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## Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination

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### ► To cite this version:

S. Lambert-Niclot, C. Allavena, M. Grude, P. Flandre, S. Sayon, et al.. Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination. *Journal of Antimicrobial Chemotherapy*, 2016, 10.1093/jac/dkw146 . hal-01332106

**HAL Id: hal-01332106**

**<https://hal.sorbonne-universite.fr/hal-01332106>**

Submitted on 15 Jun 2016

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1 Usefulness of an HIV DNA Resistance Genotypic Test in Patients Who Are Candidates  
2 for a Switch to the rilpivirine/emtricitabine/tenofovir Combination

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16 Keywords :antiretroviral resistance, DNA genotype, rilpivirine

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18 Running title: Switch to the RPV/TDF/FTC and DNA genotype

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25  
26 Abstract

27 **BACKGROUND:** In the context of a rilpivirine/emtricitabine/tenofovir switch in HIV-1  
28 infected patients with at least one year of virologic success, we determined whether proviral  
29 DNA is an alternative to plasma HIV-RNA for resistance genotyping.

30 **METHODS:** Resistance associated mutations (RAM) in DNA after at least one year of virologic  
31 success (viral load (VL) <50 copies/mL) were compared with those identified in the last  
32 plasma RNA genotype available. Rilpivirine/emtricitabine/tenofovir RAM studied were:  
33 K65R, L100I, K101E/P, E138A/G/K/R/Q, V179L, Y181C/I/V, M184V/I, Y188L, H221Y, F227C  
34 and M230I/L in reverse transcriptase. We studied patients without virologic failure (VF) and  
35 with  $\geq 1$  VF (two consecutive VL>50 copies/mL). Kappa's coefficient was used to measure  
36 agreement between the DNA and RNA genotypes.

37 **RESULTS:** In patients without (n=130) and with VF (n=114), RNA and DNA showed resistance  
38 to at least one drug of the rilpivirine/emtricitabine/tenofovir combination in 8% and 9% and  
39 in 60% and 45%, respectively. For rilpivirine RAM, correlation between RNA and DNA was  
40 higher in patients without VF than in patients with VF (kappa= 0.60 versus 0.19, p=0.026).  
41 Overall, prevalence of RAM was lower in DNA than in RNA.

42 **CONCLUSION:** The incomplete information provided by DNA genotypic test is more notable  
43 in patients with VF, suggesting that all resistance mutations associated with prior VF have  
44 not been archived in the proviral DNA or decreased to a level below threshold of detection.  
45 In the case where no historical plasma genotypic test is available, DNA testing might be  
46 useful to rule out switching to rilpivirine/emtricitabine/tenofovir.

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## 51 INTRODUCTION

52

53 The second generation NNRTI rilpivirine (RPV) formulated in a single tablet regimen (STR)  
54 with tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) was approved by the  
55 European Medicines Agency and the US Food and Drug Administration as a once-daily oral  
56 treatment for adults infected with HIV-1 without mutations associated with resistance to  
57 TDF, FTC, or the NNRTI class, and harboring a viral load (VL)  $\leq 100\ 000$  HIV-1 RNA copies/mL.  
58 Current antiretroviral treatment guidelines recommend switching therapy in virologically  
59 suppressed patients to improve adherence or tolerability or to allow for treatment  
60 simplification<sup>1-3</sup>. The SPIRIT study showed maintenance of virologic suppression at W48 for  
61 89.3% of patients switching to rilpivirine/emtricitabine/tenofovir (RPV/FTC/TDF) from a  
62 ritonavir-boosted protease inhibitor (PI/r)-based regimen, compared with those who  
63 continued treatment with a PI/r regimen with a low risk of virologic failure (VF)<sup>4</sup>. A study  
64 demonstrated switching from efavirenz (EFV/FTC/TDF to RPV/FTC/TDF) was safe and  
65 effective for virologically suppressed HIV-infected patients with EFV intolerance<sup>5</sup>. Cohort  
66 studies have also shown efficacy and tolerability of switching to RPV/FTC/TDF<sup>6</sup>. Thus

67 RPV/FTC/TDF is considered as an appropriate therapy for switch for simplification in  
68 virologically suppressed HIV infected patient.

