

1 **Viro-Immunological Response in HIV-1 Infected Patients with Multiple**
2 **Treatment Failures Receiving Raltegravir and Optimized Background Therapy,**
3 **ANRS CO3 Aquitaine Cohort[‡]**

4 Linda Wittkop^{1,2*}, Dominique Breilh^{3*}, Daniel Da Silva⁴, Pierre Duffau⁵, Patrick Mercié⁵,
5 Isabelle Raymond⁵, Guerric Anies⁴, Hervé Fleury⁴, Marie-Claude Saux³, Francois Dabis^{1,2},
6 Catherine Fagard^{1,2}, Rodolphe Thiébaud^{1,2}, Bernard Masquelier⁴, Isabelle Pellegrin^{4,†}

7 1: Inserm, Unit 897 Epidemiology and Biostatistics, Bordeaux, France

8 2: University Victor Segalen Bordeaux 2, ISPED, Bordeaux school of Public Health,
9 Bordeaux, France

10 3: Department of clinical Pharmacokinetics and clinical Pharmacy & EA 2968, University
11 Victor Segalen, Bordeaux, France and Pharmacy Haut-Lévêque Hospital, Bordeaux
12 University Hospital, Pessac, France

13 4: Department of Virology & EA 2968, Bordeaux University Hospital, University Victor
14 Segalen, Bordeaux, France.

15 5: Department of Internal Medicine and Infectious Diseases, Bordeaux University Hospital,
16 University Victor Segalen, Bordeaux, France

17 †: current affiliation: Department of Immunology & EA 2968, Bordeaux University Hospital,
18 University Victor Segalen, Bordeaux, France.

19 *The first two authors contributed equally to this study.

20 **Short title: Response to raltegravir in heavily pre-treated patients**

21 **Corresponding author:**

22 Linda Wittkop

23 Mailing address: ISPED (Case 11), 146 rue Leo Saignat, 33076 Bordeaux Cedex;

24 E-mail: Linda.Wittkop@isped.u-bordeaux2.fr

25 ‡ ANRS CO3 Aquitaine Cohort (see Appendix).

26 **Abstract:**

27 **Background:** The efficacy of raltegravir plus optimized background therapy (OBT)
28 has been demonstrated for antiretroviral (ARV)-experienced HIV-1 infected patients
29 in randomized clinical trials. We studied viro-immunological response,
30 pharmacokinetic parameters, and genotypic test results in an observational cohort of
31 multiple ARV class-experienced patients starting a raltegravir-based regimen.

32 **Methods:** Already enrolled ANRS CO3 Aquitaine Cohort patients with virologic
33 failure were included in this study after starting a raltegravir-based regimen (400 mg
34 twice a day, week 0). Virologic success was defined by plasma HIV-1 RNA level [viral
35 load (VL)] $<2.7 \log_{10}$ copies/mL at M3 and $<1.7 \log_{10}$ copies/mL at M6. One patient
36 was excluded from further analysis (no follow-up after W4).

37 **Results:** Fifty-one patients (male/female = 43/8, median age = 48 [interquartile range
38 = 43; 55] years) were included. At week 0, median CD4 count was 244 [110;
39 310]/mm³ and median VL was 4.2 [3.6; 4.7] \log_{10} copies/mL. At week 24, 39 (78%)
40 patients experienced virologic success: 4 (44%), 14 (82%) and 21 (87%) of patients
41 with a genotypic sensitivity score <1 , [1-2] and ≥ 2 ($P=0.02$), respectively. Raltegravir-
42 related mutations emerged in 9 of 11 failing patients (82%): Q148H/R (n=5),
43 N155S/H (n=3) and S230N (n=1). Median CD4 rise from week 0 to week 4 and week
44 24 were 28 [-4; 85] and 57 [0; 156] cells/mm³, respectively. A poor immune response
45 was independently associated with a lower VL decline (week 0 to week 12) [odds
46 ratio (OR): 3.5 95% confidence interval (CI): 1.4; 8.4 for 1 \log_{10} less] and CD4+% at
47 baseline (OR: 2.6, 95% CI: 0.97; 8.3 for 10% lower).

48 **Conclusions:** Raltegravir plus OBT provided a good virologic success rate in highly
49 pre-treated patients under clinical routine conditions.

