



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Etravirine Plasma Exposure is Associated with Virological Efficacy in Treatment-experienced HIVpositive Patients.

This is the author's manuscript			
Original Citation:			
Availability:			
This version is available http://hdl.handle.net/2318/147788 since 2016-06-08T23:42:21Z			
Published version:			
DOI:10.1016/j.antiviral.2014.05.009			
Terms of use:			
Open Access			
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright			

(Article begins on next page)

protection by the applicable law.





This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in ANTIVIRAL RESEARCH, 108 (1), 2014, 10.1016/j.antiviral.2014.05.009.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

(1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.

(2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.

(3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en), 10.1016/j.antiviral.2014.05.009

The publisher's version is available at: http://linkinghub.elsevier.com/retrieve/pii/S0166354214001387

When citing, please refer to the published version.

Link to this full text: http://hdl.handle.net/2318/147788

This full text was downloaded from iris - AperTO: https://iris.unito.it/

This is a pre-copyedited, author-produced PDF of an article accepted for publication in Antiviral Research following peer review. The version of record Antiviral Res. 2014 Aug;108:44-7. doi: 10.1016/j.antiviral.2014.05.009 is available online at:

http://www.sciencedirect.com/science/article/pii/S0166354214001387

Etravirine Plasma Exposure is Associated with Virological Efficacy in Treatment-experienced HIV-positive Patients

A Calcagno^{*1}, L Marinaro¹, S Nozza², C Aldieri¹, A Carbone², V Ghisetti³, A Trentalange¹, A D'Avolio¹, A Castagna² and G Di Perri¹ and S. Bonora¹

¹Unit of Infectious Diseases, Department of Medical Sciences, University of Torino;²San Raffaele Scientific Institute Milano; ³Laboratory of Microbiology and Molecular Biology, Ospedale Amedeo di Savoia, ASLTO2, Torino, Italy.

Running Head: Etravirine PK/PD

Type of article: Original Paper

Word count: 1467

Figures: 2

Tables: 1 supplementary table

Funding: This work was supported by internal funding.

*Corresponding Author:

Andrea Calcagno, Department of Infectious Diseases, University of Torino c/o Ospedale Amedeo di Savoia, C.so Svizzera 164 10159, Torino, Italy +390114393884, fax +390114393818 andrea.calcagno@unito.it

Key words: etravirine; pharmacokinetics; experienced patients; inhibitory quotient; resistance.

Etravirine is a non-nucleoside reverse transcriptase inhibitor used in combination with other antiretrovirals for the treatment of HIV infection. Given previous conflicting results aim of this study was to investigate whether etravirine plasma exposure was associated with virological outcome.

Adult HIV-positive patients starting etravirine with detectable HIV viral loads were included if highly adherent (<90% of the doses) and if steady-state plasma concentrations were available (measured through a validated HPLC-PDA method). Virological success was defined as reaching and maintaining viral suppression (HIV RNA <50 copies/mL) during follow up.

Fifty-nine (84.7% male) patients were included: baseline CD4+ T-lymphocyte and HIV RNA were 276 cells/uL (101-419) and 3.99 Log₁₀ copies/mL (3.11-4.91), respectively. Darunavir/ritonavir (n=21, 35.6%) and raltegravir plus maraviroc (n=33, 55.9%) were the most common associated antiretrovirals. 240 trough samples were available (3-7 per patient); etravirine trough concentrations (Ctrough) and weighted genotypic inhibitory quotients (wgIQ) were 426 ng/mL (266-763) and 408 ng/mL/mutation (227-663), respectively. Virological success was observed in 49 patients (83.1%). Genotypic sensitivity of associated drugs (GSS) ≥ 2 (p=0.03), etravirine Ctrough >300 ng/mL (p=0.02) and etravirine wgIQ >276 ng/mL/mutation (p=0.02) were associated with virological success; at multivariate Cox proportional analysis etravirine wgIQ <276 ng/mL/mutation (p=0.012) and baseline CD4 <200 cell/uL (p=0.043) were independently associated with virological failure.

In a cohort of experienced patients etravirine exposure as well as immune status were associated with virological success; two cut off values (300 ng/mL and 276 ng/mL) were proposed for etravirine Ctrough and wgIQ and need to be confirmed in prospective studies.

Etravirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that demonstrated satisfactory efficacy and tolerability in treatment-experienced and naïve patients (Katlama et a. 2010, Gazzard et al. 2011). Unlike first-generation NNRTIs, genotypic resistance to etravirine requires the accumulation of more than one nucleoside change in viral reverse transcriptase gene: genetic barrier to resistance is usually deemed high. Etravirine is metabolized by the hepatic cytochrome P450 (CYP) 2C9, 2C19, and 3A4 enzymes; its pharmacokinetics profile is characterized by long elimination half-life (40 hours) and by high inter-individual variability partially explained by food- and drug-to-drug interactions (Yanakakis and Bumpus 2012, Lubomirov et al. 2013, Kakuda et al. 2010, Schöller-Gyüre et al. 2008). A recent study reported a relationship between etravirine plasma exposure and efficacy: patients with etravirine 12-hour Area Under the Curve (AUC₁₂) or concentration at the end of the dosing interval (Ctrough) in the lowest quartiles (≤ 2712 ng·h/mL or ≤ 160 ng/mL) had the lowest response rates (Kakuda et al, 2012). Furthermore etravirine genetic barrier warrants investigating the used of genotypic inhibitory quotient (drug exposure compared to drug resistance) effect on antiviral efficacy: it has been demonstrated for several protease inhibitors (Gonzalez de Requena et al. 2011). Aim of this study was to investigate if either etravirine plasma exposure or weighted genotypic inhibitory quotient (wgIQ) could be associated with virological outcome in HIV-positive treatment-experienced patients.

This is a retrospective study including adult HIV-positive patients starting etravirine-containing HAARTs (200 mg twice-daily with food) after virological failures. Patients were either enrolled in one clinical study (Nozza et al, 2010) or in routine clinical care; they signed a specific written informed consent. This protocol was approved by San Raffaele Scientific Institute Ethics Committee and ASLTO2 Ethics Committee. Patients were excluded if baseline genotypic resistance test and etravirine steady-state concentrations were not available, if self-reported adherence was less than 90% or if interacting drugs were administered.

Plasma concentrations were measured through a validated High Performance Liquid Chromatography coupled with photo diode array (HPLC-PDA) method with a lower limit of detection and a lower limit of quantification respectively of 11 ng/mL and 50 ng/mL (D'avolio et al. 2008). Individual etravirine concentrations were calculated as the geometric mean of available trough levels (collected 10 to 14 hours after drug intake).

Genotypic sensitivity score (GSS) of the co-administered drugs was calculated according to Stanford algorithm using historical cumulative genotypes. Etravirine weighted score was obtained according to resistance associated mutations (RAMs) (Vingerhoets et al. 2012): each RAM was assigned a score of 1 (V90I, V179D, K101E, K101H, A98G, V179T, G190A), 1.5 (E138A, V106I, G190S, V179F), 2.5 (K101P, L100I, Y181C, M230L) or 4 (Y181I, Y181V). Etravirine weighted genotypic inhibitory quotient (wgIQ) was calculated as the ratio between etravirine Ctrough and etravirine weighted resistance score: for instance a patient with etravirine Ctrough of 426 ng/mL and whose virus was harbouring both Y181C and V179D have a wgIQ of 121.7 ng/mL/mutation [426/(2.5+1)]. Virological success was defined as reaching and maintaining viral suppression (HIV RNA <50 copies/mL) during follow up.

Concentrations and inhibitory quotients cut offs were obtained through Receiver Operator Curves identifying levels with adequate sensitivity (above 70%) and testing the obtained dichotomous variables with Chi-square tests. Data are described as medians (interquartile ranges) and non-parametric tests were used (as described in the text). A Cox proportional hazard model was used to test factors independently associated with virological efficacy over time. All statistical analyses were performed with SPSS version 20.0 (IBM Corp.).

Fifty-nine patients [male (50, 84.7%), aged 48 years (43-56), with body mass index of 21.3 Kg/m² (19.2-23.2)] were included. Eighteen (30.5%) and eight patients (13.6%) presented HCV or HBV-related chronic hepatitis. Patients had a long history of antiretroviral therapy [12.5 years (9.8-14.8)] and a CD4+ T lymphocyte nadir of 190 cells/uL (70-298). At baseline CD4+ T lymphocyte were 276 cells/uL (101-419) and HIV RNA was 3.99 log₁₀ copies/mL (3.11-4.91). Drugs associated with

etravirine are presented in supplementary table 1: patients received PI-sparing (n=36, 61%) or PIbased (n=23, 39%; mainly darunavir/ritonavir n=21, 35.6%) regimens.

