

Longitudinal Analysis of RT and Protease Mutations In HIV Subtype C Patients Receiving Antiretroviral Therapy

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(See appendix for a complete list of participating investigators and centers)

INTRODUCTION

HIV-1 consists of a variety of clades (subtypes) that are distributed in geographic clusters. These clades differ in their genetic sequence, producing distinct patterns of polymorphism in the viral proteins. Clade B virus is the predominant subtype in the United States and Western Europe, but is responsible for only a minority of HIV infections worldwide. Research on HIV resistance has been largely confined to clade B viruses. Therefore, much is known about clade B virus polymorphisms and resistance mutations but very little about the non-clade B strains. Differences between clades in baseline polymorphism, or the presence of unrecognized resistance mutations, may lead to the development of unique resistance patterns and/or reduce the efficacy of specific drugs for specific clades. Knowledge of these possible differences and their impact on drug utility is a prerequisite for a more rational treatment of patients infected with non-clade B viruses.

Israel has a mixed population of HIV infected patients including an indigenous population primarily infected with clade B virus, immigrants from Ethiopia infected with clade C, and immigrants from Europe, South America and Asia infected with clades A, D and F. All have access to the same medical care and antiretroviral drugs. In an effort to improve care of our non-clade B patients, we are studying the resistance patterns in the protease and reverse transcriptase genes.

We performed longitudinal genotypic analysis of clade C viruses from patients receiving antiretroviral therapy (ART). Here we evaluate the temporal development of patterns of drug-resistance mutations in clade C patients under treatment. We also compared the overall pattern to that in clade B.

METHODS

Plasma samples were collected from patients attending seven HIV clinics throughout Israel. The entire protease gene and codons 37 - 248 of the reverse transcriptase gene were sequenced (TrueGene™ Visible Genetics, Toronto, Canada with experimental CS-1 primers, Visible Genetics Corp. Atlanta, Ga). Sequences were obtained from 172 samples. These included 91 clade C and 81 clade B (Table 1). Eligibility criteria were: VL>1000 cp/ml and availability of clinical data and treatment history (Tables 2 and 3). Clade C patients were tested 2-6 times and clade B once or twice.

RESULTS

Table 1: Samples & Patients

108 patients, 172 samples		
	Patients	Samples
Clade B:	67	81
Clade C:	41	91
	108	172

Table 2: Longitudinal Study – Clade C Clinical Data at Testing

Viral Load (log ₁₀ copies/ml)	CD4 (cells/μl)	Follow-Up (months)
5.3	361	31
(3.0 – 6.7)	(11 - 1210)	(4 – 62)

