



HAL
open science

Resistance to integrase inhibitors: a national study in HIV -1-infected treatment-naive and -experienced patients

Anne-Geneviève Marcelin, Maxime Grude, Charlotte Charpentier, Pantxika Bellecave, Laura Le Guen, Coralie Pallier, Stéphanie Raymond, Audrey Mirand, Laurence Bocket, Djeneba Bocar Fofana, et al.

► **To cite this version:**

Anne-Geneviève Marcelin, Maxime Grude, Charlotte Charpentier, Pantxika Bellecave, Laura Le Guen, et al.. Resistance to integrase inhibitors: a national study in HIV -1-infected treatment-naive and -experienced patients. *Journal of Antimicrobial Chemotherapy*, 2019, 74 (5), pp.1368-1375. 10.1093/jac/dkz021 . hal-02154049

HAL Id: hal-02154049

<https://normandie-univ.hal.science/hal-02154049>

Submitted on 30 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Resistance to integrase inhibitors: A National Study 2 in HIV-1-Infected Naïve and Experienced Patients

3
4 Anne-Genevieve Marcelin^{1*}, Maxime Grude¹, Charlotte Charpentier², Pantxika Bellecave³, Laura Le Guen⁴,
5 Coralie Pallier⁵, Stéphanie Raymond⁶, Audrey Mirand⁷, Laurence Bocket⁸, Djeneba Bocar Fofana⁹, Constance
6 Delaugerre¹⁰, Thuy Nguyen¹, Brigitte Montès¹¹, Hélène Jeulin¹², Thomas Mourez¹³, Samira Fafi-Kremer¹⁴,
7 Corinne Amiel¹⁵, Catherine Roussel¹⁶, Julia Dina¹⁷, Mary-Anne Trabaud¹⁸, Hélène Le Guillou-Guillemette¹⁹,
8 Sophie Vallet²⁰, Anne Signori-Schmuck²¹, Anne Maillard²², Virginie Ferre²³, Diane Descamps², Vincent
9 Calvez¹, Philippe Flandre¹ on behalf of ANRS AC43 resistance group

10
11 1 AP-HP, Hôpital Pitié-Salpêtrière, INSERM-Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Paris,
12 France; 2 INSERM, IAME, UMR 1137, F-75018 Paris, France; Univ Paris Diderot, Sorbonne Paris Cité, F-
13 75018 Paris, France; AP-HP, Hôpital Bichat, Laboratoire de Virologie, F-75018 Paris, France; 3 CHU de
14 Bordeaux, Laboratoire de Virologie, Univ. Bordeaux, CNRS UMR 5234, F-33076 Bordeaux, France ; 4 CHU de
15 Nantes, Laboratoire de Virologie, France ; 5 CHU Paul Brousse, Villejuif, France ; 6 INSERM U1043 Toulouse,
16 F-31300 France and Laboratoire de Virologie, CHU Toulouse Purpan, Toulouse, F-31300 France ; 7 CHU de
17 Clermont-Ferrand, France ; 8 CHU de Lille, Lille, France ; 9 AP-HP, CHU Saint Antoine, INSERM-Sorbonne
18 Universités, UPMC Univ Paris 06, UMR_S 1136, Paris, France; 10 CHU Saint Louis, Paris, France ; 11 CHU
19 Saint-Eloi, Montpellier, France ; 12 Laboratoire de Virologie, CHRU de Nancy Brabois, Vandoeuvre-lès-Nancy,
20 France; 13 CHU de Rouen, Rouen, France ; 14 CHU de Strasbourg, Strasbourg, France ; 15 AP-HP, CHU
21 Tenon, Paris, France; 16 CHU d'Amiens, Amiens, France ; 17 CHU de Caen, Caen, France ; 18 Hôpital de la
22 Croix-Rousse, Hospices Civils de Lyon, Lyon, France; 19 Laboratoire de Virologie, CHU Angers, France ; 20
23 CHRU La Cavale Blanche, Brest ; 21 CHU Grenoble-Alpes, Grenoble, France ; 22 CHU de Rennes, France ; 23
24 CHU Nantes, Laboratoire de Virologie, CIC Inserm 143, France.

25
26 * Corresponding author: AG Marcelin, PharmD, Ph.D.

27 Mailing address: Department of Virology, Pitié-Salpêtrière Hospital, 83 Boulevard de l'Hôpital, 75013 Paris,
28 France

29 Phone: 33142177401, Fax: 33142177411

30 e mail: anne-genevieve.marcelin@aphp.fr

31 ^ÄMembers are listed in the Acknowledgement section

32 **Running title:** HIV resistance to integrase inhibitors

33 **Key words:** integrase, inhibitors, mutations, patterns, resistance

34 **Summary:** This work described the resistance patterns in a large population of patients failing an
35 integrase inhibitor-based regimen. We showed that dolutegravir exhibited the highest robustness
36 regarding resistance selection in case of virological failure in real world clinical setting.

37
38 Word count: abstract 248, text 2941

41 **Synopsis**

42 **Introduction:** It is of importance to describe integrase strand transfer inhibitors (INSTIs)
43 resistance profiles and factors associated with, in naïve- and experienced-patients failing an
44 INSTI-based regimen in clinical practice.

45 **Methods:** Data were collected from patients failing an INSTI-containing regimen in a
46 multicentre french study between 2014 and 2017. Failure was defined by 2 consecutive
47 plasma viral load (VL) > 50 copies/mL. Reverse transcriptase, protease and integrase genes
48 were sequenced at baseline and failure. INSTIs resistance-associated mutations (RAMs)
49 included in the ANRS genotypic algorithm were investigated.

