Antiretroviral resistance at virologic failure in the NEAT 001/ANRS 143 trial: Raltegravir + Darunavir/ritonavir or Tenofovir/Emtricitabine + Darunavir/ritonavir as first line antiretroviral therapy

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32 Running Head: Resistance of first-line raltegravir + darunavir/r at virologic failure

33 Keywords: retrovirus, integrase inhibitors, resistance mutations, raltegravir, darunavir

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41 SYNOPSIS (250 words)

42 OBJECTIVES: To describe the pattern of drug resistance at virologic failure (VF) in the

43 NEAT001/ANRS143 trial (first-line treatment with ritonavir-boosted darunavir plus either

44 tenofovir/emtricitabine or raltegravir).

45 METHODS: A genotypic testing was performed at baseline for reverse transcriptase (RT) and

46 protease genes and for RT, protease and integrase (IN) genes for patients with a confirmed

47 viral load (VL) > 50 copies/mL or any single VL > 500 copies/mL at or after week 32.

48 RESULTS: A resistance test was obtained for 110/805 (13.7%) randomised participants

49 qualifying for resistance analysis (61/401 of RAL arm and 49/404 of TDF/FTC arm). No

50 resistance associated mutation (RAM) was observed in the TDF/FTC+DRV/r arm, and all

51 further analyses are limited to the RAL+DRV/r arm. In this group, 15/55 (27.3%) participants

52 had viruses with IN RAM (12 N155H alone, 1 N155H + Q148R, 1 F121Y and 1 Y143C), 2/53

53 (3.8%) with NtRTI RAM (K65R, M41L), and 1/57 (1.8%) with primary protease RAM (L76V).

54 The frequency of IN mutations at failure was significantly associated with baseline VL: 7.1%

55 for VL <100,000 copies/mL, 25.0% for VL ≥100-500,000 copies/mL, and 53.8% for VL

56 ≥500,000 copies/mL (P_{TREND}=0.007). Of note, 4/15 participants with IN RAM had a VL <200

57 copies/mL at time of testing.

58 CONCLUSION: In the NEAT001/ANRS143 trial, there were no RAM at VF in the standard

59 DRV/r+TDF/FTC regimen, contrasting with rate of 29.5% in the DRV/r+RAL NtRTI-sparing

60 regimen (mostly IN mutations). Cumulative risk of IN RAM after 96 weeks follow-up in

61 participants initiating antiretroviral therapy with DRV/r + RAL was 3.9%.

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INTRODUCTION

In Europe, a combination of 2 nucleoside or nucleotide analogue reverse transcriptase inhibitors (NtRTI) and a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI), a ritonavir boosted protease inhibitor (PI) or an integrase strand transfer inhibitor (ISTI) is recommended for initial therapy for HIV-1 infected patients. [1] The tolerability and toxicity profile of NtRTIs in particular the cardiovascular risk with abacavir and bone and renal toxicity with tenofovir has led to the research of NtRTI-sparing alternative antiretroviral combinations. [2-6] NEAT 001/ANRS 143 was an European open-label, non-inferiority, phase III randomised trial that evaluated the efficacy of the NtRTI-sparing regimen raltegravir plus darunavir and ritonavir (RAL+DRV/r) versus a standard of care regimen tenofovir/emtricitabine plus darunavir and ritonavir (TDF/FTC+DRV/r) in treatment-naïve adults. This study showed the non-inferiority of the NtRTI sparing strategy (RAL+DRV/r arm) versus the standard arm but only in participants with baseline CD4 cell counts > 200 cells/mm^3. [7] As described in the main study report, genotypic analysis was done at screening and at all visits from 32 weeks onwards for participants who had HIV-1 RNA ≥ 500 copies/mL. Among participants who underwent genotype testing to assess emerging resistance at the time of virological failure, treatment-emergent resistance was seen in no participants in the standard of care-group and in six (21%) of 29 in the NtRTI sparing group, five of whom had resistance to integrase (IN) and one to NtRTI. [7] While IN-associated resistance frequency and profile are somewhat well characterized with RAL, when used in combination with TDF/FTC, [8,9] there is little information when RAL is combined with DRV/r in a randomised study. Therefore, the objective of the present study, was to describe the full resistance profile at virological failure and to determine factors associated with the development of IN-resistance mutations.

