No pol mutation is associated independently with the lack of immune recovery in patients infected with HIV and failing antiretroviral therapy

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No pol mutation is associated independently with the lack of immune recovery in patients infected with HIV and failing antiretroviral therapy

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Keywords:	CD4 T-lymphocytes, drug resistance, resistance to protease inhibitors, resistance to nucleoside reverse transcriptase inhibitors, resistance to non-nucleoside reverse transcriptase inhibitors

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Table I. Demographic and clinical characteristics of the study subjects

		<i>Overall</i>	<i>Lack of immune</i>	<i>Immune</i>	<i>P value</i>
		(<i>n</i> = 826)	<i>recovery</i>	<i>recovery</i>	
			(<i>n</i> = 375)	(<i>n</i> = 451)	
Demographic and clinical characteristics at baseline					
Gender, <i>n</i> (%)	Male	577 (69.8)	271 (72.3)	306 (67.9)	0.171
	Female	249 (30.2)	104 (27.7)	145 (32.1)	
Median age at baseline, years (<i>Q1</i> , <i>Q3</i>)		40 (37, 45)	40 (36, 45)	40 (37, 44)	0.707
AIDS diagnosis before baseline, <i>n</i> (%)					
		24 (2.9)	9 (2.4)	15 (3.3)	0.411
Median duration of antiretroviral treatment, years (<i>Q1</i> , <i>Q3</i>)		4.5 (3.7, 5.5)	4.4 (3.6, 5.4)	4.5 (3.7, 5.6)	0.623
Median baseline CD4+ count, cells/mm ³ (<i>Q1</i> , <i>Q3</i>)		358 (211, 524)	418 (257, 593)	310 (195, 454)	<0.0001
Median baseline HIV-RNA, log ₁₀ copies/ml (<i>Q1</i> , <i>Q3</i>)		3.9 (3.4, 4.5)	3.9 (3.3, 4.6)	3.9 (3.4, 4.5)	0.552
Genotype characteristics at baseline					
Year of genotyping, <i>n</i> (%)	2000	107 (3.0)	62 (16.5)	45 (10.0)	0.003
	2001	175 (21.2)	88 (23.5)	87 (19.3)	
	2002	157 (19.0)	72 (19.2)	85 (18.9)	
	2003	387 (46.9)	153 (40.8)	234 (51.9)	
Median total number of mutations (<i>Q1</i> , <i>Q3</i>)		7 (4, 10)	7 (4, 11)	7 (4, 10)	0.0001
Presence of at least 1 NRTI mutation, <i>n</i> (%)					
		711 (86.1)	328 (87.5)	383 (84.9)	0.314
Median number of TAMs (<i>Q1</i> , <i>Q3</i>)		2 (0, 4)	2 (1, 4)	2 (0, 4)	0.392

1					
2					
3	Presence of at least 1 TAM, <i>n</i> (%)				
4					
5		614 (74.3)	284 (75.7)	330 (73.2)	0.424
6					
7	TAMs, <i>n</i> (%)				
8					
9					
10	None	212 (25.7)	91 (24.3)	121 (26.8)	
11					
12	Pattern = 1	361 (43.7)	175 (46.7)	186 (41.2)	0.411
13					
14	Other	253 (30.6)	109 (29.1)	144 (31.9)	
15					
16	Presence of at least 1 NNRTI mutation,				
17					
18	<i>n</i> (%)	444 (53.8)	202 (53.9)	242 (53.7)	0.999
19					
20	Median number of major PI mutations	1 (0, 2)	1 (0, 2)	1 (0, 2)	0.029
21					
22	(<i>Q1</i> , <i>Q3</i>)				
23					
24					
25	Presence of at least 1 PI mutation, <i>n</i>				
26					
27	(%)	460 (55.7)	222 (59.2)	238 (52.8)	0.068
28					
29	Median PI genotypic sensitivity score	7 (5.5, 8)	6.5 (5, 8)	7.5 (5.5, 8)	0.030
30					
31	(<i>Q1</i> , <i>Q3</i>)				
32					
33	Median NNRTI genotypic sensitivity	3 (1, 3)	3 (1, 3)	3 (1, 3)	0.690
34					
35	score (<i>Q1</i> , <i>Q3</i>)				
36					
37	Median NRTI genotypic sensitivity	5 (3, 5)	5 (3, 5)	5 (3, 5.5)	0.387
38					
39	score (<i>Q1</i> , <i>Q3</i>)				
40					
41					
42					
43					
44	Follow-up characteristics				
45					
46	Median years of follow-up (<i>Q1</i> , <i>Q3</i>)				
47					
48		3.1 (3.1, 3.3)	3.1 (3.1, 3.3)	3.1 (3.1, 3.2)	0.021
49					
50	Median 3-year HIV-RNA, <i>log</i> ₁₀				
51					
52	<i>copies/ml</i> (<i>Q1</i> , <i>Q3</i>)	2.5 (1.7, 4.1)	3.6 (1.9, 4.5)	1.7 (1.7, 3)	<0.0001
53					
54	Median change in HIV-RNA, <i>log</i> ₁₀				
55					
56	<i>copies/ml</i> (<i>Q1</i> , <i>Q3</i>)	-1.2 (-2.1, -0.1)	-0.5 (-1.4, 0.4)	-1.7 (-2.4, -0.7)	<0.0001
57					
58	Median viremia detectability ratio (<i>Q1</i> ,				
59					
60	<i>Q3</i>)	0.7 (0.4, 1)	0.9 (0.5, 1)	0.6 (0.3, 0.9)	<0.0001