50 **Results:** Among the 674 patients, 359 were failing raltegravir, 154 elvitegravir and 161
51 dolutegravir. Overall, 389 (58%) patients showed no INSTI RAMs at failure. At failure, 36%
52 of patients failing raltegravir exhibited viruses considered genotypically resistant to
53 raltegravir, 44% of patients failing elvitegravir exhibited viruses resistant to elvitegravir, 14%
54 and 7% of patients failing dolutegravir exhibited viruses resistant to dolutegravir once per day
55 and twice daily, respectively. Patients with high VL at failure and low Genotypic Sensitivity
56 Score had a higher risk to select at least one INSTI RAM. Patients failing dolutegravir had
57 significantly less INSTI RAMs at failure than patients failing raltegravir (OR=0.57, p = 0.02)
58 or elvitegravir (OR=0.45, p = 0.005). Among the sixty eight patients failing a first-line
59 regimen: 11/41 (27%) patients failing raltegravir had at failure viruses with emergent INSTI
60 RAMs, 7/18 (39%) with elvitegravir and 0/9 with dolutegravir.

61 **Conclusions:** These results confirmed the robustness of dolutegravir regarding resistance
62 selection in case of virological failure in routine clinical care.

63

64 **Introduction**

65 Integrase strand transfer inhibitors (INSTIs), which actively block the integration of the HIV
66 genome into the host DNA, represent the latest antiretroviral (ARV) class to be approved for
67 treatment of HIV-infected individuals ¹. There are currently four INSTIs approved for the
68 treatment of HIV infection: raltegravir, elvitegravir, dolutegravir and more recently
69 bicitegravir. Although highly efficacious in the management of HIV, both raltegravir and
70 elvitegravir are susceptible to the development of resistance mutations in case of virological
71 failure. The main resistance pathways that have been reported as selected both *in vitro* and *in*
72 *vivo* with raltegravir are Y143, Q148 and N155. ² It is evident now that raltegravir and
73 elvitegravir share both the Q148 and N155 major resistance pathways. ³ However, T66 and
74 E92 pathways are predominantly selected by elvitegravir. ⁴ In contrast to raltegravir and
75 elvitegravir that share a common resistance profile, dolutegravir has a markedly distinct
76 resistance profile and appears to have a higher genetic barrier to resistance. Indeed, in clinical
77 trials it has not been shown to select for any resistance-associated mutations in treatment
78 naïve patients when used in triple therapy. ^{5,6,7} However, one case of emergence of integrase
79 resistance mutation (Q148K + M184V) during virologic failure in a treatment-naïve man who
80 initiated tenofovir disoproxil fumarate/emtricitabine plus dolutegravir has been recently
81 published. ⁸ In addition, there have been some cases of treatment failure with resistance
82 mutations in treatment-experienced but INSTI-naïve patients, in particular with the emergence
83 of the R263K mutation. ⁹ Finally, in the particular setting of dolutegravir monotherapy in
84 treatment-experienced patients, the selection of other substitutions at positions E92, Q148,
85 N155 and S230 have been reported. ¹⁰ Bicitegravir is the most recent INSTI and there is few
86 information available in regard to resistance against this drug. Given its similar chemical
87 structure with dolutegravir and the fact that bicitegravir selected for 263K during *in vitro*
88 passages, we can assume that bicitegravir share similar resistance profile as dolutegravir. ¹¹

89 Although INSTIs mutation pathways have extensively been studied, most of existing data
90 arises from *in vitro* experiments or clinical trials with a limited number of patients and
91 specific inclusion criteria. In this study, we focused on integrase genotypic resistance tests
92 performed in real world clinical setting by the French national ANRS network in order to
93 better characterize the profile of INSTI resistance among specimens obtained for clinical
94 decision making and to identify factors associated with the selection of integrase resistance
95 mutations.

96

97 **Patients and methods**

98 Patients and antiretroviral regimens. HIV-1-infected patients who experienced virologic
99 failure to an INSTI-containing regimen between 2014 and 2017 were allowed to be included
100 in the study. Patients were treated with raltegravir, elvitegravir or dolutegravir with a
101 background regimen comprising mainly NRTIs, NNRTIs, and/or PIs. Virological failure was
102 defined as two consecutive HIV-1 viral loads (VL) > 50 copies/mL. Clinical data and
103 treatment histories were collected for all patients recruited. Inclusion criteria and all data were
104 checked by the study monitor. The 21 participating laboratories belong to the Agence
105 Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) AC43 network and
106 participate in the annual ANRS quality control assessment of HIV-1 drug resistance
107 sequencing.¹² The study was approved by the scientific committee of the ANRS AC43.

108 Genotypic resistance testing. The sequences of the protease (PR), reverse transcriptase (RT)
109 and integrase (IN) genes were determined at baseline and failure (on confirmation plasma
110 failure) in each laboratory using the ANRS consensus technique
111 (<http://www.hivfrenchresistance.org/>), the Abbott ViroSeq kit, or an in-house method. For
112 resistance interpretation, we used RT, PR and IN mutations present in the ANRS algorithm
113 (Version 28) to determine whether patients receiving a particular NRTI, NNRTI or PI, had
114 resistant, intermediate or susceptible virus strains. (www.hivfrenchresistance.org). List of
115 INSTIs associated mutations used in the study is: T66AIK, L74FIM, V75I, E92Q, T97A,
116 G118R, F121Y, E138AKT, G140ACS, Y143ACGHRS, P145S, S147G, Q148EGHKR,
117 V151L, S153FY, N155HST, E157Q, S230R, R263K.

