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The rate of accumulation of NNRTI-resistance in patients kept on a virologically failing regimen containing NNRTI: a EuroSIDA study

A Cozzi-Lepri[1], B Clotet[2], R Paredes[2], J Kjær[3], AN Phillips[1] and JD Lundgren[3,4] for the EuroSIDA study group.

[1]Department of Infection & Population Health Division of Population Health Hampstead Campus Royal Free & University College Medical School, University College London, London, UK
[2]HIV Unit and irisCaixa Retrovirology Lab, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain
[3]Copenhagen HIV Programme, University of Copenhagen, Panum Institute, Copenhagen, Denmark
[4]Centre for Viral Diseases/KMA, Rigshospitalet, Copenhagen, Denmark

Alessandro Cozzi-Lepri
HIV Epidemiology and Biostatistics Group,
Dept Primary Care and Population Sciences,
Royal Free and University College Medical Schools,
Rowland Hill St
London, NW3 2PF,
UK
Tel: +44 20 7794 0500 x 36763
Fax: +44 20 7794 1224
email: a.cozzi-lepri@pcps.ucl.ac.uk

BACKGROUND

Etravirine (ETR) is a potent new NNRTI that retains antiviral activity against NNRTI-resistance viruses. This activity, however, declines as NNRTI-associated resistance mutaions accumulate. An understanding of the rate of NNRTI resistance accumulation under the pressure of nevirapine or efavirenz in presence of detectable viral load is essential to predict the activity and potential benefit of subsequent use of ETR in this clinical setting. The results of this analysis may have also implications for therapy planning in the resource limited setting (RLS).

OBJECTIVES

To describe the population of patients in EuroSIDA who remained on a failing NNRTI-based regimen some time after experiencing virological failure to an NNRTI and for whom pairs of genotypic tests were available while they were viraemic (i.e. VL>500 copies/mL) and still receiving this NNRTI-based regimen. To estimate the rate of accumulation of drug resistance over time and the predicted potential loss of susceptibility to ETR associated with this accumulation.

STUDY DESIGN - METHODS

Patients: patients of EuroSIDA with ≥2 available genotypic resistance test (GRT) provided the first GRT was after the date of first virological failure of nevirapine (NVP) or efavirenz (EFV) and that the same NNRTI was kept until the time of the 2nd GRT (the other drugs could be different at the two time points). Viral load (VL) had to be >500 cps/mL at the time of the first GRT and in between GRTs. In addition, at least one IAS NNRTI mutations had to be detected at the first genotype in a pair to insure that the study population included only patients who had genuinely virologically failed on NNRTI, with resistance. The statistical unit was pairs of GRT and patients with j GRT (j≥2) contributed j-1 pairs (e.g. a patient with 4 eligible genotype tests contributed 3 pairs, a patient with 3 eligible genotype tests contributed 2 pairs, etc.). **Figure 1** illustrates a typical patient included in this analysis and contributing 2 pairs of genotypes while on a virologically failing nevirapine-containing regimen.

STATISTICAL ANALYSIS

Patients' characteristics at time of first GRT (t0) were described and average changes in laboratory markers from to to t1 (time of 2nd GRT) were evaluated. The Rega interpretation system (IS version 7.1) was used to predict the number of active drugs in the ART regimen at to. In this analysis, it was assumed that IAS NNRTI-associated mutations identified at to was still present at t1. The rate of NNRTI-resistance accumulation was calculated as number of new mutations per person years of follow-up (PYFU). Analyses were repeated in patients in which PI were not included in the failing regimen and in those with 1-3 NNRTI mutations detected at first GRT. A multivariable Poisson regression model was performed to identify independent predictors of TAM accumulation. The model properly accounted for the fact that a patient could contribute more than one pair of genotypes and that pairs coming from the same patients could not be assumed to be independent observations. Rega-predicted susceptibility to NNRTIs was calculated at to and t1, the change calculated for each pair and the mean predicted reduction in susceptibility was estimated.

RESULTS

Main characteristics

We included 154 patents who contributed 326 pairs of genotypic resistance test (GRT), -the first at time to and the second at time t1- with the following distribution: 87 one pair, 35 two, 12 three, 20 greater than three pairs, **Table 1** (also showing a description of the other main characteristics of these patients).

