Resistance Associated Mutations to Etravirine (TMC-125) in Antiretroviral Naïve Patients infected with non-B HIV-1 subtypes

1 Almoustapha Issiaka Maïga, 2 Diane Descamps, 1 Laurence Morand-Joubert, 3 Mamadou Cisse, 4 Andre Capt, 1 Christine Katlama, 1 Dominique Costagliola, 5 Bernard Masquelier,1 Vincent Calvez and 1 Anne-Genevieve Marcelin

1 INSERM U943, Pitie-Salpetriere Hospital, Paris, France ; 2 Bichat-Claude Bernard Hospital, Paris, France ; 3 CESAC, Bamako, Mali ; 4 Virco, Belgium ; 5 CHU Bordeaux, France.

CROI 2010 SAN FRANCISCO FEBRUARY 16-19, 2010

553

Poster will be available online on the CROI website

E-mail: anne-genevieve.marcelin@psl.aphp.fr

Abstract

Background: Susceptibility to etravirine (ETR), a second generation non-nucleoside reverse transcriptase inhibitor (NNRTI), is dependent on the type and number of NNRTI resistance-associated mutations (RAMs). Studies have shown that some HIV-1 subtypes may have natural polymorphisms described as ETR RAMs. This study addresses the prevalence of ETR RAMs in treatment-naïve patients infected with HIV-1 non-B subtypes and its potential impact on ETR susceptibility.

Methods: The prevalence of ETR RAMs was studied in 726 antiretroviral naïve patients infected with non-B HIV-1 subtypes. ETR genotypic resistance was interpreted according to ANRS and Stanford algorithms. NNRTIs phenotypic susceptibility of samples with at least one ETR RAMs was measured. Phenotypic tests were done with a phenotypic assay (Antivirogram, VIRCO BVBA, Mechelen, Belgium). Fold changes (FC) in IC50 cut-off for normal susceptible range were 6.0, 3.3 and 3.2 for nevirapine, efavirenz and ETR, respectively.

Results: 75/726 (10.3%) of the sequences harbored at least one ETR RAM: 71 (9.8%) patients had one ETR RAM and 4 (0.5%) had two ETR RAMs (V90I+Y181C, E138A+V179E and V90I+A98G in 2 cases). None of the viruses had three or more ETR RAMs and consequently classified as resistant to ETR. All sequences with 2 ETR RAMs belong to CRF02_AG. The presence of one ETR RAM was statistically more frequent in CRF02_AG than in other non-B subtypes ($p = 0.004$). Among the strains harbouring at least one ETR RAM, 3 were phenotypically resistant to ETR: 1 with 2 ETR RAMs and 2 with 1 ETR RAM associated to another NNRTI resistance mutation. Indeed, these new mutations profiles showed decreased ETR phenotypic susceptibility E138A+V179I (FC = 5.2), Y181C+H221Y (FC = 11.1) and V90I+Y181C (FC = 3.3) were identified.

Conclusions: Although the prevalence of ETR RAMs in treatment-naïve patients infected with non-B HIV-1 subtypes was 10%, this had in most of cases no significant impact on ETR susceptibility. However, the transmission of drug resistant viruses with Y181C in a non-B genetic background has a potential impact on ETR susceptibility. In addition, our results suggest that mutations V179I and H221Y should be considered as ETR RAMs.

Objective

Some studies have shown that non-B subtypes may have natural polymorphisms described as etravirine (ETR) Resistance Associated Mutations (RAMs). Although ETR previously showed comparable activities against different group M subtypes (A to H), including several CRFs, the testing was done with few strains, and few data concerning the impact of the HIV-1 subtype on the virological response to ETR are currently available.

The aim of this study was to evaluate the prevalence of ETR RAMs in a large panel of patients infected by various non-B HIV-1 subtypes and who never received antiretroviral treatment, and to study the ETR phenotypic susceptibility of their strains.

Patients and methods

Patients: HIV-1 seropositive individuals infected with non B subtype were eligible for this study if they had never been exposed to antiretroviral drugs before the time of sampling. Briefly, samples were collected at time of HIV diagnosis or before the start of antiretroviral treatment. In total, 726 patients were included from the following centers (no. of patients): CESAC, Centre d'Ecoute, de Soins, d'Animation et de Conseils in Bamako, Mali (163); Nianankoro Fomba Hospital in Ségou, Mali (118); Pitié-Salpêtrière Hospital in Paris, France (192), Bichat Claude-Bernard Hospital in Paris, France (182); and Saint-Antoine Hospital in Paris, France (71). For each patient a single HIV-1 sequence was included.