118 The genotypic sensitivity score (GSS) of the current regimen (without INSTI) was calculated
119 according to the ANRS resistance algorithm. For each antiretroviral drug, patients with drug-
120 susceptible viruses were assigned a GSS of 1, and those with intermediate-level and high-
121 level resistance were assigned scores of 0.5 and 0, respectively.

122 Statistical analysis.

123 Quantitative variables are described by use of median and Interquartil Range (IQR) while
124 categorical variables are described in percent. HIV-1 RNA at failure, viral subtype (B versus
125 CRF02_AG and other non-B), baseline CD4 cell count, CD4 cell count at failure, nadir CD4,
126 age, duration of infection, duration of INSTI treatment, the ongoing treatment (dual therapy,
127 triple therapy and four and more therapy) and GSS were investigated as potential factors of
128 occurrence of INSTIs mutations by the use of Cochran-Armitage test. A logistic regression
129 model was also used to investigate whether previous variables were independent predictors of
130 occurrence of INSTIs resistance associated mutations (RAMs). All variables tested with a P-
131 value <0.10 in the univariate analysis were retained for the construction of the multivariate
132 model. The latter only keeps the variables significantly associated with the occurrence of
133 INSTIs mutation with a p-value <0.05 .

134

135 **Results**

136 Overall 674 patients failing an INSTI-containing regimen were included in the study from 21
137 French centres of the ANRS network. Patients were failing while receiving raltegravir (n =
138 359), elvitegravir (n = 154) or dolutegravir (n = 161) containing regimen and 10% of them
139 were failing their first-line treatment. The main characteristics of the global study population
140 are presented in Table 1. The average age was 48.5 years (IQR: 39.9-55.4 years) and the
141 majority (65%) of patients were male. Regarding HIV-1 subtypes, 55.8% harboured subtype
142 B and the most frequent non-B subtype was CRF02_AG (18%). The most prescribed
143 combinations with INSTI were 2 NRTIs (55%) and 1 NRTI + 1 PI (13%). Patients were
144 receiving 1, 2, 3 and more than 3 antiretrovirals including the INSTI in 1%, 17%, 66% and
145 15%, respectively.

146 Virologic failure occurred after a median time of 10.7 months (IQR: 5.7-30) following
147 administration of INSTI-containing regimen. At failure, median viral load was 2.9 log₁₀
148 copies/mL (IQR: 2.3-4). Overall, viruses harboured no known INSTIs RAMs and were thus
149 considered as fully genotypically susceptible to all INSTIs in 58% (n = 389) of cases. Thus,
150 42% of viruses harboured at least 1 INSTI RAM: 1, 2 and at least 3 mutations in 25% (n =
151 170), 10% (n = 71) and 6.5% (n = 44) of cases, respectively.

152 Regarding INSTIs RAMs in our dataset, the most frequent observed integrase mutations were
153 N155H/S/T (n = 112; 16.6%), L74F/I/M (n = 82; 11.9%), Q148H/K/R (n = 54; 8.0%) and
154 T97A (n = 53; 7.9%). The other detected INSTIs mutations were in less than 5% of cases:
155 T66A/I/K (n = 15 ; 2.1%), V75I (n = 6 ; 0.9%), E92Q (n = 26 ; 3.9%), E138A/K/T (n = 22 ;
156 3.3%), G140A/C/S (n = 33 ; 4.9%), Y143A/C/G/H/R/S (n = 25 ; 3%), P145S (n = 3 ; 0.5%);
157 S147G (n = 10; 1.5%), V151L (n = 1 ; 0.2%), S153F/Y (n = 2 ; 0.3%), E157Q (n = 22 ;
158 3.3%), S230G/R (n = 7 ; 0.6%) and R263K (n = 2 ; 0.3%). Q148H/K/R mutations were
159 selected significantly more frequently in B subtypes versus non-B subtypes (p = 0.0135). In

160 patients harboring viruses with 2 or 3 INSTIs RAMs, the most common combinations were
161 G140S/Q148H (12%), T97A/G140S/Q148H (6%) and L74I/E92Q (5%).

162 Interpretation of resistance to the different INSTIs is described in Figure 1. At failure, 36% of
163 patients failing raltegravir exhibited plasma viruses considered genotypically resistant to
164 raltegravir, 44% of patients failing elvitegravir exhibited plasma viruses considered resistant
165 to elvitegravir, 14% and 7% of patients failing dolutegravir exhibited plasma viruses
166 considered resistant to dolutegravir once per day (OD) and twice daily (BID), respectively.

167 We aimed to characterize clinical and virological factors associated with the emergence of
168 INSTIs RAMs (Table 2). The final multivariate model shows a higher risk of occurrence of at
169 least one INSTI RAM associated with a higher level of VL at failure (Odd Ratio (OR) = 1.2
170 per 1 log₁₀ copies/mL increase) (Figure 2) and a lower risk of occurrence of at least one
171 INSTI RAM with a higher level of GSS (OR = 0.29 for GSS = 1-1.5, OR= 0.12 for GSS = 2-
172 2.5 and OR = 0.08 for GSS>3 versus GSS = 0-0.5). In addition, patients failing dolutegravir
173 had viruses with significantly less INSTIs RAMs at failure than patients failing raltegravir
174 (OR = 0.57, p = 0.02) and patients failing elvitegravir (OR = 0.45, p = 0.005).

