Evaluation of the Genotypic Pattern of HIV-1 Resistance in AIDS Patients Failing Antiretroviral Therapy

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We analyzed the first 96 patients tested for HIV resistance to antiretroviral therapy in three Brazilian states. The HIV-1 reverse transcriptase (RT) and protease (PR) were sequenced by using the ABI ViroSeq system. The drugs previously used for each patient were recorded and correlated with the mutations found in the samples. Viral load (VL) and CD_4 count were also recorded. Only one patient had the wild type sequence. The most prevalent mutations were: 184V (59%), 41L (47.9%), 63P (53%), 215Y (50%), 36I (46%), 10I (35%), 67N (42%), 77I (37%), 90M (36%) and 210W (33%). A positive correlation between the number of previously used ARVs and the number of mutations was observed ($p < 0.05$). Associations between mutations and ARV drugs were identified at positions 69, 118, 184 and 215 with previous exposure to NRTI, mutations at positions 98, 100, 103, 181 and 190 with previous NNRTI use and at positions 10, 20, 30, 46, 53, 54, 71, 73, 82, 84, 88 and 90 with previous PI therapy ($p < 0.05$). Previous exposure to ARV drugs was associated with previous genotypic resistance to specific drugs, leading to treatment failure in HIV patients. Genotypic resistance was clearly associated with virological and immunological failure.
Key Words: HIV-1, mutations, resistance, antiretrovirals.

In patients under highly active antiretroviral therapy (HAART) for HIV-1, viral resistance is the main cause of antiretroviral therapy failure and of failure of subsequent treatment options [1-4]. Experience based on clinical trials has led to the suggestion that genotypic or phenotypic tests can help physicians guide therapeutic management of HIV-1 infected patients failing HAART [5-9].

The high replication rate of HIV-1, in addition to its high degree of genetic variation, allows the onset of viral mutations that confer resistance to all currently available antiretroviral drugs [10,11]. There are more than 200 different mutations interacting in many ways, leading to viral resistance, making the interpretation of genotypic and phenotypic tests very complex [12].

The Brazilian Ministry of Health started a program to evaluate the use of HIV genotyping in the management of antiretroviral therapy (RENAGENO). Patients failing first or second protease inhibitor (PI) containing regimens, or first non-nucleoside based therapy (NNRTI), or double therapy, are eligible for testing. We analyzed the genotypic profile of 96 patients tested for resistance in three Brazilian states in order to determine the frequency of mutations and its association with previous ARV drugs used by patients.

Materials and Methods

Patients living in the states of Bahia (BA), Sergipe (SE) (Northeast region) and Espírito Santo (ES) (Southeast region), eligible for genotyping, were tested between April 2002 and February 2003.

Laboratory evaluation

The HIV-1 reverse transcriptase (RT) and protease (PR) genes were sequenced by using AppliedBiosystems ViroSeq 1, according to manufacturer's instructions. Demographic data, previous use of antiretrovirals, viral load and CD_4 counts were recorded. Four results of viral load and CD_4 count were obtained: before antiretroviral therapy, after antiretroviral therapy, after treatment failure and the lowest result of viral load and CD_4 count.

Statistical analysis

Prevalence ratio was used to compare differences in frequencies. A comparison of mean CD_4 count and viral load was made with the paired sample Student's t test. We studied the association between resistance to ARV drug and a mutation at a given position, using a chi-square test, or a Fisher test if necessary. Logistic regression analysis was performed to study the linear relationship between the number of ARVs and mutation prevalence. We used a significance level of 0.05 and a Confidence Interval (CI) of 95%.

Results

A total of 96 patients were evaluated (69% males). The mean age was 40.7 ± 12.5 years. Seventy-eight (81%) were from the state of Bahia, 15 (16%) from Espírito Santo and 3 (3%) from Sergipe. The mean CD_4 counts and viral load were calculated (Table 1). All means, when compared at each time point, using paired Student's t tests, were significantly different (p<0.05), except for the comparison between CD_4 count after treatment failure/before treatment and CD_4 count after treatment/after treatment failure (p=0.99 and p=0.24 respectively). All viral load measurements was significantly different (p<0.05), except for VL before treatment and after treatment failure (p=0.65) (Table 1).