50

51 **Key words:** Raltegravir, antiretroviral treatment experienced patients, viro-
52 immunological response, integrase resistance mutations.

53 **Introduction:**

54 Raltegravir is a HIV-1 integrase inhibitor which has been successfully used in both
55 treatment-naïve patients ¹ and heavily treated patients ^{2,3}.

56 We studied the viro-immunological response of raltegravir with optimised background
57 therapy (OBT) in an observational prospective cohort of multiple antiretroviral class
58 experienced patients under clinical routine conditions. Furthermore, we evaluated
59 pharmacokinetic (PK) parameters, genotypic test results of integrase, reverse
60 transcriptase and protease genes at baseline and in failing patients.

61

62 **Patients and Methods:**

63 **Study population:**

64 The patients were selected from the ANRS Co3 Aquitaine Cohort, a prospective
65 hospital-based cohort of HIV-1 infected patients in south-western France. Informed
66 consent was obtained for all patients. The Aquitaine Cohort has an Institutional
67 Review Board (IRB) approval from the Bordeaux University IRB.

68 Patients who experienced multiple virological failure on highly active antiretroviral
69 therapy (HAART) {plasma HIV-1 RNA level viral load (VL) $>1.7 \log_{10}$ copies/ml} were
70 consecutively enrolled in the present study between October 2006 and February
71 2008 after onset of raltegravir (400 mg twice a day)-based HAART (week 0). The
72 patients were monitored at weeks 0, 4, 12 and 24. Clinical, biological and therapeutic
73 data were collected prospectively at each visit.

74

75 **Virological and immunological outcomes:**

76 Virological success was defined as plasma VL $<2.7 \log_{10}$ copies/mL at week 12 and
77 $<1.7 \log_{10}$ copies/mL at week 24, quantified using the CobasTaqman HIV assay

78 (Roche Diagnostics, Basel, Switzerland). Patients with missing VL values at week 24
79 were considered as virologic success if the VL was $<2.7 \log_{10}$ copies/mL at week 12.
80 One patient had no follow up visit after week 4 and was excluded from further
81 analysis. A poor immune response was defined as a gain of CD4⁺ cells ≤ 50
82 cells/mm³ from week 0 to week 24.

83

84 **Genotype-resistance testing:**

85 Sequencing procedures used for reverse transcriptase, protease and integrase are
86 available on the HIV French resistance website ⁴. Complete integrase gene
87 sequence was determined at baseline, and in patients with virologic failure.

88 We calculated the genotypic sensitivity score (GSS) that represents the sum of
89 genotypic sensitivities (according to the ANRS genotype-interpretation algorithm) to
90 the drugs in the OBT.

91

92 **Determination of plasma Raltegravir concentrations:**

93 Blood samples were drawn to determine plasma raltegravir concentrations at the PK
94 steady-state 4 weeks after starting raltegravir as well as at week 12 and week 24.

95 Minimum (C_{\min}) and maximum (C_{\max}) serum drug concentrations, corresponding to
96 around 12 h and 3 h after raltegravir ingestion, respectively, were measured using a
97 validated HPLC with mass spectrometry detection⁵.

98

99 **Statistical analyses:**

100 Analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC). Patients'

101 characteristics were compared between groups using a Fisher's exact test for

102 qualitative variables, and a Wilcoxon-Mann-Whitney test or a Kruskal-Wallis test for

103 quantitative variables. Distributions are described as medians (25th; 75th percentiles),
104 unless stated otherwise. We tested the following variables for their association with
105 virological failure: patients' characteristics, prior treatments, baseline viro-
106 immunological parameters, number of PI, nucleoside reverse transcriptase inhibitor
107 (NRTI), NNRTI resistance-related mutations, GSS, PK parameters and integrase
108 polymorphisms (PMs) having a prevalence >10%. Factors associated with virological
109 success and with a poor immune response were analysed using logistic regression.

110

111 **Results**

112 **Baseline patient characteristics:**

113 The baseline characteristics of the 51 patients enrolled in this observational cohort
114 study are reported in table 1. Most of the patients (72%) received raltegravir as part
115 of an expanded access program in France (Autorisation Temporaire d'Utilisation).
116 ARV drugs frequently prescribed as OBT were ritonavir-boosted darunavir (n=36,
117 71%), etravirine (n=22, 43%) and enfurvitide (n=13, 28%). Raltegravir in combination
118 with ritonavir-boosted darunavir and etravirine was prescribed in 15 patients (29%).
119 The most frequently NRTIs co-prescribed with raltegravir were tenofovir (n=20, 39%),
120 emtricitabine (n=18, 35%) or lamivudine (n=11, 22%).