175 Among the 674 patients, 68 were failing a first-line INSTI-based regimen: 41 containing
176 raltegravir, 18 elvitegravir and 9 dolutegravir. Among the 41 patients failing to a raltegravir-
177 based regimen, 11 (27%) harboured INSTI RAMs on their genotypic resistance test at failure:
178 4 with emergent mutations (1 L74I/M, 1 T97A, 1 Y143R, 1 V75I) and 7 for whom no
179 baseline test was available: 3 L74I, 1 T97A, 1 E138K, 1 N155H, 1 E92Q + N155H, 1 T97A
180 + N155H + E157Q. Among the 18 patients failing to an elvitegravir-based regimen, 7 (39%)
181 harboured INSTI RAMs on their genotypic resistance test at failure: 5 with emergent
182 mutations (1 T66I, 2 N155H, 1 E92Q + E157Q, 1 E92Q + S153Y + N155H) and 2 for whom
183 no baseline test was available: 1 L74I + P145S, 1 N155H + S230R. Among the 9 patients

184 failing to a dolutegravir-based regimen, 3 harboured INSTI RAMs on genotypic resistance
185 test at failure but none were considered as emergent: 2 mutations were already present at
186 baseline (1 L74I and 1 E157Q) and 1 E138K for which no baseline test was available.
187 Interestingly, 7/41 (17%) of the patients failing a first-line raltegravir-based regimen had
188 plasma viruses with M184V (4 M184V alone and 3 with INSTI mutation). Among the 18
189 patients failing of a first-line elvitegravir-based regimen, 7 (39%) had INSTI RAMs and all of
190 them also displayed a M184V mutation, while it was 0/9 in patients failing a dolutegravir fist-
191 line regimen. However, the Fisher test did not show a significant association between the
192 emergence of the M184V mutation and INSTI treatment ($p = 0.07$).

193

194

195 **Discussion**

196 The development and expanding use of integrase inhibitors in ARV-naïve and ARV-
197 experienced patients makes it increasingly important to survey INSTIs resistance in the
198 context of large clinical settings.¹³ Here, we provide one of the largest data that characterizes
199 INSTI resistance among INSTI failing patients obtained for clinical indications and in which
200 collection of clinical and virological parameters were available.

201 Overall, our results show that 42% of patients' viruses experiencing failure to INSTI harbor
202 viruses with at least one INSTI RAM. This rate is higher compared to a study that aimed to
203 characterize INSTI resistance among integrase resistance testing obtained for clinical
204 indications in the United States in which the investigators found that only 15.6% of viruses
205 harbored INSTI major mutations.¹⁴ However, our results are similar to a more recent study
206 showing that 39% of patients' viruses at time of failure to raltegravir harbor at least one
207 INSTI resistance mutation.¹⁵ Methodological differences between studies can be noticed, as
208 the predefined list of INSTI RAMs has evolved with the inclusion of new mutations over
209 time. In addition, in the present study, we have analyzed failures to 3 different INSTIs and not
210 only to raltegravir, as compared in the French study¹⁵ and in another study where the
211 laboratory did not obtain data on the patient's treatment status (naïve or experienced) or
212 history of prior ARV exposures.¹⁴ This point is crucial as INSTIs have different resistance
213 profile and genetic barrier. Indeed, second-generation INSTIs, including dolutegravir display
214 a more robust resistance profile than either raltegravir or elvitegravir and offer a higher barrier
215 to resistance compared to the first-generation class.¹⁶ The resistance profile of dolutegravir
216 has been extensively characterized during the past few years and high-level dolutegravir
217 resistance requires multiple INSTI first-generation resistance mutations.³ This is supported
218 by our results showing that at failure, only 14% and 7% of patients failing dolutegravir
219 exhibited viruses considered genotypically resistant to dolutegravir OD and BID, respectively,

220 whereas 36% of patients failing raltegravir exhibited viruses considered resistant to raltegravir
221 and 44% of patients failing elvitegravir exhibited viruses considered resistant to elvitegravir.
222 Indeed, dolutegravir efficacy has been initially investigated in the VIKING Phase IIB study
223 where antiretroviral-experienced patients, with raltegravir and/or elvitegravir resistant viruses,
224 received DTG 50 mg either OD (Cohort I) or BID (Cohort II).¹⁷ In spite of the positive
225 results, the VIKING-3 study also highlighted how the dolutegravir response was most reduced
226 in subjects carrying viruses with resistance-associated mutations at position G140 and Q148.
227¹⁸ This mutation complex is known to cause up to a 10620-fold reduced susceptibility to
228 dolutegravir and, furthermore, subjects harboring viruses with Q148 + × 2 mutations have
229 96% lower odds of achieving VL <50 copies/mL at week 24 if compared with those with no
230 Q148 mutations.^{19,20} In addition, our results reinforce the robustness of dolutegravir
231 regarding selection of resistance in clinical practice as patients failing dolutegravir had
232 significantly less INSTI resistance mutations at failure as compared to patients failing
233 raltegravir or elvitegravir.

