



The Role of Innate Polymorphisms in Drug Selected Reverse Transcriptase (RT) Mutations in HIV-1 and HIV-2 Subtypes

Michel Ntemgwa*, Bluma G. Brenner, Thomas d'Aquin Toni, Maureen Oliveira, Daniela Moisi, Bonnie Spira, Eugene L. Asahchop, Jordana R. Schachter, Jorge L. Martinez-Cajas and Mark A. Wainberg

McGill University AIDS Centre, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada



For further information:
Michel Ntemgwa
3755 Chemin Côte-St-Étienne, room #318
Montreal, Quebec, H3T 1E2 Canada
Tel.: (514) 340-8222 ext. 3454
E-mail: michel.ntemgwa@mail.mcgill.ca

Abstract

Background

The presence of innate resistance mutations and polymorphisms in drug naive, HIV infected persons may impact on subsequent responses to antiviral therapy. Recent findings suggest bidirectional antagonisms between K65R and thymidine analogue mutations (TAMs) in treatment experienced patients. Our studies show facilitated development of K65R in subtype C due to a signature KKK motif at codons 64, 65 and 66. This present study addressed the effects of innate polymorphisms (T69N, V75I, V118I, L210N, T215S and K219E) in HIV-2 on emergent resistance to nucleoside/nucleotide analogues.

Methods

Emergent drug resistance profiles in HIV-2 subtype A (n=3) and B (n=1) were compared to HIV-1 subtypes B and C. Drug resistance was evaluated in cord blood mononuclear cells (CBMCs), using selective pressure with tenofovir (TFV), zidovudine (ZDV), stavudine (d4T), didanosine (ddI), abacavir (ABC) and lamivudine (3TC), and dual combinations of TFV-ddI, TFV-ABC, TFV-3TC, ZDV-3TC, and d4T-ddI. Culture fluids were analyzed weekly for virus production by RT assay. Resistance was evaluated using conventional and ultra-sensitive sequencing approaches.

Results

In agreement with our previous findings, dual drug combinations of TFV, ddI, ABC, d4T, ZDV and 3TC preferentially selected for the K65R mutation in HIV-1 subtype C isolates within 22 weeks. In single drug selections, K65R was observed with TFV and ddI and M184V with 3TC. In HIV-1 subtype B, TFV-3TC and ZDV-3TC selected for M184I and D67N respectively. In stark contrast, selections with all four HIV-2 cultures favored development of M184I in all dual drug combinations that included 3TC. With single drugs, M184V appeared within 6 weeks while some HIV-2 isolates developed S134A, V167I and A174V under TFV selective pressure. Since HIV-2 cultures did not develop K65R, an ultra-sensitive codon-specific real-time PCR assay was developed and the latter distinguished the presence of the K65R mutation from the wild type HIV-2 plasmid by 16 cycles (ΔCT). Furthermore, TAMs were antagonistic to the development of K65R in HIV-2.

Conclusions

These results underscore potential differences in emergent drug resistance pathways in HIV-1 and HIV-2 and show that polymorphisms may determine the resistance pathways that emerge. These studies may have implications in the design of TAM-sparing regimens used against HIV-1 subtype C.

Baseline Genotype of HIV-2 RT: Polymorphisms

nRTI			NNRTI		
T69N, V75I, V118I, L210N, T215S, K219E	V90I (3/4), K101A, K103R (1/4), V106I, V179I, Y181I, Y188L, G190A,				

nRTI: nucleoside or nucleotide reverse transcriptase inhibitor, NNRTI: nonnucleoside reverse transcriptase inhibitor.

Natural polymorphisms in HIV-2 RT at positions known to be implicated in HIV-1 resistance to nRTIs and NNRTIs

Polymorphisms at codons 64, 65 and 66 in HIV RT

	64	65	66	K65K	K65R
HIV-1 subtype B	AAG	AAA	AAA	AAA	AGA
HIV-2 subtype A	AAG	AAA	AAG	AAA	AGA
HIV-1 subtype C	AAA	AAG	AAG	AAG	AGG
HIV-2 subtype B	AAA	AAG	AAG	AAG	AGG

Our studies have shown facilitated development of K65R in HIV-1 subtype C due to a signature KKK motif at codons 64, 65 and 66.