69 In this context, the use of previous plasma resistance genotypes was recommended to  
70 determine the susceptibility to this combination <sup>3</sup> because of the possible presence of pre-  
71 existing drug resistance mutations leading to VF. Studies have shown that resistance testing  
72 performed on HIV DNA lacks sensitivity compared with accumulated drug resistances from  
73 previous plasma genotypes.<sup>7,8</sup> However in patients fully virologically suppressed, the  
74 previous plasma genotypic test could be unavailable.

75 In the perspective of a switch to the combination RPV/TDF/FTC, the aim of this study was to  
76 determine whether proviral DNA is a potential relevant alternative to HIV-RNA for resistance  
77 genotyping in HIV-1 infected treated patients with at least one year of virologic suppression.

78

## 79 MATERIAL and METHODS

80 In 244 HIV-1 infected patients treated in 2 centers (Nantes University Hospital and Pitié-  
81 Salpêtrière Hospital) with a prior available RNA resistance test, we retrospectively analyzed  
82 HIV DNA resistance genotype generated in PBMC after at least one year of virologic success  
83 (VL <50 copies/mL). Bulk sequences of the reverse transcriptase (RT) on RNA and DNA were  
84 determined using the ANRS consensus technique primer sequences described at  
85 <http://www.hivfrenchresistance.org>. We compared prevalence of HIV resistance mutations in  
86 the DNA and RNA genotype generated from the last detectable VL. We studied 2 groups of  
87 patients: 130 patients without previous VF (pre-therapeutic plasma genotype) and 114  
88 patients with at least one previous VF (genotype on the more recent detectable VL)

89 regardless of the treatment they received (VF was defined as two consecutive VL>50  
90 copies/mL).

91 RPV/TDF/FTC combination resistance mutations studied were defined according to the IAS  
92 list (K65R, K101E/P, E138A/G/K/R/Q, V179L, Y181C/I/V, M184V/I, Y188L, H221Y, F227C and  
93 M230I/L) in RT<sup>9</sup>. Resistance to RPV was defined according the ANRS  
94 <http://www.hivfrenchresistance.org/>. Kappa's coefficient was used to measure agreement  
95 between the DNA and RNA genotypes with values <0, 0.-0.2, 0.21-0.4, 0.41-0.6, 0.61-0.8, and  
96 0.81-1.00 indicating no, very low, low, middle, high, and very high concordance,  
97 respectively.

98 We studied factors associated to the correlation between DNA and RNA mutations: VL, CD4  
99 number at the time of both genotypes, number of treatment lines, number of previous VF  
100 and mean time between last plasma RNA genotype and proviral DNA genotype.

101

## 102 RESULTS

103 We collected 244 pairs of DNA and RNA sequences for the RT gene (130 for patients without  
104 VF and 114 for patients with at least one VF). Characteristics of patients are presented in  
105 table 1. The mean time between last plasma RNA genotype and proviral DNA genotype was  
106 46 and 37 months for patients without VF and with  $\geq 1$  VF, respectively. There are significant  
107 differences between the 2 groups for VL RNA ( $p \leq 0.0001$ ), numbers of lines of treatment  
108 ( $p \leq 0.0001$ ) and time between RNA and DNA genotypes ( $p = 0.0025$ ).

109 In patients without VF, the prevalence of studied resistance associated mutation (RAM) was  
110 very low in both RNA and DNA RT sequences (Figure 1a), with resistance to at least one drug  
111 of the RPV/FTC/FTC combination in 8.0% and 9.0%, respectively.

112 In patients with at least one prior VF, the prevalence of at least one RPV RAM was 24.6% and  
113 18.4 % in RNA and DNA genotype, respectively. The most prevalent RPV RAMs were  
114 Y181C/I/V (12.3%) and E138A/G/K/Q/R (7.8%) in RNA genotype; and in DNA,  
115 E138A/G/K/Q/R (7.9%) and M230I/L (6.1%) (Figure1b). Resistance to FTC and TDF was  
116 detected in 52.6% (M184V/I) and 0.9% (K65R) in RNA genotype and 37.7% (M184V/I) and  
117 0.9% (K65R) in DNA genotype. Resistance to at least one drug of the RPV/FTC/FTC  
118 combination was 60.0% in RNA and 45.0% in DNA.