Laboratory markers

At to VL in patients was 4.11 log (range: 2.74-6.23) and CD4 count 224 cells/µl (range: 1-1,355). In 35 patients with a VL measured before any ART was initiated (used as a rough measure of patients' VL set point), the median VL suppression below this value at to was 0.78 log (IQR:0.16-1.38, **Table 2**). Over the interval to-t1 (with a median of 5 months between tests and a median number of 2 VL values over this time period) we observed a very stable VL [mean change: +0.05 log copies/mL (SD:0.54)] and CD4 count [mean change:+5.73 cells/µl, SD:88.0]. The corresponding figures for 118 patients who were not receiving a PI at either of the two time points were +0.09 (0.48) for VL and +19.7 (87.0) for CD4 count.

Figure 1

Typical patient contributing 2 genotypic test-pairs

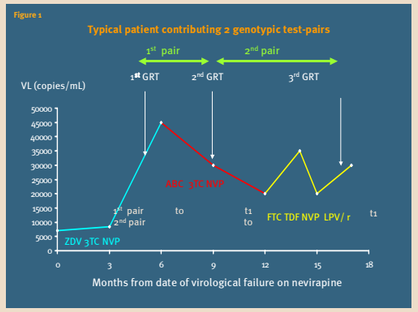

Table 1

Patients characteristics (n=154)

Age, median (range)	39 (20-69)
Female, n(%)	24 (16%)
Homosoexual contacts, n(%)	75 (49%)
Heterosexual contacts, n(%)	32 (21%)
IDUs, n(%)	20 (13%)
Number of contributing pairs	87 (57%)
2	35 (23%)
3	12 (8%)
4	10 (6%)
>4	10 (6%)

Table 2

Characteristics of genotype pairs (n=326)

	Average value	
Time from VF of NNRTI to to, months	13	(0-69)
Date of to	Oct 2000	(Feb 97-Oct 05)
VL suppression < set-point, log cps/mL (n=35)	0.78	(0.16;1.38)
VL at to, log copies/mL, median (IQR)	4.11	(3.49;4.72)
CD4 count at to, cells/µl, median (IQR)	224	(127;390)
Time between tests, months	5	(1-63)
Time on NVP-based regimens from VF to to, months	12.3	(SD:11.3)
Time on EFV-based regimens from VF to to, months	10.2	(SD:10.8)
Time on thymidine analogues from VF to to, months	22.6	(SD:16.5)
	n (%)	
NVP-based regimens	174 (53%)	
EFV-based regimens	152 (47%)	

VF=virological failure; to=time of 1st GRT, t1=time of 2nd GRT

Antiretroviral drugs

For 174 pairs, the patient was on a NVP-, and for 152 on a EFV-containing regimen (Table 2). At first GRT in a pair, the median number of drugs in regimen was 4 (range:2-10) and the most frequently used nucleosides at to together with NVP/EFV were lamivudine (56%), stavudine (47%) and didanosine (42%). In 199 (61%) of the failing regimens at to a protease inhibitors was used. The frequency of use of antiretrovirals besides NVP/EFV at t1 was similar to that observed at to, suggesting that these patients had been kept on mostly the same drugs over to-t1 despite virological failure.

HIV drug resistance

Table 3 shows the distribution of NNRTI mutations and groups of RT mutations detected in major virus populations at to and the estimated proportions at t1 (under the assumption of indefinite persistence of mutations once detected).

Rate of accumulation of resistance

The number of pairs for which there was at least one NNRTI mutation that was detected at t1 but not at to was 66 (20%). Overall, 78 IAS NNRTI resistance mutations were accumulated over 180 PYFU (0.43 per year, 95% CI:0.36-0.51) and the rate was 0.36/year (95% CI: 0.29-0.44, 62 mutations over 171 PYFU) in those with a virus predicted to be resistant, 1.52/year (95% CI:0.72-2.72, 9 over 5.9 PYFU) in those predicted to have intermediate resistance and 2.71/year (95% CI:1.15-4.93, 7 mutations over 2.6 PYFU) in those predicted to be susceptible to the NNRTI used.

The estimated rate of accumulation of IAS ETR-specific mutations was slower than the overall NNRTI accumulation: 41 new ETR-specific mutations were observed at t1 for a rate of 0.23 mutations/year (95% CI:0.17-0.30). As a consequence of this accumulation the predicted ETR activity dropped from a mean of 59% to a mean of 55% (absolute mean change of 4% over to-t1 or 8%/year, **Table 4**).