METHODS

Study design

NEAT 001/ANRS 143 was an European open-label, non-inferiority, phase III randomised trial conducted in 15 European countries. Eight hundred five participants were randomised in a 1/1 ratio to receive 400 mg twice daily raltegravir plus 800 mg darunavir and 100 mg ritonavir once daily (n=401) or tenofovir/emtricitabine in a 245 and 200 mg fixed dose combination once daily plus 800 mg of darunavir and 100 mg of ritonavir once daily (n= 404). Eligible individuals had baseline plasma VL > 1000 copies/mL and no evidence of major IAS-USA resistance mutations [10] on genotype testing, historically or at screening. The primary endpoint was the time to virological or clinical failure, with preplanned subgroup analyses of the primary endpoint by baseline CD4 cell count and HIV-1 RNA concentration. Ethics committee approval was obtained from all participating centres, in accordance with the principles of the Declaration of Helsinki. All trial participants gave written informed consent.

Genotypic resistance analyses and interpretation

The criteria for genotypic testing was a confirmed viral load (VL) > 50 copies/mL or any single VL > 500 copies/mL at or after W32. In addition, insufficient virological response was defined as decrease <1 $\log_{10}$ copies per mL in HIV-1 RNA concentration at week 18, or an HIV-1 RNA concentration ≥ 400 copies/per mL at week 24. In this situation of insufficient virological response before week 32, decision to perform genotypic testing and/or change in treatment was optional and left to the clinician.[7] Although protocol-defined virological failure was considered at or after W32, genotypes done before because of insufficient virological response were included in the resistance analysis. In patients with multiple virological

111 failures, we analysed all available resistance tests available, resistance developed on second-

112 line therapy was not considered in the analysis. Bulk sequences of the reverse transcriptase

113 (RT), protease and integrase (IN) genes on RNA were determined using the ANRS consensus

114 technique primer sequences described at http://www.hivfrenchresistance.org . In the main

115 results paper,[7] resistance mutations were interpreted according to the 2009 IAS-USA list of

116 mutations (reference list used at time of inclusion) and in the present study with the 2014

117 IAS-USA version [11]. A genotypic testing at baseline was performed for RT and protease

118 genes, at each site local laboratory and for RT, protease and IN genes at virological failure,

119 mainly in the Pitié-Salpêtrière Virology Laboratory. Only data from participants with a

120 successful genotypic test were available for the analyses. To assess potential factors

121 associated with resistance development in participants treated with DRV/r + RAL, the

122 baseline characteristics of viral load and CD4+ T-cell count were evaluated.

123

124 **Statistical analyses**

125 The Kaplan Meier method was used to estimate cumulative proportion of patients with IN

126 resistance in the NtRTI-sparing strategy, assuming that patients who did not virologically fail

127 did not develop resistance. Chi squared tests, rank sum tests and tests for trend were used

128 to compare characteristics at baseline and failure between participants who developed at

129 least one IN resistance mutation and those who did not.

