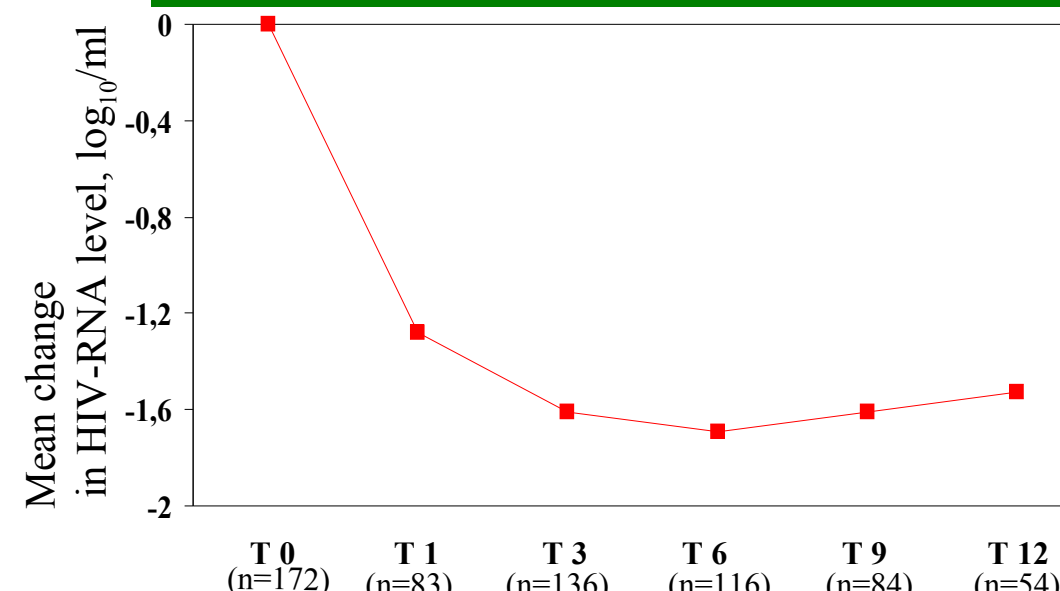
RT genotype at baseline



Percentage of patients with HIV-RNA <500/50 c/ml at various times during follow-up



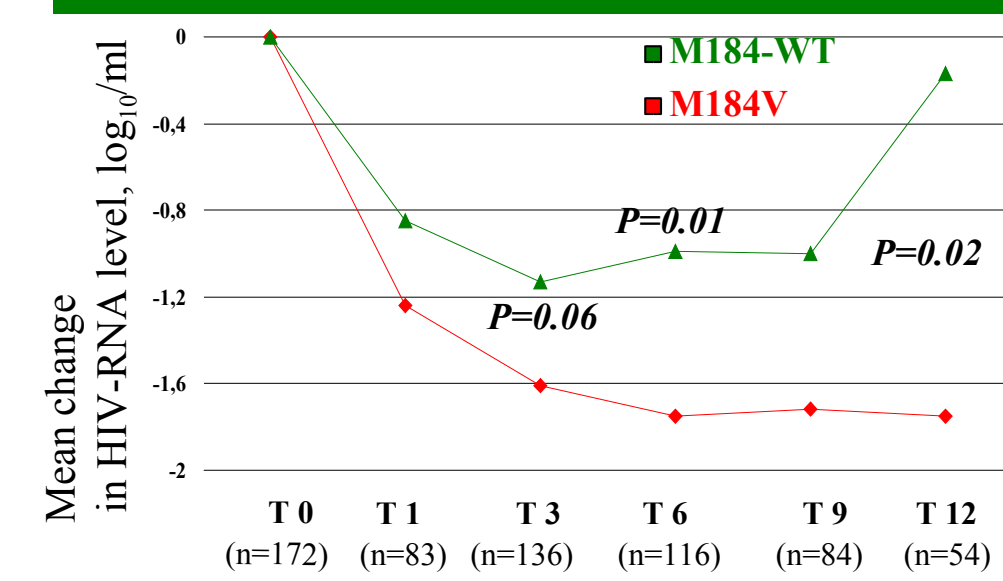
Mean logarithmic changes of HIV-RNA from baseline



