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Thymidine Analogue Mutation Profiles: Factors Associated with Acquiring Specific Profiles and Their Effect on Virologic Response to Therapy

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BACKGROUND

Small studies have suggested that HIV-1 develops TAMs by one of two distinct pathways defined as TAM1 (including 41L, 210W, and215Y) or TAM2 (including 67N, 70R, and 219E/Q).

The determinants of one or the other pathway remain unclear (Figure 1).

Similarly, the virological response to zidovudine/stavudine-containing regimens in the presence of TAM1/TAM2 has been poorly evaluated.

METHODS

Study Population

We considered the first chronological genotypic test result showing ≥1 TAMs in the EuroSIDA database.

Frozen plasma samples stored in the EuroSIDA repository were selected and sent to two central laboratories for genotypic analysis (IrsiCaixa Foundation & Lluita contra la SIDA Foundation, Badalona, Spain and International Clinical Virology Center, Buckinghamshire, UK).

TAM1 and TAM2 genotypic profiles were defined in accordance with previous literature (Figure 2).

Sequencing

HIV-1 RNA was isolated from patient blood plasma using QIAamp kit according to manufacturer's instructions. Sequence analysis of HIV-1 PR and RT reading frames was performed using the Trugene HIV-1 Genotyping Kit and OpenGene DNA Sequencing System according to the manufacturer's recommendations.

Statistical analysis

The factors associated with carrying a TAM2 profile (vs. carrying a TAM1 profile) were investigated using a logistic regression analysis.

Month 6 viral load reductions upon starting a new regimen including zidovudine/stavudine were also compared using a linear regression analysis for censored data (due to some viral load values at 6 month being below the lower limit of the assay).

RESULTS

Study Population

We originally included 733 patients with a genotypic sequence in which one or more TAMs had been detected.

Among the 625 patients with ≥2 TAMs, 343 (54.9%) fell naturally into the defined TAM1 profile, 114 (18.2%) into a TAM2 profile and the remaining 168 (26.9%) individuals could not be classified as either of these profiles ('mixed profiles').

Our analysis focussed on 565 patients classifiable as TAM1 or TAM2 profile (including 108 with a single TAM who were classifiable by default, Table 1) while the 168 patients (22.9%) with mixed profile were not further assessed.

TAM Clustering

Table 2 compares the observed probabilities of falling into TAM1/TAM2 profiles with those that could be expected to occur by chance alone for a total number of TAMs of 2 or 3. Thus, for example, since for TAMs up to a total number of 3 we are considering the mutations 41L, 210W, 215Y, 67N, 70R, 219E and 219Q and given that 219E and 219Q cannot be detected simultaneously, 30 possible triplets including these mutations can be constructed (i.e. 7 choose 3 - 5 = 7!/4!3! - 5 = 7x6x5/3x2 - 5 = 30). Of these one would be of type TAM1 (41L, 210W, 215Y), two of type TAM2 (67N, 70R, 219E and 67N, 70R, 219Q) and the remaining 27 mixed profiles. Similar considerations were used to construct all possible profiles including two TAMs. It is clear from the table that TAM1/TAM2 profiles were detected in our case study more frequently than would be expected by chance alone and that, in contrast, the mixed profiles should have occurred more frequently if the suggested clustering was not a real phenomenon.

Figure 1

TAMs pathways

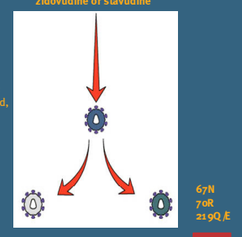

Figure 2

Number of TAMs

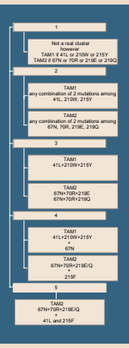

Table 1

Classifiable profiles according to total number of TAMs

Number of TAMs		TAM 1	n (%)	TAM 2	n(%)
1	n=108	41L	21 (19.4%)	67N	3 (2.8%)
		210W	3 (2.8%)	70R	35(32.4%)
		215Y	43 (39.8%)	219E /Q	3 (2.8%)
2	n=168	210W+215Y	19 (11.3%)	67N+70R	12 (7.1%)
		41L+215Y	103 (61.3%)	70R+219E /Q	4 (2.4%)
		41L+210W	6 (3.4%)	67N+219E /Q	5 (2.9%)
3	n=204	41L+210W+215Y	114 (55.9%)	67N+70R+219E /Q	37 (18.1%)
4	n=172	41L+**67N**+210W+215Y	101 (58.7%)	67N+70R+**215F**+219E /Q	30 (17.4%)
5	n=60			**41L**+67N+70R+**215F**+219E /Q	26(43.3%)
Total			410 (55.9%)		155 (21.1%)
Total ≥ 2 TAMs			343 (54.9%)		114 (18.2%)

Other characteristics of study population

Overall, plasma samples had been collected, on average, on January 1998 (range: February 1992-March 2003). Eighty-two patients were females (14.5%) and 324 homosexual men (57.4%). At time of sampling the viral load was 4.18 $\log_{10}$ copies/mL (range: 2.70-7.00) and CD_4 count was 205 cells/µl (range:1-1,123), patients had been previously treated with antiretroviral therapy for a median of 46 months (r: 0-108), and with triple combination therapy for 13 months (range: 0-79). This is the actual time spent on a potent combination therapy (i.e. interruptions have been taken into account). Forty patients (7.1%) were antiretroviral naïve at the time of the test so, potentially, the TAM(s) had been transmitted at time of infection in this group. At the time of the test, the median exposure to zidovudine mono/dual therapy was 22 months (IQR: 8-43) and 6 months (IQR: 0-15) to stavudine therapy. Overall, only 12.2% of patients (n=69) had not previously received either stavudine or zidovudine mono- or dual-containing therapy while 2.0% (n=11) had previously received only stavudine, 63.5% (n=359) only zidovudine and 22.3% (n=126) both stavudine and zidovudine. The previous total exposure to abacavir-containing therapy in our patients was 527 months was and patients have previously used tenofovir for a total of 13 months.