Treatment interruption during follow-up,

<i>n</i> (%)	160 (19.4)	80 (21.3)	80 (17.7)	0.216
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Change in any antiretroviral drug in the

3 months following GRT, <i>n</i> (%)	362 (43.8)	153 (40.8)	209 (46.3)	0.121
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Use of a new drug after GRT, <i>n</i> (%)	105 (12.7)	41 (10.9)	64 (14.2)	0.174
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GSS: genotypic sensitivity score; GRT: genotypic resistance testing; TAM: thymidine analogue mutations; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

Table II. Univariable and multivariable logistic regression: unadjusted and adjusted odds ratios for lack of immune recovery.							
		Unadjusted	95% confidence		Adjusted	95% confidence	
		OR	limits	P value	OR	limits	P value
Gender	F vs. M	0.810	0.599–1.092	0.169	0.826	0.589–1.156	0.267
Age (per 5-year increase)		0.999	0.919–1.087	0.988	1.009	0.990–1.029	0.344
Baseline CD4+ (per 50-cell increase)		1.086	1.054–1.119	<0.0001	1.104	1.069–1.142	<0.0001
Viremia detectability ratio (per 0.1 increase)		1.129	1.083–1.179	<0.0001	1.145	1.093–1.202	<0.0001
Year of genotyping				0.003			0.0007
	2000 vs. 2003	2.107	1.368–3.268	0.015	2.619	1.611–4.300	0.002
	2001 vs. 2003	1.547	1.080–2.218	0.049	1.684	1.130–2.515	0.039
	2002 vs. 2003	1.295	0.890–1.883	0.462	1.215	0.805–1.829	0.129
PI GSS (per unit increase)		0.925	0.856–0.998	0.046	0.990	0.884–1.110	0.868
Treatment interruption during follow-up							
	Yes vs. no	1.258	0.890–1.776	0.194	1.337	0.917–1.952	0.131
Change in any antiretroviral drug in the 3 months following GRT							
	Yes vs. no	0.798	0.890–1.776	0.194	0.974	0.717–1.323	0.865
Use of a new drug after GRT							
	Yes vs. no	0.742	0.486–1.124	0.163	0.714	0.430–1.175	0.188

L10I							
	Yes vs. no	1.238	0.922–1.661	0.157	0.981	0.668–1.438	0.922
I54V							
	Yes vs. no	1.600	1.124–2.288	0.009	1.366	0.826–2.264	0.224
V82A							
	Yes vs. no	1.629	1.143–2.331	0.007	1.148	0.712–1.855	0.572
T69D							
	Yes vs. no	1.379	0.855–2.232	0.187	1.195	0.713–2.007	0.498
A98G							
	Yes vs. no	1.490	0.854–2.625	0.161	1.517	0.838–2.768	0.170
M184V							
	Yes vs. no	1.261	0.959–1.661	0.097	1.311	0.972–1.770	0.076

GSS: genotypic sensitivity score; GRT: genotypic resistance testing; PI: protease inhibitor.

Drug resistance mutations at baseline by antiretroviral class in patients showing immune recovery (IR) or lack of immune recovery (LIR). By univariable analysis, the presence at baseline of the I54V mutation (OR: 1.6; 95% CI: 1.124–2.288) and the V82A mutation (OR: 1.629; 95% CI: 1.143–2.331) was associated with increased odds of lack of immune recovery.

