Disparities in HIV-1 transmitted drug resistance detected by ultradeep sequencing between men who have sex with men and heterosexual populations

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Running head: HIV-1Transmitted Drug Resistance

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23   **Keywords:** HIV; Deep Sequencing; Minority resistant variants; Transmitted Drug Resistance.

24

25 **Abstract**

26 **Objectives**

27 Transmitted Drug Resistance (TDR) can impair first-line antiretroviral therapy response. It

28 has been shown by Sanger sequencing that TDR was more common among Men having Sex

29 with Men (MSM) in treatment-naive patients chronically infected with HIV type 1 (HIV-1).

30 We aimed to compare the presence of TDR mutations between two groups of HIV-1

31 transmission.

32 **Methods**

33 We studied, by Sanger Sequencing and UltraDeep Sequencing (UDS), the presence of

34 resistance mutations, both in majority (>20%) and in minority (1-20%) proportions, among

35 70 treatment-naive MSM and 70 treatment-naive heterosexual patients recently screened

36 positive for HIV-1.

37 **Results**

38 Between the two groups, global prevalence of TDR was not significantly different either by

39 Sanger or by UDS. Nevertheless, a higher frequency of nucleoside reverse transcriptase

40 inhibitors-TDR was observed among heterosexual patients (p=0.04). There was also a trend

41 for a higher frequency of TDR among MSM infected with HIV-1 subtype B compared to MSM

42 infected with HIV-1 subtype non-B (p=0.06).

43 **Conclusions**

44 UDS allows sensitive monitoring of TDR, and highlights some disparities between groups of

45 transmission.

46

47

INTRODUCTION

Transmitted Drug Resistance (TDR) detection can impair first-line antiretroviral therapy response. Indeed, the presence of TDR-mutations can conduct to a higher risk of virological failure if the affected drugs are introduced [1]. The surveillance of HIV type 1 (HIV-1) TDR is widely recommended and it has been shown that TDR was more common among Men having Sex with Men (MSM) and among patients infected with subtype B virus in the 2010/2011 French survey study conducted in antiretroviral-naïve chronically HIV-1-infected patients by standard sequencing techniques [2]. Standard sequencing detects viral populations accounting for more than 15-20% of viral population. However, HIV-1 minority resistant variants can be a source of virological failure: it was mainly shown for first line regimens based on first generation Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [3,4]. The next generation sequencing technologies are able to detect these minority variants. Nevertheless, few data are available on presence of minority TDR variants in different groups of HIV transmission, treatment-naive patients [5]. The use of UltraDeep Sequencing (UDS) could evidence larger difference of TDR between groups of transmission than Sanger sequencing does.

The aim of the study was to compare the presence of TDR mutations, both in majority (>20%) and in minority (1-20%) proportions, between treatment-naive MSM and treatment-naive heterosexual HIV-1 chronically infected patients.

METHODS

Patients. The study enrolled 70 treatment-naïve heterosexual patients and 70 treatment-naïve MSM recently diagnosed for HIV-1. We performed Sanger Sequencing (n=140; Reverse Transcriptase gene (RT) and Protease gene (PR)) and UDS (n=70 RT and n=70 PR sequences among MSM group; n=54 RT and n=67 PR sequences among heterosexual group). Patients

72 were followed by Department of Infectious Diseases of Pitié-Salpêtrière and Bichat Claude

73 Bernard hospitals (Paris, France). Informations were obtained from the existing electronic

74 database or medical record. Patients were informed that their demographic and clinical data

75 will be recorded during their follow up and could be used for retrospective studies and gave

76 their consent. This study was approved by the Agence Nationale de Recherches sur le SIDA

77 et les hépatites virales (ANRS) AC11 Ethics Committee.

78 *Sanger sequencing*. The first sample of plasma positive for HIV-1 was used for performing

79 genotypic resistance test. RT and PR genotypic analysis was conducted according to the

80 ANRS consensus method [6]. PR and RT mutations were identified using the consensus

81 statement of the list for the TDR genotypic surveillance. [7] Additional interpretations were

82 performed with the International AIDS Society (IAS) list (figure) and the last version of ANRS

83 algorithm (www.hivfrenchresistance.org). Any sequences found to have a mixture of wild

84 type and mutant amino acid residues at single positions were considered to have the

85 mutant.

86 The subtype determination was performed using the HIV Module of SmartGene (SmartGene,

87 Zug, Switzerland) whose methodology is based on Basic Local Alignment Search Tool

88 (BLAST).

