Hot Spots of Integrase Genotypic Changes Leading to HIV-2 Resistance to Raltegravir[▽]

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Received 12 July 2010/Returned for modification 8 December 2010/Accepted 16 December 2010

We studied seven heavily pretreated HIV-2-infected patients exhibiting a virological failure while receiving a salvage raltegravir-containing regimen. At the time of virological failure, different resistance genetic pathways were observed: T97A-Y143C, Q148K, Q148R, G140S-Q148R, E92Q-Y143R-N155H, and T97A-N155H. Thus, despite a 40% difference in integrase genes between HIV-1 and HIV-2, the genetic pathways leading to raltegravir resistance are similar.

Raltegravir (RAL), the first-licensed compound of the integrase inhibitor (INI) drug class, is active against HIV type 2 (HIV-2), since the phenotypic susceptibility to RAL of wild-type HIV-2 clinical isolates was found in a range similar to that for HIV-1 (12). Previous studies have established HIV-1 integrase (IN) mutations involved in RAL resistance, which are reported in the different genotypic interpretation algorithms (<http://www.hivfrenchresistance.org>; <http://hivdb.stanford.edu>) with primary (Y143H/R/C, Q148K/R/H, N155H) and secondary resistance-associated mutations. Preliminary results for HIV-2 showed that RAL resistance involved the two main resistance mutations, Q148K/R/H and N155H, already described in HIV-1 RAL resistance, with an increase in 50% inhibitory concentration (IC₅₀) for both RAL and elvitegravir (5, 11, 13).

The aim of the study was to determine genetic mutational pathways associated with the development of resistance to RAL in HIV-2-infected patients.

Seven heavily pretreated HIV-2-infected patients receiving a salvage RAL-containing regimen exhibited incomplete viral suppression or virological failure, defined as plasma HIV-2 RNA level of >100 copies/ml 3 months after initiation of regimen. HIV-2 viral load quantification was determined by an in-house assay (2). All of the patients were identified from the French HIV-2 Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) cohort CO5. The median duration of the RAL-based regimen was 14 months (interquartile range [IQR], 12 to 16). The complete IN gene was retrospec-

tively amplified by population-based sequencing from plasma samples collected at baseline and at time of virological failure of RAL-containing treatment, as previously described (12). RAL resistance-associated mutations were identified among the following: H51Y, T66I/A/K, V72I, L74I/A/M, E92Q, T97A, T112I, F121Y, T125K, A128T, E138K/A/D, G140A/S/R/C/H, Y143C/H/R, Q146K/P, S147G, Q148K/R/H, V151I, S153Y/A, M154I, N155S/H, K156N, E157Q, K160D/N, G163R/K, V165I, V201I, I203M, T206S, S230N/R, V249I, R263K, and C280Y (7). HIV-2 protease and reverse transcriptase sequencing was performed using previously described procedures (4, 10). Then, mutations known to be involved in HIV-1 and/or HIV-2 resistance were interpreted using the French algorithm (www.hivfrenchresistance.org).

At baseline, the median HIV-2 viral load and CD4 cell count were 4.28 log₁₀ copies/ml (IQR, 4.00 to 4.50) and 48 cells/mm³ (IQR, 37 to 109), respectively. Four patients were infected with HIV-2 subtype A, two with subtype B, and one with subtype H. The optimized background therapy associated with RAL for each patient is described in Table 1. All patients received darunavir boosted with ritonavir. The nucleoside reverse transcriptase inhibitors (NRTI) lamivudine (or emtricitabine), abacavir, tenofovir, and zidovudine were received by six, three, four, and one patient, respectively. Baseline plasma virus exhibited a median number of eight mutations described as associated with resistance in HIV-1 infection (Table 1). The median number of NRTI resistance-associated mutations was 3, with the individual numbers as follows: N69D/S (*n* = 4), K65R (*n* = 3), Q151M (*n* = 3), M184V (*n* = 3), A62V (*n* = 2), Y115F (*n* = 2), D67N (*n* = 1), K70R (*n* = 1), S215W (*n* = 1). The median number of protease inhibitor resistance-associated mutations exhibited by baseline virus was 4, with the individual numbers as follows: V71I (*n* = 5), I54L/M (*n* = 4), I50V (*n* = 3), L90M (*n* = 3), V33I (*n* = 2), I82F (*n* = 2), V20I

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[▽] Published ahead of print on 28 December 2010.

TABLE 1. Baseline characteristics of HIV-2-infected patients initiating a raltegravir-based regimen^a

Patient	HIV-2 subtype	HIV-2 viral load (log ₁₀ copies/ml)	CD4 cell count (cells/mm ³)	Therapeutic regimen	RT mutations	PR mutations
1	A	4.50	274	3TC, ABC, DRV/r, RAL	A62V, K65R, N69S	I54M, V71I, I84V, L90M
2	A	4.50	56	ABC, TDF, DRV/r, RAL	K65R, N69S, Q151M	V33I, I46V, I54M, V71I, I82F, L90M
3	A	4.75	197	3TC, DRV/r, RAL	A62V, D67N, Y115F, Q151M, M184V	I50V, V71I, I82F
4	A	3.80	27	3TC, ABC, DRV/r, RAL	K70R, Q151M, S215W	None
5	B	4.00	40	3TC, AZT, DRV/r, RAL	K65R, N69S, Y115F, M184V	I50V, I54M, V71I, L90M
6	B	4.00	37	3TC, DRV/r, RAL	N69D, M184V	V20I, V33I, I50V, I54L, V71I, I89V
7	H	4.30	109	ABC, FTC, TDF, DRV/r, RAL	Not amplified	Not amplified

^a 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; DRV/r, darunavir boosted with ritonavir; FTC, emtricitabine; PR, protease; RAL, raltegravir; RT, reverse transcriptase; TDF, tenofovir.