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***No pol* mutation is associated independently with the lack of immune recovery in patients infected with HIV and failing antiretroviral therapy**

Nicola Gianotti^{1*}, Laura Galli¹, Maurizio Zazzi², Valeria Ghisetti³, Stefano Bonora⁴, Valeria Micheli⁵, Paola Meraviglia⁶, Paola Corsi⁷, Bianca Bruzzone⁸, Stefano Menzo⁹, Simona Di Giambenedetto¹⁰, Andrea De Luca^{10, 11}, Gaetano Filice¹², Giovanni Penco¹³, Antonella Castagna¹ on behalf of the ARCA database initiative.

¹Department of Infectious Diseases, IRCCS San Raffaele, Milan, Italy; ²Department of Molecular Biology, University of Siena, Siena, Italy; ³Microbiology & Virology Laboratory, Ospedale Amedeo di Savoia, Turin, Italy; ⁴Infectious Diseases Clinic, Ospedale Amedeo di Savoia, Turin, Italy; ⁵Microbiology Laboratory, Ospedale L. Sacco, Milan, Italy; ⁶Second Division of Infectious Diseases, Ospedale L. Sacco, Milan, Italy; ⁷Division of Infectious Diseases, Ospedale Careggi, Florence, Italy; ⁸Health Laboratory, Ospedale San Martino, Genoa, Italy; ⁹Virology Laboratory, Ospedale di Ancona, Ancona, Italy; ¹⁰Institute of Infectious Diseases, Sacro Cuore Catholic University, Rome, Italy; ¹¹Infectious Diseases Unit, University Hospital of Siena, Siena, Italy; ¹²Infectious and Tropical Diseases Clinic, Policlinico San Matteo, Pavia, Italy; ¹³Infectious Diseases, Ospedali Galliera, Genoa, Italy.

Correspondence to:

Dr. Nicola Gianotti

Divisione di Malattie Infettive, Istituto Scientifico San Raffaele

Via Stamira d'Ancona, 20

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20127 - Milano, Italy

Tel.: +390226437906

Fax: +390226437030

E-mail: nicola.gianotti@hsr.it

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ABSTRACT

An investigation was undertaken to determine whether specific *pol* mutations hinder long-term immune recovery regardless of virological response. In total, 826 patients with >50 HIV RNA copies/mL, who underwent genotypic resistance testing between 1 January 2000 and 31 December 2003 after >3 years of antiretroviral treatment, and were followed up for >3 years after genotypic resistance testing, were analyzed retrospectively. The outcome of the study was the lack of immune recovery after >3 years of follow-up, defined as a slope by linear regression ≤ 0 . The viremia detectability ratio was defined as the number of HIV RNA values of >50 copies/mL divided by the number of HIV RNA measurements during follow-up. Logistic regression was used for univariable and multivariable analysis. Median (Q1, Q3) values at baseline were the following: age 40 (37, 45) years, years on antiretroviral therapy 4.45 (3.65, 5.47), HIV RNA 3.91 (3.39, 4.53) $\log_{10}$ copies/mL, CD4+ T-cell 358 (211, 524)/ μ L. After 3.13 years of follow-up, 375 patients (45.4%) showed a lack of immune recovery. The risk of lack of immune recovery increased independently with increasing baseline CD4+ counts (OR = 1.104 per 50-cell increase, 95% CI = 1.069–1.142, $p < 0.0001$), increasing viremia detectability ratio during follow-up (OR = 1.145 per 0.1-unit increase, 95% CI = 1.093–1.202, $p < 0.0001$), and with earlier calendar years of resistance testing (overall effect: $p = 0.0007$). In conclusion, no *pol* mutation is associated independently with the lack of immune recovery.

KEY WORDS: CD4+ T-lymphocytes; drug resistance; resistance to protease inhibitors; resistance to nucleoside reverse transcriptase inhibitors; resistance to non-nucleoside reverse transcriptase inhibitors.

INTRODUCTION

Extensive antiretroviral resistance is associated with a blunted virological response to subsequent regimens; in particular, there is a gradient of response depending on the number of active drugs, with greater efficacy being found in the patients who receive more active drugs (Hicks et al., 2006; Clotet et al., 2007; Lazzarin et al., 2007; Madruga et al., 2007; Fätkenheuer et al., 2008; Cooper et al., 2008).

