Title: Lack of evidence for the selection of E138 mutations by first-generation NNRTIs in patients infected with HIV-1 non-B subtypes: a comment on Crawford et al.

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Word Count: 749 words

Funding: This work was supported by the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (G.0692.14N), by the AIDS Reference Laboratory of Leuven that receives support from the Belgian Ministry of Social Affairs through a fund within the Health Insurance System, by BEST HOPE: Bio-Molecular and Epidemiological Surveillance of HIV Transmitted Drug Resistance, Hepatitis Co-Infections and Ongoing Transmission Patterns in Europe (project funded through HIVERA: Harmonizing Integrating Vitalizing European Research on HIV/Aids, grant 249697) and by L'Oréal Portugal Medals of Honor for Women in Science 2012 (financed through L'Oréal Portugal, Comissão Nacional da Unesco and Fundação para a Ciência e Tecnologia). KT is funded as a Postdoctoral Researcher by the Research Foundation - Flanders (FWO).

31 **Text**

32 Understanding the mechanisms and predictors of drug resistance development to HIV-1
33 antiretroviral treatment (ART) is a key challenge to further improve HIV-1 treatment strategies
34 and prevent epidemic spread. The extensive genetic diversity characterizing the HIV-1
35 pandemic, classified into groups, subtypes and recombinants, has been shown to affect viral
36 escape from drug selective pressure and influence treatment decisions [1]. In previous issues of
37 AIDS, Crawford and colleagues reported the selection of mutations E138A/G/K/Q upon
38 treatment failure with first-generation non-nucleoside reverse transcriptase (RT) inhibitors
39 (NNRTIs) efavirenz (EFV) or nevirapine (NVP) in patients infected with subtypes A1, D and
40 recombinant form A2D [2,3]. Amino acid substitutions at polymorphic position E138 in the RT
41 enzyme recently acquired clinical relevance by their association with reduced *in vitro* activity
42 and *in vivo* response to second-generation NNRTIs etravirine (ETR) and rilpivirine (RPV)
43 [4,5,6,7]. Crawford and colleagues reported their findings as unique given that, in their dataset,
44 mutations at E138 were selected 1) in non-B subtype viruses and 2) upon treatment exposure
45 with first-generation NNRTIs [2].

46
47 HIV-1 genotypic complexity challenges the identification and interpretation of resistance-
48 associated mutations, and we feel that the confounding effect of HIV-1 epidemiology weakens
49 the authors' claims, which are not sufficiently supported provided the reported study design.
50 The authors report the occurrence of mutations E138A/G/K/Q in 12.1% (respectively 6.1%,
51 2.6%, 1.7% and 1.7%) of 115 patients failing therapy with resistance mutations at resistance
52 testing. It is well established that evidence for selection of a mutation by a specific drug
53 requires genotypic information both before drug exposure and at viral breakthrough. In
54 Crawford *et al.*, data on baseline mutational patterns in drug-naïve patients were missing and
55 the authors did not acknowledge the polymorphic nature of this codon position, with mutations
56 occurring naturally at frequencies that vary according to viral subtype [8,9]. Therefore, from
57 the information presented, it is not possible to discriminate between two plausible hypotheses:
58 independent selection of these mutations due to convergent evolution under drug pressure with
59 first-generation NNRTIs or their presence as natural polymorphisms prior to therapy initiation.

60

61 Before the advent of second-generation NNRTIs, E138 mutations were not systematically
62 monitored in HIV-1 resistance studies. By then, selection of E138 mutations by NVP and EFV
63 was less well documented, and only patchy information was available on a phenotypic impact
64 towards first-generation NNRTIs [10,11]. The wide and long-standing use of EFV and NVP have
65 resulted in abundant genotypic data on NNRTI-containing regimens, with E138 mutations
66 present at the time of treatment failure. However, this is no proof for its selection upon
67 treatment exposure [8]. In fact, a recent study by Sluis-Cremer et al., comparing E138A
68 prevalence according to treatment-experience in large patient populations infected with subtype
69 B and C viruses, showed that E138A is polymorphic in drug-naïve patients and that E138A
70 frequency is not strongly affected by prior RTI exposure [9]. Besides drug pressure, a high
71 prevalence of E138 mutations in specific patient populations could be due to HLA-restricted CTL
72 pressure, with certain HLA-carrying individuals having higher prevalence of the natural
73 polymorphism E138A [12], due to local transmission clusters of a particular viral variant [11] or
74 due to founder effects created by subtype-specific epidemics.

75

76 While we argue that evidence for selection with first-generation NNRTIs is insufficiently
77 supported, the selection of E138 mutations by second-generation NNRTIs has been observed
78 and described in clinical isolates. However, evidence is available for its selection in various HIV-
79 1 subtypes, and not exclusively in subtype B, in contrast to what the authors state [1]. Whereas
80 research and drug design were historically skewed towards HIV-1 subtype B, dominant in
81 developed countries, there has been an increased attention for the challenges of HIV-1 diversity
82 and specifically the impact of globally predominating non-B subtypes on resistance
83 development. As such, knowledge on subtype-specific mutations and pathways has been
84 acquired from genotypic and phenotypic analyses of major HIV-1 subtypes, with subtype
85 differences being acknowledged by expert-based drug resistance interpretation systems
86 [14,15,16]. We therefore feel that the paradigm claimed by the authors that antiretroviral drug
87 resistance mutation patterns and resistance algorithms are derived from *in vitro* and clinical
88 studies of subtype B viruses and infections is no longer valid.

89

90 In conclusion, insufficient evidence was provided for the original claim of E138 mutation
91 selection by first-generation NNRTIs in non-B subtypes given that a baseline drug-naïve
92 population was not studied and that potential subtype, geographical origin or host effects were
93 not considered. We suggest that additional analyses should be performed to validate the
94 hypothesis of subtype-dependent selection of E138A/G/K/Q by *in vivo* treatment with EFV and
95 NVP.

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