Predictors of accumulation of NNRTI-associated resistance

Table 5 shows that the greater was the susceptibility to the NNRTI used in the failing regimen, the faster was the rate of accumulation of NNRTI resistance.

After restricting the analysis to pairs in which 1-3 IAS NNRTI mutations were detected at the first genotype in the pair there was a 58% (95% CI:22-77, p=0.006) reduction in the rate of accumulation of resistance comparing pairs failing on NVP with those failing on EFV. The only two factors associated with the rate of accumulation of ETR mutations were the predicted susceptibility to the NNRTI used (again, the rate of accumulation was 3-fold faster if viruses were predicted to be intermediate or sensitive compared to resistant) and gender (the rate of accumulation tended to be slower by 80% in females compared to male, p=0.05).

CONCLUSIONS

The overall rate of accumulation of NNRTI resistance (0.43/year) was double that of previously estimated accumulation of TAM in this cohort. This rate was particularly fast if the initial level of NNRTI resistance was low (2.71/year if susceptible viruses). This may reflect the fact that mutations were already present at to but not sufficiently prevalent to be detected by population sequencing. There was a relatively slow reduction in ETR susceptibility (8%/year) associated with accumulation of NNRTI resistance estimated over an average of 5 months with VL>500 copies/mL and no change in NNRTI.

LIMITATIONS

It is possible that our patients are a selected sample of patients whose therapy has not been switched because viral load did not dramatically change between GRTs. The impact of resistance accumulation over a long period on the response to ETR-containing regimens remains to be seen.