It has been shown that extensive resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs), and protease inhibitors (PIs) increases the risk of progression to AIDS or death (Ormaasen et al., 2004; Hogg et al., 2006; Zaccarelli et al., 2007; Lohse et al., 2007), and that there are associations between clinical progression and single *pol* mutations (De Luca et al., 2006; Zaccarelli et al., 2007). One possible reason for these findings is that extensive drug resistance and some mutations in particular hamper the virological efficacy of subsequent regimens, thus leading to a weaker immune response and increasing eventually the rate of disease progression. However, this view has been challenged by one study (Lohse et al., 2007) that found mortality to be independent of CD4+ values at the time of death.

The relationship between the degree of drug resistance (or the number of active drugs) and the extent of immune recovery after the failure of an antiretroviral regimen has not been investigated, and it is not known whether specific *pol* mutations can hinder long-term immune recovery regardless of the virological efficacy of new antiretroviral regimens after drug failure.

This study was aimed at identifying the predictors of a lack of immune recovery in this clinical setting and at investigating whether specific *pol* mutations or their combinations may be associated independently with hindered immune reconstitution.

METHODS

The Antiretroviral Resistance Cohort Analysis (ARCA) database is a public database that has been developed as a means of investigating resistance to antiretroviral drugs, and contains data concerning the patients' treatment, longitudinally collected CD4+ cell counts, plasma HIV-RNA measurements, and HIV sequences. The data are provided by the individual centres, and descriptive statistics can be consulted in the public access area of the database at www.hivarca.net.

For the purposes of this retrospective study, we considered all of the HIV-infected patients in the database with >50 HIV RNA copies/ml while on antiretroviral treatment (ART), who underwent genotypic resistance testing (GRT) between 1 January 2000 and 31 December 2003 after at least three years of ART, and who were followed up for at least three years after GRT. The date of the GRT was considered the study baseline, and baseline values were those recorded on the date nearest to the GRT, provided that this was within the 120 days preceding it; the end-of-follow-up values were those closest to three years after GRT.

HIV genotypes were assessed at each centre on the basis of local procedures and checked for consistency upon sequence uploading into the ARCA database, which stores the sequences and automatically extracts the mutations with respect to HIV-1 consensus B using a built-in local alignment script based on ClustalW. PI mutations were defined as major or minor according to the IAS list (Johnson et al., 2009), and thymidine analogue mutations (TAMs) were profiled as previously described (De Luca et al., 2006). Genotypic sensitivity scores (GSSs) were calculated as the number of active drugs according to the ANRS version 18 algorithm

(<http://www.hivfrenchresistance.org/>) from among zidovudine, didanosine, stavudine, lamivudine, abacavir, tenofovir, emtricitabine, nevirapine, efavirenz, etravirine, ritonavir, indinavir, nelfinavir, saquinavir/ritonavir, amprenavir/ritonavir, lopinavir/ritonavir, atazanavir/ritonavir, tipranavir/ritonavir, and darunavir/ritonavir.

Statistical analysis

The continuous variables were described by means of median values and quartiles (Q1, Q3) or frequencies (%), as appropriate.

The study outcome was immune recovery, stratified as immune recovery or a lack of immune recovery after at least 3 years of follow-up. All of the available CD4+ values, obtained between baseline and the end of follow-up, were retained and used in the univariable linear regression to estimate the mean change (slope) in CD4+ values within each study patient. Lack of immune recovery was defined as a mean change in CD4+ count during follow-up of ≤ 0 (slope ≤ 0); conversely, immune recovery was defined as a mean change in CD4+ count during follow-up of > 0 (slope > 0). Univariable linear regression was also applied to HIV RNA values.

The viremia detectability ratio was calculated as the number of HIV RNA values of > 50 copies/mL divided by the number of HIV-RNA values measured during follow-up.

The characteristics of the subjects with lack of immune recovery or immune recovery were compared using Wilcoxon's rank-sum test and the chi-square test.

Logistic regression was used for the univariable and multivariable analyses to study the associations between the risk (odds ratio) of lack of immune recovery and the demographic, clinical, and genotypic parameters. Univariable logistic regression was used for all of the clinical and demographic characteristics considered in the study and

the individual *pol* mutations observed in at least 5% of the patients. The multivariable model included only those mutations and patient characteristics associated with the lack of immune recovery in the univariable logistic regression at a level of $p < 0.2$, with the exception of age and gender, which were retained as fixed covariates. The odds ratios (OR) and corresponding 95% confidence intervals were calculated to estimate the risk of lack of immune recovery.

All of the statistical tests were two-sided at the 5% level and were made using SAS Software (release 9.1; SAS Institute).