Virological methods: RT sequences were determined by bulk sequencing. We studied the prevalence of ETR RAMs according to the latest international AIDS Society (IAS)-USA panel list (www.iasusa.org, last update in December 2008): V90I, A98G, L100I, K101E, K101H, K101P, V106I, E138A, V179D, V179F, V179T, Y181C, Y181I, Y181V, G190A, G190S, and M230L. In case of presence of ETR RAMs, resistance genotypic tests were interpreted according to the last version of Agence Nationale de Recherches sur le SIDA (ANRS) (www.hivfrenchresistance.org) and Stanford algorithms (http://www.hivfrenchresistance.org/;http://hivdb6.stanford.edu/asi/deployed/hiv_central.pl?program=hivalg&action=showSequenceForm). Samples with at least one ETR RAMs were tested for phenotypic susceptibility to nevirapine (NVP), efavirenz (EFV) and ETR. Phenotypic tests were done with a commercial phenotypic assay (Antivirogram, VIRCO BVBA, Mechelen, Belgium). Fold changes (FC) in IC50 cut-off for normal susceptible range were 6.0, 3.3 and 3.2 for NVP, EFV and ETR, respectively. The definitions for resistance are those as defined by Virco: below these values samples were considered within normal susceptible range and above these values, samples were considered above normal susceptible range or resistant.

This study was supported by ANRS (French National Agency for AIDS Research) and the European Community's Seventh Framework Programme (FP7/2007-2013) under the project "Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)" - grant agreement n° 223131.

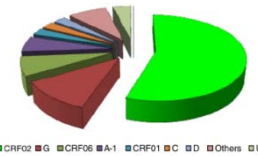
Resistance-associated mutations to etravirine (TMC-125) in antiretroviral-naïve patients infected with non-B HIV-1 subtypes.
Maïga AI et al. Antimicrob Agents Chemother. 2010 Feb;54(2):728-33.

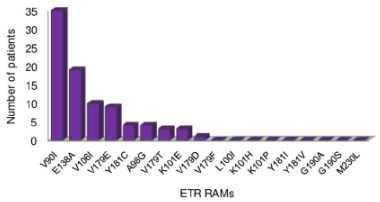
Collaborative HIV and Anti-HIV Drug Resistance Network

Results

Characteristics of patients (n = 726)

Distribution of HIV-1 subtypes

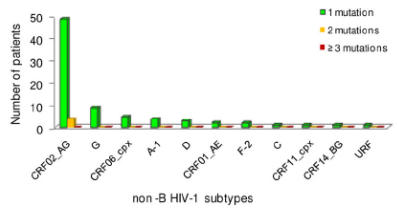




Distribution of ETR RAMs in non-B subtypes

Prevalence of ETR RAMs in non-B subtypes

- According to the December 2008 IAS-USA list of RAMs, **75 (10.3%)** of 726 sequences harbored at least one ETR RAM: sequences from 72 patients (10%) each had one ETR RAM, and sequences from 3 patients (0.4%) each had two ETR RAMs (V90I and Y181C in one case and V90I and A98G in two cases). No patient had a sequence with three or more ETR RAMs.
- According to the Stanford list, 88 (12.1%) of 726 sequences harbored at least one ETR RAM: sequences from 84 patients (11.6%) had one ETR RAM, and sequences from 4 patients (0.5%) had two ETR RAMs (V90I and Y181C, E138A and V179E, and in two cases, V90I and A98G). Also, no patient had three or more ETR RAMs.
- All sequences with two ETR RAMs belonged to CRF02_AG. The presence of one ETR RAM was statistically more frequent in CRF02_AG than in other non-B subtypes ($P = 0.004$) .