89 *UDS*. The steps until pyrosequencing on GS Junior (Roche 454® Life Sciences, Branford, CT,

90 United States) were previously described [8]. Primers used are available on

91 *hiv.frenchresistance.org*. Pyrosequencing was performed according to manufacturer

92 recommendations [9]. GS Amplicon Variant Analyzer (Roche 454® Life Sciences, Branford,

93 CT, United States) was used to analyze the UDS results. Alignments were checked.

94 *Statistical Analysis.* To compare MSM and heterosexual populations baseline

95 characteristics, Chi 2 and Mann-Whitney tests were used for categorical (subtype) and

96 continuous variables (age, viral load, CD4 cells count), respectively.

97 Global prevalence and prevalence of TDR mutations by antiretroviral classes were compared

98 by a Chi 2 test between the two groups.

99 **RESULTS**

100 An average of 5198 reads per nucleotide position was amplified and the average error rates

101 in controls (cellular clone 8E5) were 0.0012 and 0.0032 for RT and PR, respectively. These

102 results allowed for accurate detection of variants down to 1% [10, 11].

103 Characteristics of patients are as follows: the median age was 36.3 years among the 70 MSM

104 patients and 35.4 years among the 70 heterosexual patients (60% female), without

105 significant difference between the two populations. Sixty percent of the patients were

106 infected by HIV-1 subtype B among the MSM group whereas 94% were infected by HIV-1

107 subtype non-B among the heterosexual group. Among the MSM patients, median viral load

108 (VL) was 4.9 $\log_{10}$ copies/mL (IQR=4.4-5.4) and median CD4 cell count was 498/mm^3

109 (IQR=347-585). Among the heterosexual patients, median VL was 4.9 $\log_{10}$ copies/mm^3

110 (IQR=4.3-5.3) and median CD4 cell count was 348/mm^3 (IQR=208-497). The levels of HIV-1 VL

111 were similar between the two groups, but median CD4 cell count was higher among MSM

112 than among heterosexual patients (p= 0.0016).

113 Global prevalence of TDR and prevalence by drug classes are presented in Table 1. A total

114 concordance was found between Sanger sequencing and UDS for all mutations detected on

115 bulk.

116 Sanger sequencing detected two Nucleoside Reverse Transcriptase Inhibitors (NRTI)-TDR

117 mutations in virus genome of one MSM patient (M41L and T215C) and one in virus genome

118 of three heterosexual patients (M41L or T215E or M184I/V); one NNRTI-TDR mutation was

119 detected in virus genome of two MSM patients (K103N) and one in virus genome of three

120 heterosexual patients (K103N or Y181I or Y188L) and one Protease Inhibitors (PI)-TDR

121 mutation was detected in virus genome of one heterosexual patient (I85V).

122 Within each group, we retrieved a higher prevalence of TDR mutations when UDS was

123 performed: prevalence of TDR was 18.6% (95%CI=9.4%-27.7%) with UDS *versus* 4.3%

124 (95%CI=0.0%-9.1%) with Sanger Sequencing among MSM and 22.8% (95%CI=12.7%-33.0%)

125 with UDS *versus* 7.1% (95%CI=0.9%-13.4%) with Sanger Sequencing among heterosexual

126 population.

127 Among the MSM, the increased TDR detected by UDS was observed especially for NNRTI and

128 PI (10.0% *versus* 2.9% by Sanger sequencing and 7.1% *versus* 0.0% by Sanger sequencing,

129 respectively) while it was mainly for NRTI and PI among the heterosexual patients (14.8%

130 *versus* 4.3% by Sanger sequencing and 10.4% *versus* 1.4% by Sanger sequencing,

131 respectively).

132 The rate of TDR mutations only detected by UDS was low: between 1.1% and 4.8% for NRTI,

133 1.1% and 1.5% for NNRTI and 1.1% and 7.0% for PI (Figure 1).

134 Between the two groups, global prevalence of TDR was not significantly different either by

135 Sanger or by UDS. Nevertheless, a higher frequency of NRTI-TDR was observed among

136 heterosexual patients than among MSM (14.8% *versus* 4.3%, respectively; p=0.04).

137 We also retrieved a trend for a higher frequency of TDR among MSM patients infected with

138 HIV-1 subtype B compared to MSM patients infected with HIV-1 subtype non-B (26.2%

139 (n=11/42) *versus* 7.1% (n=2/28) with UDS (*p*=0.06), data not shown).