RESULTS

A total of 826 patients fulfilled all of the inclusion criteria and entered the study. Table I shows their baseline demographic and clinical characteristics in detail, grouped on the basis of their immune recovery status (lack of immune recovery vs. immune recovery).

The drugs used most frequently for genotyping were lamivudine (797; 96.5%), zidovudine (782; 94.7%), stavudine (723; 87.5%), didanosine (657; 79.5%), indinavir (560; 67.8%), saquinavir (479; 58.0%), and nelfinavir (403; 48.8%). Tenofovir was used more frequently among patients who showed immune recovery in the next three years of follow-up ($p = 0.02$); statistically significant differences were not observed with regard to other drugs.

Figure 1 illustrates the proportion of patients with individual *pol* mutations (excluding those observed in fewer than 5%, which were not considered in the analysis). Forty-seven patients (5.7%) had no *pol* mutations at baseline.

During follow-up, there was no statistically significant difference in the drugs prescribed, except for atazanavir, which was included more frequently ($p = 0.002$) in the regimens received by patients with immune recovery.

After 3.13 years of follow-up, the overall mean (SE) slope in CD4+ cell count was 7.90 (1.85) cells per year of follow-up (lack of immune recovery: -33.2 (1.67); immune recovery: 42.1 (1.97); $p < 0.0001$), and the mean (SE) slope in HIV RNA levels was -0.18 (0.01) $\log_{10}$ copies/ml per year (lack of immune recovery: -0.06 (0.02); immune recovery: -0.28 (0.01); $p < 0.0001$). Overall, 375 patients (45.4%) showed lack of immune recovery. The median (Q1, Q3) CD4+ count at the end of follow-up was 377 (232, 561) cells/ μ L (lack of immune recovery: 322 (177, 503); immune recovery: 411 (277, 613); $p < 0.0001$). The virological outcome at the end of follow-up is shown in Table I.

Table II shows the results of the univariable and multivariable logistic regression. By multivariable analysis, the risk of lack of immune recovery increased with increasing baseline CD4+ cell count (OR = 1.104 per 50-cell increase, 95% CI = 1.069–1.142, $p < 0.0001$) and increasing viremia detectability ratio during follow-up (OR = 1.145 per 0.1 increase, 95% CI = 1.093–1.202, $p < 0.0001$). In addition, the odds of lack of immune recovery decreased with decreasing calendar year of genotyping (overall effect: $p = 0.0007$).

None of the resistance mutations included in the multivariable model resulted in an increase or decrease in the odds of lack of immune recovery.

DISCUSSION

If specific *pol* mutations were able to hinder immune recovery in HIV-infected patients failing antiretroviral therapy, it would be wise (when possible) to avoid the use of drugs selecting preferentially these mutations. The present analysis did not detect any *pol* mutation that predicted a long-term lack of immune recovery regardless of virological response to the regimens that the patients received after GRT: this implies that, in terms of the risk of lack of immune recovery, there should be no reason to prefer one PI rather than another or one RTI rather than another.

The results of the present study also suggest that, in patients harbouring drug-resistant HIV variants, disease progression is not just a direct consequence of hindered immune recovery following subsequent regimens or of the *pol* mutations selected at failure, but the result of a complex interplay between various factors (including virological and immunological factors as well as adherence to treatment after GRT).

Immune recovery after treatment failure was predicted independently by the ability of the new regimen(s) to suppress viremia, baseline CD4+ cell counts, and the year of HIV genotyping. These factors seem thus to be more important than individual mutations selected by the failing regimen in driving immune recovery during the next years of therapy.

A gradient of immune responses relating to the degree of virological response was observed previously (Wood et al., 2000). The viremia detectability ratio has been used to measure virological responses to the regimens prescribed after GRT because it was believed that, unlike point observations, this could provide detailed information concerning the cumulative exposure to HIV throughout the follow-up. A positive association between the viremia detectability ratio and lack of immune recovery is also

consistent with the results of clinical trials showing that virological and immune responses are related directly in treatment-experienced patients (Lalezari et al., 2003; Lazzarin et al., 2003; Hicks et al., 2006; Clotet et al., 2007; Lazzarin et al., 2007; Fätkenheuer et al., 2008; Gulick et al., 2008; Steigbigel et al., 2008). Furthermore, these findings also underline the importance of pursuing the goal of HIV RNA values of <50 copies/mL also in this clinical setting (Hammer et al., 2008).