119 In patients without VF, concordance between resistance in RNA and DNA was not  
120 significantly higher than in patients with VF (kappa= 0.57 versus 0.43 respectively,  $p=0.36$ ).

121 For RPV RAM, correlation between RNA and DNA was significantly higher in patients without  
122 VF than in patients with at least one VF (kappa= 0.60 versus 0.19 respectively,  $p=0.026$ ).

123 Overall, prevalence of rilpivirine associated mutations was lower in DNA than in RNA  
124 genotypic test, except for mutations at positions E138 and M230 that are APOBEC driven  
125 mutations (G to A) (Figure 1b). The factors associated with a good correlation between  
126 resistance according RNA and DNA genotype were a higher VL at RNA genotype ( $p=0.0124$ ),  
127 a shorter mean time between last plasma RNA genotype and proviral DNA genotype  
128 ( $p=0.0468$ ) and a higher number of treatment lines ( $p=0.0006$ ). Number of VF, subtypes, CD4  
129 at the time of RNA genotype or DNA genotype were not associated with correlation of  
130 resistance in RNA and DNA.

131

132 DISCUSSION

133 In the context of switch to RPV/TDF/FTC therapy in HIV-1 infected treated patients, this  
134 study shows a good concordance between DNA and RNA genotypes in patients without prior  
135 VF and who are successfully suppressed for at least one year. However, DNA genotype is less  
136 informative than RNA genotype in patients with at least one prior VF. The good concordance  
137 in patients with no prior VF is in accordance with results of studies on naive patients showing  
138 that DNA genotype could be useful and even more informative than standard RNA  
139 genotyping<sup>10,11</sup> .

140 In patients with at least one prior VF, the rate of selected resistance associated mutations to  
141 RPV in RNA was somewhat lower than in previous studies conducted in patients pre-exposed  
142 to an NNRTI-based regimen but naive to RPV<sup>12-14</sup> . Indeed, in our study the 3 main RPV  
143 RAMs at codons 181, 101 and 138 had a frequency of 12.3, 2.6 and 5.3% versus 18 to 22.6%,  
144 7 to 20.5% and 5.3 to 14% in these 3 studies, respectively. This lower frequency could be due  
145 to the fact that our studied population not exclusively contained NNRTI failing patients,  
146 nevertheless the 3 mains RPV RAM are similar to those observed in others studies. In the HIV  
147 DNA, we showed a lower prevalence of the RPV RAM: 101, 181 and 221 with 0.9%, 3.5% and  
148 0.9% versus 7%, 18% and 4% in the study of Gallien et al<sup>12</sup> . However, patients of this study  
149 were selected to have a prior VF especially to NVP or EFV and had a prior history of triple  
150 class failure.

151 In patients with prior VF, our study generally confirmed results of previous studies on the  
152 discordance between DNA and RNA genotypes. Indeed, in a large number of patients with  
153 undetectable or low VL under treatment, a study showed a concordance between DNA and  
154 RNA of 26.3% for NNRTI mutations<sup>7</sup>. Another study demonstrated that mutations conferring

155 resistance to at least one antiretroviral drug were detected exclusively by RNA genotyping or  
156 exclusively by DNA genotyping in 47% and 1% of patients for NNRTIs, respectively<sup>8</sup>.

157 Overall, prevalence of RAM was generally lower in DNA than in RNA genotypic tests, except  
158 for mutations at positions E138 and M230 that are APOBEC driven mutations. Indeed,  
159 APOBEC induces G to A viral mutation and this mechanism could explain the persistence of  
160 mutations in archived cellular proviral DNA<sup>15</sup>.