The ARV drugs that were previously used were: AZT (95.8%), 3TC (87.5%), DDI (72.9%), D4T (55.2%), IDV (53.1%), NFV (45.8%), RTV (34.4%), NVP and EFV (26% each), SQV (19.8%), DDC (5.2%), APV and LPV (4.2% each) and ABC (1%).

Only one patient presented with a wild type sequence. The most prevalent mutations were 184V (59%), 63P (53%), 215Y (50%), 41L (48%), 36I (46%), 67N (42%), 77I (37%), 90M (37%), 10I (35%) and 210W (33%). The frequencies of all mutations associated with ARV drugs are listed in Tables 2, 3 and 4 divided by ARV categories (NRTI, NNRTI and PI).

A positive correlation between the number of ARVs previously used and the number of mutations was observed. The mean number of NRTIs used was 3.2 ± 0.9 drugs/patients. Forty-four patients used NNRTI (mean number of drugs used: 1.1 ± 0.3/patient). Among 88 patients who used PI, the mean number of drugs used was 1.8 ± 0.9. Figures 1 and 2 show a positive correlation with the number of drugs (NTRI and PI) used and the number of mutations identified by genotyping. Specific analysis of NNRTI was not done due to the characteristics of this class of drug (cross-resistance), which did not allow the use of a second drug after failure of the first one used.

Chi-square analysis revealed associations between some mutations and previous exposure to ARV drugs. Mutations 69D and 118I were associated with previous exposure to Stavudine (D4T), mutation 184V was associated with previous exposure to Lamivudine (3TC) and Didanosine (DDI), and mutation 215F/Y, with prior use of D4T and DDI (all p values < 0.05, Table 5).

We found an association between mutations 181I/C and 190A/S and exposure to Nevirapine (NVP). Although we detected a significant association between mutation 98G and NVP resistance (p=0.016) and mutation 100I and EFZ resistance (p=0.001), it was not possible to define the prevalence ratio, since at least one cell was equal to zero (Table 6).

Mutations at positions 10, 20, 30, 46, 53, 54, 71, 73, 82, 84, 88 and 90 were associated with prior therapy with specific protease inhibitors (Table 7).

Discussion

We analyzed genotyping tests for 96 patients from three Brazilian states (BA, SE and ES). Only one

Table1. Evolution of mean CD_4 count and viral load over time in patients under HAART

Evolution	CD_4 (cells/mm³) $\pm$ SD	VL (log 10) $\pm$ SD
Before HAART	276.7 ± 204.2	4.8 ± 0.9
1st value post-HAART	331.2 ± 239.2	4.1 ± 0.9
Lowest value during HAART	122. 4 ± 107.8	3.4 ± 1.0
After treatment failure	276.9 ± 213.3	4.6 ± 0.7

All p value <0.05 for comparison of mean CD_4 and VL at each time point.

Table 2. Frequency of mutations according to previous use of nucleoside reverse transcriptase inhibitors (NRTI)

No. Patients/ Mutations	NRTI							
	92		70		84		53	
	AZT	(%)	DDI	(%)	3TC	(%)	D4T	(%)
41L	44	(47.8)	35	(50)	41	(48.8)	29	(54.7)
44A	1	(1.1)	1	(1.4)	1	(1.2)	1	(1.9)
44D	14	(15.2)	13	(18.6)	14	(16.7)	9	(17.0)
62V	2	(2.2)	2	(2.9)	2	(2.4)	2	(3.8)
65R	2	(2.2)	2	(2.9)	2	(2.4)	2	(3.8)
67N	38	(41.3)	30	(42.9)	34	(40.5)	26	(49.1)
69D	9	(9.8)	7	(10)	9	(10.7)	8	(15.1)
70R	25	(27.2)	20	(28.6)	22	(26.2)	13	(24.5)
74I	1	(1.1)	2	(2.9)	2	(2.4)	2	(3.8)
74V	5	(5.4)	3	(4.3)	5	(6.0)	3	(5.7)
75I	5	(5.4)	5	(7.1)	5	(6.0)	3	(5.7)
75M	1	(1.1)	1	(1.4)	1	(1.2)	1	(1.9)
75T	1	(1.1)	1	(1.4)	1	(1.2)	1	(1.9)
77L	1	(1.1)	1	(1.4)	1	(1.2)	1	(1.9)
115F	1	(1.1)	1	(1.4)	1	(1.2)	1	(1.9)
116Y	3	(3.3)	2	(2.9)	3	(3.6)	2	(3.8)
118I	20	(21.7)	14	(20)	19	(22.6)	16	(30.2)
151M	4	(4.3)	3	(4.3)	4	(4.8)	2	(3.8)
184I	2	(2.2)	1	(1.4)	3	(3.6)	3	(5.7)
184V	56	(60.9)	38	(54.3)	56	(66.7)	29	(54.7)
210W	30	(32.6)	26	(37.1)	28	(33.3)	21	(39.6)
215F	15	(16.3)	13	(18.6)	14	(16.7)	10	(18.9)
215Y	46	(50)	38	(54.3)	41	(48.8)	29	(54.7)
219E	10	(10.9)	10	(14.3)	9	(10.7)	5	(9.4)
219N	0	(0)	1	(1.4)	1	(1.2)	1	(1.9)
219Q	14	(15.2)	9	(12.9)	13	(15.5)	8	(15.1)
333D	1	(1.1)	0	(0)	1	(1.2)	0	(0.)
333E	7	(7.6)	4	(5.7)	7	(8.3)	6	(11.3)

