

Berta Rodés¹, Carlos Toro¹, Raffaella Colombatti², Ainhoa Simón¹, Fabio Riccardi², Cesaltina Vieira², Alessandra Coin² and Vicent Soriano¹

¹Infectious Diseases Department, Hospital Carlos III, Madrid, Spain. ²Ospedale Comunità di Sant'Egidio, Bissau, Guinea Bissau



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I. Background

The development of resistance to nucleoside analogs in HIV-2 has shown some differences with respect to HIV-1, mainly a higher rate of selection of mutation Q151M, which unfortunately results in multinucleoside resistance. However, since most series of HIV-2 patients on antiretroviral therapy are small, information on drug resistance patterns is often inaccurate. In drug-naïve HIV-1 patients, selection of K65R in the RT is generally restricted to patients failing tenofovir, although in the presence of K65R the susceptibility to other drugs such as lamivudine, didanosine or abacavir may be diminished. Herein, we provide evidence for selection of K65R in HIV-2 patients failing abacavir and never exposed to tenofovir.

II. Patients

22 HIV-2 infected patients attended in a clinic in Bissau who initiated antiretroviral therapy as part of the DREAM program (Drug Resource Enhancement against AIDS and Malnutrition) were identified and followed for 12 months.

HAART regimens available and received by the patients as first or second line were:

- **Duovir (AZT+3TC) + Abacavir** (17 patients)
- **Indinavir + Abacavir + ddI** (3 patients)
- **Duovir-N** (5 patients)
- **Indinavir + ddI+ d4T** (1 patient)
- **Lamivir-S + Abacavir** (1 patient)
- **ddI + Abacavir + Kaletra** (1 patient)

III. Methods

Viral load determination

Plasma HIV-2 RNA was measured using Nuclisens EasyQ v1.1 (Rodes et al. JCM 2007; 45: 88-92).

Genotypic resistance analysis

A fragment of the RT gene was amplified (980 bp) from plasma RNA of each patient before and after initiation of treatment. PCR products were sequenced using Rhodamine terminator cycle sequencing kit in an ABI 3100 sequence analyzer (Applied Biosystems) following manufacturer's instructions. DNA sequences were analysed, edited and translated using the Sequence Navigator software. Encoded HIV-2 RT proteins were then aligned and compared with wild type reference sequences from GenBank using Clustal W and GeneDoc. Observed mutations were compared with those associated with resistance in HIV-1 and listed in the latest International AIDS Society (IAS) resistance guidelines.

Phylogenetic analysis

HIV-2 subtype was determined for each patient using the same RT sequences. Sequences were aligned with GenBank HIV-2 reference sequences from different subtypes. Analyses were performed using Phylip software package.

IV. Results

The majority of patients (15/22, 68%) were treated with AZT+3TC+abacavir as first-line therapy. The indinavir+abacavir+didanosine regimen was used as second line therapy. Few patients received the combination Duovir-N (AZT+3TC+nevirapine) which is suboptimal for HIV-2 and treatment was changed when this problem was detected. Mean baseline plasma HIV-2 RNA was 3.92 log copies/mL (range: 1.9-6.36). Overall, 7/22 (68%) of patients never reached viral load <200 cop/mL and 19 out of 22 (86%) failed therapy within 12 months of treatment.

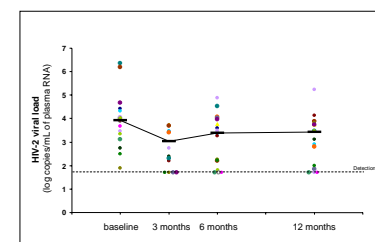


Figure 1: Viral load of HIV-2 patients before treatment and at months 3, 6 and 12 after initiation of HAART. — Indicate mean viral load at each time point.

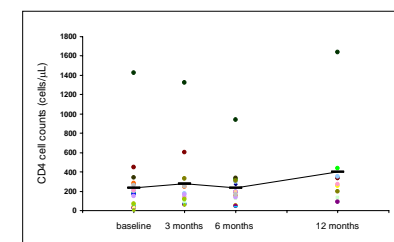


Figure 2: CD4 cell counts of HIV-2 patients before treatment and at months 3, 6 and 12 after initiation of HAART. — Indicate mean CD4 counts at each time point.

All patients were HIV-2 subtype A and carried wildtype viruses before treatment. As expected, the majority of patients failing therapy, 16/19 (84%), developed M184V in the RT. Interestingly, K65R was selected in 3 patients, 2 of whom failing AZT+3TC+abacavir and 1 receiving indinavir+abacavir+didanosine. Surprisingly, none of these patients developed Q151M, which has been reported to be frequently seen in HIV-2 patients failing AZT. Of the 5 patients receiving protease inhibitors, resistance genetic analysis could be performed in only 3; all of them carried mutations in the protease (I82F in 2 patients receiving Indinavir and L90M in 1 patient who received Indinavir and Kaletra).

Table 1: Resistance mutations in patients failing therapy.

Failure #	Treatment	1 Month	3 Months	6 Months	12 Months	Failure #	Treatment	1 Month	3 Months	6 Months	12 Months
1	Duovir-N IDV+Abacavir+ddI	2	WT	WT	WT	12	Duovir+Abacavir	0	WT	WT	WT
		3	M184V	---	---			3	M184V	---	---
		6	M184V	---	---			12	M184V	---	---
		12	L100M	---	---			6	M184V	---	---
2	Duovir+Abacavir	0	WT	WT	---	13	Duovir+Abacavir	0	WT	WT	WT
		6	M184V+I256K	---	---			6	M184V	---	---
3	Duovir-N	3	WT	WT	---	16	Duovir+Abacavir	0	WT	WT	WT
		6	M184V	---	---			3	M184V	---	---
		6	M184V	---	---			6	M184V	---	---
4	Duovir-N	0	WT	WT	---	21	Duovir+Abacavir	0	WT	WT	WT
		6	M184V	---	---			6	K65R+M184V	---	---
		12	M184V	---	---			6	K65R+M184V	---	---
5	Duovir+Abacavir	0	WT	WT	---	24	Duovir+Abacavir	0	WT	WT	WT
		12	M184V	---	---			6	M184V	---	---
6	Duovir+Abacavir	0	WT	WT	---	25	Duovir+Abacavir	0	WT	WT	WT
		12	M184V	---	---			6	M184V	---	---
7	Duovir+Abacavir	0	WT	WT	---	37	Duovir+Abacavir	0	WT	WT	WT
		3	M184V	---	---			3	M184V	---	---
8	Duovir+Abacavir	0	WT	WT	---	43	Duovir+Abacavir	0	WT	WT	WT
		12	M184V	---	---			6	M184V	---	---

V. Conclusions

Complete viral suppression in HIV-2 is not readily achieved and sustained over 1 year with triple antiretroviral regimens and drug resistance develops rapidly. Different patterns of resistance mutations in the RT of HIV-2 compared to HIV-1 seem to exist. In this study, K65R was selected