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RESULTS

Overall , 127 participants (69/401 in the RAL+DRV/r arm and 58/404 in the TDF/FTC+DRV/r arm) met the criteria for genotypic testing with, at or after W32, either a confirmed viral load (VL) > 50 copies/mL or at least one VL > 500 copies/mL. Baseline characteristics of participants are reported in table 1. At least one resistance test was obtained for 110 participants (61 in the RAL+DRV/r arm and 49 in the TDF/FTC+DRV/r arm), although not all tests were successful in all genes. Median (IQR) HIV RNA at time of genotype testing was significantly different in participants who failed between the 2 arms: 373 copies/mL (IQR: 110-1064) in the RAL+DRV/r arm vs 133 copies/ml (IQR: 67-568) in the TDF/FTC+DRV/r arm; p-value=0.02). In the TDF/FTC+DRV/r arm, among the 49 participants who met criteria for genotypic testing and successfully had genotypic resistance test, no major IAS-USA resistance mutations were observed; thus all further analyses are limited to the RAL+DRV/r arm. Of the 61 genotypes tested in the RAL+DRV/r arm, we obtained 55, 53 and 57 sequences for IN, RT and protease gene, respectively. At baseline none had major IAS RT and protease resistance mutations detected by Sanger sequencing. In those with at least one successful genotypic test, 15/55 (27.3%) in the RAL DRV/R arm had viruses with IN resistance mutations (12 N155H alone, 1 N155H + Q148R, 1 F121Y and 1 Y143C), 2/53 (3.8%) with NtRTI resistance mutations (K65R, M41L), and 1/57 (1.8%) with a primary protease mutation (L76V) (Table 2). Three patients presented minor IN resistance mutations (L74M or T97A) that could be interpreted as polymorphisms. The cumulative risk in patients in the DRV/r +RAL to experience virological failure and emergent IN resistance associated mutations was 2.1% (95% CI 1.0-4.1) at week 48 and 3.9% (95% CI 2.4-6.4) at week 96. HIV-1 RNA values at failure were not significantly different in those who failed with or without an IN mutation (median 731 copies/mL (IQR: 192, 14864) *vs.* 351 copies/mL (IQR: 134-904); p=0.17. The

157   proportion of patients in the RAL arm who achieved full virological success when switched to

158   a different regimen (mostly RAL changed to TDF/FTC) was similar in those who switched

159   after failure with resistance (13/15 = 86.7 %) and in those those who switched after failure

160   without resistance (27/34 = 79.4 %). The frequency of IN mutations at failure was

161   significantly associated with baseline VL: 7.1% (1/14) for participants harbouring a baseline

162   VL < 100,000 copies/mL, 25.0% (7/28) for a baseline VL ≥ 100-500,000 copies/mL, and 53.8%

163   (7/13) for a baseline VL ≥ 500,000 copies/mL (P_{TREND}=0.007). Although prespecified subgroup

164   analysis showed that the NtRTI-sparing regimen was inferior to the standard regimen group

165   in patients with baseline CD4 count of <200 cells/ μL there was no statistically significant

166   difference in the proportion of IN resistance between patients with a baseline CD4 count

167   <200 cells/μL compared to those above (36.8 % *vs.* 22.2%, p-value=0.25). [7] Of note, 4/15

168   participants with IN resistance mutations had a VL < 200 copies/mL at the time of testing.

169   Figure 1 shows the time to detection of IN resistance mutations on RAL+DRV/r (based on all

170   participants in this arm), that tended to emerge early (between 19 and 96 weeks).

171

172   DISCUSSION

173   NEAT 001/ANRS 143 was a phase 3 trial of NtRTI sparing regimen which compared an

174   integrase strand transfer inhibitor (raltegravir) to a NtRTI standard backbone

175   (tenofovir/emtricitabine) in first line therapy with a boosted protease inhibitor (darunavir/r).

176   This trial showed that RAL+DRV/r regimen was overall non inferior to standard treatment for

177   antiretroviral-naïve participants, but inferior for those with a CD4 count < 200 cells/μL.

178   Through week 96, a high proportion of participants treated with either regimen had viral

179   load suppression (HIV-1 RNA < 50 copies/mL in 78.6% and 82.2% for NtRTI-sparing group and

180   standard group)[7]. However the NtRTI-sparing regimen RAL+DRV/r was associated with

181   higher rates of virological failure in those with baseline CD4 counts < 200 cells/µL [7] and was

182   associated with selection of resistance mutations at virological failure, especially to IN.