Table 3. Frequency of mutations according to previous use of NNRTI

No. Patients Mutations	NNRTI			
	25		25	
	NVP	(%)	EFZ	(%)
98G	3	(12)	1	(4)
100I	0	(0)	5	(20)
101E	2	(8)	1	(4)
103N	7	(28)	8	(32)
106A	1	(4)	0	(0)
108I	3	(12)	3	(12)
179D	0	(0)	1	(4)
181I	2	(8)	0	(0)
181C	7	(28)	2	(8)
188L	1	(4)	1	(4)
190A	4	(16)	1	(4)
190S	1	(4)	1	(4)
225H	0	(0)	0	(0)

patient presented with a wild-type sequence. The most prevalent mutations were 184V, 63P, 215Y, 41L, 36I, 67N, 77I, 90M, 10I, and 210W.

The analysis of an association between previous exposure to antiretroviral drugs and resistance associated mutations show the impact of the use of these drugs in HIV-1 drug resistance [12]. A positive correlation between the number of ARVs previously used and the number of mutations was observed for the NRTI and PI drug classes. This correlation was not seen for NNRTI, due to the characteristics of this class of drug. The presence of only one mutation may lead to cross-resistance to all drugs of this class of anti-retroviral drug (Nevirapine, Efavirenz and Delavirdine) and do not allow the use of a second drug after initial failure [13].

The associations detected between mutations and exposure to specific drugs were statistically significant: prior use of D4T was associated with mutations 69D, 118I and 215F/Y; exposure to DDI was associated with mutation 215F/Y; and prior use of 3TC was associated with mutation 184V. There was a negative association between mutation 184V and exposure to DDI, with a prevalence ratio = 0.3 (95% CI:0.1-0.8) and p value =0.02. Probably, this is a result of sequential use of ARVs, since DDI has a greater incidence of adverse events and dietary limitations compared to 3TC which is prescribed more frequently as first line therapy [14]. No specific mutation associated with Zidovudine (AZT) was found: this probably reflects the high frequency of previous use of this drug (96% of the patients), so the detection of specific mutations associated with resistance to AZT was not possible.

The evaluation of mutations associated with resistance to NNRTI revealed a significant association between NVP exposure and mutations 98G, 181I and 190A/S, and of previous use of EFZ and mutations 100I and 103N. These findings are similar to those described previously [15].

Specific mutations associated with PI resistance were found in patients previously exposed to this class of drug. Previous exposure to Nelfinavir (NFV) was associated with mutations 30N, 88D and 90M (p<0.05 for each). Exposure to Saquinavir (SQV) was associated with mutations 53L, 71V, 73S and