Figure 1: Antiretroviral Drugs in Use While Testing: Clade B vs. Clade C

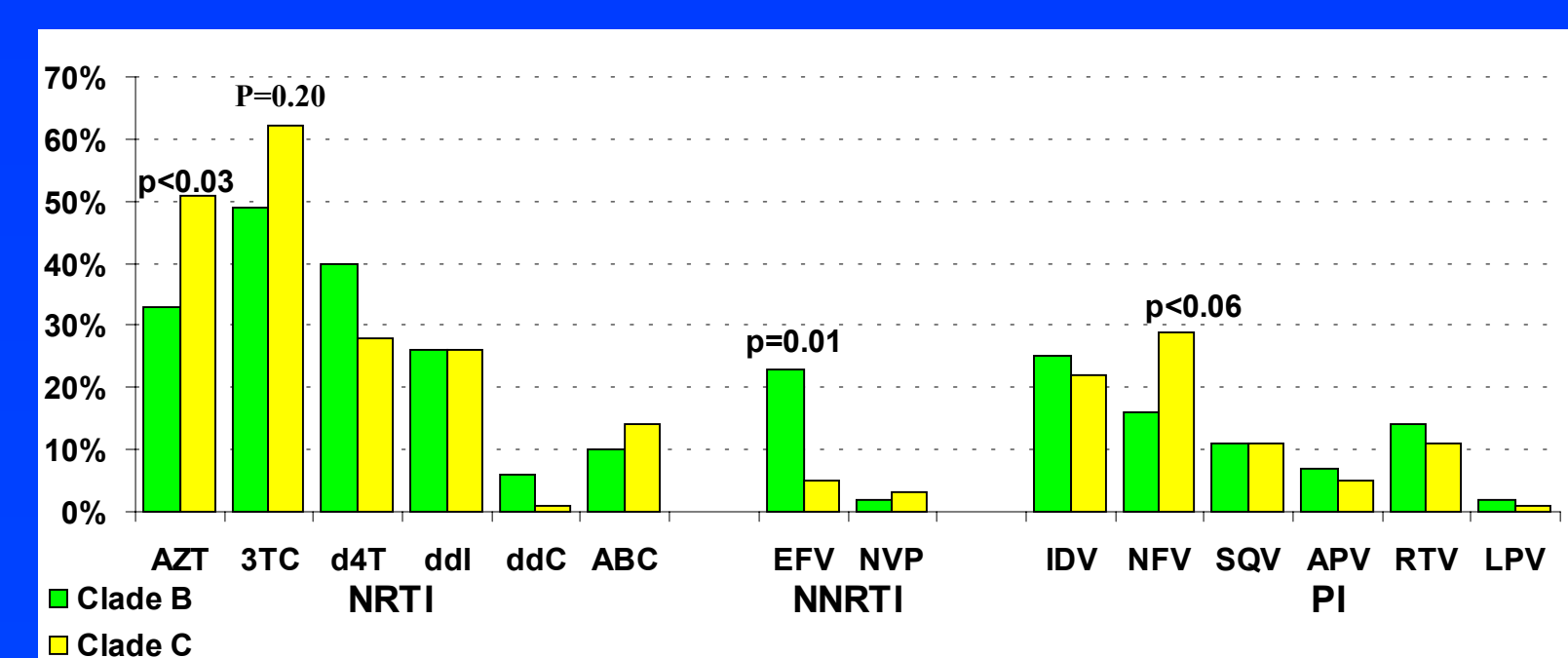


Table 3: HAART regimens until first change (clade C)

Drug	Patients (samples)	Treatment Range (months)	Mean +/- SEM (months)	
PI	NLF	13 (20)	3 - 20	10.0 +/- 1.0
	IDV	10 (15)	2 - 24	11.2 +/- 1.7
	SQV	2 (4)	3 - 28	17.5 +/- 2.6
	RTV	1 (2)	27 - 31	29.0 +/- 2.0
RTI	AZT + 3TC	23 (32)	1 - 32	13.1 +/- 1.8
	3TC + d4T	1 (1)	14	NA
	AZT + ddC	1 (1)	32	NA
	AZT + ddI	1 (2)	3 - 5	4.0 +/- 1.0

Table 4: Most prevalent mutations at first change*

NLF	L63P (4/20), K20R, D30N (2/20)
IDV	L63P (7/15), L90M (3/15)
SQV	M46I (1/4)
AZT+3TC	M184V (13/32)
EFV**	
NVP	K103N (4/7)
EFV+NVP	

* M36I was present in all samples
** NNRTIs were usually introduced as 4th regimen or higher

Mutations and Polymorphisms in the Protease and RT

Characteristic primary and secondary Pr and RT resistance mutations developed in both groups (Fig 2 & 3)