Factors associated with selection of TAM2 vs. TAM1

Longer duration of exposure to zidovudine mono/dual therapy showed a strong independent association with an increased probability of observing a TAM2 profile (OR=1.25 per year longer of exposure, 95% CI: 1.14-1.36, p=0.0001. We also found a strong correlation between TAM2 profiles and the concomitant absence of mutation 118I (OR=0.34, 95% CI: 0.20-0.60, p=0.0002). In addition, a higher viral load at the time of test was also associated with a lower chance of observing a TAM2 profile (OR=0.81 per $\log_{10}$ higher 95% CI: 0.65-1.00; p=0.05, Table 3).

Virological response to zidovudine/stavudine-containing regimens according to profile

The crude 6 month viral reduction was comparable between patients who started a stavudine-containing regimen (0.71 $\log_{10}$ copies/mL, 95% CI: 0.43-1.10) and those starting a zidovudine-containing regimen (1.17, 95% CI: 0.11-1.56). On the other hand, in the presence of TAM2 profiles, the crude reductions were 1.27 $\log_{10}$ copies/mL (95% CI: 0.89-2.31) in patients treated with stavudine but only 0.19 $\log_{10}$ copies/mL (95% CI: -0.37; +1.29) in those treated with zidovudine. The adjusted mean difference was -0.96 $\log_{10}$ copies/mL (95% CI: -1.73; -0.20) in favour of stavudine (Table 4).

CONCLUSIONS

This study provides evidence that the suggested TAM clustering is a real phenomenon and, most importantly, suggests that stavudine, unlike zidovudine, may still be active against TAM2-resistant viruses.

The interpretation of our results is limited by the fact that the allocation of zidovudine and stavudine was not determined at random. So, even if the estimates have been adjusted for a number of potential confounders, including the number of active drugs started besides the thymidine analogue, residual confounding due to lack of randomisation cannot be ruled out.

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Table 2

Evidence of TAM clustering

	Total Number of TAMs	
	2	3
Total number of profiles that can be constructed	20	30
Probability of TAM1		
Observed	128/168 (76.2%)	114/204 (55.0%)
Expected by chance alone	3/20 (15.0%)	1/30 (3.3%)
Probability of TAM2		
Observed	19/168 (11.3%)	37/204 (18.1%)
Expected by chance alone	5/20 (25.0%)	2/30 (6.7%)
Probability of 'mixture profiles'		
Observed	12/168 (7.1%)	27/204 (13.2%)
Expected by chance alone	12/20 (60.0%)	27/30 (90.0%)

Table 3

Factors associated with a TAM2 profile (vs. TAM1) from fitting a logistic regression model

Covariate	Crude OR (95% CI) p-value	Adjusted OR (95% CI) p-value
Exposure to zidovudine mono/dual-therapy per year longer	1.18 (1.08-1.28) p=0.0001	1.25 (1.14-1.36) p=0.0001
Male	1.00	1.00
Females	1.48 (0.93-2.35) p=0.10	1.18 (0.68-2.07) p=0.54
Exposure to stavudine per year longer	0.80 (0.65-0.99) p=0.04	0.87 (0.68-1.11) p=0.26
Viral load at time of test Per $\log_{10}$ higher	0.72 (0.59-0.89) p=0.002	0.81 (0.65-1.00) p=0.05
On zidovudine at time of test	1.67 (1.14-2.46) p=0.009	1.38 (0.86-2.22) p=0.18
Presence of 118I	0.33 (0.20-0.56) p=0.0001	0.34 (0.20-0.60) p=0.0002

Other factors considered (that failed to show an association) included: age, mode of HIV transmission, CD_4 count at time of test, months on each other NRTI as mono/dual therapy prior to test, total months on each other NRTI prior to test, months of HAART prior to test, specific NRTI contained in first ever therapy, calendar year of genotypic test, presence of other concomitant mutations (i.e. 44I/A, 69I, 74V, 151M, 184V)

Table 4

Month 6 viral load reduction ($\log_{10}$ copies/mL)

Regimen containing	Crude analysis mean (95% CI)	Adjusted analysis mean difference (95% CI) between zidovudine and stavudine
	Patients with TAMs profile (n=174)	
Stavudine	+0.71 (+0.43; +1.10)	-0.08 (-0.59; +0.42)
Zidovudine	+1.17 (+0.11; +1.56)	
	Patients with TAM2 profile (n=68)	
Stavudine	+1.27 (+0.89; +2.31)	-0.96 (-1.73; -0.20)
Zidovudine	+0.19 (-0.37; +1.29)	

Test for interaction p=0.02

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