The M184V mutation was not associated with immune recovery: one hypothesis is that this mutation slows the CD4+ cell loss as compared to treatment interruption (Castagna et al., 2006), but it does not necessarily favour immune recovery when antiretroviral therapy is continued (Fox et al., 2006).

Year of genotyping was another independent predictor of lack of immune recovery: this likely reflects the improved ability of interpreting genotypic drug-resistance testing and constructing more effective new regimens after treatment failure in more recent years.

In addition to those typical of all retrospective analyses, the present study has some specific limitations. Patients who were lost to follow-up or who died during the 3-year follow-up period were excluded from the analysis. It is possible that they had the worst immune response, but this analysis was aimed at studying the association between *pol* mutations and long-term immune responses rather than the rate of clinical progression.

Another limitation is the fact that data concerning adherence to therapy before and after study entry were not uniformly collected: poor adherence is a relevant predictor of lack of immune recovery (Gazzola et al., 2009), and so it is likely that patients with lower viremia detectability ratios were those more adherent to treatment.

In conclusion, no *pol* mutation was associated with a lack of immune recovery in viremic patients who underwent GRT and were followed up for at least three years,

1
2
3
4 regardless of the virological outcome of their subsequent regimens. Lack of immune
5
6 recovery was independently predicted by CD4+ cell counts at the time of failure of the
7
8 previous regimen, the viremia detectability ratio during follow-up, and the year of HIV
9
10 genotyping. Gaining knowledge of the role of these virus- and patient-related factors
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12 may help to optimise immune recovery in patients harbouring drug-resistant HIV
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Maurizio Zazzi designed the study, collected and updated data, and contributed to the writing of the manuscript.

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Valeria Micheli collected and updated data.

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Andrea De Luca collected and updated data.

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Giovanni Penco collected and updated data.

Antonella Castagna designed the study and contributed to the writing of the manuscript.

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1
2
3
4 Università, Nino Romano; Palermo - Servizio Riferimento Regionale Diagnosi AIDS,
5
6 Salvatrice Mancuso; Parma - Divisione Malattie Infettive ed Epatologia, Azienda
7
8 Ospedaliera, Carlo Calzetti; Pavia - Ambulatorio Clinica Malattie Infettive, S. Matteo
9
10 Renato Maserati; Pavia - Clinica Malattie Infettive e Tropicali, Gaetano Filice; Pavia –
11
12 Virologia, S. Matteo, Fausto Baldanti; Perugia - Malattie Infettive, Daniela Francisci;
13
14 Pescara - Malattie Infettive, Giustino Parruti; Pescara – Virologia, Ennio Polilli;
15
16 Piacenza - Malattie Infettive, Daria Sacchini; Pisa - Malattie Infettive, Chiara
17
18 Martinelli; Pisa - Pediatria I, Università, Rita Consolini; Pisa – Virologia, Linda
19
20 Vatteroni; Pistoia - Malattie Infettive, Angela Vivarelli; Prato - Malattie Infettive,
21
22 Alessandro Nerli; Prato – Virologia, Lucia Lenzi; Reggio Emilia - Malattie Infettive,
23
24 Giacomo Magnani; Rimini - Malattie Infettive, Patrizia Ortolani; Roma - Cattedra
25
26 Malattie Infettive, Tor Vergata, Massimo Andreoni; Roma - IRCCS S. Gallicano, Guido
27
28 Palamara; Roma - Immunologia Clinica, Umberto I, Caterina Fimiani; Roma - Istituto
29
30 Superiore di Sanità, Lucia Palmisano; Roma - Istituto di Clinica Malattie Infettive,
31
32 Cattolica, Andrea De Luca; Roma - Laboratorio virologia, Cattolica, Simona Di
33
34 Giambenedetto; Roma - Malattie Infettive, INMI Spallanzani, Andrea Antinori; Roma -
35
36 Malattie Infettive e Tropicali, La Sapienza, Umberto I, Vincenzo Vullo; Roma -
37
38 Medicina Sperimentale e Patologia, Sezione Virologia, La Sapienza, Ombretta
39
40 Turriziani; Roma - Monitoraggio Terapie Antivirali e Antineoplastiche, INMI
41
42 Spallanzani, Carlo Federico Perno; Roma - Virologia DMS, Tor Vergata, Carlo
43
44 Federico Perno; Roma - Virologia Malattie Infettive, Tor Vergata, Marco Montano;
45
46 Sanremo - Malattie Infettive, Giovanni Cenderello; Siena - Malattie Infettive, Angela
47
48 Gonnelli; Siena – Virologia, Laura Romano; Terni - Malattie Infettive, Michele
49
50 Palumbo; Torino - Laboratorio di Virologia, Ospedale Amedeo di Savoia, Valeria
51
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Ghisetti; Torino - Malattie Infettive, Amedeo di Savoia, Stefano Bonora; Trento -
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For Peer Review