Number of ETR RAMs in different non-B subtypes

Phenotypic study

Patient	HIV-1 subtype	ETR mutations	Other RT mutations	Fold change in IC_{50} NVP	EFV	ETR
38	CRF02_AG	V90I	V35T, E36D, K43R, V60I, K101R, K122E, D123S, I135V, S162A, K173T, Q174K, D177E, **V179I**, G196E, T200A, Q207E	0.9	1.2	1.4
675	CRF02_AG	V90I	V35T, T39L, K122E, D123N, S162A, E169T, K173E, Q174E, D177E, T200A, I202V, Q207E	2.0	1.9	1.3
948	CRF02_AG	V90I	V35T, K64R, K102Q, D123E, S162A, K173T, Q174K, D177E, G196E, T200A, Q207E, R211K	1.4	2.1	2.0
954	CRF02_AG	V90I	V35T, T39N, K46Q, V60I, S68G, D123E, I135V, S162A, K173A, Q174K, D177E, T200A, Q207E, R211K	1.3	1.7	1.6
967	CRF02_AG	V90I	V35T, E36D, T39K, I50V, V60I, S68G, I135V, S162A, K173V, Q174K, D177E, T200A, E203Q, Q207D, R211K	1.1	1.2	1.0
1344	CRF02_AG	V90I	V35T, E36D, V60I, K122E, D123S, I135V, E138D, S162A, K173T, Q174S, D177E, I178M, V189I, T200A, I202V, Q207E, F214L, V245Q	0.7	1.2	1.3
1500	CRF02_AG	A98G	V35T, V60I, I135L, S162A, K173A, Q174K, D177E, I178M, T200A, Q207E, R211K, V245Q, D250E	**8.3**	**3.6**	2.9
907	CRF02_AG	K101E	K30E, I31P, V35Q, T39S, E44G, E53G, A62G, N81H, Q91H,I135V, S162A, K173S, Q174R, D177E, V179I, T200I, Q207N, R211K, F214L	2.2	1.9	1.3
1362	CRF02_AG	K101E	V35T, E36D, K49R, V60I, K122P, I135R, S162A, E169R, K173T, Q174E, D177E, V189I, T200A, I202V, Q207E, R211K, V245Q	0.6	1.0	1.1
1491	CRF06_cpx	V106I	V21I, V35T, V60I, K122T, D123R, I135V, S162A, K173T, Q174K, D177E, I178L, T200A, Q207D, R211K, V245Q, E248D	4.4	**3.8**	2.6
879	CRF02_AG	E138A	G18V, Q23P, V35T, E40D, V60I, S68G, D123E, I135V, S162A, K173T, Q174N, T200A, Q207E, R211K, F214L	0.9	1.0	1.1
883	CRF02_AG	E138A	A33E, V35T, T39A, D123E, S162A, Q174G, T200E, I202V, Q207E	2.5	2.1	1.9
937	CRF02_AG	E138A	V35T, K122E, I135V, T139A, S162A, K173T, Q174K, N175Y, D177E, T200A, Q207E, R211K	0.8	1.2	0.8
955	CRF02_AG	E138A	K22N, V35T, T39A, K122E, I135V, T139A, S162A, K173T, Q174A, D177E, T200A, Q207E, R211K, F214L	1.4	2.6	1.5
1016	CRF02_AG	E138A	K32R, V35E, T39S, S48T, V60I, D121Y, K122E, I135T, S162A, K173A, Q174K, D177E, **V179I**, T200E, Q207D, R211K	2.9	2.9	**5.2**
1022	CRF02_AG	E138A	K20R, V35T, E36A, T39D, V60I, I135V, T139I, K173A, Q174K, T200E, Q207A, R211K, F214L	0.5	2.0	3.0
1067	CRF02_AG	E138A	K20R, V35T, E36D, T39N, V60I, D123N, I135V, S162A, K173T, Q174K, D177E, T200A, Q207E, R211K	0.4	0.8	1.7
80	CRF02_AG	Y181C	V35T, V60I, I135V, S162A, K173T, Q174K, N175Y, D177E, T200A, Q207E, R211K, **H221Y**	**>40.6**	**9.9**	**11.1**
1370	CRF02_AG	V90I, Y181C	V35T, T39M, P119S, D123E, S162A, E169D, K173T, Q174K, D177E, T200A, Q207E, K219N, V245Q, E248D	**>70.9**	3.0	**3.3**
1051	CRF02_AG	V90I, A98G	V35T, E36D, T39K, S48A, I50V, V60I, S68G, I135V, S162A, K173T, Q174K, D177E, G196E, T200A, E203G, Q207D, R211K, Q222P	2.4	1.5	1.2

ETR, etravirine ; RT, reverse transcriptase ; NVP, nevirapine ; EFV, efavirenz. Mutations in bold are not ETR RAMs but belong to the compiled list of mutations associated with resistance to NNRTIs.

Phenotypic results were available for 20 clinical samples harboring at least one ETR RAM

- Mutations V90I, A98G, K101E, V106I, and E138A alone were not associated with increased ETR resistance.
- Two samples with only one ETR RAM (mutations V179I and H221Y are not considered as ETR RAMs) were associated with an increase (> 3.2-fold) in the ETR IC50:

➢ **E138A** and V179I (patient no. 1016): 5.2 fold
This genetic profile is not considered to be resistant to ETR according to ANRS and Stanford algorithms

➢ **Y181C** and H221Y (patient no. 80): 11.1 fold
This genetic profile is not considered to be resistant to ETR according to ANRS algorithm and intermediate resistant according to Stanford algorithm.

- One sample with 2 ETR RAMs was associated with an increase (> 3.2-fold) in the ETR IC50:

➢ **V90I and Y181C** (patient no. 1370): 3.3-fold
This genetic profile is not considered to be resistant to ETR according to ANRS algorithm and intermediate resistant according to Stanford algorithm.

Conclusions

Non-B HIV-1 subtypes in naïve patients exhibit some naturally-occurring ETR RAMs (some of them considered as medium-impact ETR RAMs). The overall prevalence was 10% and this had a limited impact on ETR susceptibility.

Only 3 cases were associated with phenotypic resistance to ETR and in 2/3 cases this was in a context of Y181C transmitted drug resistance. Our results also show that V179I mutation could have an impact on ETR FC only in combination with some specific mutations such as E138A and that the concomitant presence of Y181C and H221Y, which is not considered as an ETR RAM, dramatically increases ETR FC suggesting that the role of H221Y, alone and in combination, on ETR resistance should be further investigated.