121

122 **Responses to the raltegravir containing therapy:**

123 **Virological response:**

124 Virologic success was observed for 39 patients (78%) (Table 2). According to a GSS
125 of <1, [1-2[and ≥2 virologic success occurred in 4 (44%), 14 (82%) and 21 (88%)
126 patients ($P=0.02$) with a VL decline (from baseline to week 24) of -0.64 (-2.8; -0.3), -
127 2.4 (-3.1; -1.1) and -2.2 (-2.9; -1.6) log₁₀ copies/mL ($P=0.27$), respectively. Among the

128 26 Pms having a prevalence >10% from the baseline genotype the PM T206S (n=8,
129 18%) was significantly associated with a lower response rate ($P=0.02$). Furthermore,
130 the clinical AIDS stage [odds ratio (OR):0.2, 95% confidence interval (CI): 0.05; 0.80
131 C vs. A/B, $P=0.02$), the nadir of CD4+ cell count (OR: 1.6, 95% CI: 1.1; 2.1 for a
132 difference of 10 cells/mm³, $P=0.006$), the absolute value of CD4+ cell count at
133 baseline (OR: 5.9, 95% CI: 1.9; 18.1 for a difference of 100 cells/mm³, $P=0.002$) and
134 the HIV-1 RNA level at baseline (OR: 0.1, 95% CI: 0.04; 0.5 for a difference of 1 log₁₀
135 copies/mL, $P=0.002$) were significantly associated with virologic response in
136 univariable analysis. In the adjusted analysis initial VL (OR: 0.2, 95% CI: 0.06; 0.98
137 for 1 log₁₀ copies/mL higher, $P=0.046$) and the nadir of CD4⁺ cell count (OR: 1.6,
138 95% CI: 1.0; 2.7 for 10 cells/mm³ higher, $P=0.049$) were independently associated
139 with virological response.

140

141 **Immune response:**

142 A CD4⁺ gain (baseline to week 24) ≤ 50 cells/mm³ was found in 20 patients. In
143 adjusted logistic regression, a poor immune response was independently associated
144 with a lower VL decline (W0week 0-12) (OR: 3.5 95% CI: 1.4; 8.4 for 1 log₁₀ less,
145 $P=0.009$) and CD4+% at baseline (OR: 2.6 95% CI: 0.97; 8.3 for 10% lower, $P=0.08$),
146 but did not explain the whole variability of CD4⁺ response ($R^2=0.29$). At week 24, 32
147 patients had a VL <1.7 log₁₀ copies/mL (one patient with missing values for CD4⁺
148 cells) and for 12 of them we observed a CD4⁺ gain of ≤ 50 cells/mm³ (38%, 95% CI:
149 22%, 56%). These patients had similar baseline CD4+ cell counts (249 versus
150 246/mm³), almost similar nadir of CD4⁺ cell count (146 versus 117/mm³) and lower
151 VL at week 0 (3.4 versus 4.3 log₁₀ cp/mL) than patients with both virologic and
152 immunological success (n=20).

153

154 **Pharmacokinetic parameters:**

155 Raltegravir PK parameters were stable during follow-up. Median C_{\min} was 250 (150;
156 350), 300 (200; 350) and 290 (150; 350) ng/mL at weeks 4, weeks 12 and weeks 24,
157 respectively. Median C_{\max} was 1000 (870; 1400) ng/mL at week 4, 990 (800; 1200)
158 ng/mL at week 12 and 980 (780; 1200) ng/mL at week 24. The minimal observed
159 value of C_{\min} was 50 ng/mL (0.10 μM) for each follow-up visit exceeding the 95%
160 inhibitory concentration (IC_{95}) of 0.033 μM . Patients with etravirine in the OBT had
161 slightly lower PK parameters but there was no statistical significant interaction of
162 etravirine on raltegravir PK parameters.