183   Whereas no resistance mutations were found in genotype of participants with virological

184   failure from the standard arm, IN mutation resistance was observed in more than one-

185   quarter of samples at failure in the RAL+DRV/r arm. Our results confirm very well established

186   data on the almost absence of development of protease resistance-associated mutations at

187   virological failure in patients on a first-line ritonavir boosted protease inhibitor combined

188   with 2 NtRTI, [12,13] while such resistance mutations is more likely when a ritonavir-boosted

189   protease inhibitor is combined with NNRTI, [14] or, to a lesser extent, with integrase strand

190   transfer inhibitor. [15] These data suggest a mutual bidirectional protection of NtRTI and PI/r

191   when combined with regards to resistance selection, [16] as illustrated by the total absence of

192   selection of reverse transcriptase or protease resistance-associated mutations in the 49

193   virological failures on ritonavir-boosted darunavir + tenofovir/emtricitabine.

194   However we cannot exclude that resistance mutations are selected outside the protease

195   gene such as gag-pol cleavage sites and gp41[16–18] and this question should be examined in

196   future studies. In NEAT 001, the cumulative risk of integrase resistance at virological failure

197   in patients treated with DRV/r + RAL at W48 was 2.1%, which is higher than the ~~cumulative~~

198   risk of resistance development reported in other studies with raltegravir +

199   tenofovir/emtricitabine given as first-line therapy, ranging from 0.2% [19] to 1.4% [20] at W48.

200   Such higher rate of integrase resistance has been reported in previous studies of raltegravir

201   + ritonavir-boosted protease inhibitor. In the Spartan study, a randomised controlled

202   multicentre pilot study in 94 naïve HIV infected participants received atazanavir plus RAL or

203   ritonavir-boosted atazanavir plus TDF/FTC. After 24 weeks of follow-up, 4 (6.3%) participants

204   in the NRTI-sparing arm failed with development of IN resistance mutations, while no

205   resistance mutations were observed in the control arm. Three of the 4 participants with

206   resistance at failure had baseline HIV-1 RNA > 500,000 copies/mL.[21]

207   In the PROGRESS pilot study, comparing the NtRTI sparing regimen of lopinavir/r plus RAL

208   with the standard of care regimen of lopinavir/r plus TDF/FTC in naïve HIV infected patients,

209   8 subjects in the LPV/r+RAL failed, 3 of them with IN resistance mutations (3.7%). One of

210   them had also an emergent major protease mutation; conversely, in the TDF/FTC arm only

211   1/5 patients who failed had a M184V mutation. [15] Whether these differences are related to

212   the different backbones, 2 NtRTI or ritonavir-boosted protease, in combination with

213   raltegravir, or to differences in resistance testing and analysis is unknown. One could

214   hypothesize that, similarly to what is observed with PI/r therapy, TDF/FTC confers some

215   protection to the risk of resistance emergence at virological failure with raltegravir therapy.

216   The mechanism of this NtRTI protection could be an undiscovered molecular interaction

217   within the HIV replication cycle or more probably a consequence of the very long half-life of

218   intracellular tenofovir and emtricitabine, providing forgiveness to the great variability of

219   raltegravir exposure. On the contrary, despite its high genetic barrier to resistance,

220   darunavir/ritonavir, with relative short half-life, might confer less forgiveness to raltegravir,

221   especially in situations of partial or intermittent non-adherence. Further analyses will assess

222   adherence and raltegravir plasma concentrations in NEAT 001 to elucidate reasons for the

223   high rate of resistance emergence, especially in patients with high baseline viral load. On the

224   other hand, differences in assays used for resistance testing in the various studies should be

225   considered, and more importantly, different timepoints of analysis (first of confirmed

226   virological failure sample) and level of viral load at the time of genotyping, which might

227   greatly influence genotype results. [22] This renders cross study comparisons hazardous with

228 regards to the prevalence of resistance at virological failure. Indeed, in NEAT 001, resistance

229 analysis population differed from those of previous studies of raltegravir +

230 tenofovir/emtricitabine,[19,20] or of a pilot uncontrolled study of raltegravir +

231 darunavir/ritonavir. [23] In the latter study, ACTG 5262, rate of integrase resistance at virologic

