

Original Article

GENOTYPIC EVALUATION OF ETRAVIRINE SENSITIVITY OF CLINICAL HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) ISOLATES CARRYING RESISTANCE MUTATIONS TO NEVIRAPINE AND EFAVIRENZ

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ABSTRACT

Background: Etravirine is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with a pattern of resistance mutations quite distinct from the current NNRTIs.

Methods: We collected all routine samples of HIV-1 patients followed in the AIDS reference laboratory of UCLouvain (in 2006 and 2007) carrying resistance-associated mutations to nevirapine (NVP) or efavirenz (EFV). The sensitivity to Etravirine was estimated using three different drug resistance algorithms: ANRS (July 2008), IAS (December 2008) and Stanford (November 2008). We also verified whether the mutations described as resistance mutations are not due to virus polymorphisms by the study of 58 genotypes of NNRTI-naïve patients.

Results: Sixty one samples harboured resistance to NVP and EFV: 41/61 had at least one resistance mutation to Etravirine according to ANRS-IAS algorithms; 42/61 samples had at least one resistance mutation to Etravirine according to the Stanford algorithm. 48 and 53 cases were fully sensitive to Etravirine according to ANRS-IAS and Stanford algorithms, respectively. Three cases harboured more than three mutations and presented a pattern of high-degree resistance to Etravirine according to ANRS-IAS algorithm, while one case harboured more than three mutations and presented high degree resistance to Etravirine according to the Stanford algorithm. The V106I and V179D mutations were more frequent in the ARV-naïve group than in the NNRTI-experienced one.

Conclusions: According to the currently available algorithms, Etravirine can still be used in the majority of patients with virus showing resistance to NVP and/or EFV, if a combination of other active drugs is included.

Key words: HIV-1, NNRTI, Etravirine, resistance-associated mutations

BACKGROUND

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) belong to a class of structurally diverse and highly efficient anti-HIV agents (1). NNRTIs are non-competitive inhibitors that bind allosterically to the hydrophobic cavity of HIV reverse transcriptase (RT) near to its catalytic site, leading by this way to a dramatic reduction in enzyme efficiency. The disadvantages of the currently available NNRTIs, Efavirenz (EFV) and Nevirapine (NVP), are the low genetic barrier required for HIV to become resistant and the high level of cross-resistance between these two compounds (2,3). TMC 125 (Etravirine) is a new NNRTI recommended for use in HIV-1 infected patients who failed previous NNRTI treatment. In vivo, Etravirine demonstrates activity against viruses resistant to the currently available NNRTIs in treatment-experienced patients, with a similar tolerability profile to that of the control group treated with another inhibitor (4-8). In vitro, Etravirine is highly active against both wild-type and NNRTI-resistant HIV strains and has a higher genetic barrier to the development of resistance in comparison to EFV and NVP (9,10). This feature was attributed to the molecular flexibility of this molecule allowing it to bind multiple conformations of the RT (11). Susceptibility and virological response to Etravirine is dependent on the type and number of resistance associated mutations emerging after preceding EFV or NVP treatment (5,6). Few studies exist in Belgium on the sensitivity of Etravirine on strains with resistance to other NNRTI and different polymorphisms. We evaluate here whether the mutations accumulated after EFV or NVP treatment influence virus sensitivity to Etravirine. The sensitivity to Etravirine was evaluated using the latest versions of three different algorithms (ANRS, Stanford, IAS). We also determined

whether the mutations involved in resistance to Etravirine according to these algorithms, represent polymorphisms by analysis of virus from untreated patients.

