



Clonal analyses of HIV quasiespecies in patients harbouring plasma genotype with K65R mutation associated with TAMs or L74V



M WIRDEN^{*1}, I MALET¹, A DERACHE¹, AG MARCELIN¹, B ROQUEBERT¹, A SIMON¹, M KIRSTETTER¹, L MORAND JOUBERT², C KATLAMA¹ and V CALVEZ¹.

¹Pitié-Salpêtrière and ²Saint-Antoine Hospital, Paris, France.

Background:

Incompatibility between K65R and thymidine analogue mutations (TAMs) or L74V mutation on the same virus has been described in previous studies. At the contrary, other studies have suggested a link between K65R and reverse transcriptase (RT)-mutations such as S68G or V75I. In clinical practice, some patients can harbour a genotypic resistance test with a combination of these different mutations. In most of these cases, the K65R and/or the others associated mutations are present as mixtures with wild-type. We wanted to know precisely the RT genotype of individual molecular clones derived from these samples and show whether these all mutations are really linked with K65R on the same strain.

Methods:

We analysed 5 samples harbouring plasma genotype with K65R associated with TAMs, L74V, S68G and/or V75I in 3, 1, 3 and 2 cases, respectively. In all of them, K65R emergence was observed after a Tenofovir regimen failure. For the clonal analyses, the RT fragments were cloned into pCR2.1-TOPO vector, before sequencing (automated population-based-full sequence analysis).

Results:

Clonal analyses of 2 patients showed that K65R can be linked with TAMs such as M41L, D67N, L210W and K219E in several clones, and more rarely with the T215Y mutation in a sole clone. At the contrary, analysis of another patient showed that K65R was never linked with the L74V mutation. The S68G was always or never or sometimes linked with the K65R, depending on the case. The V75I was not linked with K65R in one patient, while it was sometimes in the other case with the additional Q151M mutation.

Conclusion:

The coexistence of the both L74V and K65R mutations seems produce a too strong replicative impairment to be observed in vivo. However, if these two mutations are incompatible on the same virus, they can be selected on different strains in plasma of the same patient. The incompatibility between K65R and TAMs are more relative and perhaps more likely depends on the presence of antiretroviral compounds as Zidovudine in the treatment. The V75I mutation is not necessarily linked with K65R. The same observation is done about the S68G substitution that is not necessarily a compensatory mutation of the K65R as some authors have suggested.

TABLE 1 RT mutations harbored by the plasma genotype (bulk) performed before Tenofovir initiation and after failure with details of treatment.

Patient	Baseline RT mutations	RT mutations at failures	Treatment (duration of TDF exposure in months)
1	M41L, S68G, MI84V, L210W, T215D	M41L, K65R/K, S68G, MI84V, L210W, T215Y	TDF, ABC, 3TC (2)
2	MI84V	K65R/K, D67G/D, S68G, K70R, V75I, MI84V, K219E,	TDF, ABC, 3TC (5)
3	D67N, K70R, MI84V	K65R, D67N, MI84V, K219E	TDF, ABC, 3TC (13)
4	MI84V	K65R/K, S68S/G, L74V/L, Y115F, MI84V	TDF, ABC, 3TC, ddI (6)
5	T69D, Q151M, MI84V	K65R/K, T69D, V75I, F77L, Y115F, V118I, Q151M, MI84V	TDF, 3TC, LPV (16)

TABLE 2. RT mutations harbored by the bulk genotype at failure compared with those harbored by the different clones for each patient.

Patient 1		TAMs observed				Other RT mutations				Patient 4		Other RT mutations			
	K65	M41	L210	T215	E44	S68	V118	MI84		K65	S68	K70	L74	Y115	MI84
Bulk	R/K	L	W	Y	D	G	I	V	Bulk	R/K	S/G		V/L	F	V
No.clones									No.clones						
22/27		L	W	Y	D	G	I	V	10/30				V	F	V
3/27	R	L	W	D	D	G	I	V	8/30	R				F	V
1/27	R	L	W	Y	D	G	I	V	4/30	R	G			F	V
1/27		L	W	D	D	G	I	V	4/30	R	N			F	V
									3/30			Q	V	F	V
									1/30					F	V

Patient 2		TAMs observed				Other RT mutations			Patient 5		Other RT mutations					
	K65	D67	K70	K219	S68	V75	MI84		K65	T69	V75	F77	Y115	V118	Q151	MI84
Bulk	R/K	G/D	R	E	G	I	V	Bulk	K/R	D	I	L	F	I	M	V
No.clones								No. clones								
10/29		G	G		G	I	V	5/9		D	I	L	F	I	M	V
6/29		G	R	E	G	I	V	4/9	R	D	I	L	F	I	M	V
6/29		G	R		G	I	V									
2/29	R						V									
2/29							V									
1/29				E			V									
2/29		G	G	E	G	I	V									

Patient 3		TAMs observed		Other RT mutation	
	K65	D67	K219		MI84
Bulk	R	N	E		V
No.clones					
18/18	R	N	E		V