Table 4. Frequency of mutations according to previous use of PI

No. patients Mutations	PI							
	51		44		19		33	
	IDV	(%)	NFV	(%)	SQV	(%)	RTV	(%)
10F	5	(9.8)	5	(11.4)	1	(5.3)	3	(9.1)
10I	28	(54.9)	13	(29.5)	11	(57.9)	19	(57.6)
10V	4	(7.8)	4	(9.1)	2	(10.5)	4	(12.1)
20M	1	(2.0)	1	(2.3)	2	(10.5)	2	(6.1)
20R	9	(17.6)	2	(4.5)	3	(15.8)	6	(18.2)
24I	4	(7.8)	2	(4.5)	0	(0)	2	(6.2)
30N	3	(5.9)	13	(29.5)	1	(5.3)	1	(3)
32I	4	(7.8)	0	(0)	0	(0)	3	(9.1)
36I	24	(47.1)	20	(45.5)	10	(52.6)	19	(57.6)
46I	15	(29.4)	6	(13.6)	6	(31.6)	11	(33.3)
46L	5	(9.8)	4	(9.1)	1	(5.3)	1	(3)
47V	2	(3.9)	0	(0)	0	(0)	0	(0)
48V	6	(11.8)	3	(6.8)	4	(21.1)	4	(12.1)
50	0	(0)	0	(0)	0	(0)	0	(0)
53L	8	(15.7)	2	(4.5)	5	(26.3)	6	(18.2)
54I	1	(2)	0	(0)	0	(0)	1	(3)
54L	1	(2)	0	(0)	1	(5.3)	2	(6.1)
54T	2	(3.9)	0	(0)	1	(5.3)	1	(3)
54V	16	(31.4)	7	(15.9)	6	(31.6)	12	(36.4)
63L	0	(0)	1	(2.3)	0	(0)	1	(3)
63P	27	(52.9)	22	(50)	7	(36.8)	16	(48.5)
71I	1	(2)	0	(0)	1	(5.3)	1	(3)
71T	3	(5.9)	6	(13.6)	2	(10.5)	2	(6.1)
71V	20	(39.2)	8	(18.2)	8	(42.1)	13	(39.4)
73S	6	(11.8)	1	(2.3)	3	(15.8)	4	(12.1)
77I	17	(33.3)	18	(40.9)	6	(31.6)	8	(24.2)
82A	20	(39.2)	5	(11.4)	6	(31.6)	15	(45.5)
82T	1	(2)	0	(0)	0	(0)	1	(3)
84N	0	(0)	0	(0)	0	(0)	1	(3)
84V	12	(23.5)	3	(6.8)	8	(42.1)	10	(30.3)
88D	4	(7.8)	11	(25)	1	(5.3)	1	(3)
88N	0	(0)	0	(0)	0	(0)	1	(3)
90M	24	(47.1)	13	(29.5)	14	(73.7)	19	(57.6)
90N	0	(0)	0	(0)	0	(0)	1	(3)
93N	0	(0)	0	(0)	0	(0)	1	(3)

Figure 1. Mean frequency of mutations according to the number of NRTI previously used (p=0.027)
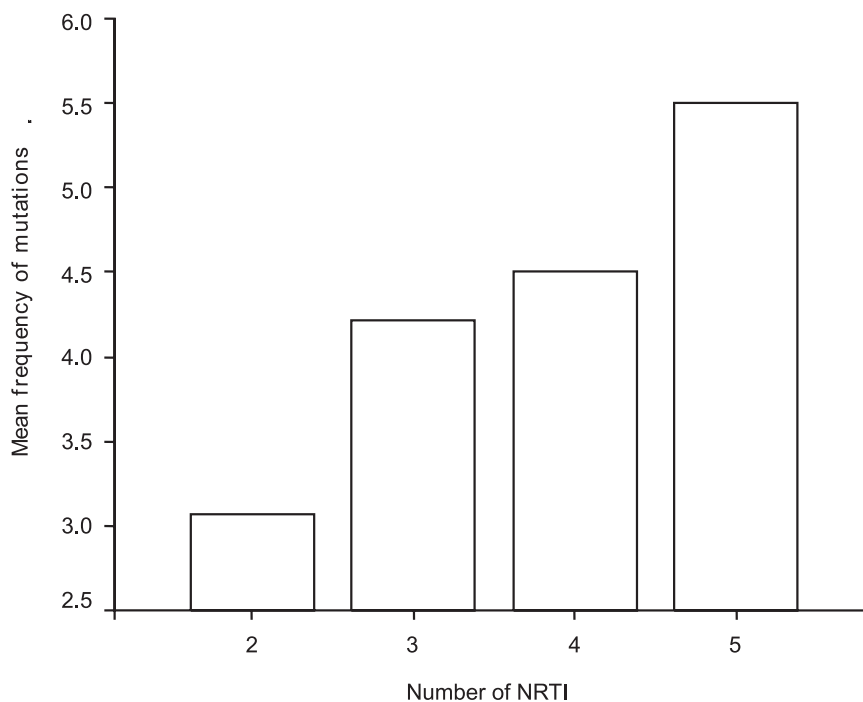
Table 5. Mutations significantly associated with previous use of NRTI

Mutation	ARV	PR (95%CI)	p
69D	D4T	7.4 (0.9-62)	0.02
118I	D4T	3.3 (1-10)	0.02
184V	DDI	0.3 (0.1-0.8)	0.02
184V	3TC	26 (3-212)	0.00
215F/Y	D4T	2.2 (0.9-5.2)	0.05
215F/Y	DDI	3 (1.2-7.9)	0.01

PR – Prevalence ratio.