MATERIAL AND METHODS

We collected sequence data on all routine samples of HIV-1 patients followed in the AIDS reference laboratory of UCLouvain (in 2006 and 2007) carrying resistance-associated mutations to NVP or EFV. Resistance to EFV and NVP was defined using the ANRS algorithm of 2006 and 2007, depending of the time of diagnosis. Thirteen baseline resistance-associated mutations (RAMs) having an effect on the virological response to Etravirine have been described by Vingerhoets *et al.* (12): V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V and G190A/S. The presence of more than three RAMs is considered as resistance to Etravirine and three RAMs as intermediate resistance to Etravirine. The sensitivity to Etravirine was estimated using three different drug resistance algorithms: ANRS (July 2008), IAS (December 2008) and Stanford (November 2008). The ANRS algorithm includes the 13 RAMs described above. The IAS rules share the same list, with the addition of K101H, E138A, V179T and M230L. The Stanford algorithm, however, includes more mutations: V179E, Y188LHC, F227C, G190E, M230L and K238T.

Association of some mutations to virus polymorphisms was excluded by analysis of the mutation prevalence in 58 genotypes of NNRTI-naïve patients, matched for subtype in the first group.

RESULTS

Among the samples we analysed, 61 genotypes harboured resistance to NVP and EFV. The majority of cases had been treated with NVP.

Among these 61 cases, 48 were considered sensitive to Etravirine according to the algorithm of ANRS, 20 of which did not have any Etravirine resistance mutation, while 18 cases showed one mutation and 10 cases two mutations. According to the algorithm of IAS, 48 were considered sensitive to Etravirine, 20 of which did not have any Etravirine resistance mutation, while 17 cases showed one mutation and 11 cases two mutations. In comparison, 53 cases were sensitive according to the Stanford algorithm, 19 with no mutation, 19 with one mutation and 15 with two mutations. Ten cases presented an intermediate resistance after analysis with the ANRS and IAS algorithms, against 7 cases according to the Stanford algorithm. Finally, 3 cases harboured more than three mutations and presented a pattern of high-degree of resistance to Etravirine according to the algorithms of ANRS and IAS, while one case harboured more than three mutations according to the Stanford algorithm.

In that NNRTI-experienced group, 41 out of 61 patients had at least one resistance mutation to Etravirine according to the ANRS and IAS algorithms, and 42 according to the Stanford one. The Y181C, K101E, G190A and V90I were the most frequent RAMs, the latter being not considered by the Stanford algorithm (Figure 1).

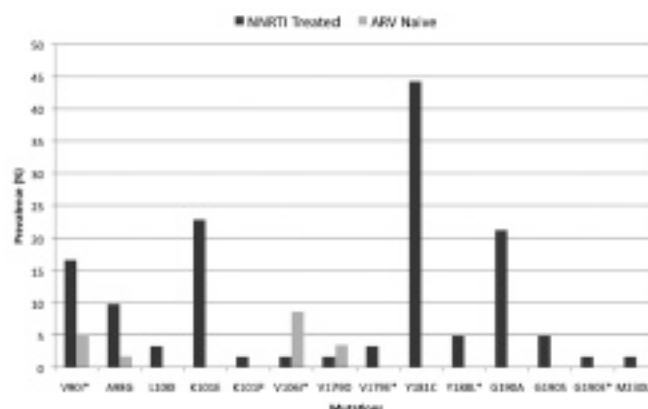


Figure 1: Prevalence (%) of Etravirine resistance associated mutations (RAM) in two groups of patients: the first included 61 patients failing an EFV or NVP based regimen and the second 58 ARV naïve patients.

*: ETR-RAMs that are not described in all 3 algorithms considered (2008 versions of ANRS, IAS and Stanford): the V179E, Y188L and G190E mutations are included only in the Stanford list, the V90I and V106I mutations are not included in the Stanford list, and the M230L mutation is not included in the ANRS list.

We then analyzed samples from 58 ARV-naïve subtype-matched patients in order to define whether some mutations we observed were due to polymorphisms. Four ETR RAMs were found in that group: V106I, V90I, V179D and A98G, respectively from the most to the least frequent (Figure 1). The V106I and V90I mutations are not included in the Stanford rules. The majority of mutations were found in samples from subtypes B and C. Among B subtype, mutations are observed in 7 cases using the ANRS and IAS algorithms, and in 2 cases using the Stanford algorithm. Among C subtype, mutations are observed in 2 cases using the ANRS and IAS algorithms and one case using the Stanford one. As V106I and V179D mutations were more frequent than in the NNRTI-experienced group, these could be considered as polymorphisms.