161 The incomplete information provided by the DNA test is more notable in patients with at  
162 least one prior VF, suggesting that all resistance mutations associated with the prior VF may  
163 not have been archived in the proviral DNA or not detected with classical Sanger sequencing.  
164 A good correlation between prior RNA genotype and current DNA genotype was significantly  
165 associated with a higher VL at RNA genotype and a shorter mean time between last plasma  
166 RNA and proviral DNA genotype. This suggest that DNA genotypic testing, with current  
167 techniques, might be suboptimal in case of low intracellular VL of resistant viruses and that  
168 archived resistant viruses might decrease over time. Further studies would be warranted to  
169 explore the interest of Ultra Deep Sequencing on DNA and the clinical relevance of minority  
170 variants.

171 From a clinical perspective, we recommend that before switching to RPV/TDF/FTC, one takes  
172 into consideration full treatment history and available past plasma genotypic testing, and in  
173 the absence of prior plasma genotype, avoid use of RPV/TDF/FTC if RAM to this combination  
174 are detected using a DNA genotype in a virologically suppressed patient.

175

176 **Acknowledgements**



177 We thank G. Le Mallier and P. Grange for their technical assistance.

## 178 **Funding section**

179 This work was supported by the Agence Nationale de Recherches sur le SIDA (ANRS).

## 180 **Transparency declarations**

181 F. R. has received honoraria for advisories or invited talks or conferences and research grants  
182 from Abbvie Labs, Bristol-Myers Squibb, Gilead Sciences, Merck Laboratories, MSD, Janssen  
183 Pharmaceuticals and ViiV Healthcare. A. G. M. has received honoraria for advisories or  
184 invited talks or conferences and research grants from Abbvie Labs, Bristol-Myers Squibb,  
185 Gilead Sciences, Merck Laboratories, MSD, Janssen Pharmaceuticals and ViiV Healthcare. V.  
186 C. has received honoraria for advisories or invited talks or conferences and research grants  
187 from Abbvie Labs, Bristol-Myers Squibb, Gilead Sciences, Merck Laboratories, MSD, Janssen  
188 Pharmaceuticals and ViiV Healthcare. The other authors have none to declare.

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## 191 REFERENCES

192 1. Guidelines-7.1-english.pdf. Available at: [http://www.eacsociety.org/files/guidelines-7.1-](http://www.eacsociety.org/files/guidelines-7.1-english.pdf)  
193 [english.pdf](http://www.eacsociety.org/files/guidelines-7.1-english.pdf).

194 2. HIV/AIDS Guidelines - adultandadolescentgl.pdf. Available at:  
195 <https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf>.

196 3. Rapport\_Morlat\_2013\_Mise\_en\_ligne.pdf. Available at:  
197 [http://www.sante.gouv.fr/IMG/pdf/Rapport\\_Morlat\\_2013\\_Mise\\_en\\_ligne.pdf](http://www.sante.gouv.fr/IMG/pdf/Rapport_Morlat_2013_Mise_en_ligne.pdf).

198 4. Palella FJ, Fisher M, Tebas P, *et al*. Simplification to rilpivirine/emtricitabine/tenofovir  
199 disoproxil fumarate from ritonavir-boosted protease inhibitor antiretroviral therapy in a  
200 randomized trial of HIV-1 RNA-suppressed participants. *AIDS Lond Engl* 2014; **28**: 335–44.