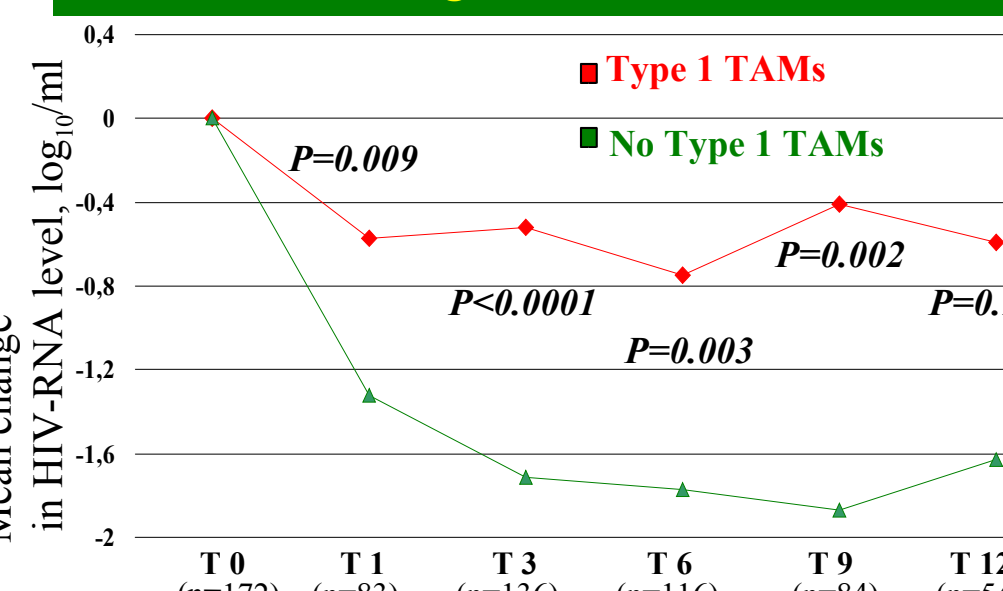
Effect of RT-mutation on HIV-RNA change at 6 months of therapy by linear regression

Variables	Coefficient Beta	P
M41L	+0.969	0.001
D67N	+0.582	0.051
K70R	-0.027	0.934
M184V	-0.759	0.019
L210W	+0.967	0.003
T215Y	+1.250	<0.0001
K219Q	-0.938	0.083

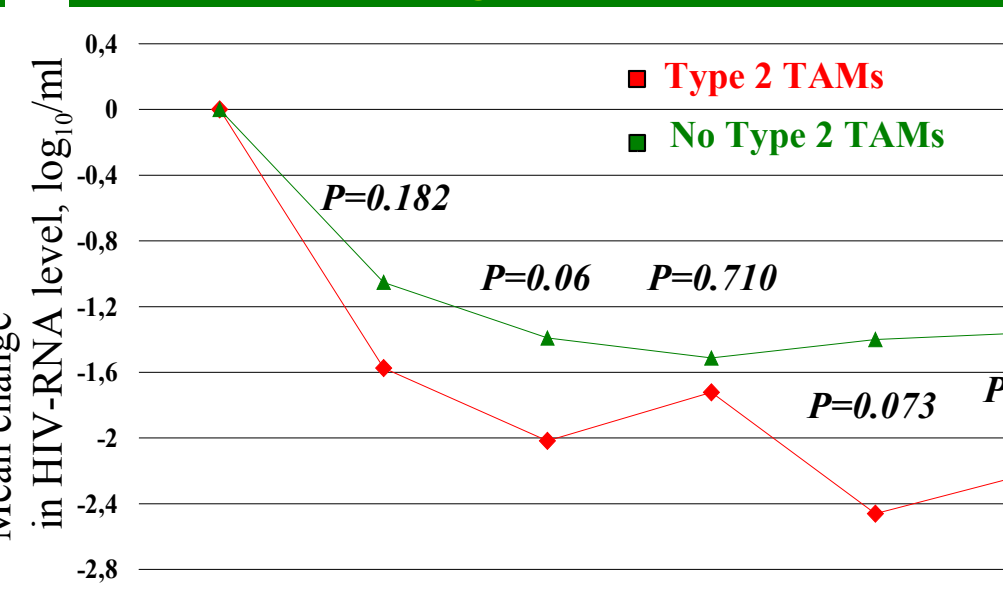
Mean logarithmic changes of HIV-RNA from baseline according with M184V at baseline