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Argentina: (M Losso), A Duran, Hospital JM Ramos Mejia, Buenos Aires. Austria: (N Vetter) Pulmologisches Zentrum der Stadt Wien, Vienna. Belarus: (I Karpov), A Vassilenko, Belarus State Medical University, Minsk, VM Mitsura, Gomel State Medical University, Gomel; O Suetnov, Regional AIDS Centre,_Svetlogorsk. Belgium: (N Clumeck) S De Wit, B Poll, Saint-Pierre Hospital, Brussels; E Colebunders, Institute of Tropical Medicine, Antwerp Bulgaria: K Kostov, Infectious Diseases Hospital, Sofia. Croatia; J Begovac, University Hospital of Infectious Diseases, Zagreb. Czech Republic: (L Machala) H Rozsypal, Faculty Hospital Bulovka, Prague; D Sedlacek, Charles University Hospital, Plzen. Denmark: (J Nielsen) J Lundgren, T Benfield, O Kirk, Hvidovre Hospital, Copenhagen; J Gerstoft, T Katzenstein, A-B E Hansen, P Skinhøj, Rigshospitalet, Copenhagen; C Pedersen, Odense University Hospital, Odense, L Oestergaard, Skejby Hospital, Aarhus. Estonia: (K Zilmer) West-Tallinn Central Hospital, Tallinn, Jelena Smidt, Nakkusoosakond Sneklinik_Kohtla-Järve. Finland: (M Ristola), Helsinki University Central Hospital, Helsinki. France: (C Katlama) Hôpital de la Pitié-Salpêtière, Paris; J-P Viard, Hôpital Necker-Enfants Malades, Paris; P-M Girard, Hospital Saint-Antoine, Paris; JM Livrozet, Hôpital Edouard Herriot, Lyon; P Vanhems, University Claude Bernard, Lyon; C Pradier, Hôpital de l'Archet, Nice; F Dabis, Unité INSERM, Bordeaux. Germany: (J Rockstroh) Universitäts Klinik Bonn; R Schmidt, Medizinische Hochschule Hannover; J van Lunzen, O Degen, University Medical Center Hamburg-Eppendorf, Infectious Diseases Unit, Hamburg; HJ Stellbrink, IPM Study Center, Hamburg; S Staszewski, JW Goethe University Hospital, Frankfurt; J Bogner, Medizinische Poliklinik, Munich; G. Fätkenheuer, Universität Köln, Cologne. Greece: (J Kosmidis) P Gargalianos, G Xylomenos, I Perdios, Athens General Hospital; G Panos, A Filandras, E Karabatsaki, 1st IKA Hospital; H Sambattakou, Ippokration Generea1 Hospital, Athens. Hungary: (D Banhegyi) Szent László Hospital, Budapest. Ireland: (F Mulcahy) St. James's Hospital, Dublin. Israel: (I Yust) D Turner, M Burke, Ichilov Hospital, Tel Aviv; S Pollack, G Hassoun, Rambam Medical Center, Haifa; S Maayan, Hadassah University Hospital, Jerusalem. Italy: (A Chiesi) Istituto Superiore di Sanità, Rome; E Esposito, I Mazeu, C Mussini, Università Modena, Modena; C Arici, Ospedale Riuniti, Bergamo; R Pristera, Ospedale Generale Regionale, Bolzano; F Mazzotta, A Gabbuti, Ospedale S Maria Annunziata, Firenze; V Vullo, M Lichtner, University di Roma la Sapienza, Rome; A Chirianni, E Montesarchio, M Gargiulo, Presidio Ospedaliero AD Cotugno, Monaldi Hospital, Napoli; G Antonucci, F Iacomi, P,Narciso, C Vlassi, M Zaccarelli, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, Rome; A Lazzarin, R Finazzi, Ospedale San Raffaele, Milan; M Galli, A Ridolfo, Osp. L. Sacco, Milan; A d'Arminio Monforte, Istituto Di Clinica Malattie Infettive e Tropicale,_Milan. Latvia: (B Rozentale) P Aldins, Infectology Centre of Latvia, Riga. Lithuania: (S Chaplinskas) Lithuanian AIDS Centre, Vilnius. Luxembourg: (R Hemmer), T Staub, Centre Hospitalier, Luxembourg. Netherlands: (P Reiss) Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam. Norway: (J Bruun) A Maeland, V Ormaasen, Ullevål Hospital, Oslo. Poland: (B Knysz) J Gasiorowski, Medical University, Wroclaw; A Horban, Centrum Diagnostyki i Terapii AIDS, Warsaw; D Prokopowicz, A Wiercinska-Drapało, Medical University, Bialystok; A Boron-Kaczmarska, M Pynka, Medical University, Szczecin; M Beniowski, E Malarska, Osrodek Diagnostyki i Terapii AIDS, Chorzow; H Trocha, Medical University, Gdansk. Portugal: (F Antunes) F Valadas, Hospital Santa Maria, Lisbon; K Mansinho, Hospital de Egas Moniz, Lisbon; F Maltez, Hospital Curry Cabral, Lisbon. Romania: (D Duiculescu) Spitalul de Boli Infectioase si Tropicale- Dr. Victor Babes, Bucarest. Russia: (A Rakhmanova), Medical Academy Botkin Hospital, St Petersburg; E Vinogradova, St Petersburg AIDS Centre, St Peterburg; S Buzunova, Novgorod Centre for AIDS, Novgorod. Serbia: (D Jevtovic), The Institute for Infectious and Tropical Diseases, Belgrade. Slovakia: (M Mokráš) D Staneková, Dérer Hospital, Bratislava. Spain: (J González-Lahoz) V Soriano, I. Martin-Carbonero, P Labarga, Hospital Carlos III, Madrid; B Clotet, A Jou, J Conejero, C Tural, Hospital Germans Trias i Pujol, Badalona; JM Gatell, JM Miró, Hospital Clinic i Provincial, Barcelona; P Domingo, M Gutierrez, G Mateo, MA Sambeat, Hospital Sant Pau, Barcelona. Sweden: (A Karlsson), Karolinska University Hospital, Stockholm; PO Persson, Karolinska University Hospital, Huddinge; L Flamholc, Malmö University Hospital, Malmö. Switzerland: (B Ledergerber) B Weber, University Hospital, Zürich; P Francioli, M Cavassini, Centre Hospitalier Universitaire Vaudois, Lausanne; B Hirschel, E Boffi, Hospital Cantonal Universitaire de Geneve, Geneve; H Furrer, Inselspital Bern, Bern; M Battegay, L Elzi, University Hospital Basel, Ukraine: (E Kravchenko) N Chentsova, Kiev Centre for AIDS, Kiev. 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Virology group: B Clotet (Central Coordinators) plus ad hoc virologists from participating sites in the EuroSIDA Study.
Steering Committee: F Antunes, B Clotet, D Duiculescu, J Gatell, B Gazzard, A Horban, A Karlsson, C Katlama, B Ledergerber (Chair), A D'Arminio Montforte, A Phillips, A Rakhmanova, P Reiss (Vice-Chair), J Rockstroh
Coordinating Centre Staff: J Lundgren (project leader), O Kirk, A Mocroft, N Friis-Møller, A Cozzi-Lepri, M Bannister, M Ellefson, A Borch, D Podlekareva, C Holkmann Olsen, J Kjær, L Peters, J Reekie