FIGURE TITLES AND LEGENDS

Figure 1. Drug resistance mutations at baseline by antiretroviral class in patients showing immune recovery (IR) or lack of immune recovery (LIR). By univariable analysis, the presence at baseline of the I54V mutation (OR: 1.6; 95% CI: 1.124–2.288) and the V82A mutation (OR: 1.629; 95% CI: 1.143–2.331) was associated with increased odds of lack of immune recovery.

For Peer Review

REFERENCES

Castagna A, Danise A, Menzo S, Galli L, Gianotti N, Carini E, Boeri E, Galli A, Cernuschi M, Hasson H, Clementi M, Lazzarin A. 2006. Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study). *AIDS* 20:795-803.

Clotet B, Bellos N, Molina JM, Cooper D, Goffard JC, Lazzarin A, Wöhrmann A, Katlama C, Wilkin T, Haubrich R, Cohen C, Farthing C, Jayaweera D, Markowitz M, Ruane P, Spinosa-Guzman S, Lefebvre E; POWER 1 and 2 study groups. 2007. Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials. *Lancet* 369:1169-78.

Cooper DA, Steigbigel RT, Gatell JM, Rockstroh JK, Katlama C, Yeni P, Lazzarin A, Clotet B, Kumar PN, Eron JE, Schechter M, Markowitz M, Loutfy MR, Lennox JL, Zhao J, Chen J, Ryan DM, Rhodes RR, Killar JA, Gilde LR, Strohmaier KM, Meibohm AR, Miller MD, Hazuda DJ, Nessly ML, DiNubile MJ, Isaacs RD, Teppler H, Nguyen BY; BENCHMRK Study Teams. 2008. Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med* 359:355-65.

De Luca A, Di Giambenedetto S, Cingolani A, Bacarelli A, Ammassari A, Cauda R. 2006. Three-year clinical outcomes of resistance genotyping and expert advice: extended follow-up of the Argenta trial. *Antivir Ther* 11:321-7.

De Luca A, Di Giambenedetto S, Romano L, Gonnelli A, Corsi P, Baldari M, Di Pietro M, Menzo S, Francisci D, Almi P, Zazzi M; Antiretroviral Resistance Cohort Analysis Study Group. 2006. Frequency and treatment-related predictors of thymidine-analogue mutation patterns in HIV-1 isolates after unsuccessful antiretroviral therapy. *J Infect Dis* 193:1219-22.

Fätkenheuer G, Nelson M, Lazzarin A, Konourina I, Hoepelman AI, Lampiris H, Hirschel B, Tebas P, Raffi F, Trottier B, Bellos N, Saag M, Cooper DA, Westby M, Tawadrous M, Sullivan JF, Ridgway C, Dunne MW, Felstead S, Mayer H, van der Ryst E; MOTIVATE 1 and MOTIVATE 2 Study Teams. 2008. Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection. *N Engl J Med* 359:1442-55.

Fox Z, Dragsted UB, Gerstoft J, Phillips AN, Kjaer J, Mathiesen L, Youle M, Katlama C, Hill A, Bruun JN, Clumeck N, Dellamonica P, Lundgren JD; COLATE study group. 2006. A randomized trial to evaluate continuation versus discontinuation of lamivudine in individuals failing a lamivudine-containing regimen: the COLATE trial. *Antivir Ther* 11:761-70.

Gazzola L, Tincati C, Bellistri GM, Monforte A, Marchetti G. 2009. The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. *Clin Infect Dis* 48:328-37.

Gulick RM, Lalezari J, Goodrich J, Clumeck N, DeJesus E, Horban A, Nadler J, Clotet B, Karlsson A, Wohlfeiler M, Montana JB, McHale M, Sullivan J, Ridgway C, Felstead S, Dunne MW, van der Ryst E, Mayer H; MOTIVATE Study Teams. 2008. Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* 359:1429-41.

Hammer SM, Eron JJ Jr., Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P, Fischl MA, Gatell JM, Hirsch MS, Jacobsen DM, Montaner JS, Richman DD, Yeni PG, Volberding PA; International AIDS Society-USA. 2008. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 300:555-70.