Figure 2: Mutations and Polymorphisms in the Protease

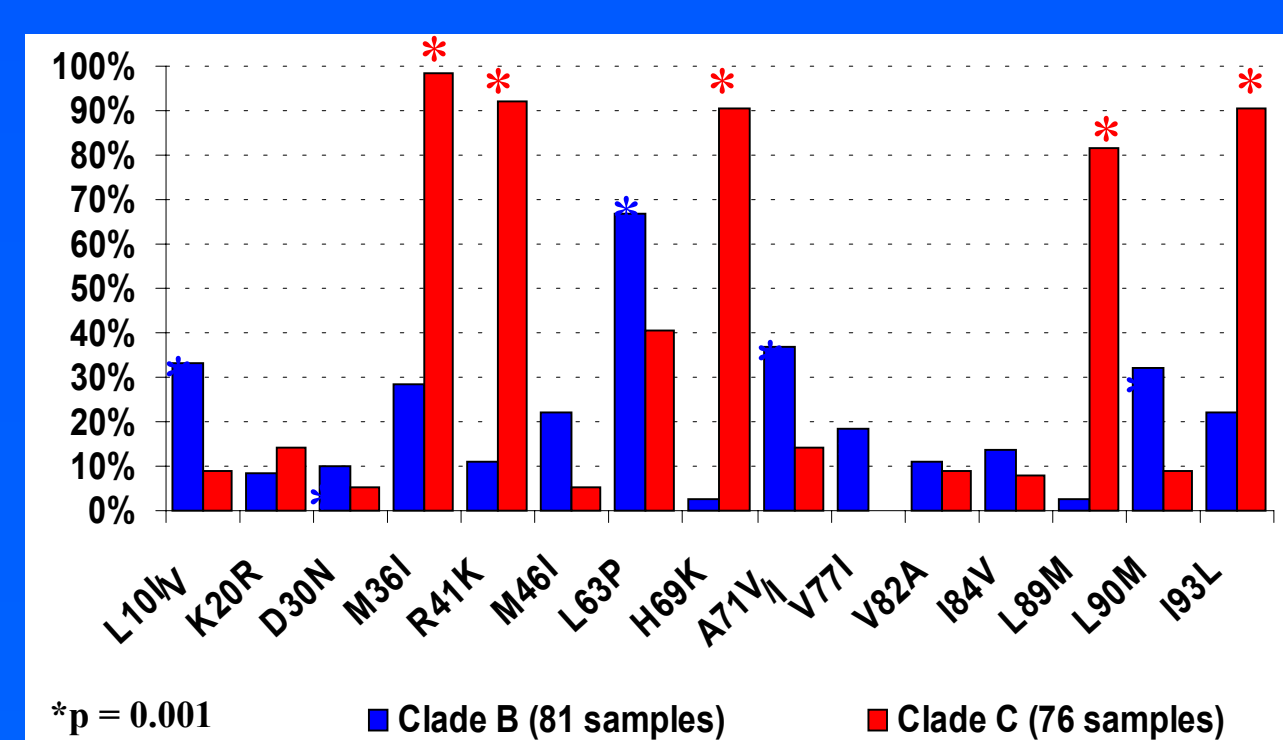