234 The most common resistance pathways identified in the present study were N155H/S/T,
235 L74F/I/M, Q148A/C/G/H/R/S and T97A. In addition, our findings corroborate previous
236 observations, indicating the unique propensity of subtype B to the development of the
237 Q148+G140 mutation pathway.²¹ A glycine to serine substitution at integrase position 140
238 requires only one nucleotide change in subtype B and two nucleotide changes in all non-B
239 clades, thus raising the genetic barrier to the emergence of G140 mutants. As mutations at
240 codon 140 play a key role in restoring the fitness of Q148 mutants, their occurrence can also
241 influence the emergence of Q148H/R/K, thus explaining the reduced prevalence of Q148
242 mutants observed in non-B subtypes. In the present study, some rare mutations have been also
243 evidenced, as the R263K mutation in two cases. The R263K mutation was the first mutation
244 rarely found selected at time of virological failure in experienced patients failing a first-line

245 dolutegravir -based treatment.⁹ Further *in vitro* studies on R263K mutants showed a moderate
246 increase in phenotypic resistance level and a drastic reduction in viral replicative capacity.
247 More recently, it has been shown that in both single and multiple rounds of HIV-1 infections,
248 bicitegravir and cabotegravir, two more recent INSTIs remained active against R263K mutant.
249 ²² Other mutations (i.e G118R and F121Y), rarely described in patients failing on raltegravir,
250 ²³ have been also shown to induce broad cross-resistance to dolutegravir *in vitro*.²⁴ However,
251 we did not see evidence of either G118R or F121Y in this study.

252 Another interesting mutation is the E157Q mutation that is polymorphic, found between 1.7%
253 and 5.6% of viral sequences issued from ART-naïve patients depending on the viral subtype;
254 as well as acquired resistance emerging at failure of a raltegravir-based regimen in two case
255 reports.²⁵ Data on phenotypic resistance level of E157Q mutants and virological response of
256 patients harboring an E157Q virus initiating an INSTI-based regimen, showed that
257 dolutegravir might be the most recommended INSTI in such patients.^{26,27} However, in the
258 present study, 1/9 patients who failed DTG had a virus already harbouring a E157Q at
259 baseline, thus it is difficult to give strong recommendations.

260 In clinical practice, it has been shown that after previous exposure to first-generation INSTIs,
261 treatment with dolutegravir showed long durability and that subjects infected with a non-B
262 HIV-1 subtype had a greater risk of having detectable VL at the last observation.²⁸ It is also
263 important to determine, in case of virological failure, which factors are associated with the
264 development of resistance mutations. In a previous study, we showed that a low GSS was
265 associated with the presence of raltegravir-associated mutations and that a high HIV-1 VL
266 level at failure (>1000 copies/mL) was associated with the presence of raltegravir-associated
267 mutations.¹⁵ Here we reinforce this message showing that patients with high VL (> 3 log
268 cp/mL) at failure and low GSS have a higher risk to select at least one INSTI RAM. This has

269 clinical consequences suggesting that careful attention should be paid to patients with
270 detectable viral load under an INSTI regimen.

271 In this study we have made a special focus on failures in treatment-naïve patients. At failure,
272 27% of patients receiving raltegravir had emergent or not previously evidenced INSTI RAMs,
273 39% with elvitegravir and none with dolutegravir. In addition, 17% of patients failing
274 raltegravir had plasma viruses with a M184V mutation (4 alone and 3 with INSTI mutation),
275 39% of patients failing elvitegravir (always associated with INSTI mutation) and none in
276 patients failing dolutegravir. Our results corroborate data from clinical trials showing that
277 raltegravir and elvitegravir have relatively low genetic barrier to the development of
278 resistance with an overlapping resistance profile and do not protect NRTI backbone.²⁹ In
279 treatment-naïve patients, data from clinical trial showed neither resistance mutation to INSTIs
280 nor to NRTIs in the rare patients experiencing virological failure in the dolutegravir arm up to
281 96 weeks.⁶ Thus our data corroborate that the use of dolutegravir as first-line therapy in
282 clinical practice should also prevent the development of INSTI and associated-NRTI drug
283 resistance. However, this should be carefully monitored because despite a high barrier to
284 resistance, no ARV agent is impervious to resistance and even it is extremely rare to date,
285 dolutegravir failure and resistance in treatment naïve patients is possible.⁸

286 Overall, this paper describes one of the largest studies characterizing INSTI resistance among
287 resistance testing obtained for clinical indications from naïve and experienced patients failing
288 to raltegravir, elvitegravir and dolutegravir and reveals factors associated with resistance to
289 INSTIs that should be taken into consideration in clinical management. The results confirmed
290 the robustness of dolutegravir regarding resistance selection in case of virological failure in
291 routine clinical care.

292

293 **Acknowledgments**

294 This work was presented in part as an oral presentation at the European AIDS Clinical Society
295 Meeting, 2017, Milan, Italy (abstract PS3/1).