Hicks CB, Cahn P, Cooper DA, Walmsley SL, Katlama C, Clotet B, Lazzarin A, Johnson MA, Neubacher D, Mayers D, Valdez H; RESIST investigator group. 2006. Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the Randomized Evaluation of Strategic Intervention in multi-drug reSistant resistant patients with Tipranavir (RESIST) studies: an analysis of combined data from two randomised open-label trials. *Lancet* 368:466-75.

Hogg RS, Bangsberg DR, Lima VD, Alexander C, Bonner S, Yip B, Wood E, Dong WW, Montaner JS, Harrigan PR. 2006. Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART. *PLoS Med* 3:e356.

Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. 2009. Update of the drug resistance mutations in HIV-1: December 2009. *Top HIV Med* 17:138-45

Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, Walmsley S, Cohen C, Kuritzkes DR, Eron JJ Jr, Chung J, DeMasi R, Donatacci L, Drobnes C, Delehanty J, Salgo M; TORO 1 Study Group. 2003. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *N Engl J Med* 348:2175-85.

Lazzarin A, Clotet B, Cooper D, Reynes J, Arastéh K, Nelson M, Katlama C, Stellbrink HJ, Delfraissy JF, Lange J, Huson L, DeMasi R, Wat C, Delehanty J, Drobnes C, Salgo M; TORO 2 Study Group. 2003. Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *N Engl J Med* 348:2186-95.

Lazzarin A, Campbell T, Clotet B, Johnson M, Katlama C, Moll A, Towner W, Trottier B, Peeters M, Vingerhoets J, de Smedt G, Baeten B, Beets G, Sinha R, Woodfall B; DUET-2 study group. 2007. Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet* 370:39-48.

Lohse N, Jørgensen LB, Kronborg G, Møller A, Kvinesdal B, Sørensen HT, Obel N, Gerstoft J; Danish HIV Cohort Study. 2007. Genotypic drug resistance and long-term mortality in patients with triple-class antiretroviral drug failure. *Antivir Ther* 12:909-17.

Madruga JV, Cahn P, Grinsztejn B, Haubrich R, Lalezari J, Mills A, Pialoux G, Wilkin T, Peeters M, Vingerhoets J, de Smedt G, Leopold L, Trefiglio R, Woodfall B; DUET-1 study group. 2007. Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet* 370:29-38.

Ormaasen V, Sandvik L, Asj B, Holberg-Petersen M, Gaarder PI, Bruun JN. 2004. An algorithm-based genotypic resistance score is associated with clinical outcome in HIV-1-infected adults on antiretroviral therapy. *HIV Med* 5:400-6.

Steigbigel RT, Cooper DA, Kumar PN, Eron JE, Schechter M, Markowitz M, Loutfy MR, Lennox JL, Gatell JM, Rockstroh JK, Katlama C, Yeni P, Lazzarin A, Clotet B, Zhao J, Chen J, Ryan DM, Rhodes RR, Killar JA, Gilde LR, Strohmaier KM, Meibohm AR, Miller MD, Hazuda DJ, Nessly ML, DiNubile MJ, Isaacs RD, Nguyen BY, Tepler H; BENCHMRK Study Teams. 2008. Raltegravir with optimized background therapy for resistant HIV-1 infection. *N Engl J Med* 359:339-54.

Wood E, Yip B, Hogg RS, Sherlock CH, Jahnke N, Harrigan RP, O'Shaughnessy MV, Montaner JS. 2000. Full suppression of viral load is needed to achieve an optimal CD4 cell count response among patients on triple drug antiretroviral therapy. *AIDS* 14:1955-60.

1
2
3
4 Zaccarelli M, Tozzi V, Lorenzini P, Forbici F, Narciso P, Ceccherini-Silberstein F,
5
6 Trotta MP, Bertoli A, Liuzzi G, Marconi P, Mosti S, Perno CF, Antinori A. 2007. The
7
8 V118I mutation as a marker of advanced HIV infection and disease progression. *Antivir*
9
10 *Ther* 12:163-8.
11
12

13
14
15
16 Zaccarelli M, Forbici F, Lorenzini P, Ceccherini-Silberstein F, Tozzi V, Trotta MP,
17
18 Marconi P, Pasquale N, Perno CF, Antinori A. 2007. Continuous evidence of fast HIV
19
20 disease progression related to class-wide resistance to antiretroviral drugs: a 6 year
21
22 follow-up analysis of a large observational database. *AIDS* 21:1824-6.
23
24
25
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27
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