232 failure was 4.5%; 5 out of 25 patients with virological failure and genotype testing had

233 integrase resistance mutations at virological failure and a baseline viral load > 100,000

234 copies/ml. In NEAT 001, the proportion of participants in the DRV/r + RAL group with

235 baseline viral load > 100,000 copies/ml who experienced virological failure and emergent

236 integrase resistance-associated mutations was 9.6% versus 10.4% in ACTG 5262.[23] Initiating

237 antiretroviral therapy with the combination of ritonavir-boosted darunavir + raltegravir in

238 patients with high baseline viral load is associated with an unacceptable high risk of

239 raltegravir resistance on treatment, particularly in those with HIV-1 RNA > 500,000

240 copies/ml; 27.3% developed resistance on treatment in our study. The main selected IN

241 mutation in our study was the N155H raltegravir signature mutation alone, so most viruses

242 at virological failure remained, in theory, susceptible to dolutegravir, except for the one

243 harbouring the F121Y mutation which confers phenotypic resistance to dolutegravir as

244 well.[24]The uncontrolled pilot VIKING 3 study have shown the efficacy of dolutegravir twice a

245 day on raltegravir failure with the mutation N155H alone. [25] However, great caution and

246 more clinical studies are needed, as recent data suggest that dolutegravir might also select

247 for N155H and that viruses harbouring such mutation might have diminished susceptibility

248 to dolutegravir when used once daily. [26] One limitation of our study is the absence of

249 genotypic information, due to either absence of available sample or failure to obtain

250 sequence in 12% of participants qualifying for resistance testing in the RAL + DRV/r arm. This

251 proportion was 16% in the TDF/FTC + DRV/r arm. Another limitation of our study is that the

252 protocol did not ask for IN gene sequence at baseline, as at the time of recruitment there

253 was little clinical use of integrase inhibitors and a risk of transmitted drug resistance was

254 very low for the integrase class (1.7% for IN resistance mutation in the PRIMO cohort of

255 recently infected patients).[27] Although we cannot formally exclude that some participants

256 might have had IN resistance pre-existing to initiation of therapy, this is highly unlikely, as

257 N155H mutation confers high level phenotypic resistance to raltegravir and in such

258 circumstance, virological failure would have occurred much more rapidly, without the early

259 virological suppression seen in 9/13 patients with N155H mutation. Although none of the RT

260 (n = 2) and protease (n = 1) resistance mutations evidenced at failure were detected at

261 baseline using Sanger sequencing, ultradeep sequencing on those baseline samples could

262 help to determine if these emergent RT (M41L, K65R) and protease (L76V) mutations are

263 due to selection or re-emergence of transmitted minority resistant variants. Of clinical

264 relevance, IN resistance was seen in patients (4/15) with very low-level viremia (HIV RNA

265 between 50 and 200 copies/mL), a phenomenon already described in the ACTG 5262 study.

266 [23] In another study on risk factors for raltegravir resistance development in clinical practice,

267 we showed that 7.7 % (6/78) of patients with HIV RNA between 50 and 200 copies/mL had

268 IN resistance mutations. [28] Thus, viral rebound with 2 consecutive HIV RNA values > 50

269 copies should be considered as definite virological failure in patients receiving DRV/r + RAL,

270 and genotypic resistance testing should be performed without delay in these patients

271   In summary, during 96-weeks of follow up, resistance to IN was detected in 15/401

272 participants randomised to DRV/r+RAL (3.7%). One quarter (27%) of samples at failure had

273 IN resistance mutations, with risk of resistance related to baseline HIV RNA. Most patients

274 with resistance mutations achieved complete suppression when switched to other regimens,

275 most often TDF/FTC instead of RAL, with continuation of ritonavir-boosted darunavir.

