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Short communication

In-vivo selection of the mutation F121Y in a patient failing raltegravir containing salvage regimen

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ABSTRACT

Raltegravir is an integrase inhibitor (INI) licensed for clinical use and other INI are in advanced stage of development. Different resistance mutations in HIV integrase from patients using these antiretroviral drugs have been described and G148H/R/K, N155H and less frequently Y143C/H/R are considered major resistant mutations to raltegravir. Both Stanford Database and Geno2Pheno list F121Y as conferring intermediate resistance "in vitro" both to raltegravir and elvitegravir. We report for the first time the "*in vivo*" selection F121Y and evolution to Y143R in a 31 years old male clade B HIV-1 infected patient failing a raltegravir-containing salvage regimen. Plasma samples nine months prior to raltegravir (RAL-Naïve) and at weeks 32, 40 and 88 after RAL-containing regimen were analyzed. Antiretroviral susceptibility was evaluated at Stanford and Geno2Pheno from sequences obtained with RT-PCR. After a Viral load at week 12 below 50 copies/mL, viremia raised at week 20 to 4.5log10. The emergence of F121Y was observed at week 32 and 40, alongside with L74I, T97A, Q137H and V151I. At week 88 F121Y was no longer detected, L74I and T97A were maintained and Y143R emerged. F121Y might be an alternative pathway to Y143R. Changing of RAL-containing regimen upon the identification of F121Y might avoid the evolution of raltegravir resistance.

Integrase inhibitors (INIs) are an important addition to the HIV infection treatment armamentarium. Licensed in 2007, raltegravir (RAL, Merck Laboratories) is the first INI approved for clinical use (FDA, 2007). Other drugs in this class, such as elvitegravir (Gilead Sciences) and dolutegravir (GlaxoSmithKline), are in advanced stage of development (van Lunzen et al., 2012; Molina et al., 2012). In Brazil, raltegravir is used as part of salvage regimens of patients with documented resistance to multiple antiretroviral drugs (http://www.aids.gov.br/sites/default/files/folder_consenso.pdf).

Despite its high activity when combined with optimized background therapy (OBT), different mutations confer loss of susceptibility to raltegravir, as well as to other INIs (Steigbigel et al., 2008; Shimura et al., 2008; Rowley, 2008; Malet et al., 2009; Mbisa et al., 2011). The pathways G148H/R/K, N155H and, less frequently Y143C/H/R, lead to an important viral resistance to raltegravir (Cooper et al., 2008). Also, some of these mutations also confer cross-resistance to other INIs, such as Q148H/R/K to elvitegravir (Shimura et al., 2008; Malet et al., 2009; Mbisa et al., 2011; Blanco et al., 2011). Dolutegravir seems less susceptible to genetic resistance (Canducci et al., 2011), but different combinations of substitutions Q148H/K/R, G140S/A and E138 K/A may reduce its susceptibility by 10- to 20-fold (http://hivdb.stanford.edu/pages/drugSummaries.html). Along substitutions associated to the loss of susceptibility to raltegravir, non polymorphic accessory mutations can emerge during therapy as result of selective pressure. Moreover, natural polymorphisms of the integrase gene, such as V151I and I72 V, have been associated to a small decrease in susceptibility to INIs (Passaes et al., 2009; Low et al., 2009). Marinello et al. (2008) documented the negative impact of the F121Y substitution on integrase strand transfer activity, while integration patterns remains unchanged. Moreover, albeit never described in clinical isolates, HIV Stanford Resistance Database and Geno2Pheno both list 121Y as conferring a 5–10-fold decrease in raltegravir (Kobayashi et al., 2008; Rowley, 2008; Blanco et al., 2011) and elvitegravir (Shimura et al., 2008) susceptibility, leading to an intermediate resistance profile. Identification of potential mutational pathways is important to understand the evolution of resistance patterns and the drug susceptibility in HIV-1 infection.

In the present study we report the *in vivo* selection of the non-polymorphic substitution F121Y in a 31 years old male patient, diagnosed in August 1998 with HIV-1 infection, who underwent six

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treatment regimens (starting with HAART: zidovudine, lamivudine and nevirapine) prior to the use of RAL-containing therapy. We evaluated the evolution of the viral integrase from a genotype previous fully susceptible to integrase inhibitors to a F121Y intermediate resistant strain, and subsequently to an Y143R fully resistant profile analyzing protease/reverse transcriptase (referred as "polymerase") and integrase genotypic population sequencing from blood samples prior to raltegravir exposure, collected in 2009 (RAL-NAÏVE), and at weeks 32, 40 and 88 after introduction of RAL-containing regimen. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to manufacturer instructions. Viral load was determined using bDNA method (Versant 3.0 Siemens, Germany) and CD4 + T-cells were measured by flow cytometer (FACS Calibur, BD, USA) during regular clinical follow up at the local laboratory. The study was approved by the ethical committees of the institutions involved.