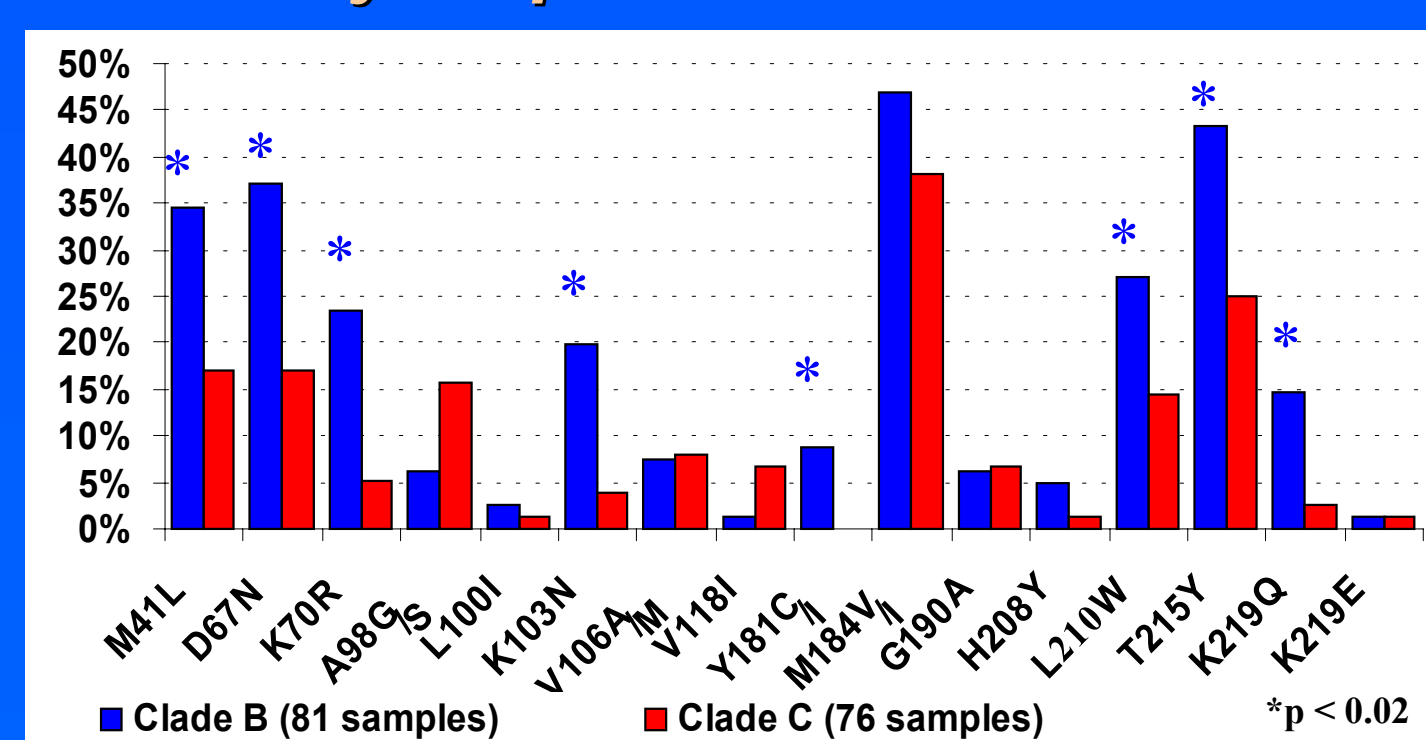
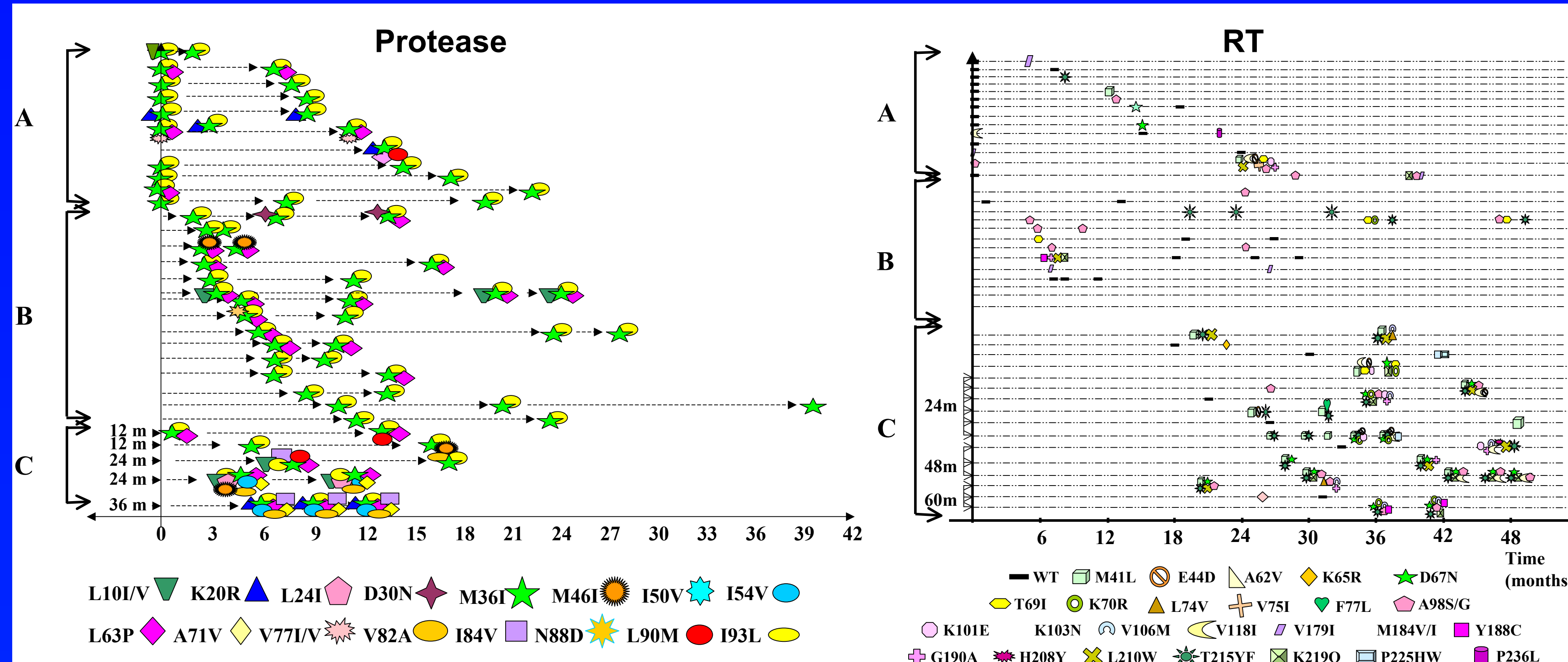


