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Accumulation of drug-related mutations in HIV-1 genome and virus replicative capacity in multi-drug failure subjects

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Sir,

Reverse transcriptase (RT) and protease (PRO) are the major target of antiretroviral compounds currently employed in HIV-1-infected individuals.[1] Multiple-drug combinations with agents active against both enzymes show more benefits than monotherapy, as witnessed by a decrease in plasma HIV-1 RNA levels and an increase in CD4 cell counts.[2] However, even following combination therapies with several drugs (nucleoside RT inhibitors, NRTI; non-nucleoside RT inhibitors, NNRTI; and protease inhibitors, PI), an incomplete viral suppression not infrequently arises with viruses showing a reduced susceptibility to more than one inhibitor in different classes.[3] Deeks *et al.*[4] recently reported the advantage of maintaining antiretrovirals even in the presence of resistance. A suggested scenario in patients who fail different therapeutic regimens requires phenotypic and genotypic monitoring of drug resistance in order to tailor antiretroviral therapy (www.hivatis.org). This approach could be successful or conversely, if a suboptimal regimen is chosen, select for viral strains with an improved replicative capacity in the presence of drugs, rendering them less susceptible to different regimens directed against both enzymes.

We investigated nine HIV-1 isolates, at three different time-points for three patients (ZU, SA and CB). These viruses were isolated after patients presented a virological failure using antiretroviral regimens including a PI. First and second time-points were obtained in 1997 and 1999, and two out of three patients were subjected to a further treatment shift from an NNRTI to lamivudine in 2001, whereas the third patient was shifted to a PI-sparing regimen. Drug susceptibilities and *pol* gene sequences were determined as described previously.[5]

Viral isolates remained drug-susceptible to those compounds not included in their current regimen and exhibited an intermediate level of cross-resistance among PI. In each patient we detected a difference between the three time-points in RT and PRO genes (Table). GenBank accession numbers for RT: AY065954–AY065962; for PRO: AY154955 and AY065946–AY065953. The resistance-associated mutations and drug pressure were critical variates for HIV-1 replication. In all patients, the replicative capacity of the isolate at the first and third time-points was higher in the presence of lamivudine and lower in the presence of an NNRTI. The opposite effect was detected at the second time-point. A dose–response profile was maintained with those drugs that were not experienced *in vivo* by our three patients, including RT and PIs, contrary to previously experienced compounds.

We have shown a viral evolution in three heavily drug-experienced patients. The genotypic and phenotypic patterns of their resistant virus mirrored the therapeutic regimen used over time. In all three patients, the viral fitness as measured in their viral isolate was higher in the presence of resistant drugs and lower when the isolate was challenged in the presence of unexperienced compounds. Endorsing the observations by Deeks *et al.*[4] we have underlined the risk of resistance accumulation in patients with suboptimal viral suppression who experience therapy changes over time. We believe this phenomenon should be considered by updated HIV-1 treatment guidelines.

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This manuscript is dedicated to the memory of our friend Izi, to his strength and courage.

Table. Evolution of genotypes at different time-points

Sequence analysed	RT codons														PRO codons												
	41	67	69	70	103	108	118	181	184	188	210	211	215	219	10	20	24	32	36	46	48	54	63	64	71	82	84	90
Clade B consensus sequence	M	D	T	K	K	V	V	Y	M	Y	L	R	T	K	L	K	L	V	M	M	G	I	L	I	A	V	I	L
SA 1st time-point (ddC, 3TC, IDV)	L	N	D	R	–	–	–	–	V	–	–	K	F	Q	–	–	–	–	–	–	–	–	P	–	–	–	–	–
SA 2nd time-point (ZDV, EFV, NFV)	–	N	D	R	K/N	–	–	C	–	–	–	K	F	Q	–	–	–	–	–	–	–	–	P	I/V	–	V/I	–	M
SA 2001 (CBV, IDV)	–	N	D	R	–	–	–	C	M/V	–	–	K	F	Q	I	–	–	–	–	I	–	–	P	L/V	A/V	–	V	M
ZU 1st time-point (3TC, d4T, SQV)	–	–	–	R	–	–	–	–	V	–	–	K	–	Q	I	R	–	–	I	–	V	V	–	–	–	A	–	–
ZU 2nd time-point (NVP, d4T, SQV)	–	–	–	R	–	–	–	–	–	L	–	K	–	Q	I	R	–	–	–	I	V	V	–	–	V	A	–	–
ZU 2001 (CBV, IDV)	L	–	N	R	–	–	–	–	V	L	–	K	F	Q	I	R	–	–	I	–	V	–	–	–	V	A	–	–
CB 1st time-point (ZDV, 3TC, IDV)	L	–	–	–	–	–	–	–	V	–	–	–	Y	–	–	R	L/I	–	I	M/L	–	–	P	–	–	A	–	–
CB 2nd time-point (d4T, ddI, NFV)	L	–	–	–	–	–	–	–	–	–	W	–	Y	–	I	–	I	–	I	I	–	V	P	–	V	A	–	–
CB 2001 (CBV, EFV)	L	N	–	–	N	I	I	–	–	–	W	–	–	–	I/F	R	L/I	V/I	I	M/L	–	I/V	P	–	V	A	–	L/M

Amino acid sequences at the positions in RT and PRO that are known to cause drug resistance. Drugs at different time-points are defined in the table, and 2001 sequences were derived after a treatment shift from an NNRTI to lamivudine in the first two individuals, whereas the third patient was shifted to a PI-sparing regimen. M, methionine; D, aspartic acid; T, threonine; K, lysine; Y, tyrosine; L, leucine; R, arginine; V, valine; G, glycine; I, isoleucine; A, alanine; N, asparagine; C, cysteine; F, phenylalanine; Q, glutamine; P, proline. Antiretroviral therapy is included in brackets: ddC, zalcitabine; 3TC, lamivudine; IDV, indinavir; ZDV, zidovudine; EFV, efavirenz; NFV, nelfinavir; CBV, combivir (zidovudine plus lamivudine); d4T, stavudine; SQV, saquinavir; NVP, nevirapine.

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