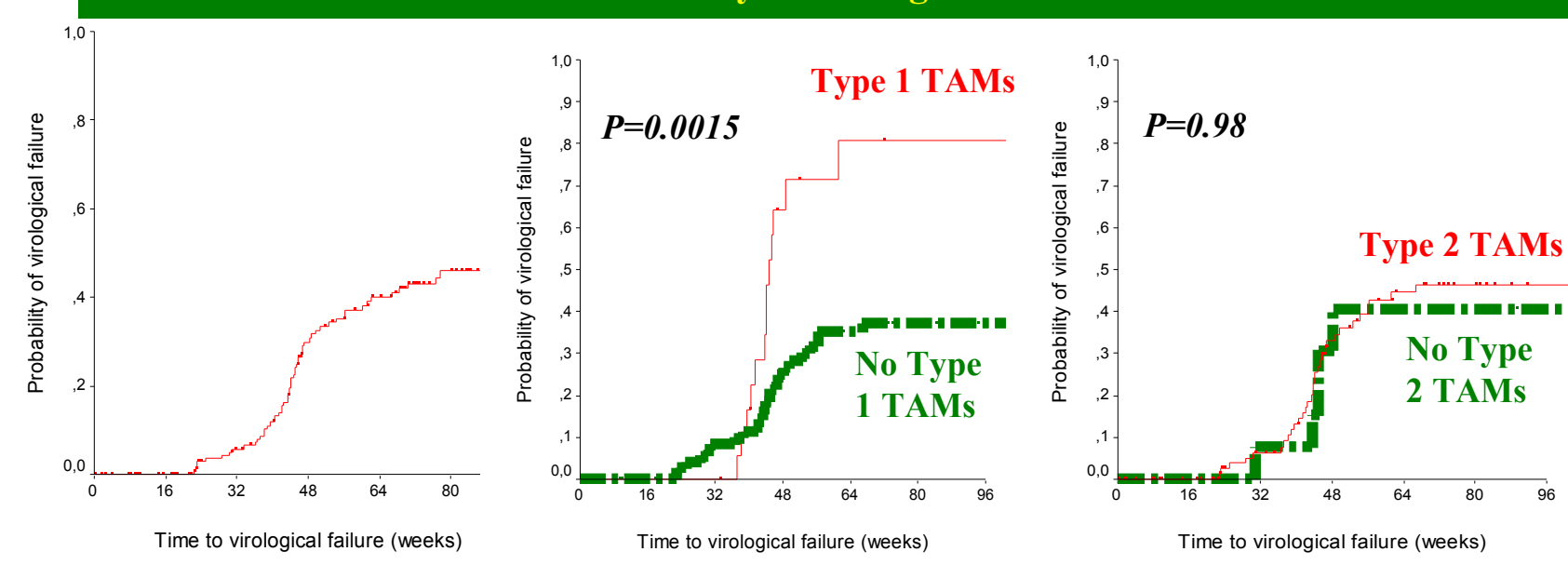
Mean logarithmic changes of HIV-RNA from baseline according with TAMs1 at baseline



Mean logarithmic changes of HIV-RNA from baseline according with TAMs2 at baseline



Probability of virological failure



Genotypic predictors of virological failures

Variables	Model 1 (at least 1 TAMs)		Model 1 (entire TAMs pattern)	
	HR (95%CI)	P	HR (95%CI)	P
Previous exposure to TDF	0.63 (0.26-1.53)	0.309	0.74 (0.31-1.76)	0.496
Previous exposure to d4T	0.88 (0.18-4.27)	0.877	1.01 (-0.21-4.81)	0.989
HIV-RNA at baseline, log ₁₀ /ml	1.21 (0.65-2.28)	0.548	1.79 (0.64-2.16)	0.596
CD4 at baseline (*50 cell/mm ³)	1.04 (0.94-1.16)	0.451	1.05 (0.95-1.16)	0.381
Number of previous failures	1.19 (0.95-1.49)	0.139	1.18 (0.94-1.49)	0.159
Type 1 TAMs	1.65 (1.19-2.29)	0.003	2.53 (1.01-6.33)	0.048
Type 2 TAMs	0.96 (0.65-1.39)	0.817	0.80 (0.27-2.35)	0.689
M184V	0.36 (0.15-0.88)	0.024	0.32 (0.13-0.79)	0.076
Number of PI mutations	1.18 (0.99-1.40)	0.07	1.18 (0.98-1.41)	0.013

GRT evolution at virological failure (17/58)

Mutation	Prevalence at baseline (%)	Prevalence at failure (%)
M41L	61.5	53.8
K65R	0	0
D67N	23.1	46.2
K70R	23.1	23.1
M184V	69.2	38.5
L210W	30.8	66.7
T215Y	69.2	69.2
K219Q	7.7	15.4

Conclusions

- The combination TDF and d4T appears to be effective in patients with previous exposure to antiretrovirals in the context of salvage therapy among patients with previous extended exposure to NRTIs
- Genotypic pattern at baseline may predict virologic success. TAMs (especially type 1) negatively affect efficacy whereas M184V has a protective role
- At failure, no K65R mutation were selected and accumulation of TAMs seems to be the most relevant GRT pattern. This finding suggest that combining thymidine-analogues with TDF could have a positive effect on virological response perhaps through a prevention of the K65R emergence.
- Additional specific studies are required to investigate whether combination of TDF with d4T could determine pharmacological interactions or interactions at the virus replication level more favourable than other NRTIs combined up to now with TDF