276 It would be interesting to investigate other NtRTI sparing strategies combining an ISTI with a

277 higher genetic barrier to resistance and a longer half-life such as dolutegravir , in

278 combination with a boosted-protease inhibitor. Based on these results on resistance,

279 initiation of antiretroviral therapy with the alternative regimen of ritonavir-boosted

280 darunavir and raltegravir in patients with CD4 > 200/µL should be limited to patients with

281 HIV RNA < 500,000 copies/ml, and discussed in patients with HIV RNA between 100,000 and

282 500,000 copies/ml.

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- **Spain**: Juan Berenguer and Santiago Moreno (Madrid)

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461   Sidonie Lambert, Anne-Geneviève Marcelin, Carlo Federico Perno, Deenan Pillay, Ellen White
462   (statistician)

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468   Rodolphe Thiébaut (statistician)

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481   Francesco Castelli (coordinator), Marco Braggion (statistician), Emanuele Focà

482   *Asterisk (*) indicates staff who left during the trial.*

483

484

485   **Transparency declarations**

486   FR has received honoraria for advisories or invited talks or conferences and research grants
487   from Abbvie Labs, Bristol-Myers Squibb, Gilead Sciences, Merck Laboratories, MSD, Janssen
488   Pharmaceuticals and ViiV healthcare.

489   AP (A Pozniak) has been an advisor and invited speaker and received honoraria, research
490   grant, travel and education from Abbvie, GSK, VIIV, BMS, Gilead Sciences, Janssen
491   Pharmaceuticals, Merck & Company, Tobira.

512

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601   Table 1 : Baseline characteristics of patients meeting criteria for genotypic testing

	RAL+DRV/r (n=69)	TDF+FTC+DRV/r (n=58)
Sex		
Male	65 (94%)	50 (86%)
Median (IQR) age (years)	37 (32-44)	39 (31-52)
Ethnic origin		
White	53 (77%)	45 (78%)
Black	12 (17%)	10 (17%)
Asian	1 (1%)	2 (3%)
Other	3 (4%)	1 (2%)
Mode of HIV infection		
Homosexual/bisexual sex	40 (63%)	32 (58%)
Heterosexual	23 (37%)	20 (36%)
IVDU	0	2 (4%)
Other	0	1 (2%)
HIV CDC clinical stage		
A	54 (78%)	47 (81%)
B	10 (15%)	7 (12%)
C	5 (7%)	4 (7%)
Median (IQR) CD4 cell count (cells per μL)	295 (150-378)	316 (205-379)
CD4 cell count category (cells per μL)		
<50	3 (4%)	3 (5%)
50-199	19 (28%)	11 (19%)
200-349	24 (35%)	21 (36%)
350-499	20 (29%)	22 (38%)