Polymerase genotyping was performed using TRUGENE® HIV-1 Genotyping Assay or OpenGene® DNA System (Siemens, USA) and a one step RT-PCR using proof reading enzyme, adapted from Van Laethem et al. 2008, followed by a nested PCR to amplify the complete integrase gene. The PCR product was then submitted to direct sequencing using BigDye® v3.1 Cycle Sequencing kit (Applied Biosystems, USA), resolved in an ABI3130XL (Applied Biosystems, USA). Three independent replicate integrase sequences were obtained from each sample. The sequences were assembled and edited using Sequencher 4.7 (GeneCodes, USA). Sequences Accession numbers: JQ797715 to JQ797734.

Resistance mutations and susceptibility to antiretroviral drugs were analyzed according to Stanford Resistance Database (Supplementary data 1, SD-1), Geno2pheno$_{[resistance]}$, IAS 2011 mutation list (Johnson et al., 2011) and the ANRS algorithm. Sequences were aligned with HXB2 reference sequence using BioEdit v.7.0.9. Subtype screening was done at NCBI Genotyping and REGA BioAfrica websites, confirmed by phylogenetic reconstruction of Neighbor Joining and Maximum Likelihood trees using Paup$*$ 4.10b (SD-2). Viral load, CD4, antiretroviral treatment, resistance mutations and sampling time points are depicted in SD-3.

Polymerase genotyping (see SD-1) prior to raltegravir exposure predicted a high-level resistance profile to all NNRTI and NRTI except for etravirine, which showed a potential low-level resistance score according to Stanford Database (G190A). As the patient had no prior exposure to the drug and did not use other NNRTI in the year preceding this sampling, the drug was considered here as fully active. The virus had high-level resistance to all PI drugs except for darunavir/r, which exhibited an intermediate resistance profile (I47V, I50V, I84V, L89V). Therefore, the patient started raltegravir regimens at best with one additional active drug (etravirine) and one partially active drug, darunavir/r. This fact may have been determinant for the virological failure within a few weeks. Samples weeks 40 and 88 showed high resistance to etravirine (E138Q, Y181C and G190A). Therefore, after 40 week of exposure the regimen contained only a partially active darunavir.

On the first available sample obtained after raltegravir introduction on the regimen (week 32) the substitution F121Y was observed on all replicate sequences. Alongside this mutation, the emergence of L74I, T97A, Q137H and V151I was observed, as well as synonymous polymorphisms in codon 167. Mutations previously described with F121Y "in vitro", as T124A and T125 K (Menéndez-Arias, 2010) were not found in these sequences. No relevant change was observed from week 32 to week 40. At week 88, a reversion was observed at positions F121Y, Q137H and V151I to the wild type amino acid, maintaining T97A and showing the emergence of K42R, V72I, L234I, V258I and the major resistance mutation Y143R. T97A is a polymorphic substitution, selected by raltegravir and is related to Y143R/C (Canducci et al., 2009). Although not directly associated to resistance, this mutation is synergic to Y143 resistant mutants, as it is capable of restoring the replication capacity of the virus (fitness), and it is expected to emerge after the fixation of 143R (Delelis et al., 2010; Reigadas et al., 2011). The viral load documented during the presence of F121Y and T97A is over half log below historical values. However, it was also documented during previous regimens (SD-3) and therefore cannot associate these mutations to a change in replicative fitness.

To determine the proportion of polymorphic positions in the integrase gene and contextualize the amino acid substitutions of the patient's virus, all 5102 complete integrase sequences available at LANL were downloaded. Subtype B sequences ("B global" alignment, $n = 2523$) were selected for amino acid composition comparison. As expected, the consensus of those sequences was identical to the Consensus B available at LANL. The RAL-NAÏVE sample did not exhibit resistance mutations to integrase inhibitors, but had mutations both in polymorphic positions, as E11D, observed in 26.9% of the B global alignment, as well as in non polymorphic positions, such as Q164 K, occurring in only 0.0004% of sequences. See Supplementary data 4 for amino acid alignment of all study time points. In addition to amino acid substitutions, silent nucleotide substitutions were observed. In a total of 13 nucleotide substitutions in 143-strains, five were observed only in 121-strains. This could indicate an evolution of 143-strains from a 121-strain precursor. Analysis of the phylogenetic reconstruction shows an evolutionary pattern, with the RAL-Naïve sequences situated closer to the main subtype B branches, with raltegravir resistant strains further away on the branch. However, the weeks 32/40 (121-strains) and week 88 sequences (143-strains) are located at two separate terminal branches (bootstrap 89), which may suggest an independent evolution of both "121Y" and "143R" strains. Our data therefore cannot determine if a 121Y variant is the origin of the 143R variants of if it evolved directly from other precursors.

In conclusion, this study documents the association of the emergence of F121Y plus L74I, T97A, Q137H and V151I mutational pattern to the virological failure of RAL-containing regimen, followed by a reversion of the F121Y substitution and appearance of Y143R after continuous exposure to the drug. Thus, these results reinforce the role of F121Y on raltegravir resistance, and suggest that F121Y could be an alternative resistance pathway to raltegravir, less fit and therefore substituted by 143R strains. F121Y and these secondary mutations could also be an intermediate step towards the emergence of Y143R. However new generation sequencing or clonal studies are necessary to clarify the mutational pathways and phenotypic studies are necessary to elucidate the impact of these mutations on drug susceptibility and on integrase activity. In either way the change of RAL-containing regimen upon the identification of F121Y might avoid the evolution of raltegravir resistance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2012.04.007.

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