140 In addition, some resistance mutations, not considered in the list for TDR genotypic

141 surveillance, but considered as major in some genotypic algorithms, were identified as

142 minority variants. For instance, three K65E mutations were detected by UDS. With the last

143 version of ANRS algorithm, the prevalence of resistance to the recommended drugs

144 (European AIDS Clinical Society EACS) detected by UDS for NRTI, NNRTI and IP were 2.9%,

145 12.8% and 7.1% among MSM patients and 11.1%, 5.6% and 11.9% among heterosexual

146 patients, respectively.

147 **DISCUSSION**

148 Higher prevalence of TDR was observed in antiretroviral-naive chronically HIV-1-infected

149 MSM and heterosexual populations when UDS was used compared to population

150 sequencing. Indeed, these powerful techniques could improve the detection of HIV-1-TDR.

151 In the present study, UDS allowed to detect a higher frequency of NRTI-TDR among

152 heterosexual population than among MSM patients, whereas based on the results of CD4

153 cell count, the date of transmission seems to be older in the heterosexual population.

154 Usually, the more time goes by and the less TDR are detected. However, most thymidine

155 analogue mutations were found to be highly stable without selection pressure. It has been

156 shown that NNRTI and PI mutations were, globally, less persistent than NRTI mutations,

157 maybe because of a negative impact on viral fitness [11,12].

158 Otherwise, UDS evidenced a higher frequency of TDR in subtype B versus subtype non-B

159 viruses among MSM patients. This is consistent with what has been previously shown by

160 Sanger sequencing in the Odyssee study on antiretroviral-naive chronically HIV infected

161 patients and in the study on French patients diagnosed at the time of primary HIV-1

162 infection [2,13].

163   We obviously need further investigations for NRTI, second generation NNRTI and PI-based

164   regimen, as the impact of minority TDR mutations on first generation NNRTI is already well

165   known [3,4]. Furthermore, minority TDR mutations on integrase strand transfer inhibitors

166   have not been studied in this work. As few data are available on this subject and still

167   controversial, more studies are needed [14,15]. Nevertheless, an increase of TDR is

168   predictable for this therapeutic class with the extensive use of these drugs and should be

169   monitored in the future.

170   In conclusion, UDS allows sensitive monitoring of TDR, and is able to evidence some

171   disparities of TDR between HIV groups of transmission. In the present study, next generation

172   sequencing technologies probably detect minority resistant variants that are disappearing.

173   The impact of these minority TDR mutations for certain therapeutic classes and in particular

174   for the more recent drugs is unknown and should be further evaluated.

175

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Table 1: Global prevalence of Transmitted Drug Resistance and by drug classes among the two groups: MSM and heterosexual patients.

	Sanger sequencing			UltraDeep sequencing		
	MSM n=70	**Heterosexual population n=70**	**p value**	**MSM n=70**	**Heterosexual population RT n=54 Protease n=67**	**p value**
Global prevalence of TDR [95% CI]	n= 3 4.3% [0.0%-9.1%]	n= 5 7.1% [0.9%-13.4%]	0.47	n= 13 18.6% [9.4%-27.7%]	n=16 22.8% [12.7%-33.0%]	0.53
Prevalence of NRTI-TDR [95% CI]	n= 1 1.4% [0.0%-4.2%]	n= 3 4.3% [0.0%-9.2%]	0.31	n= 3 4.3% [0.0%-9.1%]	n= 8 14.8% [5.3%-24.4%]	0.04
Prevalence of NNRTI-TDR [95% CI]	n= 2 2.9% [0.0%-6.8%]	n= 3 4.3% [0.0%-9.2%]	0.65	n= 7 10.0% [2.9%-17.1%]	n= 4 7.4% [0.4%-14.5%]	0.61
Prevalence of PI-TDR [95% CI]	n=0 0.0%	n= 1 1.4% [0.0%-4.3%]	0.32	n= 5 7.1% [1.1%-13.2%]	n= 7 10.4% [3.1%-17.8%]	0.49

CI: Confidence Interval; MSM: Men Men having Sex with Men; NRTI: Nucleoside Reverse Transcriptase Inhibitors; NNRTI: Non NRTI; PI: Protease Inhibitors; TDR: Transmitted Drug Resistance