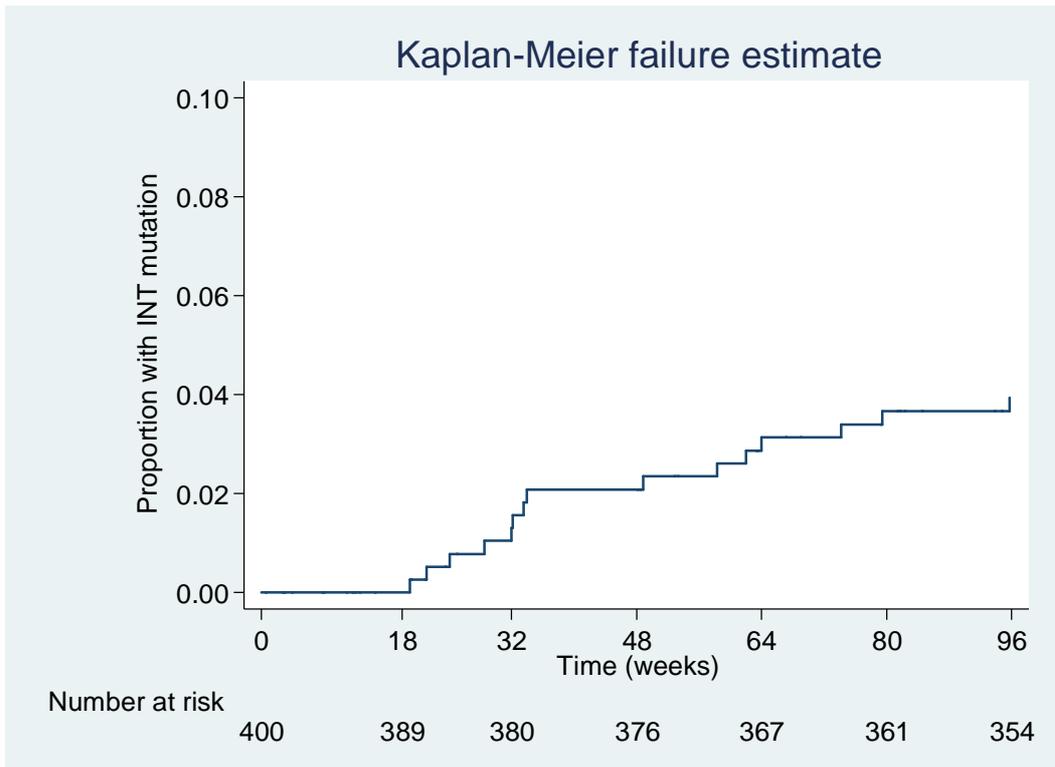|		
≥500	3 (4%)	1 (2%)
Median (IQR) HIV-1 RNA concentration at baseline (log$_{10}$ copies per mL)	5.25 (4.85-5.58)	5.19 (4.80-5.54)
Baseline HIV-1 RNA category		
≥100 000 copies per mL	49 (71%)	36 (62%)
≥500 000 copies per mL	14 (20%)	9 (16%)
HCV co-infection	3 (4%)	4 (7%)

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607   Figure 1: time to detection of IN resistance mutations, RAL + DRV/r arm, NEAT 001/ANRS 143

608   trial

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619 Table 2 : Resistance mutations in the RAL+DRV/r arm

Patients	Genotypic testing		RT	PROT	IN	Subsequent regimen	VL at W96 (copies/mL)	Suppressed before resistance test
	Time	VL (copies/mL)						
1	W47	247		L76V		RAL+DRV/r	<50	Y
2	W38	340	M41L			TDF/FTC+EFV then TDF/FTC+DRV/r	<50	N
3	W65	3800	K65R			TDF/FTC+DRV/r	<50	Y
4	W24	64041			N155H	TDF/FTC+DRV/r	107	N
5	W58	60			Y143C	TDF/FTC+DRV/r	90	Y
6	W32	85			N155H	TDF/FTC+DRV/r	<50	N
7	W34	148			N155H	No treatment after W67	227185	Y
8	W64	192			N155H	TDF/FTC+DRV/r	68	Y

9	W62	406	N155H	TDF/FTC+DRV/r	<50	Y
10	W29	442	N155H +Q148R	Missing data	Missing data	Y
11	W49	498	N155H	RAL+DRV/r	<50	Y
12	W79	731	N155H	ABC/3TC+DRV/r	<50	Y
13	W32	1311	N155H	TDF/FTC+DRV/r	<50	Y
14	W34	1900	N155H	TDF/FTC+ETR	<50	Y
15	W21	14864	N155H	TDF/FTC+EFV	<50	N
16	W19	52857	N155H	AZT/3TC+DRV/r+NVP	<50	N
17	W74	129000	N155H	RAL+DRV/r	50	Y
18	W96	1470	F121Y	RAL+DRV/r	1470	Y

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621 RT : reverse transcriptase ; PROT : protease ; IN ; integrase ; VL : viral load ; W96 : 96 week ;
622 copies/mL : copies/mililiter ; Y : yes ; N : No ; RAI : raltegravir ; DRV/r : darunavir/ritonavir ;
623 TDF : tenofovir ; EFV : efavirenz ; FTC : emtricitabine ; ABC/3TC : abacavir/lamivudine ; ETR :
624 etravirine ; NVP : nevirapine ; RPV : rilpivirine