296

297 Members of the ANRS AC43 Resistance Study Group by location

298 Amiens, C. Roussel; Angers, H. Le Guillou-Guillemette, A. Ducancelle ; Argenteuil, L.
299 Courdavault; Avicenne, C. Alloui, P. Honore; Besançon, Q. Lepiller, D. Bettinger; Bordeaux,
300 P. Bellecave, P. Pinson-Recordon, C. Tumiotto, S. Reigadas; Brest, S. Vallet, C Payan, JC.
301 Duthe; Caen, M. Leroux, J. Dina, A. Vabret; Clermont-Ferrand, A. Mirand, C. Henquell;
302 Créteil-Henri Mondor, M. Bouvier-Alias; Dijon, A. Simohamed ; Fort de France, G. Dos
303 Santos; Genève, S. Yerly, C. Gaille, W. Caveng, S. Chapalay, A. Calmy; Grenoble, A.
304 Signori-Schmuck, P Morand; HU Paris Sud, C. Pallier, M. Raho-Moussa, M. Mole, M-J.
305 Dulucq; LilleóTourcoing, L. Bocket, K.Alidjinou; Limoges, S. Ranger-Rogez; Lyon, M. A.
306 Trabaud, V Icard, J.C. Tardy; Marseille, C. Tamalet; Metz/Thionville, C. Delamare;
307 Montpellier, B. Montes; Nancy, E. Schvoerer, H. Fenau; Nantes, A. Rodallec, E. André-
308 Garnier, V. Ferré; Nice, A. De Monte, J. Dufayard; Orléans, A. Guigon, J. Guinard; Paris-
309 Bichat Claude Bernard, D. Descamps, C. Charpentier, B Visseaux, G. Peytavin; Paris-Necker,
310 M. Fillion; Paris-Pitié-Salpêtrière, C. Soulié, I. Malet, M. Wirden, A. G. Marcelin, V. Calvez,
311 P. Flandre, L. Assoumou, D. Costagliola; Paris-Saint Antoine, L. Morand-Joubert, S.
312 Lambert-Niclot, D. Fofana; Paris-Saint Louis, C. Delaugerre, ML Chaix, N. Mahjoub; Paris-
313 Tenon, V. Schneider, C. Amiel; Poitiers, G. Giraudeau, A. Beby-Defaux, D. Plainchamp;
314 Rennes, A. Maillard; Rouen, E. Alessandri-Gradt, M. Leoz, J. C. Plantier; Strasbourg, P.
315 Gantner S. Fafi-Kremer, P. Fischer ; Toulouse, S. Raymond, J. Izopet, J Chiabrando; Tours,
316 F. Barin, G. Fajole, O. Burgault; Versailles, S. Marque Juillet.

317 Members of the ANRS Clinical Centres by location

318 Angers, P. Abgueguen, V. Rabier, Y.M. Vandamme; Besançon, B. Hoen; Bordeaux, M.
319 Dupon, P. Morlat, D. Neau; Brest, M. Garré, V. Bellein; Caen, R. Verdon, A. De la
320 Blanchardière, S. Dargère, A. Martin, V. Noyou; Clermont-Ferrand, C. Jacomet; Créteil, J.D.
321 Lelièvre, J.L. Lopez-Zaragoza.; Dijon, B. Lorcerie; Fort de France, A. Cabié; Genève, S.
322 Yerly; Grenoble, P. Leclercq, M. Blanc; Le Kremlin-Bicêtre, C. Goujard; LilleóTourcoing, O.
323 Robineau; Limoges, P. Weinbreck; Lyon, L. Cotte; D. Makhloufi; Marseille, I. Poizot-Martin,
324 I. Ravaud; Montpellier, J. Reynes; Nancy, H. Fenau; Nantes, F. Raffi; Nice, E. Cua, J.
325 Durant, P. Pugliese; Orléans, L. Hocquelloux, T. Prazuck; Paris-Bichat Claude Bernard, Y.
326 Yazdanpanah, R. Landman, S. Legac; Paris-HEGP, L. Weiss, M. Karmochkine; Paris-Jean-
327 Verdier, S. Tassi; Paris-Necker-Enfants Malades, C. Duvivier ; HU Paris-Sud, C. Bolliot, M.
328 Malet, D. Vittecoq, M. Raho-Moussa, M. Mole; Paris-Pitié-Salpêtrière, C. Katlama, A.
329 Simon; Paris-Saint Antoine, P. M. Girard, J. L. Meynard; Paris-Saint Louis, J. M. Molina, N.
330 Mahjoub; Paris-Tenon, V. Berrebi, G. Pialoux; Pointe à Pitre, I. Lamaury, Fort de France , A.
331 Cabié; Poitiers, G. Le Moal, D. Plainchamp; Rennes, C. Michelet, J-C. Duthe; Rouen, F.
332 Caron, Y. Debab, G. Unal Strasbourg, M. Partisani, D. Rey, P. Fischer; Toulouse, B.
333 Marchou, P. Massip, P Delobel; Tours, G. Gras, G. Fajole; Versailles, A. Greber Belan, Ruel,
334 O. Beletry, F. Granier.

335 **Funding**

336 The research leading to these results has received funding from the Agence Nationale de Recherches
337 sur le SIDA et les Hépatites virales (ANRS) and ViiV Healthcare.

338

339 **Transparency declarations**

340 The authors have no conflict of interest.

341

342 **References**

343 1. Saag MS, Benson CA, Gandhi RT, *et al.* Antiretroviral Drugs for Treatment and Prevention
344 of HIV Infection in Adults: 2018 Recommendations of the International Antiviral Society-
345 USA Panel. *JAMA* 2018; **320**: 379696.

346 2. Malet I, Delelis O, Valantin M-A, *et al.* Mutations associated with failure of raltegravir
347 treatment affect integrase sensitivity to the inhibitor in vitro. *Antimicrob Agents Chemother*
348 2008; **52**: 135168.

349 3. Anstett K, Brenner B, Mesplede T, Wainberg MA. HIV drug resistance against strand
350 transfer integrase inhibitors. *Retrovirology* 2017; **14**: 36.

351 4. Shimura K, Kodama E, Sakagami Y, *et al.* Broad antiretroviral activity and resistance
352 profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-
353 303/GS-9137). *J Virol* 2008; **82**: 764674.

354 5. Molina J-M, Clotet B, van Lunzen J, *et al.* Once-daily dolutegravir versus darunavir plus
355 ritonavir for treatment-naïve adults with HIV-1 infection (FLAMINGO): 96 week results
356 from a randomised, open-label, phase 3b study. *Lancet HIV* 2015; **2**: e127-136.