163

164 **Emerging integrase mutations**

165 Four different patterns of emerging mutations were observed: i) five patients
166 presented the emergence of Q148H/R with secondary mutations (V72I, L74M,
167 G140A/S, E138A, K156N, K160N, V201I and T206S), ii) the N155S/H mutation
168 emerged in three patients and was replaced in the following three to five month by a
169 pattern including the mutation Y143C/H/R and secondary mutations (L74M, T97A,
170 G163R, V151I, S230R), iii) the S230R mutation was selected in one patient and iv)
171 two patients had virologic failure without emerging mutations.

172

173 **Discussion**

174 We observed a potent antiretroviral effect in patients failing multiple previous
175 antiretroviral regimens before. Our findings were comparable with virological success
176 rates observed in similar populations with heavily treatment-experienced patients^{2, 3}
177 on raltegravir-based HAART. Despite a rapid VL suppression, the overall median

178 CD4⁺ cell rise was 57 (0; 156)/mm³ between baseline and week 24 comparable to
179 that observed in the P005 and Benchmrk studies. A poor immune response was
180 associated with VL decline and CD4⁺% at baseline, but explained only around 30%
181 of the entire variability of the CD4⁺ response. We found a discordant response (VL
182 <1.7 log₁₀ copies/mL and CD4⁺ gain ≤50 cells/mm³) in 38% of patients at week 24.
183 Further investigation is needed to evaluate other hypotheses such as permanent
184 immune activation, host factors or thymus exhaustion for a poor immune response
185 despite virological success.

186 Factors associated with virological response at week 24 were the VL at baseline and
187 the nadir of CD4⁺ cell counts. These findings are in agreement with the fact that most
188 of the patients were already in an advanced disease stage.

189 The high proportion of integrase resistance mutations that developed in patients who
190 failed therapy (9/11, 82%) in this study was consistent with findings in Benchmrk
191 studies (68%)^{3,6} and the protocol 005 study (92%)⁷. Our findings confirm the low
192 genetic barrier of raltegravir. The low genetic barrier may have an influence on future
193 drug options especially in comparable patients, as cross-resistance to elvitegravir
194 and other integrase inhibitors under investigation have already been reported⁸.

195 PK parameters did not provide a statistically meaningful predictive value for
196 virological success, probably due to the fact that observed C_{min} values were quite
197 homogeneous and exceeded the IC₉₅ of raltegravir in all patients. Etravirine co-
198 prescription did not influence raltegravir PK parameters, confirming the negligible PK
199 interaction between etravirine and raltegravir observed in healthy subjects¹⁰.

200

201 **Conclusion**

202 Raltegravir plus OBT provided a good virological success rate in HIV-1-infected
203 heavily pre-treated patients with multiple treatment failures under clinical routine
204 conditions comparable to that reported in randomized clinical trials.

205

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211

212 **Transparency declaration**

213 None to declare.

214 **References**

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249 **Table 1:** Baseline characteristics of 51 patients receiving raltegravir-based HAART.
 250 ANRS CO3 Aquitaine Cohort, 2007-2008.

Characteristic	Value
Sex (M/F), n (%)	43/8 (84/16)
Age, years	48 (43; 55)
Clinical CDC stage (A/B/C), n (%)	10/24/17 (20/47/33)
Follow up time, month	8 (6; 13)
Drugs in the OBT	3 (2; 4)
HIV-1 infection duration, years since diagnosis	17 (14; 20)
HIV-1 RNA zenith, log ₁₀ copies/mL	5.4 (4.8; 5.8)
CD4 ⁺ nadir, cells/mm ³	90 (33; 175)
Baseline HIV-1 RNA level, log ₁₀ copies/mL	4.2 (3.6; 4.7)
Baseline CD4 ⁺ count, cells/mm ³	244 (110; 310)
Previous antiretroviral therapy	
Duration of exposure, years	10 (6; 12)
Previous NRTI, nb	6 (5; 7)
Previous PI, nb	5 (4; 6)
Previous NNRTI, nb	2 (1; 2)
Raltegravir co-prescribed antiretrovirals, n (%)	
+ 2 NRTIs	18 (35)
+ 1 PI	40 (80)
+ 1 NNRTI	22 (43)
Genotype characteristics at baseline	
NRTI resistance-related mutations	5 (4; 6)
PI resistance-related mutations	
Minor ^a	9 (8; 11)
Major ^a	4 (3; 5)
Total (minor + major)	13 (11; 15)
NNRTI resistance-related mutations	2 (1; 3)
GSS ANRS (<1/≥1&<2/≥2)	9/17/25 (18/33/49)