GSS was 1, 2 and above 2 respectively in 5 (8.5%), 40 (67.8%) and 14 patients (23.7%). Etravirine RAMs were present in 13 patients (22%): Y181C (5, 8.5%), K100I (3, 5.1%), G190A (3, 5.1%) were the most frequently reported.

Median etravirine concentration (out of 284 samples) was 449 ng/mL (261-721), with nine (3.2%) undetectable samples (figure 1). Marginal direct correlations (Spearman's test) emerged between etravirine concentrations and age (rho=0.24, p<0.001), BMI (rho=0.26, p<0.001) and time post-dose (rho=0.15, p=0.009). 240 measurements were trough values and median Ctrough was 438 ng/mL (261-713; coefficient of variation 0.83).

Median individual Ctrough (calculated using 3-7 samples per patient) was 426 ng/mL (interquartile range 266-763): it was significantly higher when raltegravir was co-administered (470 ng/mL vs. 230 ng/mL, p=0.01) and border-line lower when associated with boosted protease inhibitors (310 ng/mL vs. 437 ng/mL, p=0.08). Etravirine wgIQ was 408 ng/mL (227-663).

After 48.3 weeks (47.8-205.7) of follow up virological success was noted in 49 patients (83.1%); 2 (3.4%) and 8 patients (13.5%) had non-response and virological rebound, respectively. Etravirine Ctrough was higher in patients with virological success (428 ng/mL vs. 258 ng/mL, Mann-Whitney p=0.04) (figure 2) and by ROC analysis a cut off was identified at 299 ng/mL [sensitivity 77.6%, specificity 60% (AUROC 0.70, p=0.04)]. Etravirine weighted gIQ was border-line higher in patients with virological success (428 ng/mL/mutation vs. 231 ng/mL/mutation, Mann-Whitney p=0.06) (figure 2) and ROC curve identified 276 ng/mL/mutation as a possible cutoff (73.5% sensitivity and 70% specificity; AUROC 0.69, p=0.06). 17 patients (28.8%) and 20 patients (33.9%) respectively presented etravirine plasma levels below 300 ng/mL and etravirine wgIQ below 276 ng/mL/mutation.

At Log-rank univariate analysis, determinants of virological success were GSS ≥ 2 (88.9% vs. 60% in failures, p=0.02), etravirine Ctrough >300 ng/mL (90.5% vs. 64.7% in failures, p=0.02) and

etravirine wgIQ >276 ng/mL/mutation (92.3% vs 65%, p=0.008). At Cox proportional hazard analysis (including age, gender, anti HCV positivity, viral load, GSS) etravirine wgIQ <276 ng/mL/mutation (p=0.012, aOR 8.57, 95%CI 1.61-45.4) and CD4 <200 cells/uL (p=0.043, aOR 8.48, 95%CI 1.06-67.5) were independently associated with virological failure (Chi-square of the model=15.2).

The analysis here reported confirms a concentration-dependent effect of etravirine on virological success; two possible targets are suggested in etravirine Ctroughs (300 ng/mL) and in etravirine wgIQ (276 ng/mL/mutation). Similarly to what observed in the GRACE study a relationship between etravirine exposure and efficacy was confirmed (Kakuda et al, 2012); the absence of a high genetic barrier drug may unmask etravirine pharmacokinetic/pharmacodynamic correlation. The cut off here proposed is higher than what observed in the aforementioned study: we should highlight that 160 ng/mL was the lowest quartile values and that no threshold value was evaluated. The observation of a wgIQ association with efficacy confirms the hypothesis of similarities between etravirine and boosted PIs as for genetic barrier to resistance; this might suggest the possibility of higher doses to overcome partial resistance.

Etravirine Ctroughs are slightly higher than in previous studies (426 ng/mL versus 393 ng/mL and 280 ng/mL) (Kakuda et al 2010, Kakuda et al 2012) where it was associated with boosted PIs while in our study 61% of regimens are PI-free. Moreover we report higher etravirine concentrations in patients coadministered with raltegravir confirming previous data (Cmax, AUC and Cmin respectively 4%, 10% and 17% higher) although the mechanism is currently unknown (Calcagno et al. 2011). However unlikely the DUET studies, our patients are less experienced and less frequently co-administered with a PI/r: this mirrors clinical practice where patients are successfully treated with etravirine without darunavir/ritonavir. (Katlama, et al. 2010)