357 6. Raffi F, Jaeger H, Quiros-Roldan E, *et al.* Once-daily dolutegravir versus twice-daily
358 raltegravir in antiretroviral-naïve adults with HIV-1 infection (SPRING-2 study): 96 week
359 results from a randomised, double-blind, non-inferiority trial. *Lancet Infect Dis* 2013; **13**:
360 927635.

361 7. Walmsley S, Baumgarten A, Berenguer J, *et al.* Brief Report: Dolutegravir Plus
362 Abacavir/Lamivudine for the Treatment of HIV-1 Infection in Antiretroviral Therapy-Naïve
363 Patients: Week 96 and Week 144 Results From the SINGLE Randomized Clinical Trial. *J*
364 *Acquir Immune Defic Syndr* 2015; **70**: 51569.

365 8. Fulcher JA, Du Y, Zhang T-H, Sun R, Landovitz RJ. Emergence of Integrase Resistance
366 Mutations During Initial Therapy Containing Dolutegravir. *Clin Infect Dis* 2018; **67**: 79164.

367 9. Cahn P, Pozniak AL, Mingrone H, *et al.* Dolutegravir versus raltegravir in antiretroviral-
368 experienced, integrase-inhibitor-naïve adults with HIV: week 48 results from the randomised,
369 double-blind, non-inferiority SAILING study. *Lancet* 2013; **382**: 70068.

- 370 10. Blanco JL, Marcelin A-G, Katlama C, Martinez E. Dolutegravir resistance mutations:
371 lessons from monotherapy studies. *Curr Opin Infect Dis* 2018; **31**: 237645.
- 372 11. Tsiang M, Jones GS, Goldsmith J, *et al.* Antiviral Activity of Bictegravir (GS-9883), a
373 Novel Potent HIV-1 Integrase Strand Transfer Inhibitor with an Improved Resistance Profile.
374 *Antimicrob Agents Chemother* 2016; **60**: 7086697.
- 375 12. Descamps D, Delaugerre C, Masquelier B, *et al.* Repeated HIV-1 resistance genotyping
376 external quality assessments improve virology laboratory performance. *J Med Virol* 2006; **78**:
377 153660.
- 378 13. Volberding PA. HIV Treatment and Prevention: An Overview of Recommendations From
379 the IAS-USA Antiretroviral Guidelines Panel. *Top Antivir Med* 2017; **25**: 17624.
- 380 14. Hurt CB, Sebastian J, Hicks CB, Eron JJ. Resistance to HIV integrase strand transfer
381 inhibitors among clinical specimens in the United States, 2009-2012. *Clin Infect Dis* 2014;
382 **58**: 423631.
- 383 15. Fourati S, Charpentier C, Amiel C, *et al.* Cross-resistance to elvitegravir and dolutegravir
384 in 502 patients failing on raltegravir: a French national study of raltegravir-experienced HIV-
385 1-infected patients. *J Antimicrob Chemother* 2015; **70**: 1507612.
- 386 16. Hightower KE, Wang R, Deanda F, *et al.* Dolutegravir (S/GSK1349572) exhibits
387 significantly slower dissociation than raltegravir and elvitegravir from wild-type and integrase
388 inhibitor-resistant HIV-1 integrase-DNA complexes. *Antimicrob Agents Chemother* 2011; **55**:
389 455269.
- 390 17. Eron JJ, Clotet B, Durant J, *et al.* Safety and efficacy of dolutegravir in treatment-
391 experienced subjects with raltegravir-resistant HIV type 1 infection: 24-week results of the
392 VIKING Study. *J Infect Dis* 2013; **207**: 74068.
- 393 18. Castagna A, Maggiolo F, Penco G, *et al.* Dolutegravir in antiretroviral-experienced
394 patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III
395 VIKING-3 study. *J Infect Dis* 2014; **210**: 354662.
- 396 19. Naeger LK, Harrington P, Komatsu T, Deming D. Effect of dolutegravir functional
397 monotherapy on HIV-1 virological response in integrase strand transfer inhibitor resistant
398 patients. *Antivir Ther (Lond)* 2016; **21**: 48168.
- 399 20. Castagna A, Ferrara M, Galli L, *et al.* Long-term efficacy of dolutegravir in treatment-
400 experienced subjects failing therapy with HIV-1 integrase strand inhibitor-resistant virus. *J*
401 *Antimicrob Chemother* 2018; **73**: 177682.
- 402 21. Doyle T, Dunn DT, Ceccherini-Silberstein F, *et al.* Integrase inhibitor (INI) genotypic
403 resistance in treatment-naïve and raltegravir-experienced patients infected with diverse HIV-1
404 clades. *J Antimicrob Chemother* 2015; **70**: 308066.
- 405 22. Hassounah SA, Alikhani A, Oliveira M, *et al.* Antiviral Activity of Bictegravir and
406 Cabotegravir against Integrase Inhibitor-Resistant SIVmac239 and HIV-1. *Antimicrob Agents*
407 *Chemother* 2017; **61**.