DISCUSSION

This study evaluated the theoretical activity of Etravirine on HIV infected patients who had already been treated, and whose virus was genotypically resistant to NVP or EFV. Resistance to ETV was defined using the 2008 versions of the ANRS, IAS and Stanford algorithms. The most frequent mutations according to the algorithms of ANRS and IAS 2008 were Y181C, K101E, G190A and V90I. Lapadula *et al.* and Llibre *et al.* had analogous results using the IAS algorithm of 2007 (13, 14). Di Vincenzo *et al.* also found G190A and K101E as the most frequent mutations in Italy using the IAS algorithm of 2007, but not Y181C and V90I (8). These mutations were involved in resistance to Etravirine if the number of mutations was higher than 3, according to Vingerhoets *et al.* (12). This could be explained by their larger sample size. We found 3 cases of resistance and 10 cases labelled as intermediary resistance to Etravirine according to the ANRS and IAS. Shanmugham *et al.* have found similar results in 2008 in a cohort of South Indian patients using the

IAS algorithm of 2007 (15). The mutations Y181C, K101E and G190A were the most frequent in our study using the Stanford algorithm. Di Vincenzo *et al.* (8) and Scott *et al.* (16) found that K103N was the most frequent mutation in their trial on patients pretreated with EFV or NVP. We have not evaluated the influence of mutation K103N on the response to Etravirine, but other studies have shown that this mutation did not affect the response to Etravirine (5,6). We have found one case of resistance to Etravirine according to the Stanford algorithm and 7 cases of intermediary resistance. We noticed a small decrease in cases of intermediary resistance and resistance with the Stanford algorithm of 2008 as compared to the IAS and ANRS. The Stanford algorithm accumulated more cases with one or two mutations.

Along with our study samples of treated patients, we included a control group of 58 patients chosen according to the subtype with a sensitive genotype to NVP and EFV. The majority of mutations were found in the subtypes B and C. The most frequent mutations observed in the control panel were, in order of frequency, V106I, V90I, V179D and A98G. Shanmugham *et al.* did a similar study mainly with subtype C but they did not find any mutation in South Indian naïve patients using the IAS algorithm of 2007 (15). The mutations V106I and V179D could be related to a polymorphism of the virus. We considered only mutations that were most frequent in the ARV-naïve group as polymorphisms: the mutations V90I and A98G were more frequent among the NNRTI treated group. Yet, some studies consider the V106I and V179D mutations to be associated with a decrease of the antiviral response to Etravirine (5,6).

CONCLUSIONS

Etravirine can be used in patients with virus showing resistance to NVP and/or EFV since only 3 cases of resistance out of 61 were observed according to the ANRS and IAS algorithms and only one case of resistance according to the Stanford algorithm. Nevertheless, those algorithms were mainly constructed with data obtained from the DUET trials, where Etravirine was supported by a backbone containing several other drugs. The list of mutations and the scores may be revised when the susceptibility and the virological response data using three drugs regimens will be available.

The number of patient samples that accumulated one or two mutations in this evaluation should not be neglected, some were scored as intermediate resistance. It is therefore essential to have a genotype for patients who failed current NNRTIs (NVP/EFV) before administering Etravirine.

Etravirine could thus be a solution for patients with NNRTI resistance, but may not be more efficient than a switch to a PI-based regimen in patients failing current NNRTIs (17).