Time to the development of mutations associated with resistance in HIV-1 and HIV-2 using single nRTIs

DRUG	WEEK	HIV-1		HIV-2			
		Subtype C BG05	Subtype B SS12	Subtype A CBL-20	Subtype B CBL-23	MVP-15132	Subtype B CDC310319
ABC	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	L74V, M184V or K65R	wt	wt	wt	wt	wt
d4T	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	wt	wt	wt	wt	wt	wt
ddI	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	K65R	wt	wt	wt	wt	wt
TFV	1-5	wt	wt	wt	wt	wt	wt
	12	K65R	wt	wt	wt	wt	wt
	22-30	K65R	wt	A174V	S134A, V167I	wt	wt
ZDV	1-5	wt	wt	wt	wt	wt	nd
	22-30	wt	wt	wt	wt	wt	wt
3TC	1-5	wt	wt	wt	wt	wt	wt
	6	nd	M184V	M184V	M184I	M184V	M184V

In single drug selections, K65R was observed with TFV and ddI in HIV-1 subtype C. Some HIV-2 isolates developed S134A, V167I and A174V under TFV selective pressure. M184V developed within 6 weeks with 3TC in HIV-1 and HIV-2 cultures. The K65R mutation was not selected in HIV-2 cultures. End point drug concentrations in μM: ABC 5.0, d4T 20, ddi 20, TFV 10, ZDV 10 & 3TC 1.0

Time to the development of mutations associated with resistance in HIV-1 and HIV-2 using dual nRTI combinations

DRUG	WEEK	HIV-1		HIV-2			
		Subtype C BG05	Subtype B SS12	Subtype A CBL-20	Subtype B CBL-23	MVP-15132	Subtype B CDC310319
ZDV-3TC	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	K65R	D67N	M184I	nd	M184I	M184I
TFV-ddI	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	K65R	wt	wt	wt	wt	wt
TFV-ABC	1-5	wt	wt	wt	wt	wt	wt
	12	wt	wt	wt	wt	wt	wt
	22-30	K65R	wt	wt	wt	wt	wt
d4T-ddI	1-5	wt	wt	wt	wt	wt	wt
	12	K65R	M184I	M184I	M184I	M184I	M184I
	22-30	K65R	wt	wt	wt	wt	wt

HIV-2 subtype A: CBL-20, CBL-23 and MVP-15132; HIV-2 subtype B: CDC310319
HIV-1 subtype B: SS12; HIV-1 subtype C: BG05; Wt: wild type

Dual drug combinations of TFV, ddI, ABC, d4T, ZDV and 3TC preferentially selected for the K65R mutation in HIV-1 subtype C isolates within 22 weeks. In HIV-1 subtype B, TFV-3TC and ZDV-3TC selected for M184I and D67N respectively. Selections with all four HIV-2 cultures favoured development of M184I in all dual drug combinations that included 3TC. The K65R mutation was not selected in HIV-2 cultures.
End point drug concentrations in μM: ZDV/3TC 0.1/1.0, TFV/ddI 2.5/5.0, TFV/ABC 2.5/2.5, TFV/3TC 2.5/5.0, d4T/ddI 2.0/5.0