Table 3

Frequency and average number of IAS RT-mutations detected at to and estimated at t1 (assuming indefinite persistence of mutations after detection at to)

	to	t1
IAS-NNRTI, mean (SD)	2.79 (1.63)	3.02 (1.65)
90I	30 (9%)	33 (10%)
98G	53 (16%)	58 (18%)
100I	32 (10%)	34 (10%)
101EP	48 (15%)	51 (16%)
103N	200 (61%)	210 (64%)
106A|M	65 (20%)	69 (21%)
108I	78 (24%)	88 (27%)
179DF	4 (1%)	5 (2%)
181CIV	171 (53%)	183 (56%)
188CHL	20 (6%)	26 (8%)
190AS	137 (42%)	143 (44%)
225H	7 (2%)	13 (4%)
IAS-Nevirapine, mean (SD)	2.12 (0.96)	2.27 (0.93)
IAS-Efavirenz, mean (SD)	2.11 (1.07)	2.29 (1.04)
IAS-Etravirine, mean (SD)	1.68 (1.39)	1.81 (1.41)
TAM, mean (SD)	3.41 (1.70)	3.52 (1.67)

Table 4

Rega estimated susceptibility to Nevirapine, Efavirenz and Etravirine and GSS of failing regimen received at to

	to	t1	Reduction in predicted Susceptibility
Rega-nevirapine			
Susceptible	5 (2.0%)	1 (0.3%)	
Intermediate	1 (0.3%)	1 (0.3%)	
Resistant	320 (98%)	324 (99.0%)	
Mean (SD)	0.02 (0.13)	0.005 (0.06)	0.01 (0.11)
Rega-efavirenz			
Susceptible	4 (1.0%)	1 (0.3%)	
Intermediate	55 (17%)	41 (13%)	
Resistant	267 (82%)	284 (87%)	
Mean (SD)	0.10 (0.22)	0.07 (0.17)	0.03 (0.14)
Rega-etravirine			
Susceptible	90 (28%)	74 (23%)	
Intermediate	203 (62%)	212 (65%)	
Resistant	33 (10%)	40 (12%)	
Mean (SD)	0.59 (0.29)	0.55 (0.29)	0.04 (0.13)
Rega-GSS regimen at to			
0	54 (17%)	61 (19%)	
0.5-1.5	254 (75%)	246 (75%)	
2-2.5	22 (7%)	15 (5%)	
3	5 (2%)	3 (1%)	
Mean (SD)	0.80 (0.63)	0.71 (0.58)	0.09 (0.35)

Table 5

Adjusted relative rate of accumulation of NNRTI-resistance from fitting a Poisson regression model

Factor	ARR (95% CI)	p-value
Gender		
Male	1.00	
Female	0.47 (0.18-1.20)	0.12
Duration of exposure to NNRTI		
With VL>500 up to to		
1-2 years vs. <1 year	0.49 (0.25-0.98)	0.04
Viral load at t1		
Per log copies/mL higher	0.77 (0.54-1.11)	0.17
NNRTI in failing regimen		
EFV	1.00	
NVP	0.62 (0.35-1.09)	0.10
Number of drugs in failing regimen		
Per one additional	0.80 (0.58-1.08)	0.14
Number of TAMs at to		
Per one additional	1.14 (0.96-1.37)	0.14
Rega-susceptibility to NNRTI		
Resistant	1.00	
Intermediate	3.77 (1.59-8.90)	0.002
Susceptible	7.48 (2.66-20.9)	0.0001

Also adjusted for: age, modality of HIV transmission, duration of exposure to thymidine analogues with a VL>500, CD4 at to, detection of 184V at to and (or) use of lamivudine at to, Rega GSS for drugs besides NNRTI and calendar year of to, which all failed to show an association

CHIP COPENHAGEN HIV PROGRAMME

EuroSIDA