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REFERENCES

1. Delaugerre C. Résistance du VIH aux inhibiteurs non nucléosidiques de la transcriptase inverse (INNTI). *Virologie* 2006; 10: 255-266.
2. Gazzard BG. BHIVA Treatment Guidelines Writing Group. British HIV Association Guidelines for the treatment of HIV-1 infected adults with antiretroviral therapy 2008. *HIV Med* 2008; 8: 563-608.
3. Gazzard BG, Pozniak AL, Rosenbaum W, et al. An open-label assessment of TMC 125-a new, next-generation NNRTI, for 7 days in HIV-1 infected individuals with NNRTI resistance. *AIDS* 2003; 17: 49-54.
4. Montaner J, Yeni P, Clumeck NN, et al. Safety, tolerability, and preliminary efficacy of 48 weeks of etravirine therapy in a phase IIb dose-ranging study involving treatment-experienced patients with HIV-1 infection. *Clin Infect Dis* 2008; 47: 969-78.
5. Lazzarin A, Campbell T, Clotet B, et al. Efficacy and safety of TMC 125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24 week results from a randomised, double-blind, placebo-controlled trial. *Lancet* 2007; 370: 39-48.
6. Madruga JV, Cahn P, Grinsztejn B, et al. Efficacy and safety of TMC 125 (etravirine) in treatment-experienced HIV-1 infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet* 2007; 370: 29-38.
7. Tambuyzer L, Vingerhoets J, Azijn H, Daems B, Picchio G and De Bethune M. Emerging mutations in patients with virological failure on TMC 125 in the DUET-1 and DUET-2 clinical trials. *6th European HIV Drug Resistance Workshop*, 26-28 March 2008, Budapest, Hungary Abstract N°47
8. Di Vincenzo P, Rusconi S, Adorni F, et al. Prevalence of mutations and determinants of genotypic resistance to etravirine (TMC 125) in a large Italian resistance database (ARCA). *6th European HIV Drug Resistance Workshop*, 26-28 March 2008, Budapest, Hungary Abstract N°59.
9. Vingerhoets J, Hilde A, Franssen E, et al. TMC 125 Displays a High genetic Barrier to The development of Resistance: Evidence from *in vitro* selection experiments. *J Virol* 2005; 79: 12773-82.
10. Andries K, Azijn H, Thielemans T, et al. TMC 125, a novel next-generation non nucleoside reverse transcriptase inhibitor active against non nucleoside reverse Transcriptase inhibitor resistant human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 2004; 48: 4680-86.
11. Das K, Clark AD Jr, Lewi PJ, et al. Roles of conformational and positional adaptability in structure-based design of TMC 125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild type and drug-resistant HIV-1 variants. *J Med Chem* 2004; 47: 2550-60.
12. Vingerhoets J, Buelens A, Peeters M, et al. Impact of baseline NNRTI mutations on the virological response to TMC 125 in the phase III clinical trials DUET-1 and DUET-2. *XVIth international HIV Drug resistance Workshop*, 12-16 June 2007, Barbados, Abstract N°32.
13. Lapadula G, Calabresi A, Castelnovo F, et al. Prevalence and risk factors for etravirine resistance among patients failing to non-nucleoside reverse transcriptase inhibitors. *Antivir Ther* 2008; 13: 601-5.
14. Llibre JM, Santos JR, Puig T, et al. Prevalence of etravirine-associated mutations in clinical samples with resistance to nevirapine and efavirenz. *J Antimicrob Chemother* 2008; 62, 909-913.
15. Shanmugham S, Madhavan V, Pachamuthu B, et al. Etravirine Associated Mutations And NNRTI Hypersensitizing Mutations Among HIV-1 Subtype-C Infected Etravirine naïve South Indian Patients Failing Therapy. *6th European HIV Drug Resistance Workshop*, 26-28 March 2008, Budapest, Hungary. Abstract N° 65
16. Scott C, Grover D and Nelson M. Is there a role for etravirine in patients with non nucleoside reverse transcriptase inhibitor resistance? *AIDS* 2008; 22: 989-992.
17. Ruxrungtham K, Pedro RJ, Latiff GH, et al. Impact of reverse transcriptase resistance on the efficacy of TMC 125 (etravirine) with two nucleoside reverse transcriptase inhibitors in protease inhibitor-naïve, non nucleoside reverse transcriptase inhibitor-experienced patients: study TMC125-C227. *HIV Med* 2008; 10: 883-96.