Table 6. Mutations significantly associated with previous exposure to NRTI

Mutation	ARV	PR (95% CI)	p
98G	NVP	-	0.016
100I	EFV	-	0.001
103N	EFV	2.8 (0.0-8.4)	0.051
181I/C	NVP	19.4 (3.8-98.6)	<0.001
190A/S	NVP	17.5 (1.9-158)	<0.001

PR – Prevalence ratio.

Table 7. Mutations significantly associated to previous PI use

| Mutation	ARV	PR (95%CI)	p
10I/F/V	IDV	4.3 (1.8-10)	0.001
10I/F/V	RTV	4.6 (1.7-12)	0.001
20M/R	NFV	0.24 (0.064-0.9)	0.026
30N	NFV	6.8 (1.8-26)	0.002
30N	IDV	0.1 (0.04-0.5)	0.003
30N	RTV	0.1 (0.01-0.8)	0.007
46I/L	IDV	2.9 (1-7)	0.018
53L	SQV	6.5 (1.5-27)	0.014
53L	IDV	8 (0.9-68)	0.024
53L	RTV	4.4 (1-19)	0.04
53L	LPV	12 (1.5-99)	0.04
54V	NFV	0.3 (0.1-0.9)	0.03
54V	IDV	5.1 (1.7-15)	0.002
54V	RTV	5.6 (2.1-15)	0.000
54V	APV	9.5 (0.9-96)	0.05
71V	SQV	2.8 (1-7.9)	0.038
71V	IDV	2.4 (1-5.7)	0.032
73S	SQV	7.2 (2.1-24)	0.002
73S	LPV	17 (2-150)	0.026
82A	NFV	0.1 (0.06-0.5)	0.001
82A	IDV	5.6 (1.8-16)	0.001
82A	RTV	5 (2-13)	0.001
84V	NFV	0.2 (0.06-0.9)	0.026
84V	IDV	4.3 (1-16)	0.021
84V	RTV	7.3 (2-26)	0.001
84V	APV	20 (1.9-208)	0.01
88D	NFV	4 (1-14)	0.02
88D	IDV	0.2 (0.07-0.8)	0.025
90M	NFV	10 (1.6-59)	0.013
90M	SQV	7 (2.2-22)	0.000
90M	IDV	2.4 (1-57)	0.032
90M	RTV	4.5 (1,8-11)	0.001

PR – Prevalence ratio.

90M and mutations 10I, 46I, 53D, 54V, 71V, 82A, 84V, 88D and 90M were associated with previous use of Indinavir (IDV). Mutations 53L and 88D are associated with use of Ritonavir (RTV) and Nelfinavir, respectively, as we found in this study. Mutations 10I, 53L, 54V, 82A, 84V and 90M were significantly associated with resistance to Ritonavir, confirming the available data [16-18].

Despite the low frequency of use of Amprenavir (APV) and Lopinavir (LPV), some mutations were significantly associated with previous exposure to these drugs. Although mutations 54V and 84V are