Figure 1. Percentage of Transmitted Drug Resistance mutations only detected by UDS among the two groups: MSM and heterosexual patients

Mutations only detected by UltraDeep Sequencing [a]
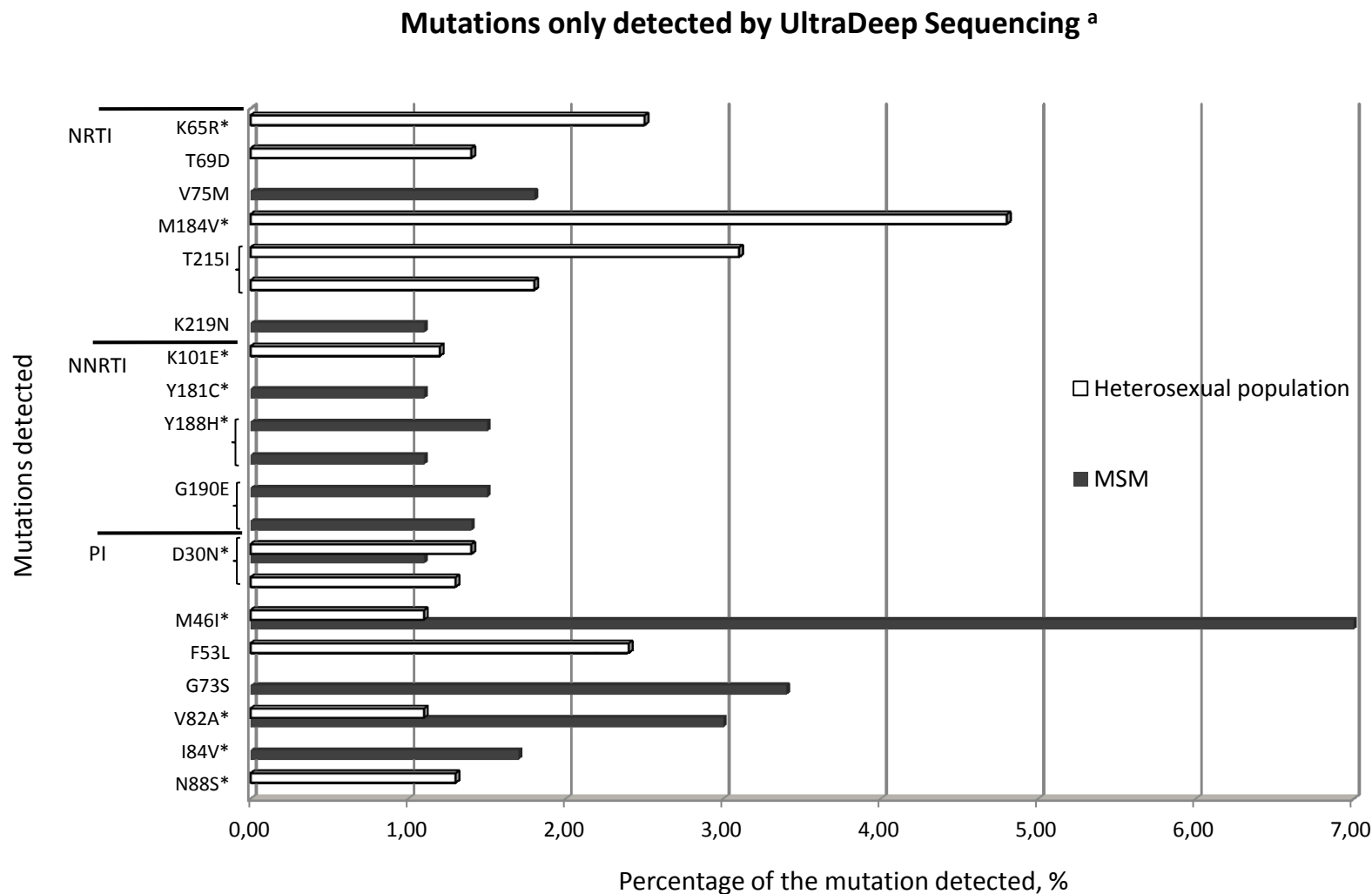
[a]: Each bar represents one patient; * Major IAS drug resistance mutations

MSM: Men having Sex with Men; NRTI: Nucleoside Reverse Transcriptase Inhibitors; NNRTI: Non NRTI; PI: Protease Inhibitors; UDS: UltraDeep Sequencing