($n = 1$), I46V ($n = 1$), I84V ($n = 1$), I89V ($n = 1$). No RAL resistance-associated mutations were found at baseline by bulk sequencing.

Although baseline viral loads were initially high, especially for HIV-2-infected patients, five of seven patients showed a rapid and remarkable decrease in HIV-2 RNA titers. Indeed, within 3 months after initiation of RAL-containing regimen, the HIV-2 viral load was below the limit of detection (100 copies/ml) in five patients. For the two remaining patients, decreases of 0.26 and 1.00 log₁₀ copies/ml in HIV-2 level were observed, failing to reach an undetectable level.

A virological rebound was observed at a median of 8 months after RAL treatment initiation among the five patients who initially reached HIV-2 RNA undetectable levels. At the time of virological failure, three different genotypic patterns in the IN gene were observed. Population-based sequencing technology prevented the determination of whether drug resistance mutations are present on the same viral genome.

First, the residue 148 pathway was observed in virus from three patients, with the presence of the Q148R, Q148K, and G140S-Q148R mutations in patients 1, 2, and 4, respectively (Table 2). The mutation Q148H, the most frequent mutation detected in HIV-1, was not detected in this series of HIV-2-infected patients. IN genotypic evolution was observed at further time points under continued RAL drug pressure in virus from two patients (1 and 2), showing the addition of the secondary mutation G140S in one case and the switch from

Q148K to the Q148R mutation followed by the addition of the G140S mutation in the second case. Second, for the residue 155 pathway, the N155H mutation was present with the T97A mutation in virus from patient 6. This genetic resistance profile remained stable during the follow-up. Finally, we observed the residue 143 pathway, with the selection of the T97A and Y143C mutations, in virus from two patients (3 and 7). A genotypic evolution was observed, with a switch to the Y143G mutation, in patient 3. Plasma virus from the remaining patient, patient 5, exhibited RAL mutations linked to two distinct genetic pathways, N155H and Y143H/R with the E92Q mutation. IN direct sequencing at further time points showed the disappearance of the Y143H/R mutation and a mixture at residue 92 (E92A/E/P/Q) with the N155H mutation.

The kinetics of development of resistance did not seem to be related to a specific genotypic resistance profile. Of note, the N155H pathway was observed only in the two patients infected with HIV-2 subtype B virus.

The selection of resistance mutations in protease and reverse transcriptase, concomitantly with RAL resistance development, was assessed in the four patients for whom successful genotypic resistance tests were obtained. Compared to the baseline genotype, additional mutations in protease and reverse transcriptase were observed in three patients (patients 2, 3, and 5) at the time of virological failure. The selection of the I15V mutation in protease was observed in virus from two patients (patients 2 and 5), both receiving darunavir boosted with ritonavir. In virus from patient 3, I89V and L90M mutations emerged in protease and the A62V mutation disappeared in reverse transcriptase at the time of virological failure.

This study showed, among seven highly antiretrovirus-experienced HIV-2-infected patients displaying virological failure while receiving a RAL-containing regimen, the role in the development of HIV-2 resistance to INIs of the three main resistance pathways: Q148H/K/R, N155H, and Y143C/H/R.

This study pointed out the genetic profiles of HIV-2 resistance to RAL. Despite an approximately 40% amino acid difference between HIV-2 and HIV-1 in wild-type IN genes, these profiles seemed to be quite similar to those found in HIV-1 infection (1), involving similar residues at the same key positions in the development of resistance among HIV-1 and HIV-2 infections. One explanation could be the 100% conser-

TABLE 2. Integrase genotypic evolution in HIV-2-infected patients failing on a raltegravir-containing regimen

Genetic pathway	Patient	Raltegravir resistance-associated mutations at time of virological failure	Integrase genotypic evolution under continued raltegravir drug pressure
143	3	T97A-Y143C	T97A-Y143G
	7	T97A-Y143C	T97A-Y143C
148	1	Q148R	G140S-Q148R
	2	Q148K	G140S-Q148R
	4	G140S-Q148R	G140S-Q148R
155	5	E92E/Q-Y143H/R-N155H	E92E/A/P/Q-N155H
	6	T97A-N155H	T97A-N155H

vation of the catalytic triad DDE and the HHCC and RKK motifs between HIV-1 and HIV-2 (7).

Genotypic evolution of RAL resistance profiles was observed over time in patients who remained on a failing RAL-containing regimen, mainly corresponding to the selection of secondary RAL resistance-associated mutations, known to improve the viral replicative capacity (6). In HIV-2-infected patients assessed in our study, no switch of genotypic resistance profile from residue 155 to 148 or from residue 155 to 143 was evidenced, conversely to what was observed in different HIV-1 RAL resistance studies (3, 8, 9). Studies assessing HIV-2 viral replicative capacity and phenotypic resistance level are needed to better describe the impact of RAL resistance-associated mutations in this genetic context.

Nucleotide sequence accession numbers. Integrase sequences were submitted to GenBank and given the following accession numbers: HQ694834 to HQ694845.

We thank Juliette Leleu (clinical research assistant) for excellent technical assistance.

The research leading to these results has received funding from the French National Agency for Research on AIDS and Viral Hepatitis (Agence Nationale de Recherches sur le SIDA et les hépatites virales [ANRS]) and the European Community's Seventh Framework Programme (FP7/2007–2013) under the project Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN) (grant no. 223131).

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