Figure 2. Mean frequency of mutations according to the number of PI previously used (p=0.001)
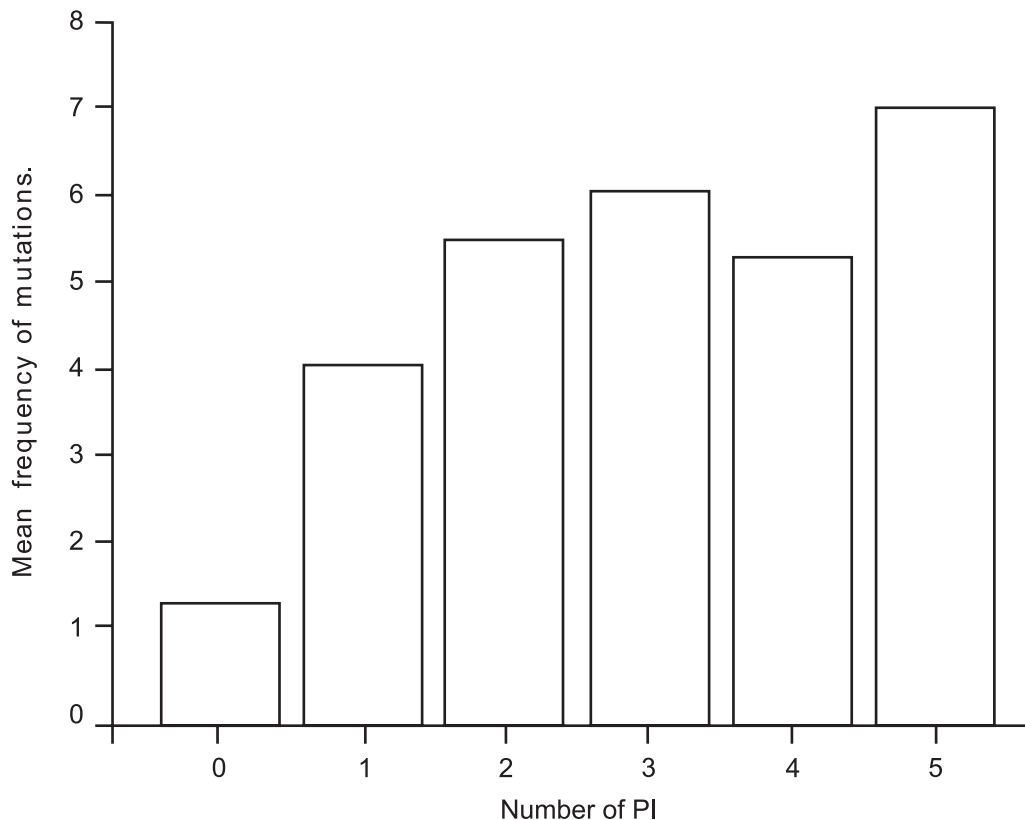
not induced only by these drugs, the high value of the prevalence ratio (PR) suggests a strong association between some mutations and these ARVs: APV and 84V – PR=20 (95%CI: 1.9-208); IDV and 84V – PR=4.3 (95%CI:1-16); RTV and 84V – PR= 7.3 (95%CI: 2-26). Mutations 53L and 73S were associated with previous exposure to LPV/r (PR=12, 95%CI (1.5-99) and PR=17, 95%CI (2-150) respectively), but these mutations are also reported to be associated with RTV (53L), and SQV or IDV (73S) [11]. Again, the higher PR observed in the analysis of mutations associated with LPV/r, suggest a potential role for induction by this drug. We cannot discard a cumulative effect due to sequential use of these protease inhibitors during therapy, even though the criteria of RENAGENO excluded genotyping in patients failing more than two regimes containing PIs.

HIV drug resistance is the main cause of virological and clinical failure in HIV-1 infected patients [1,19]. The results of this study revealed the impact of HIV-1 genotypic resistance on prognostic markers (CD_4 count and viral load). Viral load mean before treatment (log 4.85 RNA copies) decreases after the initiation of HAART (log 4.13), reaching the lowest value during treatment (log 3.41). However, after treatment failure, mean viral load returned to baseline values (log 4.67), as a consequence of the loss of activity of anti-retroviral drugs. An inverse correlation was observed between CD_4 counts and therapy failure: there was an increase of 101 cells/mm^3 after HAART, with return to baseline values after treatment failure.

In Brazil, there is little data on HIV-1 drug resistance [20-24]. It is important to evaluate the prevalence rate and mutation pattern for drugs in both naive and treated patients, in order to design better strategies that make

better management of antiretroviral therapy possible. This study provides some insights on this kind of problem, and it adds some new information about the potential impact of the introduction of genotyping as a strategy to recognize and minimize drug resistance among AIDS patients under therapy.

Acknowledgments

References

1. Moyle G.J. Resistance to antiretroviral compounds: implications for the clinical management of HIV infection. Imuunol & Infec Dis **1995**;5:170-82.

2. Condra J.H., Emini EA. Preventing HIV-1 Drug Resistance. Science & Medicine, **1997**;Jan-Feb:2-11.

3. Larder B.A., Kemp S.D. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to Zidovudine (AZT). Science **1989**;246:1155-8.