Differential amplifications observed using the K65R-specific PCR assay in HIV-2

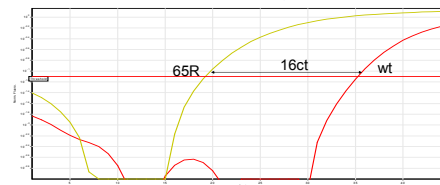


FIG 1a

Since HIV-2 cultures did not develop K65R, this mutation was introduced into an HIV-2₂₂₀ plasmid by site-directed mutagenesis and an ultra-sensitive codon-specific real-time PCR assay was developed. We used 3' locked nucleic acid (LNA) allele specific (AS) PCR primers and SYTO 9, an intercalating dye. The run was carried out in the Rotor-Gene™ 6000 real-time rotary analyzer (Corbett Life Sciences.) As shown in FIG 1a, this assay distinguished the presence of the K65R mutation from the wild-type HIV-2 plasmid by 16 cycles (ΔCT) (65636) fold decrease in efficiency of amplification of the incorrect target. FIG 1b shows the High Resolution melt (HRM) analysis and confirms the results obtained in FIG 1a. HRM is a simpler and much more cost effective way to characterise DNA samples according to their sequence compared to probes.

High resolution Melt Analysis

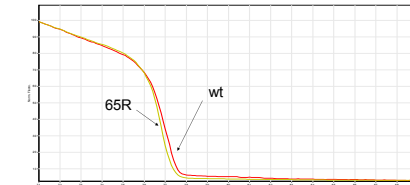


FIG 1b

Amplification plots of K65R mutant plasmid using the K65R AS-PCR primer.

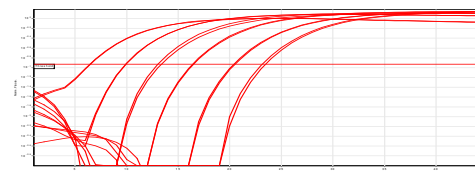


FIG 2a

10 fold dilutions of mutant plasmid was prepared in duplicates from 10⁸ to 10⁵. The amplification curves are shown in FIG 2a. Similar curves were obtained when primers for wild-type and total virus population were used (data not shown). The standard curve generated from FIG 2a is shown in FIG 2b. The efficiency was 0.97 and gradient -3.4

Standard curve of K65R mutant plasmid using the K65R AS-PCR primer

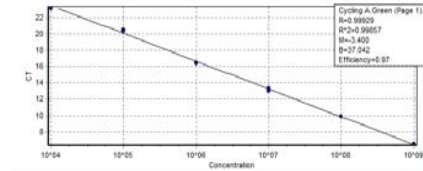


FIG 2b

Amplification plots of CBL-20 TFV after 30 weeks of drug pressure using different AS primers

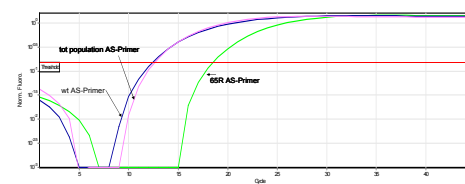


FIG 3a

High resolution Melt Analysis

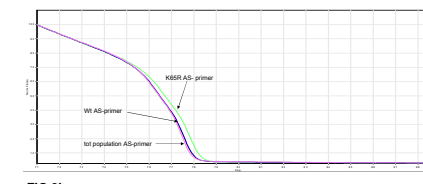


FIG 3b

We tested HIV-2 cultures after 30 weeks of culture in CBMCs from both single drug and dual drug combinations for the presence of the K65R mutation using our assay. We used three primer pairs for each sample. One pair for the mutation specific reaction, one for wild-type specific reaction, and another for the total (tot) virus population reaction. As indicated in the amplification plots (FIG 3a) and HRM analysis (FIG 3b), the K65R mutation was absent even after 30 weeks of culture under TFV drug pressure. In FIG 3a, total virus population and wild type curves appeared at the threshold level at the same point indicating the absence of K65R. Similar results were obtained for other samples (data not shown)

Acknowledgments

This project was funded by CANFAR and CHRR. The work was performed in partial fulfillment of Michel Ntemgwa's PhD project in McGill University, Department of Experimental Medicine. MN is the recipient of a CHRR Doctoral Research Award. We thank Dr. Andrew Lever of Cambridge University, UK and Dr. Tom Schulz Hannover University, Germany for providing us with HIV-2₂₂₀ plasmid.