These findings might be useful in order to optimize the treatment of experienced patients with limited therapeutic options. Considering drug-to-drug interactions as well as food-drug interactions may allow for higher exposures (etravirine exposure is reduced by 51% in the fasted state)

(Schöller-Gyüre et al. 2008). Furthermore once-daily etravirine pharmacokinetics may be beneficial since it was associated with higher AUCs and equivalent Ctroughs (Gutiérrez-Valencia et al. 2012). Some limitations must be highlighted: the retrospective study design, the limited sample size, the short follow up, the heterogeneity of patients and follow ups (partially corrected using a Cox proportional hazard model) and the relatively low number of viruses carrying etravirine RAMs (22%). Furthermore patients without RAMs were assigned a value of 1 (as if they had 1 mutation): this correction, usually adopted in gIQ studies, has probably a limited effect since a similar virological efficacy was noted in patients with RAMs scores ranging from 0 to 2 (Vingerhoets et al. 2012).

In conclusion, in a cohort of experienced patients etravirine plasma exposure was associated with virological success: etravirine weighted genotypic inhibitory quotient as well as CD4 cell count are independently associated with the achievement and maintenance of HIV RNA undetectability. Two targets were proposed (300 ng/mL for etravirine Ctrough and of 276 ng/mL/mutation for etravirine wgIQ) and they need to be confirmed in prospective studies.

Transparency declaration

All authors declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years OR A. C. has received travel grants or speaker's honoraria from Abbvie, Boehringer- Inghelheim, BMS, MSD, Gilead, Janssen-Cilag, Viiv AND S. N. has received travel grants or speaker's honoraria from BMS, Abbott, Pfizer, Janssen-Cilag and GlaxoSmithKline (GSK) AND A.Ca. has received travel grants or speaker's honoraria from BMS, Mbott, Pfizer, Roche and Janssen-Cilag AND G. D.P. has received grants, travel grants and consultancy fees from Abbvie, Boehringer-Inghelheim, BMS, Gilead, Viiv AND S. B. has received grants, travel grants and consultancy fees from Abbvie, Boehringer, Janssen-Cilag, Viiv AND S. B. has received grants, travel grants and consultancy fees from Abbvie, Boehringer, Janssen-Cilag, Viiv in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

References

Calcagno A, Nozza S, Bonora S, Castagna A, Gonzalez de Requena D, D'Avolio A, Lazzarin A, Di Perri G. Pharmacokinetics of the raltegravir/maraviroc/etravirine combination. J Antimicrob Chemother. 2011;66(8):1932-4.

D'Avolio A, Baietto L, Siccardi M, Sciandra M, Simiele M, Oddone V, Bonora S, Di Perri G. An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other antiretroviral agents in the plasma of HIV-infected patients. Ther Drug Monit. 2008;30(6):662-9.

Gazzard B, Duvivier C, Zagler C, Castagna A, Hill A, van Delft Y, Marks S. Phase 2 double-blind, randomized trial of etravirine versus efavirenz in treatment-naive patients: 48-week results. AIDS. 2011;25(18):2249-58.

Gonzalez de Requena D, Bonora S, Viganò O, Calcagno A, Cometto C, D'Avolio A, Baietto L, Ghisetti V, Magnani S, Ferramosca S, Vitiello P, Galli M, Rusconi S, Di Perri G. Comparative evaluation of seven resistance interpretation algorithms and their derived genotypic inhibitory quotients for the prediction of 48 week virological response to darunavir-based salvage regimens. J Antimicrob Chemother. 2011;66(1):192-200.

Gutiérrez-Valencia A, Martin-Peña R, Torres-Cornejo A, Ruiz-Valderas R, Castillo-Ferrando JR, López-Cortés LF. Intracellular and plasma pharmacokinetics of 400 mg of etravirine once daily versus 200 mg of etravirine twice daily in HIV-infected patients. J Antimicrob Chemother. 2012;67(3):681-4.

Kakuda TN, Wade JR, Snoeck E, Vis P, Schöller-Gyüre M, Peeters MP, Corbett C, Nijs S, Vingerhoets J, Leopold L, De Smedt G, Woodfall BJ, Hoetelmans RM. Pharmacokinetics and pharmacodynamics of the non-nucleoside reverse-transcriptase inhibitor etravirine in treatment-experienced HIV-1-infected patients. Clin Pharmacol Ther. 2010;88(5):695-703.