4. Iversen A.K.N., Shafer R.W., Wehrly K., et al. Multidrug-resistant human immunodeficiency virus type I strains resulting from combination antiretroviral therapy. J Virol **1996**;70(2):1086-90.

5. Durant J., Clevenbergh P., Halfon P., et al. Drug-resistance genotyping in HIV-1 therapy: the VIRAD APT randomized controlled trial. Lancet **1999**;353:2195-9.

6. Meynard J.-L., Vray M., Morand-Joubert L., et al. Phenotypic and genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. AIDS **2002**;16:727-36.

7. Hirsch M.S., Conway B., D'Aquila R.T., et al. Antiretroviral drug resistance testing in adults with HIV infection. Implications for clinical management. JAMA **1998**;279(24):1984-91.

8. Ruiz L. Interpreting resistance test results to optimize treatment regimens. Advanced Studies in Medicine **2002**;2(23):823-6.

9. Mayers D.L. Prevalence and incidence of resistance to Zidovudine and other antiretroviral drugs. Am J Med **1997**;102(5B):70-5.

10. Coffin J.M. HIV population dynamics *in vivo*: implications for genetic variation, pathogenesis and therapy. Science **1995**;267:483-9.

11. Clotet B., Menendez-Arias L., Ruiz L., et al. Guide to management of HIV drugs resistance and pharmacokinetics of antiretroviral therapy. 2 edition, Editora TAISA, **2002**, pp 269.

12. Villalba N., Gómez-Cano M., Holguin A., et al. Multiple drug resistance genotype causing failure of antiretroviral treatment in an HIV-infected patient heavily exposed to nucleoside analogues. Eur J Clin Microbiol Infect Dis **1999**;18:372-5.

13. Casado J.L., Hertogs K., Ruiz L., et al. Non-nucleoside reverse transcriptase inhibitor resistance among patients failing a nevirapine plus protease inhibitor-containing regimen. AIDS **2000**;14(2):F1-F7.

14. Carpenter C.C.J., Cooper D.A., Fischl M.A., et al. Antiretroviral therapy in adults. Update recommendations of the international AIDS Society-USA Panel. JAMA **2000**;283(3):381-91.

15. Deeks S.G. Nonnucleoside reverse transcriptase inhibitor resistance. JAIDS **2001**;26:S25-S33.

16. Race E., Dam E., Obry V., et al. Analysis of HIV cross-resistance to protease inhibitors using a rapid single-cycle recombinant virus assay for patients failing on combination therapies. AIDS **1999**;13:2061-8.

17. Miller V. Resistance to protease inhibitors. JAIDS **2001**;26:S34-S50.

18. D'Aquila R.T., Schapiro J.M., Brun-Vézinet F., et al. Drug resistance mutations in HIV-1. Inter AIDS Society-USA **2002**;10(5):21-5.

19. Rizzardi G.P., de Boer R.J., Hoover S., et al. Predicting the duration of antiviral treatment needed to suppress plasma HIV-1 RNA. J Clin Invest **2000**;105(6):777-82.

20. Dumans A.T., Soares M.A., Pieniazek D., et al. Prevalence of protease and reverse transcriptase drug resistance mutations over time in drug-naïve Human Immunodeficiency Virus type –1 positive individuals in Rio de Janeiro, Brazil. Antimicrob Agents Chemother **2002**;46(9):3075-9.

21. Sucupira M.C., Souza I.E., Costa L.J., et al. Antiretroviral treatment failure and HIV-1 genotypic resistance in São Paulo, Brazil. Antivir Ther **2001**;6(4):263-4.

22. Tanuri A., Caridea E., Dantas M.C., et al. Prevalence of mutations related to HIV-1 antiretroviral resistant in Brazilian patients failing HAART. J Clin Virol **2002**;25(1):39-46.

23. Brindeiro R., Vanderborght B., Caride E., et al. Sequence diversity of the reverse transcriptase of human immunodeficiency virus type 1 from untreated Brazilian individuals. Antimicrob Agents Chemother, **1999**;43(7):1674-80.

24. Brites C., Bahia F., Gilbert M., et al. Evaluation of viral resistance to reverse transcriptase inhibitors (RTI) in HIV-1 infected patients before and after 6 months of single or double antiretroviral therapy. Braz J Infect Dis **2001**;5(4):177-82.