- 201 5. Mills AM, Cohen C, Dejesus E, *et al.* Efficacy and safety 48 weeks after switching from  
202 efavirenz to rilpivirine using emtricitabine/tenofovir disoproxil fumarate-based single-tablet  
203 regimens. *HIV Clin Trials* 2013; **14**: 216–23.
- 204 6. Cazanave C, Reigadas S, Mazubert C, *et al.* Switch to Rilpivirine/Emtricitabine/Tenofovir  
205 Single-Tablet Regimen of Human Immunodeficiency Virus-1 RNA-Suppressed Patients,  
206 Agence Nationale de Recherches sur le SIDA et les Hépatites Virales CO3 Aquitaine Cohort,  
207 2012–2014. *Open Forum Infect Dis* 2015
- 208 7. Wirden M, Soulie C, Valantin M-A, *et al.* Historical HIV-RNA resistance test results are  
209 more informative than proviral DNA genotyping in cases of suppressed or residual viraemia.  
210 *J Antimicrob Chemother* 2011; **66**: 709–12.
- 211 8. Delaugerre C, Braun J, Charreau I, *et al.* Comparison of resistance mutation patterns in  
212 historical plasma HIV RNA genotypes with those in current proviral HIV DNA genotypes  
213 among extensively treated patients with suppressed replication. *HIV Med* 2012; **13**: 517–25.
- 214 9. Complera\_pi.pdf. Available at:  
215 [http://www.gilead.com/~media/files/pdfs/medicines/hiv/complera/complera\\_pi.pdf?la=en](http://www.gilead.com/~media/files/pdfs/medicines/hiv/complera/complera_pi.pdf?la=en)
- 216 10. Ellis GM, Page LC, Burman BE, Buskin S, Frenkel LM. Increased detection of HIV-1 drug  
217 resistance at time of diagnosis by testing viral DNA with a sensitive assay. *J Acquir Immune*  
218 *Defic Syndr* 1999 2009; **51**: 283–9.
- 219 11. Kabamba-Mukadi B, Duquenne A, Henrivaux P, *et al.* HIV-1 proviral resistance mutations:  
220 usefulness in clinical practice. *HIV Med* 2010; **11**: 483–92.
- 221 12. Gallien S, Charreau I, Nere ML, *et al.* Archived HIV-1 DNA resistance mutations to  
222 rilpivirine and etravirine in successfully treated HIV-1-infected individuals pre-exposed to  
223 efavirenz or nevirapine. *J Antimicrob Chemother* 2015; **70**: 562–5.
- 224 13. Anta L, Llibre JM, Poveda E, *et al.* Rilpivirine resistance mutations in HIV patients failing  
225 non-nucleoside reverse transcriptase inhibitor-based therapies. *AIDS Lond Engl* 2013; **27**:  
226 81–5.
- 227 14. Lambert-Niclot S, Charpentier C, Storto A, *et al.* Rilpivirine, emtricitabine and tenofovir  
228 resistance in HIV-1-infected rilpivirine-naïve patients failing antiretroviral therapy. *J*  
229 *Antimicrob Chemother* 2014; **69**: 1086–9.
- 230 15. Mulder LCF, Harari A, Simon V. Cytidine deamination induced HIV-1 drug resistance. *Proc*  
231 *Natl Acad Sci U S A* 2008; **105**: 5501–6.

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234

235 **Table 1:** Characteristics of patients according the 2 groups: without previous virologic failure  
 236 (VF) and patients with  $\geq 1$  VF. \*corresponding to pretreatment VL for patients without  
 237 previous VF and VL at failure for patients with at least one VF

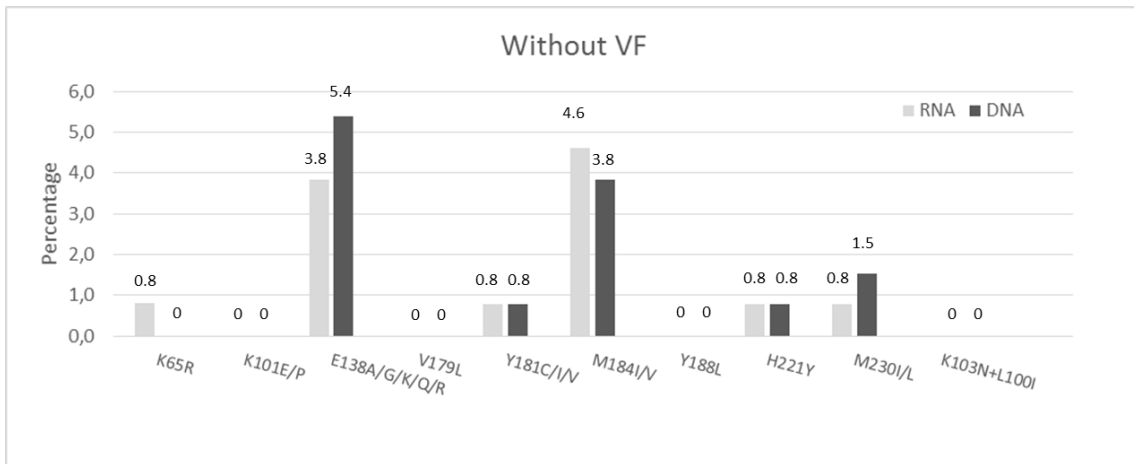
	<b>Without VF N=130 Median (standard deviation)</b>	<b>With at least one VF N=114 Median (standard deviation)</b>
<b>VL RNA log copies/mL*</b>	4.5 (1.2)	3.0 (1.1)
<b>CD4 at time of RNA genotype Number of cells/mm3</b>	347 (230.2)	358 (251.6)
<b>CD4 at time of DNA genotype Number of cells/mm3</b>	586 (311.6)	537 (338.2)
<b>Time between RNA and DNA genotypes (month)</b>	43 (31)	29.5 (36.8)
<b>Number of lines of treatment</b>	3 (3.3)	12 (7.5)