Kakuda T, Sekar V, Vis P, Coate B, Ryan R, Anderson D, De La Rosa G, Mrus J. Pharmacokinetics and Pharmacodynamics of Darunavir and Etravirine in HIV-1-Infected, Treatment-Experienced Patients in the Gender, Race, and Clinical Experience (GRACE) Trial. AIDS Res Treat. 2012;2012:186987.

Katlama C, Clotet B, Mills A, Trottier B, Molina JM, Grinsztejn B, Towner W, Haubrich R, Nijs S, Vingerhoets J, Woodfall B, Witek J. Efficacy and safety of etravirine at week 96 in treatmentexperienced HIV type-1-infected patients in the DUET-1 and DUET-2 trials. Antivir Ther. 2010;15(7):1045-52.

Lubomirov R, Arab-Alameddine M, Rotger M, Fayet-Mello A, Martinez R, Guidi M, di Iulio J, Cavassini M, Günthard HF, Furrer H, Marzolini C, Bernasconi E, Calmy A, Buclin T, Decosterd

LA, Csajka C, Telenti A; Swiss HIV Cohort Study. Pharmacogenetics-based population pharmacokinetic analysis of etravirine in HIV-1 infected individuals. Pharmacogenet Genomics. 2013;23(1):9-18.

Nozza S, Galli L, Bigoloni A et al. Durability and safety of a novel salvage therapy in R5-tropic HIV-infected patients: maraviroc, raltegravir, etravirine. J Acquir Immune Defic Syndr 2011; 56: e113–5.

Schöller-Gyüre M, Boffito M, Pozniak AL, Leemans R, Kakuda TN, Woodfall B, Vyncke V, Peeters M, Vandermeulen K, Hoetelmans RM. Effects of different meal compositions and fasted state on the oral bioavailability of etravirine. Pharmacotherapy. 2008;28(10):1215-22.

Vingerhoets J, Nijs S, Tambuyzer L, Hoogstoel A, Anderson D, Picchio G. Similar predictions of etravirine sensitivity regardless of genotypic testing method used: comparison of available scoring systems. Antivir Ther. 2012;17(8):1571-9.

Yanakakis LJ, Bumpus NN. Biotransformation of the antiretroviral drug etravirine: metabolite identification, reaction phenotyping, and characterization of autoinduction of cytochrome P450-dependent metabolism. Drug Metab Dispos. 2012;40(4):803-14.



Figure 1. Etravirine plasma concentrations (ng/mL) according to time post-dose (hours). Steady-state plasma concentrations (measured through a validated HPLC-PDA method) plotted with self-reported hours after drug intake. Etravirine was administered at the standard dose of 300 mg twice-daily with food (not standardized meals).



Figure 2. Etravirine individual trough concentrations (left) or weighted score genotypic inhibitory quotient (right) according to virological outcome. Comparison of individual trough concentrations (geometric mean of available plasma concentrations collected 10-14 hours after drug intake per each patient) in patients showing virological success (n=49) versus those with virological failure (n=10) (left, Mann-Whitney p value 0.04). Comparison of individual weighted score genotypic inhibitory quotients (individual C troughs divided by etravirine resistance score) in the same groups of patients (right, Mann-Whitney p value 0.06). Boxes represent interquartile range with medians (central lines); whiskers represent 10th and 90th percentile while single dots are outliers.

Not including PIs	36 (61%)	
RAL	3	
RAL + MVC	32	
RAL + MVC + ENF	1	
Including PIs	23 (39%)	
PI	6	DRV 5, LPV 1
PI + RAL	5	DRV 5, ATV 1
PI + ENF	2	DRV 2
PI + MVC	2	DRV 2
PI + RAL + ENF	4	DRV 3, TPV 1
PI + MVC + ENF	3	DRV 3
PI + RAL + MVC	1	DRV 1
Receiving NRTIs	11 (18.6%)	
TDF/FTC	6	
3TC	2	
Other	3	TDF1, AZT 1, ddI + FTC 1

Supplementary table 1: Antiretroviral regimens associated with etravirine. PIs "Protease inhibitors", "RAL" raltegravir, "MVC" maraviroc, "ENF" enfuvirtide, "DRV" darunavir, "LPV" lopinavir, "ATV" atazanavir, "TDF" tenofovir disoproxil fumarate, "FTC" fluorcitosine, "3TC" lamivudine, "AZT" zidovudine, "ddI" didanosine.