Figure 3: Mutations and Polymorphisms in the RT



ZDV mutations (41, 67, 70, 210, 215, 219) were significantly higher (p<0.02) in B compared to C (except for L210W: p<0.08). No significant difference between the two groups was found in the frequency of M184V (p=0.3)

Figure 4: Emergence of resistance mutations in HIV-C patients under ART



A: Naïve at first genotyping test; B: First sample taken within a year on HAART; C: HAART more than one year

DISCUSSION

Frequent mutations in RT and protease genes accumulate in HIV-C as a function of exposure to HAART. On the background of M36I, K41R, K69R and L93I in HIV-C, other major mutations emerge in the PI exposed C group. The exception is D30N which is relatively rare in C in spite of high exposure to Nelfinavir. However, fine differences do exist.

M184V is the most prevalent RT mutation in clade C patients, and usually emerges first. Although C patients received AZT significantly more than clade B patients, the NAM mutations appear more frequently in the B group.

The most prevalent mutation following NNRTI treatment in clade C patients is K103N (p<0.001). V106A, 188C and G190A are seen occasionally but Y181C is rare (p<0.001). Consistent with the temporal pattern (Table 4; Fig 4), we have lately observed mutation K103N in recently seroconverted naïve clade C patients.

Non-compliance is widespread and is demonstrated by the lack of resistance mutations in 12/32 (37%) of NRTI-exposed patients who failed therapy.

ABSTRACT

Background: Little is known about patterns of drug resistance of clade-C HIV and their relationship to treatment regimens. Differences in baseline sequence among clades may result in the selection of different mutations under the pressure of particular drug combinations. We compared the development of resistance mutations in clade C patients treated with different anti-retroviral drugs.

Methods: We performed a longitudinal study of HIV clade C patients receiving therapy and compared the development of resistance mutations in these patients to our clade B patients. Samples with VL >1,000 were sequenced (TRUGENE™, HIV-1 genotyping kit and CS-1 experimental primers for RT & amplification, Visible Genetics Inc). Patients had two or more sequential tests performed.

Results: 91 plasma samples from 41 clade C patients (pts) were analyzed. Of these, in 12 the initial sample was taken prior to HAART, in 15 during the first year of HAART and in 11 >1 year. The mean follow-up time was 31 (4-62) months. Data were compared with 81 samples from 67 local Clade B pts. The mean VL was 5.3 (3.0-6.7) log₁₀ copies/ml, mean CD4 361 (11-1210) cells/μl. The most common drug combinations included AZT+3TC (23 pts, 32 samples), NFV (13 pts, 20 samples) and IDV (10 pts, 15 samples). Drug use, VL and CD4 values were similar in the Clade B and C groups, but more B pts received EFV (23% vs. 4.5%), and more children were in the clade C group (8 vs. 2). The longest drug exposure times on first-line treatments were documented for AZT/3TC (17 pts, mean follow-up (f/u) 13.1 +/-1.8 m), Nelfinavir (13 pts, mean f/u 10 +/- 1m), and Indinavir (8 pts, mean f/u 11.2 +/-1.7 m).

Characteristic primary and secondary PI resistance mutations developed in both groups, although L10I/V, 30N, 63P 71V/I 77I and 90M were significantly higher in B, and 36I, 41K, 69K, 89M and 93L in C (P < 0.001 for all). Patients were commonly on ZDV/3TC as the first line of NRTI. 33% and 51% of samples of clade B and C, respectively, received ZDV (p<0.03). The frequency of characteristic ZDV mutations (or NAMS: 41, 67, 70, 210, 215, 219) were significantly higher (p<0.02) for all except L210W (p<0.08) in B compared to C. No significant difference was found in the frequency of M184V between the two groups (p=0.333).

Conclusion: During antiretroviral therapy mutations frequently arise in clade C and appear to follow a general pattern similar to that in clade B infected patients. The background mutations in the Protease differ in C vs. B. The clinical significance is not known. The M184V/I mutation rate was similar in C and B while the characteristic ZDV mutation rate was significantly lower in C. To what degree this was due to adherence or other factors remains to be determined.

Appendix

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