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239

240 Figure 1 : Prevalence of resistance mutations to RPV/TDF/FTC in RNA and in DNA : a) in patients  
 241 without previous virologic failure. b) in patients with at least one virologic failure.\* correspond to  
 242 statistical significant difference of prevalence of resistance mutations between DNA and RNA  
 243 ( $p < 0.05$ )

244 a)



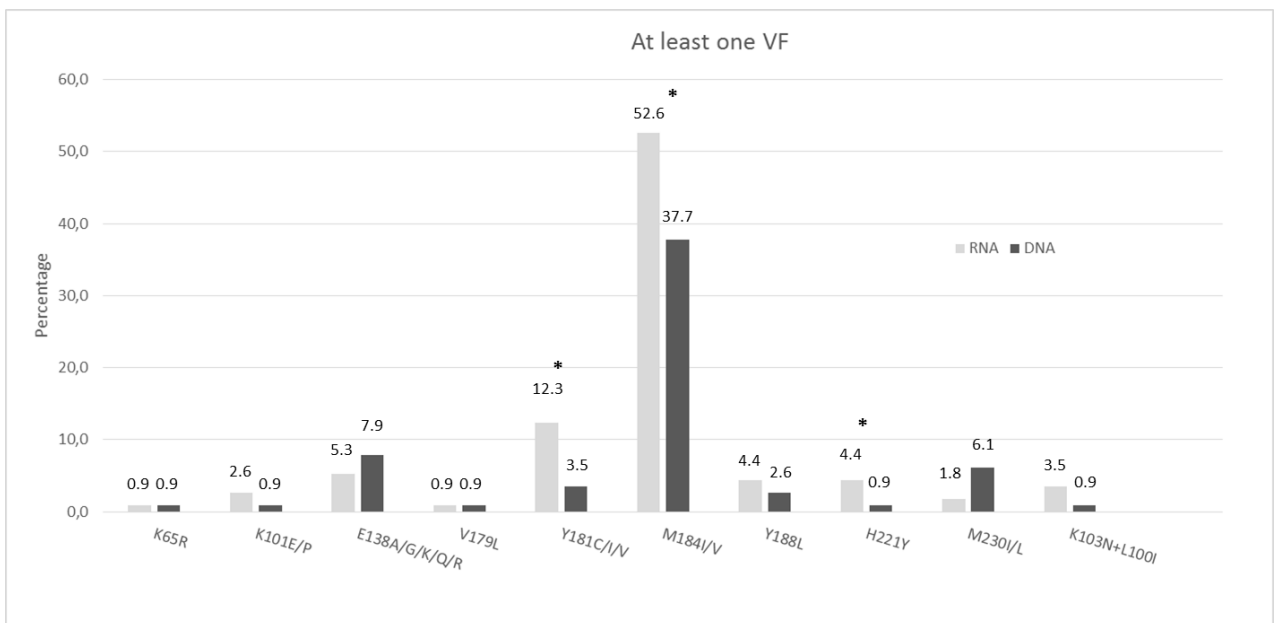
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249 b)



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