- 408 23. Munir S, Thierry E, Malet I, *et al.* G118R and F121Y mutations identified in patients
409 failing raltegravir treatment confer dolutegravir resistance. *J Antimicrob Chemother* 2015; **70**:
410 739649.
- 411 24. Malet I, Gimferrer Arriaga L, Artese A, *et al.* New raltegravir resistance pathways induce
412 broad cross-resistance to all currently used integrase inhibitors. *J Antimicrob Chemother*
413 2014; **69**: 2118622.
- 414 25. Charpentier C, Descamps D. Resistance to HIV Integrase Inhibitors: About R263K and
415 E157Q Mutations. *Viruses* 2018; **10**.
- 416 26. Charpentier C, Malet I, Andre-Garnier E, *et al.* Phenotypic analysis of HIV-1 E157Q
417 integrase polymorphism and impact on virological outcome in patients initiating an integrase
418 inhibitor-based regimen. *J Antimicrob Chemother* 2018.
- 419 27. Saladini F, Giannini A, Boccuto A, Tiezzi D, Vicenti I, Zazzi M. The HIV-1 integrase
420 E157Q polymorphism per se does not alter susceptibility to raltegravir and dolutegravir in
421 vitro. *AIDS* 2017; **31**: 230769.
- 422 28. Rusconi S, Adorni F, Tau P, *et al.* Dolutegravir (DTG)-containing regimens after
423 receiving raltegravir (RAL) or elvitegravir (EVG): Durability and virological response in a
424 large Italian HIV drug resistance network (ARCA). *J Clin Virol* 2018; **105**: 11267.
- 425 29. Blanco J-L, Varghese V, Rhee S-Y, Gatell JM, Shafer RW. HIV-1 integrase inhibitor
426 resistance and its clinical implications. *J Infect Dis* 2011; **203**: 1204614.

427

428

429 **Table 1. Baseline characteristics of the study population (n = 674)**

430

Male	65 %
Subtype B	56 %
Median time since HIV-1 diagnosis, years (IQR)	15.7 (6.74-22.4)
Median duration of current INSTI regimen, months (IQR)	10.7 (5.7-30)
Median baseline plasma HIV-1 RNA log ₁₀ copies/mL (IQR)	3.1 (1.9-4.9)
Median failure plasma HIV-1 RNA log ₁₀ copies/mL (IQR)	2.9 (2.3-4)
Median baseline CD4 cell count/mm ³ (IQR)	371 (173-649)
Median failure CD4 cell count/mm ³ (IQR)	418 (223-670)
INSTI co-treatment (%):	
NRTIs	55.3 %
NRTIs + PIs	13.2 %
NNRTIs	7 %
PIs	5.6 %
NNRTIs + PIs	4.9 %
NRTIs + NNRTIs	3.8 %
Other	8.7 %
431 GSS Score (%):	
432 0-0.5	16.11%
433 1-1.5	27.22%
434 2-2.5	44.07%
435 >=3	12.59%

436

437 IQR, interquartile range; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse

438 transcriptase inhibitors; PIs, protease inhibitors; INSTI, integrase strand transfer inhibitors, GSS, genotypic

439 sensitivity score.

440

441

442

443

444

445

446

447

448

449

450

451

452 **Table 2. Factors associated with the occurrence of INSTIs resistance associated**
 453 **mutations**

454

	Univariate Analysis			Multivariate Analysis			
	OR	95% IC	P-value	OR	95% IC	P-value	
Age (per 10 years increase)	1.115	0.977-1.273	0.1065				
CD4 baseline (per 100 cells/mm3 increase)	1.007	0.960-1.056	0.7764				
CD4 Failure (per 100 cells/mm3 increase)	0.988	0.941-1.038	0.6387				
Nadir CD4 (per 100 cells/mm3 increase)	0.99	0.902-1.087	0.8338				
Duration of Infection (per years increase)	1.018	1.001-1.035	0.0393				
Duration of INSTI treatment (per years increase)	1.052	0.982-1.126	0.1519				
LOG HIV RNA baseline (per 1 log10 copies/ml increase)	0.956	0.850-1.074	0.4478				
LOG HIV RNA Failure (per 1 log10 copies/ml increase)	1.345	1.165-1.553	<0.0001	1.223	1.027-1.456	0.0242	
Viral subtype	CFR02 VS B	0.869	0.572-1.319	0.5425			
	NON B VS B	0.971	0.677-1.394	0.8239			
	1 or 1.5 VS 0 or 0.5	0.29	0.156-0.540	0.0715	0.293	0.156-0.551	0.1326
GSS	2 or 2.5 VS 0 or 0.5	0.101	0.056-0.184	<0.0001	0.116	0.063-0.213	<0.0001
	>=3 VS 0 or 0.5	0.075	0.035-0.162	<0.0001	0.079	0.036-0.174	<0.0001
	Dual Therapy VS Triple Therapy	0.545	0.361-0.822	0.2545			
Dual Therapy VS Four and more Therapy	0.437	0.253-0.754	0.0235				
DTG VS RAL	0.406	0.270-0.610	<0.0001	0.567	0.345-0.931	0.0251	
DTG VS EVG	0.362	0.226-0.581	<0.0001	0.448	0.254-0.789	0.0055	

455

456

457

458 INSTI, integrase strand transfer inhibitors; OR, odds ratio; GSS, genotypic sensitivity score; DTG, dolutegravir;

459 RAL, raltegravir; EVG, elvitegravir

460

461 **Figure 1. Genotypic interpretation of resistance to different integrase strand transfer**
462 **inhibitors (INSTIs) among the 674 patients failing an INSTI-containing regimen.**
463 Predicted resistance to raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) once
464 per day (OD) or twice daily (BID) according to the ANRS algorithm.

465

466

467

468

469

470

471

472

473

474

475

476

477

478

479 **Figure 2. Association between level of HIV viral load at failure and the selection of**
480 **integrase strand transfer inhibitors (INSTIs) resistance associated mutations (RAMs).**

481