251 Baseline: Initiation of raltegravir-based HAART. Values are medians (IQR) unless
 252 stated otherwise. NRTI: nucleoside reverse-transcriptase inhibitor; NNRTI: non-
 253 nucleoside reverse-transcriptase inhibitor; PI: protease inhibitor; nb: numbers; OBT:
 254 Optimised background therapy; ^aMajor and minor protease mutations were defined
 255 according to the IAS–USA panel ⁹; ^bGSS: Genotypic sensitivity score of optimized
 256 background therapy according to the ANRS algorithms.

257 **Table 2:** Viro-immunological response in patients receiving raltegravir-based
 258 HAART, ANRS CO3 Aquitaine Cohort, 2007-2008.

	Follow-up		
	W4 (n=43)	W12 (n=50)	W24 (n=45)
<1.7 log₁₀ copies/mL			
n	26	27	33
%	60	54	73
95% CI	44; 75	39; 68	58; 85
<2.6 log₁₀ copies/mL			
n	36	42	35
%	84	84	78
95% CI	69; 93	71; 93	63; 89
Delta HIV-1 RNA			
log₁₀ copies/mL			
median	-2.1	-2.1	-2.1
IQR	-2.6; -2.1	-2.9; -1.2	-2.9; -1.1
Virological success*			
n			39
%			78
95% CI			64; 88
CD4+ cells/mm³			
median	255	298	286
IQR	165; 376	176; 396	164; 400
Delta CD4⁺ cells/mm³			
median	28	40	57
IQR	-4; 85	2; 95	0; 156

259 CI: confidence interval; IQR: Interquartil range; W4: week 4; W12: week 12;
 260 W24: week 24; Delta CD4+: difference of CD4+ cell counts between W4, W12,
 261 W24 and baseline, respectively; Delta HIV-1 RNA: difference of HIV-1 RNA
 262 between baseline and W4, W12 and W24, respectively. *HIV-1 RNA level less
 263 than 2.7 log₁₀ copies/mL at W12 and HIV-1 RNA level less than 1.7 log₁₀
 264 copies/mL at W24, patients with missing data at W24 and HIV-1 RNA level
 265 less than 2.7 log₁₀ copies/mL at W12 were considered with virologic success;
 266 One patient had neither a HIV-1 RNA value at W12 nor at W24.
 267

268 **Appendix**

269 The Groupe d'Epidemiologie Clinique du Sida en Aquitaine (GECSA) steering the
270 ANRS CO3 Aquitaine Cohort is organized as follows:

271 Scientific committee: F. Dabis (Chair and Principal Investigator), M. Dupon, M.
272 Longy-Boursier, P. Morlat, JL. Pellegrin, and JM. Ragnaud.

273 Epidemiology, Methodology: M. Bruyand, G. Chêne, F. Dabis, S. Lawson-Ayayi, R.
274 Thiébaud.

275 Infectious diseases, Internal Medicine: M. Bonarek, F. Bonnal, F. Bonnet, N. Bernard,
276 O. Caubet, L. Caunègre, C. Cazanave, J. Ceccaldi, FA Dauchy, C. De La Taille, S.
277 De Witte, M. Dupon, P. Duffau, H. Dutronc, S. Farbos, MC Gemain, C. Greib, D.
278 Lacoste, S. Lafarie-Castet, P. Loste, D. Malvy, P. Mercié, P. Morlat, D. Neau, A.
279 Ochoa, JL. Pellegrin, JM. Ragnaud, S. Tchamgoué, JF. Viillard.

280 Immunology: P. Blanco, JF. Moreau. I. Pellegrin.

281 Virology: H. Fleury, ME. Lafon, B. Masquelier.

282 Pharmacology: D. Breilh.

283 Pharmacovigilance: G. Miremont-Salamé.

284 Data collection: MJ. Blaizeau, M. Decoin, S. Delveaux, S. Gillet, C. Hannapier, O.
285 Leleux, B. Uwamaliya-Nziyumvira.

286 Data management: S. Geffard, G. Palmer, D. Touchard.

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